

AN ABSTRACT OF THE THESIS OF

Theodosius Marmaduke Velloza for the degree of Doctor of Philosophy in Crop Science presented on November 4, 1997.

Title: Performance of Perennial Ryegrass (*Lolium perenne* L.) Seed Crops Under Water Stress Conditions.

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Thomas G. Chastain

Summer drought is a major factor limiting the regrowth of perennial ryegrass seed crops. This phase of crop development has a strong influence on seed yield because most of the tillers that contribute towards next season's seed crop are produced or regrown during this period. In recent years many seed fields have exhibited premature decline probably because of extensive drought after harvesting. Therefore, this study was undertaken to (i) assess how post-harvest leaf and tiller development is impacted by the timing and severity of water stress and (ii) identify potential relationships of water stress to flowering and seed yield.

Rain-out shelters were used to exclude rainfall from two cultivars which received either no irrigation or 2.5 cm of simulated rainfall in mid-August or mid-September or both. These were compared to an ambient treatment. No rainfall decreased total tiller production by approximately 30% in 1995 and 50% in 1996. There were also moderate reductions in tiller dry weight, tiller height, and slight decreases in number of leaves and the basal diameters. The trend showed that the cultivar Affinity responded quicker to an

early irrigation whereas the cultivar Buccaneer had a longer period of summer dormancy. Total soluble sugars concentrations increased as tiller number decreased suggesting the potential for rapid compensatory growth upon alleviation of drought. Limited irrigation during the post-harvest period of regrowth did not generally affect fertile tiller number nor seed yields. Fewer vegetative tillers as the stands aged, together with other changes in plant parameters, may mark the beginning of the dieback problem.

In greenhouse studies, four cultivars were rapidly stressed using vermiculite as a growth medium. Though little differences were observed among cultivars when physiological responses were evaluated, stomatal diffusive resistance and leaf temperatures increased, whereas plant water potential and leaf transpiration decreased as stress was prolonged. Plant survival following water stress was largely dependent on cultivar and gravimetric water content of vermiculite. This technique did not reasonably simulate natural drought conditions in terms of plant physiological performances nor soil characteristics, but it was useful to differentiate the ability of different genotypes to survive a drought-induced dieback.

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Performance of Perennial Ryegrass (*Lolium perenne* L.) Seed Crops Under Water Stress Conditions

by

Theodosius Marmaduke Velloza

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Theodosius Marmaduke Velloza, Author

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TMV.

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PERFORMANCE OF PERENNIAL RYEGRASS (*Lolium perenne* L.) SEED CROPS UNDER WATER STRESS CONDITIONS

INTRODUCTION

Deficiency of water limits growth of grasses (Powers, 1971). Often, water is adequate during winter and spring but deficient during the summer and early fall, when evapo-transpiration increases and precipitation decreases.

It is well known that soil water deficits -- even small deficits of a few cm -- reduce the yield of temperate grass crops (Stiles and Williams, 1965; Leafé et al., 1977). Nevertheless, grasses can show a remarkable resistance to severe water stress; they are able to survive very large soil water deficits even though yields are drastically reduced.

During drought, a number of interrelated factors can limit growth of the sward. These include soil water stress, atmospheric stress (high radiation causing a high evaporation load), shortage of available nutrients and supra-optimal temperature (Morrison et al., 1974 as cited by Norris, 1982).

There are, as yet, no reliable physiological criteria for selecting temperate grasses for resistance to drought or subsequent recovery (Thomas and Evans, 1991). The alternative approach, direct selection in the field, is complicated by unpredictable weather and the confounding effects of management and heading date (Hughes et al., 1977, as cited by Thomas and Evans 1991).

Irrigation is recognized to be expensive, and grass, being considered a low-valued crop, has led to the opinion that irrigation of grasslands, while economically valid in some cases, is probably unprofitable in others (Stiles and Williams, 1965).

Determination of the conditions for the optimum response of herbage yield to irrigation, and of the response under optimum conditions, should lead to a more efficient use of water, which tends to be least available when it is most needed, and to a better assessment of the expedience of irrigation in particular conditions of climate and crop use (Stiles and Williams, 1965).

Under natural conditions, water stress usually does not develop suddenly; rather, it increases gradually (changes requiring several hours are gradual, relative to molecular events in the cell) (Hsiao and Acevedo, 1974). Therefore, the process most sensitive to stress is normally altered first, and such alterations, in turn, may lead to many secondary and tertiary changes.

Water deficit affects processes such as cell growth, cell wall synthesis, protein synthesis, stomatal opening, nitrate reductase level, abscisic acid accumulation, carbon dioxide assimilation, respiration, proline accumulation, and sugar accumulation.

The harmful effects of rather severe water stress can be both metabolic and strictly physical, e.g., cavitation (vapor blockage) of xylem vessels, resulting in increased xylem resistance to water flow. In general, a water stress during vegetative growth that is not severe and long merely postpones plant growth and development to a later date (Hsiao and Acevedo, 1974).

Loss of tissue water may be expected to have the following physical and chemical impacts:

1. The chemical potential or activity of cellular water is reduced
2. Turgor pressure decreases in the cell

3. Small molecules and macro-molecules become more concentrated and spatial relations in the plasmalemma, tonoplast, and membranes of organelles are altered as cell volume is reduced, and
4. Macro molecules may be affected through the removal of water of hydration or through modifications of the structure of adjacent water.

Quantification of yield and the relationship with water stress is very difficult since casual connections between many detailed processes and yield are unknown and the effects are dependent on severity, duration, and the time of occurrence of stress within the growth cycle (Hsiao, 1973).

The tiller is the fundamental demographic unit of perennial grass populations. Tillering is a continuous process, and thus at any time the grass plant comprises a collection of tillers, differing in age, position on the plant, and size (Langer, 1980). As floral induction and initiation take place only a portion of all tillers become reproductive, while the others will be subjected to increasing competitive stress as stem elongation and seed development increase the need for assimilates in fertile tillers (Langer, 1980).

Tillering in grass plants is primarily dependent on two internal plant factors: leaf appearance rate and site filling (Davies, 1974). Site filling is a measure of the readiness of the leaf axillary bud to develop new tillers, and is expressed by the number of new tillers per tiller per leaf appearance interval (Davies, 1974).

Due to growth-limiting factors, tiller formation from leaf axillary buds can be delayed or suppressed entirely. Neuteboom and Lantinga (1989) emphasized that leaf

appearance rate can only be determined by marking and counting leaves on single tillers on consecutive dates.

The components of seed yield in a grass seed crop can be sub-divided as seed weight, number of fertile florets per spikelet, number of spikelets per tiller, number of reproductive tillers per plant and the stand density (Marshall, 1985). It seems logical that most environmental stress can impact adversely on one or more of these components resulting in a reduction in seed yield. There is a dearth of information on the effect of water supply on fertile tiller number, but irrigation could be beneficial on very shallow soils. However, the time at which fertile tillers arise does not generally coincide with periods of water stress probably because it is the most recently formed tillers that tend to die first (Hill and Watkin, 1975).

Fertile tiller number are the most important component of seed yield, hence, any management system designed to stimulate early tillering and tiller survival should result in improved yields (Hill and Watkin, 1975; Langer, 1980). These early tillers contribute the major proportion of the inflorescences, and are individually larger, thus, more valuable (Langer, 1980).

The Pacific Northwest (PNW) region of the U.S., including the states of Idaho, Oregon and Washington, is uniquely adapted to seed production of temperate grasses (Youngberg, 1980). The Willamette Valley of Oregon is the most concentrated area of seed production and produces all of the U.S. seed of *Lolium perenne* L. (perennial ryegrass) (Youngberg, 1980).

The connection between crop development and the environment can be established through observation of phenological events. The benefits of this approach include (i) better understanding of the impacts of management and environment on growth, development, and seed yield and quality, (ii) improved timing of water application for increased efficacy, (iii) improving the present management practices, and (iv) developing better cultural strategies for the future.

The post-harvest regrowth period is a critical phase of seed crop development that can have a strong influence on seed yield. Perennial grass plants enter a dormant period of 35-40 days after harvest as a result of the dry summer conditions (Youngberg, 1980). This dormancy is an important adaptive response for summer survival (McWilliam and Kramer, 1968). Extremely dry summers as experienced in the PNW no doubt impose a low water stress in the field and delay regrowth of the grass. Apparently, tillers must reach a critical size and height before they are capable of receiving the flower induction signal (Heide, 1994). It seems plausible therefore, that any factor adversely affecting tiller growth will lower induction and subsequent flowering and seed yield.

REVIEW OF LITERATURE

Plant responses to water stress vary with the stage of growth of the plant. For example, the leaf tissues of some cultivars of sorghum were less susceptible to desiccation injury prior to anthesis than at the post-anthesis stage (Sullivan and Eastin, 1974). Water deficit reduced leaf extension rates, rates of leaf appearance, tiller number, leaf length (Garwood and Williams, 1967; Norris, 1982; Barker et al., 1989) and tiller height (Busso and Richards, 1995), but increased root initiation and elongation (Jupp and Newman, 1987a).

Both growth (size) and development (timing of tiller emergence, foliar stage of tiller at the end of the experiment) were affected by a period of cessation of watering in wheat. The number of primary tillers was little affected, but there were drastic effects on secondary tillers; the presence of previously formed side shoots was not beneficial for recovery of the main shoot (Cabeza et al., 1993). Yield and yield components may also be influenced by timing of water stress. For instance in wheat, the consequence of water stress applied at heading was more severe than a stress at tillering (Moustafa et al., 1996).

Drought has diverse effects on different kinds of grasses. For instance, water deficit affected leaf area proportionately more than net assimilation rate (NAR) in Altai wildrye (*Leymus angustus*) and crested wheatgrass (*Agropyron cristatum*), but not in smooth brome grass (*Bromus inermis*) (Bittman and Simpson, 1987).

Changes in leaf morphology appear to play an important part in the response of perennial ryegrass to water stress in the field. For instance, leaves that grew under slowly

developing conditions (as occur in a field), were smaller, thicker, had higher stomatal density, smaller epidermal cells, and a more pronounced leaf ridging on their adaxial surface (Leafe et al., 1977; Jones et al., 1980b). Therefore, the adaptation to water stress was a combination of osmotic and structural adaptation associated with corresponding physiological adaptations. In general, stress developed more rapidly (over 5 days) in simulated swards, but gradually (over 4 wk) in field-grown plants. Thus, plants in the field are able to undergo physiological and morphological changes that help minimize the adverse effects of water stress.

Leaf extension may be reduced considerably by water shortage (Thomas and Evans, 1991; El Nadi et al., 1969; Jones et al., 1980b; Volaire, 1995). The response, however, varies with the kind of plant. The leaves of *Vicia faba* elongated almost immediately after watering (El Nadi et al., 1969), while perennial ryegrass had a fairly lengthy recovery period (Thomas and Evans, 1991). After only two weeks of drought leaf extension had declined considerably but turgor was maintained in perennial ryegrass (Thomas, 1991). This suggested that leaf growth in the early stages of drought was reduced, not by turgor, but by limitations in water supply to the expanding cells or in cell wall extensibility. Plant response to water stress in terms of cell growth is a complex interaction of a decrease in threshold turgor, a degree of osmotic adjustment to maintain turgor, and possibly an increase in gross extensibility of the cells (Jones, 1988).

Initial diurnal variation in the rate of leaf extension was small but the leaves tended to grow faster at night (Jones et al., 1980b). As stress became more severe, leaf extension was markedly faster at night although the overall rate of extension had declined. In

rapidly stressed simulated swards, leaf extension was unaffected until four days after irrigation had stopped, but subsequently the rate of extension declined rapidly and progressively (Jones et al., 1980b).

Rates of leaf extension have been correlated with temperature (Peacock, 1975; Baker and Younger, 1987). The association between soil temperature and leaf extension rate in spring was such that for every 1 °C rise in temperature, leaf extension rate increased by approximately 0.4 mm day⁻¹ over a range of 0.2 to 6.7 °C (Baker and Younger, 1987). However, since measurements of soil and air temperatures are closely related it is difficult to distinguish whether it is the temperature of the soil or air at a particular level in the canopy, that is influencing leaf growth (Peacock, 1975). Factors such as soil fertility and previous management also have a modifying influence on this response.

The interval between the appearance of new leaves was increased in stressed field swards (Jones et al., 1980b). New leaves appeared more than twice as fast in the irrigated than non-irrigated swards, and it was observed that a combination of slower leaf appearance and leaf extension contributed to a marked decrease in the leaf area expansion in the stressed field swards.

As water becomes limiting, leaf conductance generally decreased with leaf water potential. However, much of the variation (in conductance) was not related to leaf water potential suggesting that the rate of water loss from the leaf affected stomatal response to tissue water loss (Bittman and Simpson, 1989a). Perennial ryegrass stomates began

closing at a leaf water potential below -13 bars resulting in a decrease in transpiration and an increase in leaf resistance (Jones et al., 1980b).

Although the grass plant is perennial, its component tillers are short-lived. Persistence is thus determined by the plant's capacity to replace dying tillers. The tillers form a hierarchy of sinks in which competition for assimilates increases with time as the sward becomes established. Competition is especially acute during stem elongation and results in the death of a high proportion of the small, non-flowering tillers (Colvill and Marshall, 1984). The flowering tillers become dominant and their stems form the major sink for assimilate from the leaves even during seed filling.

The capacity of a grass plant to regrow after defoliation depends on factors relating both to past management and genotype, one of which is the amount and proportion of green leaf tissue remaining in the stubble. For example, when less leaf sheath remained, leaf length was as much affected as leaf appearance. On the other hand, no reduction of leaf length occurred when the tubes of leaf sheaths remained (i.e., removal of leaf blade only), and the removal of lower leaves had little effect on the relative growth rate of the plant (Davies, 1974). When combined with drought, clipping produced the lowest regrowth and herbage accumulation (Busso and Richards, 1995), probably because of low carbohydrate reserves (Boschma et al., 1997). However, it had been suggested (Davies, 1974) that regrowth in vegetative plants related more to stubble weight rather than to the leaf area.

The main shoot and primary tillers produced during summer had slightly longer inflorescences with one or two additional spikelets compared with tillers produced during

the autumn and winter, but the greatest difference was in the number of florets/spikelet (Colvill and Marshall, 1984). The main shoots and primary tillers had almost 50% more florets/spikelets than tillers appearing in the first month of the following year. Thus, older flowering tillers produced significantly more florets/inflorescence than younger tillers, and as there was about 70% seed set by tillers of all ages and origin, older tillers produced a greater number of seeds/inflorescence. The mean seed weight was also higher in older tillers.

Different selection lines of perennial ryegrass were also greatly affected by drought. Even though high-tillering lines had higher regrowth potential when irrigated, they recovered from drought no better than low-tillering lines (Thomas and Evans, 1991). Tiller number also declined during water stress in both lines. This range of genetic variation in osmotic and water potentials was also observed within a single cultivar of perennial ryegrass during drought (Thomas, 1987).

Herbage (dry matter) yields were reduced with corresponding reduction in leaf area index (LAI) caused by a slower rate of leaf expansion, resulting in smaller leaves, and a slower rate of appearance (Leafe et al., 1977). Conversely, as the frequency of irrigation increased the dry matter yields also increased, and there were consistently lower yields in under-irrigated fields compared with the fully-irrigated fields watered at the same time (Stiles and Williams, 1965). However, in crested wheatgrass (*Agropyron desertorum*) and blue bunchgrass (*Pseudoroegneria spicata*), three consecutive years of drought treatment were required before dry matter declined (Busso and Richards, 1995).

In the field, herbage growth rate was negatively correlated with osmotic potential when plants were well watered, i.e., faster growing plants had a higher concentration of osmolytes (Amin and Thomas, 1996; Thomas and Evans, 1989). However, in controlled environment, there was a positive correlation between osmotic potential and hydration (Thomas and Evans, 1990) but, hydration was negatively correlated with herbage growth rate for both irrigated and drought-exposed plants. These plants with an inherently low ratio of tissue water to dry matter grew faster.

In stressed fields, there was little reduction in the rate of photosynthesis of individual leaves with the lower rate of photosynthesis being due to a reduction in leaf area and hence a lowering of light interception. In contrast, in a controlled environment, a decline in canopy photosynthesis was due to a decline in individual leaf photosynthesis caused by stomatal closure (Leafe et al., 1977; Sheehy et al., 1975). As a result, caution should be taken with regards to extrapolating from controlled environment to the field, in order to have a full understanding of the effects of water stress on crops (Amin and Thomas, 1996).

Under slow drought the decline in tissue water content was due mostly to a change in hydration and the relative water content (RWC) declined significantly after 8 weeks. In rapid drought there is little opportunity for developmental changes in hydration to occur, and RWC declined rapidly after only a few days (Thomas, 1991). Since there was a significant difference, during the recovery period, between lines of high and low water potential (Thomas and Evans, 1991), selecting lines with low water potential led to a low rate of autumn growth and no improvement in drought tolerance or in recovery.

Higher canopy temperatures were indicative of greater water stress (Frank, 1994, Ehrler et al., 1978). Ehrler et al. (1978) found that moisture content in the root zone of wheat controlled plant water potential which, in turn, influenced the temperature difference between the canopy and the air.

The drier the soil the deeper roots grow, i.e. soil water influences root distribution in the profile but hardly affects root weight. For example, with beans, roots continued to grow at the same rate during flowering (but no pods were developing) as in the vegetative phase, leading to the conclusion that fruit setting and fruit growth, not flowering, reduced root growth (El Nadi et al., 1969). However, in perennial ryegrass drought reduced the relative growth rate of the root system more than that of the shoot system, resulting in a decrease in the root:shoot ratio (Gales, 1979). It has been established that there was a large concentration of roots of perennial ryegrass in the 0-10 cm horizon although the effective rooting depth of utilization of water for maximum dry matter yield was approximately 80 cm (Garwood and Sinclair, 1979).

Phosphorus uptake is reduced immediately after a period of drought, and recovery in the rate of uptake occurred 2-3 weeks after rewetting. This reduction occurred regardless of whether the plants were mycorrhizal (Jupp and Newman, 1987b). The inability of a grass sward to regrow after cutting when available moisture has been removed from the uppermost horizon is probably due to a deficiency of plant nutrients, the major deficiency being nitrogen (Power, 1971; Garwood and Williams, 1967). In fact, if nutrients were available from the sub-soil some of the effects of drought in depressing growth could be alleviated (Garwood and Williams, 1967). For instance, it has been

suggested that, in some circumstances, the availability of phosphate in the soil may influence the effect of drought on root:shoot ratios (Gales, 1979). It may be concluded that most of the available nutrients are concentrated in the surface horizon of the soil and are unavailable as the upper horizons dry out.

Drought affects many physiological processes in plants (Chaves, 1991; Hsiao, 1973; Levitt, 1972; Morgan, 1984), with consequent changes to the metabolic pathways. Changes in carbohydrates levels are of particular interest to researchers because of the direct relationship to processes such as translocation, respiration and photosynthesis (Kameli and Lösel, 1993). Water stressed plants tend to have higher levels of carbohydrates than non-stressed plants because growth is impaired before photosynthesis declines (Hsiao, 1973). For instance, both total nonstructural carbohydrates (TNC) (Busso et al., 1990) and water soluble carbohydrates (WSC) contents (Frank, 1994) were higher in drought-stressed than in irrigated grasses.

In wheat, fructose and glucose levels were found to be more sensitive than sucrose to the degree of water stress (Kameli and Lösel, 1993). This rapid increase in the accumulation of low molecular weight carbohydrates in wheat stems suggested that fructan depolymerization may be a sensitive test for drought stress (Virgona and Barlow, 1991). The increase in low molecular weight carbohydrates (possibly caused by depolymerization) led to a large decline in osmotic potential. Together with the hexoses, the sugar alcohols, mannitol and arabitol, accumulated during drought in endophyte-infected tall fescue (*Festuca arundinacea*) (Richardson et al., 1992).

Although WSC content increased in perennial ryegrass during drought, most of the WSC were found in the leaf bases with little in the laminae (Thomas, 1991). Increase in WSC was also observed in orchardgrass (*Dactylis glomerata*) during summer drought (Volaire, 1995) or under simulated conditions (Volaire and Thomas, 1995). However, the levels of starch (Chaves, 1991) and small fructan molecules (degrees of polymerization of 3-5) (Thomas, 1991) decreased in leaf bases during prolonged water stress. This led Thomas (1991) to suggest that the fructan to oligosaccharide ratio may be important in regulating osmotic potential during drought.

The WSC reserves are considered an important source of carbon for the regrowth of perennial grasses following defoliation (White, 1973; Daer and Willard, 1981) or summer drought (Volaire, 1994, 1995). A close correlation existed between WSC accumulation and recovery from summer drought in the fall. The concentrations of WSC in the leaf bases of *Dactylis glomerata* followed a seasonal pattern with increases in summer (drought) but a decline in spring (Volaire, 1994). Fructan levels showed similar trends (Waite and Boyd, 1953). These fluctuations may be attributed to changes in the rate of growth, slow in summer or fast in spring (White, 1973). In *Lolium perenne* defoliation had a greater effect than drought on carbohydrate reserves during the summer-fall period of regrowth (Boschma et al., 1997). Recovery growth in autumn was closely related to tiller density, WSC and fructan contents in leaf bases recorded in August, but dry matter was highly correlated with glucose and fructose contents in leaf bases in September (Volaire, 1995).

The presence of a deep tap root system was of major importance for the survival of summer drought of perennial *Phalaris spp.* when compared to its annual relatives. The deep root system enabled the dormant culms and buds to be maintained at a favorable water balance (high moisture content) throughout summer drought (McWilliam and Kramer, 1968). Severing of these roots caused the death of the perennial plants.

The relative contribution of solute concentration and osmotic adjustment to changes in osmotic potential vary depending on grass species (Bittman and Simpson, 1989b). For example, osmotic adjustment contributed more than solute concentration to changes in osmotic potential in Altai wildrye and smooth brome grass but not in crested wheatgrass (Bittman and Simpson, 1989b). Though the leaves of tropical grasses adjust osmotically (Wilson et al., 1980) soluble sugars play only a minor role in osmotic adjustment, the inorganic ions (Na, Cl and K) being of greater importance (Ford and Wilson, 1981).

MANUSCRIPT I: WATER STRESS IMPACTS ON DIEBACK OF PERENNIAL RYEGRASS SEED FIELDS

ABSTRACT

Tiller number, height, dry weight, basal diameter and number of leaves of two cultivars of perennial ryegrass were measured prior to and after an irrigation in late-summer or early-fall over two years. After seed harvest and straw removal, plants were given 2.5 cm of simulated rainfall either mid-August (SU) or mid-September (FA) or both (SF). These were compared to no rainfall (NR) or an ambient (AM) treatment. Polyethylene rain-out shelters were used to manually exclude natural rainfall from selected plots.

The cultivar Affinity responded quickly to the early irrigation while the cultivar Buccaneer showed a longer period of summer dormancy. At the end of the early-fall period of regrowth the NR plots had 30% (1995) and 50% (1996) less tillers than the SF plots, however, compensatory growth following the onset of the rainy season resulted in little differences in fertile tiller number and seed yield. The gradual decline in total tillers as the stand aged may be the beginning stages of the dieback phenomenon that eventually results in great losses in stand and seed yield in commercial fields of perennial ryegrass.

At the end of the rainfall treatments, no rainfall caused a decline in total tiller dry weight from 20 - 40% in 1995 to greater than 50% in 1996. There were also reductions in tiller height while the number of leaves per tiller and the basal diameter were the least

sensitive to drought. These data indicate a late-summer rainfall is more beneficial than one in early-fall.

As tiller number was reduced there were increases in the concentration of total soluble sugars, which indicated the potential for exponential growth at the onset of the rains. It is suggested that where feasible farmers should irrigate in summer to prolong stand life.

Keywords: Perennial ryegrass, soluble sugars, drought and dieback.

INTRODUCTION

The premature decline of perennial ryegrass seed fields, commonly known as dieback or dieout, is a widespread problem of economic importance to seed producers. Dieback generally occurs in the third or fourth year of production, but in several instances as early as the second year (Mellbye et al., 1994). This loss in stand is estimated at about \$10 million annually in Oregon's Willamette Valley. In recent years the sales of ryegrass seed amounted to \$100 million (Anon.).

The incidence and severity of the dieback symptoms appear to vary among cultivars of perennial ryegrass, but the severity of dieback also increases as the stand ages. One possible explanation for this phenomenon is that as the plant ages it is increasing less capable of withstanding water stress and producing the critical regrowth required for the development of the next season's crop. Plant persistence is dependent on the ability of new tillers to replace the older ones (Jewiss, 1972). When the plant is placed under stress, the water and nutrient supply to the young tillers is reduced or halted in an effort to save the parent plant. Thus, it is the youngest tillers that are most vulnerable in stress situations. The plant's ability to replace older tillers is drastically reduced under drought or other stress conditions. The impact of water deficit at critical times in the regrowth of the crop cannot be underestimated.

Research to date has eliminated the association of the dieback incidence with pathogens, insect pests, nematodes or soil fertility problems (Mellbye and Young, 1994). Nearly all of Oregon's perennial ryegrass seed crop is cultivated under rain-fed conditions

and most of the vegetation needed to contribute towards the next year's seed crop has to be produced in the summer-fall post-harvest period of regrowth. However, the often dry summer-early-fall period can impose severe moisture stress on this shallow-rooted crop, hence delaying the production of tillers. Therefore, continual water stress during this period of regrowth may be a contributing factor to the dieback problem. Irreversible damage may have occurred even though there is an abundance of water from late fall rains. It seems plausible that any factor affecting growth of tillers in the fall may also reduce flowering and seed yield. Since there is a dearth of information on the effect of water supply on flowering and seed yield, we designed this study to assess the impact of summer and early-fall rainfall on the field performance of perennial ryegrass seed crops. The findings of this project could also be used to assist in irrigation management of perennial ryegrass seed crops.

