Physiological Response of Turf Grasses to Trampling Pressure.

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Abstract

Some responses of turfgrasses to trampling stress were examined using simulated trampling methods in greenhouse and laboratory studies. Subsidiary experimental field studies indicated that the laboratory results were relevant to the natural situation. Shoot yield, CO_2 exchange and water relation parameters were measured, concentrating on the short term response to simulated trampling. A range of turfgrass species and cultivars were included so comparison of wear tolerant and wear susceptible cultivar response was possible.

An electrolyte release method was developed to ascertain plant injury following simulated trampling. Differences in injury index were related to levels of wear tolerance of the species and cultivars.

Shoot yield was reduced with simulated trampling in all species and cultivars tested. Decrease in shoot yield correlated significantly with increasing intensity of trampling and increased injury index. Differences in amount of shoot decrease between species and cultivars related to ratings of wear tolerance in the literature.

Net photosynthesis of *Lolium perenne* S23 decreased significantly with simulated trampling and a slight increase in respiration was recorded. Net photosynthesis correlated with injury level. Continuous monitoring over the initial few hours after treatment revealed a sharp decline in photosynthesis rates, followed by gradual recovery.

Selected cultivars had a lower shoot water content two hours after treatment, the higher the intensity of simulated trampling the lower was the water content. SEM studies indicated disruption of epicuticular wax, therefore reducing cuticular resistance to water loss. Transpiration rates were reduced with simulated trampling and calculations showed a reduced hydraulic conductivity.

These findings were related to relevant observations of response of plants to other stresses, particularly wind. A model is presented suggesting how observed and hypothetical responses, both short term and long term, may contribute to the survival or death of grass following trampling.

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CHAPTER 1

Introduction

A report in 1977 by the Natural Environment Research Council summarised the importance of amenity grassland as a national resource. Covering an area of nearly 4% of the UK, amenity grassland was defined as all grassland which has recreational, functional or aesthetic value, but which is not used primarily for agricultural production (NERC 1977). Within amenity grasslands there is a variety of different categories representing different levels and purpose of usage and different levels of maintenance and therefore of costs. Extensive tables represented this data (NERC 1977) which has also been summarised by Rorison (1980). Less than half (45%) of the total amenity grassland was categorised as "intensively managed grassland" or "man-made trampled open space" yet the cost of maintenance of these areas represented 90% of the total expenditure on amenity grasslands. The NERC report (1977) therefore highlighted the major research needs in order to decrease maintenance costs yet maintain and improve the social value of the various types of amenity grassland. High priority was given to research on the problem of wear and trampling including the effects of different types of wear, wear in relation to various factors (soil type and moisture, shade, usage, maintenance, plant communities, specific situations) and fundamental reasons for the variation in trampling tolerance (anatomy, morphology and physiology, effect of environmental factors, species interaction, plant communities and recuperative potential). Of these, fundamental reasons for the variance in trampling tolerance appeared to be of major priority. The current study was undertaken, in light of this and other comments (e.g. Liddle 1975a, Canaway 1975a) that there is little information particularly about the physiology of plant response to wear.

Analytical and Experimental studies of plant response to trampling.

Early observations of the response of vegetation to recreational use (Jeffreys 1917, Bates 1935) pioneered the study of plant response to trampling. Most of the work concentrated on the observed differential response of species to treading. Jeffreys (1917) noted poor survival of tall stemmed plants on footpaths. Bates (1935) made an intensive survey of the floristic composition of the vegetation of footpaths from a very wide range of habitats. Using both quantitative and qualitative data, he showed that trampling eliminated susceptible species, leaving only those with a certain morphology or phenology. Grasses such as Poa pratensis and Lolium perenne (which are cryptophytes and have a conduplicate stem and folded leaves) dominated footpaths. Morphology such as the rosette form of Plantago major also enabled survival on footpaths. Plants tended to remain in the vegetative state. Poa annua for example only flowered when trampling was at a relatively low level. Bates also noted the compacting effects of trampling on the soil and that this indirectly contributed to the observed response to trampling.

Bates (1935) further investigated the tolerance of certain species to treading with various experiments. In one, strips of different species of grass were trodden daily. While Poa pratensis, Lolium perenne and Dactylis glomerata persisted, species such as Anthoxanthum odoratum, Alopecurus pratensis, Agrostis stolonifera and Agropyron repens were eliminated. He concluded that the mechanical effect of treading exerts a selective influence, eliminating those species not structurally adapted to withstand the injury, but that certain life form, leaf and stem structure of other species enabled them to persist.

It is therefore appropriately considered that Bates (1935) was the pioneer of the use of both analytical and experimental approaches to determine plant response to trampling (Liddle 1975a).

The analytical approach has been concerned with ecological surveys of areas of recreational use, particularly paths (Plate 1). The differences between the worn areas and the adjacent vegetation are attributed solely to the effects of trampling, based on assumptions that the whole area was homogenous before the path was created, that the adjacent taller vegetation is undisturbed by trampling and that there has not been any overall change in the environment since the route was introduced (Liddle 1975a).

Interest in this area was limited to a few studies such as those of Bates (1935) and Davies (1938) until in the 1960's, increasing mobility, leisure time and pursuit of outdoor activity (Wagar 1964) threatened over-use and damage to species-rich semi-natural areas of high conservation value and also high susceptibility to damage (Liddle 1975a). Numerous studies resulted, for example Goldsmith *et al.* (1970), Bayfield (1971a), Chappell *et al.* (1971), Burden and Randerson (1972), Bayfield (1973), Dale and Weaver (1974), Leney (1974), Liddle and Greig-Smith (1975a,b), Crawford and Liddle (1977), Blom (1979). Observations have consisted of recording the vegetational characteristics such as species presence/absence and frequency, ground cover, biomass and also associated factors such as soil compaction, soil bulk density, soil porosity and soil moisture.

Methods of estimating trampling intensity have included direct observations of numbers of people (Goldsmith *et al.* 1970, Bayfield 1971a, Burden and Randerson 1972, Bayfield 1973, Leney 1974) and even questionnaires (Burden and Randerson 1972) or of indirect methods such as the trampleometer of Bayfield (1971b, 1973) or soil compaction parameters (e.g. Chappell *et al.* 1971, Liddle and Greig-Smith 1975a).

The vegetational differences have frequently been related to the

Plate 1 A typical footpath with short vegetation and patches of exposed soil, flanked by taller untrampled vegetation.

Springpool Plantation, Keele.

estimates of intensity of wear by means of transects across paths (e.g. Goldsmith *et al.* 1970, Bayfield 1971a, Chappell *et al.* 1971, Dale and Weaver 1974, Liddle and Greig-Smith 1975b, Crawford and Liddle 1977) using measurements of either ground cover, frequency or biomass. Alternatively in some studies, a small number of plots have been observed (e.g. Leney 1974).

In worn areas there is usually a reduction in the ground cover of the vegetation and reduction in the number of species present (Liddle 1975a). Leney (1974) noted that even light activity such as lying or sitting on vegetation has been shown to have considerable effects. In particular, these analytical studies have identified those species able to withstand heavy wear such as *Lolium perenne*, *Poa pratensis*, *Poa annua*, *Bellis perennis* and species which are susceptible to wear such as *Calluna vulgaris*, *Mercurialis perennis*, *Viola hirta*. Comprehensive lists (e.g. Speight 1973) have been made in this respect. Morphological and anatomical features often found in trampling tolerant plants such as procumbent form, leaves in a basal rosette, flexible leaves and stems, have also been summarised (Speight 1973).

The concept of carrying capacity was introduced by Wagar (1964) and later defined by Burden and Randerson (1972) as "the maximum intensity of use an area will continue to support under a particular management regime without inducing a permanent change in the biotic environment maintained by that management". Smith (1978) noted that carrying capacity should take into account variations in growth rate during the period of wear and he re-defined carrying capacity as "the trampling pressure at which net production becomes zero since at this pressure gross production is in equilibrium with losses due to trampling and represents a situation of stability". This would seem to depend not only on the species present but also on factors of soil and environmental conditions (Crawford and Liddle 1977) and even on details such as angle of slope (Bayfield 1973).

The experimental approach involves identifying methods to assess changes and quantifying the processes in trampling induced changes in an ecosystem. In some experiments (e.g. Wagar 1964, Liddle 1975a,b) regression equations predicting ecosystem changes have been attempted, in order to ultimately try and predict the carrying capacity of a recreational site.

Experiments by Edmond (1958, 1962, 1963, 1964) considered the effect of sheep treading on pasture species. Other studies using the experimental approach (but not in the agricultural context) include work by Bates (1935), Wagar (1964), Bayfield (1971a), Kellomaki (1973), Leney (1974), Liddle and Greig-Smith (1975b), Smith (1978) and Blom (1979). In the more specific context of turfgrass response to wear, there have been various studies such as Youngner (1961), Shearman and Beard (1975a,b,c), Canaway (1976b, 1978, 1981a,b).

In experimental studies there have been two basic approaches to applying trampling stress. The first is actual human trampling where people of known weight and boot size walk or run over defined areas of vegetation (e.g. Bayfield 1971a, Kellomaki 1973, Leney 1974, Smith 1978). The second approach employes simulated trampling methods which divide into three categories. These are compaction/trampling machines (e.g. Youngner 1961, Goss and Roberts 1964, Shearman et al. 1974, Canaway 1976a), horizontal wear/shear strength test apparatus (Canaway 1975b, 1981a) and the vertical falling tamp system (Wagar 1964, Bayfield 1971a, Kellomaki 1973, Smith 1978, Blom 1979). Each approach has advantages but also disadvantages as will be mentioned later.

As with analytical studies, differences in species response to trampling have been demonstrated in all of these studies. Further, two components of the response have been clarified by Canaway (1975a): "wear tolerance", which is the ability to withstand wear and "recuperative potential", which is the ability to recover from wear. The combined effect of wear tolerance and recuperative potential is the "durability" (or "wear resistance") which is the overall response of a plant to wear. Both analytical and experimental studies mentioned above have usually essentially recorded plant durability.

The level of plant durability depends on the plant response to direct injury from wear and also the indirect effect of soil compaction. These have been reviewed by Speight (1973) and Canaway (1975a). Direct effects include bruising, crushing, tearing and abrasion of shoots. Roots may be broken and growing points may be killed, leading to eventual death of the plant. Plants may even be buried. Different species (and cultivars) have different degrees of wear tolerance (e.g. Bates 1935, Youngner 1961, Bayfield 1971a, Shildrick 1971, 1973, Leney 1974, Liddle 1975a, Liddle and Greig-Smith 1975b, Shearman and Beard 1975a, Canaway 1978, 1981a, 1983, Smith 1978, Blom 1979, STRI 1980) which may vary according to the type of wear (Shearman and Beard 1975a).

Environmental factors influence the response and usually those factors which reduce sward growth and those which also decrease plant wear tolerance (Canaway 1975a). Examples are shading, drought and excessively wet conditions (Beard 1973). Management practices also influence wear tolerance (Canaway 1975a). Cutting height for example has a marked effect. A decrease in wear tolerance with lower cutting height has been demonstrated by Youngner (1961). It may be because lower cutting heights reduce root biomass (Dickinson and Polwart 1982, Parr _et al._ 1984) and also because of depletion of carbohydrates (Madison 1971) which may otherwise have contributed to plant recuperative potential. Application of fertiliser may increase wear tolerance by increasing sward growth (Canaway 1984a,b) but an optimum level of application has been identified above which wear tolerance is reduced (Canaway 1984a, 1985a,b).

Indirect effects of wear include soil compaction, soil smearing and soil structure damage (Canaway 1975a). There is little information about the latter two although a typical football pitch goal-mouth (see Plates 2 and 3) frequently exhibits these. Effects of soil compaction include mechanical impedance to root penetration (Rosenberg 1964, Beard 1973, Goss 1977, Goss and Scott Russell 1980), reduction in soil aeration (Canaway 1981a) which has an effect because there is a direct link between root development and oxygen diffusion rate (Letey et al. 1966), and changes in soil temperature and soil moisture (Rosenberg 1964, Blom 1979).

The relative contributions of direct mechanical injury or soil compaction are not clear as various opinions are recorded. Gupta (1933), Bates (1935) and Edmond (1962) suggested direct damage was most important while Edmond (1963) and Wood (1973) thought soil compaction effects were most dominant. Leney (1974) examined the relative effects and found differences of response by different species. As pointed out by Rosenberg (1964), response will depend on original conditions of the soil, season and stage of the plants.

The attributes that have been ascribed as giving good wear tolerance and/or recuperative potential have been mainly anatomical and morphological, based on qualitative observations. Speight (1973) listed such features characteristic of species resistant to trampling. Some studies have however looked experimentally at these characteristics, attempting to quantitatively relate various attributes such as amounts of cellulose or fibre to wear tolerance (e.g. Shearman and Beard 1975b,c, Canaway 1978, 1981a). Few studies, however, have attempted to relate plant physiology to wear tolerance

Plate 2 A typical football pitch goalmouth showing poor ground cover where intensity of use is high.

University of Keele Playing Fields.

Plate 3 Football pitch goalmouth - close up of soil smearing and puddling and very sparse, partly buried grass.

and/or recuperative potential (Canaway 1975a, Liddle 1975a). Shearman and Beard (1975c) investigated the influence of percent moisture content and percent relative turgidity but found no correlation with wear tolerance using a variety of different turfgrass species. Smith (1978) however, found that severe water deficits developed in Calluna vulgaris following trampling. Significantly lower RWC was recorded within 6 hours of treatment and remained at this reduced level for up to 6 months after treatment (when measurements were terminated). Smith (1978) also found reduction of photosynthetic capacity of Calluna vulgaris with water stress. He concluded that trampling-induced water stress in Calluna vulgaris may have caused reduced photosynthesis, which along with other factors may explain the observed reduced net primary productivity. Experiments using Phleum bertolonii and Plantago lanceolata, however, did not demonstrate such reductions in photosynthesis. At 7 and 21 days after simulated trampling, photosynthesis rates were higher, the greater the intensity of simulated trampling. Smith had no data for water status or assimilation over the initial few hours after simulated trampling, however. Evidently there was a different response between woody and non-woody plants, with Calluna vulgaris exhibiting prolonged water deficits and reduced photosynthetic capacity. Phleum bertolonii and Plantago lanceolata showed increased photosynthetic capacity presumably as a result of growth of new leaves, high photosynthesis rates (Jewiss and Woledge 1967), perhaps rejuvenation of tissue (Hodgekinson 1974) and possibly premature death of senescing tissue (Thomas and Stoddart 1980).

As little information seems to be available on the immediate plant physiological responses to trampling, the present study sought to elucidate the situation. Certain beneficial management practices may be indicated from results of such studies and as the most relevant area of application (in view of practical application and finance) is in the intensively used amenity grassland areas such as sports pitches, it was decided to concentrate on the responses in turfgrass species.

Examining aspects of plant physiology tended to dictate the type of approach and method employed. Actual human trampling treatments give the most realistic situation and therefore most valid results but disadvantages include problems of quantifying the forces applied (Harper _et al._ 1961). Thus treatments are not reproducible, the treatment of a particular part of the trial area is unknown (as not every plant will necessarily receive trampling according to where the foot lands) and variability in the environment will cause variation in the force applied (for example, where there is variation in soil water content over the trial area (Liddle 1975b)). Simulated trampling methods were therefore chosen for the main part of the study. Although only a limited number of components of real trampling are applied (Canaway 1975b), they have the advantage of being quantifiable, reproducible (Youngner 1961) and very usable in the greenhouse situation and over a wide range of experimental situations (Bayfield 1971a). Of the three main simulated trampling methods, the falling tamp method was chosen because it is relatively easy to construct, forces applied are easily calculated and reproducible and a wide range of forces can be applied.

Problems arise in relating greenhouse experimental results to real trampling results (Smith 1978). Leney (1974) found that some species in laboratory experiments had very different results compared to her findings in the field. Relating monoculture pot experimental results to findings from natural environments where there may be a number of different species present must be approached with great caution. Clearly many more factors such as effects of interspecies competition, environmental conditions and stresses will complicate the response in a natural plant community. These factors are in addition to the recognised deficiencies of simulated trampling compared to real trampling. Experiments relating simulated trampling to actual trampling under the same conditions are restricted to those of Kellomaki (1973) and more recent sports pitch studies by Canaway (1976b, 1981b). Kellomaki concluded that "despite certain deficiencies, the simulated trampling gave parallel results to those obtained from real trampling". Canaway found that real and artificial football pitch wear related closely. In the present study, field investigations were also attempted to provide indication that physiological results from laboratory investigations were also likely in the natural situation.

Measurements in both laboratory studies and in the field were based on three main parameters: shoot yield response, carbon dioxide exchange and water relations. The response of these three parameters are now considered in relation to various other stresses.

Response of plant shoot yield to stress.

The annual pattern of growth of *Lolium perenne* is largely affected by the seasonal weather pattern (Sheehy *et al.* 1979). A model was therefore developed by Sheehy *et al.* (1979, 1980) to investigate the influences of some environmental factors on the growth of perennial ryegrass. The model consisted of the following steps: generation of assimilate, partitioning of assimilate, respiration of assimilate, death of tissue, redistribution of assimilate following defoliation, daily redistribution of assimilate for emerging leaves and transformation of assimilate into plant tissue.

Shoot growth is dependent on factors influencing all these components which include effects of temperature, light, water and nutrients. Extremes of these factors may impose stress (mild or severe) on a plant and therefore inhibit growth by its effects on one or more of the listed components. Considerable information has been gathered about stresses such as freezing, chilling and high temperature, high and low solar energy, nutrient deficiencies and excesses, water deficits and waterlogging. Full reviews of plant response to most stresses are given by Levitt (1980a,b).

The results of all these stresses is to inhibit growth because by definition (Levitt 1980b) "all stresses are similar in the sense that they all may induce potentially injurious strains on the plant, either reversibly by inhibiting metabolism and growth or irreversibly by injuring or killing the cells". Different plant responses to stress in terms of susceptibility, avoidance and tolerance mechanisms are well discussed (Levitt 1980b). Indeed, there have been proposals of a unifying concept of plant response to stress (e.g. Steponkus 1980) because of observations that tolerance of one stress may confer a tolerance to another stress (Levitt 1980b, Steponkus 1980). This is by no means universal and it has been shown that even the avoidance and tolerance of a single stress require completely different mechanisms (Levitt 1980b).

Plant adaptation to the stress is by developing resistance, either by stress avoidance or stress tolerance.

Water stress is well documented (see reviews by Hsaio 1973, Levitt 1980b, Hanson and Hitz 1982, Morgan 1984). Reduction in shoot yield with water stress is very evident (Slatyer 1973) and appears to be mediated by many factors such as stomatal and nonstomatal effect on CO_2 exchange (e.g. Pasternak and Wilson 1974, Farquhar and Sharkey 1982, Kramer 1983), and reduction of cell elongation (e.g. Hsaio 1973, Hsaio and Acevedo 1974). Lawlor (1972a,b) investigated aspects of growth of Lolium perenne in response to periods of increasing water stress. He (Lawlor 1969) had established that small reductions in turgor greatly decreased extension growth. He found (1972b) that reduction of plant water potential from -3 to -6 bar reduced root and leaf extension growth by 50%. Stomata closed at -4 bar and growth completely stopped at -16 bar. Interestingly, on re-watering severely stressed plants, the rate of elongation was observed to increase for several days until after 3 days, growth was four times faster than controls. By the eighth day, control rates were resumed. Watts (1974) commented that as leaf elongation in graminaceous plants is controlled by the water status in the embryonic region at the base of the leaves, the situation may be complicated. Water status at the leaf base may be very different to that in older and more exposed central and terminal regions. This suggests that the waer potential recorded as causing cessation of growth may in fact be incorrect, using measurements of leaf water potential. Barlow et al. (1981) suggested that leaf expansion in grasses is not necessarily related to bulk leaf turgor. Both comments have been confirmed by Michelena and Boyer (1982) who demonstrated lost turgor and eventual wilting in the leaf blade but accumulation of solutes in the elongating regions, thus maintaining turgor. Leaf elongation, however did decrease despite turgor maintenance.

Mechanical stress, such as effects of shaking, has also been demonstrated as affecting plant growth (Neel and Harris 1971, Turgeon and Webb 1971, Jaffe 1973, Rees and Grace 1980a). Reduced plant growth by even gentle mechanical stimulation has been shown to cause decrease in growth (Jaffe 1973, 1980, Jaffe and Biro 1979). This phenomenon is termed thigmomorphogenesis. Associated to some extent is wind stress which has similarly been shown to decrease shoot yield (e.g. Whitehead 1963, Russell and Grace 1978a, 1979a, Fluckiger et al.

1978, Rees and Grace 1980b, Armbrust 1982).

Response of plant photosynthesis and respiration to stress.

It was evident from the work of Sheehy *et al.* (1979, 1980) that plant photosynthesis (and respiration) was the major component in the growth model for *Lolium perenne* and as reviewed by Levitt (1980a,b) various stresses have a major influence.

Freezing causes an abrupt cessation of CO_2 uptake and on thawing, photosynthesis remains depressed in relation to the depth and frequency of the frost (Bauer *et al.* 1975).

Effects of water stress on photosynthesis are well documented (Levitt 1980b, Farquhar and Sharkey 1982). In particular, from various work such as Brix (1962) and Ng *et al.* (1975) there appears to be unchanged rate of photosynthesis from zero leaf water potential to a few bars below this (0 bars to -6, -8 bars) then a rapid decline in rate over a further drop of a few bars (-8 to -14 bars) to zero net photosynthesis. Much of this dehydration induced inhibition of photosynthesis has been attributed to stomatal resistance (e.g. Troughton 1969, Moldau 1973, Brady *et al.* 1975) since at full stomatal closure, net photosynthesis is zero.

There also appears to be an effect of so-called mesophyll resistance which encompasses all nonstomatal leaf factors inhibiting photosynthesis due to dehydration. These nonstomatal factors consist of metabolic disturbances such as decrease in ribulose bisphosphate carboxylase activity (Jones 1973, O'Toole *et al.* 1976, O'Toole *et al.* 1977), chlorophyll loss (Alberte *et al.* 1977, Bengtson *et al.* 1978), and decrease in photorespiration (Lawlor and Fock 1975, 1977, Lawlor 1976a,b,). Decreased intercellular space has also been suggested by Levitt (1980b) in view of work such as Wooley (1973) and Todd *et al.* (1974).

Plant respiration response to water stress is not so clear with some recordings of an increased rate followed by a decline (e.g. Koeppe et al. 1973) or of only decreases (e.g. Brix 1962). The influence of stomatal closure and metabolic changes on plant respiration are discussed by Levitt (1980b).

Other factors influence photosynthesis and respiration such as light (e.g. Gaastra 1959, Monteith 1965b, Gloser 1976, 1977, Biscoe et al. 1977), nitrogen deficiency (e.g. Robson and Parsons 1978) and temperature (e.g. Lawlor 1979, Berry and Bjorkman 1980). Mechanical stimulation was demonstrated by Audus (1935) to cause increased respiration rates. In subsequent investigation of thigmomorphogenesis (Jaffe 1980) a reduction in photosynthesis was suggested (but not demonstrated). Wind stress has been shown to cause decreased photosynthesis in grass by Grace and Thompson (1973), Grace (1977) and Armbrust (1982). No effect of wind on photosynthesis however, was recorded by Russell and Grace (1978a,b, 1979a,b). Where decreased photosynthesis was shown, this was largely attributed to water stress effects. Reduction in growth in the findings of Russell and Grace (1978b, 1979b) were attributed to thigmomorphogenesis. At present, evidence for an effect of wind stress on photosynthesis is conflicting.

Defoliation has also been shown to influence photosynthesis. Hodgekinson (1974) demonstrated a rejuvenation effect in mature and even senescing leaves following partial defoliation so that in young and mature leaves, rates comparable with those of newly expanded leaves were attained. Gifford and Marshall (1973) have shown a change in stomatal resistance after partial defoliation. Other studies (Wareing et al. 1968, Meidner 1970) have shown stomatal and intracellular resistance to CO_2 transfer are affected by partial defoliation and that changes in net photosynthesis rate are positively correlated with ribulose bisphosphate activity. Along with this observed effect of defoliation on leaf photosynthesis is a further factor of the observed increase in sward photosynthesis (e.g. Ollerenshaw and Incoll 1979). It is attributed to removal of old leaf tissue and hence higher rates of photosynthesis because of predominantly young grass leaves (Jewiss and Woledge 1967, Woledge 1973) and also because of reduced shading. There is evidence (Woledge 1973) that shading during leaf extension reduces the leaf photosynthetic capacity.

A further factor influencing photosynthesis is the level of current assimilate. Much evidence (Sweet and Wareing 1966, Neales and Incoll 1968, Humphries 1969, Thorne and Koller 1974) has demonstrated that CO_2 fixation may be inhibited by accumulation of photosynthetic products in the leaf which may be a function of utilisation but also of slow translocation rates (Hofstra and Nelson 1969).

Response of plant water relations to stress.

Water is vital for many different processes in a plant. As summarised by Slavik (1974) "Functions vary from maintaining turgidity of the cells; facilitating opening of stomata to allow the gaseous exchange essential for photosynthesis and respiration; protection from heat injury by evapotranspiration; to being vitally involved in the many hydrolytic and metabolic processes in living cells".

Recent reviews covering aspects of plant water regulation include those of Morgan (1984) and Boyer (1985). Boyer (1985) reviewed the transport of water in the soil-plant-atmosphere continuum and Passioura (1982) particularly discussed factors that influence the movement of water. The water status of a plant depends largely on the recent history of the evaporative losses from it and the fluxes of water into and through it (Passioura 1982). These will be influenced by a variety of stresses as reviewed by Levitt (1980a,b). Unfavourable conditions may lead to water deficits, referred to by Kramer (1983) as "situations in which plant water potential and turgor are reduced enough to interfere with normal functioning". Small water deficits may occur during the daily cycle when morning transpiration rate is greater than root absorption due to considerable resistance to water flow between soil and root. Stomata close in response to lowering of plant water potential and subsequently rehydration occurs (Turner and Begg 1981).

Freezing stress causes dehydration of plant cells. In some environmental situations there may be great resistance of water flow between soil and root due to frozen soil water (Bauer *et al.* 1975).

Water stress has a severe effect on water relations, restricting the supply of water for turgor maintenance. There is much information in the literature such as reviews by Hsaio (1973), Sullivan and Eastin (1974), Turner (1979), Hanson and Hitz (1982), Morgan (1984) and Boyer (1985).

Lawlor (1972a) investigated the water relations of *Lolium perenne* exposed to water deficit. During a soil drying cycle, small changes in soil-water potential caused diminished plant water loss because stomata closed when plant-water potential decreased by 2 to 3 bars. Lawlor (1969) recorded decreasing leaf water potential in ryegrass with decreasing osmotic potential of the root medium. Wilson (1974) recorded declines in leaf water potential, osmotic potential and pressure potential with decline in leaf relative water content.

The effects of wind have also been demonstrated to influence plant water relations. Increased rates of transpiration were recorded by Grace (1974) following exposure of four different grass species to wind. This was also recorded by Fluckiger *et al.* (1978)

and Armbrust (1982). Other observations, however, such as Dixon and Grace (1984), have suggested wind causes reduced transpiration rates. Grace (1974) also recorded lower diffusive resistances for five grass species subjected to wind stress compared to that of controls. Reduced water content and high mesophyll resistance had been found by Grace and Thompson (1973) in *Festuca arundinacea*. Conclusions were that damage to epicuticular waxes by leaf buffetting as demonstrated by Thompson (1974, 1975) caused a reduced cuticular resistance and stomatal resistance, hence greater transpiration rate and reduced water content. The studies of Fluckiger *et al.* (1978), however, noted some increase in stomatal diffusive resistance but then a sudden decrease after 1.5 hours exposure to wind. Later studies by Russell and Grace (1978a,b, 1979a,b) do not support the earlier findings but results were variable. Leaf conductance was not affected in one study (Russell and Grace 1978a) but was found to increase in another study (Russell and Grace 1978b). The water potential was unaffected in both studies of Russell and Grace (1978b, 1979a). There were differences in wind speed between the various experiments. Some of the differences in response may be because in earlier work more leaf damage occurred thus reducing cuticular resistance and therefore having a marked effect on the water relations.

In the above review, response of plant shoot yield, CO_2 exchange and water relations to various stresses has been examined. It is evident that each parameter is influenced to some extent by stress and that these parameters are important in elucidating the response of plants to stress. The experiments reported later examine the response of these parameters to trampling.

CHAPTER 2

Materials and Methods

Introduction

To investigate a variety of responses by grass to trampling stress, it was necessary to be able to look at isolated mini-swards of grass where treatments and conditions are more reproducible. This was most readily facilitated by using pots of grass grown in the greenhouse and later kept in constant environment growth cabinets. The application of variable but also identically reproducible levels of trampling stress have been successfully demonstrated using a simulated system consisting of a falling tamp (Bayfield 1971a, Kellomaki 1973, Smith 1978, Blom 1979), and it was decided to use this approach.

Grass grown in pots for simulated trampling experiments.

10 cm pots of John Innes No 2 compost were seeded with the appropriate variety of grass seeds. Approximately 15 seeds were scattered in the centre of the pot and left to germinate. Greenhouse conditions of at least 16 hours day length with a minimum day temperature of 18°C (maximum of 26°C) were maintained. During months with less than 16 hours daylight, 400 W high pressure sodium lamps supplemented the day length giving an illuminance of 10 to 11 klux and an energy flux (PAR) of around 100 W m^{-2} at bench height. The dark period of 8 hours (or less) was at a minimum temperature of 13°C. It was not possible to control the air humidity.

Approximately 2 weeks after germination, the seedlings were thinned to approximately 12 plants forming a group in the centre of the pot with a margin of at least 2 cm by the pot wall left clear of any plants. This was to prevent excessive shearing off of the grass during application of simulated trampling. Preliminary experiments had shown that grass near the edge of the pot tended to shear off.

In early experiments, at the same time as thinning, the grass was cut to 3 cm and thereafter once a week to 3 cm up to and including the 8th week when the grass was ready to be used in simulated trampling experiments. However, as root and shoot growth proved to be poor and there was evidence in the literature that early cutting prevents the development of a good root system and hence the shoot system (Woledge 1977), the procedure was consequently changed so that cutting to 3 cm weekly commenced at the 4 week stage, thus first allowing for good root development and establishment.

After the 8th week stage the pots of grass were usually relocated to the constant environment growth cabinet where they were allowed to acclimatize for 6 days in conditions as described later. Then experiments proceeded as will be described in the appropriate sections.

Choice of species and varieties of grass.

It was necessary to choose a certain grass species cultivar which could be used as a standard. Because the fundamental grass of most hard-wearing sports pitches is *Lolium perenne* (STRI 1980) it was decided to concentrate on this species. The Sports Turf Research Institute (STRI) have produced comprehensive tables giving comparative ratings of different cultivars' performance relating to various aspects such as tolerance of artificial wear, persistence under mowing to certain heights, compactness, freedom from certain diseases (STRI 1980). For perennial ryegrass, comparisons were restricted to diploid cultivars of the "turf type" or cultivars of good agricultural persistence (thus satisfactory for turf). Of these, Aberystwyth S23 was given medium ratings for most of the attributes, including tolerance of artifical wear and persistence under mowing at 2 cm. This rating suggested its suitability as the standard grass species cultivar for the present study. Further, in agricultural research, Aberystwyth S23 has been frequenty used as the standard cultivar against which the performance of others is compared (NIAB 1978, STRI 1980). There is therefore, relatively much documentation on this variety. From all these considerations, it was decided to choose *Lolium perenne* S23 as the standard variety and species studied.

For comparison of performance in certain experiments in the study, other species and cultivars were required. A wide variety of the major turf species were selected (nomenclature after Hubbard 1968), namely: *Lolium perenne* L. (Perennial ryegrass), *Agrostis tenuis* Sibth (Common bent, Brown top), *Festuca rubra* L. subsp. *commutata* Gaud. (Chewings fescue), *Poa pratensis* L. (Smooth-stalked meadow grass), *Phleum pratense* L. (Timothy grass, Cat's-tail), *Phleum bertolonii* D.C. (Smaller cat's-tail), *Holcus lanatus* L. (Yorkshire fog).

Holcus lanatus was included to provide a non-sports turf species for comparison.

The performance of these different turf species relative to trampling stress is rather varied. Both *Agrostis* spp. and *Festuca* spp. are of relatively low tolerance of trampling stress, as trials at STRI (STRI 1980) amongst others, have shown. However, they have greatest persistence under close mowing, hence their importance in bowling greens, cricket wickets and golf greens. *Phleum* spp. and *Poa pratensis* are tolerant of artificial wear, although their ratings are not as high as those for *Lolium perenne*.

In addition to comparing species of different tolerance levels of trampling stress, varieties within each species showing different tolerance to trampling stress, were also sought where feasible. The comparison tables for each species based on performance trials at

STRI (STRI 1980) were consulted and suggestions direct from STRI were also received (Canaway, pers. comm.). Other factors such as availability of seed, were taken into consideration. The cultivars finally used are summarised along with the comparative performance rating, in Table 1.

No ratings of wear tolerance were available for Agrostis tenuis, Festuca rubra ssp. commutata and Phleum spp. cultivars. The relatively limited information on wear tolerance of the various Agrostis species suggests there may be more difference between cultivars than between species (STRI 1980). In this study, the cultivar Highland will be referred to as a cultivar of Agrostis tenuis, although it is suggested that it really belongs to the species Agrostis castellana (Shildrick 1976). Highland and Holfior were chosen as they had relatively high ratings for aspects of performance other than wear. For Festuca rubra ssp. commutata, response to close mowing was the most important factor. In this respect, Highlight (good) and Cascade (poor) contrasted in performance, while Wintergreen was sent for comparison along with Cascade by the seed supplier as they were phasing out Cascade in favour of Wintergreen. For Phleum spp., no ratings were available at all. Phleum pratense S48 and P. bertolonii S50 were chosen as they constituted fairly well known varieties.

Description of species and cultivars used.

Extensive information on grass turf species is given by Beard (1973), STRI (1980, 1982), and Johnson (1980) from which the following details (unless otherwise stated) have been extracted:

Lolium perenne (perennial ryegrass)

It has been a prominent grass of cultivation for well over 250 years especially in well drained fertile soils. Although it is used extensively in agriculture, varieties suitable for turf have become

Table 1 Grass species and cultivars: performance rating and supplier.

Species	Cultivar	Rating - comparative to other varieties within the species based on wear tolerance and tolerance of mowing	Supplier
Lolium perenne	S23	medium (standard)	6
	Manhattan	good (top rating)	1
	Melle	medium	2
	Sportiva	poor	1
Agrostis tenuis	Highland	no rating available	3
	Holfior		4
Festuca rubra ssp. *commutata*	Highlight	no rating available	1
	Wintergreen		3
	Cascade		3
Poa pratensis	Parade	good (top rating)	4
	Entopper	good	1
	Primo	poor	5
Phleum pratense	S48	no rating available	6
	Comet		1
Phleum bertolonii	S50		6
Holcus lanatus	-	-	4

1 Pope and Chapman Ltd., Bishops Stortford, Herts.
2 Rothwell Plant Breeders Ltd., Rothwell, Lincoln
3 Northrup King Ltd., Southend-on-Sea, Essex
4 WW Johnson & Son Ltd., Boston, Lincs.
5 Hurst Gunson Cooper Taber Ltd., Witham, Essex
6 Welsh Plant Breeding Station, Aberystwyth

well established. All the different varieties can be divided into 3 distinct groups - early heading hay types which are only moderately persistent under hard wear or frequent cutting, so they remain largely as agricultural cultivars only; late heading pasture types which are relatively prostrate and have good persistence even when mown to 1.8 cm or used for heavy wear sports fields hence these are used for sports turf; and turf or amenity types which have only been developed in recent years since 1960. These turf types are much finer leaved, short growing and are very tolerant of both close mowing and wear so they may be used on different types of sports pitches such as cricket wickets, golf greens or football pitches.

Ryegrass is a non-creeping species which forms a medium textured turf of good short density. It has a rapid rate of seed germination, establishment and leaf extension. It does not tolerate extremes of cold, heat or drought so it is well suited to the cool moist summers and mild winters experienced in Britain. It has extremely good tolerance to wear particularly for winter sports such as football.

Cultivar S23 was released in 1933 and became the standard agricultural species but it has also been extensively used for parks, open spaces, roadsides and sports fields because of its high wear tolerance (Shildrick 1973, 1974) and fairly prostrate growth habit. In the STRI (1982) ratings it has "medium" rating for wear tolerance under both high and low N, persistence when mown at both 2.0 cm and 1.0 cm but has "poor" rating for shoot density, fineness of leaf, cleanness of cut, short growth and winter hardiness.

Cultivar Manhattan was released in 1967, having originated from Central Park. It is noted for its diminutive growth habit and slow vertical shoot growth rate yet profuse tillering, leafiness and rooting at the nodes as well as extremely high wear tolerance ratings (Shildrick 1974). These are excellent characteristics for a hard wearing yet fairly fine turfgrass so its use is very extensive. STRI (1982) ratings were "good" for wear tolerance under both high and low N, persistence when mown at 2.0 cm, cleanness of cut and winter hardiness. "Medium ratings" were given for persistence when mown at 1.0 cm, shoot density, fineness of leaf and shoot growth. It also has medium tolerance of heat and shade.

Cultivar Melle is less well documented. In the STRI (1980) ratings it was given "good" rating for persistence when mown at 2.0 cm and cleanness of cut but "medium" ratings for tolerance of artificial wear, compactness, short growth and winter hardiness.

Cultivar Sportiva too is not well documented and had lower ratings than Melle and S23 in the 1980 STRI ratings. A "good" rating was given for cleanness of cut; a "medium" rating was given for persistence under mowing at 2.0 cm, compactness and short growth but "poor" ratings were given for tolerance of artificial wear and winter hardiness.

Agrostis tenuis

Naturally, this species is abundant on all types of soil but it is most frequent under poor dry acid conditions, hence it has good drought tolerance. It forms an upright fine textured dense turf under close mowing which it tolerates well due to the low growth habit (because the lower internodes are quite short). Both rhizomes and stolons are produced so it is able to colonise bare or scarred areas of turf rapidly. It has a very good establishment rate and has good low temperature hardiness. Tolerance of wear however is poor. *Agrostis tenuis* is widely used in all types of turf and becomes a major component along with Chewings fescue for the fine, closely mown bowling greens. It is also used for high quality fine textured lawns, golf greens and fairways and it may become the dominant species especially where mowing is at a height of less than 2.5 cm.

Cultivar Highland was released in 1934 and is a very distinct type that has been classed as a separate species (as has already been discussed). It has an extremely aggressive growth habit and will spread rapidly, perhaps a result of its high proportion of rhizomes relative to the other cultivars. Highland has less compact shoot density than other cultivars and its leaves are less fine. Drought tolerance is superior to the other cultivars. The STRI (1982) ratings were that it had "poor" compactness of sward, fineness of leaf, short growth but "good" winter greenness.

Cultivar Holfior was released in 1940. It is adapted to less fertile soils. Spreading is mainly by stolons. It has a very fine texture and high shoot density with a semi-erect growth habit and is best cut at heights of 1.25 to 2.5 cm. Recuperative potential is not as good as Highland. The STRI (1982) ratings were "medium" for compactness of sward, fineness of leaf, short growth and greenness.

Festuca rubra ssp. *commutata*

It is one of the distinct subspecies of *Festuca rubra* (an abundant, widely distributed natural species found in nearly every type of soil and situation) and is a relatively dwarf form with a tufted growth habit. It forms a fine textured, erect growing, high density turf. Its good tolerance of close mowing is second to that of *Agrostis tenuis*. A cutting height of 3.75 to 5.0 cm is preferred. The tufted growth habit and extensive tillering produces an extremely dense turf. It has poor tolerance to temperature extremes but fairly good drought tolerance. Tolerance of heavy winter wear is poor but with somewhat less severe trampling on summer sports pitches it has fairly reasonable tolerance.

Cultivar Highlight was released in 1959. It has very fine texture and high shoot density. Drought tolerance is relatively poor but it has good tolerance of close mowing. STRI (1982) trials gave "medium" ratings for compactness, persistence under mowing at 0.5 cm and short growth.

Cultivar Wintergreen was released in 1968. It has a very fine texture, very high shoot density, and minimal creeping habit. STRI (1982) trials indicate it has a "good" persistence when mown at 0.5 cm, "medium" compactness and "poor" shoot growth.

Cultivar Cascade was released in 1964. It has very fine texture, medium shoot density and good establishment vigour. It is well adapted to drought susceptible coarse textured soils. STRI (1982) trials gave ratings of "poor" compactness and persistence when mown at 0.5 cm but "very poor" short growth.

Poa pratensis

Naturally it occurs widely in a variety of habitats but mostly on well drained, light and loamy soils. It is initially sensitive to competition and has a relatively slow growth rate. It is however, strongly rhizomatous, giving it a very good recuperative potential. Drought tolerance and winter hardiness are good but it is not tolerant of close mowing. A cutting height of 2.5 to 5.0 cm is preferred. It has very good wear tolerance being considered second to *Lolium perenne*. It is widely used for general purpose turfs and so is found in lawns, golf greens, fairways, roughs and in low maintainence areas such as roadsides. For football pitches its attributes are good wear tolerance, good satisfactory compactness and good recuperative potential. For fairly close mown sports turf its attributes are good/satisfactory compactness, some tolerance of mowing at 2.0 cm and satisfactory fineness of leaf. For low maintainance density areas, without wear, its attributes are satisfactory compactness and short growth.

Cultivar Parade is a more recently developed variety. It has relatively fast establishment and excellent ability to cover ground.

It has a relatively broad leaf and is strongly rhizomatous enabling it to produce a very dense hard wearing turf. The STRI (1982) ratings were "good" for wear tolerance, compactness, tolerance of mowing at 2.0 cm and "medium" fineness of leaf and short growth.

Cultivar Entopper has a finer leaf than Parade. STRI (1982) ratings were "good" for wear tolerance, compactness, tolerance of mowing at 2.0 cm, fineness of leaf and "medium" for short growth.

Cultivar Primo was released in 1949. It has medium fine texture, medium shoot density, semi-erect growth habit and moderately slow vertical growth rate. The establishment rate and recuperative potential is good. STRI (1982) ratings were "medium" for compactness, tolerance of mowing at 2.0 cm, short growth and "poor" for wear tolerance and fineness of leaf.

Phleum pratense

Naturally it is widely distributed though more abundant on heavier soils where moisture stress is rarely limited. It is relatively coarse leaved and is widely used in agriculture but also occasionally in sports turf. *Phleum pratense* generally forms a coarse textured turf which is fairly wear tolerant though not as tolerant as *Lolium perenne* and *Poa pratensis*. Drought tolerance is poor. Its recuperative potential especially after water stress is poor. Germination is rapid but subsequent growth is slow. Its cold tolerance is very good, however, hence its greater use in cold climates such as Scandinavia.

The main use of *Phleum pratense* is in agriculture but where it is used in amenity/sports turf, it is usually limited to low quality turf grass areas as it is not suited to mowing below approximately 1.5 cm. No ratings were available for any cultivars.

Cultivar S48 is a commonly used agricultural variety.

Cultivar Comet was chosen as a contrast, though little information was available about it.

Phleum bertolonii

In many ways it is similar to Phleum pratense except for its finer leaves and a denser more compact growth as a result of creeping stolons. Phleum bertolonii is therefore better adapted for turf grass use especially as its tolerance to close mowing (as low as 1.5 cm) and to heavy winter wear is considerably better than P. pratense. Its recuperative potential is fairly good too. It has the good winter hardiness but poor drought tolerance of P. pratense. Phleum bertolonii is used as a component of close cut heavy wear sports turf.

Cultivar S50 was released in 1932. It has medium texture, medium high shoot density, prostrate growth habit and roots at the nodes. Wear tolerance is good and it tolerates close mowing at 1.0-1.2 cm so it is sometimes used to add wear tolerance to a fine fescue/bent turf. No performance ratings are available.

Holcus lanatus

Naturally it is a very common grass found in a great variety of situations. It is generally regarded as a weed of meadows and pastures. It is often abundant in rough grassland, wasteland and open woodland (Hubbard 1968).

Growth may vary from loosely to compactly tufted. It has no rhizomes or stolons.

Essentially Holcus lanatus is a non-sports turf species which has extremely poor tolerance of wear.

Application of trampling stress.

a) Forces involved in trampling.

In order to establish satisfactory methods of applying trampling stress it was first necessary to examine the forces involved in real human trampling. For convenience, these forces have largely been categorised into either vertical or horizontal components (Canaway 1975b) although in reality forces are applied to turf at a variety of angles.

In the most straight-forward situation where a person is static, the force exerted is entirely vertical and depends on the persons' weight and the area of shoe sole in contact with the ground. Liddle (1975a) stated that the vertical force from a standing man may exceed 0.2 kg cm^{-2} (1.96 x 10^4 N m^{-2}). Beard (1973) gave values of 0.441 kg cm^{-2} (4.33 x 10^4 N m^{-2}) for a 91 kg man standing in street shoes but the force was 20.38 kg cm^{-2} (200.0 x 10^4 N m^{-2}) when standing in football boots with 7 x 0.9 cm diameter studs.

Once there is a dynamic situation, for example walking or running, the forces involved become far more complex and more difficult to quantify and to simulate. Harper _et al._ (1961) investigated the forces involved in walking in great detail. Both for walking in a straight line and also for walking round a right-angled corner they quantified not only vertical and horizontal forces (in terms of mass per unit area) but also torque, pressures and the changing area of contact during a step. How these various forces changed with time were also closely recorded. An average of 1.1 kg cm^{-2} (10.79 x 10^4 N m^{-2}) was calculated for the force of one step averaged over the whole foot when walking in a straight line. They suggested that any simulation machine should deliver a force of 1.476 kg cm^{-2} (14.48 x 10^4 N m^{-2}) as this was their calculated upper 95% limit. They concluded that the forces involved in walking were complex and not readily simulated. This is further complicated by their observations that individual walking patterns were extremely variable.