The objectives of this study were to (1) investigate how post-harvest leaf and tiller development in perennial ryegrass seed crop is impacted by the timing, severity and length of late-summer and early-fall water stress; and (2) identify potential relationships between water stress and flowering, and seed yield.

MATERIALS and METHODS

This experiment was conducted as a randomized complete block design with four replications and five treatments for 2 yr at the Oregon State University's Hyslop Research Farm near Corvallis, OR. The soil was a Woodburn silt loam (fine-silty, mixed, mesic Aquultic Argixeroll). Trials were initiated in August 1995 after harvesting fields that were sown October, 1994. Two cultivars, Affinity and Buccaneer, differing in their responses to drought were used in the study. The entire experiment was replicated in the summer of 1996.

Rainfall was simulated by using a drip emitter system specifically designed for small plot irrigation (after Bittman et al., 1986a). The one-inch PVC pipe-constructed device was 1.8 m by 7.6 m (same size as each plot) and the irrigation tubes and drip-emitters on each line of tubing were 30.4 cm on center. Water delivery was via a Pak® tank (Rear Mfg., Eugene, OR) by a piston driven pump, passing through a screen before output to the plots. Plots were 0.91 m apart within a replication and replications were 3.04 m distant from each other. The two cultivars were sown in blocks 6.08 m apart.

The irrigation system was used to simulate rainfall events (2.5 cm precipitation) in mid-August (summer, SU), in mid-September (early-fall, FA), and the combination of rainfall (SF) events during both periods. The treatments were compared with no rainfall (NR) and ambient (AM) rainfall conditions. To deliver the equivalent of 2.5 cm of precipitation an output of 4.69 L/min for 75 min. was used. This amount increased the soil water potential from about -0.4 MPa to approximately -0.1 MPa.

Polyethylene rain-out covers were used to exclude natural rainfall from all but the ambient treatments. The covers were raised in dry but lowered in wet or inclement weather. When lowered the covers were gable shaped allowing water to run off to the sides of the plots. This was achieved by placing vinyl insulated aircraft cables (3.16 mm thick) at a height of 0.46 m down the center of plots. The covers were held in place by six J-bolts buried in the ground along the length of each side of a plot. Two persons were able to cover or uncover a plot in ≤ 1 minute. Although the manual rain-out shelters may have created an artificial microclimate with higher air temperature, lower air flow and less light, they are still effective where the effects of treatments or varieties are compared under contrasting moisture regimes (Bittman et al., 1986b).

Estimation of crop evapo-transpiration (Et_c) was determined by the summation of applied water, precipitation, and the change in soil-water content determined by time-domain reflectometry (TDR). This method can be represented by the equation:

$$ET = P + I - (RO + \Delta G + \Delta W - L)$$

where P and I are precipitation and irrigation, respectively; RO is surface runoff; ΔG is storage; ΔW is change in soil water storage; and L represents drainage loss (Carrow, 1996). Under the existing conditions (except for the ambient treatment) of this trial, the equation simplifies to,

$$ET = I - \Delta W.$$

The ΔW was determined from the TDR readings for each depth.

Weekly monitoring of canopy temperature and volumetric soil moisture content was achieved by using infrared thermometry (Hatfield, 1990) and time-domain

reflectometry (Wraith and Baker, 1991), respectively. Wave guides were buried horizontally in the center of each plot to determine average soil volumetric water content (θ_v) at 15.2 cm and 30.4 cm depths in the root zone. Sequential samples were taken at three weeks after each treatment to follow plant development through the fall period. Plant growth and developmental characteristics including the number of aerial (tillers produced from axillary buds above the soil surface) and basal (tillers produced from axillary buds at or below the soil surface) tillers, tiller dry weight, Haun stage by count (Haun, 1973), tiller basal diameter and tiller height were measured from a random selection of ten tillers. To obtain the dry weight samples were oven-dried at 65 °C for at least 48 hours.

Fertile and spring vegetative tiller numbers were measured prior to peak anthesis and their combined weights used as total biomass. For sampling, a 30.4 cm² quadrant was used in 1996 and a 22.86 cm² one in 1997. Plots were harvested in July 1996 and 1997 with a small plot swather and dried in windrows to approximately 12% moisture content. Windrows were combined with small plot machinery. The harvested area in one plot consisted of 11.6 m². Seeds were cleaned with a laboratory size Clipper M-2B air-screen cleaner (A.T. Farrell, Saginaw, MI) before weighing. Harvest index (HI) was calculated as

$$HI = \frac{\text{clean seed yield plot}^{-1}}{\text{total biomass plot}^{-1}} \times 100$$

The percent stand cover was taken in October, 1996 to assess plant establishment by noting the presence or absence of a plant at 30.4 cm intervals for 7.6 m . Three rows were measured in each plot.

Mid-morning collecting of plant samples was done to gather materials to ascertain soluble sugars in plants. Samples were refrigerated, cleaned, oven-dried, ground to a fine powder (using a Wiley Mill with a no. 20 screen, 1 mm) then again refrigerated until ready for use. Total soluble carbohydrates (TSC) were determined according to the phenol-sulfuric acid colorimetric method described by Dubois et al., 1956. Fifty milligrams of ground sample were placed in a test tube and 10 ml of 80% ethanol were added. Test tubes were placed in a 60 °C water bath for 30 minutes before adding 40 ml of double distilled water and re-submersing for another 30 minutes. Contents were then filtered through Whatman no.1 filter paper containing activated charcoal to remove pigments which may interfere with absorbance. To 1 ml aliquot of the filtrate was added 1 ml of 5% phenol and 5 ml of concentrated sulfuric acid. Tubes were covered with paraffin film to avoid evaporation. Samples were left standing for 1 hour to develop a stable color before reading the absorbance at 490 nm using a double-beamed UV-VIS scanning spectrophotometer. Blanks were prepared by substituting the sugar solution (samples) with double distilled water. The reference curve was obtained by measuring absorbance of a set of known sucrose solution ranging from 0 to 100 $\mu\text{g/ml}$ (Appendix-Fig. 2). From this working curve the spectrophotometer was able to ascertain the concentration of the samples. Two replicates were used in the analyses (after Ourry et al., 1989a).

STATISTICAL ANALYSES

All field data and soluble carbohydrates were analyzed as a randomized complete block design with five treatments and four replications. Analysis was done using SAS for Windows version 6.12 (SAS Institute, Inc. 1993). Treatments effects were tested by analysis of variance and means separated by Fisher's Protected LSD values.

RESULTS and DISCUSSION

Aerial Tillers

There was a decrease in the number of aerial tillers from the 1995 to 1996 regrowth period (Table I-1). Contrast statements were used to compare the irrigated (SU and SF) versus the non-irrigated (NR and FA) treatments prior to the application of an autumn rainfall. For Affinity in 1995, the SU and SF treatments had more aerial tillers than in NR and FA treatments. The ambient treatment also had more tillers than the NR and FA treatments at this time (Tables I-2). However, there were no differences between the ambient treatment and the SU and SF plots. The number of tillers in Buccaneer in the ambient treatment was marginally higher after summer rainfall ($p=0.06$) in 1995 than the SU and SF treatments and the NR and FA treatments ($p=0.08$). However, there were no differences between the SU and SF and NR and FA treatments (Table I-3). Both cultivars exhibited no differences among treatments at the end of the early-fall rainfall application.

Since there were differences between treatments in the production of aerial and total tillers but not in basal tillers then it appears that it is proliferation of aerial tillers that contributed to the differences in total tiller number.

In 1996, tests of contrasts also showed that after a summer rainfall treatment, SU and SF had higher aerial tiller number than NR and FA in both cultivars (Tables I-2 & I-3). Ambient rainfall produced aerial tiller number similar to other treatments. After fall rainfall, no differences existed among the treatments. However, as the regrowth season progressed, aerial tiller number increased even in plots without natural or artificial rainfall.

Table I-1. Water stress impacts on number of aerial tillers at various times during autumn regrowth in two cultivars of perennial ryegrass seed crops.

Rainfall treatment	Affinity		Buccaneer			
	8 Sept	13 Oct	8 Sept	13 Oct		
<u>1995</u>	<hr/> 10 ² m ⁻² <hr/>					
Ambient	46 a †	64 a	53 a	48 a		
No rain	30 bc	36 a	31 a	29 a		
Summer	37 ab	50 a	35 a	43 a		
Fall	24 c	43 a	42 a	33 a		
Summer+Fall	39 ab	63 a	35 a	36 a		
<u>1996</u>	18 Aug	11 Sept	11 Oct	18 Aug	11 Sept	11 Oct
Ambient	2 a	13 a	8 a	1 a	8 a	13 a
No rain	3 a	4 a	8 a	1 a	5 a	6 a
Summer	2 a	18 a	15 a	1 a	12 a	10 a
Fall	2 a	9 a	14 a	2 a	4 a	9 a
Summer+Fall	2 a	14 a	17 a	1 a	9 a	12

† Means within columns not followed by the same letters are significantly different according to Fisher's Protected LSD (P=0.05) values.

Table I-2. Contrasts for several parameters measured after summer rainfall in Affinity perennial ryegrass.

Parameter Measured	Applied rain vs no rainfall	Applied rain vs Ambient	Ambient vs no rainfall
<u>1995</u>			
Aerial tillers	**	‡	**
Basal tillers	*	NS	NS
Total tillers	**	NS	**
Dry weight	*	NS	**
Haun stage	*	NS	**
Basal diameter	NS	‡	*
Tiller height	**	**	NS
<u>1996</u>			
Aerial tillers	*	NS	NS
Basal tillers	**	**	NS
Total tillers	**	*	NS
Dry weight	**	‡	NS
Haun stage	NS	NS	*
Basal diameter	NS	NS	NS
Tiller height	**	**	NS

Applied rain = summer & summer+fall, no rainfall = no rain & fall.

‡, * and ** contrasts significant at P= 0.1, 0.05 and 0.01 levels respectively.

NS = non-significant

Table I-3. Contrasts for several parameters measured after summer rainfall in Buccaneer perennial ryegrass.

Parameter Measured	Applied rain vs no rainfall	Applied rain vs Ambient	Ambient vs no rainfall
1995			
Aerial tillers	NS	‡	‡
Basal tillers	‡	NS	NS
Total tillers	NS	NS	‡
Dry weight	NS	‡	NS
Haun stage	NS	‡	*
Basal diameter	NS	NS	NS
Tiller height	NS	*	NS
1996			
Aerial tillers	*	NS	NS
Basal tillers	**	*	NS
Total tillers	**	*	NS
Dry weight	**	NS	‡
Haun stage	NS	NS	NS
Basal diameter	NS	NS	NS
Tiller height	*	NS	NS

Applied rain = summer & summer+fall, no rainfall = no rain & fall.

‡, * and ** contrasts significant at P= 0.1, 0.05 and 0.01 levels respectively.

NS = non-significant

Basal Tillers

Contrast statements revealed differences in basal tiller production between the SU and SF and NR and FA treatments after summer rainfall in both cultivars and in both years (Tables I-2 & I-3). These differences were highly significant in all cases except for Buccaneer in 1995 where it was not significant ($p=0.055$). Basal tiller number in 1995 was not different in the ambient treatment from either the SU and SF nor NR and FA treatments. Also, at the end of autumn rainfall no differences were observed between the treatments (Table I-4). After autumn rainfall treatment was imposed in 1996 there were differences between treatments in Buccaneer but not in Affinity. In Buccaneer the SF had the greatest amount of basal tillers and the NR had the least. There were no differences amongst the ambient, fall and summer irrigated plots after autumn rainfall.

Total Tillers

The two cultivars responded differently to water stress. However, no rainfall generally produced less regrowth than other rainfall treatments (Table I-5). There were no differences in tiller number at the first sampling date (7 Aug. 1995 & 18 Aug. 1996). These dates immediately preceded the summer rainfall treatment.

In 1995 SU and SF in Affinity had more total tillers after summer rainfall than the NR and FA, but no differences were noted in Buccaneer (Tables I-2 & I-3). Total tiller number in ambient was significantly greater than in NR and FA in Affinity but was not different ($p=0.067$) in Buccaneer. There were no differences in total tiller number between ambient and the SU and SF for both cultivars.

Table I-4. Water stress impacts on number of basal tillers at various times during autumn regrowth in two cultivars of perennial ryegrass seed crops.

Rainfall treatment	Affinity		Buccaneer			
	8 Sept	13 Oct	8 Sept	13 Oct		
1995	10 ² m ⁻²					
Ambient	17 a†	36 a	14 a	56 a		
No rain	11 a	36 a	8 a	37 a		
Summer	16 a	37 a	16 a	48 a		
Fall	15 a	34 a	13 a	45 a		
Summer+Fall	20 a	34 a	14 a	62		
1996	18 Aug	11 Sept	11 Oct	18 Aug	11 Sept	11 Oct
Ambient	9 a	16 b	49 a	8 a	12 bc	37 ab
No rain	10 a	16 b	24 a	9 a	9 c	17 c
Summer	9 a	26 ab	31 a	8 a	18 ab	31 b
Fall	9 a	16 b	35 a	8 a	10 bc	37 ab
Summer+Fall	9 a	34 a	48 a	6 a	23 a	44 a

† Means within columns not followed by the same letters are significantly different according to Fisher's Protected LSD (P=0.05) values.

Table I-5. Water stress impacts on total tiller number at various times during autumn regrowth in two cultivars of perennial ryegrass seed crops.

Rainfall treatment	Affinity			Buccaneer		
	7 Aug	8 Sept	13 Oct	7 Aug	8 Sept	13 Oct
<u>1995</u>	<hr/> 10 ² m ⁻² <hr/>					
Ambient	14 a †	64 a	99 a	18 a	70 a	104 a
No rain	17 a	41 b	72 a	22 a	40 a	66 c
Summer	16 a	53 ab	87 a	15 a	51 a	91 ab
Fall	17 a	40 b	77 a	14 a	55 a	78 bc
Summer+Fall	12 a	60 a	97 a	18 a	49 a	98 ab
<u>1996</u>	18 Aug	11 Sept	11 Oct	18 Aug	11 Sept	11 Oct
Ambient	11 a	29 bc	57 a	8 a	20 bc	50 a
No rain	12 a	20 c	31 a	11 a	13 c	23 b
Summer	10 a	44 ab	46 a	9 a	30 ab	41 a
Fall	11 a	25 c	49 a	10 a	13 c	46 a
Summer+Fall	11 a	49 a	65 a	7 a	33 a	55 a

† Means within columns not followed by the same letters are significantly different according to Fisher's Protected LSD (P=0.05) values.

In 1996 SU and SF produced more total tillers after summer rainfall than both the ambient and NR and FA treatments in both cultivars. However, there were no differences in total tiller production between the ambient and NR and FA treatments.

The timing and quantity of water application was important. For instance, in 1996, up to the time of summer sampling (Sept.), ambient was not different from NR having received only 0.33 cm of natural rain. However, by the Oct. sampling date, there were an additional 6.17 cm of rainfall and this caused marked responses in tillering such that the ambient treatment was second only to the SF treatment in tiller number. Since the SF treatment outperformed the other treatments it demonstrated the importance of early rainfall to tiller production and survival.

For Affinity (in both years) there were differences between treatments after summer rainfall. No rainfall produced fewer tillers than when rainfall was provided. This reduction was about 30% in 1995 and 50% in 1996. This indicated that Affinity responded very rapidly to this early rainfall. This trend continued after autumn rainfall although the difference was not statistically significant. In 1995, Buccaneer did not respond in tiller number as quickly as Affinity. However, at the end of the fall treatment, no rain produced about 30-35% less total tillers than the summer and the summer-fall treatments. There were no differences between no rain and fall treatments. This implies that a summer rainfall may be more critical than rain in the early fall. Jones et al., (1980) also reported a reduction in the number of live tillers found in stressed swards relative to irrigated fields. Responses to rainfall for Buccaneer in 1996 were similar to that observed for Affinity.

Ambient conditions produced the highest number of total tillers in both cultivars during the first year of this study. Combined summer and fall rainfall events provided precipitation roughly equivalent to normal level during the regrowth period, but tiller production in this treatment was not different than that observed in the ambient treatment, which received nearly twice normal precipitation. The cumulative natural rainfall at the completion of the summer and early-fall periods in 1995 were 2.7 cm and 16.1 cm, respectively. For the same periods in 1996 the quantities were 0.3 cm and 6.5 cm.

It is noteworthy that where treatments received an equal amount of rainfall there was less tiller production in 1996 than in 1995 (Table I-5). This supports the contention that as plants become older they are increasingly more susceptible to stress conditions. Therefore, the ability to replace the older tillers as they die is markedly reduced. Continual summer and early-fall water stress may be a major contributing factor to the onset of dieback. The reduction in tiller production from 1995 to 1996 may be an early indicator of dieback in our experimental stands.

Climatic data over the past 20 years indicate that the average rainfall during the summer and early-fall period is about 7.5 cm. However, about 10.2 cm of rainfall may be necessary for optimum tiller production (Fig. I-1). Therefore, the crop may be under moderate to severe moisture stress during the early regrowth period in most years. This stress may have had detrimental effects on the ability of plants to recover and produce the tillers necessary for stand persistence.

Regression analysis of the total tiller number plotted against natural and applied rainfall showed that the quadratic model best explained the variation in total tiller

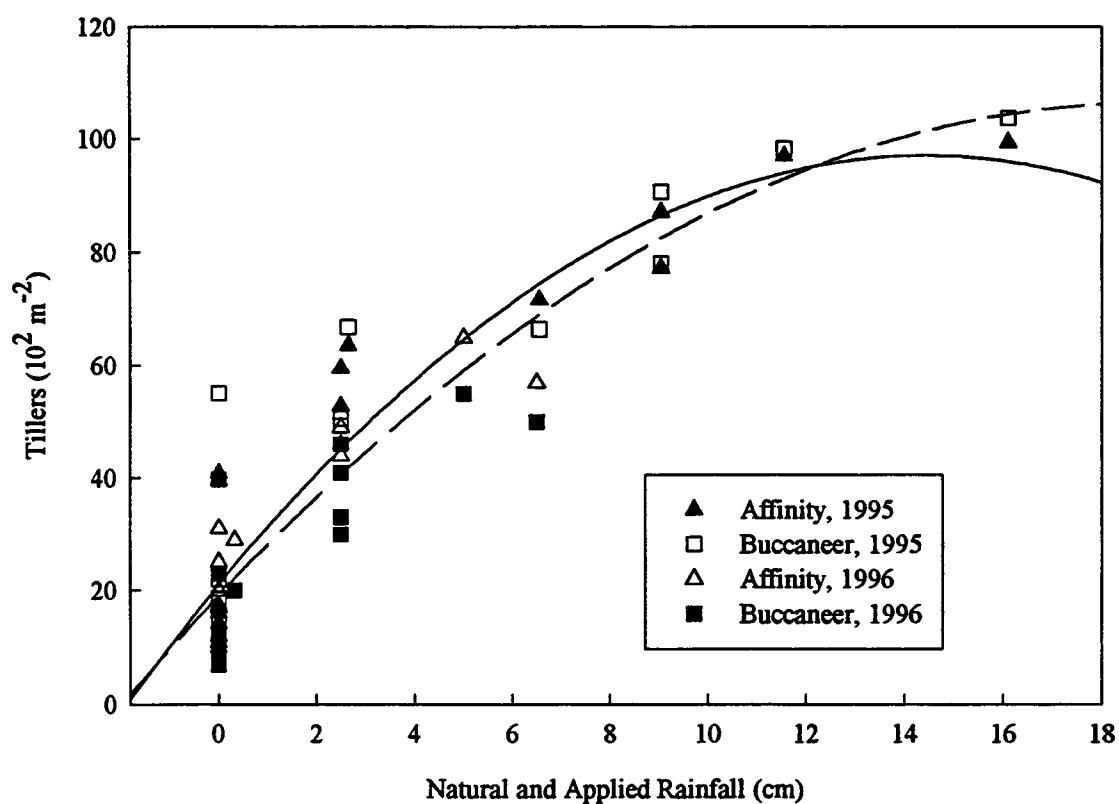


Fig. I-1. Water stress impact on autumn tiller number in two cultivars of perennial ryegrass. (Affinity $r^2 = 0.88$, $P < 0.01$; Buccaneer $r^2 = 0.83$, $P < 0.01$;) Regression equation for the fitted line for each cultivar is: Affinity, $Y = 21.14 + 10.54X - 0.37X^2$; Buccaneer $Y = 19.23 + 9.21X - 0.24X^2$.

production. By obtaining the first derivatives of these equations (for both cultivars) and equating these to zero, the value(s) of rainfall that gave maximum tiller production were obtained. These values were 14.1 cm for Affinity and 16.7 cm for Buccaneer. These values represent the point where there is no additional increase in tiller number with an added increment of rainfall. Since climatic data over the past 20 years indicated that the average rainfall amounted to a mere 7.5 cm during this period of regrowth it becomes obvious that the seed crop is under severe water stress during this time, hence detrimental effects on the ability of plants to recover and produce tillers needed for stand persistence. Over several years the cumulative effect may be devastating.

When the values of rainfall that gave maximum tillers were substituted into the response equation the predicted maximum tiller production were 9800 m⁻² and 10300 m⁻² for Affinity and Buccaneer, respectively. These values represent just under a 300% increase when compared to the amount of tiller production that occurs with zero incremental rainfall, 2500 m⁻² and 2800 m⁻² for Affinity and Buccaneer, respectively (Fig. I-1).

In 1996 there was very little natural rainfall (6.5), hence even less tillers (6000-7000 m⁻²) were produced than in 1995. However, a comparison of an equivalent (6.5 cm) quantity of rainfall from 1995 showed that there was a similar trend in tiller production to that of 1996 (Fig. I-1). It is therefore logical to assume that soil water availability was the limiting factor in tiller production in 1996 and if additional increments of rainfall were added then increased tiller production would have been evident. This corroborates the conclusion of Norris (1982) that drought is the major factor influencing the growth rate of

perennial ryegrass during the summer. From this study, it is clear that the amount of rainfall in the late-summer early-fall period is very critical to total tiller production in perennial ryegrass seeds fields.

Tiller Dry Weight

In 1995, tiller dry weight in Affinity responded more to summer rainfall while Buccaneer responded more to the early-fall rainfall. For instance, Affinity plots with no rainfall (NR and FA) after summer rainfall had the lowest dry weights but by the end of the early-fall sampling treatments were not different in dry weight (Table I-6). The reverse occurred in Buccaneer whereby dry weight was not affected by summer rainfall, but differences were observed after fall rainfall. This supported the observations noted from the total tiller number that Affinity responded rapidly to the early rainfall treatment whereas Buccaneer appeared to have more summer dormancy and responded at a later date.

At the end of the fall sampling, NR had 20% less dry weight than SF in Affinity, but 40% less dry weight in Buccaneer. This is similar to other findings where stressed swards of perennial ryegrass had a 20% (Jones et al., 1980) or a 32% (Norris, 1982) reduction in dry matter compared to irrigated fields. Tests of contrasts revealed that at the end of the summer rainfall, SU and SF did produce more tiller dry weight than NR and FA in Affinity but not in Buccaneer. Also in Affinity, ambient had greater dry weight than NR and FA, but the dry weight was similar to SU and SF. In Buccaneer, there were no differences between any of the treatments after summer rainfall. These results agreed with

Table I-6. Water stress impacts on dry weight of tillers at various times during autumn regrowth in two cultivars of perennial ryegrass seed crops.

Rainfall treatment	Affinity			Buccaneer		
	7 Aug	8 Sept	13 Oct	7 Aug	8 Sept	13 Oct
1995	<hr/> 10 ⁻³ kg m ⁻² <hr/>					
Ambient	15 a†	62 a	73 a	26 a	71 a	112 b
No rain	19 a	37 b	86 a	24 a	45 a	97 b
Summer	15 a	45 ab	93 a	22 a	52 a	112 b
Fall	17 a	39 b	75 a	17 a	67 a	103 b
Summer+Fall	11 a	60 a	108 a	24 a	47 a	164 a
1996	18 Aug	11 Sept	11 Oct	18 Aug	11 Sept	11 Oct
Ambient	8.6 a	26 b	54 a	5.4 a	20.7 ab	50.8 b
No rain	8.6 a	16 b	41 a	6.5 a	10.5 b	28.2 c
Summer	4.3 a	29 b	43 a	6.5 a	26.9 a	55.3 b
Fall	7.5 a	23 b	54 a	7.5 a	12.7 b	50.8 b
Summer+Fall	6.5 a	47 a	90 a	4.3 a	31.4 a	74.0 a

† Means within columns not followed by the same letters are significantly different according to Fisher's Protected LSD (P=0.05) values.

the findings of others (Stiles and Williams, 1965; Penman, 1962) that water deficits of even a few centimeters caused a reduction in dry matter yield of temperate grass crops. Since the stands were relatively young (2 years old) dry matter decline may be only slight at this juncture, so differences in dry matter were not substantial ($0.05 < p > 0.1$).

In 1996 the tiller dry weight, like total tiller number, was lower than in 1995 for the corresponding treatment. However, at the end of the fall sampling NR had an average dry weight less than half that of SF in both cultivars. In Buccaneer, the dry weight of NR was lower than in all other treatments. These results were consistent with those for crested wheatgrass (*Agropyron desertorum*) and blue bunchgrass (*Pseudoroegneria spicata*) (Busso and Richards, 1995) where drought-exposed plots had much lower herbage accumulation than both natural and irrigated treatments regardless of whether plots were clipped or unclipped. Their study also showed that ranking of natural and irrigated treatments were inconsistent across cultivars or clipping treatments. Contrast statements showed that for both cultivars summer rainfall resulted in higher tiller dry weight than no rainfall. However, ambient natural rainfall produced tiller dry weights similar to other treatments.

It should be noted at this juncture that a total absence of additional water did not result in death of the plants or stunted growth. In fact, there was increased production of tillers and dry weight. For example, in NR in both years and cultivars, there was more than a 300% increase in tiller dry weight throughout the autumn regrowth period. This meant that the residual moisture in the soil was sufficient to promote plant growth after an initial period of summer dormancy. However, the addition of even a small increment of

water (2.5 cm) would stimulate increased growth and development. Similar field findings were noted by Barker et al. (1989) when *L. perenne* was subjected to a progressive soil water deficit of 18.3 cm. They recorded decreases in total dry matter, leaf length, tiller density, leaves per tiller, length of leaves, and leaf area due to stressed conditions.

However, it was only after a soil water deficit of 10.0 cm were there significant changes in leaf extension and leaf appearance rates. Norris (1982) also concluded that irrigation increased herbage accumulation when potential soil water deficit was greater than 10.0 cm.

There was a very close relationship between the total number of tillers and their dry weights ($r = 0.94$, $p = 0.01$); as the total number of tillers increased there were corresponding increases in dry weight. A strong positive correlation between autumn biomass and live tiller densities also existed in *Dactylis glomerata* (Volaire, 1994).

Haun Stage of Basal Tillers

The Haun stage is a modified system used to quantify the morphological development of a tiller in perennial grasses. It is based on the number of fully emerged leaves on a tiller, and the length of the youngest unemerged leaf in relation to the length of the youngest fully emerged leaf.

After summer rainfall in 1995, NR and FA produced more leaves than both ambient and SU and SF in Affinity (Table I-2). However, in Buccaneer though NR and FA had more leaves than ambient they were not different from SU and SF after a summer

Table I-7. Water stress impacts on Haun stage of basal tillers at various times during autumn regrowth in two cultivars of perennial ryegrass seed crops.

Rainfall treatment	Affinity			Buccaneer		
	7 Aug	8 Sept	13 Oct	7 Aug	8 Sept	13 Oct
<u>1995</u>	-----leaf no. tiller ⁻¹ -----					
Ambient	2.9 a†	2.5 bc	2.7 a	2.8 a	2.6 b	3.0 a
No rain	4.0 a	3.0 a	2.9 a	2.4 a	3.2 a	3.0 a
Summer	2.9 a	2.9 ab	3.1 a	2.5 a	3.0 ab	2.9 a
Fall	3.1 a	3.1 a	2.8 a	2.8 a	3.0 ab	2.9 a
Summer+Fall	3.7 a	2.5 c	2.9 a	2.4 a	3.1 ab	2.8 a
<u>1996</u>	18 Aug	11 Sept	11 Oct	18 Aug	11 Sept	11 Oct
Ambient	3.2 a	2.8 a	2.6 a	3.2 a	2.7 a	2.6 a
No rain	3.1 a	3.4 a	2.8 a	2.9 a	2.8 a	2.6 a
Summer	2.8 a	3.0 a	2.6 a	2.7 a	2.7 a	2.4 a
Fall	3.0 a	3.0 a	2.7 a	2.7 a	2.9 a	2.6 a
Summer+Fall	2.5 a	3.1 a	2.7 a	2.5 a	2.8 a	2.5 a

† Means within columns not followed by the same letters are significantly different according to Fisher's Protected LSD (P=0.05) values.

rainfall. Comparisons of the ambient versus the SU and SF treatments revealed only marginal ($p=0.066$) differences in Buccaneer but none were apparent in Affinity.

In 1996, no differences between treatments were generally noted after summer rainfall except in Affinity where NR and FA again produced more leaves than the ambient. However, for both cultivars and in both years, no differences existed in leaf number at the end of the regrowth period after fall rainfall (Table I-7). Perennial ryegrass plants grown in compost and subjected to a reduced water supply for 28-42 days exhibited a decrease in leaf number from 3 - 4 to 1.2 - 1.8 leaves per tiller (Thomas, 1987).

Basal Diameter

After summer rainfall in 1995 ambient plots of Affinity had a significantly larger mean basal diameter than NR and FA, and also tended ($p=0.063$) to have a higher mean than SU and SF (Table I-2). Basal diameter was not different between SU and SF and NR and FA. There were no differences after summer rainfall amongst the treatments in Buccaneer (Table I-3). By the end of the autumn rainfall treatment, no differences were evident amongst treatments in both cultivars (Table I-8).

No differences in basal diameter were observed between treatments in 1996 after summer rainfall within each cultivar. This trend continued through autumn rainfall. In this study, there was very poor correlation between the basal diameter and the mean Haun stage by count. This meant that as the number of leaves on basal tillers changed a corresponding change in the tiller basal diameter did not occur.

Table I-8. Water stress impacts on basal diameter at various times during autumn regrowth in two cultivars of perennial ryegrass seed crops.

Rainfall treatment	Affinity			Buccaneer		
	7 Aug	8 Sept	13 Oct	7 Aug	8 Sept	13 Oct
<u>1995</u>	-----mm-----					
Ambient	1.4 a†	1.7 a	1.9 a	1.6 a	1.8 a	2.1 a
No rain	1.6 a	1.4 c	1.9 a	1.6 a	1.6 a	2.2 a
Summer	1.3 a	1.6 ab	2.2 a	1.8 a	1.6 a	2.0 a
Fall	1.4 a	1.6 ab	2.3 a	1.4 a	1.7 a	2.0 a
Summer+Fall	1.5 a	1.5 bc	2.2 a	1.5 a	1.5 a	2.2 a
<u>1996</u>	18 Aug	11 Sept	11 Oct	18 Aug	11 Sept	11 Oct
Ambient	1.7 a	1.8 a	2.0 a	1.6 a	1.7 a	2.0 a
No rain	1.5 a	1.7 a	2.0 a	1.5 a	1.4 a	1.8 a
Summer	1.6 a	1.6 a	1.8 a	1.5 a	1.5 a	1.8 a
Fall	1.6 a	1.8 a	1.8 a	1.5 a	1.7 a	1.9 a
Summer+Fall	1.7 a	1.8 a	2.0 a	1.5 a	1.7 a	2.0 a

† Means within columns not followed by the same letters are significantly different according to Fisher's Protected LSD ($P=0.05$) values.

Height of Basal Tillers

After summer rainfall in 1995, Affinity had taller tillers in SU and SF than in both ambient and NR and FA. There were no differences between ambient and when water was withheld (Table I-9). In Buccaneer, SU and SF had taller tillers than ambient but not NR and FA. Tiller height response in NR and FA cannot be explained since the trend in this study was for Buccaneer to be less responsive to summer rainfall but more responsive to fall rainfall. Mean height increased in all treatments as the season progressed except in NR in Buccaneer. Since this was the only exception in both years it was considered an aberrant result and not due to any specific treatment effects. Etiolation caused by covering of the plots may have increased tiller height but this effect was considered to be minimal since no difference existed between the ambient (not covered) and the non-irrigated (covered) plots. Therefore, differences between the irrigated (also covered) and the ambient were considered to be caused entirely by the changes in soil moisture content.

Though tiller height increased throughout the regrowth period, the percentage increase from the beginning to the end of the sampling period was not as great as the percentage increases in number of total or basal tillers, nor tiller dry weight. This increase was usually less than 50% across cultivars in both years. The mean height within treatment for a corresponding period also generally decreased from 1995 to 1996. After fall rainfall in 1995, SF mean height was only about 18-19% greater than where no rainfall was applied, and in 1996 this difference was even less (Table I-9).

In 1996 SU and SF had taller tillers after summer rainfall than NR and FA in both cultivars. Also, in both cultivars no difference in tiller height existed between the ambient

Table I-9. Water stress impacts on height of basal tillers at various times during autumn regrowth in two cultivars of perennial ryegrass seed crops.

Rainfall treatment	Affinity			Buccaneer		
	7 Aug	8 Sept	13 Oct	7 Aug	8 Sept	13 Oct
<u>1995</u>	cm					
Ambient	6.5 a†	9.0 c	12.3 a	7.7 a	9.9 b	12.4 ab
No rain	6.9 a	8.8 c	11.3 a	8.1 a	12.1 a	11.6 b
Summer	7.0 a	11.4 a	13.2 a	7.8 a	12.5 a	12.7 ab
Fall	6.6 a	9.4 bc	11.9 a	6.4 a	10.7 ab	13.7 ab
Summer+Fall	6.2 a	10.9 ab	13.9 a	8.7 a	12.0 ab	14.2 a
<u>1996</u>	18 Aug	11 Sept	11 Oct	18 Aug	11 Sept	11 Oct
Ambient	7.6 a	6.6 c	7.7 c	5.8 a	7.8 a	8.2 a
No rain	5.7 a	7.3 bc	9.5 ab	6.0 a	6.5 a	8.6 a
Summer	4.8 a	8.2 b	9.0 abc	6.0 a	9.1 a	10.3 a
Fall	6.0 a	7.1 bc	8.7 bc	6.6 a	8.0 a	9.1 a
Summer+Fall	5.7 a	9.8 a	10.3 a	4.9 a	9.0 a	10.1 a

† Means within columns not followed by the same letters are significantly different according to Fisher's Protected LSD ($P=0.05$) values.

and NR and FA. When SU and SF were contrasted with ambient, no differences were noted in Buccaneer, but in Affinity, SU and SF were taller. This trend continued through to the period after autumn rainfall treatment. Other studies also reported reduced height in grasses (Jones et al., 1980). Drought depressed tiller height by approximately 27% in both crested wheatgrass and bluebunch wheatgrass under field conditions (Busso and Richards, 1995). In our study, the percentage reductions in tiller height were lower. Reduced leaf elongation and leaf expansion caused by drought were also reported by other researchers (El Nadi et al., 1969; Leafe et al., 1977).

Canopy Temperature

The use of canopy temperature to detect water stress in plants is based on the assumption that transpired water evaporates and cools the leaves below the temperature of the surrounding air. As water becomes limiting, transpiration is reduced and the leaf temperature increases (Jackson, 1981).

There was poor correlation between soil moisture content at 15 cm depth and canopy temperature. However, for up to five days after rainfall treatment, NR had a higher canopy temperature than recently irrigated plots. Since the crop just commenced its regrowth, the canopy was open and tillers were small which may account for the poor correlation between canopy temperature and soil moisture content.

There was a tendency towards decreasing differences between the canopy and air temperature as volumetric water content increased at the 15 cm level. This was observed in both 1995 and 1996 (Fig. I-2 & I-3). At the 30 cm level this was not seen probably

Fig. I-2. Effect of soil moisture content at two soil depths on canopy temperature in two cultivars of perennial ryegrass in 1995. (a) Affinity $r^2 = 0.0059$, $P > 0.05$; Buccaneer $r^2 = 0.013$, $P > 0.05$. Regression equation for the fitted line for each cultivar is: Affinity, $Y = 7.94 - 0.11X$; Buccaneer, $Y = 7.97 - 0.20X$.
 (b) Affinity $r^2 = 1.83 \times 10^{-3}$, $P > 0.05$; Buccaneer $r^2 = 0.013$, $P > 0.05$. Regression equation for the fitted line for each cultivar is: Affinity, $Y = 3.83 + 0.14X$; Buccaneer, $Y = 10.7 - 0.25X$.

Fig. I-3. Effect of soil moisture content at two soil depths on canopy temperature in two cultivars of perennial ryegrass in 1996. (a) Affinity $r^2 = 0.043$, $P > 0.05$; Buccaneer $r^2 = 0.01$, $P > 0.05$. Regression equation for the fitted line for each cultivar is: Affinity, $Y = 1.45 - 0.15X$; Buccaneer, $Y = -0.06 - 0.04X$.
 (b) Affinity $r^2 = 6.83 \times 10^{-4}$, $P > 0.05$; Buccaneer $r^2 = 0.01$, $P > 0.05$. Regression equation for the fitted line for each cultivar is: Affinity, $Y = -4.74 \times 10^{-3} - 0.04X$; Buccaneer, $Y = 1.22 - 0.09X$.

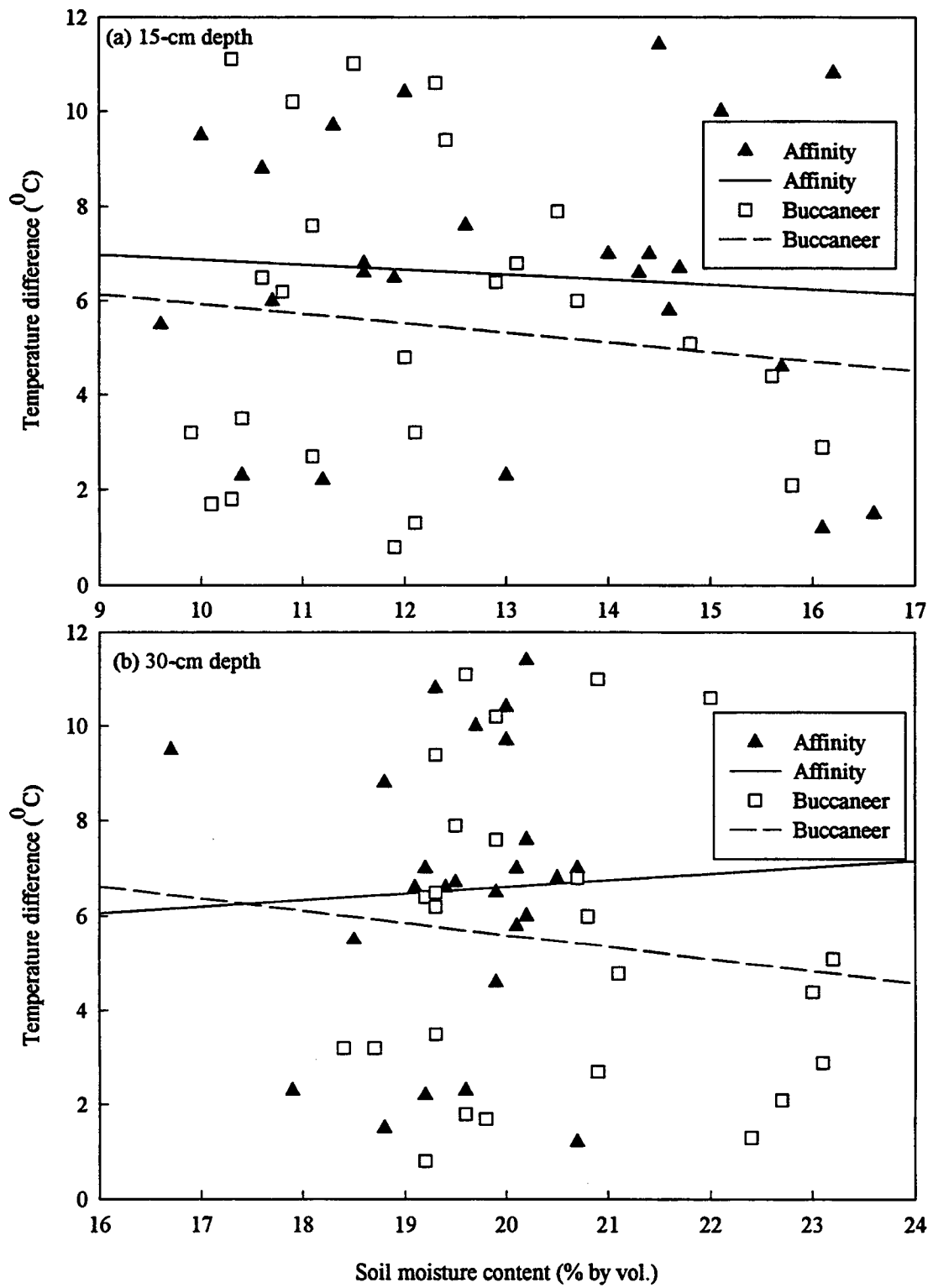


Fig. I-2.

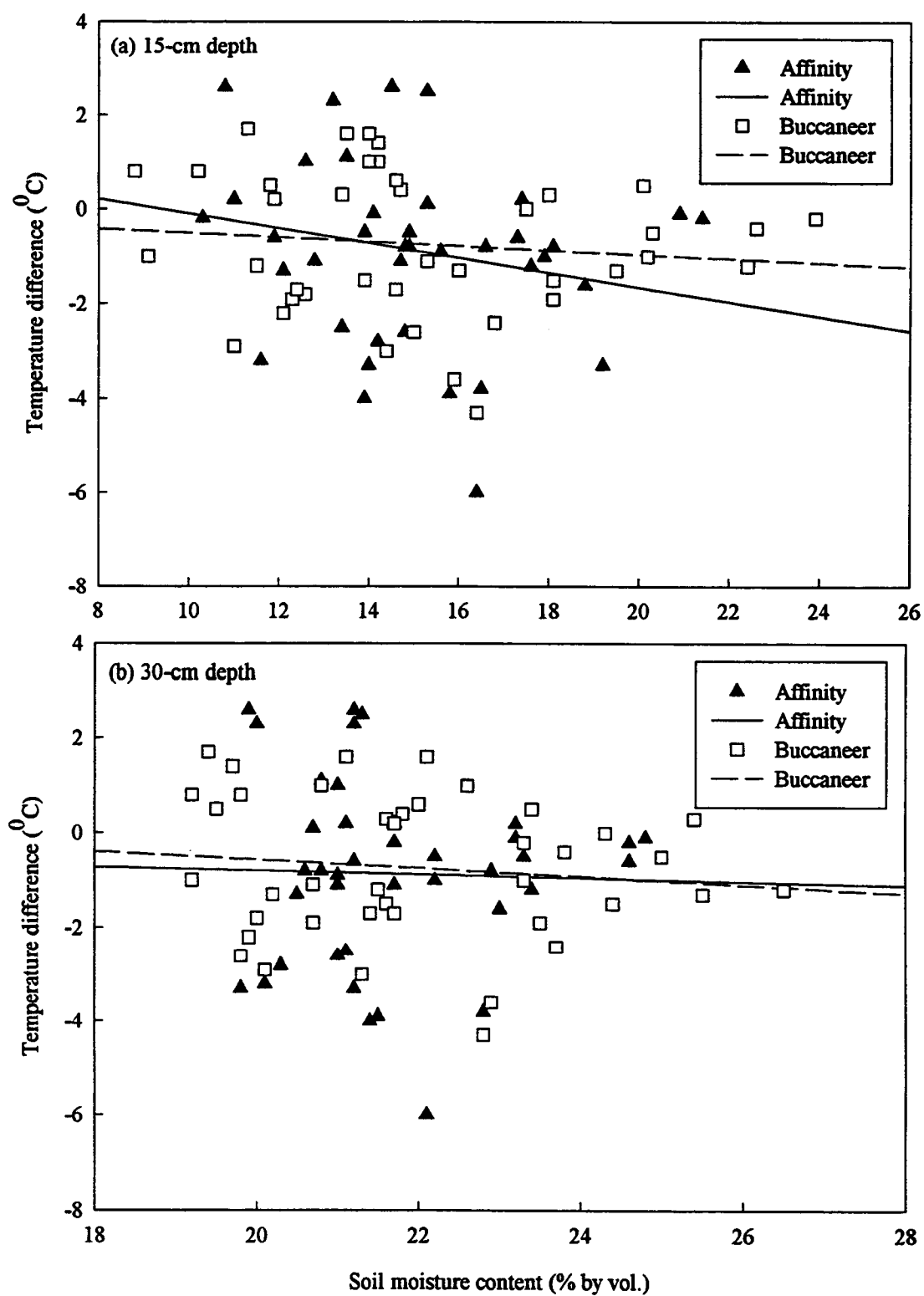


Fig. I-3.

because of the amount of water applied. Since it is impossible to half wet a soil it means that anytime a soil is watered to below field capacity (f.c.) part (top) will be wet and the bottom dry. Only when field capacity has been attained will water drain to the lower levels. Two and one-half centimeters of rainfall is nowhere close towards wetting this soil to f.c. thus, the 30-cm depth volumetric water content was not affected by the irrigation treatment. Therefore, roots in this zone of soil did not benefit from any increment of water so it can be assumed that transpiration was not much affected hence little changes in the temperature of the crop.

Stand Coverage

Stand cover was clearly affected by the water stress treatments (Table I-10). For both cultivars, the no rain and the summer/ fall treatments produced the lowest and the highest amount of plant cover respectively, while the other treatments represented the middle of the range. The no rain, ambient and the summer /fall treatments resulted in equivalent amounts of plant cover in both cultivars. However, the summer and the fall treatments produced slightly less stand in Buccaneer than in Affinity. This agrees with the observations made earlier that the two cultivars responded differently to water stress.

In all cases percent stand cover was reduced probably because of the insufficiency of water. Since death and loss of stand is the last response of the crop to water stress then it is important that the stress be less severe. No water in the late summer - early fall period of regrowth resulted in approximately 32 - 40% loss of stand. In fields ≥ 2 years old serious consideration must be given to supplemental irrigation if a dry regrowth period

Table I-10. Effect of water stress on percent cover of plant stand in two cultivars of perennial ryegrass seed crops on October 11, 1996.

Rainfall Treatment	Affinity	Buccaneer
	(% cover)	
Ambient	84.7 b†	85.0 ab
No rain	68.4 c	60.0 d
Summer	83.0 b	77.3 bc
Fall	87.6 ab	73.4 c
Summer+Fall	91.7 a	88.7 a

† Means within columns not followed by the same letters are significantly different according to Fisher's Protected LSD ($P=0.05$) values.

is forecasted. Seed growers should be advised that even a small quantity of water in summer can greatly benefit stand persistence.

Percent stand cover at the end of the irrigation period was regressed against tiller number to see how much variation in tiller number at harvest was due to field conditions in October. The regression was done using a zero-intercept model because it is prudent to assume that where no stand exists there would be no tillers in June. As expected the relationship was close in all cases with r^2 of 0.94 ($P < 0.01$), 0.97 ($P < 0.01$) and 0.99 ($P < 0.01$) for vegetative, reproductive, and total tillers in Affinity, respectively. For Buccaneer, the corresponding values were 0.90 ($P < 0.01$), 0.97 ($P < 0.01$) and 0.96 ($P < 0.01$) for vegetative, reproductive, and total tillers, respectively.

However, because of other factors including competition and self-thinning that can occur throughout the growing season, it is difficult to predict the proportion of surviving tillers that may become reproductive or remain vegetative. It is difficult to integrate the responses of an array of biological processes to a number of environmental factors and it is, therefore, not surprising that there is no simple correlation between stand cover and seed yield. Therefore, it is almost impossible to be able to predict final clean seed yields from the stand coverage in October. However, it may be argued that because tillering is a continual process throughout the year it is of interest to seed producers to know the number of tillers originating at different times of the year, what proportion become reproductive, and their individual contribution to seed yield (Hill and Watkin, 1975).

Stand cover also influences competition of the crop with weeds. More openings in the stand result in an increase in the sites available for weed plant establishment.

Total Soluble Carbohydrates

The accumulation of carbohydrate reserves in plant tissue depends upon the balance between photosynthesis and respiration (White, 1973). Changes in carbohydrate contents of the stubble is very important since this is the primary site for the storage of non-structural carbohydrates in grasses such as perennial ryegrass (Ourry et al., 1989a). In perennial grasses carbohydrate reserves are vital for winter survival, initiation of new spring and fall growth and initiation of regrowth following herbage removal (Daer and Willard, 1981).

Analysis of water soluble sugars were intriguing. There were no differences between treatments for both cultivars in 1995. However, we did observe that the summer treatment for Affinity had a somewhat lower concentration than other treatments. When it is noted that this same treatment had a lower yield than the others (Table I-12) it is interesting. The ability of a crop to adapt to stress and still continue to function at optimum is critical for its continued development, growth and production. This may be a case where the plants in the other treatment were able to osmotically adjust to the conditions and when later rains came they continued to function optimally. However, as explained before, early summer treatments may have initiated rapid growth, but subsequent withholding of water produced adverse effects.

The 1996 trend revealed that the concentration of sugars tended to be greater than in 1995 (Table I-11). This may be caused by a concentration effect since there were fewer tillers in 1996 than in 1995. More sugars would accumulate when less tillers are present. Since this period of regrowth is not a very active one (photosynthetic capacity > demand

Table I-11. Water stress impacts on total soluble sugars at the end of the autumn regrowth period in two cultivars of perennial ryegrass seed crops.

Rainfall Treatment	Affinity		Buccaneer	
	1995	1996	1995	1996
	mg g ⁻¹ dry wt.			
Ambient	59.73 a†	67.36 b	61.74 a	53.94 cd
No rain	61.14 a	83.23 a	64.92 a	69.93 b
Summer rain	49.10 a	71.59 b	60.35 a	90.49 a
Fall rain	62.12 a	57.39 c	61.55 a	51.05 d
Summer+Fall	59.26 a	81.64 a	56.97 a	64.79 bc

† Means within columns not followed by the same letters are significantly different according to Fisher's Protected LSD (P=0.05) values.

Table I-12. Water stress impacts on spring tiller number and seed yield in two cultivars of perennial ryegrass seed crops.

Rainfall treatment	Affinity			Buccaneer		
	Vegetative tillers	Fertile tillers	Seed Yield	Vegetative tillers	Fertile tillers	Seed Yield
<u>1995/6</u>	————(10 ² m ⁻²)————		(kg ha ⁻¹)	————(10 ² m ⁻²)————		(kg ha ⁻¹)
Ambient	37 a†	24 a	1337 a	39 a	25 a	1219 a
No rain	35 a	22 a	1299 a	34 a	23 a	1164 a
Summer	36 a	23 a	1035 b	34 a	21 a	1156 a
Fall	38 a	24 a	1367 a	30 a	21 a	1215 a
Summer+Fall	41 a	25 a	1295 a	32 a	22 a	1235 a
<u>1996/7</u>						
Ambient	29 a	29 a	1338 a	31 a	24 ab	1428 a
No rain	24 a	31 a	1263 a	23 a	20 b	1344 a
Summer	24 a	31 a	1292 a	30 a	24 ab	1383 a
Fall	24 a	32 a	1189 a	34 a	29 a	1355 a
Summer+Fall	26 a	37 a	1249 a	25 a	29 a	1373 a

† Means within columns not followed by the same letters are significantly different according to Fisher's Protected LSD (P=0.05) values.

for growth) for the plant, sugars may have been accumulated until ready for later use. This is evident in Affinity where the no rain treatment had the highest concentration of sugars. As noted earlier Affinity responded very rapidly to the early irrigation hence a lot of sugar will be used up during this initial growth. For instance, the plant samples were taken in October, three weeks after the early-fall irrigation, and we see that the FA treatment had the lowest net concentration of soluble sugars. There may not have been sufficient time for much sugar accumulation to occur in this treatment. The SU and SF being irrigated before (in August) had adequate time to replenish sugar reserves. These observations were validated in part by the findings of Ourry et al. (1989a) who found that there is a rapid decline of organic carbon reserves during the first week of perennial ryegrass regrowth. More than half of the TNC disappeared, most of which comprised the oligofructans. After approximately six days there is reconstitution of the carbohydrate reserves as new foliage develops, but by day 14 these levels were still 47% lower than at day 0 (Ourry et al., 1989b). Following this initial period (\approx one week), plant regrowth rate depends on other factors, e.g., leaf area, water availability and nutrient uptake (White, 1973). In our study, with the limited irrigation, plants in the FA treatment would be unable to accumulate TNC to the original level in such a short time, hence lower levels than those in the ambient treatment.