Other observations have given lower, perhaps underestimated values, as Canaway (1975b) suggested. For example Goss and Roberts

(1964) calculated that an 81 kg man exerted a force of 0.564 kg cm^{-2} (5.53×10^4 N m^{-2}) when walking. Beard (1973) estimated that a 91 kg man in football boots with 7 x 0.9 cm diameter studs would exert between 42 and 63 kg cm^{-2} ($412-613 \times 10^4$ N m^{-2}) when running. Further extrapolatians by Canaway (1975b) suggested that this may be as high as 61.3 kg cm^{-2} (601×10^4 N m^{-2}) but the effects of stud penetration however would perhaps reduce the force somewhat.

More recent work by Quinn _et al._ (1980) has investigated the mechanics of real trampling on slopes of up to 26° from horizontal. Like Harper _et al._ (1961), they monitored the forces involved in walking in great detail. Using an 80 kg person in tennis shoes (therefore applying 785 N when static), they found peaks of both horizontal and vertical forces coincided at 0.2 sec of approximately 900 N vertical and 85 N horizontal force. (No data was available to express forces in kg cm^{-2} or N m^{-2}).

Other measurements for horizontal forces are relatively few compared to the number for vertical forces. Harper _et al._ (1961) found that the horizontal force in walking may rise to 25% of the body weight. At or soon after a sprint start it may be as great as 87% of the body weight (Canaway 1975b). Horizontal forces may even reach values near to that of the body weight in running at a constant speed (Cavagna _et al._ 1964). Very large forces may be produced in sports by changing direction, stopping and starting (Canaway 1976a). The actual effects may vary too, according to the type of foot wear. Flat surface shoes will cause abrasion whereas studded soles will cause tearing and smearing of the turf (Canaway 1975a,b, 1976a). Harper _et al._ (1961) did detailed time/pressure/torque studies, revealing great variation in direction as well as amount of horizontal force during one walking step. Clearly, closely realistic simulation of such forces presents many difficulties.

Calculations can be made from the data of Harper _et al._ (1961) to obtain values of forces applied. The standard "typical" graphs given for a person walking on the flat in a straight line showed a very large vertical force initially when the heel made contact with the ground, peaking at 14.06 kg cm^{-2} (138×10^4 N m^{-2}). The average maximum heel pressure recorded from 48 subjects was 16.20 kg cm^{-2} (159×10^4 N m^{-2}) but the range was very large with the highest individual recording of 57.65 kg cm^{-2} (566×10^4 N m^{-2}) and lowest of 1.02 kg cm^{-2} (10×10^4 N m^{-2}). In contrast the average pressure for the whole foot was only 0.88 kg cm^{-2} (8.65×10^4 N m^{-2}) which is roughly 20 times less than the average maximum force applied by the heel only. (Highest individual pressure for the whole foot was 1.69 kg cm^{-2} (16.6×10^4 N m^{-2}) and lowest was 0.49 kg cm^{-2} (4.8×10^4 N m^{-2})). Clearly, considerable vertical forces may be generated by the heel during walking on a hard horizontal surface. This would suggest that the vertical heel force is the most damaging part of trampling.

It is perhaps significant however that the studies by both Harper _et al._ (1961) and Quinn _et al._ (1980) examined the forces involved in walking on hard surfaces. Where a surface has negligible resilience, the forces actually applied will be much greater than where the surface is fairly resilient. As there appears to be no work on the forces imparted to resilient surfaces such as turf, it is difficult to estimate exactly how close the values calculated from work of Harper _et al._ (1961) may be to trampling on a typical grass sward. It may be expected however that at some time, conditions such as extreme compaction and/or drying out of the soil may well result in a turf of very low resilience. These calculated values may therefore serve to indicate the maximum forces that could in reality be applied. Indeed, Youngner (1961) found greatest damage was done to turf by spiked rollers (largely vertical forces) than by scuffing rollers (mainly abrasive horizontal forces). Shearman and Beard (1975a) found however that simulated wear from dragging a sled damaged turf more than simulated wear from a tyre. It is difficult to assess these conclusions because in the work of both Youngner (1961) and Shearman and Beard (1975a) it is not very clear how the simulation of the horizontal and vertical forces relate to real wear and indeed how severe the horizontal forces are in comparison to the vertical forces. Quinn et al. (1980) established an experimental grass plot on a slope and applied real trampling. They concluded that "most damage to vegetation by walking arises from compaction by the heel in the early part of each step and shearing by toe action at the end of each step". The shearing action and therefore the horizontal force was considered to be of greatest importance. Again assessment of these findings is difficult because trampling on a slope will involve different forces to trampling on the flat.

Ideally, as suggested by Canaway (1975b), to simulate wear accurately it will be necessary ultimately to make very precise measurements of all the forces involved in each of the various sport and leisure activities. Measuring the forces using force platforms with appropriately resilient surfaces, if this is at all possible, would be important. Subsequently application of these forces should take place under experimental conditions to establish the different effects of trampling by different activities.

b) Application of simulated trampling using a falling tamp method.

In the present study where various aspects of the physiological response of grass to trampling stress was to be examined in some detail, application of simulated trampling using a falling tamp was considered to be the most appropriate approach. Grass could be grown in pots in controlled environmental conditions, receive simulated trampling from a tamp and subsequently be monitored in detail, measuring various aspects of physiology in controlled conditions.

The force exerted by a falling tamp depends on the height from which it is dropped, its weight, area and sole, and the resilience or amount of compaction of the soil/plant surface it lands on. The latter surface resilience is difficult to quantify so expressing the forces in terms of potential energy input has proved to be more useful as discussed by Canaway (1975b). He defined the potential energy input, E_{pot} as:

$$E_{pot} = mgh \qquad J$$

where m = mass (kg)

g = acceleration due to gravity (9.81 m s^{-2})

h = distance the tamp falls (m)

The tamp apparatus used is the actual one described by Smith (1978) consisting of a metal frame supporting a shaft with a circular tamp head on the end. A pulley system allowed the shaft and tamp to be lifted to a selected height and then dropped onto the pot of grass accurately located in a concrete block (see Plate 4). The concrete mount, therefore, prevented any shattering of pots from the tamp falling inadvertently on the pot rim and also helped to prevent pot fracture from severe soil compaction. For the higher intensities of trampling, however, even the concrete mount was unable to prevent pot fracture in some cases as a result of severe compaction, so a pot shaped steel holder (minus a base) was made to encase the pot to prevent pot fracture. The holder was split down one side with two joining screws to allow for widening and tightening of the holder for addition and removal of the pots.

The tamp head described by Smith (1978) consisted of a disc 10 cm in diameter with a weight of 2.64 kg (including the shaft). Additional weights could be added. A different, smaller diameter

Plate 4 Falling tamp apparatus used to apply simulated trampling treatments to pots of grass.

tamp head was used for the present study of 7.5 cm diameter with a total disc and shaft weight of 2.20 kg. It was dropped from a height of 25.4 cm thus applying 5.48 J. As the area of the disc was 44.2 cm^2 this represents a potential energy of 1239.82 J m^{-2}. The surface of the tamp head was fitted with a vibram sole so there were some localised points of higher pressure. This was minimal due to the covering of the tamp head by frequently renewed polythene bags which were necessary in order to minimise soil smearing (particularly important in the electrolyte release and photosynthesis experiments. There was also a ring of polythene placed around the grass, over the soil to further reduce soil contamination of the head).

In comparison, Smith (1978) used the 10 cm diameter head with added weights giving a total of 4 kg in weight. This represents a potential energy of 10.79 J. Calculating potential energy input per unit area, as the contact area of the disc was 57.5 cm^2, he therefore applied 1877 J m^{-2}. The forces used by other workers are very varied. Wagar (1964) applied 116.8 J m^{-2} and Kellomaki (1973) applied 137.4 J m^{-2} in their field studies whereas in their greenhouse studies Smith (1978) as already discussed applied a potential energy of 1877 J m^{-2} and Bayfield (1971a) employed 1529.1 J m^{-2}.

Calculations can be made to estimate the vertical force given by a falling weight onto turf. Indeed, Canaway (1975b) said it is desirable to measure the dynamic forces as well as the energy input in Joules so that falling weight treatments can be more closely related to human activities. Calculations were made (see Appendix 1) based on realistic assumptions and observations. From these, it was estimated that the tamp in the present study imparts a force of 49.59 $\times 10^{-4}$ N m^{-2} which is approximately one third of the average maximal heel pressure calculated from the data of Harper _et al._ (1961). In view of the fact that the surface had no resilience in the investigation by Harper et al. (1961), the vertical force imparted by the tamp appears to give a fairly realistic simulation of the vertical forces involved in trampling on turf.

Ways of introducing horizontal forces into the simulated trampling treatments were examined but it was not found to be feasible for various reasons discussed in Appendix 2.

c) Intensity and timing of simulated trampling treatments.

The range of number of impacts applied in this study was 1 to 25 at any one time. A maximum of 25 was established following trials to estimate the number of impacts required to cause death of *Lolium perenne*. Smith (1978) applied up to 40 impacts while Bayfield (1971a) used a range of 1 to 100 drops of the tamp at any one time on *Phleum bertolonii* S50. In the field situation, Wagar (1964) used up to 40 impacts per week while Kellomaki (1973) applied a range of 1 to 64 drops of the tamp per week.

Because of the variation in the force applied at each drop of the tamp and also because of the different number of impacts applied, comparison of results from the different workers may appear to be difficult and perhaps of limited value. Canaway (1975a) however observed that even where physical forces have not been quantified in any way but the treatment has been described only as a given number of passes or revolutions of a given machine, the use of such techniques is justified by the reasonable concurrence of results of all the different workers evaluation of cultivars.

Some comparison is perhaps plausible between the treatments given in the present study and those of Smith (1978) and Bayfield (1971a) as the forces, treatments and conditions were relatively similar. Both Smith (1978) and Bayfield (1971a) left the pots of grass up to 3 weeks after treatment before harvesting for dry weights. In the present study, weekly cuttings were made following treatment (see Chapter 3) so the values for the dry weights obtained at the third week were compared. The response curves for dry weight to the different ranges of impacts were very similar, in particular those of Smith (1978) and the present study. It seems that 25 impacts in the present study had similar proportional reduction in dry weights as 40 impacts in Smith's (1978) work and 100 impacts per pot in that of Bayfield (1971a). The slight variation in forces applied, species used and biomass of the material at time of treatment may account for the similar response to differing number of impacts applied.

The treatments have been applied in various ways with regard to timing. Some workers have applied regular trampling, say weekly or fortnightly over a period of time, usually with experiments in the field situation (Wagar 1964, Kellomaki 1973, Leney 1974). Blom (1979) applied treatments several times daily on Plantago species according to the severity of trampling required. Smith (1978) working with Calluna vulgaris found that the end result was similar whether a treatment was applied all at one time or applied periodically but with the final total number of tramples being equivalent. Leney (1974), however, applied various trampling treatments, both walking and running, to an area of grassland dominated by Ammophila. Her findings were that the final damage was much more severe when given all on one occasion than from fortnightly treatments that totalled more than that given on the one occasion. The different response compared to that of Calluna is probably due to graminaceous plants having relatively protected meristems at the base of the shoots and in creeping perennial grass species there are intercalary meristems on rhizomes and stolons. During the intervals between trampling treatments, significant regrowth is therefore possible, giving a different end result to where the trampling has been given all on one occasion, severely damaging the grass shoots and perhaps even the meristems (Canaway 1975a). In the present investigations, plants were treated usually on one occasion only when the grass was 8 weeks old and measurements were made immediately and/or over a period of time subsequent to the treatment.

Electrolyte release as a measurement of injury from trampling.

a) Introduction

It has been observed that trampling bruises, abrazes, crushes and tears shoots (Bayfield 1971a, Speight 1973, Beard 1973, Canaway 1975a). Further observations have shown that damaged parts of shoots frequently show subsequent loss of colour and eventual death. Shearman and Beard (1975a) found that wear damaged tissues desiccated and turned straw-coloured within three days of treatment. A short investigation into the effects of simulated trampling on shoots of intact pots of grass similarly showed rapid desiccation followed by necrosis of the damaged areas (see Appendix 3).

It would therefore appear that there is injury from trampling to the shoots at tissue level from the bruising, abrazing, crushing and tearing but also at the cellular level as a result of desiccation. The actual amount of injury incurred may be of importance in determining the overall response and potential for recovery. It may also significantly vary according to the species or cultivar and hence indicate less or more wear tolerant species and cultivars. It was therefore considered appropriate to measure the level of injury following simulated trampling. There appears, however, to be no published method for quantitatively measuring the actual trampling injury.

There are a number of methods of quantitatively measuring frost injury in plants such as depression of photosynthesis, depression of respiration, cessation of protoplasmic streaming, electrolyte release, vital staining (using reduction of colourless triphenyltetrazolium chloride), as described by Levitt (1956, 1972, 1980a) . These methods were examined for their suitability in determining injury from trampling. The electrolyte release method has also been used to quantify injury from drought (Levitt 1972, 1980b, Sullivan and Kinbacher 1967, Bannister 1970, Dlugokecka and Kacperska-Palacz 1978). It is a straight-forward method which is suitable for field as well as laboratory or greenhouse investigations so it seemed an appropriate method to use. Preliminary trial experiments further suggested its suitability for measurement of injury.

The standard method for measuring frost injury in plants by electrolyte release was pioneered by Dexter <u>et al.</u> (1930, 1932) and was subsequently used by workers such as Carrier (1951), Emmert and Howlett (1953) and Wilner (1955). Carpenter <u>et al.</u> (1963) modified the technique by showing that autoclaving was more convenient and efficient than boiling. Flint <u>et al.</u> (1967) further modified the technique by developing an index of injury using the equation:

$$I_t = 100 \frac{(R_t - R_o)}{1 - R_o}$$

where $R_t = \frac{L_t}{L_k}$, $R_o = \frac{L_o}{L_d}$

I_t = Index of injury resulting from exposure to temperature t.

R_t = Fractional release of total electrolytes from samples exposed to temperature t.

R_o = Fractional release of electrolytes from unfrozen sample.

L_t = Specific conductance of leachate from sample frozen at temperature t.

L_k = Specific conductance of leachate from sample frozen at temperature t and then heat killed.

L_o = Specific conductance of leachate from unfrozen sample.

L_d = Specific conductance of leachate from unfrozen sample, heat killed.

If samples are uniform, L_k and L_d are identical therefore the index can be simplified to:

$$I_t = 100 \frac{(L_t - L_o)}{(L_k - L_o)}$$

The Flint injury index has been successfully used in various pieces of research such as Bannister (1970) and Polwart (1970). In applying the equation of Flint _et al._ (1967) to measure injury from trampling "simulated trampling of x impacts per pot" was substituted for "temperature t" and "no simulated trampling", was substituted for "unfrozen".

Following initial trial experiments to establish details of the method, preliminary experiments were carried out to check the suitability of the Flint injury index for measuring injury from trampling. The results were unsatisfactory in that extremely widely ranging values were obtained. Closer examination showed this was largely due to the variation in control plant values being highly exaggerated because of the nature of the equation.

A much simpler ratio was therefore used:

$$\text{Index of Injury} = I = \frac{L_t}{L_k}$$

where L_t = specific conductance of leachate from sample receiving simulated trampling of x impacts per pot.

L_k = specific conductance of leachate from sample receiving simulated trampling of x impacts per pot and then heat killed.

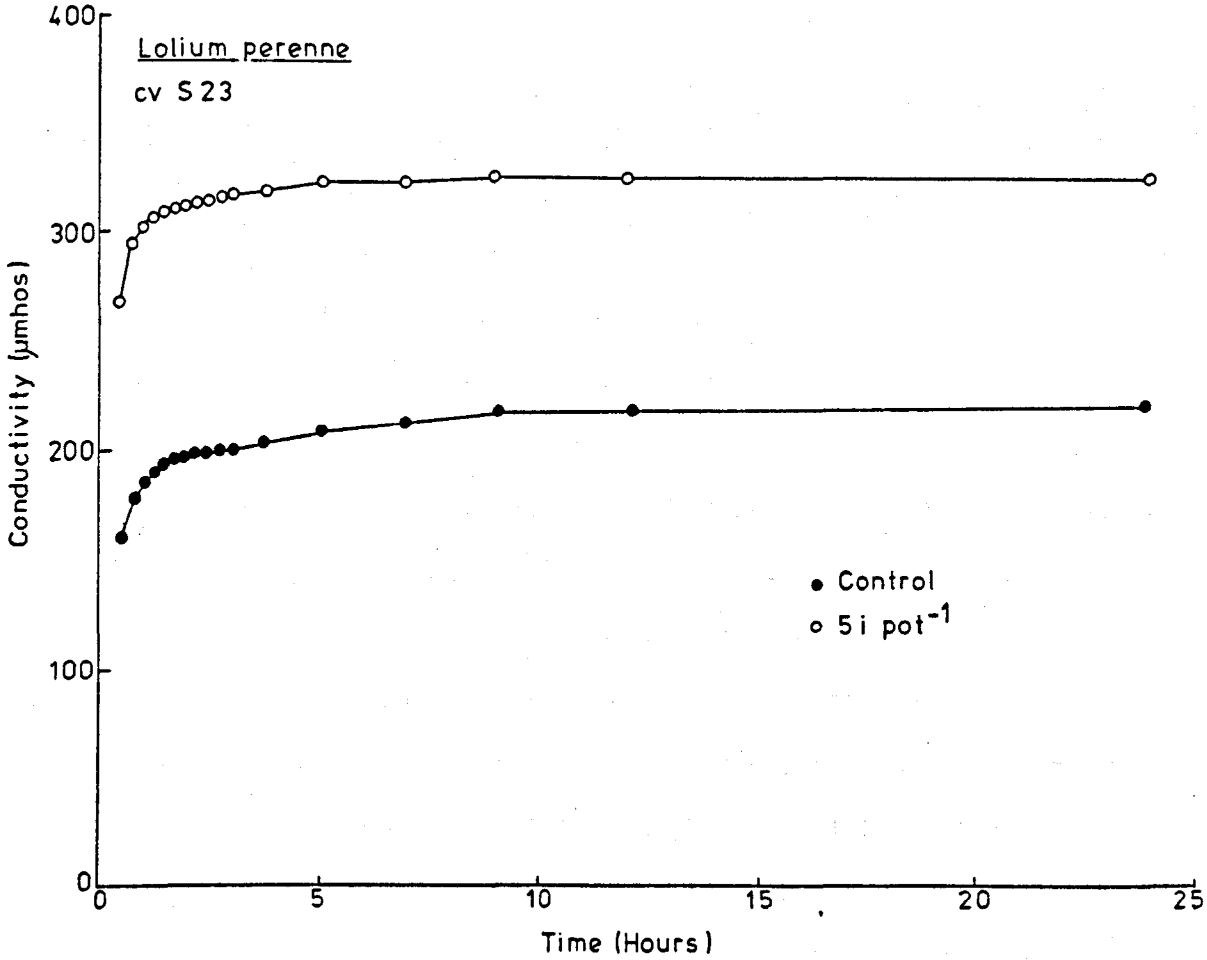
Preliminary results showed this to be very satisfactory with very small standard errors for samples of 6 replicate control plants.

b) Method

The method that was developed was as follows:
Pots of grass from the greenhouse were acclimatized for 6 days in the growth cabinets at standard conditions of 20°C, 70% r.h. for 16 hours photoperiod and 13°C, 90% r.h. for 8 hours of dark. They were regularly watered over this period to maintain the soil at near field capacity. The experimental procedure started with application of the appropriate simulated trampling treatment. The shoots were then immediately cut at the standard 3 cm level (thus not including any of the dead leaf sheaths). Fresh weight of the excised shoots was then taken. Next, the shoots were placed in a boiling tube, double glass distilled water was added using a zipette and the level of the water in the tube was marked with an indelible marker pen. The boiling tubes were then left in the dark at ambient temperature for 3 hours as established from preliminary trials (see Fig 1). After exactly 3 hours, the water level was checked to see it measured up to the pen mark, homogeneity of the solution was ensured and then it was subsampled. The conductivity of this sample was measured using a Lock bridge conductivity meter and noted. The sample was returned to the boiling tube which was then capped with silver foil to minimise evaporative losses. The sample was then subsequently heat killed by autoclaving (15 lb in^{-2} for 15 minutes). Once the sample was cold again, usually 24 hours after the original start of the procedure, a reading was taken again as outlined above. If the level of the liquid was reduced, it was made up to the level of the mark before ensuring homogeneity of the solution and then measuring the conductivity. The index of injury, $I = L_t/L_k$, as described above was calculated.

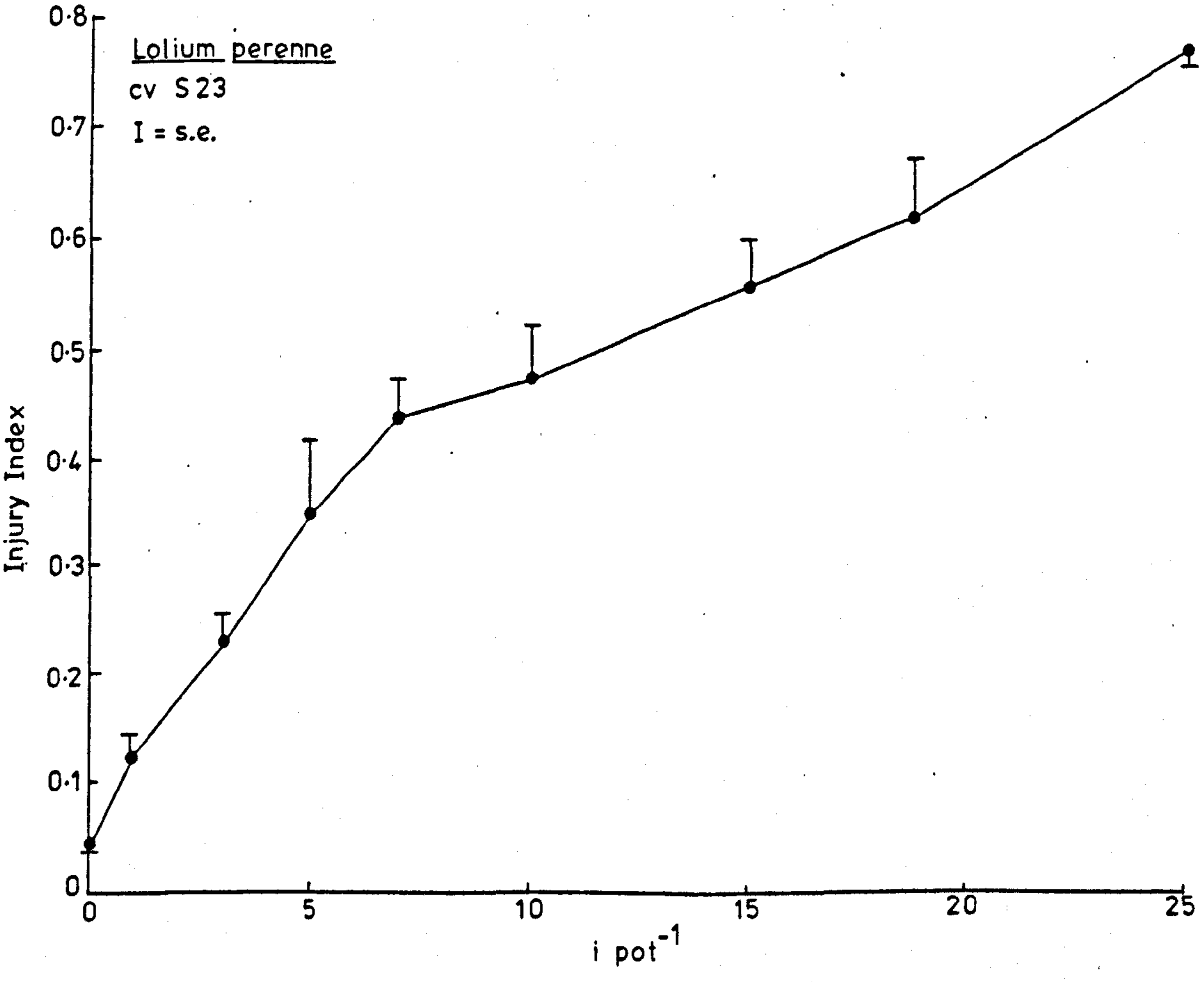
Fig. 1 Loss of electrolytes from excised grass shoots in double glass distilled water over time.

Fig. 2 Injury index at different intensities of simulated trampling: Lolium perenne S23



400
300
200
100
0
Lolium perenne
cv S23
Conductivity (μmhos)
● Control
○ 5 i pot⁻¹
0
5
10
15
20
25
Time (Hours)

Fig. 1



0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0
Lolium perenne
cv S23
I = s.e.
Injury Index
0
5
10
15
20
25
i pot⁻¹

Fig. 2

The injury index over a range of simulated trampling treatments from 0 to 25 impacts per pot was obtained for all species and cultivars as listed earlier (Table 1). The quantity of shoots and amount of double glass distilled water used varied slightly. In initial experiments with Lolium perenne S23, a standard approximate fresh weight, usually 0.3 g, was subsampled from each pot and usually 20 ml of double glass distilled water was used. In subsequent experiments, as the ratio of fresh weight to conductivity after autoclaving was found to be similar for a variety of fresh weight samples usually taken (approximately 0.6-1.0 g), the total amount of excised shoots was used in 40 ml double glass distilled water. Where, however, the total quantity of shoots per pot weighed about 0.2 to 0.4 g as for Festuca and Agrostis species the shoots were placed in 20 ml of double glass distilled water.

CHAPTER 3

The effects of simulated trampling on grass shoot yield.

Introduction

A large number of observations and experiments both in the field (e.g. Edmond 1958, Wagar 1964, Bayfield 1971a, Smith 1978, Blom 1979) and also in the laboratory (e.g. Bayfield 1971a, Smith 1978) have shown that the general effect of trampling, whether naturally by man or animal or artificially using simulated methods, is to reduce the plant shoot yield. This reduction has been found usually to be related to the intensity of trampling and at high intensities there may well be death of the plants, typically seen in the case of many footpaths (Bates 1935).

Observations have also been made that trampling bruises, abrazes, crushes and tears shoots (Bayfield 1971a, Speight 1973, Beard 1973, Canaway 1975a).

It was therefore appropriate to firstly investigate the injury sustained by grass shoots and the shoot yield response to a variety of intensities of simulated trampling and to see if a relationship between shoot yield and injury was apparent.

Methods

a) Injury of grass shoots with simulated trampling at different intensities.

As discussed in Chapter 2 (page 36), measurement of the injury sustained by grass shoots from trampling was required. An index of injury, I, using proportional electrolyte release was developed for this purpose and the method is given in Chapter 2 (page 39).

Pots of grass were grown to the 8 week stage as described in the general methods. Treatments of 0, 1, 3, 5, 7, 10, 15, 20 or 25 impacts per pot (i pot^{-1}) were applied but where poor germination or growth limited the number of pots of grass available for some species and cultivars, treatments were 0, 1, 3, 7, 15 or 25 i pot^{-1}. There were 5 replicates per treatment generally but where reduced numbers of pots were used there were 6 or 4 replicates in those cases. Results were graphed and the L_{50} was listed for all cultivars and also the value of I for 25 i pot^{-1}. Comparisons were made between species and cultivars.

b) Response of shoot yield to intensity of simulated trampling and the relationship with injury level.

Bayfield (1971a) and Smith (1978) investigated the shoot yield of plants that had received simulated trampling at various intensities. Both measured the biomass 3 weeks after treatment and found that there was a decrease in shoot biomass with increase in intensity of simulated trampling. At 3 weeks after treatment, the recuperative potential would be becoming evident so they were able to give some assessment of the plant durability and not just the wear tolerance.

Comparable experiments were carried out to determine shoot yield response to simulated trampling in the present study.

Weekly cuttings to 3 cm before and after simulated trampling treatment were collected from the pots of grass used for the measurement of injury described above (section a)) for all the species and cultivars until the experiment was terminated at the 15th week. Dry weight of cuttings each week were obtained after drying overnight at 95°C. Results were analysed using analysis of variance, and particular attention was paid to the shoot dry weights for 3 weeks after treatment. Values of L_{50} and dry weight at 25 i pot^{-1} as % of control were also listed and used to compare all species and cultivars.

Results of section a) were compared to those of this section using regression/correlation analysis. Various transformations of the data were tested to establish the relationship between I and shoot yield at 3 weeks after treatment and to see if this varied between species and cultivars.

c) Effect of simulated trampling on the proportion of dead and live shoot biomass.

Shearman and Beard (1975a) found that injured parts of grass blades desiccated and turned straw coloured within 3 days of treatment. This has been confirmed in Appendix 3. Hodgkinson (1974) found that where selected younger leaves of lucerne plants were defoliated there was a reversal of senescence in remaining old leaves and complete rejuvenation of photosynthetic capacity in remaining mature leaves between 8 and 12 days after treatment, the peak rate being maintained for only 3 or 4 days. Drought stress, however, has been shown to accelerate senescence in old and mature leaves (Ng *et al.* 1975).

To investigate if simulated trampling caused any acceleration or reversal of senescence, the following experiment was carried out. Pots of grass were grown to the 8 week stage as described in Chapter 2 (page 18). Treatments of 0, 1, 3, 5, 7, 10, 15, 20 or 25 i pot^{-1} were applied 7 days after the 7th week cut and the pots of grass were then left for 11 days in the greenhouse. Shoots were then harvested and divided into categories of live tissue, dead tissue or 3 cm shoot bases. Dry weights were obtained. There were 5 replicates for each treatment. *Lolium perenne* S23, *Lolium perenne* Manhattan, *Poa pratensis* Entopper and *Phleum pratense* Comet were used. Results were graphed and analysed using analysis of variance. Responses were compared between species and cultivars.

d) Shoot yield response of grass to soil compaction or shoot injury.

It is well documented that there are two effects of trampling (Bates 1935, Speight 1973, Canaway 1975a). Firstly, there is direct injury of the shoots and secondly, an indirect effect resulting from soil compaction.

In order to investigate the relative importance of the two components in reducing shoot yield the following experiment was carried out. Pots of grass were grown to the 8 week stage as described in chapter 2 (page 18). 7 days after the 7th week cut, the following treatments were applied: 0 i pot^{-1}, 5 i pot^{-1} to the whole pot of grass, 5 i pot^{-1} to the shoots only, 5 i pot^{-1} causing soil compaction only. The latter two treatments were achieved using apparatus (see Fig. 2a and 2b) described by Smith (1978) which is similar to that used by Leney (1974). Subsequent weekly cuttings to 3 cm were collected for 3 weeks and dry weights were obtained. There were 6 replicates for each treatment. Results were graphed and analysed using analysis of variance with particular attention to the 3rd week after treatment.

Results

a) Injury of grass shoots with simulated trampling at different intensities.

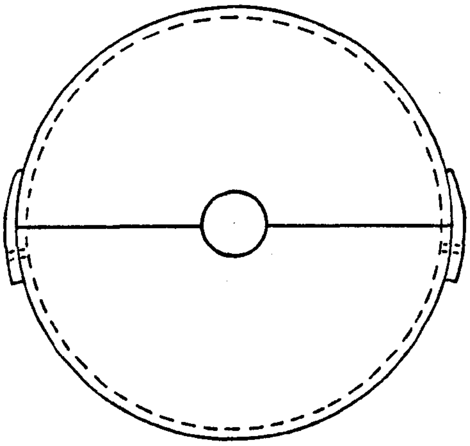
Results are shown in Figs. 2 to 17. For all species and cultivars there was a highly significant increase ($p<0.001$) in injury with increase in trampling pressure (Table 2). As it appeared that the relationship between I and i pot^{-1} was not completely linear but curvilinear in some cases (*Poa pratensis* cultivars, *Phleum bertolonii* especially), some transformations were tried. The correlation was improved by plotting I with log (i pot^{-1}+1) for all except *Lolium perenne* Manhattan and Melle. This was further improved in some cases by plotting arc sin I with log (i pot^{-1}+1) as shown in Table 2.

Values of L_{50} and I at 25 i pot^{-1} are given in Table 3. For L_{50}, *Lolium perenne* Manhattan and Melle, *Poa pratensis* Entopper and Primo, *Phleum bertolonii* S50, all had values greater than 25 suggesting a very high wear tolerance. *Lolium perenne* Sportiva, *Poa*

Fig. 2a Apparatus for applying simulated trampling to shoots only.
(Drawn to scale 1 cm ≐ 2.11 cm, some lines omitted for clarity.)

a - rubber pad simulating soil resilience
b - split steel plate with hinge to fit over pot
c - hole for protrusion of grass shoots

Fig. 2a



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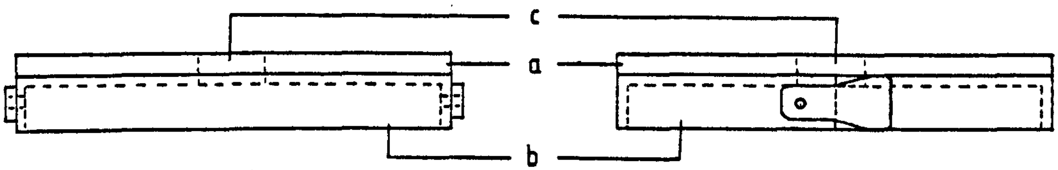

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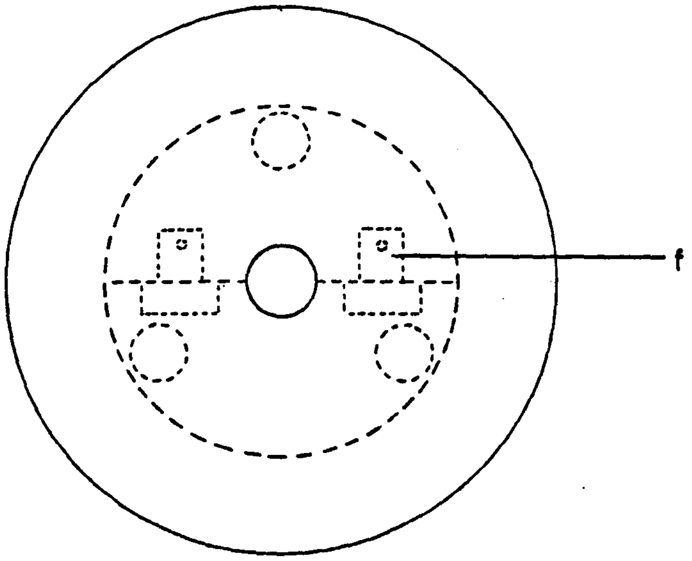
Fig. 2b Apparatus for applying simulated trampling to soil and roots only.
(Drawn to scale 1 cm = 2.08 cm, some lines omitted for clarity.)

a - steel plate receiving impact of tamp
b - split steel plate resting on soil in pot
c - hole for grass shoots
d - space for shoots(protected from impact by upper plate)
e - steel rods screwed into upper and lower plates
f - flange attached to lower half of split plate

Fig. 2b



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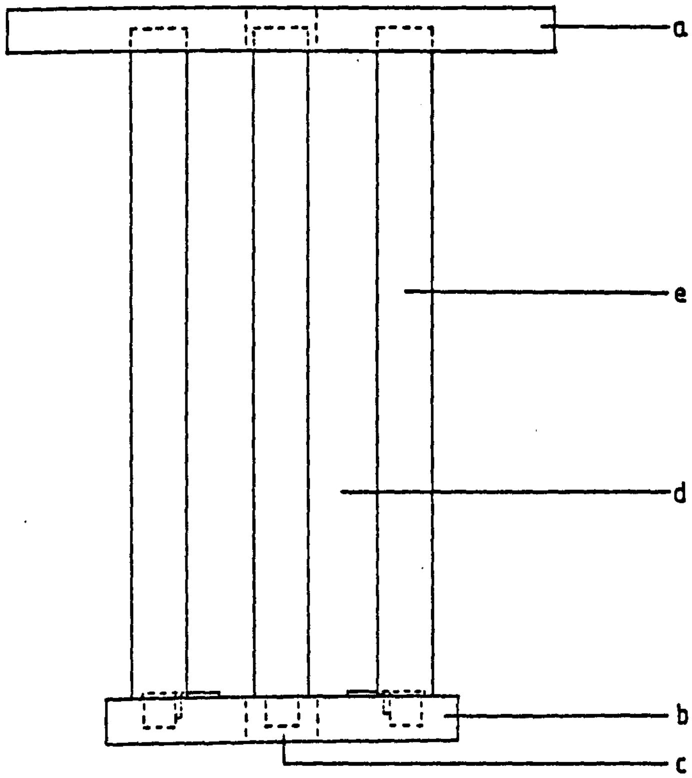

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Injury index at different intensities of simulated trampling:

Fig. 3 *Lolium perenne* Manhattan
Fig. 4 *Lolium perenne* Melle
Fig. 5 *Lolium perenne* Sportiva
Fig. 6 *Festuca rubra* ssp. *commutata* Highlight

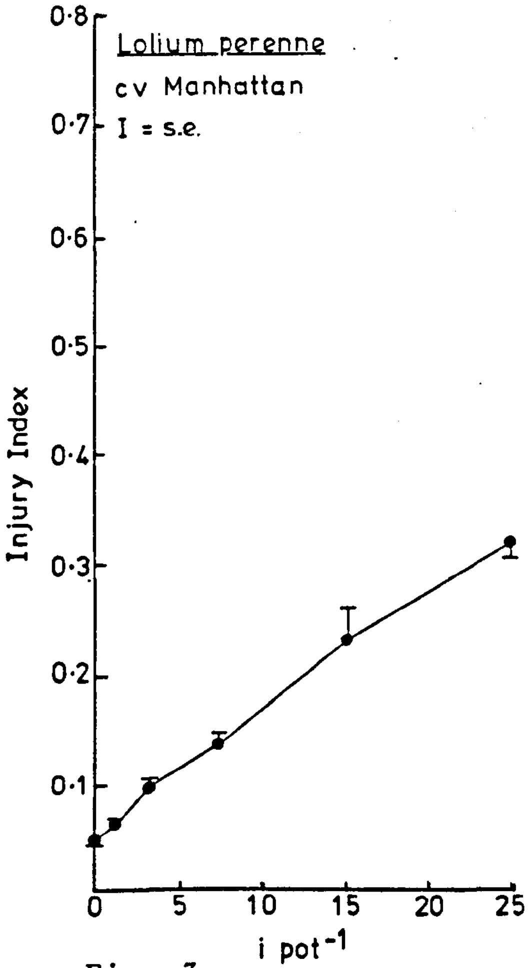


Fig. 3

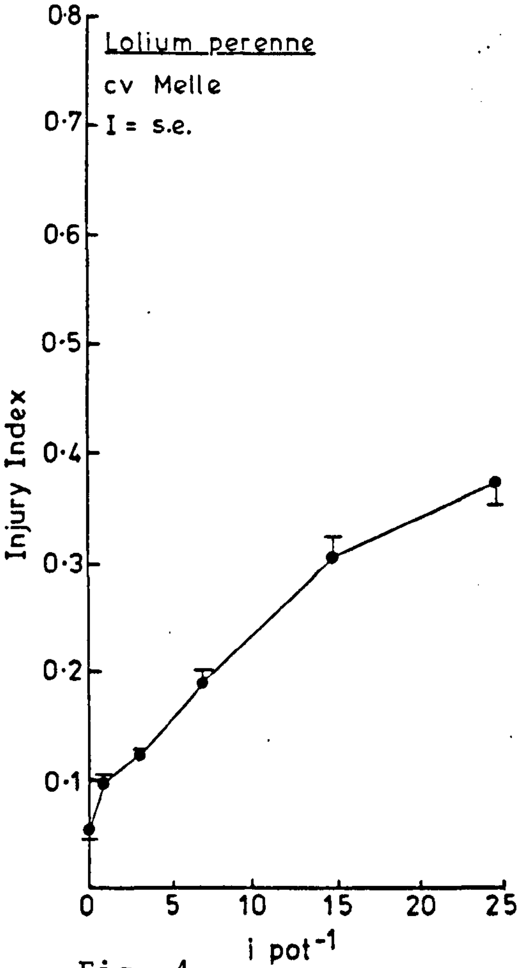


Fig. 4

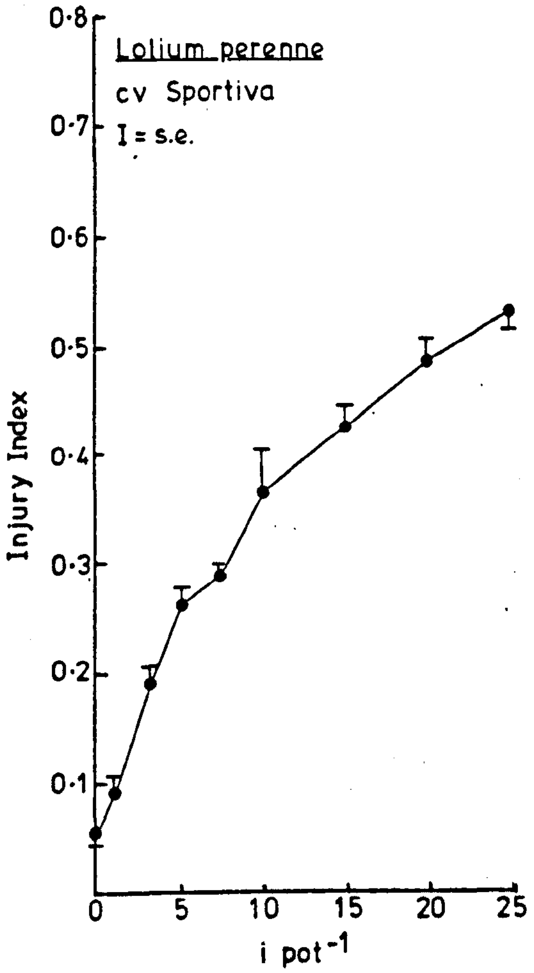


Fig. 5

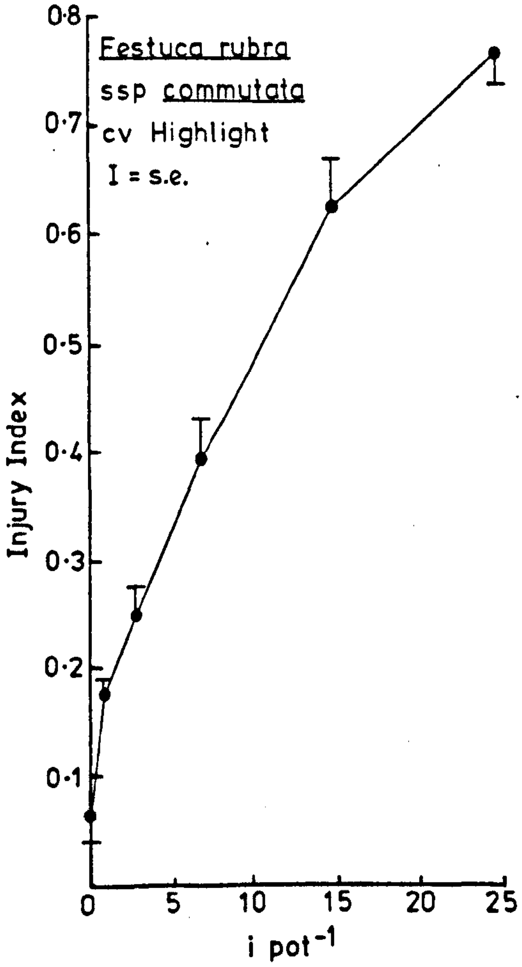


Fig. 6

Injury index at different intensities of simulated trampling:

Fig. 7 *Festuca rubra* ssp. *commutata* Cascade
Fig. 8 *Festuca rubra* ssp. *commutata* Wintergreen
Fig. 9 *Agrostis tenuis* Highland
Fig. 10 *Agrostis tenuis* Holfior

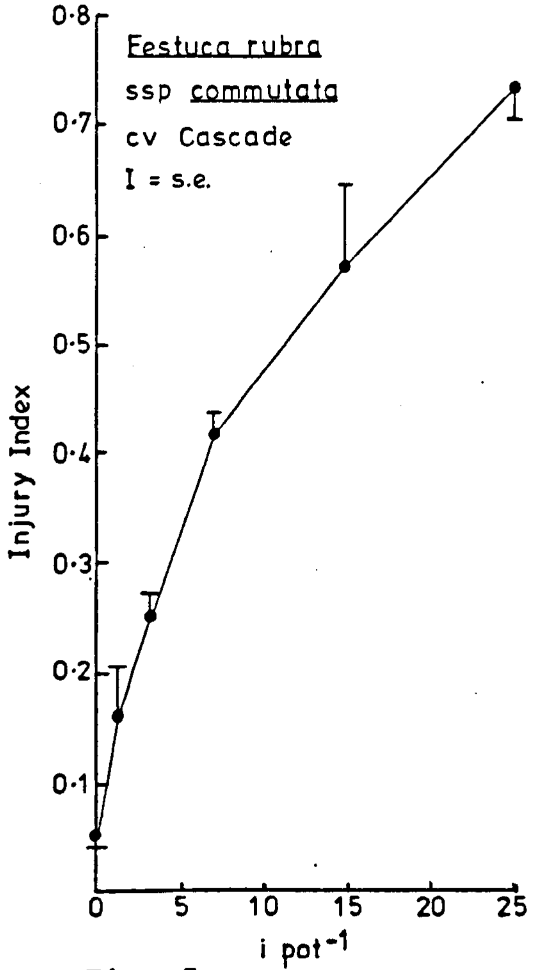


Fig. 7

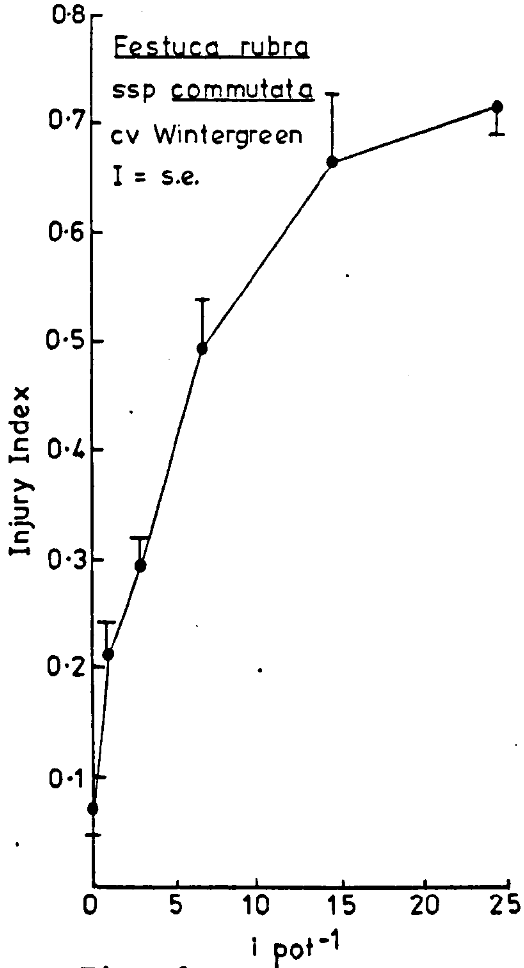


Fig. 8

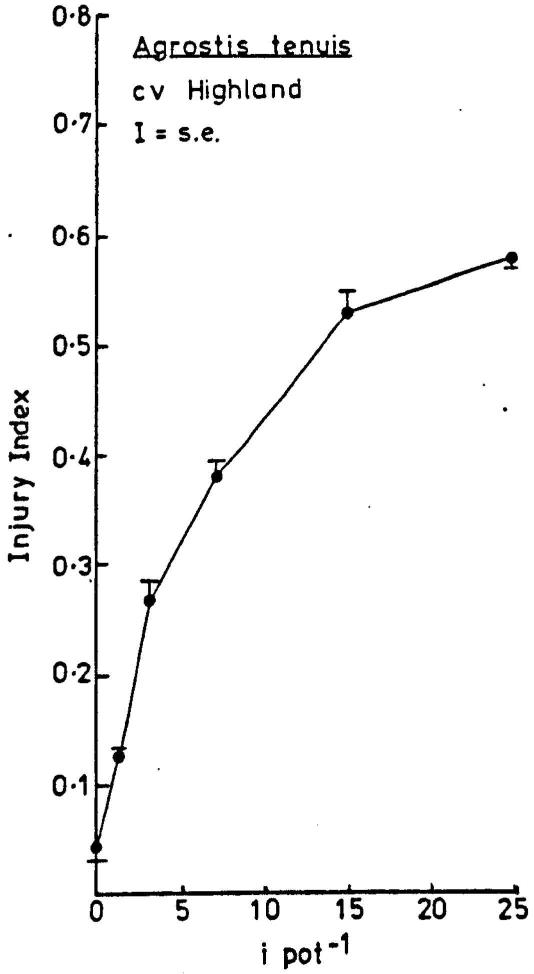


Fig. 9

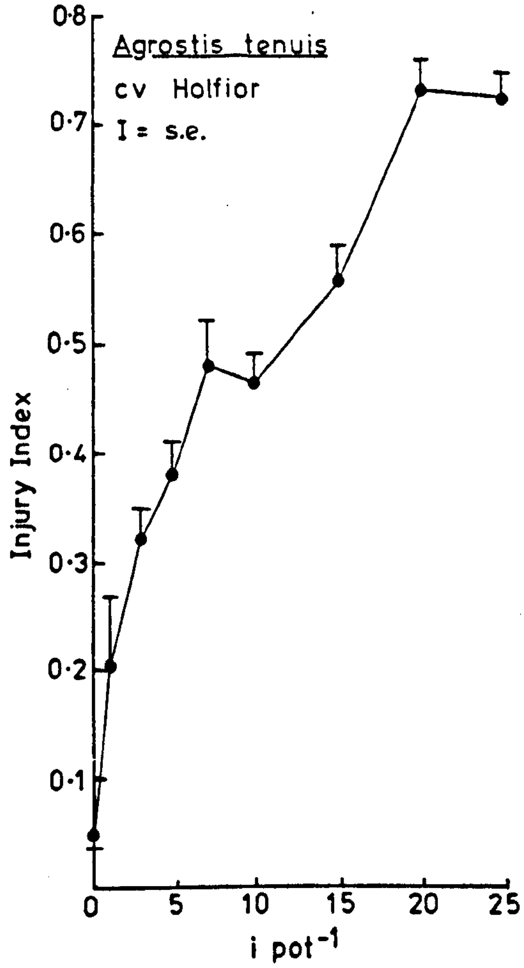


Fig. 10

Injury index at different intensities of simulated trampling:

Fig. 11 *Holcus lanatus*

Fig. 12 *Poa pratensis* Parade

Fig. 13 *Poa pratensis* Entopper

Fig. 14 *Poa pratensis* Primo

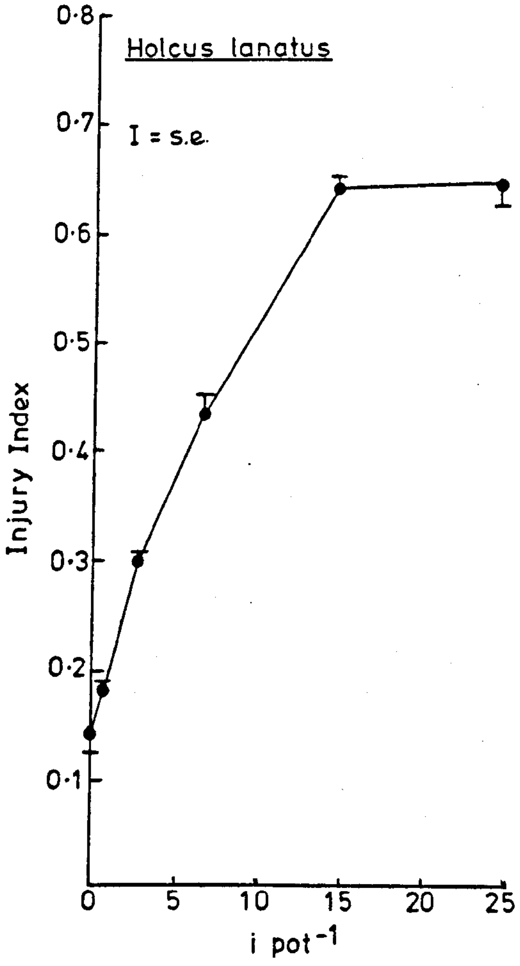


Fig. 11

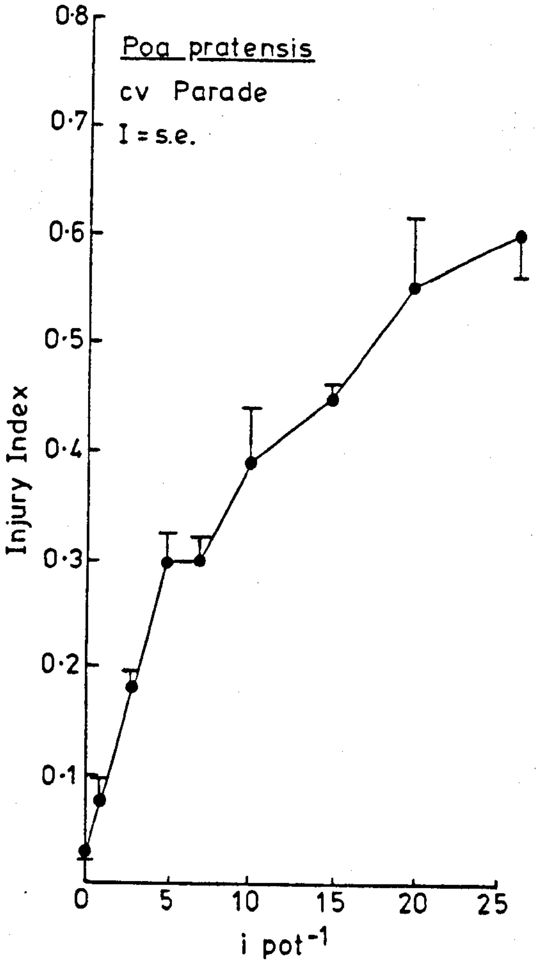


Fig. 12

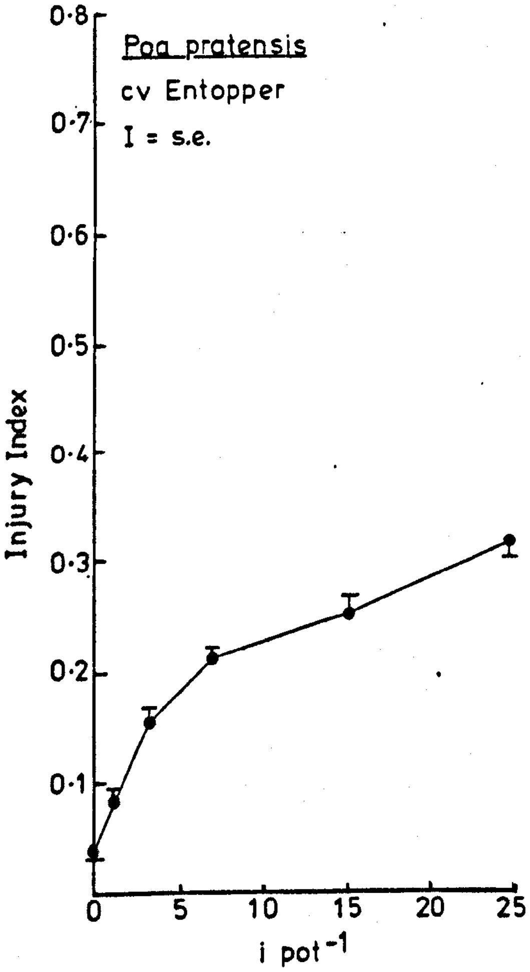


Fig. 13

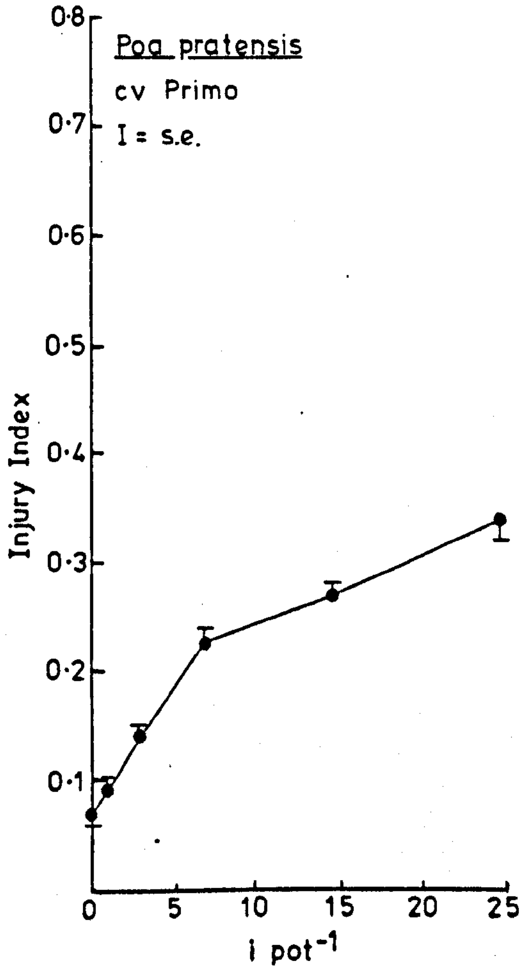


Fig. 14

Injury index at different intensities of simulated trampling:

Fig. 15 *Phleum pratense* S48

Fig. 16 *Phleum pratense* Comet

Fig. 17 *Phleum bertolonii* S50

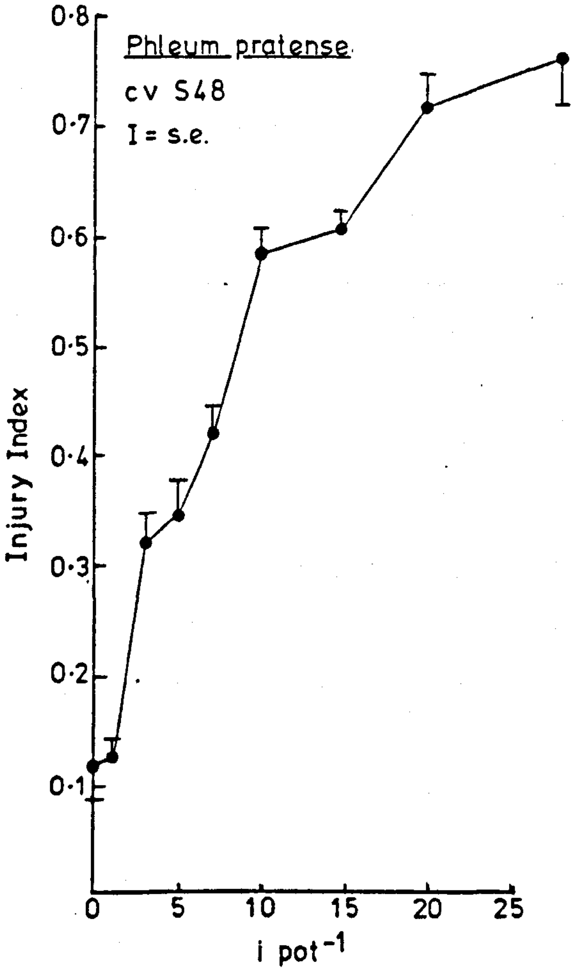


Fig. 15

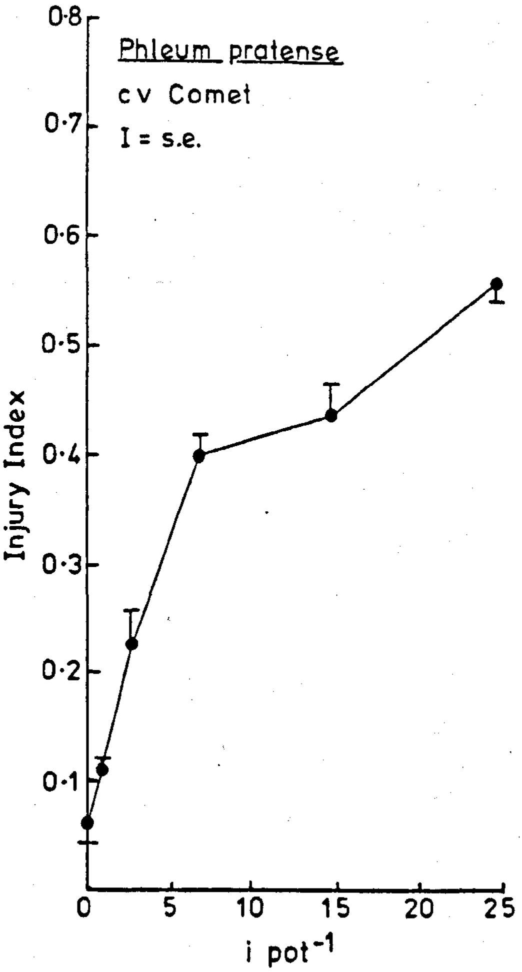


Fig. 16

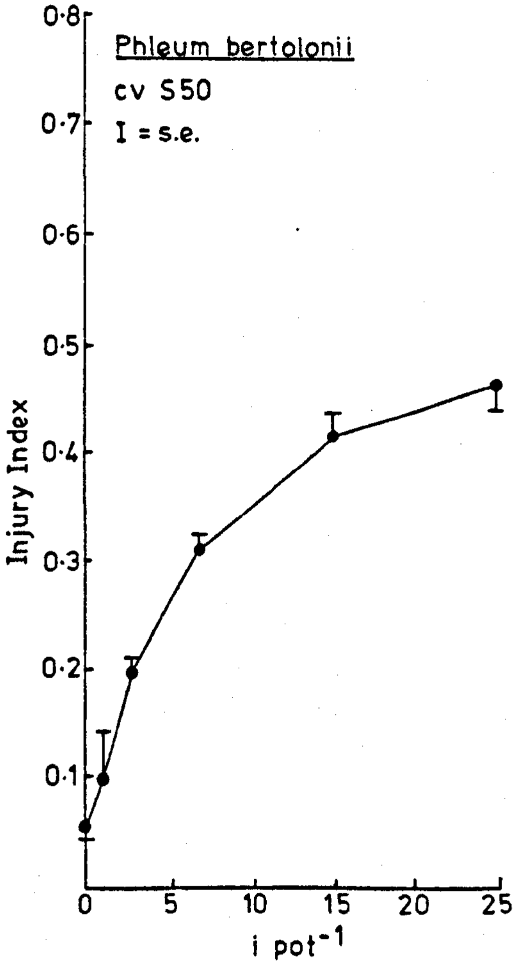


Fig. 17

Table 2 Correlation between I and $i\,pot^{-1}$.

Species/cultivar		Significance of correlation of I with $i\,pot^{-1}$	Transformation improving the correlation coefficient of I with $i\,pot^{-1}$
Lolium perenne	S23	$p < 0.001$	$\arcsin\sqrt{I}$, $\log(i\,pot^{-1}+1)$
	Manhattan	$p < 0.001$	$\arcsin\sqrt{I}$, $i\,pot^{-1}$
	Melle	$p < 0.001$	I , $i\,pot^{-1}$
	Sportiva	$p < 0.001$	$\arcsin\sqrt{I}$, $\log(i\,pot^{-1}+1)$
Festuca rubra			
ssp. *commutata*	Highlight	$p < 0.001$	$\arcsin\sqrt{I}$, $\log(i\,pot^{-1}+1)$
	Cascade	$p < 0.001$	$\arcsin\sqrt{I}$, $\log(i\,pot^{-1}+1)$
	Wintergreen	$p < 0.001$	I , $\log(i\,pot^{-1}+1)$
Agrostis tenuis	Highland	$p < 0.001$	I , $\log(i\,pot^{-1}+1)$
	Holfior	$p < 0.001$	$\arcsin\sqrt{I}$, $\log(i\,pot^{-1}+1)$
Holcus lanatus		$p < 0.001$	$\arcsin\sqrt{I}$, $\log(i\,pot^{-1}+1)$
Poa pratensis	Parade	$p < 0.001$	$\arcsin\sqrt{I}$, $\log(i\,pot^{-1}+1)$
	Entopper	$p < 0.001$	I , $\log(i\,pot^{-1}+1)$
	Primo	$p < 0.001$	$\arcsin\sqrt{I}$, $\log(i\,pot^{-1}+1)$
Phleum pratense	S48	$p < 0.001$	I , $\log(i\,pot^{-1}+1)$
	Comet	$p < 0.001$	$\arcsin\sqrt{I}$, $\log(i\,pot^{-1}+1)$
Phleum bertolonii	S50	$p < 0.001$	I , $\log(i\,pot^{-1}+1)$

Table 3 L_{50} and values at 25 i pot^{-1} with respect to I and shoot yield.

		L_{50} for I (i pot^{-1})		I at 25 i pot^{-1}		L_{50} for shoot yield (i pot^{-1})		DW at 25 i pot^{-1} as a % of control		Mean Rank
		Value	Rank	Value	Rank	Value	Rank	Value	Rank	
Lolium perenne	S23	11.5	11	0.78	16	2.5	10=	1.09	8	10
	Manhattan	>25.0	1=	0.31	1	>25.0	1=	79.80	1	1
	Melle	>25.0	1=	0.35	4	>25.0	1=	75.62	2	2
	Sportiva	22.0	6	0.52	6	5.0	9	9.22	7	8
Festuca rubra ssp *commutata*	Highlight	11.0	12=	0.77	15	2.0	12=	0.00	12=	15
	Cascade	11.0	12=	0.73	13	1.0	15=	0.01	10=	11
	Wintergreen	7.0	16	0.72	11=	1.5	14	0.00	12=	16
Agrostis tenuis	Highland	13.0	9	0.55	7	10.0	5	14.40	6	7
	Holfior	12.0	10	0.72	11=	2.5	10=	0.00	12=	12
Holcus lanatus		9.5	14	0.65	10	2.0	12=	0.01	10=	13=
Poa pratensis	Parade	17.5	8	0.60	9	6.0	6	0.00	12=	9
	Entopper	>25.0	1=	0.32	2	16.5	3	32.39	5	3
	Primo	>25.0	1=	0.34	3	5.5	7=	38.88	4	4
Phleum pratense	S48	8.0	15	0.76	14	1.0	15=	0.00	12=	13=
	Comet	20.5	7	0.56	8	11.5	4	45.18	3	5=
Phleum bertolonii	S50	>25.0	1=	0.46	5	5.5	7=	1.03	9	5=

pratensis Parade and Phleum pratense Comet had values between 15 and 25 indicating fairly good wear tolerance. The remainder had values between 7 and 13 showing relatively poor wear tolerance. Values of I at 25 i pot^{-1} showed some similarities in groupings. Lolium perenne Manhattan and Melle and also Poa pratensis Entopper and Primo had lowest values of 0.30 to 0.35. The range 0.40 to 0.60 included Lolium perenne Sportiva, Agrostis tenuis Highland, Poa pratensis Parade, Phleum pratense Comet and Phleum bertolonii S50. All the rest had values for I at 25 i pot^{-1} of between 0.65 and 0.80.

b) Response of shoot yield to intensity of simulated trampling and the relationship with injury level.

A reduction in shoot yield (third weekly cut after treatment) in response to increasing intensity of simulated trampling is shown in Figs. 18 to 33 for all species and cultivars. Table 4 gives the levels of significance for each cultivar from analysis of variance tests. All were significantly reduced except Lolium perenne Manhattan. The majority of cultivars were highly significantly reduced ($p<0.001$) with the exception of Lolium perenne Melle, Festuca rubra ssp. commutata Cascade and Wintergreen ($p<0.01$) and Phleum pratense Comet ($p<0.05$).

There were significant negative correlations of shoot yield with i pot^{-1} for all species and cultivars except Lolium perenne Manhattan as shown in Table 4. For all cultivars except Lolium perenne Manhattan and Melle and Agrostis tenuis Highland, greater correlation was obtained when shoot yield was plotted with log(i pot^{-1}+1).

There was considerable variation in L_{50} (Table 3). Lolium perenne Manhattan and Melle had values greater than 25 indicating a very high durability. Values between 10 and 17 for Agrostis tenuis Highland, Poa pratensis Entopper and Phleum pratense Comet suggest a fairly good durability. A further grouping around 5 for Lolium

Shoot yield 3 weeks after simulated trampling at different intensities:

Fig. 18 *Lolium perenne* S23

Fig. 19 *Lolium perenne* Manhattan

Fig. 20 *Lolium perenne* Melle

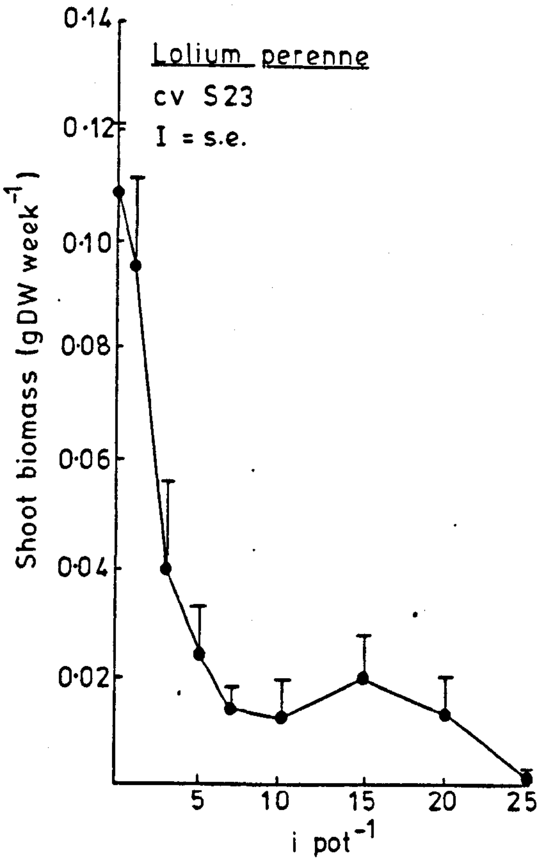


Fig. 18

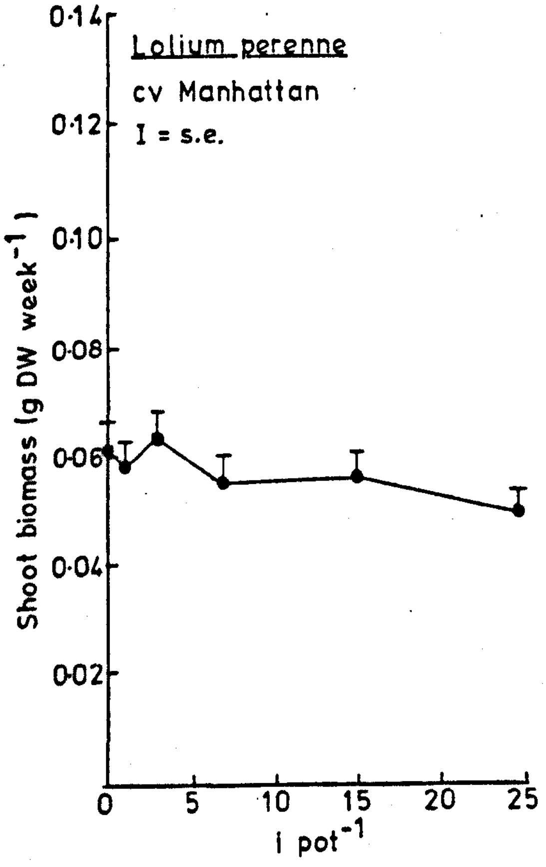


Fig. 19

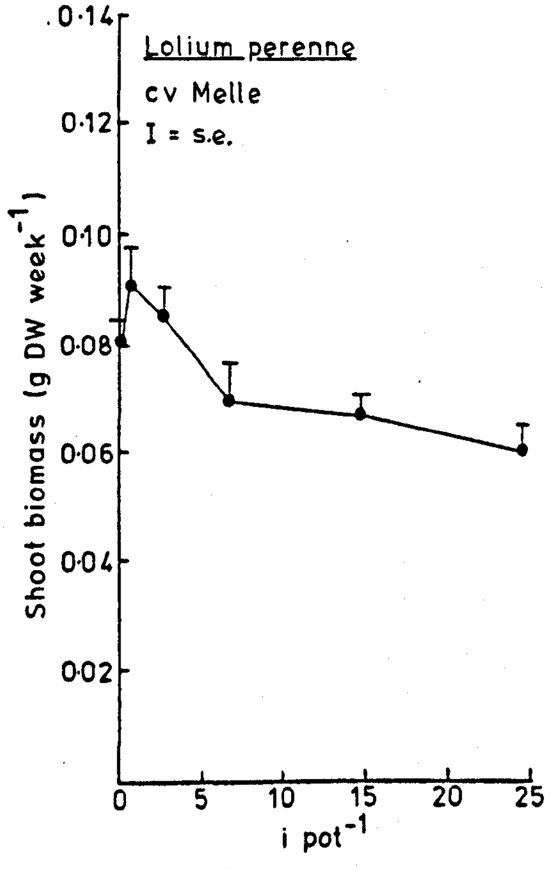


Fig. 20

Shoot yield 3 weeks after simulated trampling at different intensities:

Fig. 21 *Lolium perenne* Sportiva

Fig. 22 *Festuca rubra* ssp. *commutata* Highlight

Fig. 23 *Festuca rubra* ssp. *commutata* Cascade

Fig. 24 *Festuca rubra* ssp. *commutata* Wintergreen

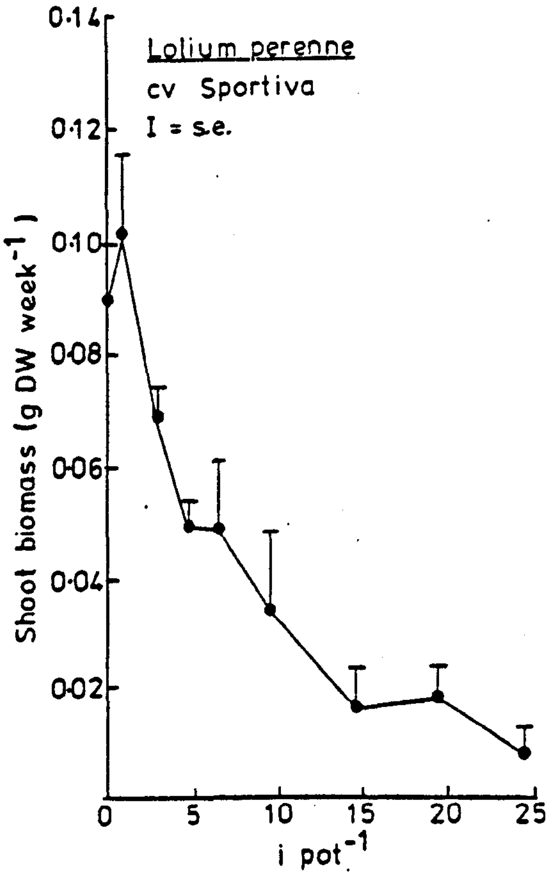


Fig. 21

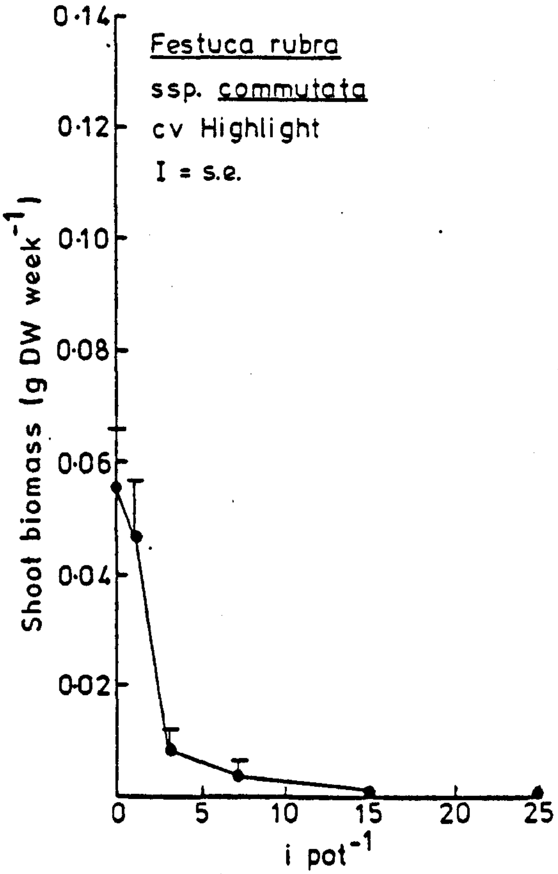


Fig. 22

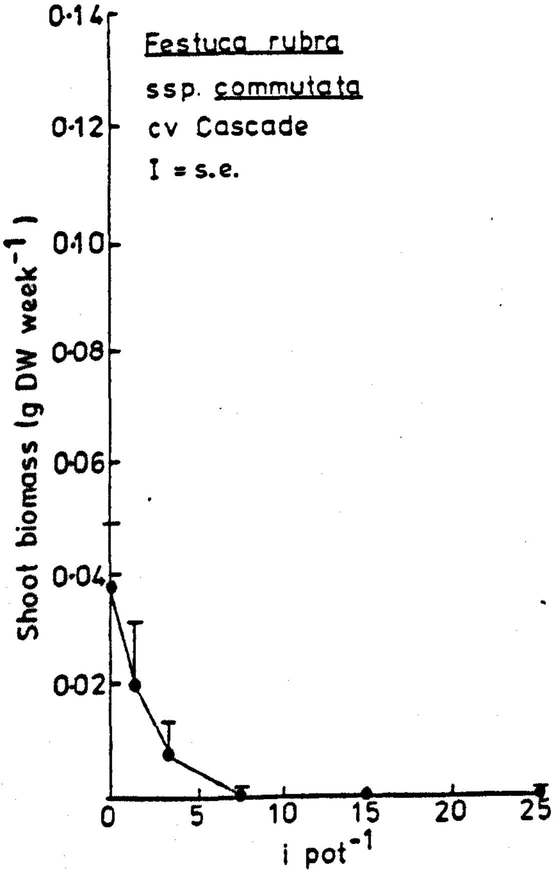


Fig. 23

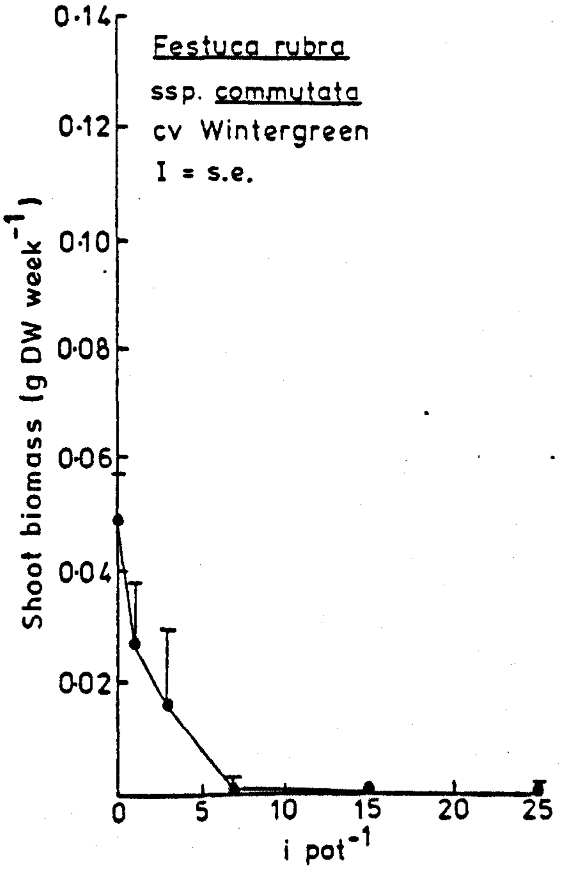


Fig. 24

Shoot yield 3 weeks after simulated trampling at different intensities:

Fig. 25 *Agrostis tenuis* Highland
Fig. 26 *Agrostis tenuis* Holfior
Fig. 27 *Holcus lanatus*
Fig. 28 *Poa pratensis* Parade

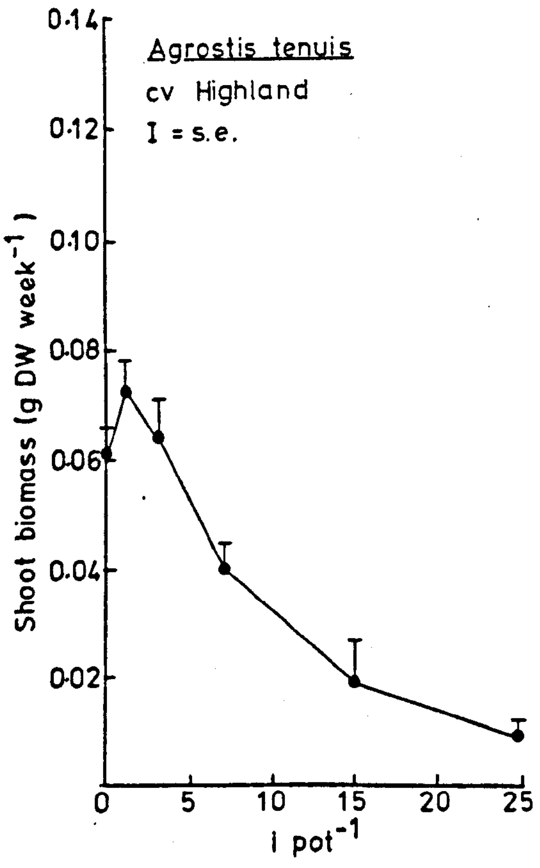


Fig. 25

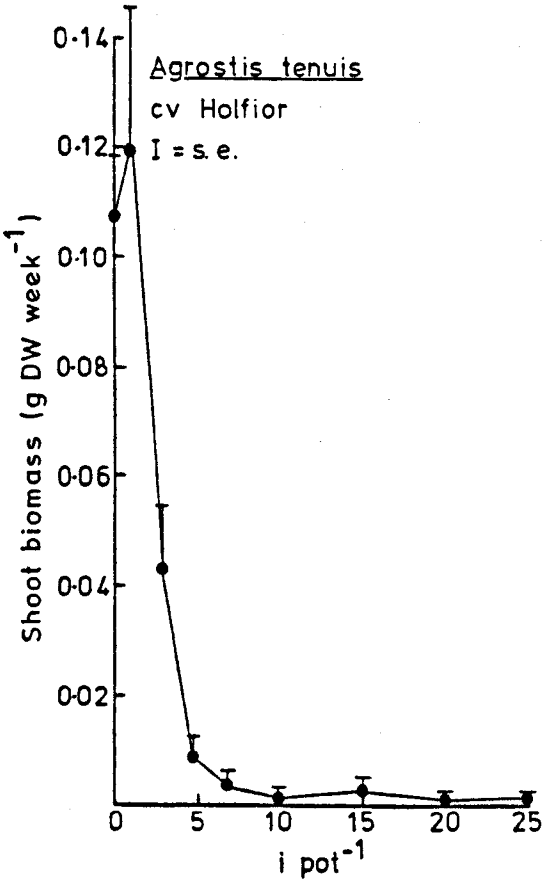


Fig. 26

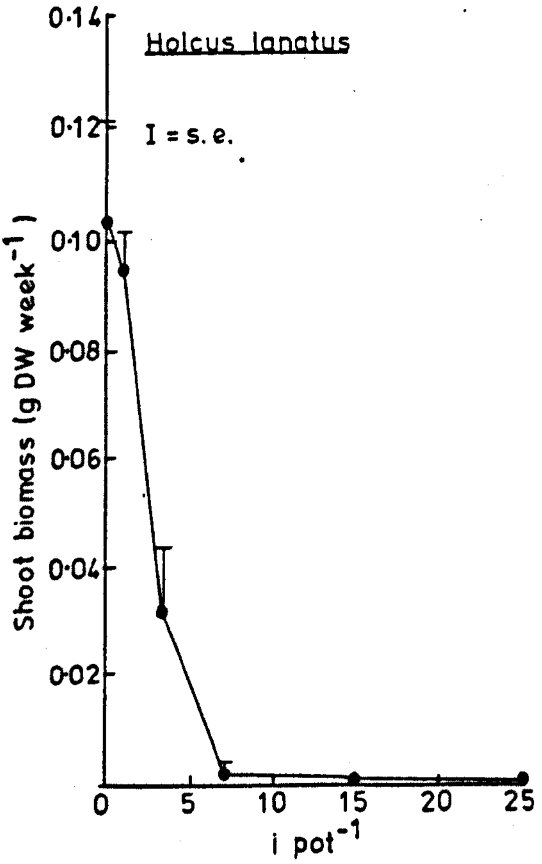


Fig. 27

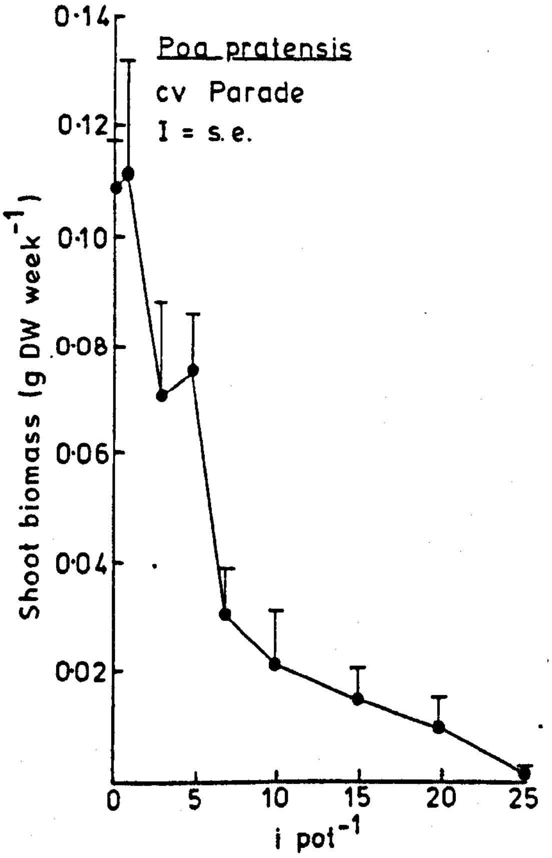


Fig. 28

Shoot yield 3 weeks after simulated trampling at different intensities:

Fig. 29 *Poa pratensis* Entopper
Fig. 30 *Poa pratensis* Primo
Fig. 31 *Phleum pratense* S48
Fig. 32 *Phleum pratense* Comet

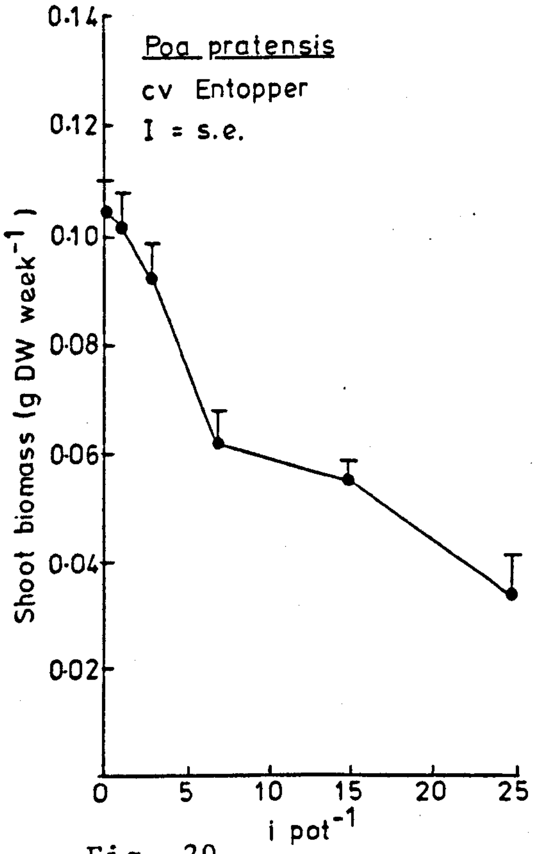


Fig. 29

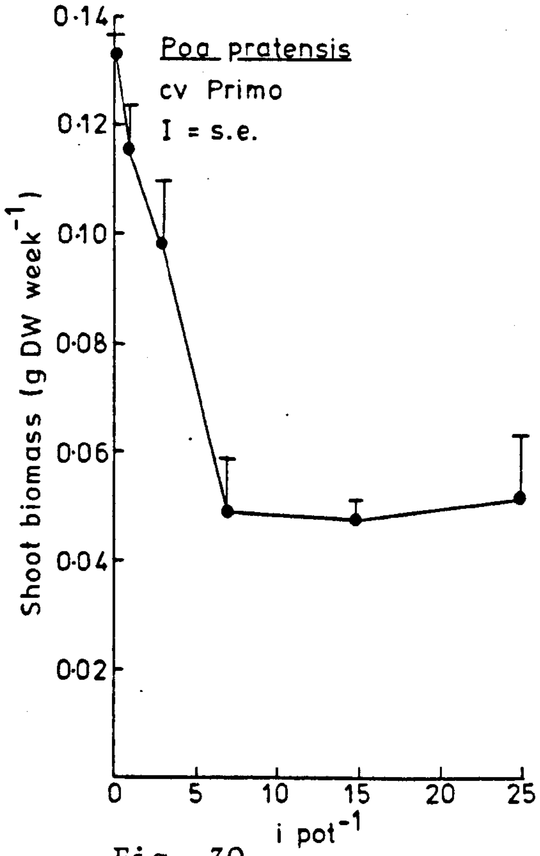


Fig. 30

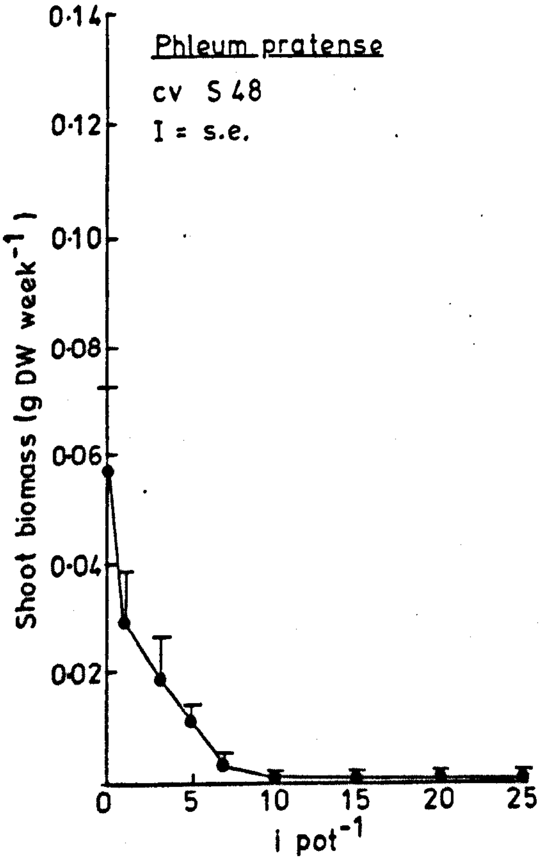


Fig. 31

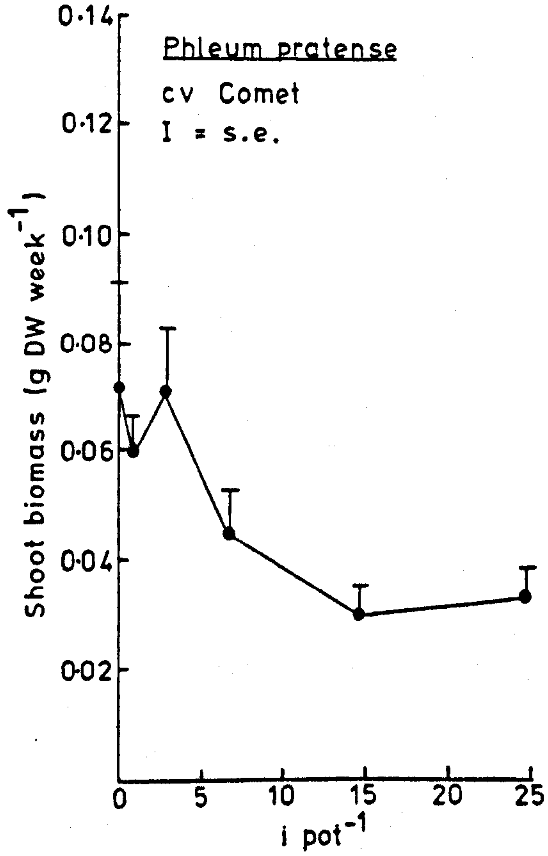


Fig. 32

Fig. 33 Shoot yield 3 weeks after simulated trampling at different intensities: *Phleum bertolonii* S50

Shoot biomass fractions after simulated trampling at different intensities:

Fig. 34 *Lolium perenne* S23

Fig. 35 *Lolium perenne* Manhattan

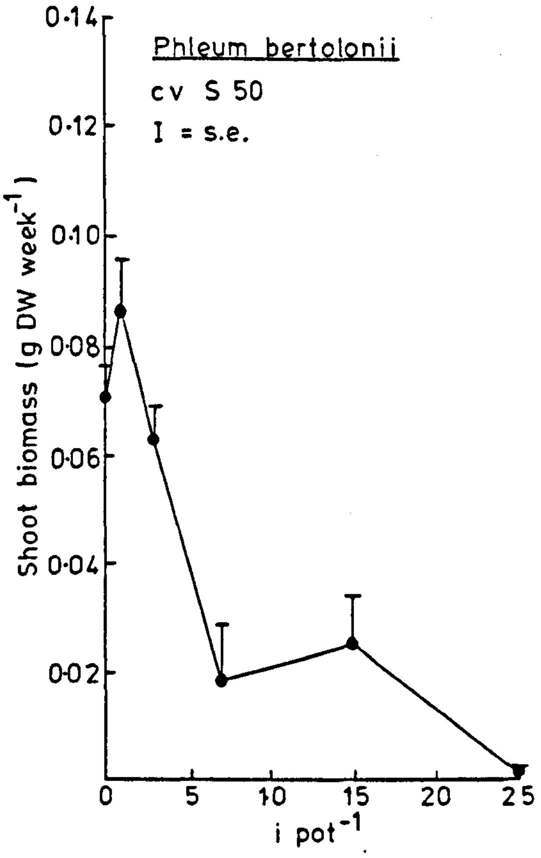


Fig. 33

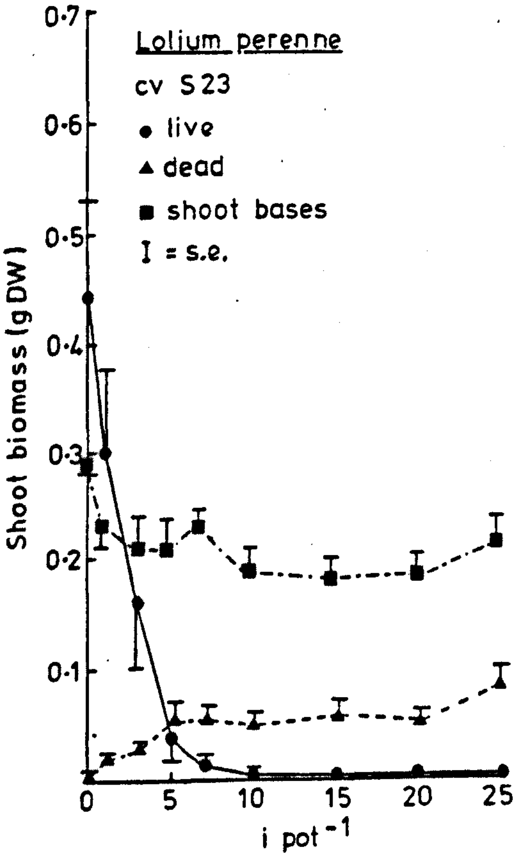


Fig. 34

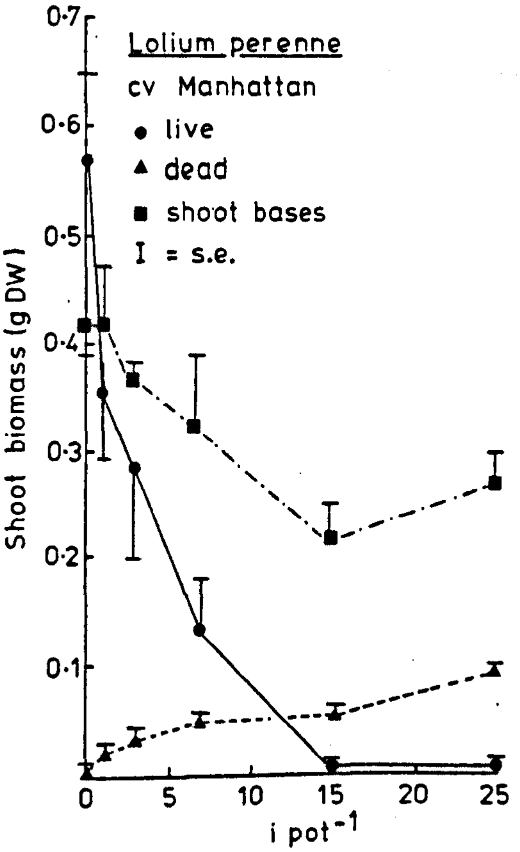


Fig. 35

Table 4 Correlation between shoot yield and i pot^{-1}.