Buccaneer responded slower than Affinity to irrigation resulting in a different rate of net accumulation of its water soluble sugars. Similar trends in wheat suggested that the rate and extent of increase in sugar content caused by drought depended on species and even on the genotype within the same species (Kameli and Lösel, 1993). The SU

treatment had the highest level of sugars with FA treatment having the lowest. The NR treatment ranked second indicating that in both cultivars, sugars did accumulate under drought stress. This interpretation is supported by the work of Busso et al. (1990) who found high accumulation of total non-structural carbohydrates in drought-exposed plants relative to irrigated plants of *Agropyron desertorum* and *Pseudoroegneria spicata*. They suggested that plants exposed to prolonged periods of drought have rapid initial regrowth upon alleviation of the stress because of the large quantities of TNC that had accumulated in their tissues. This was evident in *Dactylis glomerata* (Volaire, 1994 and 1995) where strong correlations existed between recovery growth in autumn and water soluble carbohydrates (WSC), and also between the live tiller density and WSC. On the contrary, Thomas and James (1993) observed a 30% decrease in WSC content of drought-exposed plants of perennial ryegrass. In our study, the relatively high levels of sugars in the NR treatment could be the indicator that upon the release of the stress in late fall there will be rapid tiller production. This could partly be one reason why plants in the NR treatment exhibited such high compensatory regrowth (Fig. I-4b). Barker et al. (1985) did not consider compensatory growth to be dependent on higher concentrations of soluble sugars because stressed plants, though having lower levels of sugars still exhibited compensatory growth. However, White (1973) maintained that the initial effect from the level of carbohydrate reserves can continue during subsequent exponential growth.

The growth rate of a population during and following drought is a function of its (i) potential growth rate and (ii) drought resistance (Amin and Thomas, 1996).

Undoubtedly, the accumulation of sugars will contribute towards the potential growth rate

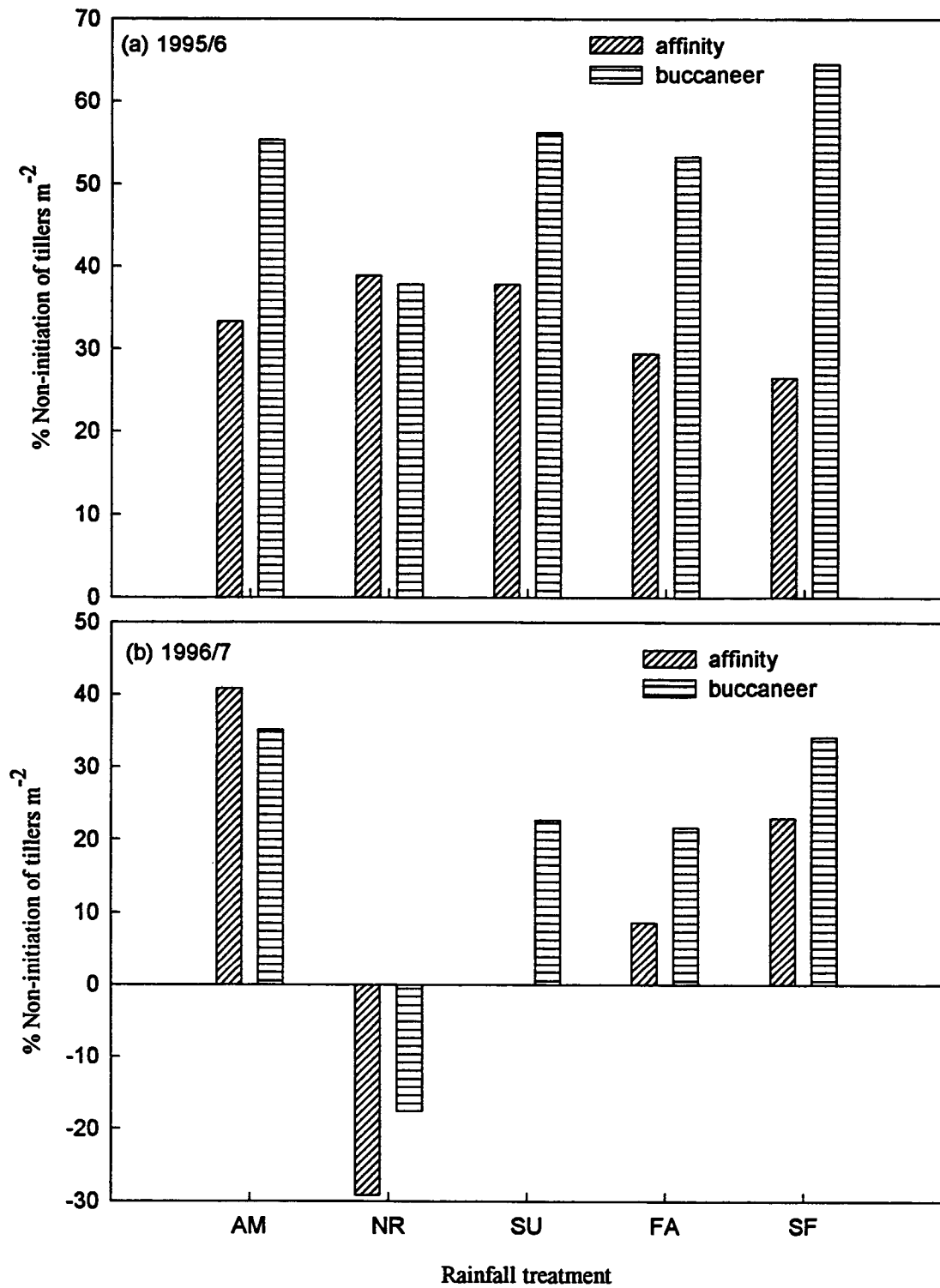


Fig. I-4. Spring fertile tillers as a percentage of basal tillers in October in two cultivars of perennial ryegrass.

leading to rapid compensatory growth following alleviation of drought. Why there was such high levels of sugar in the SU treatment is still unclear. In 1996/7 year most (95%) of the ambient rainfall occurred late September to October so, like the FA treatment, plants would be net users of sugars because of rapid growth. Thus lower levels in these two treatments. The above results confirm the judgement of White (1973) that "the degree of water stress and plant growth stage during which it occurs will variably affect carbohydrate reserve levels".

Fertile Tillers and Seed Yield

There were no differences in fertile tiller number in 1996 (Table I-12). Also, yields were similar except for the summer treatment in Affinity. Yields may have been reduced in the summer treatment because of rapid growth initiated by the early irrigation. However, as subsequent water was not forthcoming plant growth and development may have been reduced. Buccaneer responded less slowly to early irrigation; therefore, tillers formed late in fall may have contributed to yields. Since perennial ryegrass has little or no juvenility (Silsbury, 1965), the dependence on early-formed tillers in the fall to produce high numbers of inflorescences the following spring is not very great.

The decrease in total and basal tillers, which may be the beginning stages of the dieback problem, may not have been that severe so early in the life of the stand to adversely reduce the final reproductive tillers. It can be speculated that if this rate of tiller reduction continues as the stand ages there will be a point where fertile tiller numbers are affected to such an extent that seed yields will be significantly lower because the seed crop

cannot compensate. A two year-old stand of perennial ryegrass may not be old enough to show this decline because the surviving basal tillers that are induced will no doubt compensate for previous tillers losses at this early stage in the life of the crop. Based on the data obtained in this trial between 2000-3000 fertile tillers m^{-2} appear to be optimum for seed yield in perennial ryegrass seed crops. If this quantity is to be reduced (through a substantial reduction in fall tillers) then we will see a decline in yield. Since most of the tillers that contribute towards next season's seed crop is produced during this period of regrowth then the later formed tillers will not be able to compensate adequately for the substantial decrease in the fall basal tillers.

What was remarkable in this study was that when the quantity of basal tillers during fall regrowth was lower than the compensation optimum amount there was actually more production of tillers to compensate. This was observed in the NR treatment in the 1996/7 year. In 1995/6 the amount of basal tillers was greater than the compensation threshold even in the NR treatment so fewer tillers were induced. However, in 1996/7 the amount (in fall) was below the compensation threshold for NR treatment, but we observed that there was actually an increase in the tillers induced (Fig. I-4) in both cultivars. This meant that tillers which may have been produced later in the fall or even in the spring stood a greater chance of being induced to flower. We see this in the NR treatment where it is the only treatment in both cultivars to show an increase in conversion of basal tillers from the vegetative to fertile state. Compensatory growth is common in temperate grasses as noted by Amin and Thomas (1996) who stated that in very dry summer the

yield of herbage may be only 10% as in wetter years but, recovery growth may equal or exceed "normal" growth after the advent of the fall rains.

Self thinning in plants is a natural process that allows plants to select the strongest and largest tillers that are best suited to receive the floral induction stimulus and hence be initiated. Once growth conditions are favorable in the late-summer early-fall period of regrowth plants will produce as many tillers as resources will allow. However, as growth and development progress competition for light, nutrients and water forces plants to divert resources from the smaller and /or later formed tillers to the larger tillers that most likely have received the floral induction stimulus in winter. This ensures that there is an optimum quantity of reproductive tillers to be produced, and allowing for maximum yield hence assuring the plants' survival. This would elucidate why with the greater quantity of basal tillers present at the end of regrowth in 1995/6 year there was less percentage conversion to fertile tillers compared to 1996/7 (Fig. I-4).

Fertile tillers were 38 - 41% of the total tillers at the end of the 1995/6 growing season, but accounted for 44 - 59% in 1996/7. This change is no doubt caused by the decline in tiller production from year to year, but not in reproductive tillers because of the ability to compensate tiller losses to some extent. However, this ability to compensate may vary as tiller production declined thus we saw that 1996/7 had a wider range. In subsequent years, this range may be even wider!

The compensation threshold and the self-thinning strategies in perennial ryegrass seed crops are further illustrated by the total tiller mortality throughout the growing season. In 1995/6 there was a higher amount of mortality (Fig. I-5) because of the vast

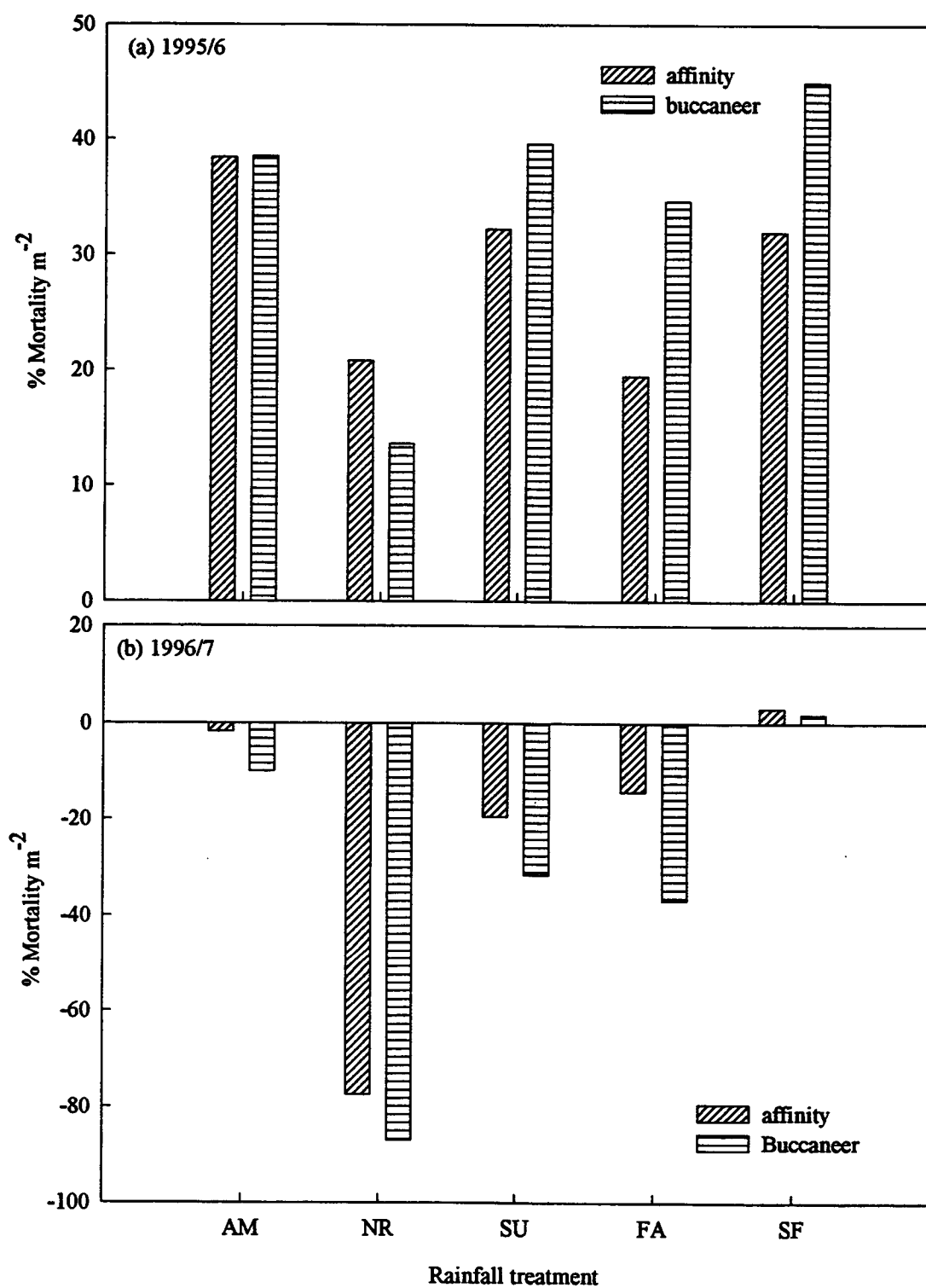


Fig. I-5. Growing season total tiller mortality in two cultivars of perennial ryegrass.

amount of tillers produced. Many of these were aerial tillers (Table I-1) that undoubtedly were self-thinned as time progressed. On the contrary, in 1996/7 the ratio of aerial tillers to basal tillers was very low and hardly any mortality was seen (Fig. I-5). This further supported the theory of more resources being allocated to fewer tillers from an early stage hence an increased, or little change, in seed yield from 1995/6 to 1996/7. Perennial ryegrass is known for its production of aerial tillers. It therefore means that any factor affecting this will eventually impact the basal tillers before seed yields decline. It appears that this is exactly what the dieback phenomenon is doing. At this incipient stage the aerial, basal and the total tillers are first affected, then as time and the dieback problem progress stand density and ultimately seed yield decline.

Further evidence that self-thinning played a role in determining yield can be deduced from a plot of seed yield against total tiller mortality throughout the growing season (Fig. I-6). In Buccaneer there was a linear relationship whereby seed yield increased with increasing mortality. When aerial tillers account for a major fraction of such mortality this relationship is readily understood. However, with r^2 of 0.32 and 0.43 (1995/6 and 1996/7) there were other factors, e.g., yield compensation for tiller losses, that determine final seed yields.

In general, there were no differences in seed yield between treatments in both years except for the summer rainfall treatment in 1996 when the yield was lower than the others (Tables I-12 & I-13). The seed yield of the Buccaneer treatments increased from 1995/6 to 1996/7 while those of Affinity treatments varied. These differences in seed yield may

Fig. I-6. Effect of total tiller mortality on the seed yield of two cultivars of perennial ryegrass. (a) Affinity $r^2 = 0.08$, $P > 0.05$; Buccaneer $r^2 = 0.32$, $P > 0.05$. Regression equation for the fitted line for each cultivar is: Affinity, $Y = 1398.16 - 4.61X$; Buccaneer, $Y = 1141.17 + 1.66X$.
 (b) Affinity $r^2 = 8.83 \times 10^{-3}$, $P > 0.05$; Buccaneer $r^2 = 0.429$, $P > 0.05$. Regression equation for the fitted line for each cultivar is: Affinity, $Y = 1269.71 + 0.16X$; Buccaneer, $Y = 1397.01 + 0.623X$.

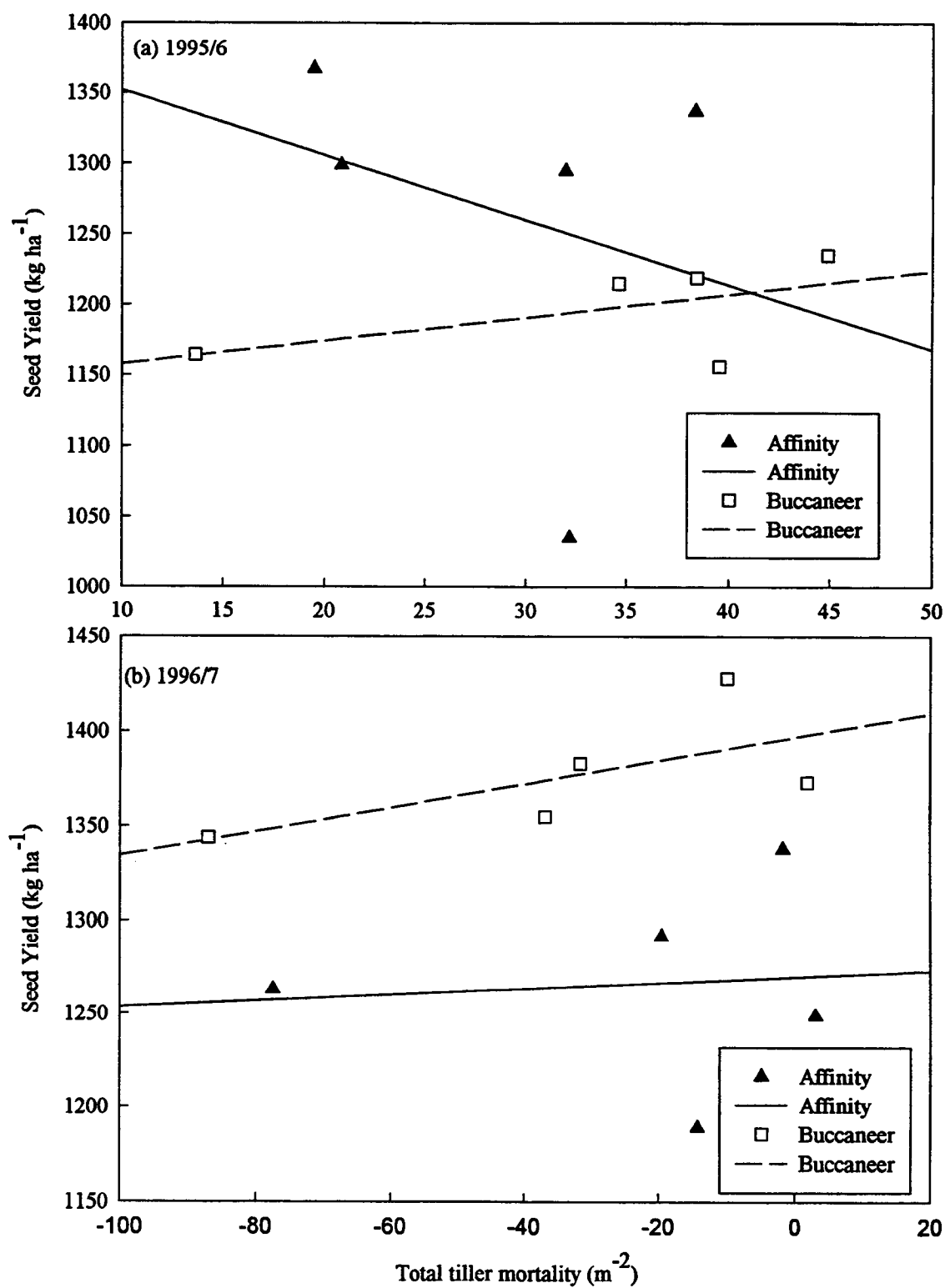


Fig. I-6.

Table I-13. Water stress impacts on spring tiller biomass, seed yield and harvest index in two cultivars of perennial ryegrass seed crops.

Rainfall treatment	Affinity			Buccaneer		
	Tiller biomass	Seed yield	Harvest index	Tiller biomass	Seed yield	Harvest index
<u>1995/6</u>	-----kg ha ⁻¹ -----			-----kg ha ⁻¹ -----		
Ambient	11527 a†	1337 a	11.60 a	13278 a	1219 a	9.18 a
No rain	11096 a	1299 a	11.71 a	11726 a	1164 a	9.93 a
Summer	12135 a	1035 b	8.53 a	11072 a	1156 a	10.44 a
Fall	11707 a	1367 a	11.68 a	10397 a	1215 a	11.69 a
Summer+Fall	12619 a	1295 a	10.26 a	11949 a	1235 a	10.34 a
<u>1996/7</u>						
Ambient	11323 a	1338 a	11.82 a	9458 a	1428 a	15.10 a
No rain	9611 a	1263 a	13.14 a	7112 a	1344 a	18.89 a
Summer	10526 a	1292 a	12.27 a	8964 a	1383 a	15.43 a
Fall	10107 a	1189 a	11.77 a	11008 a	1355 a	12.31 a
Summer+Fall	12161 a	1249 a	10.27 a	11649 a	1373 a	11.79 a

† Means within columns not followed by the same letters are significantly different according to Fisher's Protected LSD (P=0.05) values.

be linked to the production and survival of the basal tillers throughout the growing season and the eventual conversion to fertile tillers.

Aerial tillers remain vegetative (if they survived) continuing to divert essential nutrients and water from the basal tillers, and the longer they survive the greater (more negative) is their impact on seed yield. These tillers being vegetative behave as annual tillers and die before harvest (Hill and Watkin, 1975). Basal tillers on the other hand stood a greater chance of being induced to the reproductive stage. Therefore, the fewer aerial tillers and the more basal tillers present throughout the growing season the better should be the plants' chances of producing good seed yields. Colvill and Marshall (1984) observed that the earlier a tiller was produced the greater were its chances for flowering because its chances of surviving through the growing season were greater. Also, the primary tillers survived better than secondary and tertiary ones. Much of our basal tillers were primary tillers whereas the aerial tillers were comprised mainly of secondary and tertiary tillers. Therefore, fewer aerial tillers were expected to survive the growing season.

In both cultivars, it was observed that the total tiller production at the end of autumn regrowth declined from 1995/6 to 1996/7 (Table I-5). It meant that more resources were available to the existing tiller in 1996/7 than in 1995/6 (i.e., less competition between tillers). However, in Buccaneer, though the fertile tillers remain about the same from one year to the next, the quantity of basal tillers that was present in 1996/7 was far less than the previous year. This meant that there were more resources available to fewer tiller in 1996/7 than in 1995/6, thus, the percentage of basal tillers that may have been induced to fertile ones in 1996/7 was greater than in 1995/6. Such

increased representation was also observed by Colvill and Marshall (1984) who also worked with *L. perenne*. If it is assumed that the basal tillers that were not converted (Fig. I-4) remained vegetative and competed with the fertile ones then it should be expected that there will be a greater adverse effect on seed yield in 1995/6 (less % conversion to fertile tillers) than in 1996/7. This may explain why even though there were fewer total and basal tillers in 1996/7 than the previous year the seed yields were either equal to or about the same in both cultivars. We did not measure the other components of seed yield namely, spikelets tiller⁻¹, florets spikelets⁻¹, % seed set and seed weight, to ascertain if these changed significantly.

The zero-intercept model was used for the regression of seed yield on vegetative and reproductive tillers because it is assumed that in the absence of these tillers yield will be zero. Model checking revealed that (1) there was a significant interaction between vegetative and reproductive tillers for both cultivars in both years and (2) seed yield was better explained by the quadratic effects of tillers rather than a linear one. Though the regression curves were different there were similar trends between vegetative and reproductive tiller effects on yields (Fig. I-7 & I-8). What was being observed here was the production of fertile tillers was closely associated with that of vegetative tiller production. Therefore, as the number of vegetative tillers increased there were more tillers being induced to flower.

In all cases as the number of fertile tillers increased initially the yield also increased, after which there was a slight decline in yield with increasing tiller production. In general, the highest yield per tiller occurs with only a few tillers present (less competition), then as

Fig. I-7. Impact of type and number of spring tillers on seed yield of two cultivars of perennial ryegrass in 1996. (a) vegetative $r^2 = 0.01$, $P > 0.05$; fertile $r^2 = 0.21$, $P > 0.05$. Regression equation for the fitted line for each type of tiller is: vegetative, $Y = 67.06X - 0.88X^2$; fertile, $Y = 85.82X - 1.34X^2$.
 (b) vegetative $r^2 = 0.04$, $P > 0.05$; fertile $r^2 = 0.18$, $P > 0.05$. Regression equation for the fitted line for each type of tiller is: vegetative, $Y = 70.74X - 0.99X^2$; fertile, $Y = 93.56X - 1.74X^2$.