Species/cultivar		Significance of Analysis of Variance	Significance of correlation of shoot yield with i pot^{-1}	Transformation improving the correlation of shoot yield with i pot^{-1}
Lolium perenne	S23	$p < 0.001$	$p < 0.001$	log(i pot^{-1}+1)
	Manhattan	n.s.	n.s.	-
	Melle	$p < 0.01$	$p < 0.001$	-
	Sportiva	$p < 0.001$	$p < 0.001$	log(i pot^{-1}+1)
Festuca rubra ssp.				
commutata	Highlight	$p < 0.001$	$p < 0.001$	log(i pot^{-1}+1)
	Cascade	$p < 0.01$	$p < 0.01$	log(i pot^{-1}+1)
	Wintergreen	$p < 0.01$	$p < 0.01$	log(i pot^{-1}+1)
Agrostis tenuis	Highland	$p < 0.001$	$p < 0.001$	-
	Holfior	$p < 0.001$	$p < 0.001$	log(i pot^{-1}+1)
Holcus lanatus		$p < 0.001$	$p < 0.001$	log(i pot^{-1}+1)
Poa pratensis	Parade	$p < 0.001$	$p < 0.001$	log(i pot^{-1}+1)
	Entopper	$p < 0.001$	$p < 0.001$	log(i pot^{-1}+1)
	Primo	$p < 0.001$	$p < 0.001$	log(i pot^{-1}+1)
Phleum pratense	S48	$p < 0.001$	$p < 0.001$	log(i pot^{-1}+1)
	Comet	$p < 0.05$	$p < 0.01$	log(i pot^{-1}+1)
Phleum bertolonii	S50	$p < 0.001$	$p < 0.001$	log(i pot^{-1}+1)

perenne Sportiva, Poa pratensis Parade and Primo, Phleum bertolonii S50 suggests some durability. The remainder had values of around 1 to 2 indicating poor durability.

The values for relative shoot yield at 25 i pot^{-1} (Table 3) grouped cultivars similar to results of L_{50} with values between 75 and 80% for Lolium perenne Manhattan and Melle, 30-45% for Poa pratensis Entopper and Primo and Phleum pratense Comet, 9-15% for Lolium perenne Sportiva and Agrostis tenuis Highland and 0-2% for all the remaining cultivars.

The relationship between shoot yield and I is summarised in Table 5. There was a highly significant correlation ($p<0.001$) between shoot yield reduction and I for all species and cultivars except Lolium perenne Manhattan ($p<0.05$) and Phleum pratense Comet ($p<0.01$). The correlation was further improved for some cultivars by plotting shoot yield with log (I) (or in one case arc sin $\sqrt{I}$, and another case log (arc sin $\sqrt{I}$)). The linear relationship between shoot yield and I was notably the best correlation for Lolium perenne Manhattan, Melle, Sportiva, Agrostis tenuis Highland, Poa pratensis Entopper and Phleum bertolonii S50 which had indicated wear tolerance and durability in the results discussed above.

c) Effect of simulated trampling on the proportion of dead and live shoot biomass.

Results are given in Figs 34 to 37. Live shoot dry matter decreased with increasing impacts and dead shoot dry matter increased with increasing impacts. Shoot base dry weight was reduced with increasing impacts.

Analysis of variance showed highly significant ($p<0.001$) reductions in live shoot matter with increasing intensity of simulated trampling for all species and cultivars tested. For Lolium perenne S23 and Phleum pratense Comet, the decline in live dry matter

Table 5 Correlation between shoot yield and I.

Species/cultivar	Significance of correlation of shoot yield with I	Transformations improving the correlation of shoot yield with I
Lolium perenne S23	$p < 0.001$	log (I)
Manhattan	$p < 0.05$	-
Melle	$p < 0.001$	-
Sportiva	$p < 0.001$	-
Festuca rubra ssp.		
commutata Highlight	$p < 0.001$	log (I)
Cascade	$p < 0.001$	log (I)
Wintergreen	$p < 0.001$	log (I)
Agrostis tenuis Highland	$p < 0.001$	-
Holfior	$p < 0.001$	log (I)
Holcus lanatus	$p < 0.001$	log (I)
Poa pratensis Parade	$p < 0.001$	$\arcsin \sqrt{I}$
Entopper	$p < 0.001$	-
Primo	$p < 0.001$	log (I)
Phleum pratense S48	$p < 0.001$	log (I)
Comet	$p < 0.01$	$\log(\arcsin \sqrt{I})$
Phleum bertolonii S50	$p < 0.001$	-

Shoot biomass fractions after simulated trampling at different intensities:

Fig. 36 *Poa pratensis* Entopper
Fig. 37 *Phleum pratense* Comet

Fig. 38 Response of *Lolium perenne* S23 to soil compaction only, shoot injury only and complete simulated trampling.

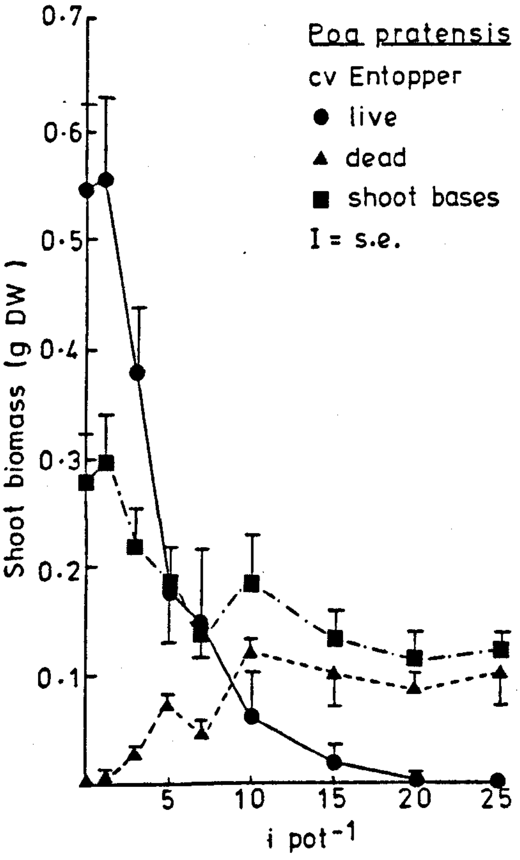


Fig. 36

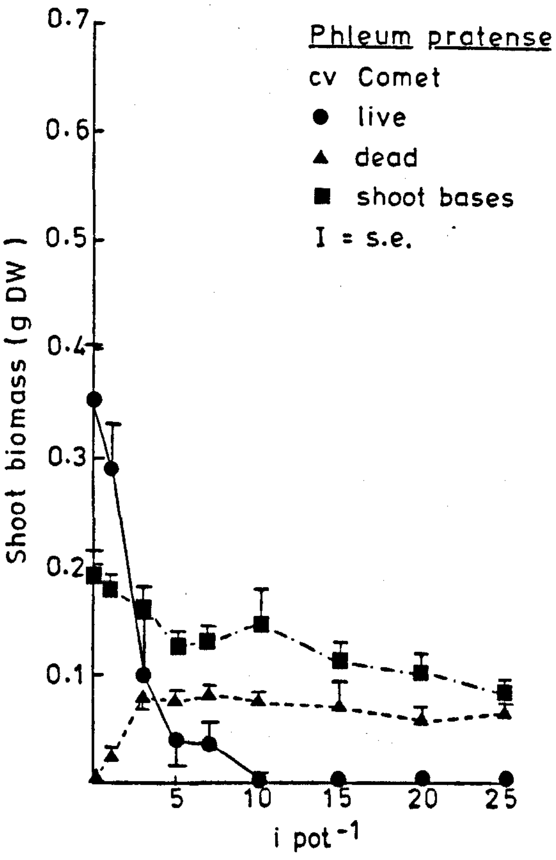


Fig. 37

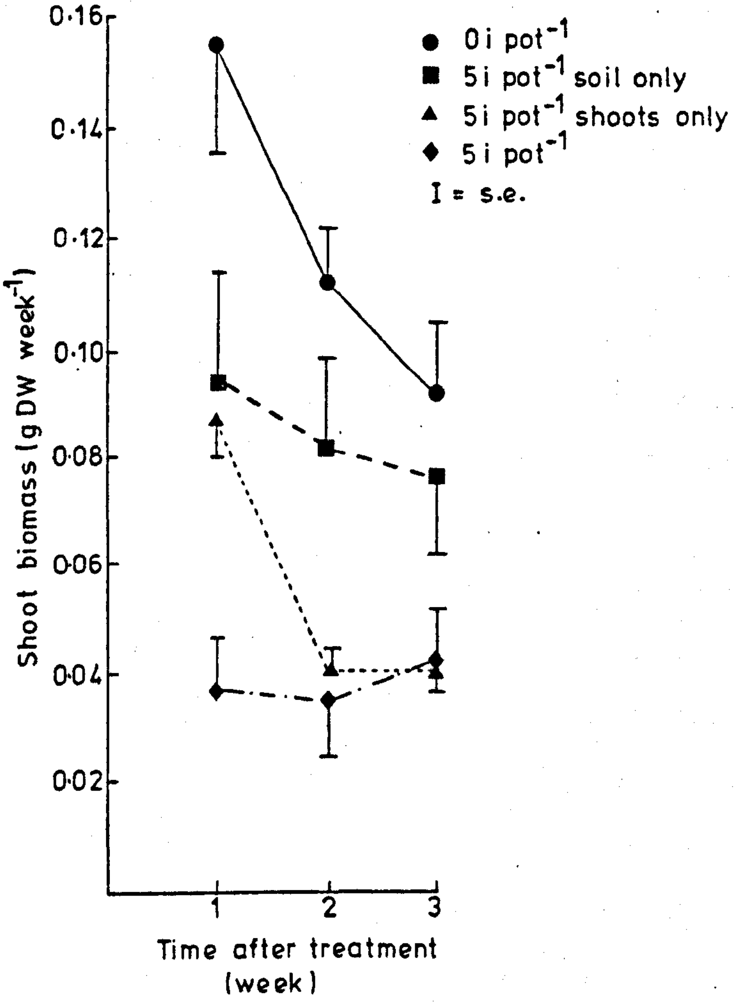


Fig. 38

with increasing simulated trampling was greater than for Lolium perenne Manhattan and Poa pratensis Entopper.

There were highly significant ($p<0.001$) increases in dead shoot matter with increasing intensity of simulated trampling for Lolium perenne S23 and Manhattan and Poa pratensis Entopper. Phleum pratense Comet, however, was non-significant. For Lolium perenne S23 and Manhattan, the increase in dead shoot dry matter with increasing impacts was more gradual than for Poa pratensis Entopper and Phleum pratense Comet which reached a plateau at 10 and 3 i pot^{-1}, respectively.

There were significant decreases in shoot base dry weight with increasing number of impacts for Lolium perenne Manhattan ($p<0.05$), Poa pratensis Entopper ($p<0.01$) and Phleum pratense Comet ($p<0.01$) but not for Lolium perenne S23. Comparing results of the species, there was a significant difference ($p<0.001$) between species for both live shoot biomass and for shoot base biomass but no significant difference between species for dead shoot biomass.

d) Shoot yield response of grass to soil compaction or shoot injury.

Results are shown in Fig. 38. There was a significant reduction ($p<0.01$) in shoot yield with simulated trampling (for the 3rd weekly cut after treatment) but there was no significant difference between 5 i pot^{-1}, 5 i pot^{-1} shoots only and 5 i pot^{-1} soil compaction only.

Discussion

Results in section b) showed a reduction in shoot yield with increasing intensity of simulated trampling. Similar results have been well recorded in the literature. Bayfield (1971a) harvested pot grown Phleum bertolonii S50, 3 weeks after simulated trampling of 0, 1, 5, 10, 20, 40 or 100 impacts. Shoot dry weight was depressed for treatments greater than 10 impacts. Smith (1978) similarly recorded shoot dry weight 3 weeks after applying 0, 2, 5, 10 or 15 i pot^{-1} to Phleum bertolonii and Plantago lanceolata and found 2 i pot^{-1} significantly decreased shoot dry weight. Significant decreases were obtained usually with 3 i pot^{-1} or more in the present study.

Field simulated trampling trials (e.g. Wagar 1964, Kellomaki 1973, Blom 1979) and field trampling trials (e.g. Kellomaki 1973, Bayfield 1971a, Smith 1978) have also demonstrated reductions in shoot biomass and/or ground cover with trampling. Observations in analytical studies of amenity grasslands have also recorded reduction in biomass and/or ground cover with trampling (e.g. Bates 1935, Edmond 1964, Bayfield 1971a, 1973, Leney 1974, Liddle & Greig-Smith 1975b, Liddle 1975a).

Variations in degree of reduction according to species are evident (e.g. Bayfield 1971a, Leney 1974, Liddle & Greig-Smith 1975a, Smith 1978). Early observations such as those of Bates (1935) have established a differential response of species resulting in "typical" footpath communities. Bates carried out experimental investigations, trampling pure stands of various grass species and thus identified their relative tolerance to trampling. Some species such as Anthoxanthum odoratum, Alopecurus pratensis and Agrostis tenuis were exterminated by daily treading whereas Poa pratensis, Lolium perenne and Dactylis glomerata persisted. These results agreed with his studies of footpaths where dominant species were Poa pratensis, Lolium perenne and Trifolium repens.

Most relevant to the present study are the comparisons of turf grass species and cultivars such as Youngner (1961, 1962), Shearman and Beard (1975a) and Canaway (1976b, 1978, 1981a) who all recorded reductions in ground cover with artificial wear.

In the present study the index of injury (section a)) and the shoot yield (section b)) related directly to the intensity of simulated trampling but with a variety of responses between the species and cultivars. The relationship between injury and number of impacts was curvilinear for most cultivars. Shoot yield and number of impacts usually had a pronounced curvilinear relationship, hence most reduction in shoot biomass was from the first 5 to 10 impacts. Injury index and shoot yield correlated significantly with a curvilinear relationship and hence, most reduction of shoot biomass was as a result of the injury from the first 5 to 10 impacts, although this was not always the case (see Table 5). Similar curvilinear responses were obtained by Bayfield (1971a) and Smith (1978).

Table 3 gives the values for each species and cultivar for four categories based on injury index and shoot yield. The rank in order of greater wear tolerance to least wear tolerance using the values in each category is also given.

Response based on L_{50} for I gave a measurement of how susceptible the leaf tissue of the particular species and cultivar was to simulated trampling which may indicate the level of tolerance of trampling.

Response based on I at 25 i pot^{-1} gave a measurement of tolerance of very heavy wear and indicated whether a cultivar had reached damage saturation point (at which further wear contributes little further injury). The more curvilinear the response, the more this effect was evident (eg. Fig. 8, 9, 11, 17).

Response based on L_{50} for shoot yield gave an overall measurement of how each cultivar performed with simulated trampling. A curvilinear response indicated large reductions at lower levels of wear and thus the more sensitive cultivars (see Figs. 22, 23, 24, 26, 27, 31).

Response based on shoot yield at 25 i pot^{-1} (as a proportion of that at 0 i pot^{-1}) indicated which cultivars were able to survive heavy wear.

Differences in rank between the categories for a particular cultivar indicate the individual component contribution dictating the response, namely, susceptibility to injury, saturation injury levels, overall shoot growth response (thus indicating how other plant functions influence the durability of a cultivar) and survival of heavy wear.

For most cultivars, rankings were similar showing a relationship between all of these factors. Averaging the rankings of the four categories (Table 3) from highest durability to lowest durability were *Lolium perenne* Manhattan, *Lolium perenne* Melle, *Poa pratensis* Entopper, *Poa pratensis* Primo, *Phleum pratense* Comet, *Phleum bertolonii* S50, *Agrostis tenuis* Highland, *Lolium perenne* Sportiva, *Poa pratensis* Parade, *Lolium perenne* S23, *Festuca rubra* ssp. *commutata* Cascade, *Agrostis tenuis* Holfior, *Phleum pratense* S48, *Holcus lanatus*, *Festuca rubra* ssp. *commutata* Highlight and *Festuca rubra* ssp. *commutata* Wintergreen.

For some cultivars, however, there were obvious differences between ranks for the four categories suggesting a differential influence of the multiple factors contributing to the overall durability of a cultivar. Cultivars with obvious differences were *Lolium perenne* S23, *Agrostis tenuis* Highland and *Phleum pratense* Comet which had higher rankings based on shoot yield than those based on injury index. This suggested they had greater durability than other cultivars with similar amounts of injury, presumably because of a more efficient repair and/or regrowth mechanism. *Lolium perenne* Sportiva and *Phleum bertolonii* S50 had higher rankings based on injury index than on shoot yield suggesting they had less durability than other cultivars with similar amounts of injury because of a less effective repair and/or regrowth mechanism.

These results were compared to other species/cultivar trials in the literature, firstly considering injury index.

Generally, assuming the value of injury index to be an indication of wear tolerance, most species results were as expected from performance ratings of wear tolerance by STRI (STRI 1980, 1982) and also from the species characteristics noted in Chapter 2 (pages 21-27). The most wear tolerant species were Lolium perenne and Poa pratensis which generally had high L_{50}/low I at 25 i pot^{-1} in the present study. The least wear tolerant species were Festuca rubra ssp. commutata, Holcus lanatus and Agrostis tenuis in performance trials. These had lower values of L_{50}/higher I at 25 i pot^{-1} in this investigation. Both Phleum pratense and Phleum bertolonii are considered of secondary wear tolerance to Lolium perenne and Poa pratensis so the high L_{50}/low I at 25 i pot^{-1} of Phleum bertolonii S50 and Phleum pratense Comet were expected, especially as Phleum bertolonii S50 is increasingly being sown to add greater durability to fine sports turf.

Lolium perenne S23, however, showed a poor performance in comparison with not only the other Lolium perenne cultivars, but also with other species in this study (based on values of injury index). Although Canaway (1981a) commented that turf type cultivars of Lolium perenne are generally superior to the traditional agricultural cultivars such as S23, the ratings of S23 in STRI trials (STRI 1980) were of good wear tolerance (greater than for Sportiva) and it was expected to be so in this study. Apart from S23, all the other Lolium perenne cultivars performed in accordance with STRI trials with Manhattan superior to Melle which was superior to Sportiva. Poa pratensis Parade had a poorer wear tolerance, based on values of I, than suggested by STRI ratings (STRI 1980) where it is given the highest rating of all Poa pratensis cultivars. Conversely Agrostis tenuis Highland had a higher tolerance rating, based on I, than had been expected, although it was noted (Beard 1973) as more tolerant of wear than other Agrostis tenuis cultivars.

Specific wear tolerance field studies by Shearman and Beard (1975a) and Canaway (1981a) both used artificial wear and measured wear tolerance using verdure and ground cover, respectively. Relevant cultivars used by Shearman and Beard (1975a) were Lolium perenne Manhattan and Festuca rubra ssp. commutata Cascade which were ranked highest and second lowest respectively for wear tolerance. This was confirmed in the present study using measurement of injury. Canaway (1981a) concluded that in order of greatest wear tolerance to least were Lolium perenne S23, Poa pratensis Baron, Phleum pratense S48, Agrostis tenuis Highland, Festuca rubra ssp. commutata Highlight and Festuca rubra ssp. rubra Boreal. Rankings of the relevant species in the current study using L_{50} from greatest to least were Agrostis tenuis Highland, Lolium perenne S23, Festuca rubra ssp. commutata Highlight and Phleum pratense S48. Using I at 25 i pot^{-1} rankings were Agrostis tenuis Highland, Phleum pratense S48, Festuca rubra ssp. commutata Highlight and Lolium perenne S23.

Both are very different to the rankings of Canaway (1981a) based on ground cover. The discrepancies indicate that factors other than just the amount of tissue injury affect the general wear tolerance of a particular grass cultivar.

The importance of ability to withstand wear and receive minimal cellular damage, however is shown by the general agreement of injury index ranking and wear tolerance ranking in the literature. There are anatomical variations between the different grass species and even between cultivars within a species (Beard 1973). Esau (1965) noted that mechanical cells, often found adjacent to the epidermis in graminaceous plants, enable the leaves to withstand various strains resulting from stretching, bending, weight and pressure without undue damage to the thin-walled softer cells. Differences in extent and position of mechanical cells will therefore influence the degree of damage when a plant is trampled. Time did not permit anatomical investigations in the present study, however.

Anatomical studies by Shearman and Beard (1975c) suggested that the percent sclerenchyma fibres and lignified cells were closely associated with wear tolerance of the species they investigated and also the combined effects of leaf tensile strength and leaf width. Edmond (1964) considered that the toughness of a pasture species may be a factor determining tolerance of animal treading. Evans (1967a,b) investigated the tensile strength of various pasture species. Cellulose was considered to be the important component in conferring leaf tensile strength. The possibility that other cell wall substances may have some influence was also mentioned.

Shearman and Beard (1975b) and Canaway (1981a) related amount of various cell wall constituents and fibre contents of certain species of turfgrass to the observed wear tolerance (Shearman and Beard 1975a, Canaway 1981a, respectively). Rankings according to cell wall component quantities by Shearman and Beard (1975b) were *Festuca arundinacea* Kentucky 31 > *Lolium perenne* Manhattan = *Poa pratensis* Merion > *Lolium perenne* Pennlawn = *Lolium multiflorum* > *Festuca rubra* ssp. *commutata* Cascade > *Poa trivialis*. On a weight to area basis, there was correlation with wear tolerance (Shearman and Beard 1975b, Canaway 1978, 1981a) but on a weight to biomass basis, both Shearman and Beard (1975b) and Canaway (1981a) found no correlation. Canaway (1981a) suggested that the dominance of biomass may be the reason for fibre per unit area being positively correlated with wear tolerance and that this may also apply to Shearman and Beard's (1975b) findings. Canaway (1981a) also commented that reported fluctuations in fibre and cellulose content with season, regrowth age, leaf water content (Evans 1967a,b, Shearman and Beard 1975b) may have influenced results. He concluded that fibre content may not be very useful for predicting wear tolerance.

Despite the inconclusive findings relating cell wall constituents and quantity of fibre with wear tolerance, such factors may have some influence on the wear tolerance as variation in injury index was related to wear tolerance in the present study. Anatomical differences would appear to have some influence on this (Esau 1965, Shearman and Beard 1975c) but further work is necessary to clarify the importance of cell wall constituents and fibre contents. It is also apparent that other factors influence the wear tolerance as seen from discrepancies between ratings of injury index and wear tolerance ratings by STRI based on ground cover and biomass. Ground cover and biomass have been the chief measurements used to quantify wear tolerance, recuperative potential and durability because plant growth and survival is dictated by a combination of all the plant processes and the interaction of these with environmental factors.

In the present study, shoot yield was also used to measure plant response to trampling. The order of durability based on L_{50} for shoot yield and on shoot yield at 25 i pot^{-1} (as a proportion of that at 0 i pot^{-1}) were compared to species ratings of Canaway (1981a) based mainly on ground cover. Ranking in order of greatest to least was *Lolium perenne* (turf type), *Lolium perenne* (pasture type), *Poa pratensis*, *Phleum pratense*, *Phleum bertolonii*, *Festuca rubra* ssp. *commutata* and *Agrostis tenuis* (from Canaway 1981a).

There are some discrepancies comparing rankings of Canaway with the present study lists (Table 3) but there were generally good agreements, *Lolium perenne*, *Poa pratensis* and *Phleum* ssp. dominating the top ratings of both. Turf type Lolium perenne Manhattan was of highest wear tolerance in Table 3, by far exceeding other species and cultivars. In fact the shoot yield of Manhattan was not significantly reduced with increasing number of impacts although a slight decline was observed (Fig. 19). In STRI ratings (STRI 1980), Lolium perenne Manhattan was of superior wear tolerance to all other Lolium perenne cultivars. Lolium perenne Melle was amongst the higher ratings in STRI trials (STRI 1980) and in this study ranked second (based on shoot yield). Melle is used both as a turf type and also for pasture (STRI 1980, NIAB 1985) due to its high wear tolerance.

In Table 3, Phleum pratense Comet ranked first below Lolium perenne Melle, along with Poa pratensis cultivars. This is in agreement with Canaway (1981a) with the exception of Phleum pratense S48 which in Table 3 ranks the lowest of all. S48 ranked third behind Lolium perenne and Poa pratensis in Canaway's investigations. Wear tolerance of Agrostis tenuis Holfior and Festuca rubra ssp. commutata cultivars were low both in the present study and the rankings of Canaway (1981a). Holcus lanatus showed poor tolerance as had been expected and reported by Watts (1978). Phleum bertolonii S50 ranked fairly high in both this study and Canaway's (1981a). Both Lolium perenne Sportiva and S23 were of lower rating in the present study than for Canaway's rankings. This is the same situation as for injury index rating (Table 3). An unexpectedly high ranking was for Agrostis tenuis Highland in this investigation as it was considered to be of similarly low wear tolerance as Festuca rubra ssp. commutata (Canaway 1981a). Some explanation is possible of these anomalies, however.

Canaway (1981a) also individually assessed some cultivars for wear tolerance based on shoot biomass. It is interesting that a rather different ranking was obtained: *Poa annua*, *Agrostis tenuis* Highland, *Poa pratensis* Baron, *Festuca rubra* ssp. *commutata* Highlight, *Festuca rubra* ssp. *rubra* Boreal, *Phleum pratense* S48 and *Lolium perenne* S23 (from greatest to least). Canaway concluded that although biomass after wear correlated with ground cover, individual species may not follow the trend, an obvious example being *Lolium perenne* S23 which was of consistently high wear tolerance but of low biomass. The same would seem to apply to *Phleum pratense* S48. *Agrostis tenuis* Highland was conversely of high biomass yet low wear tolerance. This difference in rank, based on ground cover compared to biomass was further confirmed by Canaway (1983).

It therefore seems that there are anomalies in response for some species and cultivars when shoot biomass is used, compared to wear tolerance based on ground cover. These anomalies were found in both the present study and that of Canaway's (1981a,1983) and this would appear to explain their unexpected rankings in this investigation compared to performance ratings of STRI (STRI 1980, 1982). It is also interesting that for these anomalous cultivars, the ratings based on shoot yield reflect those for injury index. Canaway (1981a) commented that there is still very limited understanding of the properties of different grass species which give rise to differences in wear tolerance. He suggested that there existed extremely complex and subtle physiological factors related to the plant response to damage and to the ability to store and mobilize reserves for repair and new growth. It might therefore appear that injury index was surprisingly consistent with shoot yield response for most species and cultivars and that shoot yield response was remarkably consistent with ratings from ground cover measurements. Indeed, Leney (1974) compared monoculture greenhouse grown plants treated with simulated trampling and found that species which performed best in these conditions did not necessarily survive well under trampling in the field. Low yield in some situations may be a selective advantage (Bradshaw et al. 1964).

Injury index gave a measurement that represented the immediate injury to shoots only, thus ignoring subsequent shoot response and effects of soil compaction. There are varied opinions about the contribution that the soil compaction component of trampling makes in reducing biomass ground cover. Gupta (1933) found that shoot dry weight was not significantly affected by compaction. Bates (1935) deemed it to be less than the effects of shoot injury. Leney (1974) found that shoot damage tended to reduce the dry weight of plants more than soil compaction but results varied with species and increase in biomass was recorded for Ammophila and Calluna. Smith (1978) found decreases in shoot biomass with soil compaction. Carrow (1980) recorded reduced percent turf cover in three turfgrass species in response to soil compaction and he concluded that Lolium perenne and Poa pratensis had greater tolerance than Festuca arundinacea.

The investigations of section d) showed that in this particular study, for Lolium perenne S23 at least, both soil compaction only and shoot injury only significantly reduced shoot yield. Soil compaction has been linked with reduced shoot yield in the agricultural situation (e.g. Rosenberg 1964).

The reduced shoot biomass with soil compaction is thought to be mediated by greater energy requirements for roots to overcome the increased mechanical impedance as they undergo cell elongation (Barley 1962) hence stunting both root and shoot growth. Stunted roots may limit the ability of the plant to exploit soil reserves (Liddle & Greig-Smith 1975a, Goss 1977) and hence further affect shoot and root growth. The compaction may even cause direct injury to the roots and hence seriously impede uptake of water, vital for cell elongation (Hsaio 1973, Hanson & Hitz 1982) and minerals, essential for plant processes and growth. Hormonal effects of reduced root cytokinin affecting shoot photosynthesis (Wareing et al. 1968) have also been suggested (Smith 1978). Goss and Scott-Russell (1980) found various indications of a hormonal mechanism causing the observed transient and long term effects on root growth. Ethylene has been noted to temporarily increase when roots were impeded (Kays et al. 1974).

Evidently, there is great scope for further research on the physiological effects of soil compaction on roots and especially how this affects the shoots. It was however decided in the present study to concentrate on the effects of trampling (combined shoot injury and soil compaction effects) on shoot processes but to bear this other seemingly important aspect of plant trampling response in mind.

Having looked at shoot injury and then shoot yield effects, the influence of simulated trampling on the amount of dead and live shoot tissue was examined. In section c) of the present study, a limited number of cultivars were analysed for components of dead and live tissue 11 days after trampling. For each cultivar, an increase in dead material was recorded with increasing number of impacts while the live biomass was reduced and the shoot base biomass was reduced. As only 4 cultivars were analysed, however, comparisons were very restricted. There were significant differences between species for live shoot biomass and shoot base biomass but not for dead shoot biomass. Lolium perenne Manhattan performed best considering live shoot biomass, along with Poa pratensis Entopper. Phleum pratense Comet and Lolium perenne S23 showed a poorer response. For shoot base biomass, Lolium perenne Manhattan again performed best with Lolium perenne S23 a close second, then Poa pratensis Entopper and lastly Phleum pratense Comet. The wear tolerance order from shoot base data appears to closely follow that of ground cover estimates (Canaway 1981a, 1983). The different order when live biomass is used is similar to total biomass results with the anomalie of low shoot biomass/high wear tolerance for Lolium perenne S23.

Other comparative work is scant, especially for the laboratory situation. Canaway (1981a) sampled well established field swards and measured the biomass of component fractions. (These do not match the ones of the present study). Canaway found significant differences between species for canopy, litter and live shoot fractions but not for shoot bases, roots and total above ground biomass. Agrostis tenuis Highland and Poa annua had largest amounts of canopy material while Phleum pratense S48 and Lolium perenne S23 had the lowest. This is similar to total biomass rankings (see above). For the litter fraction, Poa pratensis Baron had significantly more. Relating these fractions to wear tolerance did not give significant correlations, even though shoot biomass was positively correlated with ground cover and therefore wear tolerance. More work would seem to be needed to clarify the effects of wear on the component fractions on a sward and the implications of size of the fractions.

Although further needs for research especially regarding soil compaction and component fractions of a sward after trampling, have been highlighted, the values of injury index and shoot yield have shown certain components of the response to wear.

Some anomalies such as the low biomass yet high wear tolerance of Lolium perenne S23 and high biomass yet low wear tolerance in Agrostis tenuis Highland indicate that caution is needed when making conclusions based on biomass, but injury index and shoot yield have proved, in the present study, to be representative of wear tolerance. Variation of response between cultivars and species in both injury levels and shoot yield response have been evident.

In comparing injury index and shoot yield in the laboratory situation with wear tolerance based on field trials, which were assessed predominantly from measurements of ground cover after wear, some differences would be expected. Leney (1974) found some discrepancies in response of laboratory compared to field grown swards subjected to simulated trampling. Different types of simulated wear would also be likely to cause some differences between results. In the present study, a falling tamp applying only a vertical force was compared to a wear machine (Canaway 1976a) applying both horizontal and vertical forces.

Comparing the response of 8 week old pots of grass with that of a well established field sward may have an influence. Canaway (1981a) noted variations in wear tolerance with age of the sward particularly with Poa pratensis and Phleum pratense.

Environmental conditions will modify the response with some species and cultivars performing better in response to laboratory conditions compared to performance in the field under natural conditions (Leney 1974).

Height of cut may also have had an influence on results of the present study. A certain height was required to standardize cutting treatment (at 3 cm). Different species are well known for their tolerance of low cutting heights while others do not persist very well at low cutting levels (STRI 1980). Youngner (1962) noted different wear tolerance according to cutting height, with shorter height reducing wear tolerance. Amount of shoot biomass was implicated as being of importance in conferring wear tolerance and more recent trials by Shildrick (1980), showing improved wear tolerance when high levels of nitrogen are applied to turf, further strengthens this argument. Brougham (1956) had previously found that clipping practices affected the growth and development of the grass plant. The cutting height of 3 cm may therefore have restricted the durability of some species requiring a higher cutting treatment (such as Phleum pratense) or even have favoured the durability of others (such as Agrostis tenuis).

In conclusion, injury index and shoot yield results gave good indications of wear tolerance and durability although a few anomalies suggested some caution is necessary when evaluating such results. Some differences in measurements representing some components of the response and obvious variation of these between species and even between cultivars, all suggest that varied anatomy, morphology and "extremely subtle physiological factors related to the plant response to damage and to the ability to store and mobilize reserves for repair and new growth" (Canaway 1981a) dictate the final durability of grass subjected to trampling. It was therefore evident that physiological functions needed investigation. Because shoot growth was related to wear tolerance and as growth depends on the balance of photosynthesis to respiration rates, the response of CO_2 flux to trampling seemed to be in particular need of further analysis.

CHAPTER 4

Photosynthesis and Respiration of Lolium perenne S23 in response to simulated trampling.

Introduction

Experiments described in the previous chapter showed a deleterious effect of simulated trampling on shoot yield which related to levels of injury sustained. As plant growth depends directly on the balance of photosynthesis to respiration, and as these processes are complex and sensitive to a variety of physiological and environmental factors, it was appropriate to investigate both the rate of photosynthesis and respiration in response to simulated trampling.

General methods and principles.

Gasometric methods are the most widely used methods for determining the gas exchange rates of plants as they are eminently suitable for estimating photosynthesis and respiration rates in many kinds of investigation, particularly where regular measurements or continuous recordings are required. Of the various gasometric methods, comprehensively described in detail by Sestak et al. (1971), the measurement of carbon dioxide (CO_2) uptake and evolution by infra-red gas analysis provides a sensitive, accurate and widely used method of determining rates of photosynthesis and respiration.

Infra-red gas analysis requires a gas exchange system consisting of four main groups of components (Sestak et al. 1971), namely:

1) Infra-red gas analyser
2) Assimilation chamber
3) Gas handling system
4) Air conditioning system.

1) Infra-red gas analyser (IRGA)

Full descriptions of the general principles of infra-red gas analysis and detailed functioning of an infra-red gas analyser are given by Sestak *et al.* (1971). Essentially, the analyser detects the difference in CO_2 concentration of the air entering the assimilation chamber (reference air stream) to the CO_2 concentration of air leaving the assimilation chamber (sample air stream). In order to do this, the reference and sample air streams enter the analyser and pass through reference and sample absorption tubes, respectively (Fig. 39). Infra-red radiation produced from a nichrome metal heater at a temperature of 600-800°C and reflected by mirrors, passes down the same absorption tubes. The amount of infra-red radiation reaching the sample and reference detecting chambers will depend upon the amount of CO_2 present in the absorption tubes. The greater the CO_2 concentration in a tube, the greater the amount of infra-red radiation that will be absorbed and hence, less will reach the detecting chamber.

On reaching the detecting chamber, the infra-red radiation heats the gas in the chamber, applying pressure to a diaphragm between sample and reference detection chambers. A difference in CO_2 concentration between reference and sample air therefore causes differential deformation of the diaphragm. A rotational shutter interrupts the light beam at a specific frequency, causing the diaphragm to vibrate and changing the capacity of the condenser, the output voltage from which is proportional to the capacity changes.

There is a direct and individual relationship between the molecular structure of a gas and its infra-red absorption spectrum. This relationship is determined by the number of atoms, mass, molecular symmetry and inter-atomic bonding of the molecule. Gases such as nitrogen (N_2) which has two identical atoms do not absorb in the infra-red. Water (H_2O) however, does and there is an overlap in the absorption spectra of CO_2 and H_2O. Water is therefore filtered out of the air entering the analyser or alternatively the region of overlap is filtered out using an optical filter (Sestak _et_ _al._ 1971).

2) Assimilation chamber

The assimilation chamber encloses all or part of a plant to facilitate measurements of gas exchange. The plant material causes changes in CO_2 concentration of the surrounding air which is detected by the infra-red gas analyser and thus measures the rate of gas exchange. Usually the conditions of irradiance, temperature, humidity, ambient CO_2 concentration and air movement are precisely controlled.

Despite the accurately controlled conditions that are achieved, the main short-coming is that enclosure in a chamber imposes an unnatural environment on the plant. This is particularly a problem in the field where conditions in the chamber may be very different to those outside the chamber especially the factors of air movement and the fluxes of short and long wave radiation. Very sophisticated systems and chambers have been developed to precisely apply, as far as possible, the natural environmental conditions such as the system of Koch _et_ _al._ (1971) but even here, interpretation of results requires caution in applying findings to natural field situations. In the laboratory situation, plants have frequently been raised under artificial conditions which may be accurately reproduced in the chamber, so the problem is less serious. Frequently too, the aim is to obtain basic information on the functioning of the photosynthesis and respiration systems of plants in response to changes in external environmental variables (e.g. irradiance, temperature, humidity, CO_2 and O_2 concentration) and internal environment (e.g. water potential, salt content, hormone level, sugar content). In an assimilation chamber, such variables can be easily manipulated while maintaining reasonably natural conditions for the factors not under study.

As outlined by Ormrod and Krizek (1978), care in design of the system is required to avoid inadvertently imposing stress such as temperature or heat stress or CO_2 depletion.

The basic requirements for an assimilation chamber are that the environmental conditions affecting CO_2 flux should be controlled and known, particularly leaf temperature, photosynthetically active radiation (PAR) incident on leaves from all directions, ambient CO_2 and O_2 concentrations, water vapour and atmospheric pressures (Sestak *et al.* 1971). The chamber should also enable the plant material to be placed in a reproducible position in the chamber (Hardwick *et al.* 1966) and air must be evenly distributed in the chamber which is sometimes facilitated by use of fans (Natr and Spidla 1963, Smith 1978).

All the principles of assimilation chamber design are comprehensively covered by Sestak *et al.* (1971) along with a variety of examples of chamber design.

3) The gas handling system

The air is conditioned before being pumped to the assimilation chamber. The sample and reference air streams are then transported to the gas analyser. It is essential that it is more or less leak proof and the tubing is CO_2 inert and CO_2 impermeable.

Three possible systems are available, namely closed, semi-closed and open systems (Larcher 1969) and are fully described by Sestak *et al.* (1971). The closed system involves measuring the time taken for the plant material to reduce the CO_2 concentration by certain amounts in a recirculating air system. It is unsuitable for measuring rates of photosynthesis because photosynthesis varies non-linearly with CO_2 concentration and stomata respond slowly to comparatively small changes in CO_2.

The semi-closed system was also unsuitable because for small amounts of plant material the method of maintaining CO_2 levels at a certain constant level is very difficult to measure and regulate.

In an open system, air of known controlled properties and flow rate passes from a source through the assimilation chamber to the gas analyser before then flowing out from the system. It is particularly useful for investigation of photosynthesis rates when parts of plants or fairly small plants are being used and allows the continuous recording of small rapid fluctuations and changes in rate to be made with a high degree of accuracy. Other advantages are that where the air is pushed through the system, it does not have to be so completely leak proof as both the closed and semi-closed systems require. This is because there is no drawing in of air from the analysis room (which may be of very different CO_2 concentration to the air moving through the system). A further advantage is that air pressure throughout the system remains close to that of atmospheric pressure which is important because it affects the flow rates throughout the system.

4) Air conditioning system

The air conditioning system pre-determines the temperature, humidity, CO_2 and sometimes O_2 levels of air entering the assimilation chamber. Ideally, sensors in the chamber or sample air stream automatically regulate these conditions accurately (e.g. Koch *et al.* 1971).

Infra-red gas analysis system employed.

1) Infra-red gas analyser

A series 225 gas analyser (Analytical Development Co. Ltd., see Plate 5 and Fig. 39) was used. It has built in pumps to draw in both sample and reference air at a rate of 0.2 l s^{-1} (hence it was

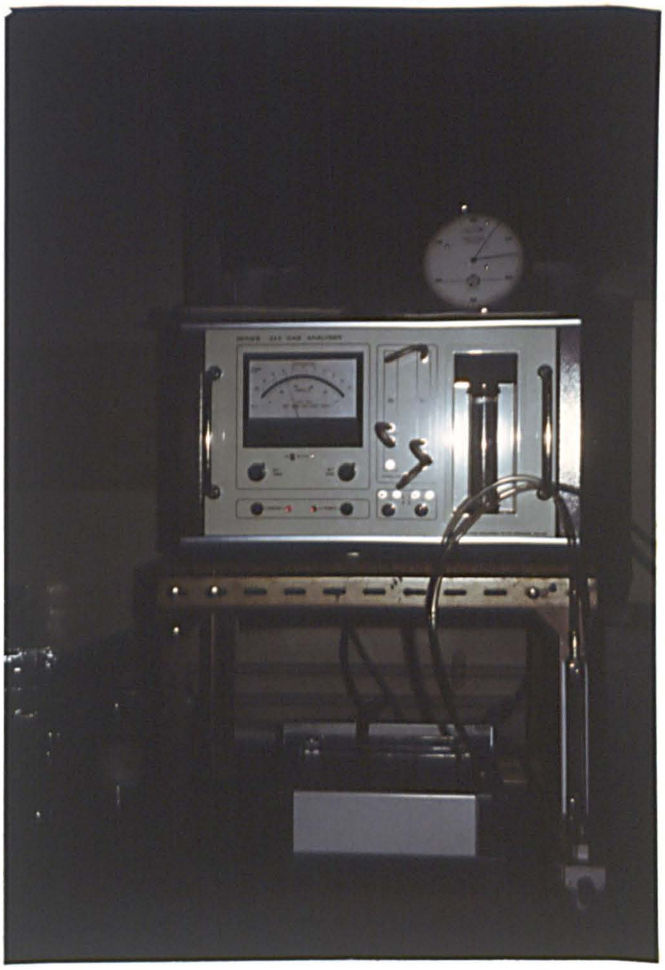


Plate 5 Infra red gas analyser, used to measure CO_2 flux.

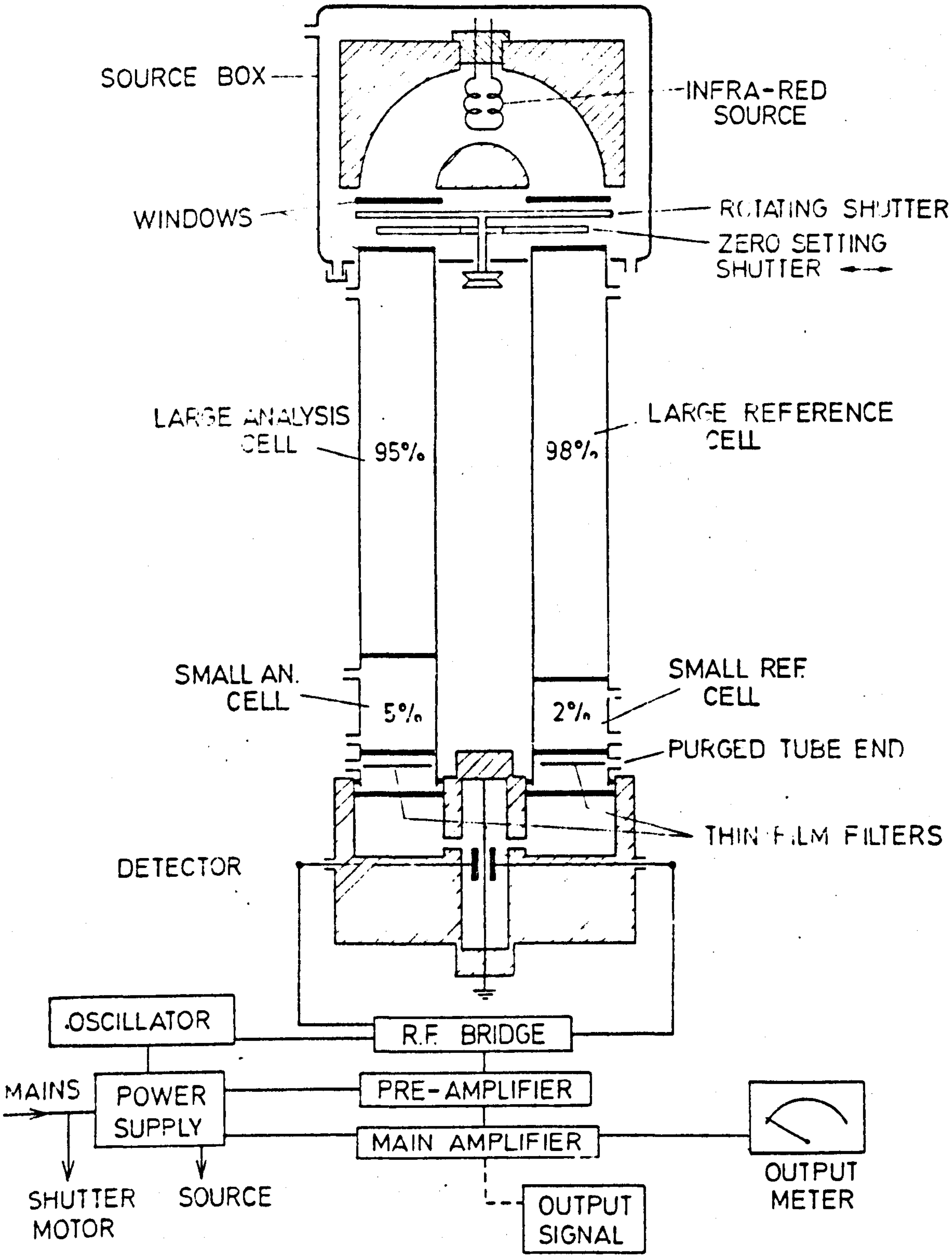


Fig. 39 Schematic diagram of infra-red gas analyser Type 225, Analytical Development Co. Ltd.

necessary that the external sample and reference air flow rates were higher than 0.2 l s^{-1} to ensure no drawing in of air from the analysis room as will be discussed below). The analyser also has a tower of self-indicating sodalime to remove CO_2 from a further stream of air drawn in from the analysis room. This CO_2-free air flows through various compartments of sample and/or reference tubes according to the measurements being taken. Optical filters are present to filter out wavelengths of infra-red light over which CO_2 and H_2O absorption spectra overlap.

The gas analyser had differentially split reference and sample tubes which give a more sensitive measurement of both absolute and differential CO_2 levels. Calibration of a split tube analyser also requires fewer standard CO_2 concentrations. The theory of this method is described by Legg and Parkinson (1968) and also in the instrument manual along with the full operating procedure.

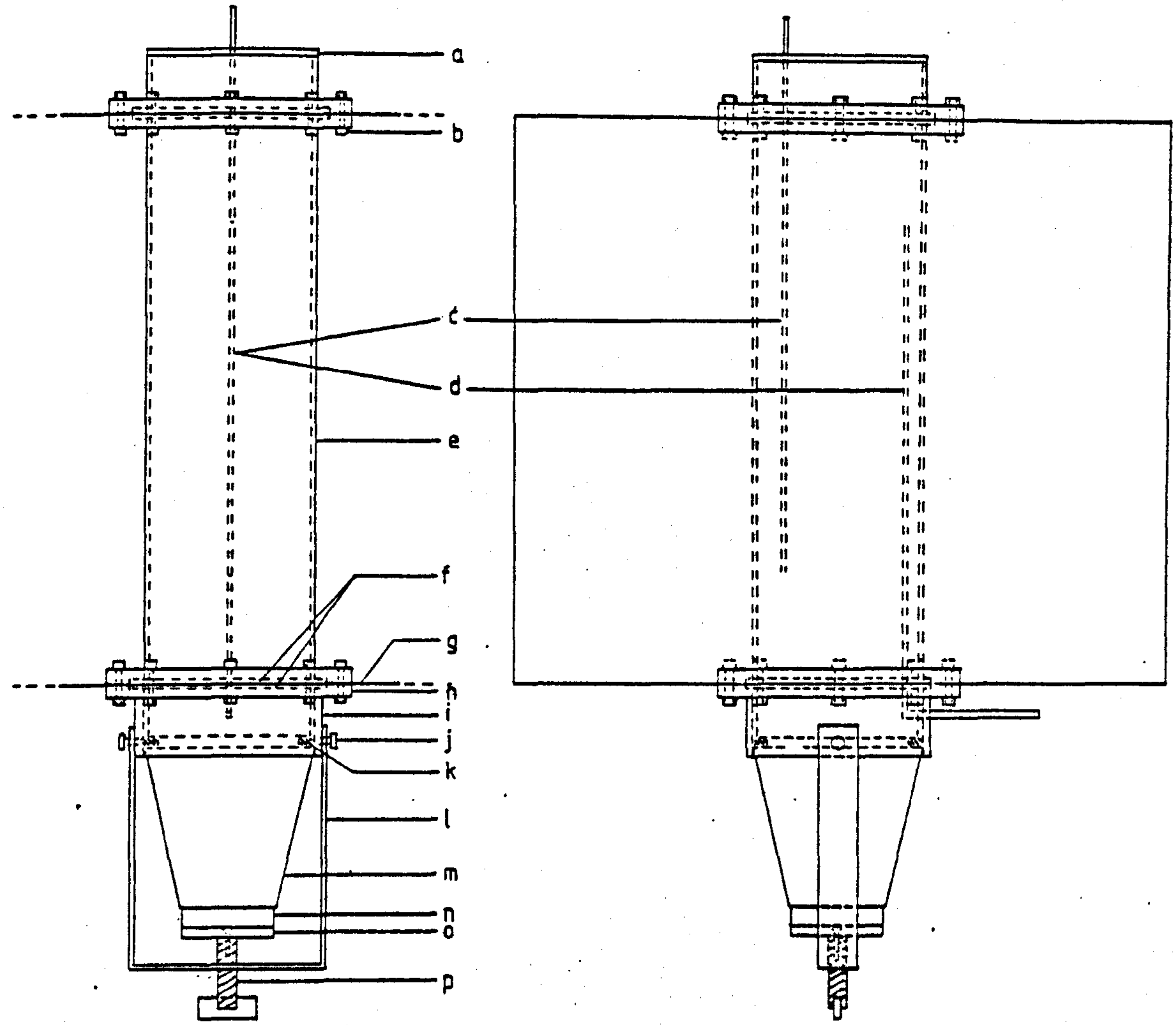
2) Assimilation chambers

Previous experiments (Chapter 3) measured shoot yield and injury index of whole pots of grass in order to see the overall response of a mini-sward of grass. In relation to these experiments, CO_2 flux of the pot of grass was to be measured. In order to do this, it was necessary to place the shoots of a pot of grass in an assimilation chamber. The chamber designed for this study is shown in Fig. 40 and Plate 6. It consists of a "perspex" (methacrylate polymer) cylinder sealed at one end by a circle of perspex through which a brass tube (the sample air outlet) passes. Perspex has a very low absorption of light (less than 0.2% per centimetre thickness) and a high uniform transmittance over the range of photosynthetically active radiation (400-700 nm wavelength) of 0.92 (Sestak et al. 1971) thus being a very suitable material for an assimilation chamber. Problems of sealing joints as experienced by others such as Smith (1978) were

Fig. 40 Assimilation/respiration chamber.

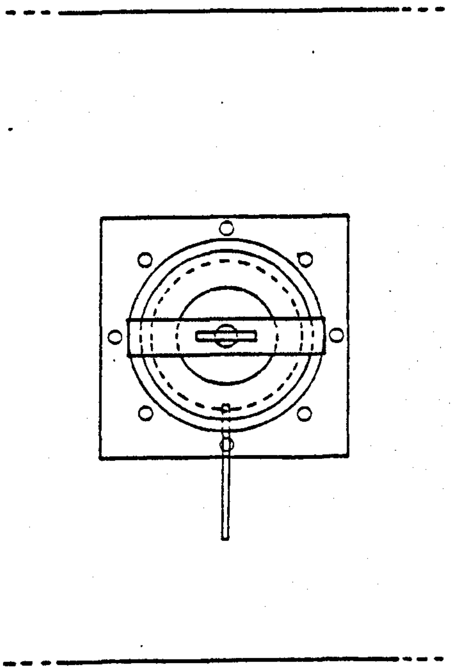
(Drawn to scale 1 cm = 4.62 cm, some lines omitted for clarity.)

a - Perspex end plate
b - Fixing nut and bolt
c - Outlet tube
d - Inlet tube
e - Perspex cylinder
f - O ring
g - Side of aluminium water bath tank
h - Supporting aluminium plate
i - Brass ring
j - Pivot screw
k - O ring
l - Brass clamping frame
m - Pot
n - Rubber pad
o - Brass disc
p - Threaded brass screw



PLAN

SIDE



END

Fig. 40

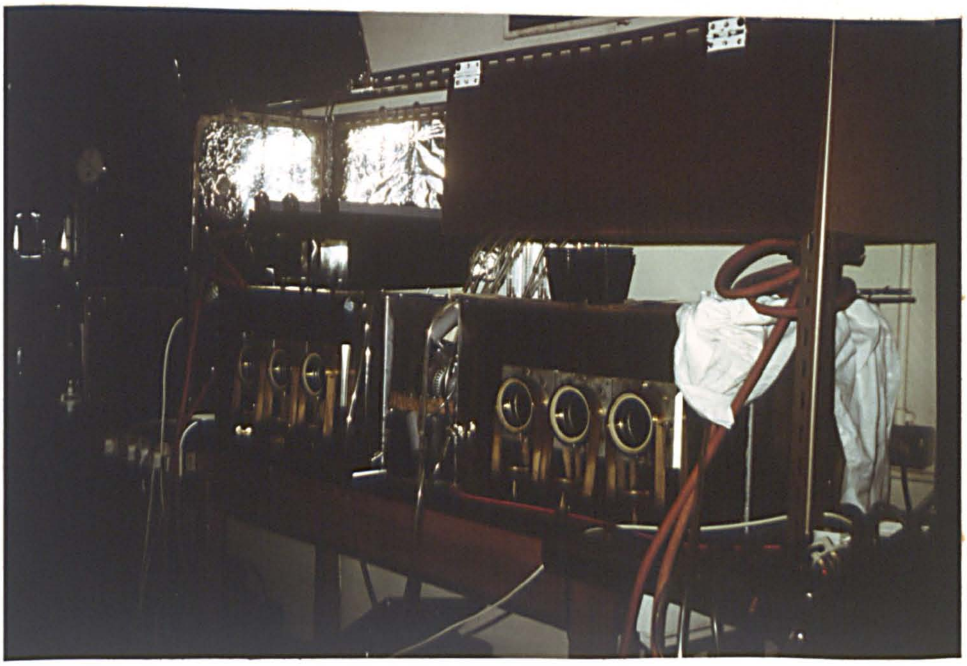


Plate 6 Equipment used to measure CO_2 flux: assimilation and respiration water baths and chambers.

Plate 7 Equipment used to measure CO_2 flux: flow meters and 6-way switch.

minimal because of few joints in the design.

To give as near as possible a curved sphere surface through which light is most naturally and efficiently transmitted (Sestak *et al.* 1971), and also to standardize the height of the plant material from the light source (in view of the observation that trampled grass shoots do not stand up as tall as untrampled grass), the chamber was placed horizontally (see Fig. 40 and Plate 6). The open end was designed so that a pot of grass was clamped horizontally against the end of the perspex cylinder by means of a brass disc (attached to a frame) over the base of the pot. The pot was therefore pressed tightly against an O ring, giving a good seal. The sample air inlet tube enters the perspex cylinder through the brass clamp support.

So that a number of replicate measurements could be made in rapid succession, 6 chambers of identical design were constructed. Two aluminium water tanks of dimensions 77 cm x 40 cm x 35 cm were made and 3 chambers were set through each water tank with rubber seals and aluminium plates around the chambers, outside and inside the tanks for strengthening and sealing. The temperature inside the chambers was controlled by maintaining water in the tanks at an appropriate temperature.

One set of 3 chambers was designated for measuring rate of photosynthesis. The other set of 3 chambers was used for measuring dark respiration and so was fitted with an aluminium lid which was further covered with towelling to exclude all light. Plate 6 illustrates the two tanks and chambers.

3) Gas handling system

Fig. 41 gives the gas handling and air conditioning systems used in the study. The system was an open system drawing in air from the atmosphere outside the analysis room by means of a M361 (Charles Austin Pumps Ltd., Sussex) membrane pump situated after the air had

Fig. 41 Diagram of gas handling system and air conditioning system used for the measurement of carbon dioxide exchange by infra-red gas analysis.

AMT - Air mixing tank
DWB - Dewpoint water bath
RU - Regrigeration unit
WB - Water bath
P - Pump
CD - Circular distributor
RC - Rectangular regulating clip
FM - Flow meter
RC - Respiration chamber
AC - Assimilation chamber
ERU - Extra regrigeration unit
FHC - Flow heater cooler
WBL - Water bath lid
L - Lights
6WS - 6-way switch
SLT - Slaked lime tower
CG - Calibration gas
IRGA - Infra-red gas analyser
CR - Chart recorder

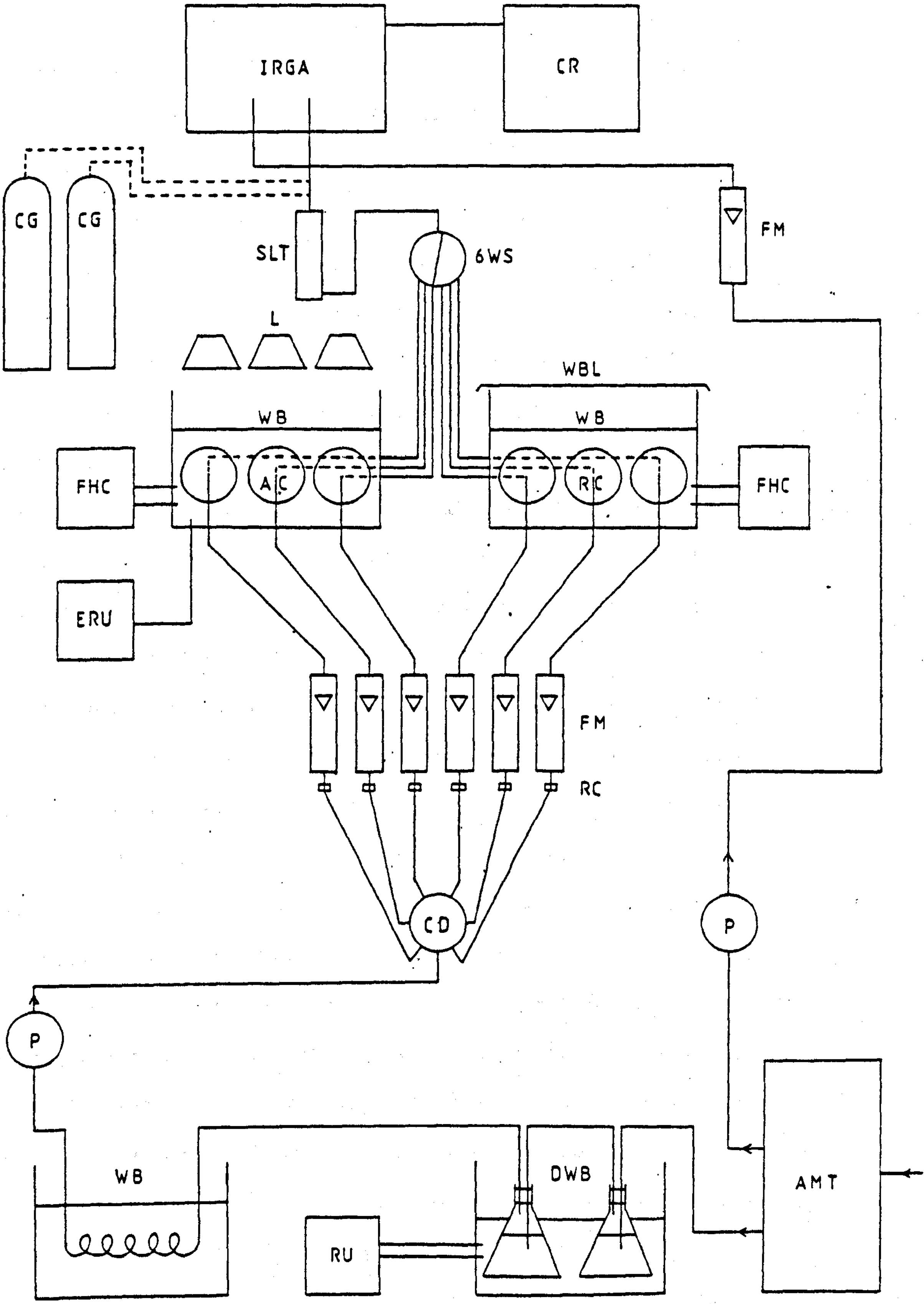

IRGA
CR
CG
CG
SLT
6WS
FM
L
WBL
WB
WB
FHC
AC
RC
FHC
ERU
FM
RC
CD
P
P
WB
DWB
RU
AMT

Fig. 41

been conditioned to the correct humidity. The pump had a maximum capacity of 17 l min^{-1}. The air was split into 6 streams on leaving the pump and flow rate was adjusted and measured by six flow meters (rotameters) (G.A. Platon Ltd.). The six air streams then entered the 6 assimilation/respiration chambers (Figs. 40,41, Plate 6). The air inlets and outlets were constructed and arranged in the chambers so that mixing of the air was facilitated (Fig. 40). The air flowed out of each chamber to a specially designed 6-way switch (Fig. 42, Plate 7), collecting all 6 air streams from the assimilation/respiration chambers. According to the position of the movable central disc, air from one chamber only was directed to the gas analyser. For the other 5 chambers, the air flowed out into the analysis room via the channels in the 6-way switch, thus not inhibiting the open system at any stage. The air stream being sampled was passed through a tower of slaked lime to remove excessive water vapour and then entered the sample air inlet of the analyser.

Reference air was drawn in from the atmosphere as for the sample air but using a Capex MkII membrane pump (Charles Austin Pumps Ltd., Sussex) which has a maximum capacity of 5 l min^{-1}. The reference air passed through a flow meter (CAP flowstat regulator, G.A. Platon Ltd., Hants) and rate of flow was adjusted so that it was greater than the flow rate of air drawn in by the gas analyser (see above), and similar to that of the sample air stream being likewise monitored. The reference air then entered the reference air inlet.

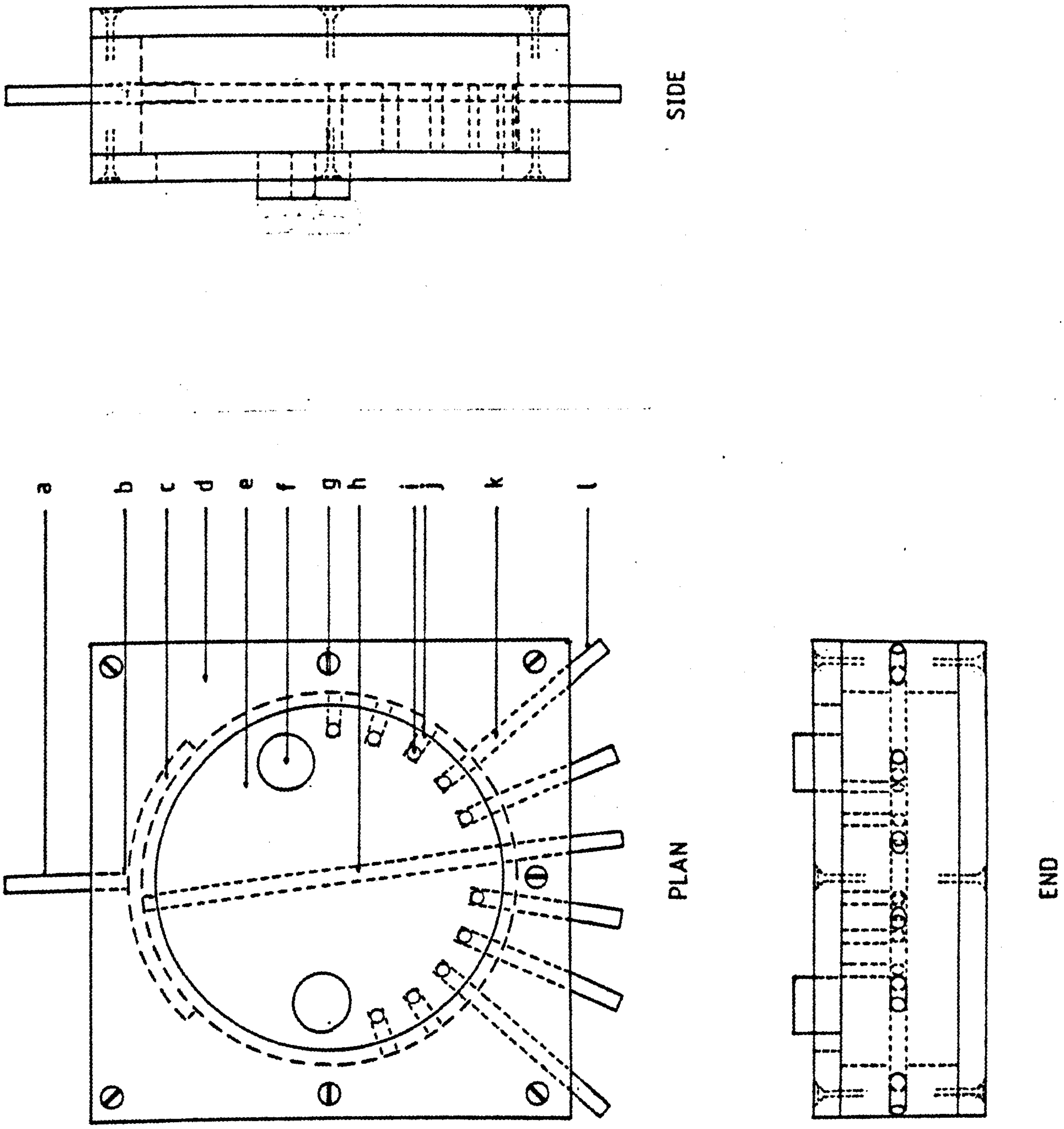
After flowing through the gas analyser absorption tubes, both streams of air passed freely out into the analysis room.

The tubing used throughout the system was thick-walled PVC tubing as it is flexible and readily available. With thick walls, CO_2 diffusion is considered to be minimal (Sesak et al. 1971) so long as relatively short lengths are utilized.

Fig. 42 6-way switch for gas handling system. (Drawn to scale 1 cm = 1.39 cm, some lines omitted for clarity.)

a - Brass outlet port
b - Short drilled 3.5 mm hole
c - 3 mm channel
d - Overlapping plate
e - Central disc
f - Knob
g - Brass screw
h - Drilled 3.5 mm hole through central disc
i - Exhaust port
j - Short drilled 3.5 mm hole
k - Drilled 3.5 mm hole
l - Brass inlet port

Fig. 42



4) Air conditioning system

The correct humidity of the air was maintained by passing air through two flasks of water submerged in a water bath maintained at the dewpoint temperature, thus saturating the air. The temperature of the air was then raised by passing the air tubing through a water bath at a specific higher temperature, so establishing the desired humidity as indicated in each individual experiment later. This is a well used method of producing a specific water vapour pressure (e.g. Bierhuizen and Slatyer 1964, Nevins and Loomis 1970, Beadle *et al.* 1974, Smith 1978) but problems of water condensation were experienced in some of the air tubes where the external air temperature was low. Similar problems have been encountered by others (Grace and Woolhouse 1970, Smith 1978). A humidity sensor (Shaw Moisture Meter) was therefore regularly used to check the actual humidity in the chambers.

The temperature of the air was controlled by passing the tubing through water baths at specific temperatures. Flow heater/coolers (Grant Instruments Ltd.) were used to control the temperature of the water around the chambers, and hence the temperature inside the chambers. As there was considerable heat from the lights over the assimilation chambers, a further cooling unit was used in the assimilation chamber water tank, to supplement the flow heater/cooler unit. Constant check was kept of both water bath temperatures and chamber air temperatures using mercury in glass thermometers.

Light system.

Radiation was provided by three 400 W high pressure sodium lamps (400 W Solarcolour SON/T high pressure sodium lamps fitted in Camplex HD71Q22S plant irradiators, Simplex of Cambridge, Sawston, Cambridge). The irradiator was adjusted to divert light more intensely on each chamber. The lamps were mounted usually with a gap of 35 cm between light bulb and base of chamber.

As discussed by Warrington (1978), high pressure sodium lamps are among the most efficient of the high pressure discharge lamps which in general give high radiant flux output, adequate spectral distribution within the visible and far-red wavelengths, long life, high luminous efficiencies but have the disadvantage of producing substantial amounts of heat (hence heat filters are necessary between lamp and chamber as above). High pressure sodium lamps, however, do have somewhat low output in the short visible wavelengths.

The frame on which the lamps were mounted had aluminium foil-lined inner sides to increase the lateral radiation. Regular measurement of quantum energy flux global radiation and illuminance were made inside the assimilation chambers, at the same position as the grass in the chamber. A Cat. No. 550 Quantum/Radiometer/Photometer with appropriate sensors (T.J. Crump, Scientific Instruments, Rayleigh, Essex) was used to measure these light radiation parameters. The aim was to supply light intensity at or above the light saturation level for photosynthesis of grasses so that slight differences between the chambers would be of negligible consequence.

The light saturation of Lolium perenne was found to be 25 klux, 158 W m^{-2} by Wilson and Cooper (1969) and 175 W m^{-2} by Woledge and Leaf (1976), so light in excess of this value was supplied. Typical readings taken at plant level were:

	Quantum flux (PAR) (μE $m^{-2}s^{-1}$)	Global radiation (PAR) (W m^{-2})	Illuminance (klux)
Chamber 1	1250	190	46
Chamber 2	1950	280	64
Chamber 3	1250	175	46

Calculation of CO_2 exchange rate.

From the IRGA, a measurement of the CO_2 concentration in μl l^{-1} was obtained. Various equations for calculating factors such as CO_2 exchange rate in volumetric units, CO_2 exchange rate in terms of mass flux of CO_2 and ambient CO_2 concentration within the chamber are available and described in detail by Sestak et al. (1971). In the present study, the CO_2 exchange rate in terms of the mass flux of CO_2 was calculated. The equation for this is based on the simpler expression for CO_2 exchange rate with volumetric units (e.g. Gaastra 1959):

$$F = \frac{\Delta C.\ J}{A}$$

where F = flux density of CO_2 ($cm^3\ cm^{-2}\ s^{-1}$)

ΔC = difference in CO_2 concentration of air streams before and after the assimilation chamber measured at the same temperature and pressure ($cm^3\ cm^{-3}$)

J = air flow rate through assimilation chamber ($cm^3\ s^{-1}$)

A = leaf area, usually of one surface (cm^2)

To express CO_2 exchange rate in terms of mass flux of CO_2 however, units of volume are converted to units of mass. Temperature and pressure in the laboratory at the time of the observation are required (Sestak et al. 1971) and their deviations from S.T.P. are corrected. Temperature and pressure at which the flow meters were calibrated is also required if, as in this case, they are of the rotameter type (Baker and Musgrave 1964, Sestak et al. 1971). All these correction factors are applicable where water vapour interference is suppressed by optical filters in the IRGA (Sestak *et al.* 1971) as is the case in the present study (otherwise allowances must be made as given by Sestak *et al.* 1971).

Thus the equation for expressing CO_2 flux in terms of mass flux of CO_2 and used in the present study is:

$$F = \frac{\Delta C}{A} \cdot \frac{J.44}{22414} \cdot \frac{273}{T} \cdot \frac{P}{1013.25} \cdot \sqrt{\frac{T}{T^1}} \cdot \sqrt{\frac{P^1}{P}} \quad g\ cm^{-2} s^{-1}$$

where F = flux density of CO_2 ($g\ cm^{-2}\ s^{-1}$)

ΔC = difference in CO_2 concentration of the air streams before and after the assimilation chamber measured at the same temperature and pressure ($cm^3\ cm^{-3}$)

A = leaf area, usually of one surface (cm^2)

J = air flow rate through the assimilation chamber ($cm^3\ s^{-1}$)

T = temperature of the flow meter at the time of observation (°K)

P = barometric pressure at the time of observation (mb)

T^1 = temperature at which the rotameter was calibrated (°K)

P^1 = pressure at which the rotameter was calibrated (mb).

As measuring the leaf surface area of a pot of grass was a very lengthy and difficult process, total shoot dry weight was most appropriate and was therefore substituted for leaf area. CO_2 flux measurements in this study are therefore expressed in terms of mg CO_2 $g\ DW^{-1}\ h^{-1}$ (milligramme of carbon dioxide per gramme dry weight per hour). Two experiments were conducted with *Lolium perenne* S23, relating leaf area to biomass, and they are given in Appendix 4. There was a very high correlation between leaf dry weight and leaf surface area ($p < 0.001$).

Methods

Pots of grass were grown as described in the general methods (Chapter 2, page 18) and at the eight week stage, were transferred to constant environment growth cabinets. Experiments were carried out after 5 or 6 days in the usual conditions of 18°C day/13°C night, 16 hour photoperiod, 70% relative humidity (unless otherwise stated), and a light intensity of 165 $\mu E\ m^{-2}\ s^{-1}$ (PAR 400-700 nm Quanta), 31 $W\ m^{-2}$ (PAR 400-700 nm horizontal global radiation), 7000 lux (CIE standard illumination) supplied by warm white fluorescent lights.

The general procedure for use of the IRGA system was that plants were sequentially, at intervals of 5 (or 10) minutes, put in the chamber following the appropriate treatment. Previous observations had established that a 20 minute equilibration time was required for the air in the chamber to be depleted of air from the analysis room after addition of a pot of grass, and for the chamber air to then level off at a steady level according to the rate of plant photosynthesis/respiration. With 6 chambers, therefore, and pots of grass added/replaced sequentially every 5 minutes, it was possible and convenient to take the reading at 30 minutes after placing the plant in the chamber. During 25 minutes of this 30 minute period, air from the chamber flowed through the 6-way switch to the analysis room. Over the last 5 minutes the air flowed to the IRGA. As soon as the 30 minute reading had been taken, the 6 way switch was moved to the next chamber to be sampled by the IRGA for the final 5 minute period, and the sequence was continued for all plants to be measured.

In practice, chambers 1 to 3 had illumination from high pressure sodium lamps as described above and so were used to measure photosynthesis rate while chambers 4 to 6 had all light excluded and were therefore used to measure respiration rate.

A variety of experiments were carried out as described below.

To elucidate results of previous experiments (Chapter 3) the CO_2 flux of whole pots of grass was required. With intact plant material, it is usually possible to seal around the base of the shoot system to exclude soil and root respiratory CO_2 (e.g. Scott et al. 1970, Catsky and Ticha 1974). Even with a single non-tillering grass plant however, this is difficult because it consists of younger leaf blades and sheaths within older leaf sheaths. With a mini-sward of grass, the situation presents even more problems. Diffusion of CO_2 from soil/root respiration into the chamber was reduced to some extent by covering the soil surface with an annulus of plastic, which also served to prevent soil falling from the pots when the pot of grass lay horizontally clamped into the chamber. In addition, in some experiments, the soil/roots was enclosed in a polythene bag and secured around the shoot bases using a rubber band, loose enough not to damage the shoots and with the polythene drawn back away from the shoot to prevent any interference of gas exchange and light interception.

Experiments were also carried out to investigate the respiration rate of soil and roots only (see section a)). In further experiments, measurements of soil and root respiration only were usually taken to correct the CO_2 flux of readings from the intact plant. A similar method was used by Smith (1978) to overcome problems of sealing round the base of Plantago lanceolata. Where readings of soil/root CO_2 were not obtained, results are expressed as CO_2 flux per pot of grass, exposed to light or dark.

a) CO_2 flux of soil and roots with simulated trampling.

The aim of this section was first to investigate the effect of simulated trampling on the CO_2 flux from soil and roots. This was because the extent of CO_2 flux from the shoots of intact pots of grass using the designed IRGA system as described above may also include CO_2 from the roots and soil (Farrar 1981). It was therefore necessary to test for any significant increase/decrease in soil/root respiration with simulated trampling (see a(i) and a(ii)) and also for an effect over a period of time following simulated trampling. Any such effects would need to be eliminated from subsequent experiments investigating the response of intact pots of grass to simulated trampling and effects of time after treatment.

As only limited numbers of pots of grass could be measured because of the time needed to obtain each reading and also to obtain sufficient replicates per treatment, it was necessary to investigate the effects of simulated trampling and time after treatment using two experiments below, a(i) looking at more immediate effects and a(ii) the more long term response.

Evidence that lower soil water contents were linked with lower soil respiration rates (Parkinson 1981, Hutchinson and King 1982) suggested it was necessary to check if soil water status had any influence on soil/root respiration rate. Simulated trampling may modify this effect as Blom (1977) showed changes in water status of trampled ground. A range of simulated trampling was used (a(iii) below) to see if there was a differential response, although the range of treatments was restricted because of time required to obtain readings for sufficient replicates.

(i) To discover if there was a differential response of soil/root respiration to the time a pot of grass was left intact following simulated trampling, the following experiment was carried out. Pots of grass were treated with 0 or 5 i pot^{-1} at 0, 0.5, 2.5 and 4 hours prior to complete excision of the shoots. Immediately after excision, each pot was placed in the chamber and a measurement of CO_2 flux was obtained after 30 minutes as described above. Results were expressed in terms of mg CO_2 pot^{-1} h^{-1}. For each treatment there were 12 replicates. Data was analysed using analysis of variance.

(ii) To examine if soil/root respiration changed according to the period of time following simulated trampling treatment before excision, pots of grass were treated with 0 or 5 i pot^{-1} at 0.5 or 24 hours prior to obtaining the CO_2 flux reading (including 30 minutes in the chamber). Of these, after treatment, the shoots were immediately excised to soil level from half of the pots. The other half were left until just before being placed in the chamber when the shoots were excised to the base. After 30 minutes in the chamber, CO_2 flux measurement was obtained. Results were expressed in mg CO_2 pot^{-1} h^{-1}. For each treatment there were 12 replicates. Data was analysed using analysis of variance.

(iii) Pots of grass, watered to field capacity or droughted for 6 days were given treatments of 0, 1, 3 or 5 i pot^{-1}. Immediately after treatment shoots were excised and the pots were placed in the assimilation chambers and a reading of CO_2 flux obtained after 0.5 hours. Results were expressed in mg CO_2 pot^{-1} h^{-1}. There were 6 replicates for each treatment. Analysis of variance was carried out to see if there was a significant effect of soil water status with intensity of simulated trampling on soil/root respiration.

b) CO_2 flux of intact plants with simulated trampling.

Experiments in Chapter 3 had shown a decrease in shoot yield with simulated trampling. This response has been demonstrated in various other pieces of work on the effects of trampling both in the laboratory (e.g. Bayfield 1971a, Smith 1978) and field situation (e.g. Leney 1974, Smith 1978). As the balance of photosynthesis to respiration largely dictates the growth of a plant, it was appropriate to investigate photosynthesis and respiration of grass that had received simulated trampling. Results may indicate if the reduction in shoot yield can be attributed to a reduction in photosynthesis or to increase in respiration or to a combination of both.

So that light was not a limiting factor, irradiance in excess of the saturation level was utilised in the following experiments, b)(i), b)(ii) and b)(iii). Again, the time required to obtain readings restricted the number of treatments of simulated trampling that could be applied in any one experiment, so the full range of treatments up to 25 i pot^{-1} was supplied using two experiments b)(i) and b)(ii) below. Both photosynthesis and dark respiration were measured.

The results were expressed in terms of whole pot CO_2 flux because the soil/root CO_2 flux was included in the measurement. Where time permitted in b)(ii), the CO_2 flux of the soil/root was also measured, enabling calculation of shoot net photosynthesis and dark respiration.

Trampling has a dual effect of soil compaction and shoot injury which has been well documented (e.g. Speight 1973, Canaway 1975a). To find out if the shoot yield response could be explained by either of the separate effects of soil compaction or shoot injury on the photosynthesis and dark respiration rates, a further experiment b)(iii) was carried out where only the shoots received simulated trampling treatment. Similar intensities of simulated trampling to those used in b)(i), were applied to facilitate comparison.

(i) The effect of intensity of trampling on both rate of photosynthesis and of dark respiration was investigated. Pots of grass were treated with either 0, 1, 3 or 5 i pot^{-1} and immediately placed in the chamber. A reading was taken after 30 minutes had elapsed. Shoots were then cut to the 3 cm level and oven-dried at 95°C overnight to obtain dry weights. There were 3 replicates for each treatment. CO_2 flux of whole pots of grass in response to light

(> 45 klux) and dark was calculated. Results were analysed using analysis of variance.

(ii) The above experiment was repeated for simulated trampling treatments of 0, 5, 10, or 25 i pot^{-1}. Soil/root CO_2 flux for each pot of grass was also obtained, so results were expressed first in terms of CO_2 flux of whole pots of grass, then by subtraction of soil/root CO_2 flux, net photosynthesis of shoots and dark respiration of shoots were calculated.

(iii) The above experiment was repeated for simulated trampling treatments of 0, 1, 3, 5 i pot^{-1} but only the shoots received treatment. This was facilitated by use of a metal annulus fitted over the pot but around the base of the grass shoots (see Smith 1978). Resilience of soil was simulated by covering the upper side of the metal plate with 0.5 cm thick rubber.

c) CO_2 flux at lower light intensities with simulated trampling.

It has been demonstrated that the light levels at which a young grass leaf is exposed to during expansion determines the subsequent photosynthetic capacity. Heavily shaded leaves show a marked reduction in photosynthetic capacity in comparison to ones that were not shaded during expansion (Woledge 1973, 1977). It is also well established that light irradiance levels less than the saturation level are a limiting factor to photosynthesis. To see therefore, how simulated trampling affects shoot net photosynthesis at limiting light intensities, the following experiments c)(i) and c)(ii) were carried out.

Jaffe and Biro (1979) recorded an immediate reaction to a stimulus such as rubbing or shaking. Injury in the form of excision has been shown to initiate a series of changed physiological functions which vary over time after injury (Macnicol 1976). It therefore seemed necessary to examine the effect of time after treatment on rates of photosynthesis and respiration (experiments c)(i), c)(ii)).

Quantity of pots of grass for any one experiment and also time required to obtain measurements, again restricted the number of treatments that could be applied and the number of times after treatment that could be measured, hence two experiments c)(i) and c)(ii) were carried out.

(i) CO_2 flux of whole pots of grass at 12 klux or in darkness, was examined using the IRGA system as described above. Pots of grass were treated with 0 or 5 i pot^{-1}. Readings were taken after 30 minutes in the chamber. To also investigate the effects of time after simulated trampling treatment on the CO_2 flux, pots of grass were treated with 5 i pot^{-1} at 1, 6 and 7 hours before the reading was taken (i.e. treatment was 0.5, 5.5 and 6.5 hours before the pots were placed in the chamber). There were 6 replicates for each treatment. Results were expressed as whole pot CO_2 flux in mg CO_2 g shoot DW^{-1} h^{-1} and were analysed using analysis of variance.

(ii) The above experiment (i) was repeated for 0 and 5 i pot^{-1} but pots of grass were treated 0.5 and 3.5 hours before the reading was taken. CO_2 flux of soil/root only were also obtained. By subtracting these values from the whole pot CO_2 flux readings, net shoot photosynthesis and dark shoot respiration were obtained and analysed using analysis of variance.

d) Continuous monitoring of CO_2 flux before and after simulated trampling.

In sections b) and c), CO_2 flux results were obtained for a certain time following simulated trampling in response to various intensities of treatment. The response over a period of time however, was not very clear so CO_2 flux from continuous monitoring of pots of grass before and after application of simulated trampling treatments was investigated at chosen intensities for both photosynthesis and respiration.

A pot of grass was randomly chosen. A continuous reading of the CO_2 flux was obtained usually over a period of a few hours. The pot of grass was then removed from the chamber, treated with the appropriate intensity of simulated trampling, replaced in the chamber and was monitored continuously, usually over a period of a few hours. At the end of the experiment, shoots were excised and dried at 95°C overnight to obtain dry weights. CO_2 flux of the whole pot (in mg CO_2 g shoot DW^{-1} h^{-1}) was then calculated from readings taken from the chart record at 10 minute intervals (unless otherwise stated). A continuous moving average was then calculated.

Only one pot of grass was monitored in each experiment because of the limitations of having only one IRGA. To ensure that the measured response was reproducible, at least 5 replications of the experiments were carried out. Representative experimental results are given of the following treatments:

(i) As previous results in section c) showed no differential effect of simulated trampling and time but the number of time intervals used had been restricted, continuous monitoring of a pot of grass before and after 5 i pot^{-1} at 11 klux was carried out to clarify c)(i) and c)(ii).

(ii) Further to d)(i), continuous monitoring of a pot of grass before and after 5 i pot^{-1} in dark conditions was carried out.

(iii) The same treatment as in experiment d)(i) was given and monitored over a more prolonged time of 30 hours (compared with 10 hours) to investigate if the trend observed in d)(ii) continued.

(iv) The effect of higher intensities of simulated trampling on photosynthesis has already been investigated at high levels of irradiance in section b). To further examine this response in these conditions over a period of time, continuous monitoring of a pot of grass before and after 25 i pot^{-1} at > 45 klux was carried out.

(v) Further to d)(iv), continuous monitoring of a pot of grass before and after 25 i pot^{-1} in dark conditions was carried out.

(vi) To see if there was any change in soil/root respiration over a period of time which would therefore affect results for whole pot CO_2 flux, especially continuous monitoring experiments described above, a pot of grass with shoots excised was monitored to obtain soil/root CO_2 flux over a period of time.

e) CO_2 flux at different relative humidities with simulated trampling.

All previous experiments in this chapter had been carried out using air with a relative humidity of 70%. It has been shown that net CO_2 uptake rates are reduced in low humidity conditions (Lange *et al.* 1971, Farquhar 1978, Bunce 1984). To see if this response was affected (increased/decreased) by simulated trampling, two experiments were carried out where treated pots of grass were exposed to four levels of relative humidity. There were slight discrepancies between the relative humidities used in each experiment because of the difficulties of very precisely controlling the air humidity with the system used. A similar range was obtained, however. As increasing intensities of simulated trampling had been shown to have a greater effect on shoot yield and CO_2 flux, it seemed appropriate to look at the combined effects of level of simulated trampling and air humidity on CO_2 flux. Response at a high (e)(i)) and a low (e)(ii)) intensity of simulated trampling was therefore investigated.

(i) CO_2 flux of whole pots of grass in light (> 45 klux) and dark was recorded using the IRGA system as above. Pots of grass were treated with 0 or 15 i pot^{-1} just prior to being placed in an assimilation chamber at either 16, 30, 64 or 82% relative humidity. Readings were taken after 30 minutes. There were 3 replicates for each treatment. Results were expressed in mg CO_2 g shoot DW^{-1} h^{-1} and analysed using analysis of variance.

(ii) The above experimental procedure was used but the treatments were either 0 or 5 i pot^{-1} and the relative humidities were either 20, 39, 62 or 80%.

f) Comparison of CO_2 flux with electrolyte release in response to simulated trampling.