Fig. I-8. Impact of type and number of spring tillers on seed yield of two cultivars of perennial ryegrass in 1997. (a) vegetative $r^2 = 0.02$, $P > 0.05$; fertile $r^2 = 0.02$, $P > 0.05$. Regression equation for the fitted line for each type of tiller is: vegetative, $Y = 94.83X - 1.68X^2$; fertile, $Y = 71.51X - 0.97X^2$.
 (b) vegetative $r^2 = 0.12$, $P > 0.05$; fertile $r^2 = 0.10$, $P > 0.05$. Regression equation for the fitted line for each type of tiller is: vegetative, $Y = 103.70X - 1.82X^2$; fertile, $Y = 108.0X - 2.04X^2$.

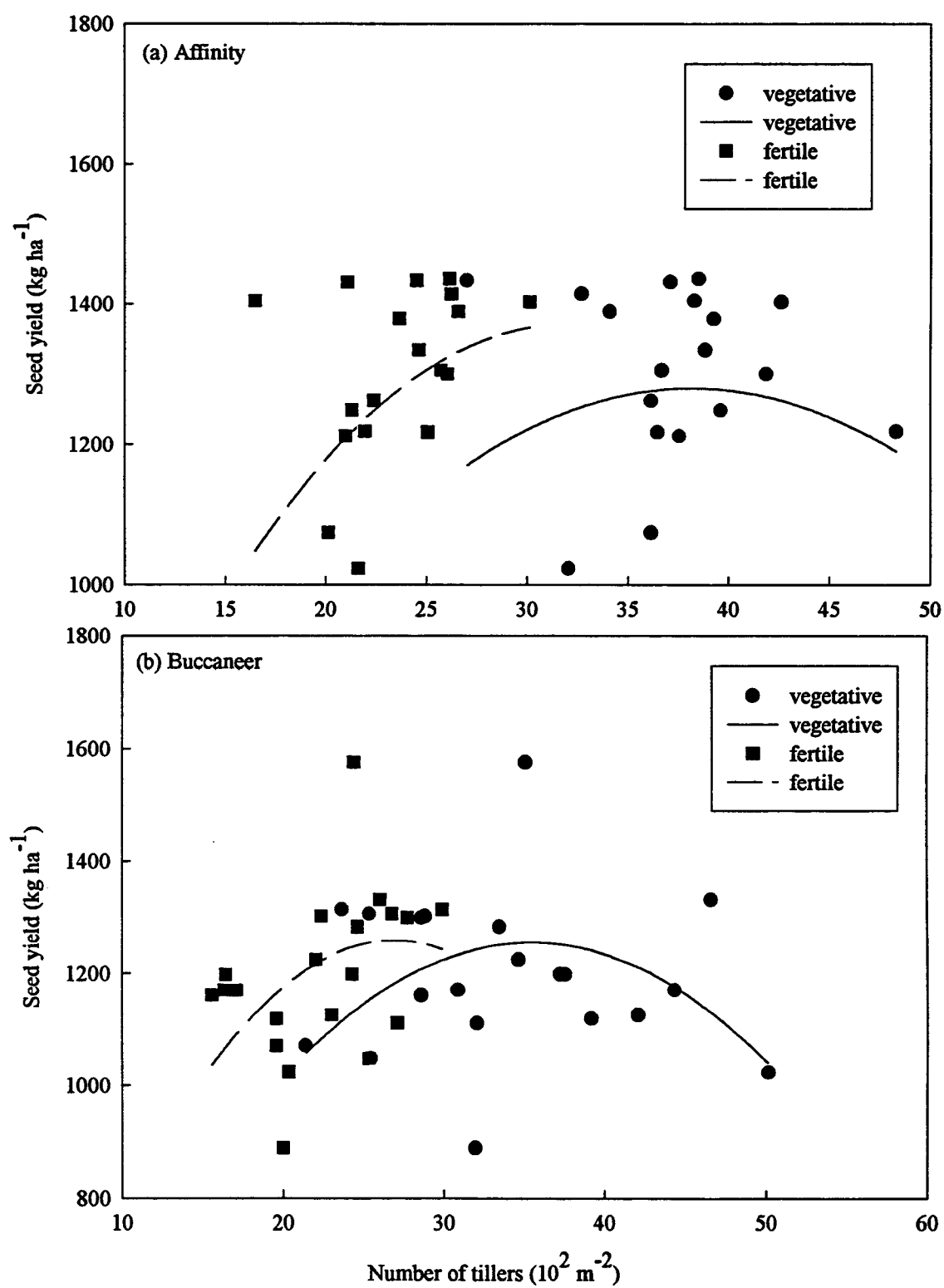


Fig. I-7.

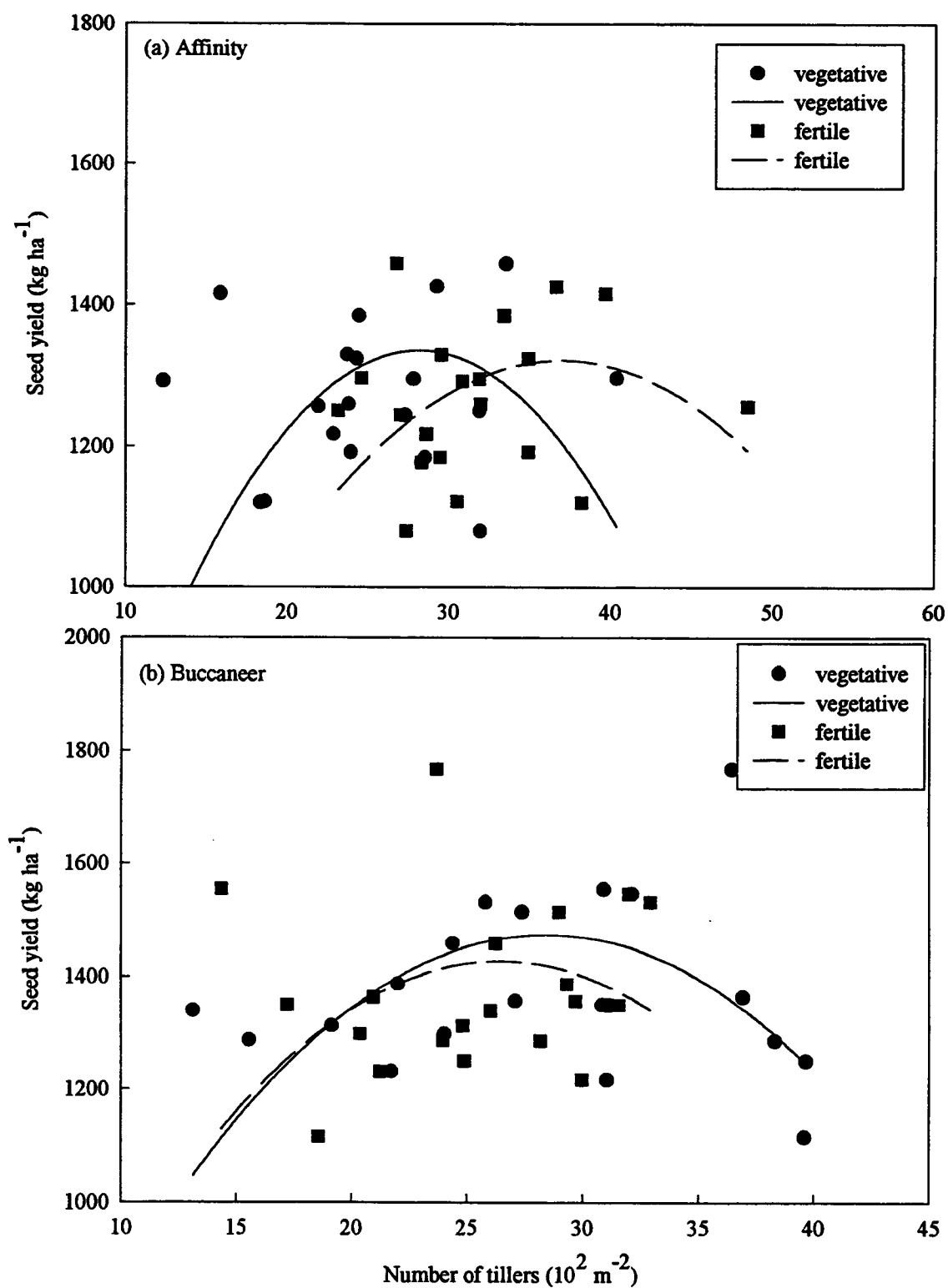


Fig. I-8.

tiller number increases yield per individual tiller decreases slightly (Colvill and Marshall, 1984). However, overall yield increases (or remains the same) because of more fertile tillers. Beyond a certain number of tillers inter-tiller competition for assimilates is so great that individual and total seed yields are adversely affected. This decline in seed yield occurs beyond the tiller compensation range. Overall, the increasing competition for assimilates at flowering exerts a major influence on the production and survival of tillers (Colvill and Marshall, 1984).

In Affinity, more fertile tillers were required in 1996/7 to produce an equivalent quantity of seed yield as in 1995/6 (Fig. I-7a and I-8a). This would substantiate the point that seed yield per tiller is decreasing from year to year. Since the fields used in this study were only two years old any decline in seed yield may only be slight at this point, becoming more pronounced in later years. This means that in subsequent years if yields were to be comparable to the present more fertile tillers will be needed, eventually overall seed yield will decline. Observations revealed that in commercial seed fields a fall in seed production is also accompanied by a loss in stand.

Unlike Affinity, Buccaneer did not exhibit such early responses to seed yield, partly supporting the observation that it is not as susceptible to dieback as the cultivar Affinity. Figures I-7 and I-8 show that in 1995/6 the number of vegetative tillers clearly outnumbered the fertile tillers but a year later they were roughly equal. It maybe that as vegetative tillers decreased a greater proportion were induced to flower to avoid a decline in seed production.

CONCLUSION

Our findings showed that though limited rainfall had a marked influence on the response of perennial ryegrass after seed harvest and during the summer and autumn periods of regrowth, there was no effect on spring fertile tillers and seed yields. Thus, if it is anticipated that the regrowth period will be very dry then irrigation is recommended to increase tiller production and also to prolong the life of the stand.

No rainfall affected all of the parameters measured. There was a decline in total tiller number, tiller dry weight, plant height, number of leaves per tiller and tiller basal diameter. However, total tiller number was affected the most and tiller basal diameter the least. Measurements of canopy temperature did not detect subtle differences in water stress levels probably because crop canopy was very open at this stage. However, total soluble sugars did increase as number of total tillers declined suggesting an accumulation of reserves to be utilized upon the advent of the rains.

Further Work: The accumulation of sugars suggests the need for a better understanding between tiller production and water stress. In particular, it would be interesting to identify and quantify the types of sugars and explore any relationship to tiller production. Changes in the ratio of hexoses to oligofructans need to be ascertained. Carbohydrate analyses should be conducted before and periodically throughout the regrowth period to establish how water stress affects the type and concentration of sugars and how these impact autumn tiller number, tiller dry weight and spring tillering patterns.

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MANUSCRIPT II: SURVIVAL AND WATER RELATIONS OF FOUR CULTIVARS
OF *Lolium perenne* EXPOSED TO DROUGHT IN A GREENHOUSE
ENVIRONMENT

ABSTRACT

Four physiological responses and the survival rates of four cultivars of perennial ryegrass were measured after clipped and unclipped plants were subjected to rapid drought under greenhouse conditions. As drought developed plant water potential and transpiration rate decreased, and leaf and canopy temperatures and stomatal diffusive resistances increased. However, there were no significant and consistent differences between the cultivars, or cutting regimes, for these parameters. It is suggested that because of the extreme shrinkage of the vermiculite there likely was poor root-soil contact which created rapid artificial drought conditions. Cultivars did not have sufficient time to adapt to the water stress hence little differences in their responses.

Plant survival was more closely linked to the gravimetric water content than the water potential. Mortality commenced from day two of stress and by day 21 very few surviving plants remained. The cultivar Linn was the most susceptible while Affinity and Delray were the least susceptible. Defoliation did not significantly alter the responses nor the rate of survival of stressed plants. Dry matter tended to decrease for the first 14 days of stress then increased. This technique did not simulate natural drought hence the physiological responses of the cultivars were not adequately evaluated.

Keywords: Perennial ryegrass, drought, survival, water relations and vermiculite.

INTRODUCTION

The growth and survival of grasses are affected by the availability of water. An understanding of how grasses respond when there is deficit of water may help improve management and selection of new, more stress-tolerant varieties (Jones, 1988). Grasses show a remarkable resistance to severe water stress in that they are able to survive very large soil water deficits even though yields are drastically reduced (Stiles and Williams, 1965). Drought leads to rapid physiological and morphological changes as plants adapt to this emerging situation. For instance, there is an increase in canopy temperature (Frank, 1994) and stomatal diffusive resistance (Sheehy et al., 1975) but decreases in plant water potential, transpiration rate (Jones et al., 1980a) and net assimilation rate (Bittman and Simpson, 1987). Plants also adapt through a reduced growth rate resulting in lower rates of leaf appearance (Garwood and Williams, 1967), leaf length (Norris, 1982) and tiller height (Busso and Richards, 1995); however, there may be stimulation and proliferation of new roots (Jupp and Newman, 1987). In general, a water stress during vegetative growth that is not severe and long merely postpones plant growth and development to a later date (Hsiao and Acevedo, 1974).

The quantity of water available to the plant is less than the soil's storage capacity and depends upon the plant's ability to maintain a concentration gradient from the soil to the roots. The lower limit of this available moisture is generally accepted to be -1.5 MPa but this may vary as the osmotic properties fluctuates hence influencing turgor pressure (Jones, 1988). The gradient that is created as roots absorb moisture depends upon

climatic conditions, nature of the rooting medium and the size, number, age and distribution of the roots.

Since drought is probably the major deterrent to the growth of grasses (Power, 1971) there is an ever present need to evaluate new and existing genotypes in their response to increasing water deficit. There are, as yet, no reliable physiological criteria for selecting temperate grasses for resistance to drought or subsequent recovery (Thomas and Evans, 1991). The preferred technique should be one that is objective, uncomplicated, inexpensive, rapid, reliable and reproducible. It would be desirable to have cultivars that continue to grow during periods of stress without many of the marked detrimental changes caused by drought and possibly heat stress. More work is needed at the physiological level to better determine the suitability and adaptability to water stress.

The responses of four perennial ryegrass cultivars (Affinity, Buccaneer, Delray and Linn) to water stress were tested in the greenhouse. These cultivars represent the range of dieback symptoms from unaffected to high incidence and severity. At present, no formal ranking of all cultivars in terms of the susceptibility to this decline has been done but it is generally accepted that Delray is more susceptible than Linn, and Affinity is more susceptible than Buccaneer. The objectives were (1) to ascertain differences between cultivars in their responses to water stress, (2) to learn whether water stress may be a causal factor in the development of dieback in perennial ryegrass seed fields and (3) to develop a simple and practical method that could be used as an evaluation criterion to identify genotypes of perennial ryegrass differing in their responses to water stress.

MATERIALS and METHODS

Seed were sown in large flats with cell packs before transplanting into size D-40 DeepotsTM (656 ml) and subjected to water stress at 3 months of age. A single tiller was placed per conetainer filled with vermiculite (Therm-O-Rock # 2, coarse) and allowed to develop before being stressed. Plants were fertilized weekly with 20-20-20 (NPK) water soluble fertilizer (with trace elements) at a rate of approximately 4 ml/L of water. There were four stress levels (1) No stress- plants watered on alternate days (W_0); (2) No water for 7 days (W_7); (3) No water for 14 days (W_{14}); and (4) No water for 21 days (W_{21}). There were three timings of stress (a) Plants cut, watered to saturation, then stressed (T_1); (b) Plants watered to saturation, stressed, then cut (T_2); and (c) Plants watered to saturation, stressed for 1 wk, cut, then stressed (T_3). The experiment was a split-split plot with level of stress as a whole plot, timing as a sub-plot and cultivars as a sub-sub plot. There were three replications (blocks) in time because of space constraints within the greenhouse.

There were three conetainers of each cultivar per treatment combination. Plants were watered to saturation the night before the treatments were implemented and allowed to drain overnight. The weight of the conetainers the following morning were considered to be that at 'field' capacity. Water retention values and curves (Fig. II-1) for vermiculite were obtained from the OSU Soil Physics Laboratory for both gravimetric and volumetric water content at varying water potential. Changes in the weights of the conetainers allowed for calculation of the vermiculite water potential at different times of stress. The

gravimetric values were used because of extreme shrinkage during desaturation of the laboratory test. Sample weights, canopy temperature, plant water potential, leaf transpiration rate and stomatal diffusive resistance were obtained bi-weekly.

Plant water measurements were made with a model 3005 plant water status console (Soil Moisture Equipment Corp., Santa Barbara, CA) utilizing compressed nitrogen gas. Water potential was measured at the same time each day. No correction was made for the osmotic potential of the xylem sap as it is consistently high (>-1 bar) (Jones et al., 1980a, and 1980b). The tiller was clipped at the soil level and immediately inserted into the chamber. Water potential was read within 3 minutes of tiller removal. One tiller was used from each container. Transpiration rate and stomatal resistance was obtained using a model Li-1600 steady state porometer (Li-cor, Inc.) with a 1 cm² narrow aperture (1600-01) attached to the sensor head. Because perennial ryegrass leaves are epistomatous, measurements were made at mid-point of the adaxial surface of the last fully expanded leaf (Jones et al., 1980a). An infrared thermometer (Teletemp Corp.) was used to monitor canopy temperature. All measurements were taken on Tuesdays and Thursday of each week. At the end of the specific period of stress the shoots were cut at the soil surface, oven-dried (65 °C for ≥ 48 hours) then weighed. Recovery of growth (after stress) was not estimated in this study.

STATISTICAL ANALYSES

Dry matter was analyzed as a split-split plot with stress level as whole plots, timing of stress as sub-plots and cultivars as sub-sub plots. Other parameters of plant response were analyzed as a split plot with repeated measures using timing of stress as the whole plot and cultivar as a sub-plot. In this type of analysis, the dates when measurements were taken were considered as the level of a third factor (date) applied to the sub-plots (cultivars) in a split-split plot type of experiment. This was because the number of treatments restricted measurements to only a portion of all plants. Analysis was done using SAS for Windows version 6.12 (SAS Institute, Inc. 1993). Treatments effects were tested by analysis of variance and means separated by Fisher's Protected LSD values.

RESULTS and DISCUSSION

Vermiculite -Physical Properties

It is important that the relationship between the gravimetric water content (gwc) and the water potential of the vermiculite be known in order to better understand the physical properties of vermiculite and its behavioral patterns as it dries. The commonly observed relationship between soil water potential and soil water content is a sigmoidal pattern where water potential decreases as the soil dries (Brady, 1990). This facilitates the gradual imposition of moisture stress on plants both in the fields and the greenhouse because water loss per day tend to be reasonably constant. However, with the vermiculite this pattern was not observed. Values from the Soil Physics Laboratory showed that there is a sharp linear relationship between gwc and water potential from approximately the point of saturation to -0.01 MPa (Fig. II-1) which represented a change in weight of about 38%, and a change in both gwc and volumetric water content of approximately 54%. Thereafter, there was a more gradual decline in all parameters to the -1.5 MPa water potential level; this latter decline approximates a quadratic pattern very well (Fig. II-1).

Extremely high temperature were used to create vermiculite such that it will expand upon wetting to hold a tremendous quantity of water in its lattice structure. This means that there will be very rapid initial desorption followed by a lower rate of water loss. As such it may be most useful as a plant growth medium where water stress is not to be imposed. Therefore, under the conditions used in this trial it imposed early and rapid water stress situations around the roots of plants. This caused high mortality, high

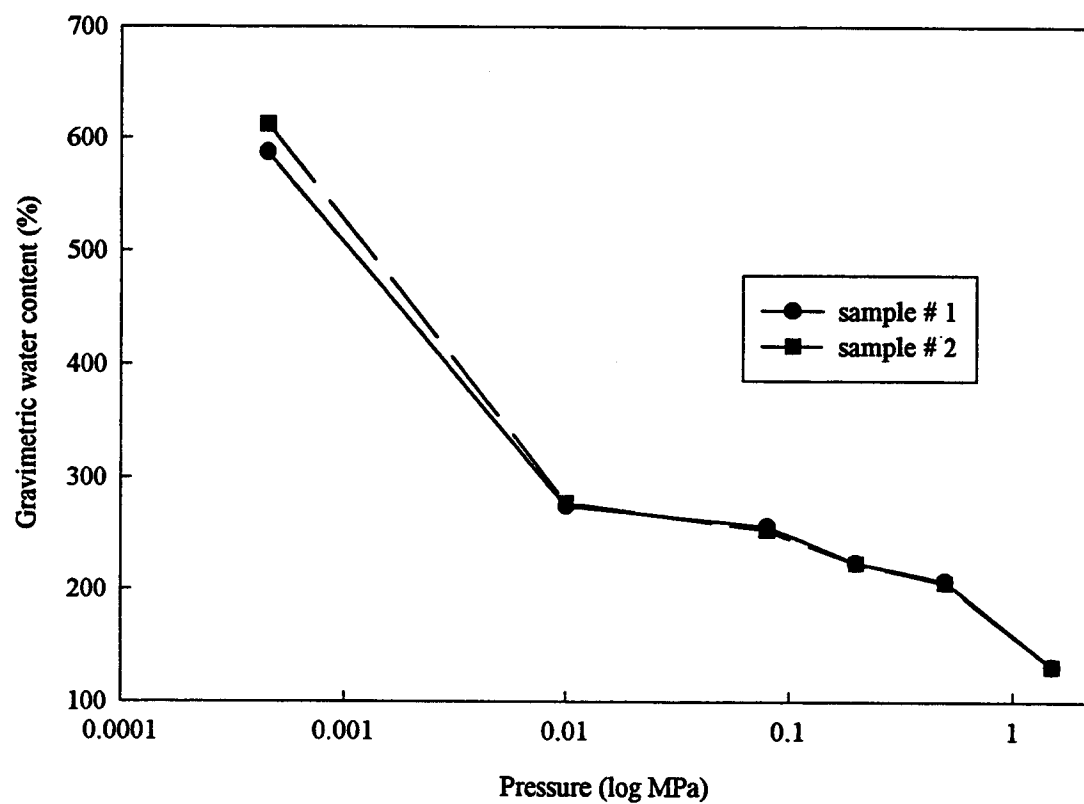


Fig. II-1. Water retention curve for vermiculite.

stomatal diffusive resistance and low plant water potential in a short time (≤ 1 wk) regardless of the cultivar or cutting regime used in the trial. This may therefore account for many of the inconclusive differences observed between cultivars and among the three cutting treatments used. This argument agreed with that of Bittman and Simpson (1989a) for sandy soils. They argued that the nature of shallow, coarse-textured soils is such that they dry rapidly, giving up their moisture easily to the plant, and maintaining little reserve. This caused rapid plant reaction when tissue moisture content began to decrease. Deep, fine-textured soils (dry slowly) maintained a reserve of water that resisted uptake by plants. This water reserve buffered against desiccation and allowed the plant to risk greater loss of tissue water content. This may be one reason why potted plants (rapid soil-water depletion) have a threshold-type stomatal response. Our measurements in this study revealed no drastic differences from the beginning of stress to the time of plant mortality. It appeared that it was the vermiculite water status, not the leaf water status, that regulated stomatal behavior. Plant hormones may have been transported from roots (sensors of stress) to the leaves in the transpiration stream to solicit the above reactions (Bittman and Simpson, 1989a; Davies et al., 1990).

The coefficient of determination (r^2 -value) determined by regression analysis showed a strong ($r^2=0.996$) linear relationship between the change in weight of vermiculite and the gwc (Appendix-Fig. -3). These data point values were obtained from the Soil Physics Laboratory at Oregon State University. However, the relationship between change in weight of vermiculite and water potential was a quadratic one ($r^2=0.965$)

(Appendix-Fig. -4). The gwc was recommended by the laboratory for use because of the extreme shrinkage underwent by the vermiculite during desaturation.

Plant Survival

Affinity and Delray were the two cultivars that succumbed last to the imposed water stress. Although in treatments T1 and T2 no plant survived beyond three weeks of water stress, plants from the two cultivars survived to 21 days of stress (Fig. II-2).

Less than one week of water stress was needed before marked changes (external appearances and internally) in plant responses were observed. For instance, by the second date of measurements of the stress period there were plants that were wilted to such an extent that a water potential measurement could not be taken with the pressure bomb (capacity of up to 4 MPa), nor the porometer used to record other parameters. Since these plants had insufficient surviving green leaf tissues they were considered to be dead. Such rapid stress is not unknown since a noticeable difference between the field and simulated swards was that the stressed fields showed no visible signs of wilting at -16 bars (Jones et al., 1980a), whereas the stressed simulated swards wilted when leaf water potential fell below -12 bars after only five days of drought. These researchers stated that when stress occurs naturally in the field it appears that changes in the leaves take place as stress is developing and these changes result in a less severe effect of water stress on leaf photosynthesis. However, when stress is imposed with unnatural rapidity, there is no chance for these changes to take place and stress leads to a rapid and severe decline in leaf photosynthesis. Under these conditions plant responses (or lack of) failed to prevent

Fig. II-2. Survival rate of perennial ryegrass under simulated drought conditions. (T1 $r^2 = 0.92$, $P < 0.01$; T2 $r^2 = 0.88$, $P < 0.01$; T3 $r^2 = 0.88$, $P < 0.01$;). Regression equation for the fitted line for each treatment is: T1, $Y = 104.4 - 4.13X$; T2, $Y = 97.99 - 4.02X$; T3, $Y = 94.89 - 3.32X$.