It was found (see Chapter 3) that intensity of trampling correlated positively with the index of injury and also with shoot yield. To discover if there was a relationship between electrolyte release, which is an indication of the level of injury sustained from simulated trampling, and subsequent CO_2 flux, the following investigation was made.

Pots of grass were treated with either 0 or 5 i pot^{-1} and were then placed in assimilation chambers. Whole pot CO_2 flux in light (11 klux) or dark conditions was recorded after 1 hour. The shoots were excised and the soil/roots only placed back in the chamber to provide a measurement of soil/root CO_2 flux after 1 hour. After excision, the shoots were immediately weighed to give fresh weights. 0.3 g fresh weight of the shoots from each pot was then subsampled and used straight away for measurement of electrolyte release, as described in Chapter 2 (page 39). The quantity of double glass distilled water per 0.3 g subsample was 20 ml. The remaining excised shoots from each pot were oven dried at 95°C overnight to give dry weights. The ratio of dry weight to fresh weight was used to give an estimated total original shoot dry weight value. For each treatment, there were 3 replicates.

Root/soil CO_2 flux was subtracted from whole pot CO_2 flux to give shoot CO_2 flux (i.e. net photosynthesis and dark respiration rates) in mg CO_2 g DW^{-1} h^{-1}. Whole pot CO_2 flux and shoot CO_2 flux were graphed in relation to the calculated index of injury, I (see page 39). Regression analysis was carried out on grouped 0 and 5 i pot^{-1} data for both dark and light CO_2 flux results.

Results

a) CO_2 flux of soil and roots with simulated trampling.

(i) Results are given in Table 6. Analysis of variance showed no significant difference between treatments.

(ii) Results are given in Table 7. Analysis of variance showed no significant differences between treatments.

(iii) Results are given in Fig. 43. Analysis of variance showed a significant difference between dry and wet conditions ($p<0.05$), the CO_2 flux being greater for wetter soil/roots than for dry soil/roots. There was no significant effect of simulated trampling treatment.

b) CO_2 flux of intact plants with simulated trampling.

(i) Increasing intensity of simulated trampling significantly ($p < 0.001$) reduced (i.e. made less negative) the CO_2 flux of whole pots of grass exposed to light greater than 45 klux (Fig. 44). There was a slight increase (i.e. more positive) in the CO_2 flux of whole pots of grass exposed to complete dark conditions but this was not significant (Fig. 44).

(ii) Like (i) above, increasing intensity of simulated trampling significantly ($p<0.001$) reduced the CO_2 flux of whole pots of grass exposed to light (Fig. 45) but there was a significant ($p<0.001$) effect also when exposed to dark conditions (Fig. 45). In the dark, the CO_2 flux for 0 i pot^{-1} was significantly lower than that for 5,

Table 6 <u>Respiration rate of soil/roots at various times after simulated trampling treatments.</u>

	Respiration (mg CO_2 pot^{-1} h^{-1})			
	0 i pot^{-1}	1h after 5 i pot^{-1}	3h after 5 i pot^{-1}	4.5h after 5 i pot^{-1}
$\bar{x}$	0.580	0.862	0.753	0.679
s.e.	0.0588	0.0781	0.0917	0.0917

Table 7 <u>Respiration rate of soil/roots at different times after simulated trampling and shoot excision.</u>

	Respiration (mg CO_2 pot^{-1} h^{-1})	
	Shoots excised 0.5h prior to reading	Shoots excised 24h prior to reading
0 i pot^{-1}	$\bar{x}$ = 0.650 s.e. = 0.094	$\bar{x}$ = 0.595 s.e. = 0.099
5 i pot^{-1} 24h prior to reading	$\bar{x}$ = 0.548 s.e. = 0.074	$\bar{x}$ = 0.534 s.e. = 0.074

Fig. 43 Response of root/soil respiration to water status and simulated trampling.

● watered to field capacity

■ droughted for 6 days

Fig. 44 Response of whole pot CO_2 flux (root/soil + shoots) to light and dark with simulated trampling at different intensities

○ assimilation at >45 klux

● dark respiration

Fig. 45 Response of whole pot CO_2 flux (root/soil + shoots) to light and dark with simulated trampling at different intensities

○ assimilation at >45 klux

● dark respiration

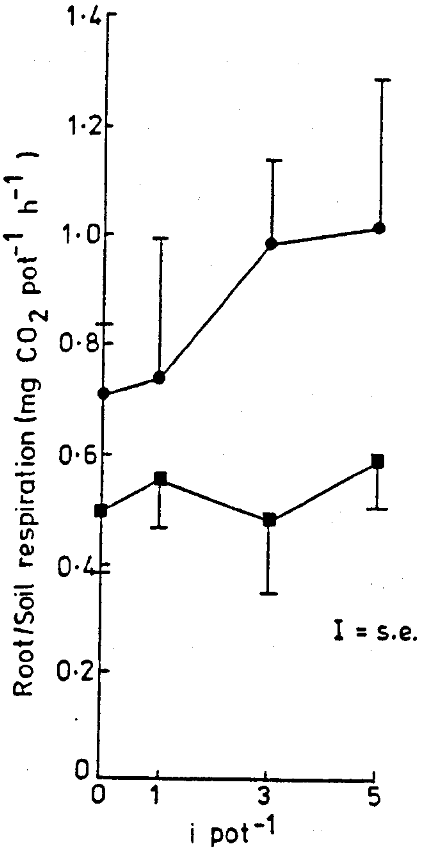


Fig. 43

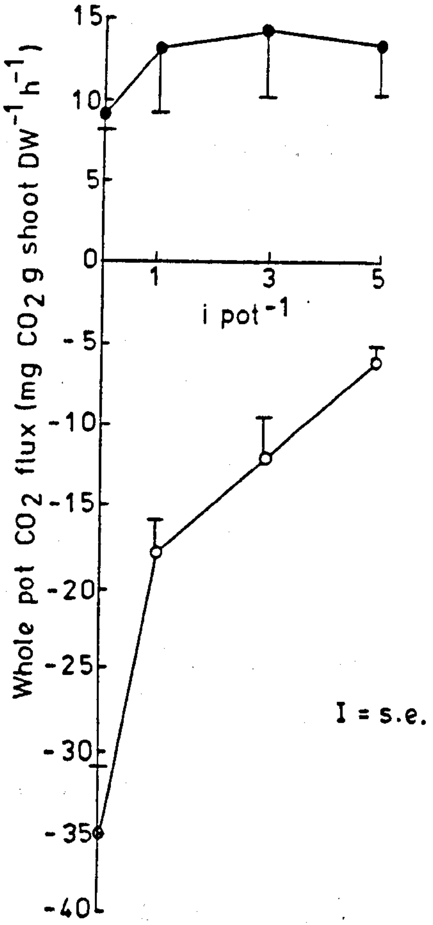


Fig. 44

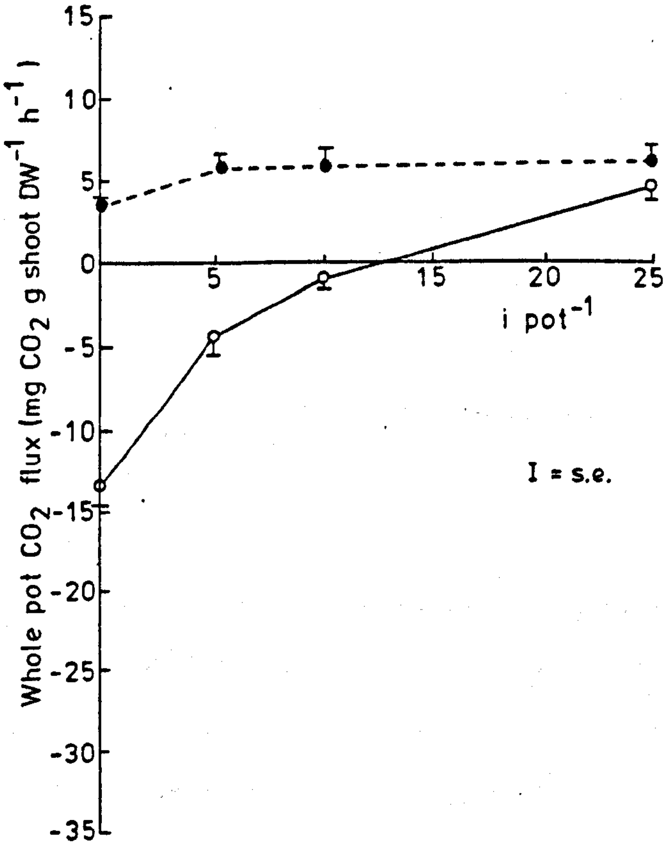


Fig. 45

10, 25 i pot^{-1}. There was no significant difference between 5, 10 and 25 i pot^{-1}. There was a significant increase ($p<0.01$) of soil/root CO_2 flux with simulated trampling but there was no significant effect of treatment intensity above 0 i pot^{-1}.

Simulated trampling had a significant effect ($p<0.001$) on net photosynthesis (whole pot CO_2 flux minus soil/root CO_2 flux), which was reduced (i.e. neared the compensation point) when simulated trampling was applied (Fig. 46). There was however, no significant difference between the treatments of 5, 10 and 25 i pot^{-1}. There was also a significant increase ($p<0.05$) in shoot dark respiration rate with simulated trampling (Fig. 46) but no significant difference between treatment intensity above 0 i pot^{-1}.

(iii) Results are given in Fig. 47. Simulated trampling reduced significantly ($p<0.01$) the rate of whole pot CO_2 flux in response to light but there was no significant difference between 1, 3 and 5 i pot^{-1}.

Rates of whole pot CO_2 flux in the dark were not significantly affected by simulated trampling although there appeared to be a slight increase with increasing number of impacts (Fig. 47).

c) CO_2 flux at lower light intensities with simulated trampling.

(i) Results are given in Fig. 48. There was a significant reduction ($p<0.001$) of rate of CO_2 flux in light following simulated trampling but no significant difference between rates measured at 1, 6 and 7 hours after treatment.

There was no significant difference between rates of CO_2 flux in the dark for pots receiving 5 i pot^{-1} at 1, 6, 7 hours before measurement and also no significant difference between treated and untreated pots of grass for CO_2 flux in the dark (Fig. 48).

Fig. 46 Response of shoot net photosynthesis and dark respiration to different levels of simulated trampling

○ net photosynthesis at >45 klux

● dark respiration

Fig. 47 Response of whole pot CO_2 flux (root/soil + shoots) to light and dark at different levels of simulated trampling applied to shoots only.

○ net photosynthesis at >45 klux

● dark respiration

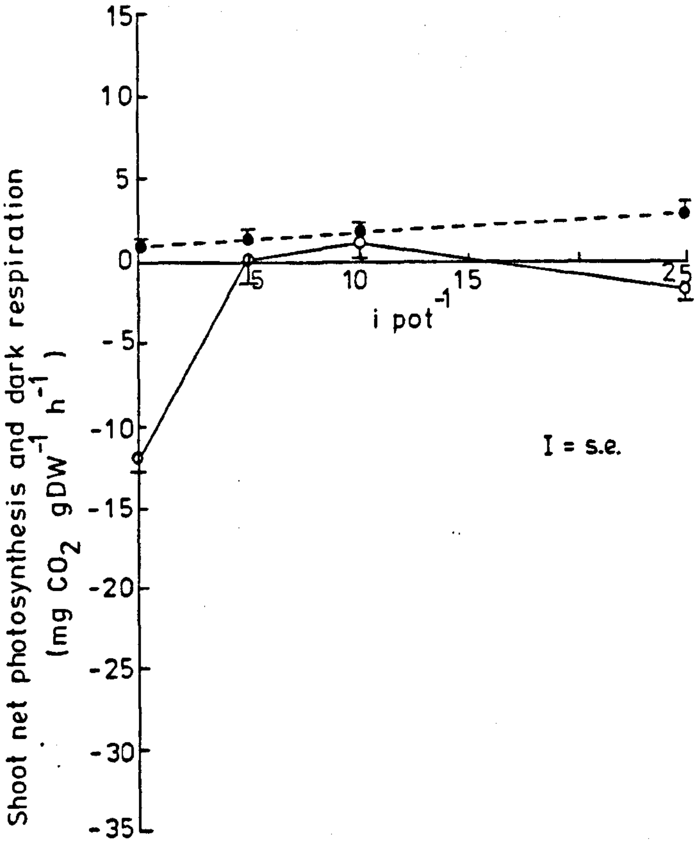

Shoot net photosynthesis and dark respiration (mg CO_2 gDW^{-1} h^{-1})
15
10
5
0
-5
-10
-15
-20
-25
-30
-35
5
10
15
25
i pot^{-1}
I = s.e.

Fig. 46

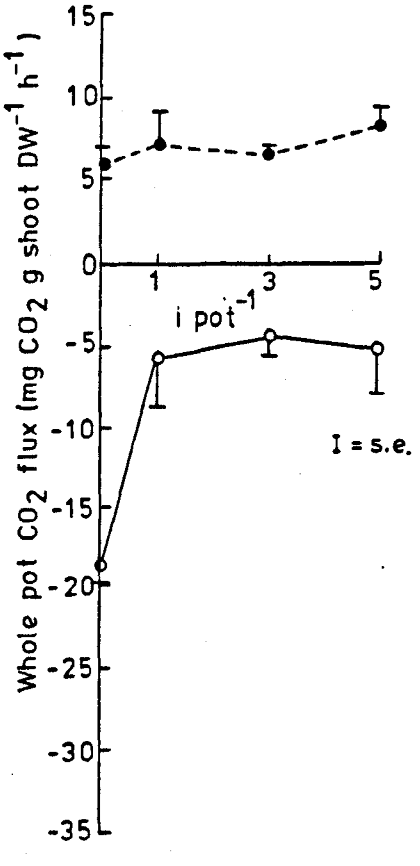

Whole pot CO_2 flux (mg CO_2 g shoot DW^{-1} h^{-1})
15
10
5
0
-5
-10
-15
-20
-25
-30
-35
1
3
5
i pot^{-1}
I = s.e.

Fig. 47

Response of whole pot CO_2 flux (root/soil + shoots) to light and dark with simulated trampling treatment:

Fig. 48 At 1, 6, 7 hours prior to measurement.

Fig. 49 At 0.5 and 3.5 hours prior to measurement.

Fig. 50 Response of shoot CO_2 flux to light and dark with simulated trampling treatment at 0.5 and 3.5 hours prior to measurement.

●—● Assimilation at 11 klux for 5 i pot^{-1}
●---● Dark respiration for 5 i pot^{-1}
▧ Assimilation at 11 klux for 0 i pot^{-1}
▨ Dark respiration for 0 i pot^{-1}
▩ Assimilation at 11 klux for 5 i pot^{-1} 0.5 hours after treatment
▤ Assimilation at 11 klux for 5 i pot^{-1} 3.5 hours after treatment
▥ Dark respiration for 5 i pot^{-1} 0.5 hours after treatment
▦ Dark respiration for 5 i pot^{-1} 3.5 hours after treatment

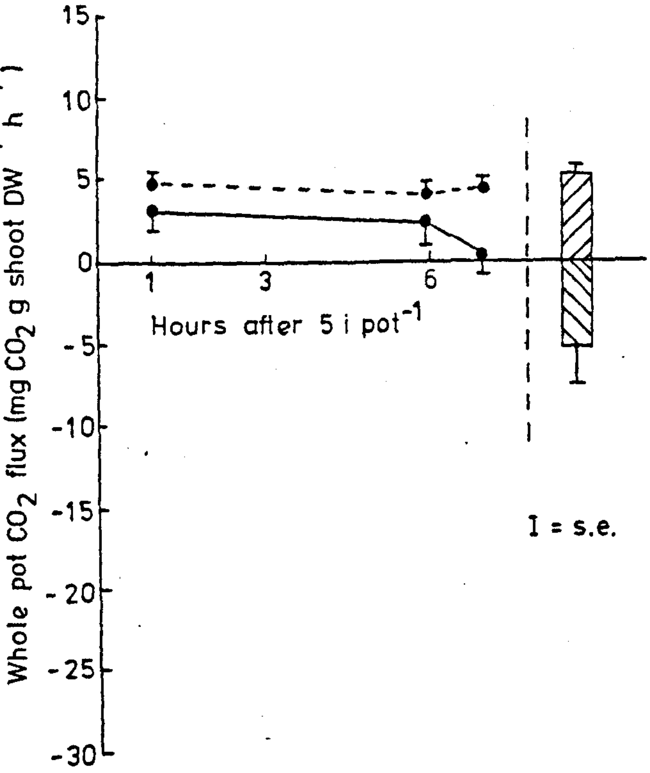


Fig. 48

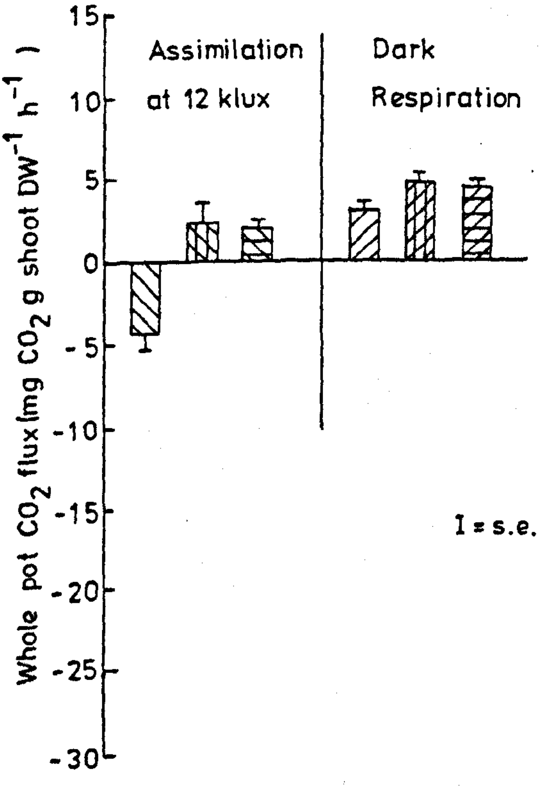


Fig. 49

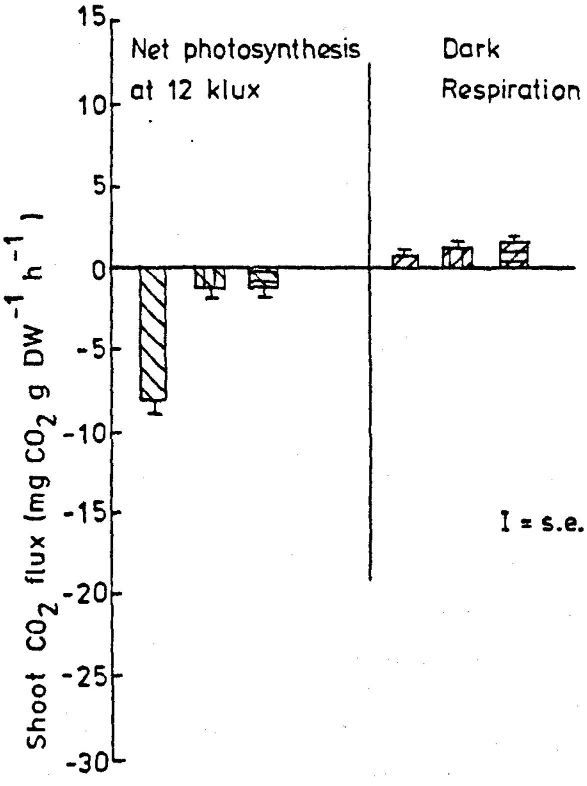


Fig. 50

(ii) There was a significant reduction ($p<0.001$) of whole pot CO_2 flux in the light with simulated trampling (Fig. 49). There was no significant difference between the rates of whole pot CO_2 flux at 0.5 and 3.5 hours after 5 i pot^{-1}.

Whole pot CO_2 flux in the dark increased significantly ($p<0.05$) with increased number of impacts (Fig. 49). Again there was no significant difference between rates of whole pot CO_2 flux at 0.5 and 3.5 hours after 5 i pot^{-1}.

There was no significant difference between soil/root CO_2 flux rates for all treatments.

For net photosynthesis (whole pot CO_2 flux minus soil/root CO_2 flux), 5 i pot^{-1} significantly reduced ($p<0.001$) the rate of CO_2 flux (Fig. 50). Dark respiration (whole pot CO_2 flux minus soil/root CO_2 flux) of shoots was increased by simulated trampling but this was not significant (Fig. 50). For 0.5 and 3.5 hours after 5 i pot^{-1}, rates of net photosynthesis and also shoot dark respiration were not significantly different.

d) Continuous monitoring of CO_2 flux before and after simulated trampling.

(i) Fig. 51 shows the change in whole pot CO_2 flux in the light when 5 impacts of the tamp were applied. There was an immediate reduction in rate followed by a slow partial recovery over the subsequent 6 hours. At 6 hours after simulated trampling, the rate was still considerably lower than the rate before treatment, approximately -1.0 compared to -6.0 mg CO_2 g shoot DW^{-1} h^{-1}.

(ii) Whole pot CO_2 flux in the dark showed virtually no change when 5 impacts of the tamp were applied (Fig. 52). Before and after treatment, the rate was approximately +0.9 mg CO_2 g shoot DW^{-1} h^{-1}.

Moving average of whole pot CO_2 flux measured continuously:

Fig. 51 Before and after 5 impacts in light at 11 klux

Fig. 52 Before and after 5 impacts in dark conditions

Fig. 53 Prolonged time before and after 5 impacts in light at 11 klux

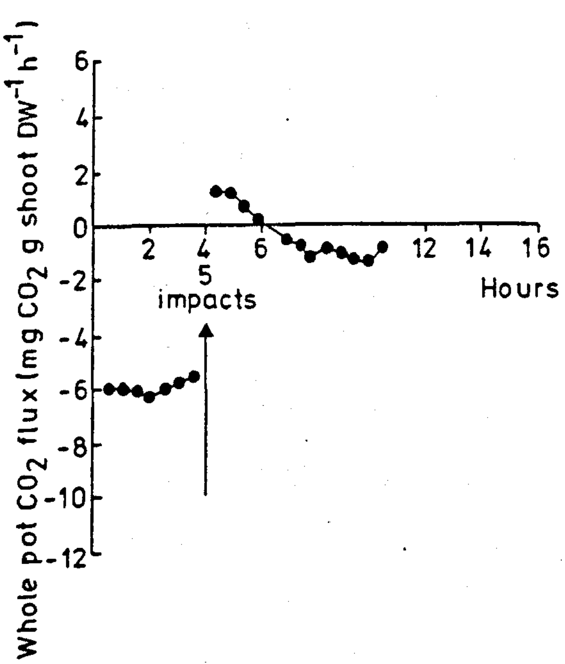


Fig. 51

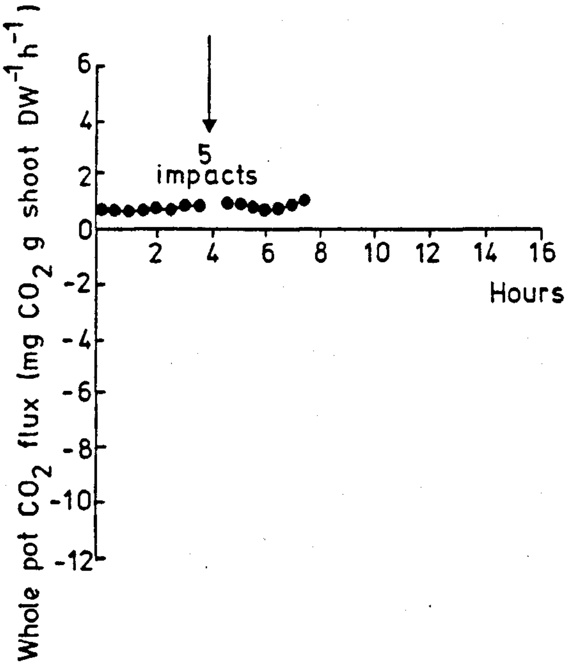


Fig. 52

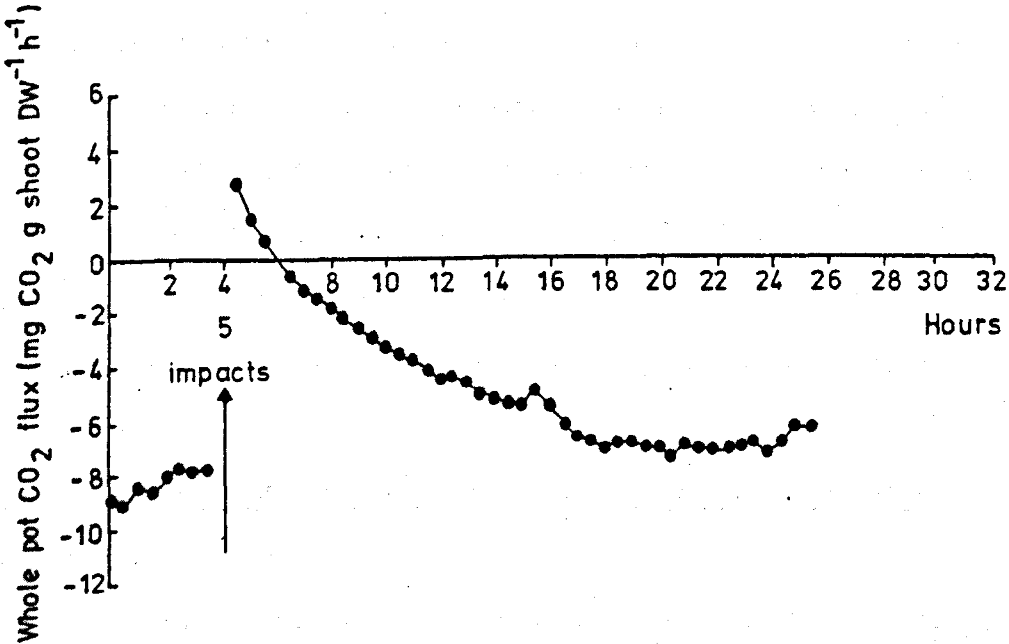


Fig. 53

(iii) Fig. 53 shows whole pot CO_2 flux in the light at prolonged times before and after 5 impacts of the tamp. Before treatment, CO_2 flux remained at a rate of approximately -8 mg CO_2 shoot DW^{-1} h^{-1}. Immediately after treatment, the rate was reduced to over +2 mg CO_2 g shoot DW^{-1} h^{-1} but slowly increased in rate over the next 20 hours to approximately -7 mg CO_2 g shoot DW^{-1} h^{-1}.

(iv) At 58 klux, whole pot CO_2 flux before treatment averaged at approximately -39 mg CO_2 g shoot DW^{-1} h^{-1} (Fig. 54). Immediately after 25 impacts of the tamp, CO_2 flux was reduced to about +10 mg CO_2 g shoot DW^{-1} h^{-1} (Fig. 54). Over the subsequent 15 hours, the rate gradually increased to around +6 mg CO_2 g shoot DW^{-1} h^{-1} at 15 hours after treatment.

(v) Fig. 55 shows the change in whole pot CO_2 flux in the dark after 25 impacts was applied. Pre-treatment rate was steady at about +10 mg CO_2 g shoot DW^{-1} h^{-1}. Immediately after treatment, the CO_2 flux rose reaching +16 mg CO_2 g shoot DW^{-1} h^{-1}, 2.5 hours later. Thereafter, the rate declined very gradually. 26 hours after treatment, the rate was approximately +11 mg CO_2 g shoot DW^{-1} h^{-1}.

Root/soil CO_2 flux was measured at the end of the monitoring period and was found to be +3.5 mg CO_2 pot^{-1} h^{-1}.

(vi) Root/soil CO_2 flux was measured over a period of 25 hours and was found to remain at a steady rate of approximately +0.83 mg CO_2 pot^{-1} h^{-1}.

e) <u>CO_2 flux at different relative humidities with simulated trampling</u>.

(i) Results are given in Fig. 56. In light, whole pot CO_2 flux was significantly decreased ($p<0.001$) by simulated trampling but there was no significant effect of humidity for either 0 or 15 i pot^{-1}. In the dark, whole pot CO_2 flux was increased significantly ($p<0.001$) by simulated trampling and there was a significant effect ($p<0.001$) of

Moving average of whole pot CO_2 flux measured continuously:

Fig. 54 Before and after 25 impacts in light at 58 klux

Fig. 55 Before and after 25 impacts in dark conditions

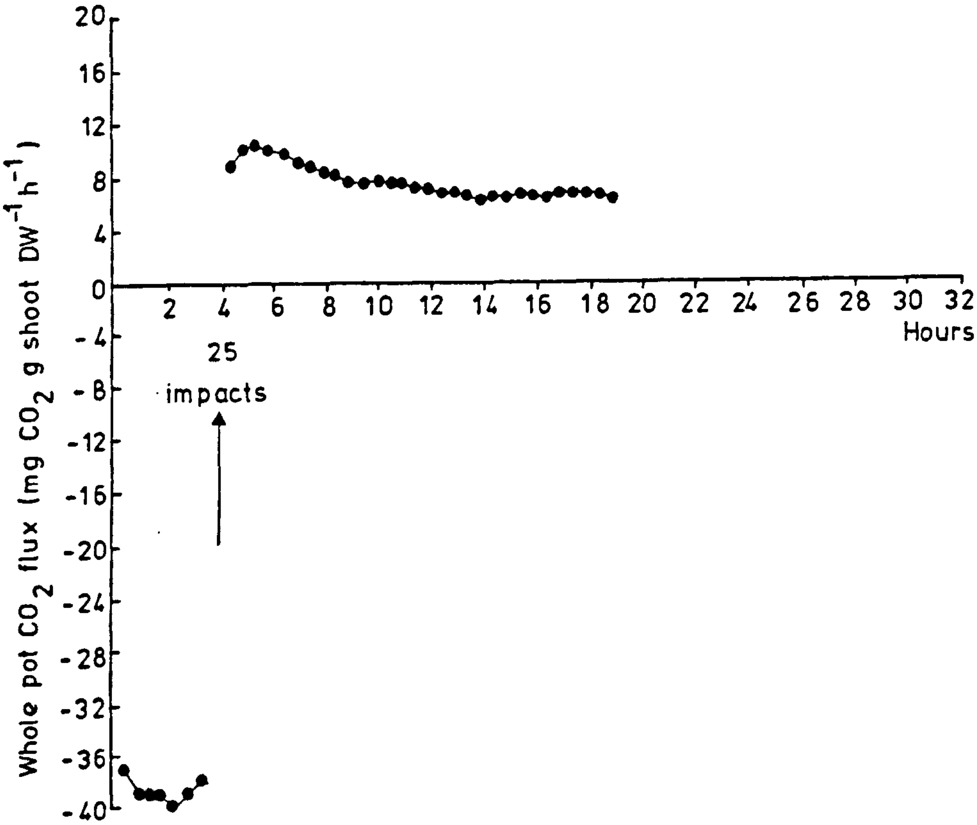

Whole pot CO$_2$ flux (mg CO$_2$ g shoot DW^{-1}h^{-1})
20
16
12
8
4
0
-4
-8
-12
-16
-20
-24
-28
-32
-36
-40
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
Hours
25
impacts

Fig. 54

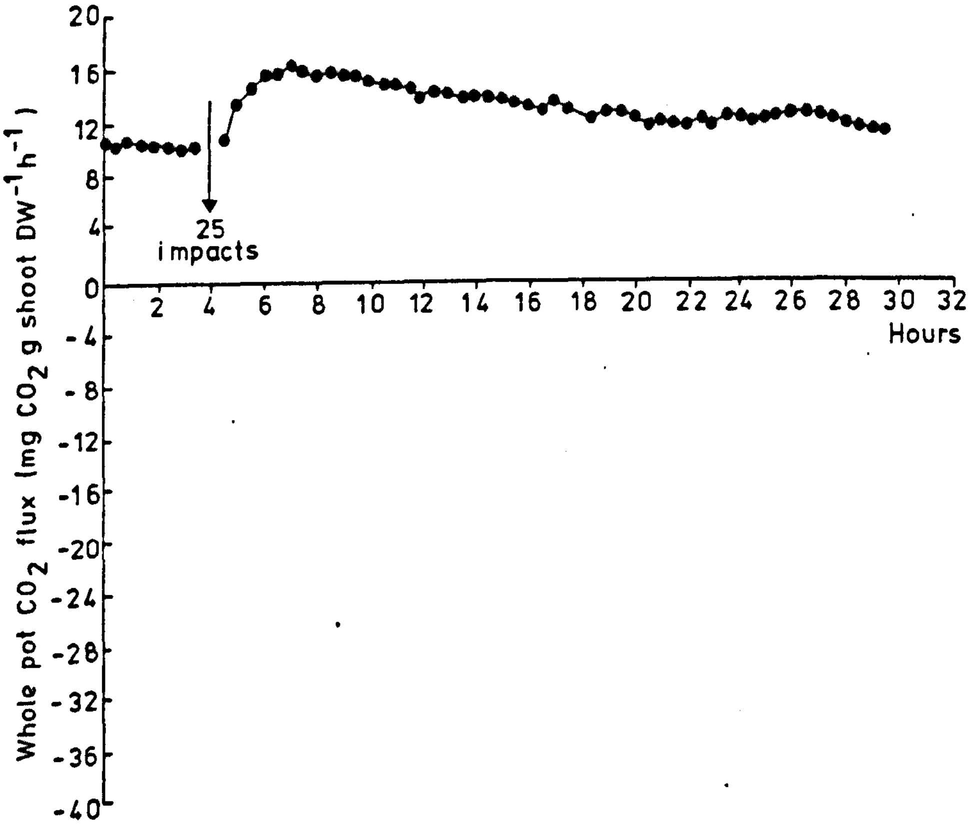

Whole pot CO$_2$ flux (mg CO$_2$ g shoot DW^{-1}h^{-1})
20
16
12
8
4
0
-4
-8
-12
-16
-20
-24
-28
-32
-36
-40
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
Hours
25
impacts

Fig. 55

Fig. 56 Response of whole pot CO_2 flux to different relative humidities with simulated trampling: 15 i pot^{-1}.

○ 0 i pot^{-1}, light
● 0 i pot^{-1}, dark
□ 15 i pot^{-1}, light
■ 15 i pot^{-1}, dark

Fig. 57 Response of whole pot CO_2 flux to different relative humidities with simulated trampling: 5 i pot^{-1}.

○ 0 i pot^{-1}, light
● 0 i pot^{-1}, dark
□ 5 i pot^{-1}, light
■ 5 i pot^{-1}, dark

Fig. 58 Relationship between injury index and whole pot CO_2 flux in light and dark conditions.

Fig. 59 Relationship between injury index and shoot CO_2 flux in light and dark conditions.

F = light (>45 klux)
R = dark
● = 0 i pot^{-1}
▲ = 5 i pot^{-1}

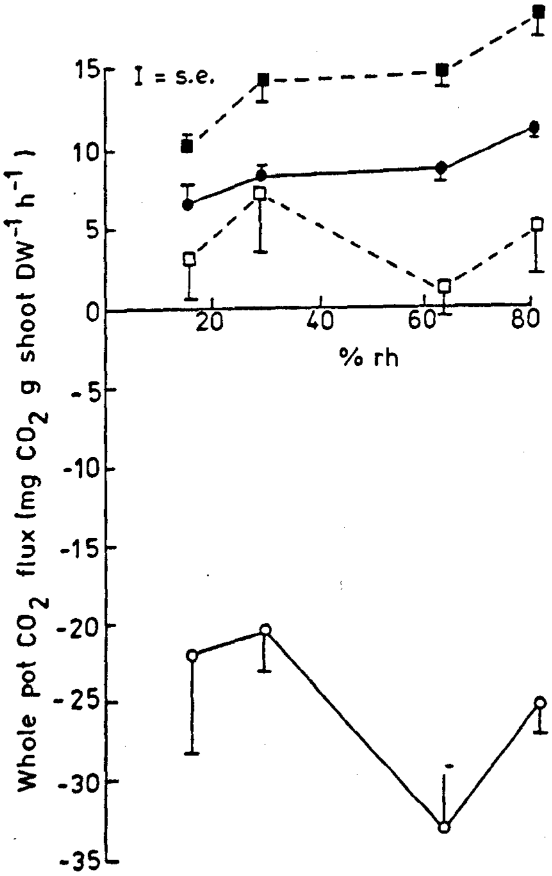


Fig. 56

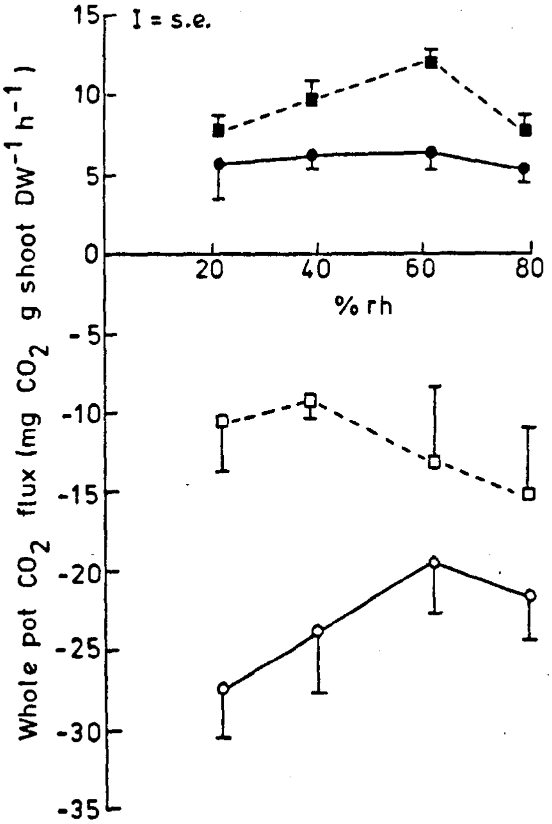


Fig. 57

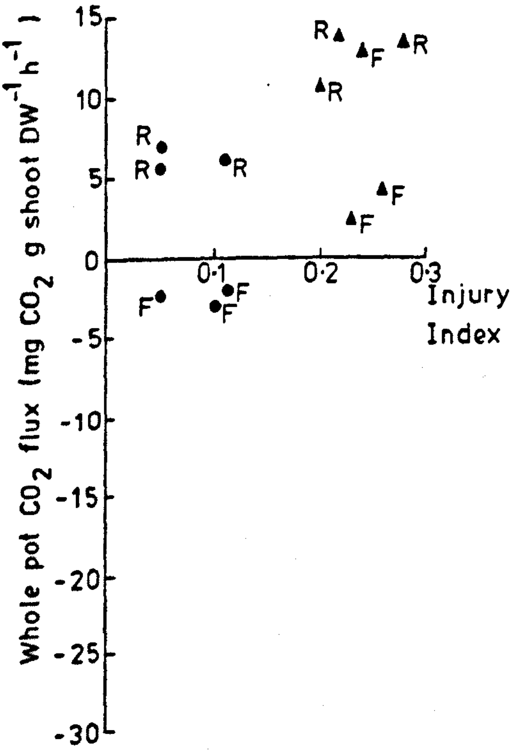


Fig. 58

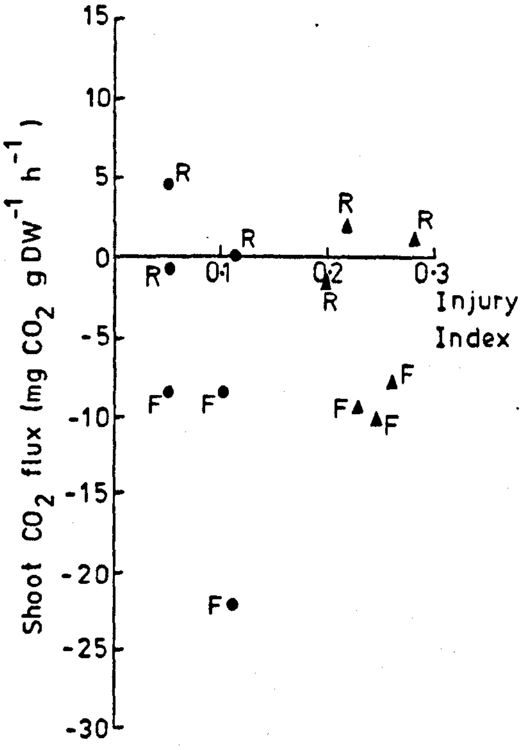


Fig. 59

humidity, rates of dark CO_2 flux increasing with increasing relative humidity.

(ii) Fig. 57 shows the response to slightly different humidities with simulated trampling at a lower intensity. Rates of CO_2 flux in light were significantly lower ($p<0.001$) for 5 i pot^{-1} compared to those of 0 i pot^{-1} although this was only at the two lower relative humidities. There was no significant effect of humidity on light CO_2 flux for either 0 or 5 i pot^{-1}.

In dark conditions, whole pot CO_2 flux was significantly lower ($p<0.001$) for 0 i pot^{-1} compared to that of 5 i pot^{-1}. At 0 i pot^{-1} there was no significant effect of humidity. For 5 i pot^{-1} there was a significant increase in CO_2 flux up to 62% relative humidity and then a sharp decline.

f) Comparison of CO_2 flux with electrolyte release in response to simulated trampling.

Results are shown in Figs. 58 and 59. For whole pot CO_2 flux, there was a positive correlation between CO_2 flux and injury index in both light and dark conditions indicating that an increase in injury related to a decrease in CO_2 flux in the light and related to an increase in CO_2 flux in dark conditions. Correlation coefficients were $R = 0.755$ for whole pot CO_2 flux in the light and $R = 0.958$ for whole pot CO_2 flux in the dark. Because of limited number of pairs of values, however, the correlation was only significant ($p<0.01$) for dark whole pot CO_2 flux with injury index.

For shoot CO_2 flux, there was little relationship between injury index and CO_2 flux, the correlation coefficient being $R = -0.194$ in light and $R = -0.123$ in dark conditions, both values being non-significant.

Discussion

Although the experiments of section a) (i), (ii) and also b) (iii), c) (ii) and d) (vi) showed a non-significant effect of simulated trampling with time and intensity on soil/root CO_2 flux following shoot excision, a small but significant increase was recorded in section b) (ii) after simulated trampling. From the results of the various sections, it would seem that simulated trampling may have caused a slight increase in soil/root respiration rate but that usually this was too small to be analysed as significant. As the increase appeared to be small, soil/root CO_2 flux was still used for interpretation of later experiments where the results were expressed only in terms of whole pot CO_2 flux, therefore assuming that changes in whole pot CO_2 flux were predominantly due to changes in shoot photosynthesis and/or respiration.

Various factors have been observed to influence soil/root respiration in the literature. Root respiration rate is apparently dependent upon the rate of growth of the plant (Hunt and Loomis 1979). Hatrick and Bowling (1973) and Farrar (1981) have demonstrated a reduction in root respiration of intact plants when the shoots were exposed to dark conditions. When returned to light conditions for the normal photoperiod, root respiration of barley plants rose (Farrar 1981). Osman (1971) observed that the effects of changed shoot conditions on root respiration were apparent within 12 minutes in wheat. Further, Farrar (1981) showed that the respiration rate of excised roots depended on the pre-excision conditions. Where the intact plant had been exposed to dark, the subsequent excised root respiration rate was 40% of that for plants that had been exposed to light. It was perhaps surprising, therefore, that there was little effect of simulated trampling and time on soil/root respiration in the present study as, from section b), reduction in light CO_2 flux of shoots with simulated trampling may have been expected to cause a reduction in soil/root CO_2 flux where grass was left for 24 hours after simulated trampling before shoot excision and soil/root CO_2 flux determination. Shoot excision may have been a significant factor.

The response of soil/root respiration to shoot excision, however, is not clear from the literature. In the field, "removal of tops" caused a reduction in root respiration (Redmann and Abouguendia 1978). Humphries (1951) showed a falling of root respiration following excision. Excision of plant material however, has been demonstrated to initiate a complex series of metabolic alterations (Ap Rees 1966, Laties 1967, Kahl 1973) including a prolonged increase in respiration rate. The initial peak in respiration rate occurred 90 minutes after cutting of leaf discs (Macnicol 1976). After this initial "wound" respiration, there was a further "induced" increased respiration rate taking 15 to 40 hours to reach its peak. The extent of increased respiration rates from the point of wounding is not clear, however. The results of the present study did not show either significant reduced or increased rates of soil/root respiration at different times after excision of shoots.

Another aspect, the soil compaction effect of simulated trampling which is proportional to intensity of treatment, may also produce a change in root respiration, because of wounding of roots. Although there appears to be no published work on effects of soil compaction on root respiration, various work (Wilkins _et_ _al._ 1976, Goss 1977, Goss and Scott Russell 1980) has shown an effect on root growth, apparently by endogenous ethylene synthesis (Goeschl _et_ _al._ 1966, Kays _et_ _al._ 1974). As wounding of leaves results in formation of ethylene (Konze and Kwiatkowski 1981), it suggests that the ethylene synthesis of roots in compacted soils is similarly a wounding response. It has been long established (Audus 1935) that mechanical stimulation causes increased respiration rates. It would therefore seem likely that soil compaction may cause increased rates of root respiration. Again, however, this was not demonstrated in the results obtained here.

It is possible that these responses were all present but not detected. A significant feature of the CO_2 flux measurement method in the present study was that an annulus of plastic was placed over the soil surface to prevent soil displacement. This cover may have prevented normal CO_2 diffusion from the soil and roots. The values of soil/root CO_2 flux obtained would therefore be reduced. This may explain lack of significant results regarding effects of simulated trampling and time on soil/root respiration. At the other extreme, the response to excision of shoots may have been large enough to mask differential responses to simulated trampling.

In experiment a) (iii) a significant effect of soil moisture on soil/root respiration was shown. This confirmed the practice of maintaining all conditions as identical as possible from one experiment to another. Again, there was no significant difference between different intensities of simulated trampling. Increases in soil moisture content (and temperature) are known to increase soil respiration rates (Hutchinson and King 1982, de Jong _et al._ 1974) due to greater microbial activity (Currie 1974, Pritchard 1979, Parkinson 1981). It has also been shown that drying of roots reduces root respiration (Crappo and Coleman 1972, Hutchinson and King 1982).