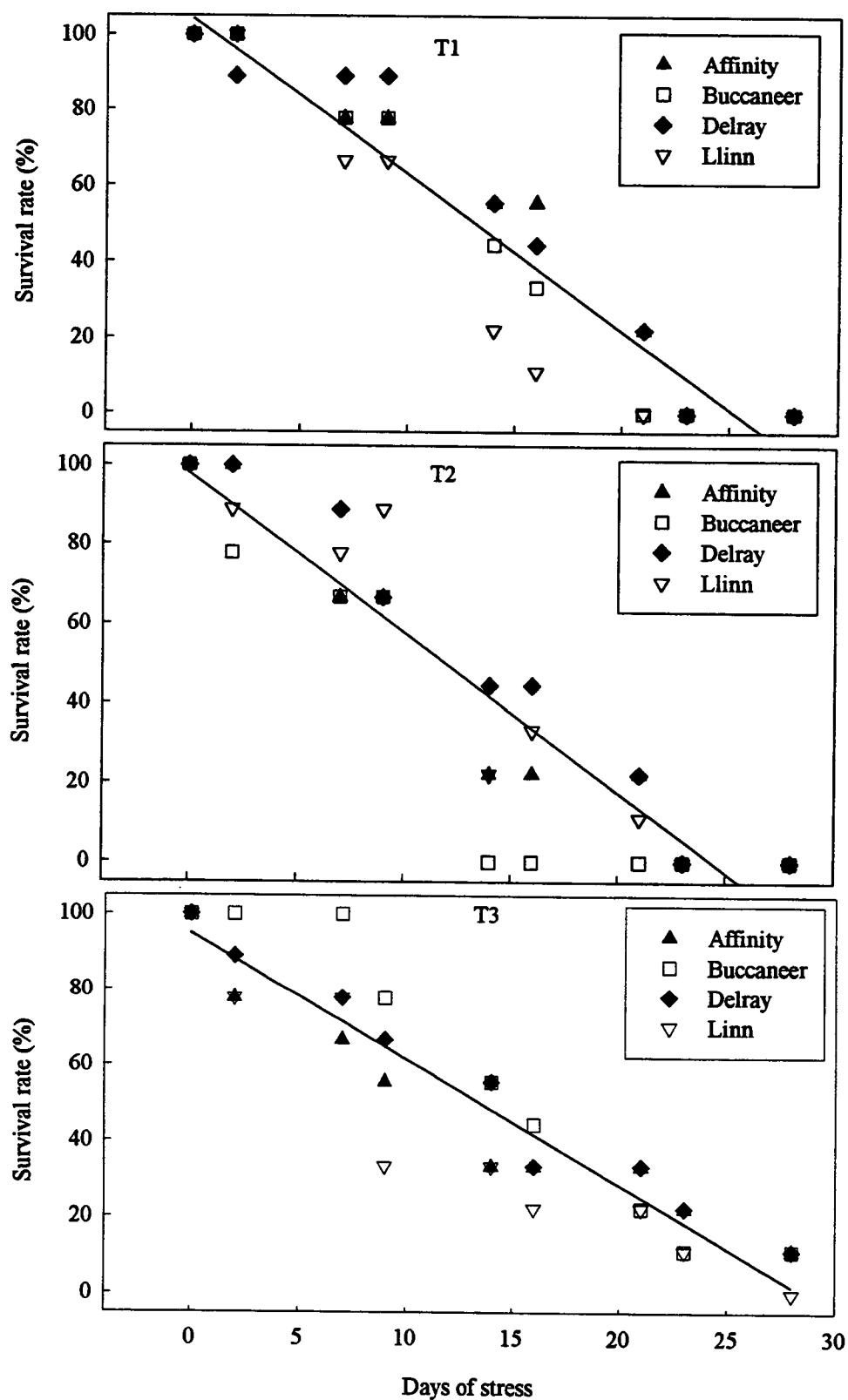


Fig. II-2.

turgor from falling to zero and the leaves wilted between four and five days after water was withheld (Jones et al., 1980b). Pronounced and rapid physiological changes caused by drought were also recorded for *Agropyron* and *Pseudoroegneria* plants (BassiriRad and Caldwell, 1992). With sand as the soil medium, severely stressed plants exhibited a 70% - 80% drop in stomatal conductance within four days of stress. However, with compost as the soil medium (Sheehy et al., 1975) perennial ryegrass showed a change in leaf resistance from 2 s cm^{-1} (control) to 6.9 s cm^{-1} in 8 days, and to 26.3 s cm^{-1} on day 15 of stress; however, for the first 7 days the leaf water potential of stressed plants did not differ significantly from those of the control (-7 bars). At day 15 the water potential in stressed plants reached a low of -15.5 bars.

In general Linn, wilted more than the others at nearly all stages of stress and regardless of the treatment. Whether or not plants were cut before being stressed did not make a difference on survival, transpiration, stomatal diffusive resistance nor the plants' water potential. This may reflect the great influence of an external factor, vermiculite. Although vermiculite holds a tremendous amount of water relative to an equal volume of soil when saturated, it also dries out and shrinks extremely rapidly (Fig. II-1). Thus by the third day of stress there was so little moisture in the soil (Fig. II-3 & II-4) that there was no significant differences between treatments. Under controlled environment other researchers, (Amin and Thomas, 1996), also found no significant variation between populations of perennial ryegrass when leaf water conductance were examined.

It is well established that drought increases the rate of leaf senescence in plants (Hsiao, 1973). The vermiculite imposed a moisture stress very rapidly resulting in wilted

Fig. II-3. Changes in vermiculite water potential under simulated drought conditions. (T1 $r^2 = 0.93$, $P < 0.01$; T2 $r^2 = 0.92$, $P < 0.01$; T3 $r^2 = 0.94$, $P < 0.01$;). Regression equation for the fitted line for each treatment is: T1, $Y = 0.074 - 0.12X$; T2, $Y = -0.073 - 0.13X$; T3, $Y = 7.66 \times 10^{-3} - 0.12X$.

Fig. II-4. Gravimetric water content of vermiculite under simulated drought conditions. (T1 $r^2 = 0.95$, $P < 0.01$; T2 $r^2 = 0.95$, $P < 0.01$; T3 $r^2 = 0.94$, $P < 0.01$;). Regression equation for the fitted line for each treatment is: T1, $Y = 551.02 - 43.21X + 0.88X^2$; T2, $Y = 541.66 - 47.22X + 1.04X^2$; T3, $Y = 535.89 - 41.99X + 0.89X^2$.

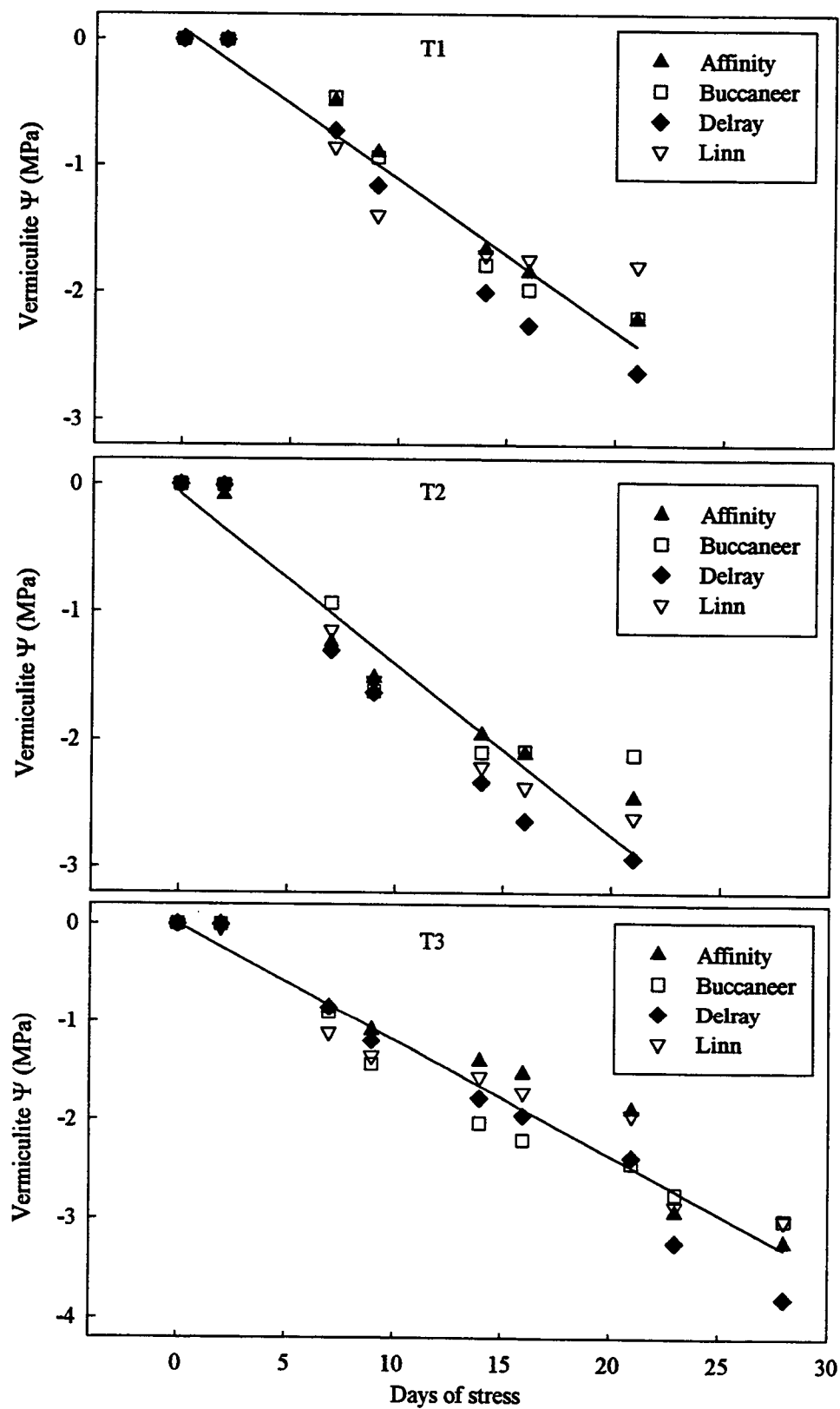


Fig. II-3.

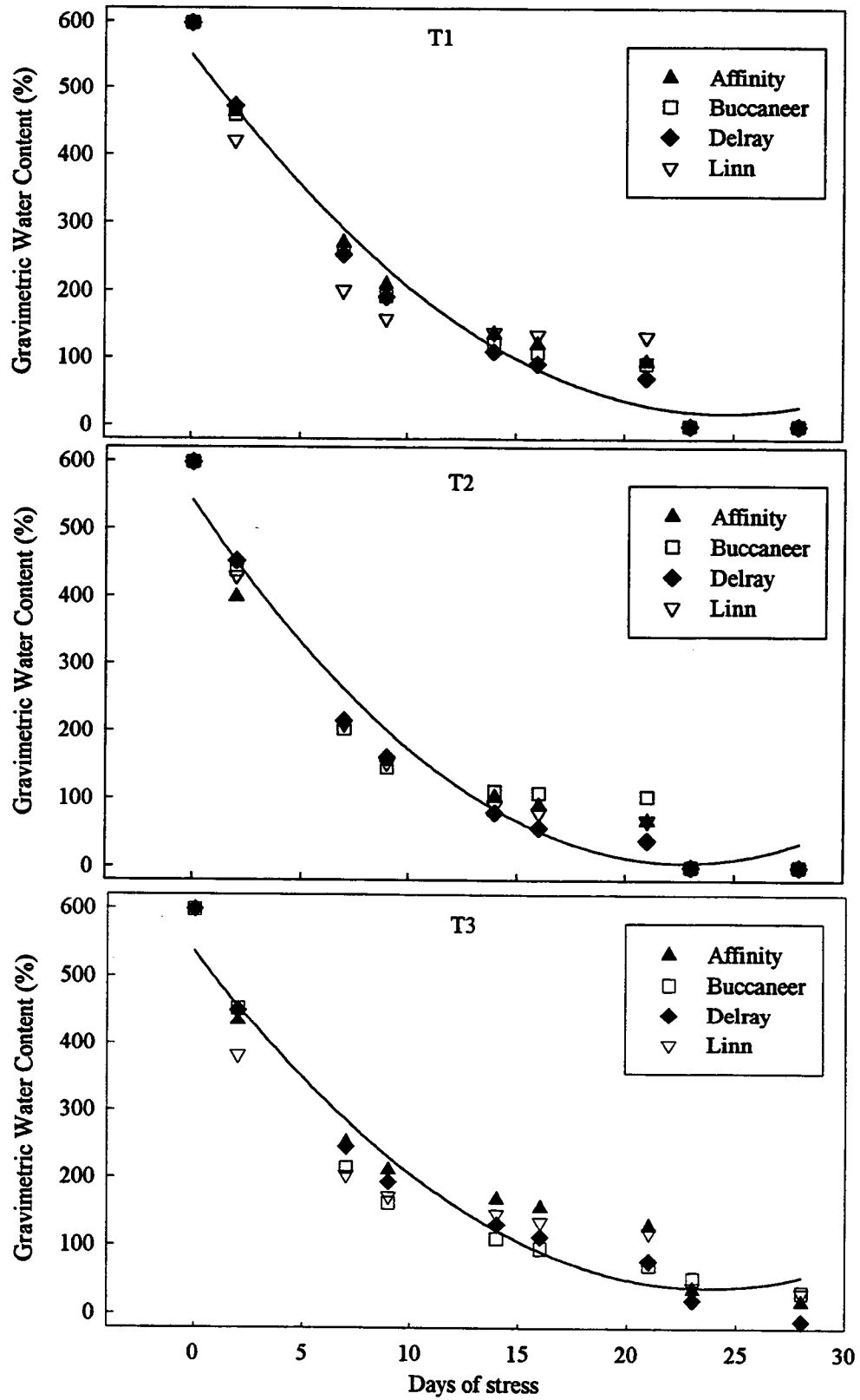


Fig. II-4.

plants that made it extremely difficult to take measurements of water potential, leaf temperature, transpiration and stomatal diffusive resistance. To maintain a constant rate of absorption the water potential gradient between the roots and the medium must remain constant. However, it is believed that as the vermiculite shrank root-soil contact was incomplete creating very high resistances at this interface. This created an early "false" drought leading to poor water absorption and early leaf senescence even though soil water potential may have been high. It is probably this inability to adjust to rapid stress that may cause conflicting results between field and greenhouse studies (Sheehy et al., 1975; Jones et al. 1980b). Restricted volume of the vermiculite and the dense root system may also be contributing factors to the rapid wilting.

Unlike most other water stress studies, plants in this experiment were not re-watered following a period of drought therefore, a high plant mortality was expected by the completion of the stress period. These very wilted plants were considered as mortalities thus creating missing values and making it difficult for statistical analyses. To have a better understanding of what was occurring and to explain responses in a more easily and concrete manner regression was done on plant survival against the gwc and water potential of the vermiculite.

As expected (regardless of treatment) plant survival depended on the gwc of the medium which also determined its water potential. However, this survival is best described quadratically by the gwc and linearly by the water potential (Fig. II-5 & II-6 resp.). This can be explained when it is observed that there is a quadratic relationship between the gwc and the water potential of the vermiculite (Appendix-Fig. -5), and also

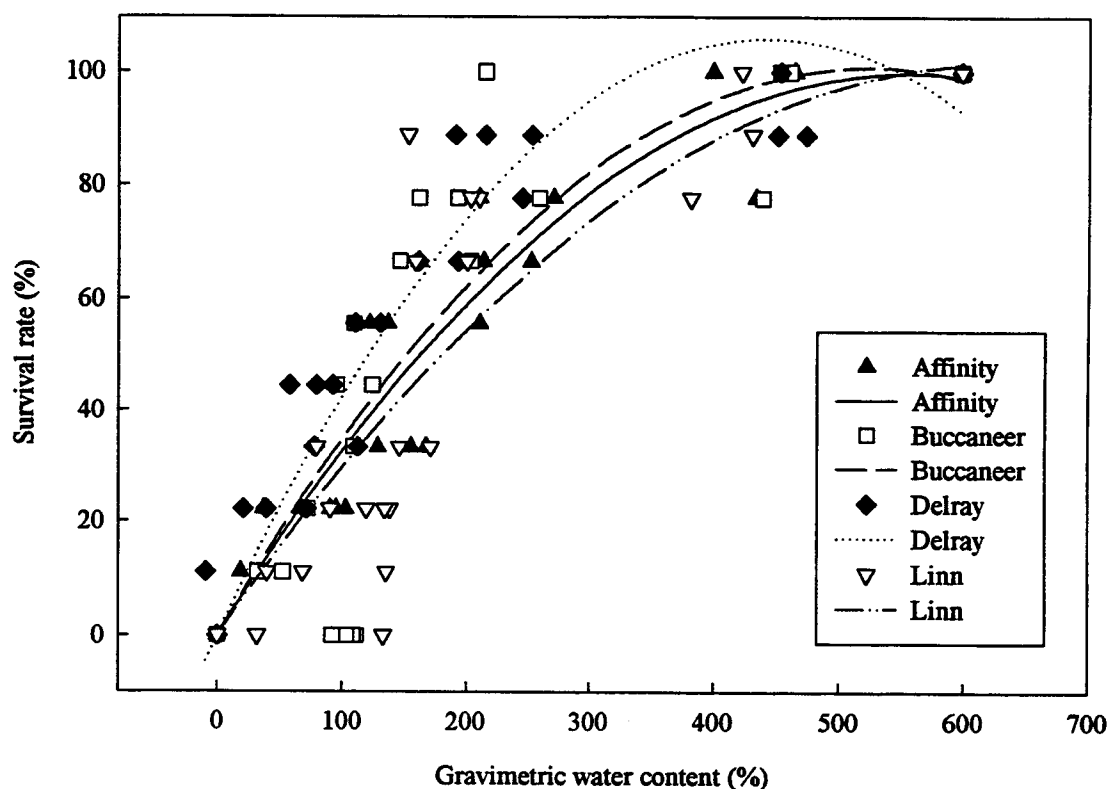


Fig. II-5. Effect of gravimetric water content of vermiculite on the mortality of perennial ryegrass under simulated drought conditions. (Affinity corrected $r^2 = 0.92$, $P < 0.01$; Buccaneer corrected $r^2 = 0.80$, $P < 0.01$; Delray corrected $r^2 = 0.92$, $P < 0.01$; Linn corrected $r^2 = 0.81$, $P < 0.01$). Regression equation for the fitted line for each cultivar is: Affinity, $Y = 0.36X - 3.24 \times 10^{-4}X^2$; Buccaneer, $Y = 0.39X - 3.68 \times 10^{-4}X^2$; Delray, $Y = 0.48X - 5.41 \times 10^{-4}X^2$; Linn, $Y = 0.32X - 2.58 \times 10^{-4}X^2$.

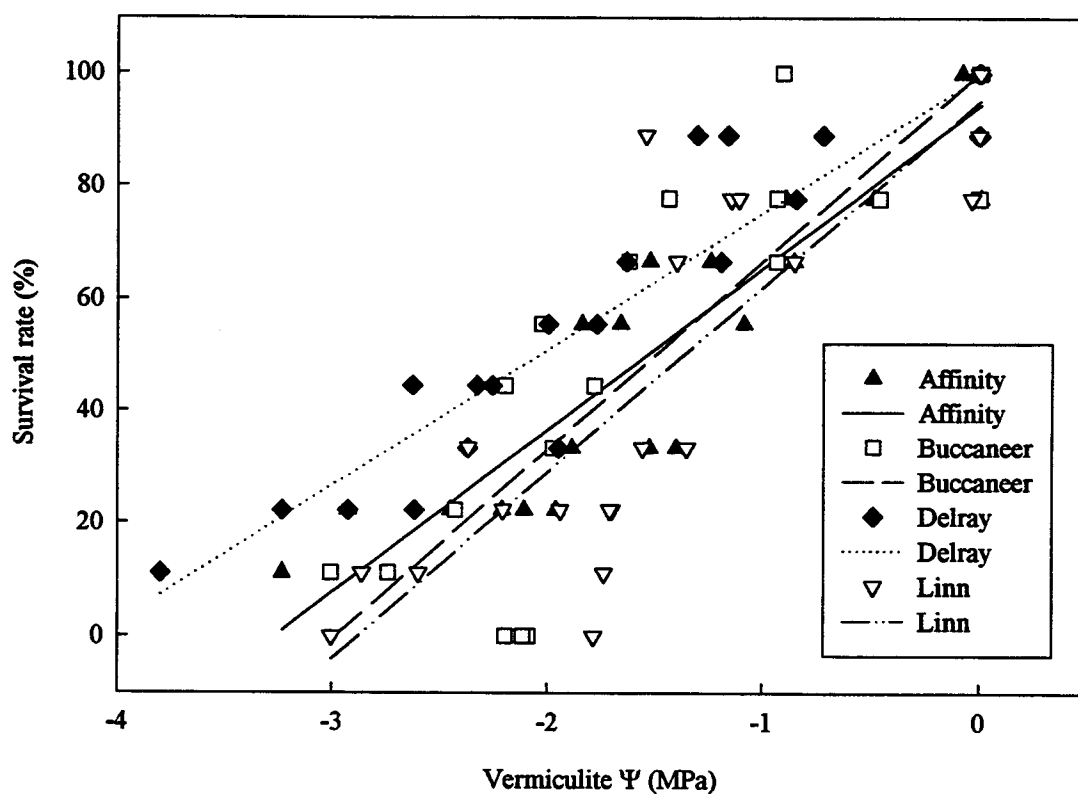


Fig. II-6. Effect of water potential of vermiculite on the mortality of perennial ryegrass under simulated drought conditions. (Affinity $r^2 = 0.87$, $P < 0.01$; Buccaneer $r^2 = 0.78$, $P < 0.01$; Delray $r^2 = 0.91$, $P < 0.01$; Linn $r^2 = 0.76$, $P < 0.01$;) Regression equation for the fitted line for each cultivar is: Affinity, $Y = 94.39 + 28.89X$; Buccaneer, $Y = 100.27 + 33.57X$; Delray, $Y = 99.51 + 24.29X$; Linn, $Y = 95.11 + 33.01X$.

between gwc and days of stress (Fig. II-4). This quadratic relationship is most likely due to the nature of the medium which underwent extreme shrinkage upon drying.

Percentage plant survival increased linearly as the growth medium water increased. From regression analyses the water potential accounted for approximately 87%, 78%, 91% and 76% of the variation in plant survival in the cultivars Affinity, Buccaneer, Delray and Linn respectively. Although all cultivars had a maximum survival rate close to a water potential of zero (Table II-1), the water potential at which 100% mortality occurred varied depending on cultivar (Fig. II-6). For instance, in Affinity this value is about -3.3 MPa, in Buccaneer and Linn it is around -3.0 MPa and in Delray it is about -3.8 MPa; plant water potential at this juncture was > 4.0 MPa. Drought-induced leaf senescence was observed in crested wheatgrass when the leaf water potential was in the range of -1.95 MPa to -2.65 MPa, and in smooth brome grass between -1.85 MPa to -2.25 MPa (Bittman et al., 1988). From the slopes of the regression lines Buccaneer and Linn had the highest mortality rates followed by Affinity then Delray over a given range of vermiculite water potential. This confirmed observations made in the field on the cultivars Affinity and Buccaneer that their responses differed to water stress. Two other cultivars of perennial ryegrass, Condesa and Wendy, (Van Loo, 1992) displayed great differences in tillering, leaf appearance rate and specific leaf area when they were grown hydroponically.

In the field Affinity responded rapidly to the addition of an increment of moisture by increasing the number of its total tillers. Since Buccaneer responded less slowly to this added moisture (following a summer drought stress) it might be expected that the reverse may be true i.e. it responds slower than Affinity to the loss of moisture from its growth

Table II-1. Regression parameters, estimated gwc, ψ and predicted survival % of four cultivars of perennial ryegrass in a controlled environment.

Cultivar	b_0	b_1	b_2	r^2	gwc (%)	ψ (MPa)	predicted survival %
Affinity	0.5996	0.3551	-3.1772×10^{-4}	0.92	558.83	-0.00167	99.82
Buccaneer	-6.636	0.4401	-4.4537×10^{-4}	0.81	494.08	-0.00357	102.09
Delray	6.8579	0.4248	-4.6572×10^{-4}	0.94	456.07	-0.00469	103.73
Linn	-5.239	0.3651	-3.1551×10^{-4}	0.81	578.59	-0.00109	100.38

Table II-2. Regression parameters, estimated gwc, ψ and predicted survival % of four cultivars of perennial ryegrass in a controlled environment when the intercept is zero.

Cultivar	b_1	b_2	r^2	corrected r^2	gwc (%)	ψ (MPa)	predicted survival %
Affinity	0.3596	-3.2390×10^{-4}	0.98	0.92	555.11	-0.00178	99.81
Buccaneer	0.3851	-3.6777×10^{-4}	0.92	0.80	523.56	-0.0027	100.81
Delray	0.4794	-5.4190×10^{-4}	0.98	0.92	442.33	-0.0051	106.03
Linn	0.3235	-2.5771×10^{-4}	0.92	0.81	627.64	-0.000355	101.52

medium. However, this was not seen probably because of the differences between field and controlled environments and also because of the unique nature of vermiculite which created a severe stress too rapidly. This agreed with the work of Amin and Thomas (1996) who found no relationship between expression of particular characteristics across environment. They correlated growth to various physiological characteristics (e.g. osmotic potential, tissue water content, membrane stability) but found inconsistencies across all environments. Furthermore, the ranking of populations changed from one environment to the next. Thus the authors warned about the dangers of conducting research solely under artificial environment. Water stress in the field, unlike other types of stress, usually develops slowly (Larcher, 1995) giving plants adequate time to adjust osmotically and physically. However, under the conditions used in this study severe water stress occurred over a period of one week or less making it unlikely for plants to adapt sufficiently to the imposed stress. This may account for the differences in responses seen between the field and the greenhouse.

The nature of the growth medium caused the gwc to be more closely related to plant survival (Fig. II-5). A quadratic model better explained the relationship between the gwc and survival percentages. Unlike the water potential where none of the quadratic terms were significant ($p=0.05$) all the quadratic terms were significant ($p=0.01$) for the gwc. The quadratic model explained approximately 93%, 80%, 94% and 81% of the variation in survival percentages for the cultivars Affinity, Buccaneer, Delray and Linn respectively.