In all experiments with irradiance both above the saturation level and at lower levels of 10 to 12 klux, simulated trampling caused a very significant decrease in whole pot CO_2 flux and shoot CO_2 flux.

At high light irradiances, whole pot CO_2 flux decreased significantly as simulated trampling increased. Shoot CO_2 flux, however, showed no significant difference to intensity of simulated trampling even where the range of treatment was 5 to 25 i pot^{-1}. This contrasting result is not easily explained as the only difference between shoot CO_2 flux and whole pot CO_2 flux was the soil/root CO_2 flux which was tested and found to have a small increase but which was usually not significant with simulated trampling treatments.

At high light irradiance and 0 i pot^{-1}, ranges of -13 to -23 mg CO_2 g shoot DW^{-1} h^{-1} were recorded for whole pot CO_2 flux compared to -11 mg CO_2 g DW^{-1} h^{-1} for shoot CO_2 flux. At lower light irradiance and 0 i pot^{-1} ranges of -4 to -7 mg CO_2 g shoot DW^{-1} h^{-1} were recorded for whole pot CO_2 flux which compared to -8 mg CO_2 g DW^{-1} h^{-1} shoot CO_2 flux. When 5 i pot^{-1} was applied and with high light irradiance, ranges of -2 to -5 mg CO_2 g shoot DW^{-1} h^{-1} for whole pot CO_2 flux and 0 mg CO_2 g DW^{-1} h^{-1} for shoot CO_2 flux were obtained. At lower light irradiance and 5 i pot^{-1} whole pot CO_2 flux ranged from +3 to +1 mg CO_2 g shoot DW^{-1} h^{-1} while shoot CO_2 flux was -2 mg CO_2 g DW^{-1} h^{-1}. Cooper and Tainton (1968) found increasing rates of photosynthesis according to light intensity up to levels of 20 to 30 klux which they considered to be light saturation levels for Lolium perenne. It appears that whole pot CO_2 flux in light is related to the light irradiance, and the effect of simulated trampling in reducing CO_2 flux is more or less superimposed upon the effects of light irradiance. There is a much less marked effect of light intensity on shoot CO_2 flux. Again, this is difficult to explain as the only difference was soil/root CO_2 flux which was not significant in most experiments.

Where shoots only received simulated trampling there was a significant effect of treatment, but between the different levels of simulated trampling there was no significant difference. This may be due to omitting the effects of soil compaction (see above) but as the range of treatments was only between 1 and 5 i pot^{-1}, the lack of any significant difference may well be attributed to similarity of treatments along with natural variation in response.

There appears to be no work published on how ryegrass photosynthesis and respiration is influenced by trampling so only studies on the effects of other stresses, particularly wind, on photosynthesis and respiration can be compared.

Russell and Grace (1978a) investigated the response of Lolium perenne to continuous wind stress. Plants exposed to a wind speed of 1.7 m s^{-1} showed a slightly reduced rate of net photosynthesis (expressed on a leaf area basis) although this was not significantly lower. The lack of significant effects was rather a surprise as it had been demonstrated in Festuca arundinacea (Grace and Thompson 1973) that wind treated plants, given 3.5 m s^{-1}, showed a reduction in net photosynthesis particularly at high irradiance. (This reduction was not due to an increase in dark respiration which was not significantly different compared to control plants.) They found that mesophyll resistance in wind treated plants was higher than controls whilst leaf surface resistance was lower than in the controls. The high mesophyll resistance in the wind treated plants was attributed to reduced water content. The difference in photosynthetic response compared to that of ryegrass may be due to the lower wind speed used to treat the ryegrass by Russell and Grace (1978a) of 1.7 m s^{-1} compared to 3.5 m s^{-1} used by Grace and Thompson (1973). Armbrust (1982) attributed reduced growth of wind and sand blast treated plants in part to a loss of viable tissue and reduced photosynthesis rate.

In virtually all experiments of sections b) and c), there were slight increases in dark whole pot CO_2 flux and of dark shoot CO_2 flux in response to simulated trampling. In some cases, the increase was significant (b)(iii), Fig. 46 and c)(ii), Fig. 49). Response of whole pot CO_2 flux and shoot CO_2 flux were similar. Rates for 0 i pot^{-1} ranged from +6 to +3 mg CO_2 g shoot DW^{-1} h^{-1} for whole pot CO_2 flux and +0.5 to +1 mg CO_2 g DW^{-1} h^{-1} for shoot CO_2 flux. For all simulated trampling treatments, rates ranged from +9 to +4 mg CO_2 g shoot DW^{-1} h^{-1} and +2 to +1 mg CO_2 DW^{-1} h^{-1} for whole pot and shoot CO_2 flux, respectively. Where shoots only were treated, there was a similar slight increase in dark whole pot CO_2 flux although this was not significant.

Todd et al. (1972) showed that a wind speed of 3.6 m s^{-1} and above caused a marked increase in dark respiration of wheat plants. Armbrust (1982) concluded that sorghum plants exposed to wind and sandblast injury showed increased respiration. Russell and Grace (1978a) found increased respiration rates in Lolium perenne, exposed to continuous wind at 1.7 m s^{-1}, yet Grace and Thompson (1973) found no significant increase in wind exposed Festuca arundinacea respiration rates. Differences in treatment severity and species susceptibility may explain the variations. It would seem that as for wind stress, simulated trampling does have some effect, increasing the dark respiration rate slightly.

Continuous monitoring further revealed the overall response to simulated trampling. With no simulated trampling, whole pot CO_2 flux remained very consistent over the time monitored (up to 9 hours, Fig. 53) and the rate was very clearly a response to the level of light irradiance: -39 mg CO_2 g shoot DW^{-1} h^{-1} at 58 klux (Fig. 54), and between -6 (Fig. 51) and -8 (Fig. 53) at 11 klux. For all experiments, on application of simulated trampling, there was an immediate reduction in whole pot CO_2 flux to +9 mg CO_2 g shoot DW^{-1}

h^{-1} after 25 i pot^{-1} at 58 klux (Fig. 54), to +1.5 mg CO_2 g shoot DW^{-1} h^{-1} (Fig. 51) and +2.5 mg CO_2 g shoot DW^{-1} h^{-1} (Fig. 53) after 5 i pot^{-1} at 11 klux. After 25 i pot^{-1} at 58 klux over a subsequent period of 15 hours the rate gradually increased to +6 mg CO_2 g shoot DW^{-1} h^{-1} (Fig. 54). After 5 i pot^{-1} at 11 klux over a subsequent period of 6.5 hours the rate slowly increased to -1 mg CO_2 g shoot DW^{-1} h^{-1} (Fig. 51) and over a subsequent period of 21.5 hours slowly increased to -7 mg CO_2 g shoot DW^{-1} (Fig. 53). It would appear that simulated trampling causes an immediate response after which there is a slow recovery which is relative to the intensity of treatment applied. It is possible that at the higher intensities, the recovery may take a very prolonged time and where injury is severe, the recovery may consist entirely of new shoot growth replacing senescing damaged shoots.

Without simulated trampling treatments, dark whole pot CO_2 flux remained very consistent over the time monitored but showed rather a wide variation between the different pots of grass: + 0.9 mg CO_2 g shoot DW^{-1} h^{-1} (Fig. 52), +9 mg CO_2 g shoot DW^{-1} h^{-1} (Fig. 55). On application of simulated trampling there was no noticeable effect at 5 i pot^{-1} but a more obvious but not very marked increase after 25 i pot^{-1} which gradually declined over the subsequent time monitored. This was very much in line with earlier observations of only a slight effect of simulated trampling on dark CO_2 flux.

The non-significant effect of a wide range of humidity on light CO_2 flux with or without trampling was surprising as reduction in net photosynthetic rate has been attributed to large leaf to air water vapour pressure differences, mediated through reduced stomatal conductance (Hall and Kaufman 1975, Long and Woolhouse 1978) and more recently there have been suggestions that there is also a nonstomatal mediated reduction in photosynthesis rate (Morrison and Gifford 1983, Bunce 1984). Bunce (1981), however, showed a wide range of sensitivities to humidity of leaf conductance in different species grown in the same environment. Schulze and Kuppers (1979) demonstrated that short-term (i.e. hours) changes in humidity appeared to have limited influence on stomatal conductance in Corylus avellana. In the present study, the humidity conditions were imposed for only the period of time while the grass was in the assimilation chamber hence the findings of Schulze and Kuppers (1979) may apply. That the dark respiration of shoots significantly increased for both untreated and treated grass with increasing humidity in the present study, was not expected. Other evidence of such a phenomenon has not been found. Further research in this area would appear to be very necessary. Comparing whole pot CO_2 flux and shoot CO_2 flux throughout the results, there are some discrepancies. Shoot CO_2 flux was calculated by subtracting soil/root CO_2 flux from whole pot CO_2 flux (section b)(ii), c)(ii), d)(vi)). Shoot CO_2 flux did not necessarily, however, record parallel results to those of whole pot CO_2 flux. Shoot CO_2 flux was based on the assumption that the respiration rate of roots following shoot excision was the same as for intact plants. Removal of shoots, however, may have altered this (see above) hence the calculated value would be erroneous. Results of shoot CO_2 flux were therefore regarded with caution.

Whole pot CO_2 flux represented a combination of soil, root and shoot responses. Root/soil CO_2 flux was only slightly and usually not significantly affected by simulated trampling and time after treatment (section a)) and therefore changes in whole pot CO_2 flux were assumed largely to be due to changes in shoot CO_2 flux. It has also been discussed above that there may have been restricted diffusion of soil/root CO_2 flux and as a result, whole pot CO_2 flux may fairly closely represent net photosynthesis of shoots (as it may consist largely of shoot net photosynthesis rather than the expected shoot net photosynthesis plus substantial soil/root respiration).

The slight increases in dark CO_2 flux with simulated trampling were not sufficient to explain the full reduction in CO_2 flux in light. It was therefore concluded that simulated trampling causes an immediate reduction in rate of photosynthesis, which is related to intensity of treatment, and a small increase in rate of respiration. This is followed by a gradual recovery over a period of hours which seems to be related to some extent to the intensity of treatment. In Chapter 3, a very significant relationship between injury index and intensity of simulated trampling was established. Correlations between injury index and whole pot CO_2 flux in the present Chapter were significant both in the light and dark conditions. Injury index and shoot CO_2 flux however did not correlate significantly. The reason for this difference is not entirely clear but may be due to the proposed errors associated with shoot CO_2 flux and the low replicate numbers used, combined with the usual variability of results and that lower light intensity and low simulated trampling intensity were applied. Alternatively it may suggest that the influence of soil/root CO_2 flux with trampling may be more than indicated from the appropriate experiments (a) (i), (ii), b) (ii), (iii), c) (ii), d) (vi)). Clearly further work is required to clarify this situation.

The decline in photosynthesis with wind stress (Grace and Thompson 1973) was attributed to reduced water content and therefore higher mesophyll resistance, suggesting that similar mechanisms may be at work in grass that has been damaged by simulated trampling. Consequently the effects of simulated trampling on water relations of grass was investigated in the following Chapter.

The effects of water stress in reducing photosynthesis are well documented in reviews such as Hsaio (1973), Hanson and Hitz (1982). Much of the reduction is due to stomatal closure impeding the influx of CO_2 but there are also nonstomatal effects. Cornic *et al.* (1983) presented a simple method to evaluate the relative contributions of stomatal and nonstomatal components during the decline in photosynthesis with rapid water stress. Work by Pasternak and Wilson (1974) had previously shown that reduced net photosynthesis in intact leaves could largely be accounted for by stomatal closure but as excised leaves started and completed stomatal closure at much lower leaf water contents, nonstomatal effects, were therefore much more important in causing decline in photosynthesis. Cornic *et al.* (1983) also concluded that with excised leaves of *Sinapsis alba*, nonstomatal effects were mainly responsible for the decline of photosynthesis. For gradual water stress of intact plants, the stomatal component of the decline was given as 80% so the conclusions of Cornic *et al.* (1983) were similar to those of Pasternak and Wilson (1974). An interesting final comment by Cornic *et al.* was that an increase in respiration rate during water stress could explain the observed nonstomatal effect. This requires further investigation but this suggestion, in light of slight increases after wind stress, as discussed above and also in the findings of the present study, may be of some consequence. As reviewed by Hsaio (1973) and Hanson and Hitz (1982) however, it has been generally found that respiration in water stressed plants is suppressed (proportionately so) but not very markedly. There are exceptions such as Brix (1962) who found an increase in respiration rate with moisture stress.

Clearly more work is required to clarify the response of dark respiration to water stress. Different results may have been obtained with use of excised or intact plants, which in light of work by Cornic *et al.* (1983) may respond differently. There may also be other factors influencing results. Such a factor affecting the response of grass CO_2 flux to simulated trampling may be the effects of wounding. Audus (1935) showed an increase in respiration rate following mechanical stimulation. Interesting effects including changes in electrical resistance and production of ethylene have been shown in response to mechanical stimulation by Jaffe and Biro (1979) and Jaffe (1980) and will be discussed later (Chapter 7).

The response of CO_2 flux when grass is exposed to simulated trampling has been shown in this Chapter to consist of a decrease in shoot photosynthesis and a slight increase in dark respiration although not sufficient to explain the relatively large reduction of photosynthesis. A slight increase in soil/root respiration is also evident. There are many similarities of this response with the response of plants to wind damage as described in the literature. There is evidence that at least the reduction in photosynthesis by wind stress is caused by a resulting induced water stress. This suggests that water stress similarly may play an important part in the response of plants to trampling stress. Aspects of water relations of grass subjected to simulated trampling therefore required investigation.

CHAPTER 5

Water Relations

Introduction

During both simulated trampling experiments on shoot yield response (Chapter 3) and during field experiment trampling treatments (Chapter 6), casual observations had been made that plants suffered some wilting and that damaged parts of leaves dehydrated.

Smith (1978) showed development of a severe water deficit in *Calluna vulgaris* following trampling, and he concluded that the water balance was the major component affected by mechanical damage. Other mechanical stresses such as wind (Grace 1974, 1977, Russell and Grace 1978a, and Fluckiger *et al.* 1978) and defoliation (Hodgekinson 1974) have been shown to cause substantial changes in the water relations of plants. Smith (1978) had also investigated the water relations of *Phleum bertolonii* following trampling. However, his results suggested that the slight changes in water relations of this grass following trampling did not contribute to its capacity to recover from trampling damage. As these conclusions conflict with other observations and as results of response of CO_2 flux to trampling (Chapter 4) suggested that altered water relations may be an important factor, investigations were carried out to examine the effects of simulated trampling on the various components of the water relations of grasses.

Slavik (1974) divided the water relations of any plant into five aspects, namely (i) water potential, (ii) water content, (iii) water exchange between roots and soil, (iv) water movement in plants and (v) water exchange between plants and atmosphere. Many different direct and indirect methods have been developed to measure these aspects (see Slavik 1974) and no one technique is suitable for every situation and specific measurement. Following consideration of the morphology of grass, many methods were found not to be feasible. It was therefore decided to concentrate on three aspects: water potential, water content and water exchange between plant and atmosphere.

Methods

Pots of grass were grown as described in the general methods (Chapter 2). After 5 or 6 days in the growth cabinets in the usual conditions of 18°C day/13°C night, 16 hours photoperiod, 70% relative humidity (unless otherwise stated) and a light intensity of 165 μE m^{-2} sec^{-1} (PAR 400-700 nm Quanta), 31 Wm^{-2} (PAR 400-700 nm horizontal global radiation), 7000 lux (CIE standard illumination) supplied by warm white fluorescent lights, the following experiments were carried out.

a) Water Loss of Excised Shoots.

(i) Evapotranspiration of grass not subjected to simulated trampling.

The standard method (Slavik 1974) for evaluating water content is Relative Water Content (RWC) where

$$RWC = \frac{FW-DW}{SW-DW} \times 100\%$$

SW = Saturated weight

FW = Fresh weight

DW = Dry weight.

All shoots from a pot of grass were excised at the 3 cm height and were immediately weighed and the cut ends placed in water in a beaker covered over with a polythene bag and left to saturate for 24 hours. 6 replicates were left to saturate at 1°C in the dark to exclude error from any extension growth by young leaves during that period (Barrs and Weatherley 1962, Milburn and Weatherley 1971).

Another 6 replicate samples were left to saturate in the growth cabinet at the standard conditions described above, for comparison with samples saturated at 1°C in the dark. Following saturation, the samples were individually blotted dry, weighed and placed in the growth cabinet. Fresh weights were recorded every 30 mins. After 4 hours the grass was dried overnight at 95°C to obtain the dry weights. Following calculation of RWC, graphs showing the decrease in RWC during the 4 hour period were obtained (Figs. 60 and 61). By plotting the rate of water loss against time, graphs were constructed from which the stomatal closure points were determined (Bannister 1964, 1976). Cuticular transpiration rates following stomatal closure were also determined from the same graph.

(ii) Evapotranspiration of grass subjected to simulated trampling.

The above standard RWC method was used on pots of grass that had received 1 impact of the tamp immediately beforehand (Fig. 60 and 61). However, the reliability of using this standard method to measure the water content of more severely damaged grass was examined. Trampling bruises, bends and breaks the grass (Speight 1973). Slavik (1974) mentioned possible errors arising from infiltration of cut edges. This had also been observed to be quite considerable in some species by Barrs and Weatherley (1962) during measurement of RWC of discs of different diameter. Such infiltration would cause higher saturated weights, thus lowering the RWC erroneously. The abraded and torn parts of grass resulting from simulated trampling would be expected, therefore, to take up more water than healthy tissue by infiltration, so giving incorrect saturated weights. Clausen and Kozlowski (1965) found that where xylem was cut and dried out, there was susbsequently a much reduced conduction of water when saturation of the tissue was attempted. This

Fig. 60 Water loss (RWC) of excised shoots: growth cabinet.

Fig. 61 Water loss (RWC) of excised shoots: $1^{\circ}C$, dark.

Fig. 62 Water loss (%W_d) of excised shoots: growth cabinet.

Fig. 63 Water loss (%W_d) of excised shoots: $1^{\circ}C$, dark.

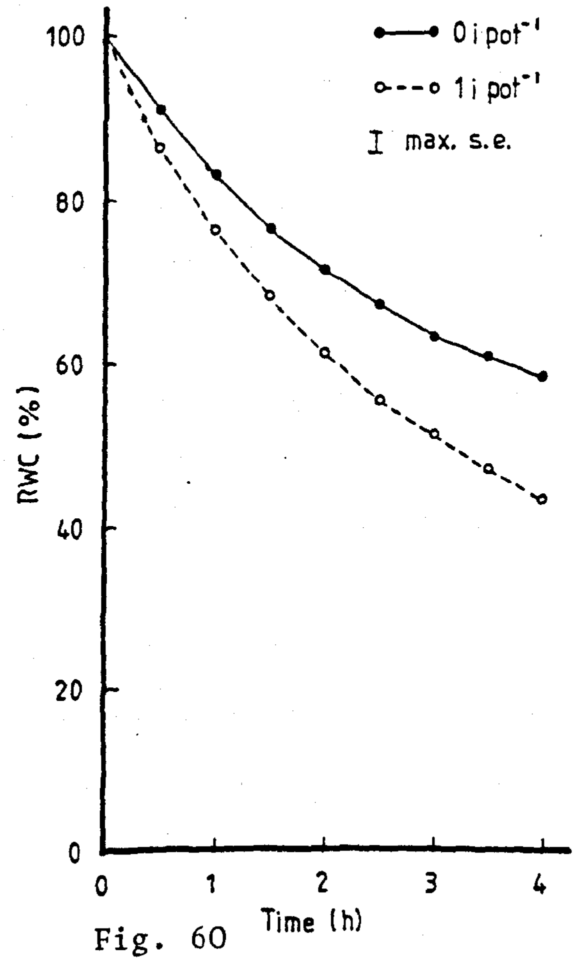


Fig. 60

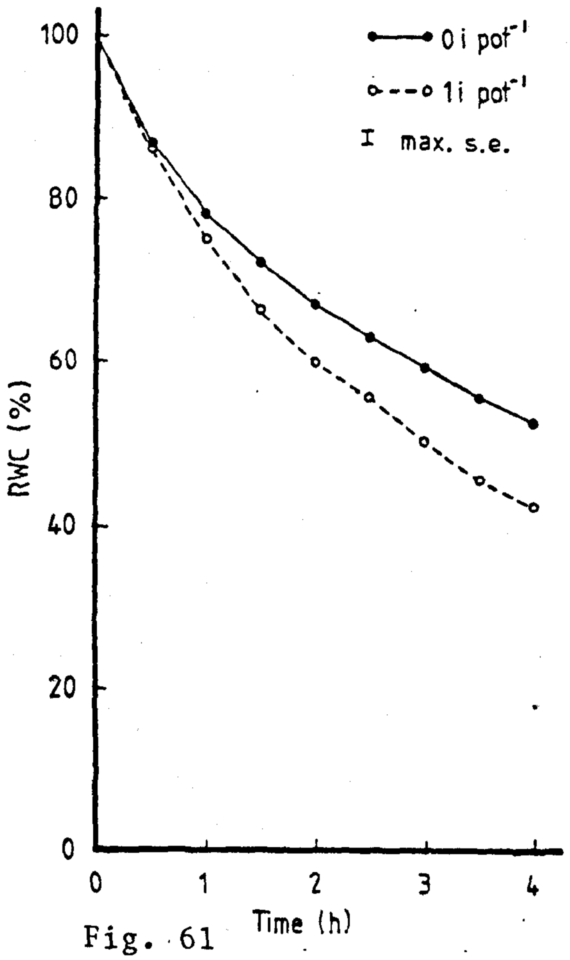


Fig. 61

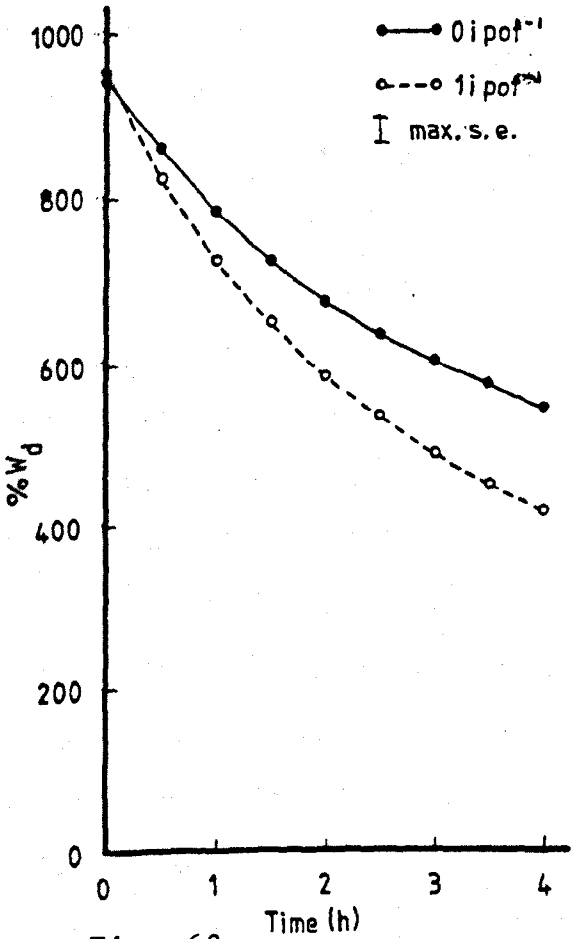


Fig. 62

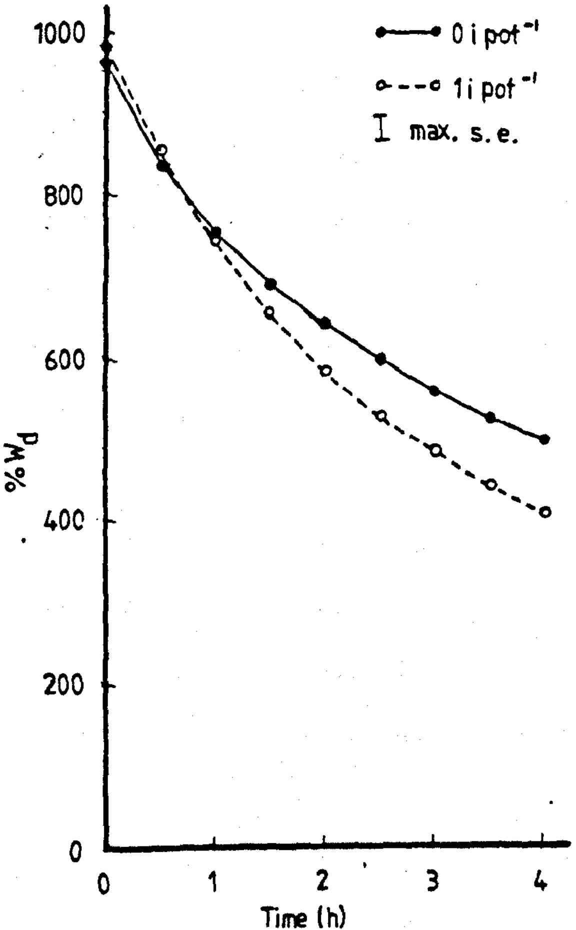


Fig. 63

suggests that where xylem vessels may have been broken from simulated trampling treatments, normal conduction of water to enable saturation of all of the grass leaf would not be totally possible and an incorrect RWC would result. General observations had indicated that the simulated trampling abraded parts of the leaf yet bruised and bent or tore other parts. These bruised, bent and torn parts were observed to dry out.

These considerations suggest that although RWC is the best method for measuring and expressing water content, in these experimental conditions, RWC would be unreliable. It was therefore decided to use a simpler method for measurement of water content in shoots, namely water content as a percent of dry weight defined by Bannister (1976):

$$\%W_d = \frac{FW - DW}{DW} \times 100\%$$

FW = Fresh weight

DW = Dry weight.

Unlike RWC, $\%W_d$ is dependent on dry weight which is prone to fluctuation diurnally and seasonally (Weatherley 1950). In order to be able to compare different experiments, they were all carried out after standard growth conditions, at the same stage of growth and at a similar time so as to minimise spurious relative results, due to these fluctuations.

For comparison of RWC and $\%W_d$, the data from the RWC experiments were recalculated to give the $\%W_d$ and graphs were plotted (Figs. 62 and 63). Similar response curves were obtained. Regression analysis of $\%W_d$ on RWC was carried out to examine the relationship between the two measurements in order to relate $\%W_d$ values to RWC.

Following the above considerations and having established a relationship between RWC and $\%W_d$, further experiments were carried out measuring $\%W_d$ in response to simulated trampling. The above RWC method without the saturation phase was repeated for treatments of 0, 2 and 7 i pot^{-1} with 6 replicates. $\%W_d$ was calculated and graphs of the change in $\%W_d$ over 5 hours were plotted. Graphs of the rate of water loss were used to find the stomatal closure point, this time in terms of $\%W_d$. In the same experiment, 6 other pots of grass were used as RWC controls. The usual RWC method as described above was carried out, obtaining RWC at the commencement of the experiment. The initial $\%W_d$ could therefore be related to RWC.

b) Water content of intact shoots at different intensities of simulated trampling.

The effect of simulated trampling on the water content of various species and cultivars of grass was investigated. For each cultivar, pots of grass were subjected to 0, 1, 3, 5, 7, 10, 15, 20 or 25 i pot^{-1} (however, where germination or growth had been poor in some species, not all these treatments could be applied). After treatment, each pot was immediately replaced in the growth cabinet (conditions as stated above) and left for 2 hours. At exactly 2 hours after treatment, each pot of grass was cut to 3 cm, the shoots immediately weighed and then oven dried at 95°C to obtain dry weights. Each treatment usually had 5 replicates (although where germination or growth had been poor in some species, fewer replicates were available). Graphs were obtained showing $\%W_d$ of shoots 2 hours after simulated trampling at different intensities. The results were analysed using GENSTAT (Lawes Agricultural Trust 1980). For each species and cultivar, polynomial regression analysis of $\%W_d$ on the number of i pot^{-1} were calculated. These showed a significant quadratic component. Log transformations were carried out on the independent variable yielding significant linear regressions.

Subsequently a general linear model using the transformed data was fitted to compare all species and varieties.

c) Transpiration of an intact plant system when subjected to simulated trampling.

To investigate the overall effect of trampling on the water relations of an intact plant system, a simple gravimetric method was used. Pots of grass, watered to field capacity, were subjected to 0, 1, 3, 5 or 7 i pot^{-1} and each treatment had 4, 5 or 6 replicates (according to the germination and growth of the particular species). After treatment, each pot was immediately covered by a polythene bag which was secured round the base of the shoots using a rubber band. (Care was taken not to damage the shoots or constrict the base of the shoots, but to secure it closely enough to prevent water loss apart from via the shoots. The polythene bag was drawn back over itself so there was no interference of water loss from the shoots.) Each pot was weighed and returned to the growth cabinet (at the above standard conditions). Subsequent weighings were taken at 3, 5 and 24 hours on a Mettler PL 1200 electronic balance. The 8 hour dark period occurred between the 5 hour and 24 hour reading. After 24 hours, the shoots were cut to 3 cm, weighed, then dried overnight at 95°C to obtain the dry weights. Transpiration rates (g H_2O g DW^{-1} h^{-1}) averaged over the full 24 hours, transpiration rates over the first 3 hours and %W_d at 24 hours were calculated and graphed. Results were first analysed using analysis of variance and then comparisons of all means were made using the Tukey-Kramer Method (Sokal and Rohlf 1981) to show all significant differences.

d) Effects of humidity on the response of water content and transpiration rates of grass subjected to simulated trampling.

The responses of transpiration rate over 24 hours, transpiration rate over the first 3 hours, %W_d at 24 hours and %W_d at 2 hours to different relative humidity following simulated trampling was examined in Lolium perenne S23. During the corresponding experiments described above in section b) (%W_d at 2 hours) and section c) (transpiration rates and %W_d at 24 hours) further pots of grass from the same batch received identical experimental procedure and conditions but with a relative humidity of 45% for the 5 days preceeding and during the experiment. The responses to 45% relative humidity were compared to those at 70% relative humidity using two-way analysis of variance. Comparisons of all means were made using the Tukey-Kramer method (Sokal and Rohlf 1981) to show all significant differences.

e) Diffusive Resistance Porometry.

As stomatal pores represent a major resistance to the diffusive flux of water vapour in transpiring leaves, measurements of the diffusive resistance of stomatal pores are therefore essential to assess the influence of stomata in relation to transpiration (Slavik 1974). Following the transpiration rate experiments in sections c) and d), diffusive resistance porometry was used to investigate the stomatal response when grass is subjected to simulated trampling.

A Li-Cor Diffusive Resistance Meter LI - 60 with a Horizontal Diffusive Resistance Sensor LI 20S was utilized. Some modifications first had to be made because the sensor is provided with a standard sized aperture pad which was several times wider than the width of any Lolium perenne S23 leaf blade. Uncut aperture pads were readily available. A much narrower aperture pad of 3 mm width was therefore made. The Horizontal Sensor Calibration Resistance Plate consists of a range of various different numbers of holes set in an area equivalent to the standard aperture. The modified pad was thus cut and fitted to the sensor head in such a position that as large a range of calibration values as possible could be obtained. Kanemasu et al. (1969) outlined the standard method of calibration for a Diffusive Resistance Porometer. From the values obtained, a calibration graph was made by plotting time against actual calculated resistance.

Intact plants of Lolium perenne S23 were used. The sensor was held by a clamp and stand. This was adjusted so the sensor could be attached to any leaf in its natural position and cause minimal disturbance. The pot of grass was placed under a 400W High Pressure Sodium light to simulate good photosynthesis and transpiration conditions. Between the light and the grass was a perspex container across which cold water flowed in order to minimise heat from the light. A constant check of air temperature around the grass was kept. Various treatments of 0, 3 or 5 i pot^{-1} were applied and individual leaves from each of the treatments were monitored over a period of time with frequent readings of stomatal diffusive resistance being taken. However there was great difficulty encountered in taking readings using Lolium perenne S23. This was largely due to the leaf being very narrow and also tending to fold lengthwise. Perfect alignment was not always achieved and hence results gave erroneously high diffusive resistances. As readings were so unreliable, this method of determining the stomatal response was not pursued further.

f) Scanning Electron Microscopy. (SEM)

The effects of simulated trampling on the aperture of stomata and also on the surface cells and waxes of grass blades were investigated further following the stomatal diffusive resistance experiments.

Various techniques are available for determining the aperture of stomata including microrelief methods, infiltration methods and direct observation methods (Slavik 1974). Adaxial surface anatomy of

Lolium perenne can be seen as a "ridge and furrow" pattern (see Plate 8) and the majority of the stomata are deeply set into the sides of the furrows. There are therefore difficulties in most direct observation methods. Micro relief methods would be erroneous because such methods are not suitable for leaves with sunken stomata (Slavik 1974). Infiltration methods are completely unsuitable for examining damaged grass, for obvious reasons. The most feasible method therefore for examining stomata was by direct observation using the SEM. This would also facilitate observation of the surface cells and waxes at the same time.

(i) Preparation of material for the SEM.

1 cm long sections were taken from the mid region of selected grass blades and were immediately placed in the recesses of a specially constructed brass container (see Fig. 64). A copper gauze lid was placed over the recesses and the brass container was quickly immersed in liquid nitrogen in a Dewar Flask. After approximately 10 minutes, the brass container was transferred from the Dewar Flask to the plate of an Edwards-Pearse EPD2 tissue dryer for accelerated freeze drying of the grass samples at -60°C for 24 hours. Subsequently, the leaf blade sections were mounted on stubs using double sided sticky-tape and kept in a desiccator. The stubs were then coated with gold palladium at 1.2 kV for 2 successive periods of two minutes duration in an Edwards S150 Sputter Coater. The stubs were examined in a Cambridge Stereoscan S L-10 (secondary electron image) at 15 kV with 2.5 A filament current.

(ii) Experimental Methods.

Initial observations were made of various leaf blade sections. These included Lolium perenne S23 and Phleum pratense S48 which had received 0, 1 or 5 i pot^{-1} at various times prior to sampling (0 hours, 8 days or 5 weeks). Presence and absence of waxes, state of

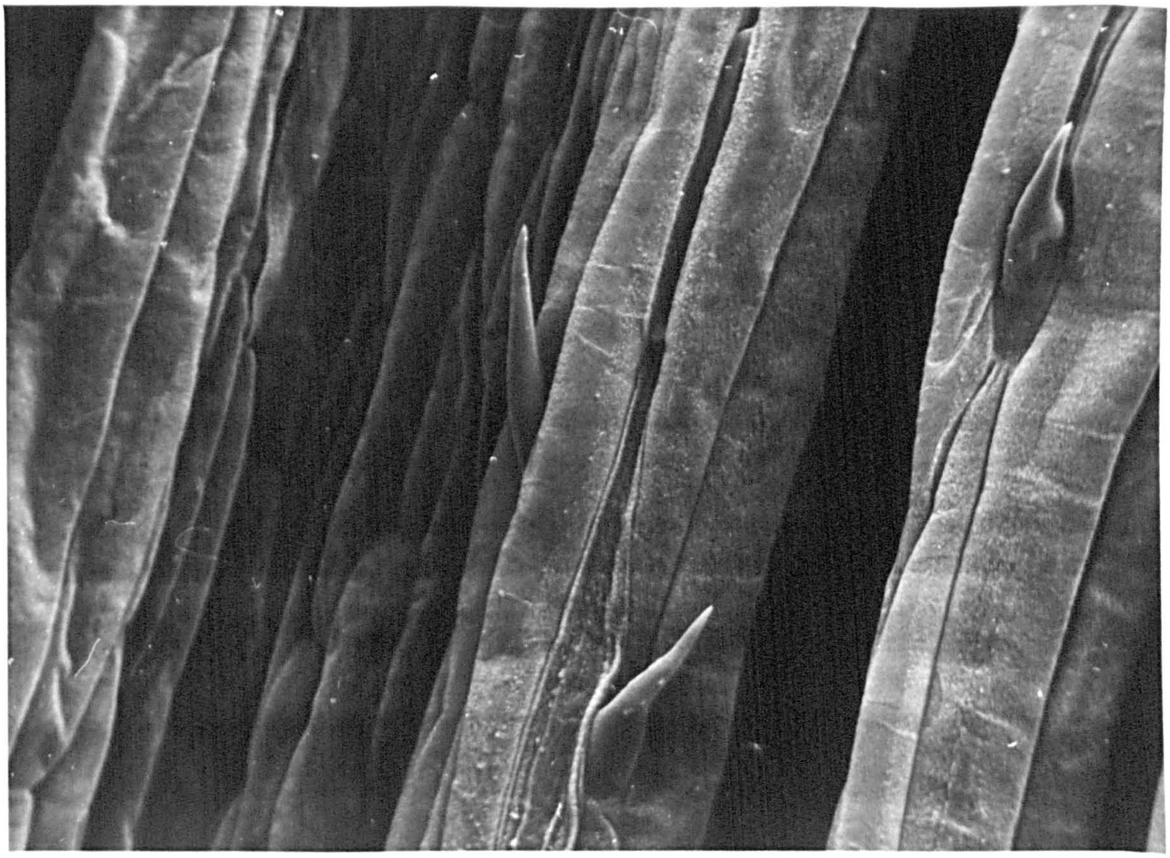


Plate 8 SEM observations:- typical "ridge and furrow" adaxial surface of Lolium perenne S23.

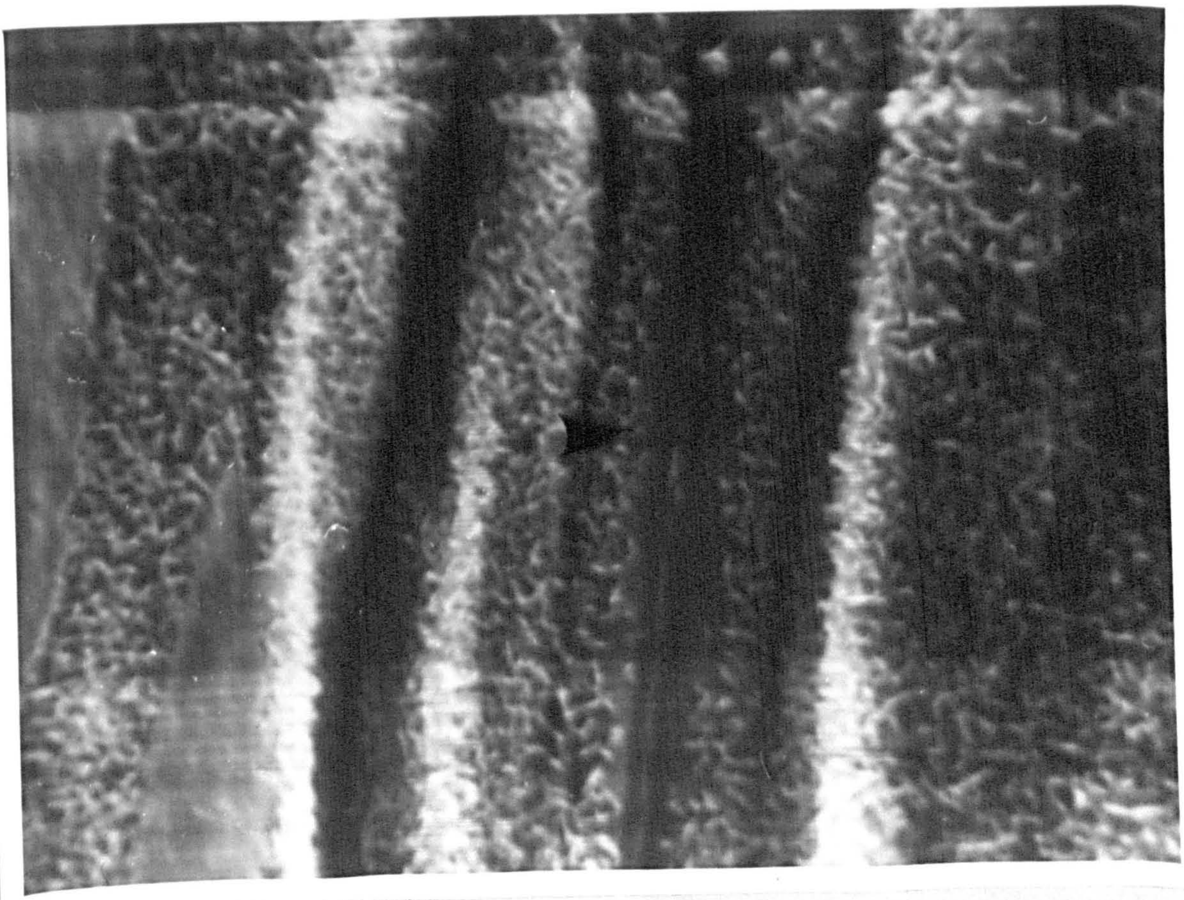


Plate 9 SEM observations:- closed adaxial stoma of Lolium perenne S23.

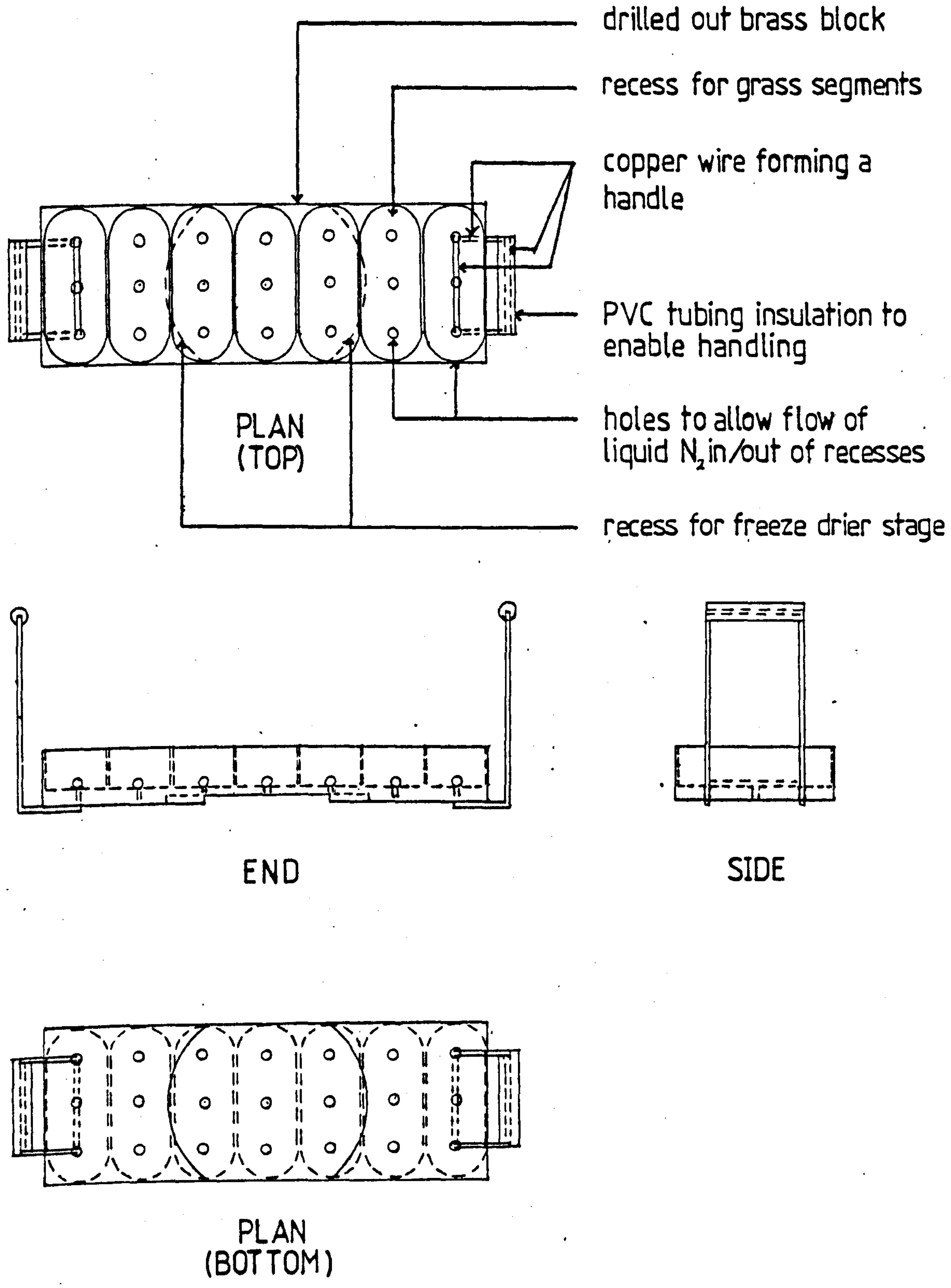


Fig. 64 Apparatus for accelerated freeze drying of grass blade segments.

(Drawn to scale, 1 cm = 1 cm)

stomata and presence of disrupted epidermal cells were noted in particular as well as other features such as presence of germinating fungal spores.

Papers by Day and Scott (1973) and Freeman *et al.* (1979) suggested it was feasible to examine plant material without first fixing in liquid nitrogen and subsequently accelerated freeze drying. Sections of *Lolium perenne* S23 were therefore sampled and immediately attached to a stub using double sided sticky-tape, sputter coated and examined in the SEM as described (section f)(i)). The same observations as above were made on sections taken from *Lolium perenne* S23 that had received either no simulated trampling, 3 i pot^{-1} just prior to sampling or 3 i pot^{-1} 72 hours prior to sampling. The method was compared to the standard fixing and freeze drying method.

Examination of the response of stomata over a period of 24 hours following simulated trampling was made. In order to achieve this, sets of 4 pots of grass were sampled at different times after receiving simulated trampling treatments. For each set of 4 pots of grass, 2 pots received 3 i pot^{-1} and the other 2 pots received 0 i pot^{-1}. Material was sampled, prepared and examined according to the standard method described in section f)(i). Stubs of control and treated leaf sections of *Lolium perenne* S23 were therefore available for a sequence of time intervals of 0, 0.5, 1, 1.5, 2, 3, 5, 7, 12, 18 and 24 hours after simulated trampling.