To ascertain the gwc at which mortality was zero the response (quadratic) equation for each cultivar was differentiated. These first derivatives of the response equations were equated to zero and the solution for X (gwc) obtained, e.g. assume the response equation is $Y = b_0 + b_1X + b_2X^2$; the first derivative will be $b_1 + 2b_2X$. From the graph, maximum percentage survival (zero mortality) will occur where the slope=0, i.e. no change in survival % with an added increment of gwc, hence $dy/dx=0$; $b_1 + 2b_2X = dy/dx = 0 \Rightarrow X = -b_1/2b_2$. This gave gwc of 558.83%, 494.08%, 456.07% and 578.59% for Affinity, Buccaneer, Delray and Linn respectively. Using the linear aspect of the relationship ($b_0 = -0.1811$ and $b_1 = 2.9421 \times 10^{-4}$) between gwc and water potential of vermiculite these values corresponded to a water potential of -0.00167, -0.00357, -0.00469 and -0.00109 MPa respectively. To verify that these values of gwc gave the predicted maximum survival % the values for X was substituted into the response equation and a solution obtained for Y. For all four cultivars this value was approximately 100% (Table II-1).

When it is considered that at "field capacity" the water potential of vermiculite is 0.00045 MPa with a gwc of 597.751% it can be seen that water stress can be very easily and readily imposed to the point where plants are severely wilted. Vermiculite is therefore suitable as a means for the rapid imposition of water stress but not for long periods of time. However, it has the advantage in being light, cheap and easily handled. Since the degree of water stress is also a function of the length of stress this medium is suitable for rapid and drastic imposition of stress. However, as previously noted such rapid imposition

of water stress is not a normal occurrence under field conditions nor is it usually desired in the greenhouse.

However, since it is logical to assume that when the vermiculite is completely dried (gwc=0) there will be 100 % mortality then the regression line should pass through the origin i.e. $b_0=0$. This increased the fit of the regression lines with r^2 of 98%, 92%, 98% and 92% for Affinity, Buccaneer, Delray and Linn respectively when the quadratic model is fitted. Again the quadratic term is significant for all four cultivars. The first derivatives of the new response equation produced X values (gwc) of 555.11%, 523.56%, 442.33% and 627.64% respectively for the cultivars as mentioned previously. Substituting the values of X into the response equations and solving for Y also gave predicted maximum survival % values of approximately 100 (Table II-2).

However, since the least squares regression is forced through the origin the r^2 is expected to be grossly inflated (Stafford & Sabin, unpubl., pp. 9-19) and this is especially obvious in Buccaneer and Linn cultivars. The inflated r^2 occurs because the total sum of squares is uncorrected for the mean in the zero intercept model. However, to correct for this inflation a new r^2 was calculated (Stafford & Sabin, unpubl. pp. 9-21) using the formula

$r^2_{\text{corrected}} = 1 - (\text{SSE uncorrected} / \text{SST corrected})$, where SSE uncorrected is the SSE from the zero intercept model and SST corrected is from the model with an intercept. This gave the following r^2 : Affinity:

$$1 - \frac{2416.044}{31915.560} = 0.924$$

Buccaneer:

$$1 - \frac{8616.465}{42551.608} = 0.797$$

Delray:

$$1 - \frac{2516.989}{33095.653} = 0.924$$

Linn:

$$1 - \frac{7415.125}{38107.048} = 0.805$$

The new r^2 are equivalent to the ones where the models had intercepts and these confirm that plant survival depends to a large extent on the gwc of the growth medium. Compared with water potential, the gwc was a better predictor, and gave less variation, of the survival percentage of the plants. It is recommended as a more useful tool when using vermiculite as the growth medium.

Regression analysis (model checking and lack of fit F-tests) revealed that there were no treatment specific responses in the relationship of plant survival % regressed against the gwc of vermiculite. Therefore, the rates of plant mortality as a function of gwc were the same regardless of whether or not plants were clipped before being stressed. Considering that mortality is the last stage of plant response to any stress condition, and because there were no differences in the rate of mortality between treatments, this may explain why we did not observe any differences (between treatments) in the other responses preceding death, specifically transpiration, stomatal diffusive resistance, water

potential and leaf and canopy temperatures as measured in this study. However, as expected, the rates of transpiration and the water potential decreased over time while the stomatal diffusive resistance, leaf and canopy temperatures increased with time. This agreed with the trials done on other grasses where leaf conductance (Bittman and Simpson, 1989a), leaf water potential (Bittman and Simpson, 1989b) and transpiration (Jones et al., 1980a) decreased, but stomatal resistance (Jones et al., 1980b) increased as stress increased; in *Vicia faba* transpiration rates also decreased as soil water was depleted (El Nadi et al., 1969). However, in our trial there were no differences between the treatments for any of the parameters measures. Cultivar differences were inconsistent, on all parameters measured, for conclusive results to be drawn and a meaningful discussion obtained. For example, there were no differences between cultivars in canopy temperature throughout the study; while for diffusive resistance Linn was more than twice that of Delray at the end of week one regardless of the treatment. For transpiration and water potential the treatment affected the cultivar response, e.g., Linn at T3, after 7 days of stress, had a higher water potential than Buccaneer at T1 and T3 but not at T2. It was also higher than Delray at T1. Delray's water potential at T3 was also higher than Buccaneer's. One week after the experiment began the rate of transpiration of Buccaneer at T1 was higher than Delray and Linn but not Affinity. On the other hand at T2, Buccaneer had a lower rate of transpiration than those of Affinity and Delray. At T3 Delray was only marginally ($p=0.054$) different than Linn.

The leaf temperatures of Delray two days after the beginning of the experiment were lower than those of Affinity and Linn regardless of the treatment. However, five

days later Delray's leaf temperatures continue to be lower than those of Linn at T3 but not at T1 and T2. Delray also had a higher leaf temperature than Buccaneer at T1. Thus the statement by Amin and Thomas (1996) that "the paucity of significant relationships between measurements of performance and physiology militates against defining robust physiological selection criteria for *L. perenne*," holds true in this study.

These inconsistencies may be because the physical characteristics of the vermiculite did not allow for a change in its water potential that was gradual enough to effect changes in the plants that may have caused differences in cultivars to be detected. Since the cultivars were bred as synthetics, instead of there being just four genotypes to be evaluated each plant represented a unique genotype and this experiment may not have been structured to determine such an array of differences among the plants. This is in agreement with the observations of Thomas (1987) who found that within a single cultivar of *L. perenne* there were wide variations between individual genotypes in constitutive and acclimatory expression of water relations characters. Thomas and Evans (1990) also noted that though experimental populations derived from a single cultivar had diverse expression of osmotic adjustment they did not differ in drought resistance. All four cultivars used in this study are considered to have moderate to high drought tolerance.

There were significant interactions between cultivars and the water treatment on the final dry weight of the shoots. However, interaction plots and contrasts statements revealed that some main effect comparisons were possible (Fig. II-7). The cultivar Delray had a lower final dry matter yield than Linn and Affinity regardless of cutting treatment or length of water stress. Delray was also less yielding over Buccaneer except in the control

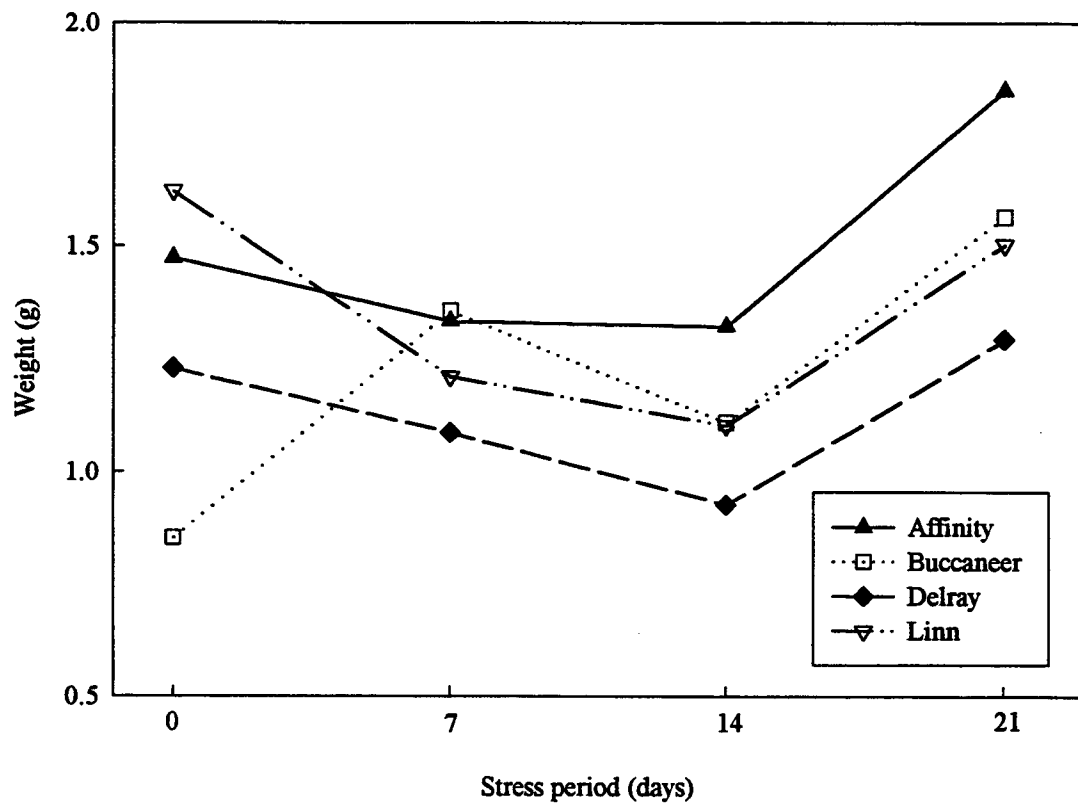


Fig. II-7. Changes in shoot dry matter for four cultivars of perennial ryegrass under simulated drought conditions.

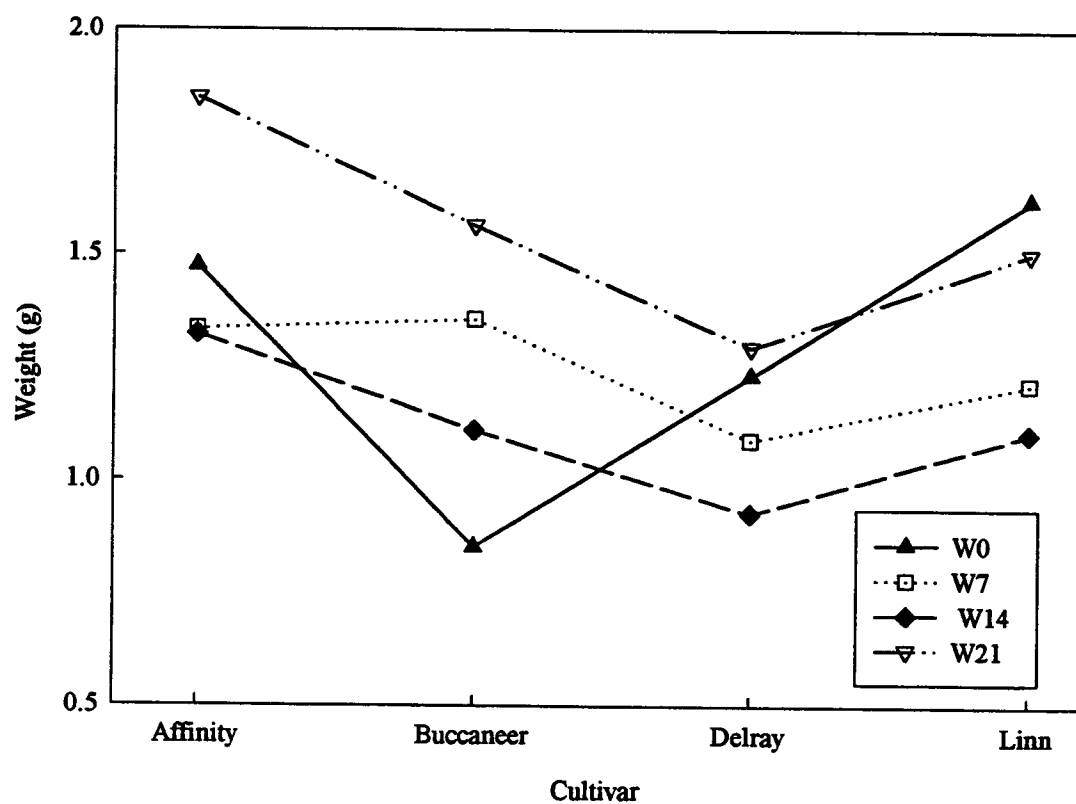


Fig. II-8. Impact of length of water stress on dry matter of four cultivars of perennial ryegrass under simulated drought conditions.

(W_0) treatment where the reverse occurred. Affinity was consistently higher than Linn at all treatments except the control (W_0) where no difference occurred. It was also observed that the W_{21} treatment was consistently higher than the W_{14} regardless of the cultivar or the cutting treatment (Fig. II-8). W_7 also had higher dry matter yields than W_{14} except under controlled conditions. The general trend (Fig. II-7) showed that the dry weight decreased initially then increased after day 14 of the stress treatment.

Stomatal closure is one of the first line of defense against desiccation because it is a more flexible alternative to processes such as changes in life cycle, root growth or leaf area, which are better suited for long-term adaptation (Chaves, 1991). However, when stomates close carbon assimilation is also reduced but respiration continues to occur for plants to survive. When the rate of respiration is greater than that for photosynthesis plants will use up their reserves hence the dry matter declines. Those plants that survive the stress would have adapted sufficiently by day 14 such that the assimilation of carbon is more than the respiration rate. Other adjustments in the plants' physiological processes would also allow for reduced respiration.

Van Loo (1992) observed that plants of perennial ryegrass grown hydroponically at a water potential of 0 MPa had 64% more final dry weight than those grown at -1.3 MPa water potential. This was because of reductions in the rates of tillering, leaf appearance and site filling (the relative increase in leaf area number per leaf appearance interval) (20%), and also lower leaf extension rate (36%). Very few tillers died at this low water potential and plants survived 57 days of this stress.

The change in dry weight over time in our study contradicts the results of Gales (1979) who found that the dry matter in both stress and control plants of perennial ryegrass continually increased from day 1 to day 24. However, from day 16 the rate of increase was slightly lower in the drought-exposed plants. In that study, soil was used as the medium and no doubt stress was more gradually imposed than in our work because of greater uniformity in the loss of soil moisture and less shrinking of the medium. Thus, plants' physiological processes would differ causing differences in the rates of both photosynthesis and respiration and ultimately dry matter production.

CONCLUSION

The water holding capacity of the growth medium had a great influence on the physiological responses and survival rate of perennial ryegrass. When vermiculite was used as the medium, water loss and shrinkage were very rapid, most likely affecting root-soil contact and slowing water uptake. This occurred over a period of a week or less and resulted in rapid senescence of leaves. Because of this rapid wilting, it was difficult to ascertain genotypic differences in stomatal diffusive resistance, leaf and canopy temperature, plant water potential and leaf transpiration. Clipping plants before the stress was implemented did not significantly and consistently affect the aforementioned responses. However, by measuring the survival rate it was possible to establish a sensitivity among the genotypes to extreme drought.

A reliable, objective and relatively inexpensive greenhouse test for evaluating the performance of grasses when subjected to water stress is still needed. Such a tool would be of immense worth to any breeding program since it would eliminate many undesirable genotypes very early in the program.

Further Work: It would be interesting to examine the recovery of plants after being subjected to such an intense exposure to drought. Maybe the ability to recover can be a genotypic characteristic and therefore, a useful screening technique. Also, daily measurements of plant responses and characteristics may provide a greater insight into the relationship between grass physiology and water stress.

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SUMMARY and CONCLUSIONS

Two studies were undertaken to determine the effects of water stress on the performance of commercial cultivars of perennial ryegrass. The major study was conducted in the field to ascertain the impact of summer drought on the occurrence of the phenomenon dieback (die-out) as observed in commercial seed fields. The second study was carried out under greenhouse conditions to monitor differences in selected physiological responses between cultivars.

In the first study two cultivars, Affinity and Buccaneer, were used in a randomized complete block design at the Hyslop Farm Field Research Laboratory. The cultivars were irrigated with 2.5 cm of water either in the late summer or early fall or in both periods. These three treatments were compared to an ambient and to a no-rainfall treatment. Plots were sampled three weeks after each treatment and examined for number of aerial and basal tillers, tiller dry weight, plant height, number of leaves and the basal diameter. Total soluble carbohydrates in the above ground tissue at the last sampling date were quantified and correlated to tiller dry matter. Fertile and vegetative tillers were sampled in spring just prior to peak anthesis and shortly thereafter seed yields were obtained. This experiment was repeated over two seasons.

The influence of water stress as manifested in a reduction in the number of total tillers indicated that this may be the incipient stage of the dieback phenomenon. The two cultivars responded differently to water stress at the different periods used. However, in general the NR treatment produced less tillers than the others though this value may or

may not be significantly different. More differences amongst the treatments for all parameters were observed in Affinity but not in Buccaneer after summer rainfall confirming the observation that Affinity responded much faster to the early irrigation whereas Buccaneer had a longer period of summer dormancy. Visual observations revealed that in Affinity the ambient plots were less green relative to the irrigated one; while in Buccaneer both looked alike. This agreed with the data that Affinity responded quicker to the early rainfall. Observations also revealed that where plots were irrigated there was faster decomposition of the straw (i.e. more brown-colored straw as against yellow-colored straw⇒less decomposition).

Though the two cultivars responded differently to water stress, optimum tiller production can be attained in fall regrowth with approximately 10.2 cm of water. No irrigation caused a reduction in tiller production by approximately 30% in 1995 and about 50% in 1996. For similar treatments, tiller production decreased as the stand aged, and canopy temperature was not a reliable indicator of water stress during the post-harvest regrowth stage of perennial ryegrass.

In Affinity the irrigated plots had a greater dry weight than the non-irrigated ones after summer rainfall in both years, but in Buccaneer this was only observed in 1996. After autumn rainfall, NR plots had an average of 20% - 40% less dry weight than the SF plots in Affinity and Buccaneer respectively. Drought also impacted the height of the basal tillers much more in Affinity than in Buccaneer. In both years it was noted that plants in the NR plots had reduced height relative to those in the AM and irrigated plots. Tiller basal diameter and the number of leaves were the least affected by the irrigated

treatments. Since there is usually a positive correlation between these two traits this was not unexpected. However, from the amount of tests of contrasts that were significant the basal diameter was the least affected of all the traits measured in this study.

In the early production cycle of the crop fertile tiller production and seed yield may be affected by water stress but this is dependent on the time of stress and the cultivar. Insufficiency of water reduced percent stand cover by varying degrees in both cultivars with approximately 32 - 40% loss with no irrigation. Laboratory analysis revealed that the soluble sugars concentrations were generally higher in the 1996 regrowth than in 1995. This may be due to fewer number of total tillers being produced and hence, reflected the potential for rapid (exponential) compensatory growth following the completion of drought. With one exception, no significant correlations occurred between total soluble sugars and tiller dry weight.

On the basis of results obtained in this study the following conclusions can be drawn

- (1) Aerial tillers decreased sharply from one growing season to the next.
- (2) There was a slight decrease in basal tiller number from one growing season to the next.
- (3) Overall, the total tiller number dropped from 1995 to 1996 and this decline appeared to be strongly dependent on the age of the stand. However, to prolong stand life irrigation of even small quantities (of water) will be beneficial.
- (4) No rainfall decreased tiller production in both cultivars in both years. This decrease may mark the beginning of the dieback problem as observed in commercial perennial ryegrass seed fields.

- (5) Drought led to an accumulation of soluble sugars in the shoots of the grasses. This accumulation facilitated rapid growth of the stand following the onset of the rains.
- (6) Plant height and dry matter were lowered by water stress. However, the percent reduction was greater for dry matter than for plant height when NR treatments were compared to irrigated plants.
- (7) The Haun stage of basal tillers and basal diameter were least affected by drought.
- (8) Canopy temperature was not a reliable indicator of water stress during the post-harvest regrowth stage of perennial ryegrass seed crops.
- (9) A high correlation existed between total tiller number and dry weight, but the association was poor when dry weight and total soluble sugars were correlated.
- (10) As total tiller number decreased the concentration of soluble sugars increased.
- (11) Compensatory growth and production of new tillers following drought removal allowed young stands of perennial ryegrass to offset any loss in seed yield which may have occurred because of an inadequacy of tiller early in the regrowth period.

Similar to the conclusions of Busso et al (1990), the results of this work are probably representative of tiller regrowth stimulation that would occur following severe natural drought during the late-summer early-fall period. Total plant regrowth would depend not only on this stimulation, but on the potentially reduced number of tillers growing after such a drought.

In the second study plant water potential, transpiration rate, stomatal diffusive resistance and leaf and canopy temperatures were measured every two or five days in four cultivars of perennial ryegrass, Affinity, Buccaneer, Delray and Linn. "Field capacity" was

attained by watering (in the evenings) to saturation then allowing for overnight drainage. Plants were either cut before or after a period of stress where no further water was given. The stress periods lasted 0, 7, 14 or 21 days. In addition there was a third treatment where plants were stressed for a week, cut, then further stressed for 0, 7, 14 and 21 days. This study further elucidated the strong influence of the soil water potential on plant survival and growth. It showed how sensitive perennial ryegrass cultivars were to rapid changes in soil medium water potential. There was a marked interaction between the effects of soil water potential and physiological responses on plant survival.

From this study, the following conclusions can be made

- (1) Plant water potential and leaf transpiration decreased as the severity of the stress increased.
- (2) Conversely, stomatal diffusive resistance, leaf and canopy temperatures increased with the length of stress.
- (3) Cultivar differences in the above parameters were inconclusive probably as a result of the rapidity of stress.
- (4) Cutting the plants before or after stress did not significantly alter the parameters measured because of the nature of the potting medium.
- (5) Diverse genotypes even within a single cultivar succumbed to water stress in varying degrees. This could be an indication that water (or lack of) plays a significant role in dieback of perennial ryegrass seed fields.
- (6) The impact of water stress on plant dry weight is influenced by the degree (length) of stress and the cultivar.

- (7) In water relations studies the nature of the potting medium has a significant influence on the imposition of stress and consequently all physiological responses.

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APPENDIX

Appendix-Table 1. Meteorological data for the late-summer early-fall period of regrowth of perennial ryegrass seed crop at Hyslop Farm, Corvallis.

Month	-----Precipitation (inches)-----		Evaporation (inches)	---Mean air temperature (°F)---	
	Total	Normal†		Maximum	Minimum
<u>1995</u>					
August	0.82	0.81	6.67	79.7	50.0
September	3.14	1.48	4.63	77.9	53.0
October	3.96	3.39	2.03	63.5	42.5
<u>1996</u>					
August	0.14	0.81	7.88	83.5	52.4
September	2.22	1.48	4.51	73.4	47.0
October	5.32	3.39	1.98	62.6	42.6

† Normal precipitation is the average of 67 years for that month.

Appendix-Table 2. Soil water contents at indicated suction values for the Woodburn silt loam at Hyslop Farm, Corvallis.

Soil layer (Inches)	Bulk density	Unit of measure	Water content at indicated tension							
			-----bars-----							
			0	0.015	0.03	0.6	2.0	5.0	15.0	
0-3	1.37	% by wt.	35.1	33.3	31.9	30.1	28.1	21.4	15.6	10.6
3-6	1.34	% by wt.	36.6	34.1	31.4	30.4	29.3	21.5	16.3	10.9
6-9	1.39	% by wt.	34.2	32.2	30.9	29.3	26.8	20.9	16.5	11.3
9-12	1.40	% by wt.	31.9	30.8	29.9	28.6	27.1	19.3	15.8	11.6

Appendix-Table 3. Volumetric water content converted to depth of water for a field under Affinity perennial ryegrass, 1995.

Treatment	Date	Water content (θ)		mm of water		% of available water at 15 cm
		15 cm	30 cm	15 cm	30 cm	
AM	AUG18	11	18.5	16.50	55.50	-13.17
AM	AUG25	9.6	18.5	14.40	55.50	-18.24
AM	SEPT1	10.6	18.8	15.90	56.40	-14.62
AM	SEPT8	10	16.7	15.00	50.10	-16.79
AM	SEPT15	11.6	19.1	17.40	57.30	-10.99
AM	SEPT22	10.4	17.9	15.60	53.70	-15.34
AM	OCT6	22.2	25.5	33.30	76.50	27.43
AM	OCT13	31.1	33.8	46.65	101.40	59.69
NR	AUG18	11.3	20.5	16.95	61.50	-12.08
NR	AUG25	10.7	20.2	16.05	60.60	-14.25
NR	SEPT1	11.3	20	16.95	60.00	-12.08
NR	SEPT8	12	20	18.00	60.00	-9.54
NR	SEPT15	11.9	19.9	17.85	59.70	-9.90
NR	SEPT22	11.2	19.2	16.80	57.60	-12.44
NR	OCT6	8.7	19.9	13.05	59.70	-21.50
NR	OCT13	19.9	24.3	29.85	72.90	19.10
SU	AUG18	17.9	21.4	26.85	64.20	11.85
SU	AUG25	15.7	19.9	23.55	59.70	3.87
SU	SEPT1	14	20.7	21.00	62.10	-2.29
SU	SEPT8	16.2	19.3	24.30	57.90	5.68
SU	SEPT15	14.6	20.1	21.90	60.30	-0.12
SU	SEPT22	13	19.6	19.50	58.80	-5.92
SU	OCT6	10.4	19.4	15.60	58.20	-15.34
SU	OCT13	26.7	24.8	40.05	74.40	43.74
FA	AUG18	13.3	20.6	19.95	61.80	-4.83
FA	AUG25	11.6	20.5	17.40	61.50	-10.99
FA	SEPT1	12.6	20.2	18.90	60.60	-7.37
FA	SEPT8	14.5	20.2	21.75	60.60	-0.48
FA	SEPT15	14.4	20.1	21.60	60.30	-0.84
FA	SEPT22	16.1	20.7	24.15	62.10	5.32
FA	OCT6	17.9	22.5	26.85	67.50	11.85
FA	OCT13	29.8	29.8	44.70	89.40	54.98
SF	AUG18	19	19.7	28.50	59.10	15.83
SF	AUG25	14.7	19.5	22.05	58.50	0.25
SF	SEPT1	14.3	19.4	21.45	58.20	-1.20
SF	SEPT8	15.1	19.7	22.65	59.10	1.70
SF	SEPT15	14.4	19.2	21.60	57.60	-0.84
SF	SEPT22	16.6	18.8	24.90	56.40	7.13
SF	OCT6	16.8	22.4	25.20	67.20	7.86
SF	OCT13	28	30.2	42.00	90.60	48.46

Appendix-Table 4. Volumetric water content converted to depth of water for a field under Buccaneer perennial ryegrass, 1995.

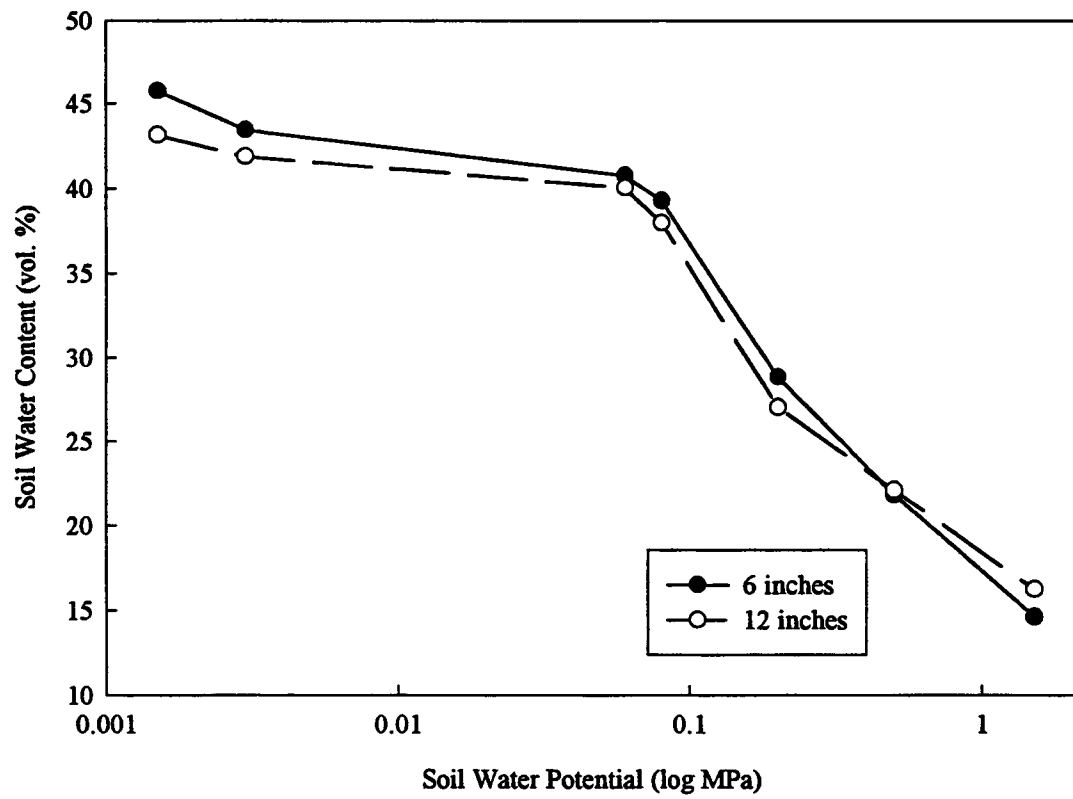
Treatment	Date	Water content (θ)		mm of water		% of available water at 15 cm
		15 cm	30 cm	15 cm	30 cm	
AM	AUG15	14.3	19.7	21.45	59.10	-1.20
AM	AUG23	13.5	19.5	20.25	58.50	-4.10
AM	AUG30	11.9	19.2	17.85	57.60	-9.90
AM	SEPT8	12.4	19.3	18.60	57.90	-8.09
AM	SEPT15	12.9	19.2	19.35	57.60	-6.28
AM	SEPT22	12.1	18.4	18.15	55.20	-9.18
AM	OCT6	25.7	28	38.55	84.00	40.12
AM	OCT13	32.4	34.9	48.60	104.70	64.40
NR	AUG15	12.4	20.4	18.60	61.20	-8.09
NR	AUG23	11.1	19.9	16.65	59.70	-12.80
NR	AUG30	10.3	19.6	15.45	58.80	-15.70
NR	SEPT8	10.3	19.6	15.45	58.80	-15.70
NR	SEPT15	10.6	19.3	15.90	57.90	-14.62
NR	SEPT22	10.4	19.3	15.60	57.90	-15.34
NR	OCT6	10.2	20.6	15.30	61.80	-16.06
NR	OCT13	22.6	28.2	33.90	84.60	28.88
SU	AUG15	15.9	20.8	23.85	62.40	4.60
SU	AUG23	13.1	20.7	19.65	62.10	-5.55
SU	AUG30	10.1	19.8	15.15	59.40	-16.43
SU	SEPT8	10.9	19.9	16.35	59.70	-13.53
SU	SEPT15	10.8	19.3	16.20	57.90	-13.89
SU	SEPT22	9.9	18.7	14.85	56.10	-17.15
SU	OCT6	11.5	15.7	17.25	47.10	-11.35
SU	OCT13	20.2	25.4	30.30	76.20	20.18
FA	AUG15	13.8	21.1	20.70	63.30	-3.02
FA	AUG23	12	21.1	18.00	63.30	-9.54
FA	AUG30	11.1	20.9	16.65	62.70	-12.80
FA	SEPT8	11.5	20.9	17.25	62.70	-11.35
FA	SEPT15	13.7	20.8	20.55	62.40	-3.38
FA	SEPT22	16.1	23.1	24.15	69.30	5.32
FA	OCT6	14	22	21.00	66.00	-2.29
FA	OCT13	26.9	28.4	40.35	85.20	44.47
SF	AUG15	17.5	23.4	26.25	70.20	10.40
SF	AUG23	14.8	23.2	22.20	69.60	0.61
SF	AUG30	12.1	22.4	18.15	67.20	-9.18
SF	SEPT8	12.3	22	18.45	66.00	-8.45
SF	SEPT15	15.6	23	23.40	69.00	3.51
SF	SEPT22	15.8	22.7	23.70	68.10	4.23
SF	OCT6	13.1	22.3	19.65	66.90	-5.55
SF	OCT13	25.9	28.1	38.85	84.30	40.84

Appendix-Table 5. Volumetric water content converted to depth of water for a field under Affinity perennial ryegrass, 1996.

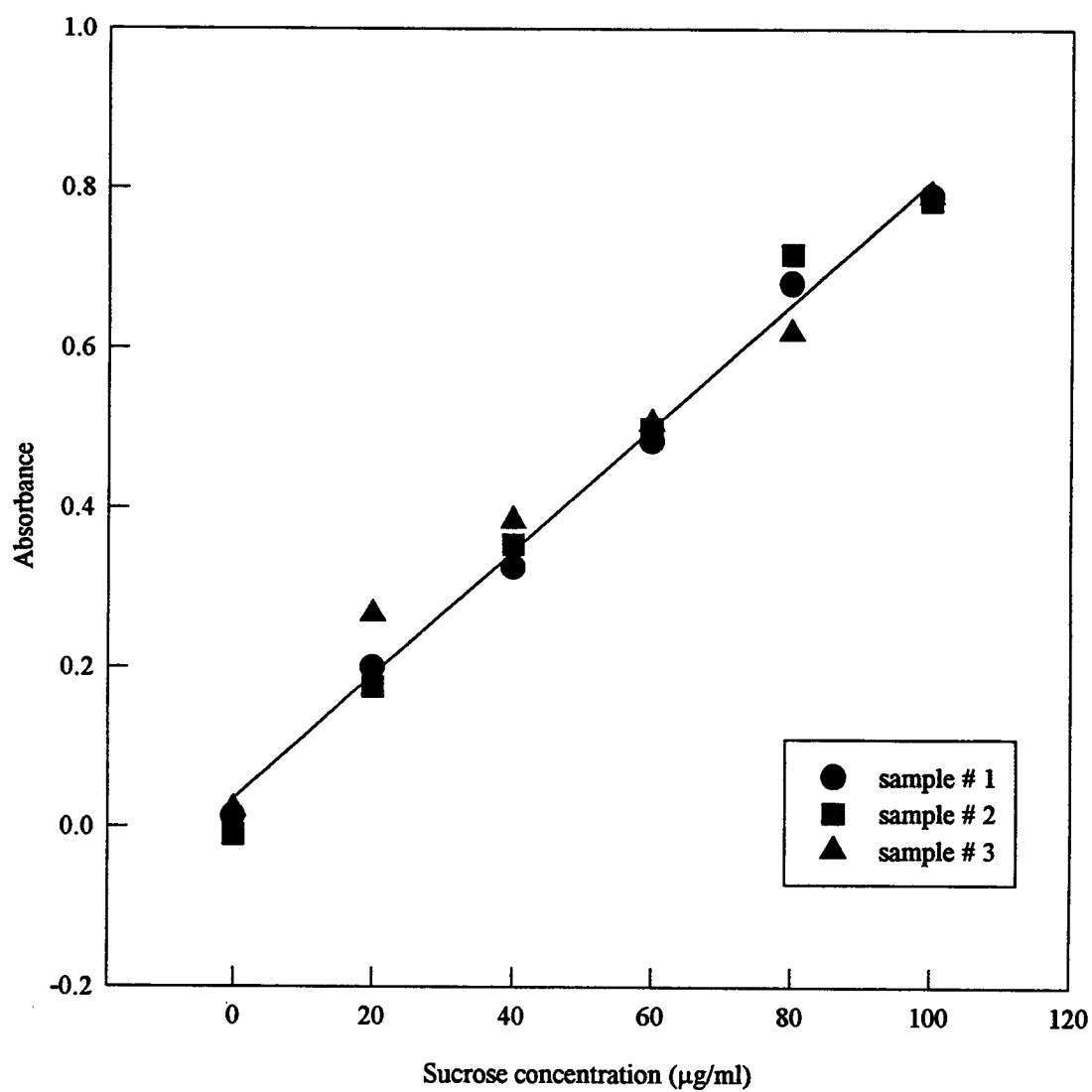
Treatment	Date	Water content		mm of water		% of available water at 15 cm
		15 cm	30 cm	15 cm	30 cm	
AM	AUG19	12.8	21	19.20	63.00	-6.64
AM	AUG28	12.1	20.5	18.15	61.50	-9.18
AM	SEPT6	11.6	20.1	17.40	60.30	-10.99
AM	SEPT11	10.8	19.9	16.20	59.70	-13.89
AM	SEPT19	19.2	21.2	28.80	63.60	16.56
AM	SEPT25	17.3	24.6	25.95	73.80	9.67
AM	OCT2	13.9	23.3	20.85	69.90	-2.65
AM	OCT9	14.1	23.2	21.15	69.60	-1.93
NR	AUG19	14.9	22.2	22.35	66.60	0.97
NR	AUG28	14.7	21.7	22.05	65.10	0.25
NR	SEPT6	13.9	21.4	20.85	64.20	-2.65
NR	SEPT11	13.2	21.2	19.80	63.60	-5.19
NR	SEPT19	13.4	21.1	20.10	63.30	-4.47
NR	SEPT25	12.6	21	18.90	63.00	-7.37
NR	OCT2	11	21.1	16.50	63.30	-13.17
NR	OCT9	10.3	21.7	15.45	65.10	-15.70
SU	AUG19	14.9	20.8	22.35	62.40	0.97
SU	AUG28	17.9	22.2	26.85	66.60	11.85
SU	SEPT6	15.8	21.5	23.70	64.50	4.23
SU	SEPT11	14.5	21.2	21.75	63.60	-0.48
SU	SEPT19	14.8	21	22.20	63.00	0.61
SU	SEPT25	13.5	20.8	20.25	62.40	-4.10
SU	OCT2	11.9	21.2	17.85	63.60	-9.90
SU	OCT9	11.9	21.7	17.85	65.10	-9.90
FA	AUG19	15.6	21	23.40	63.00	3.51
FA	AUG28	14.8	20.6	22.20	61.80	0.61
FA	SEPT6	14.2	20.3	21.30	60.90	-1.57
FA	SEPT11	13.2	20	19.80	60.00	-5.19
FA	SEPT19	14	19.8	21.00	59.40	-2.29
FA	SEPT25	21.4	24.6	32.10	73.80	24.53
FA	OCT2	18.1	22.9	27.15	68.70	12.57
FA	OCT9	17.4	23.2	26.10	69.60	10.03
SF	AUG19	15.3	20.7	22.95	62.10	2.42
SF	AUG28	18.8	23	28.20	69.00	15.11
SF	SEPT6	16.4	22.1	24.60	66.30	6.41
SF	SEPT11	15.3	21.3	22.95	63.90	2.42
SF	SEPT19	16.5	22.8	24.75	68.40	6.77
SF	SEPT25	20.9	24.8	31.35	74.40	22.72
SF	OCT2	17.6	23.4	26.40	70.20	10.76
SF	OCT9	16.6	22.9	24.90	68.70	7.13

Appendix-Table 6. Volumetric water content converted to depth of water for a field under Buccaneer perennial ryegrass, 1996.

Treatment	Date	Water content		mm of water		% of available water at 15 cm
		15 cm	30 cm	15 cm	30 cm	
AM	AUG19	15.3	20.7	22.95	62.10	2.42
AM	AUG28	16	20.2	24.00	60.60	4.96
AM	SEPT6	15	19.8	22.50	59.40	1.33
AM	SEPT11	14.2	19.7	21.30	59.10	-1.57
AM	SEPT19	23.9	23.3	35.85	69.90	33.59
AM	SEPT25	22.6	23.8	33.90	71.40	28.88
AM	OCT2	20.2	23.3	30.30	69.90	20.18
AM	OCT9	20.1	23.4	30.15	70.20	19.82
NR	AUG19	11	20.1	16.50	60.30	-13.17
NR	AUG28	12.6	20	18.90	60.00	-7.37
NR	SEPT6	12.1	19.9	18.15	59.70	-9.18
NR	SEPT11	11.3	19.4	16.95	58.20	-12.08
NR	SEPT19	11.8	19.5	17.70	58.50	-10.27
NR	SEPT25	10.2	19.2	15.30	57.60	-16.06
NR	OCT2	9.1	19.2	13.65	57.60	-20.05
NR	OCT9	8.8	19.8	13.20	59.40	-21.14
SU	AUG19	12.3	20.7	18.45	62.10	-8.45
SU	AUG28	18.1	23.5	27.15	70.50	12.57
SU	SEPT6	16.4	22.8	24.60	68.40	6.41
SU	SEPT11	14	22.1	21.00	66.30	-2.29
SU	SEPT19	14.7	21.8	22.05	65.40	0.25
SU	SEPT25	13.4	21.6	20.10	64.80	-4.47
SU	OCT2	11.5	21.5	17.25	64.50	-11.35
SU	OCT9	11.9	21.7	17.85	65.10	-9.90
FA	AUG19	13.9	21.6	20.85	64.80	-2.65
FA	AUG28	14.6	21.4	21.90	64.20	-0.12
FA	SEPT6	14.4	21.3	21.60	63.90	-0.84
FA	SEPT11	13.5	21.1	20.25	63.30	-4.10
FA	SEPT19	14.2	20.8	21.30	62.40	-1.57
FA	SEPT25	20.3	25	30.45	75.00	20.55
FA	OCT2	18.1	24.4	27.15	73.20	12.57
FA	OCT9	17.5	24.3	26.25	72.90	10.40
SF	AUG19	12.4	21.7	18.60	65.10	-8.09
SF	AUG28	16.8	23.7	25.20	71.10	7.86
SF	SEPT6	15.9	22.9	23.85	68.70	4.60
SF	SEPT11	14	22.6	21.00	67.80	-2.29
SF	SEPT19	14.6	22	21.90	66.00	-0.12
SF	SEPT25	22.4	26.5	33.60	79.50	28.16
SF	OCT2	19.5	25.5	29.25	76.50	17.65
SF	OCT9	18	25.4	27.00	76.20	12.21

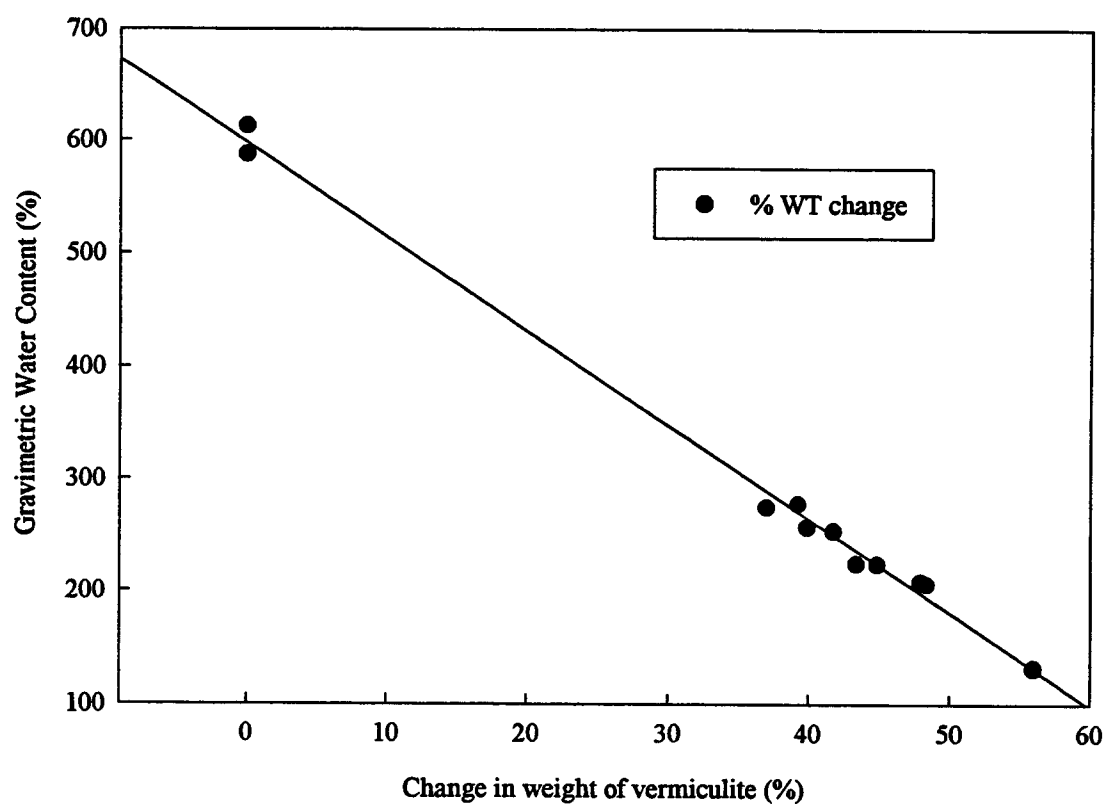


Appendix-Fig. 1. Water release curve for Hyslop Farm (Woodburn soil series).

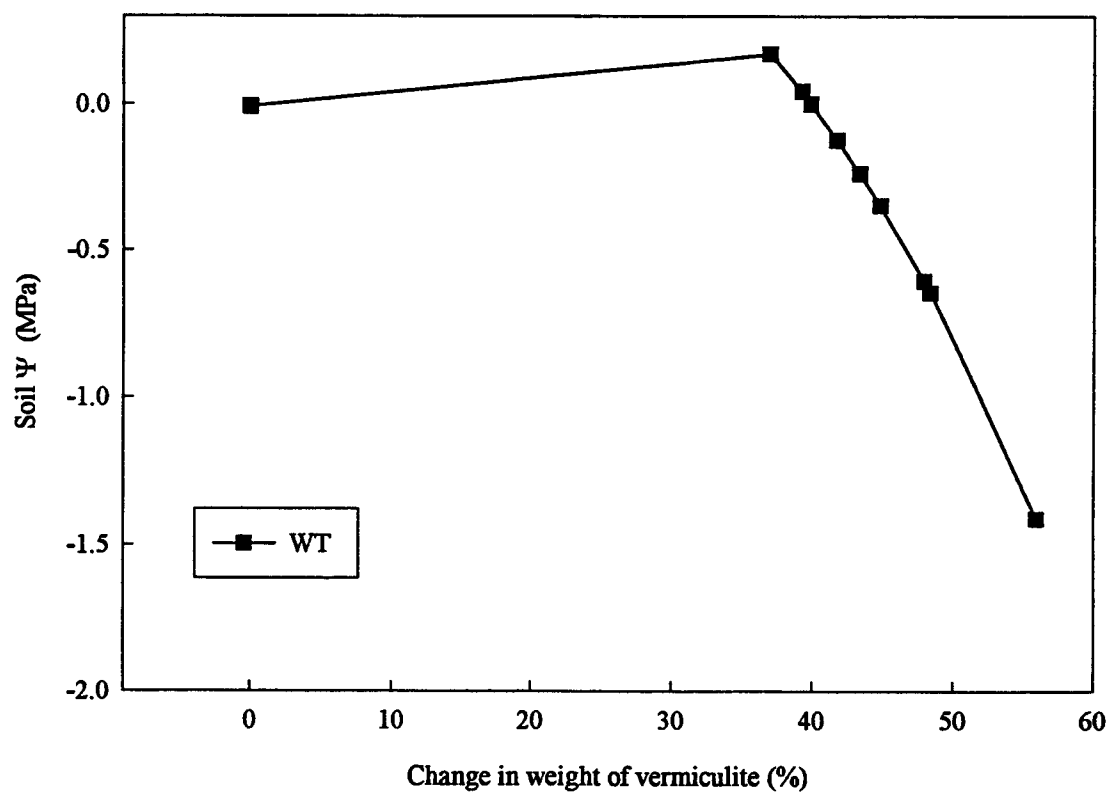


Appendix-Fig. 2. Phenol-H₂SO₄ absorbance curve. ($r^2 = 0.99$, $P < 0.01$).

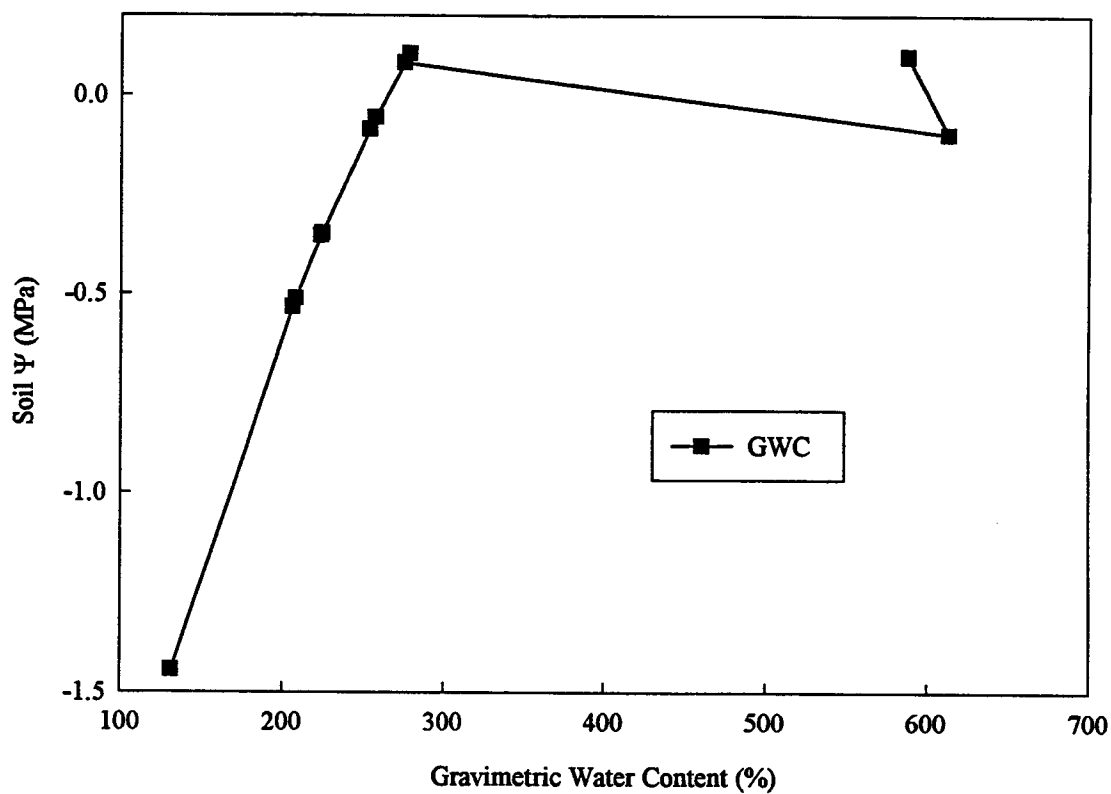
Regression equation for the fitted line is: $Y = 0.035 + 7.75 \times 10^{-3}X$.



Appendix-Fig. 3. Effect of change in vermiculite weight on gravimetric water content. ($r^2 = 0.99$, $P < 0.01$). Regression equation for the fitted line is: $Y = 597.74 - 8.33X$.



Appendix-Fig. 4. Effect of change in weight on Ψ of vermiculite. ($r^2 = 0.97$, $P < 0.01$). Regression equation for the fitted line is: $Y = -0.087 + 0.63X - 0.016X^2$.



Appendix-Fig. 5. Effect of gravimetric water content of vermiculite on its water potential. ($r^2 = 0.97$, $P < 0.01$). Regression equation for the fitted line is: $Y = -36.87 + 0.20X - 2.33 \times 10^{-4}$.