Adaxial surface stomata were examined from random fields of view and were categorised as "closed" (see Plates 9, 10, 11), "partially open" (see Plates 12, 13) and "open" (see Plates 14, 15). The stomata lie in the furrows and are often at an angle away from the horizontal. Although rotation of the stub is possible in the SEM, as the stomata are fairly sunken it was sometimes still not possible to determine their category so a further one, namely "inconclusive" (see

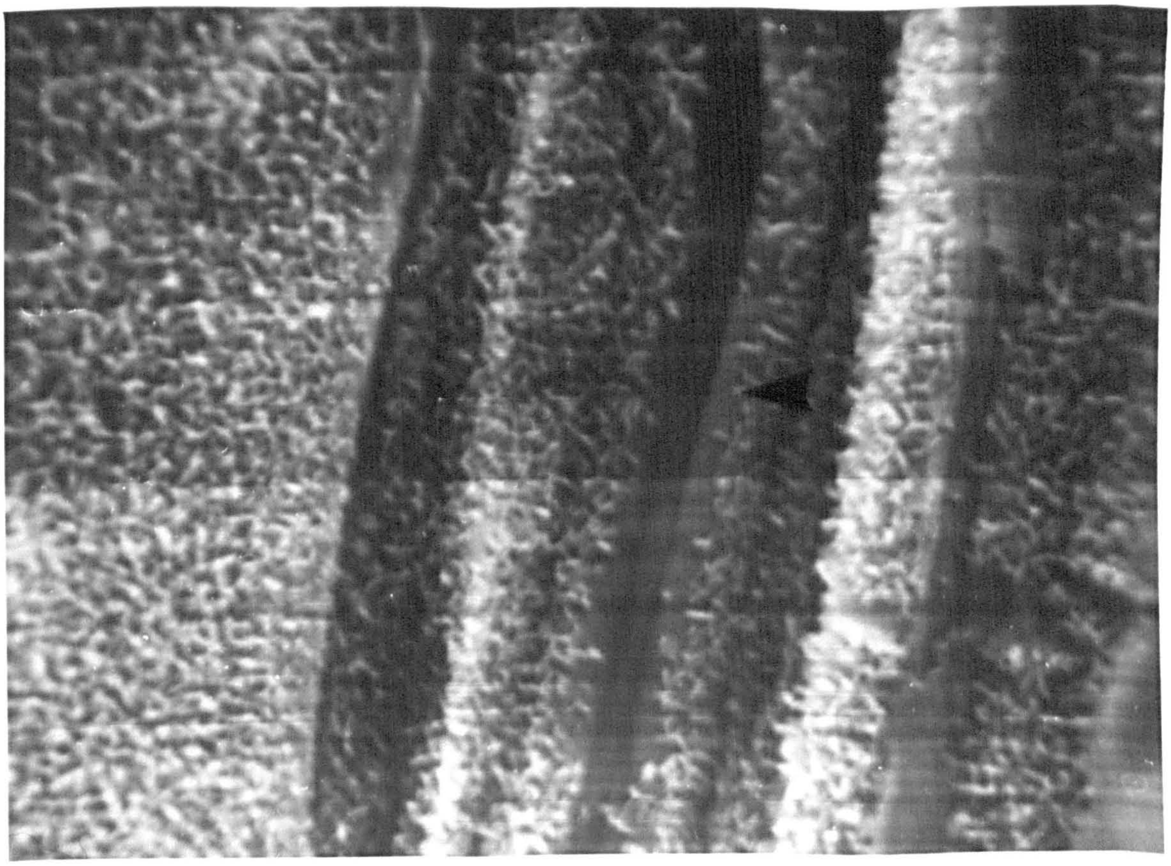


Plate 10 SEM observations:- closed adaxial stoma of *Lolium perenne* S23.

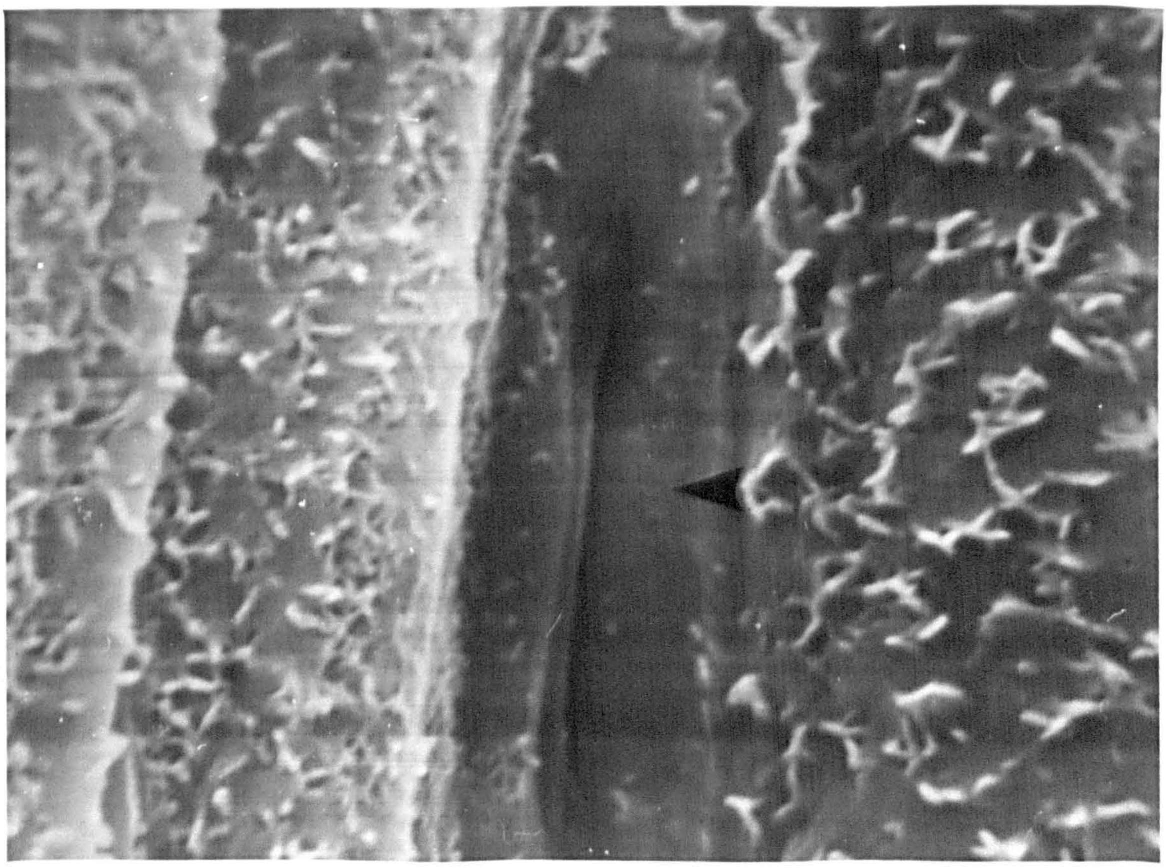


Plate 11 SEM observations:- closed adaxial stoma of *Lolium perenne* S23

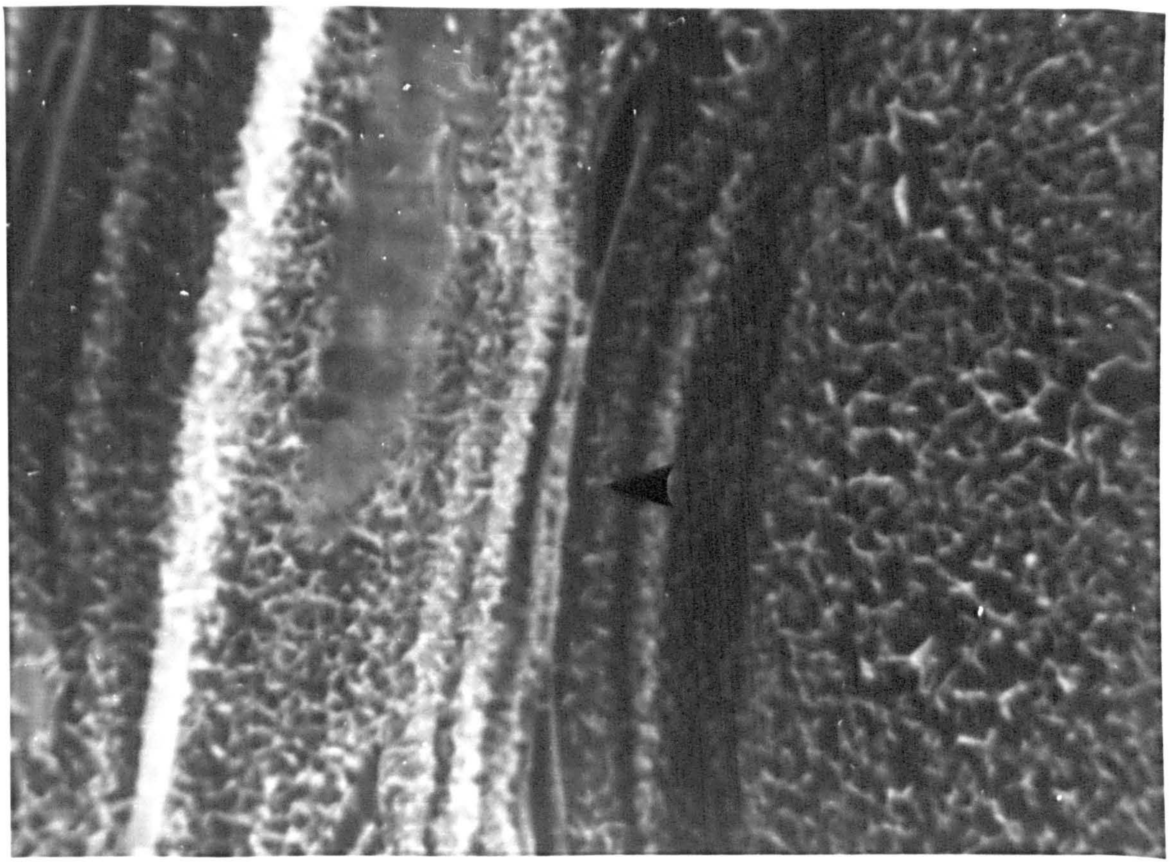


Plate 12 SEM observations:- Partially open adaxial stoma of Lolium perenne S23.

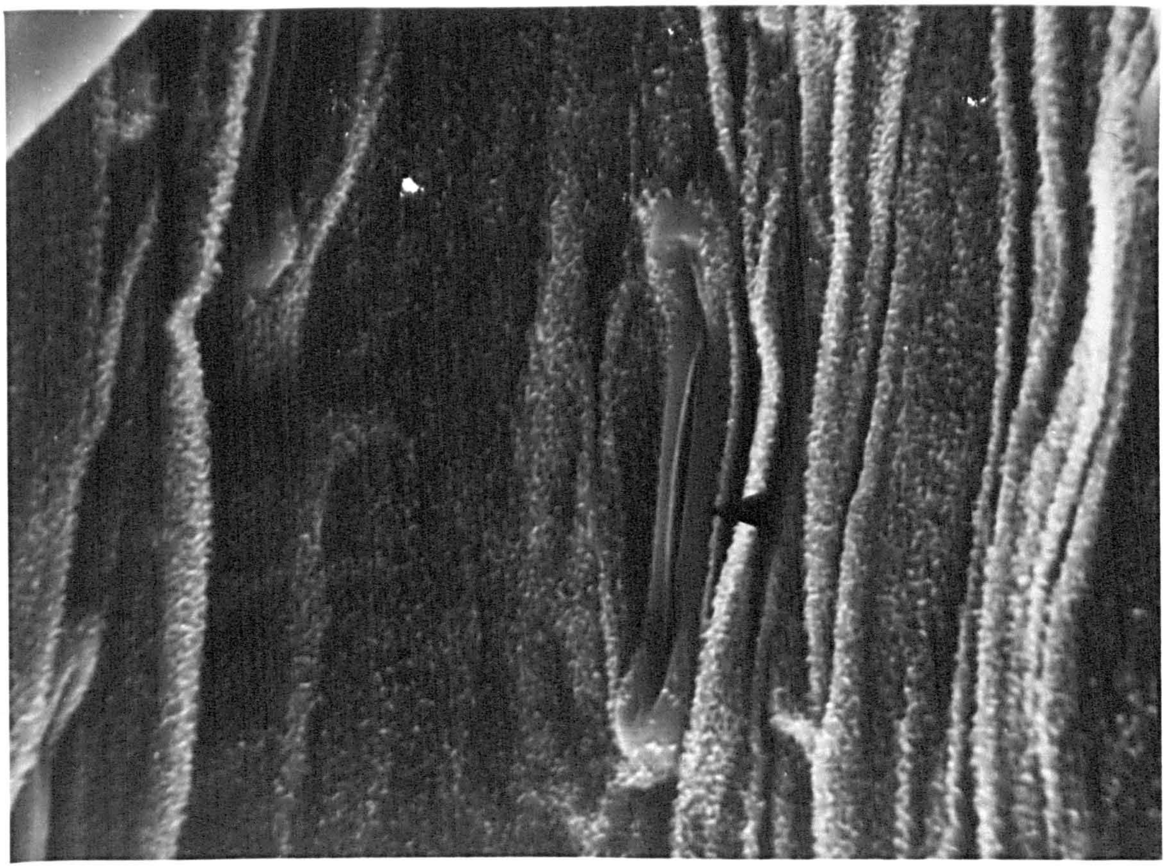


Plate 13 SEM observations:- Partially open adaxial stoma of Lolium perenne S23.

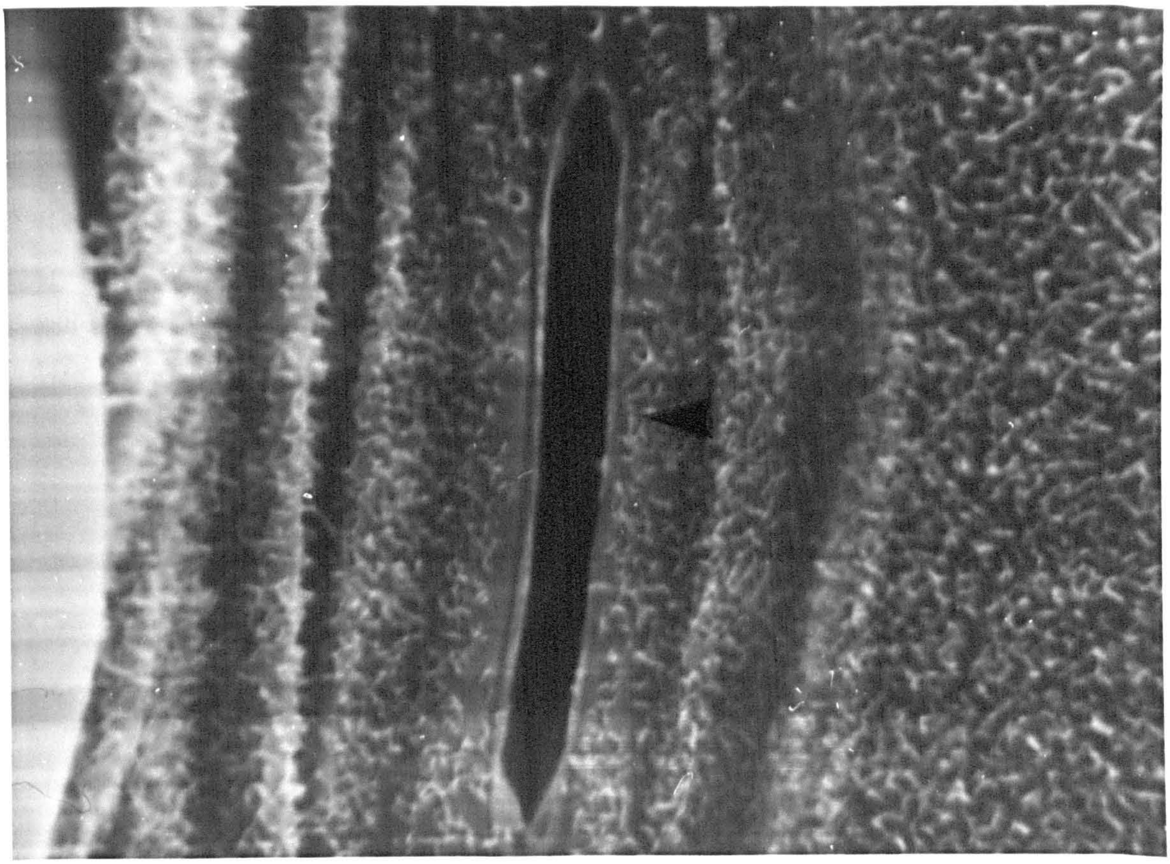


Plate 14 SEM observations:- open adaxial stoma of Lolium perenne S23.

Plate 15 SEM observations:- open adaxial stoma of Lolium perenne S23.

Plates 16, 17) was necessary. Counts were made categorising each stomata, but quantitative observations proved not to be feasible. This was because of widely varying numbers of fields of view available from one section to another. Forcep damage, overheating at high magnification in the SEM and some cracking, presumably during freeze drying were the main causes. Hence only qualitative summaries of stomatal aperture are given. Observations were also made of the general condition of the stomata and also of the epicuticular waxes. Any bacterial or fungal activity was noted.

For comparison of the effects of simulated trampling with the effects of trampling in the field, samples of leaf blades were also taken from the field plots, prepared for the SEM as described in section f)(i) and examined. Presence and absence of waxes, state of stomata, presence of disrupted epidermal cells and presence of bacteria and germinating fungal spores were noted in particular.

g) Water potential.

Attempts were made to measure the water potential of Lolium perenne S23 in order to find out the response to simulated trampling. Of particular interest was whether gradients of water stress developed, radiating out from points of injury; whether the undamaged parts of injured leaves were affected; or whether the whole plant, including undamaged leaves changed its water potential. A Dewpoint Psychrometer (Wescor Inc. HR - 33T Microvoltmeter) was used to measure water potential of attached leaves. After many unsuccessful attempts to take readings it was concluded that this would not be possible, mainly due to the morphology of Lolium perenne. It appeared that a seal was unobtainable because of the deep ridge and furrows. No further determinations were attempted.

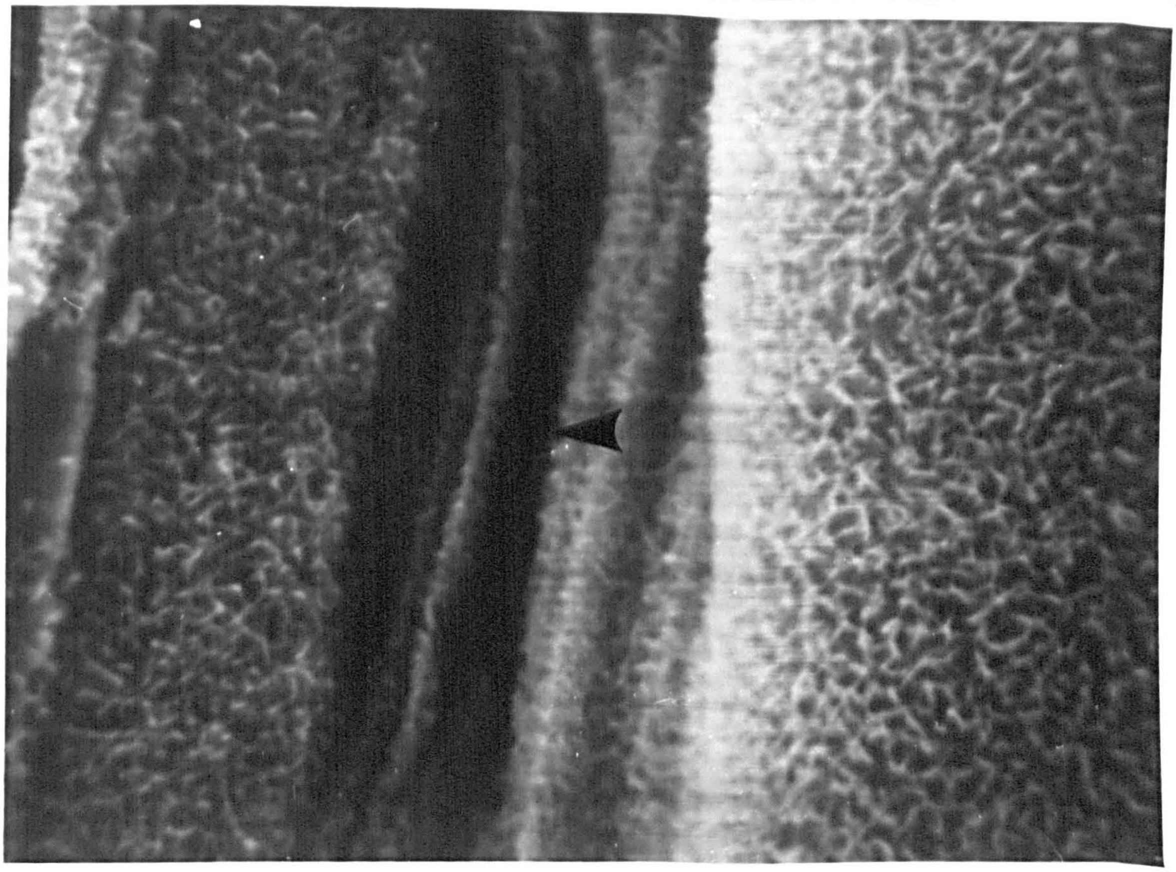


Plate 16 SEM observations:- inconclusive adaxial stoma of Lolium perenne S23.

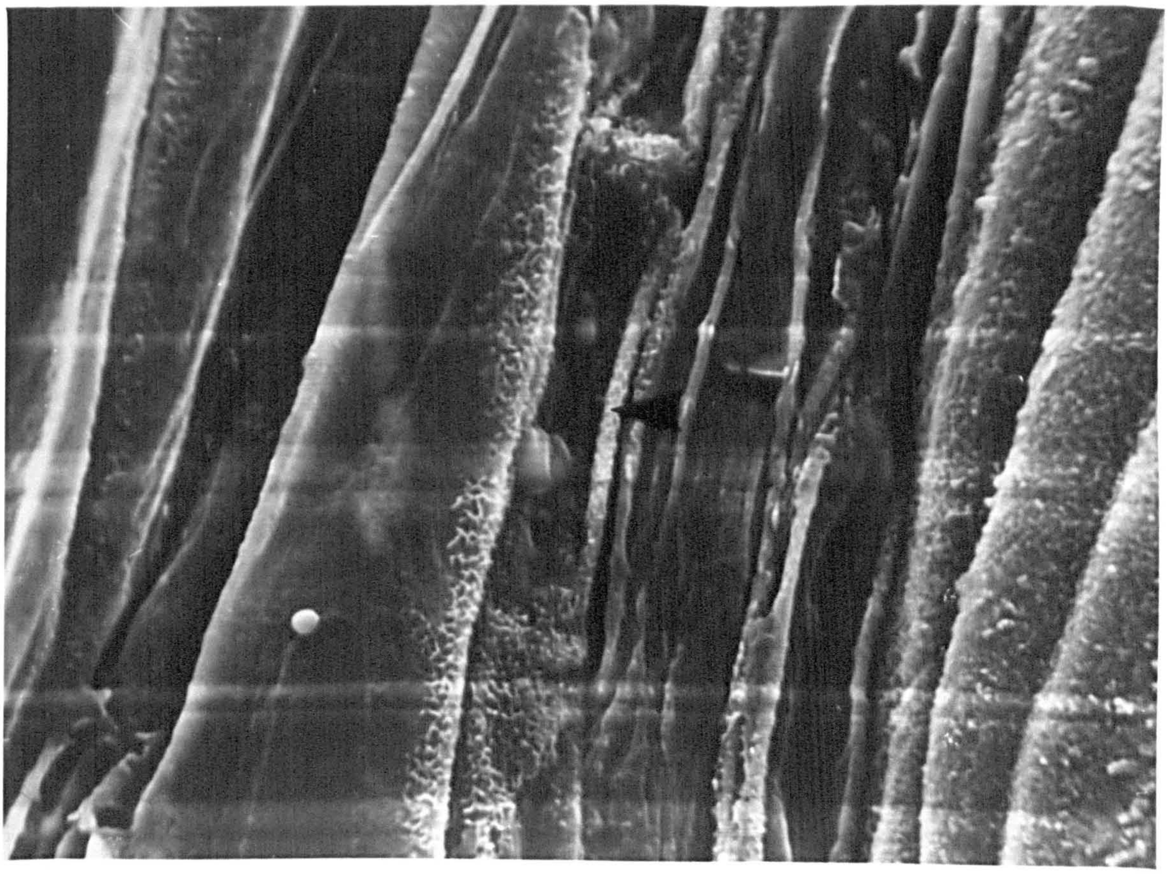


Plate 17 SEM observations:- inconclusive adaxial stoma of Lolium perenne S23.

Results

a) Water Loss of Excised Shoots.

(i) Evapotranspiration of grass not subjected to simulated trampling.

The decrease in RWC of excised shoots over the 4 hour period is shown for shoots saturated in the growth cabinet (Fig. 60) and for shoots saturated at 1°C in the dark (Fig 61). T-tests showed there was no significant difference between the two groups before saturation, at 2 hours and at 4 hours.

The calculated stomatal closure points and the subsequent rate of cuticular transpiration are listed in Table 8.

Using the same data to calculate $\%W_d$, graphs of the decrease in $\%W_d$ over the 4 hour period were plotted (Figs. 62 and 63). These showed similar response curves to the RWC curves.

(ii) Evapotranspiration of grass subjected to simulated trampling.

The decrease in RWC of excised shoots that had received 1 i pot^{-1} is shown for shoots saturated in the growth cabinet (Fig. 60) and for shoots saturated at 1°C in the dark (Fig. 61). T-tests showed there was no significant difference in RWC between the two groups before saturation, at 2 hours and at 4 hours. These curves are plotted with the appropriate curve of untreated shoots for comparison (Figs. 60 and 61). T-tests between 0 and 1 i pot^{-1} of the corresponding saturation conditions are summarised in Table 9. Grass receiving 1 i pot^{-1} has a lower RWC compared to grass receiving no simulated trampling. This is highly significant at 4 hours.

Using the same data to calculate $\%W_d$, graphs of the decrease in $\%W_d$ over the 4 hour period are shown in Figs. 62 and 63. Similar response curves were obtained.

Regression analysis of $\%W_d$ on RWC carried out using grouped 0 and 1 i pot^{-1} results for the different saturation conditions gave

Table 8 Calculated points of stomatal closure and rate of subsequent transpiration for different intensities of simulated trampling and different saturating conditions.

Saturating conditions	i pot^{-1}	RWC at estimated stomatal closure (%)	Time from saturation to stomatal closure	Cuticular transpiration following stomatal closure (loss of RWC per hour)
growth cabinet (standard)	0	67.0	2h 30 min	6
	1	60.5	2h 02 min	8
1°C dark	0	69.0	1h 48 min	6
	1	59.0	2h 03 min	8

Table 9 t-test of RWC values: 0 i pot^{-1} against 1 i pot^{-1}.

saturating conditions	time after treatment		
	0 hours	2 hours	4 hours
growth cabinet	n.s.	$p < 0.001$	$p < 0.001$
1°C in dark	n.s.	n.s.	$p < 0.01$

Table 10 Regression analysis of $\%W_d$ on RWC.

saturating conditions	i pot^{-1}	Regression coefficient	p	Regression slope (b)	p
growth cabinet	0	0.8966	$p < 0.001$	+8.6727	$p < 0.001$
	1	0.9659	$p < 0.001$	+9.7968	$p < 0.001$
	0 + 1 combined	0.9443	$p < 0.001$	+9.3490	$p < 0.001$
1°C in dark	0	0.9625	$p < 0.001$	+9.6537	$p < 0.001$
	1	0.9692	$p < 0.001$	+9.9149	$p < 0.001$
	0 + 1 combined	0.9658	$p < 0.001$	+9.7253	$p < 0.001$

highly significant regressions and are summarised in Table 10.

For treatments of 0, 2 and 7 i pot^{-1} just prior to excision and subsequently measuring %W_d at half hourly intervals for 4 hours (Fig. 65), analysis of variance and protected LSD showed significant differences between 0 and 2, 2 and 7 i pot^{-1}, both at 2 and 4 hours. For 0 i pot^{-1}, there was a significant reduction in W_d between 0 and 2 hours but the further decline between 2 and 4 hours was not significant. For both 2 and 7 i pot^{-1} there was a significant reduction in %W_d between 0 and 2 hours and between 2 and 4 hours. At 0 hours, 0 and 2 i pot^{-1} were not significantly different but there was a significant difference between 2 and 7 i pot^{-1}. This may be due to full saturation not being achieved as discussed (methods section a)(ii)).

The calculated points of stomatal closure and subsequent cuticular transpiration rates are listed in Table 11.

Results from the control RWC replicates gave the mean RWC at the start of the experiment as 82.22 (s.e. = 0.44). The mean initial %W_d for 0 i pot^{-1} was 709.95 (s.e. = 33.14).

b) Water content of intact shoots at different intensities of simulated trampling.

Graphs showing the response of %W_d of various species and cultivars of grass to a range of different intensities of simulated trampling are shown in Fig. 66 to Fig 74. For each species and variety there is a decrease in %W_d with increasing number of i pot^{-1}.

The general linear model analysis produced and compared regression coefficients for all species and cultivars. The results are summarised in Tables 12, 13 and 14. Generally, there is no significant difference in response between the Lolium perenne cultivars for both the intercept (i.e. the %W_d of undamaged plants) and also for the gradient (i.e. the response to increasing intensity

Fig. 65 Water loss (%W_d) of excised shoots at different intensities of simulated trampling.

Fig. 66 %W_d at 2 hours after simulated trampling at different intensities:
Lolium perenne S23 (a)

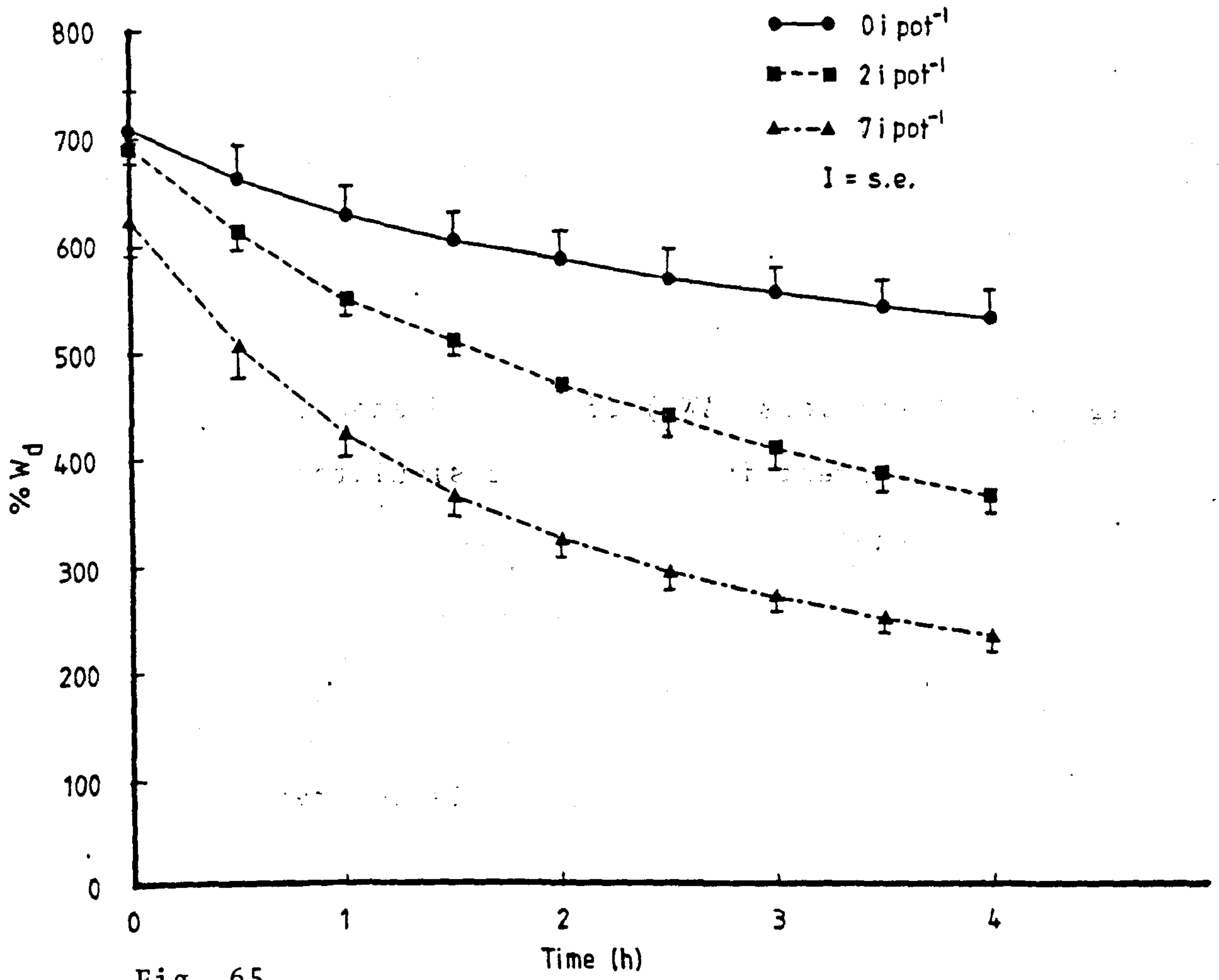

0 i pot^{-1}
2 i pot^{-1}
7 i pot^{-1}
I = s.e.
800
700
600
500
400
300
200
100
0
% W$_d$
0
1
2
3
4
Time (h)

Fig. 65

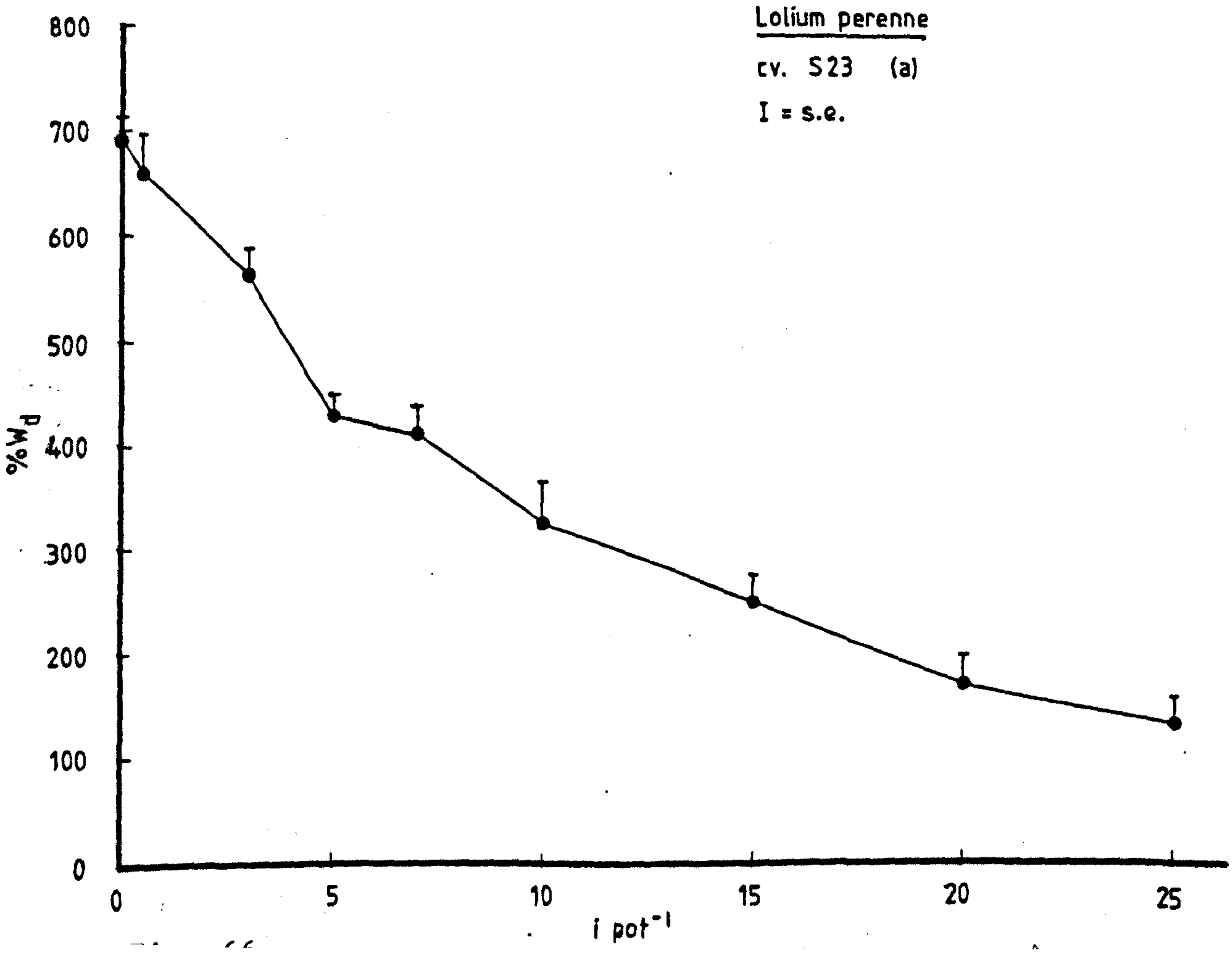

Lolium perenne
cv. S23 (a)
I = s.e.
800
700
600
500
400
300
200
100
0
%W$_d$
0
5
10
15
20
25
i pot^{-1}

Table 11 Calculated points of stomatal closure and rate of subsequent transpiration for different intensities of simulated trampling.

i pot^{-1}	$\%W_d$ at estimated stomatal closure	Time from saturation to stomatal closure	Cuticular transpiration after stomatal closure (loss $\%W_d$ h^{-1})
0	595	1h 40 min	25.56
2	490	1h 40 min	44.25
7	340	1h 50 min	39.85

%W_d at 2 hours after simulated trampling at different intensities:

Fig. 67 *Lolium perenne* S23 (b)

Fig. 68 *Lolium perenne* S23 (c)

Fig. 69 *Lolium perenne* Manhattan

Fig. 70 *Lolium perenne* Melle

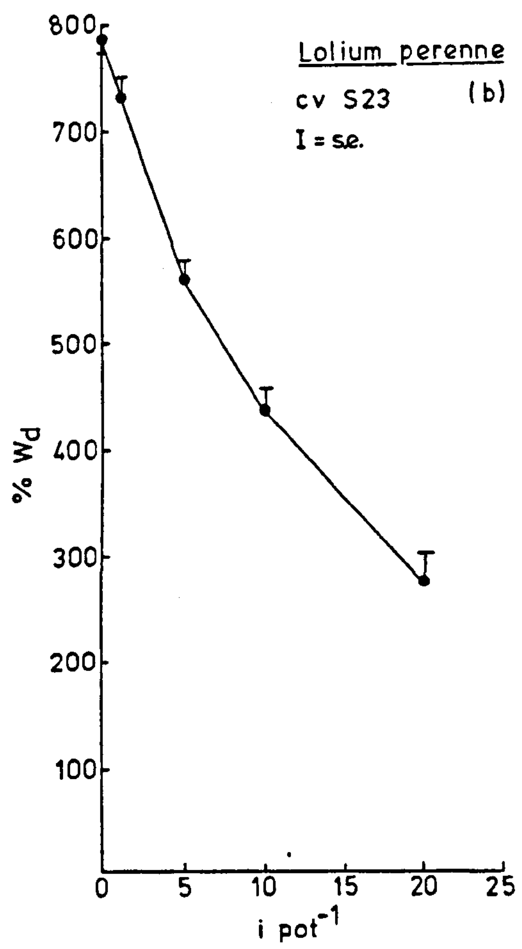


Fig. 67

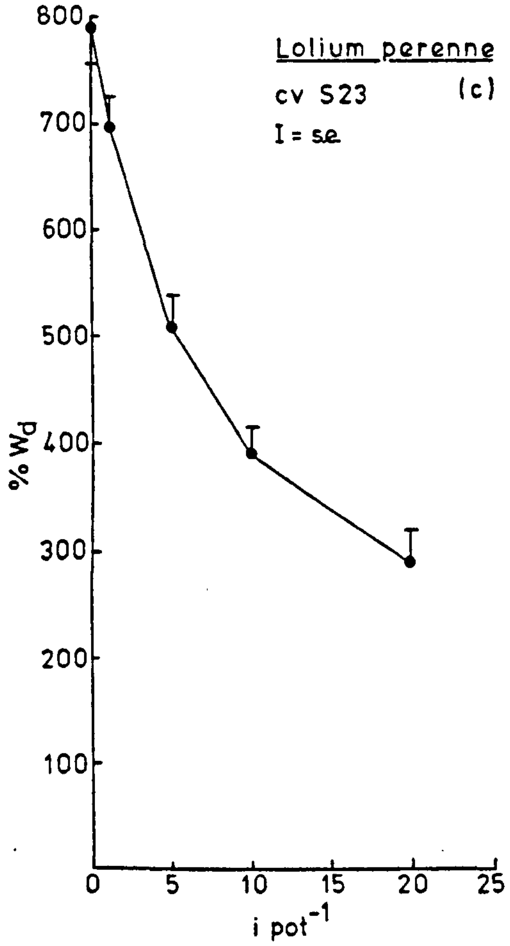


Fig. 68

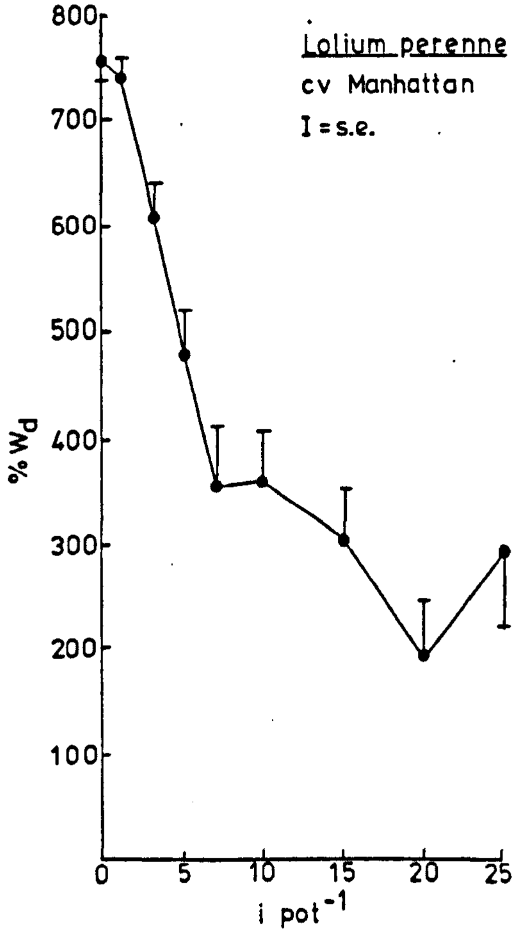


Fig. 69

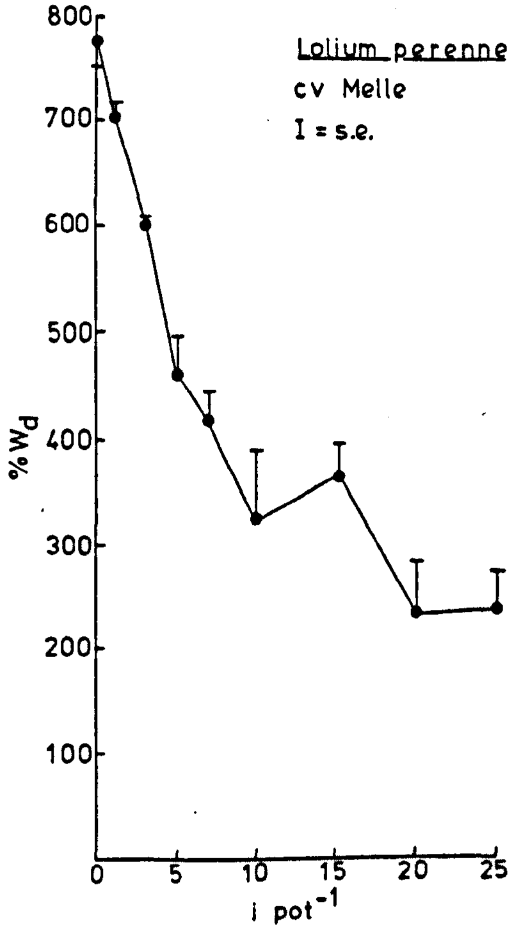


Fig. 70

%W_d at 2 hours after simulated trampling at different intensities:

Fig. 71 *Agrostis tenuis* Holfior

Fig. 72 *Poa pratensis* Parade

Fig. 73 *Phleum pratense* S48 (a)

Fig. 74 *Phleum pratense* S48 (b)

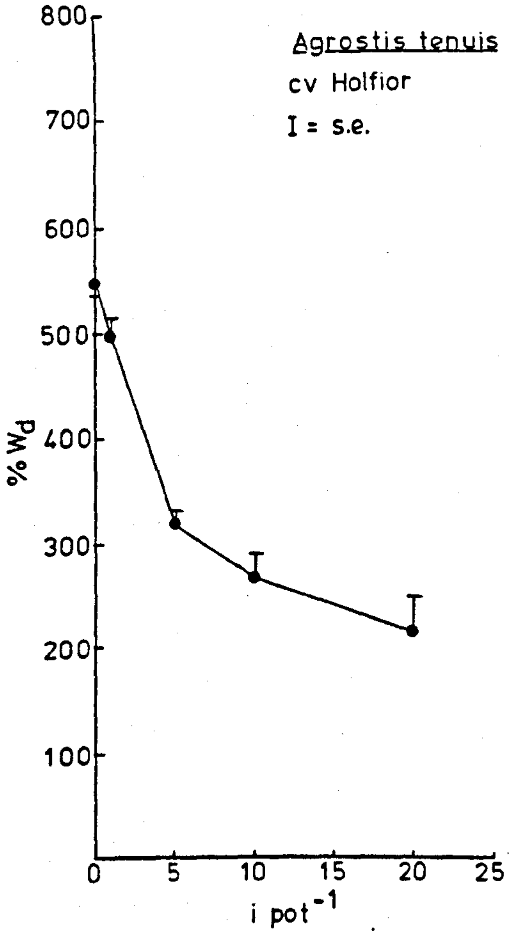


Fig. 71

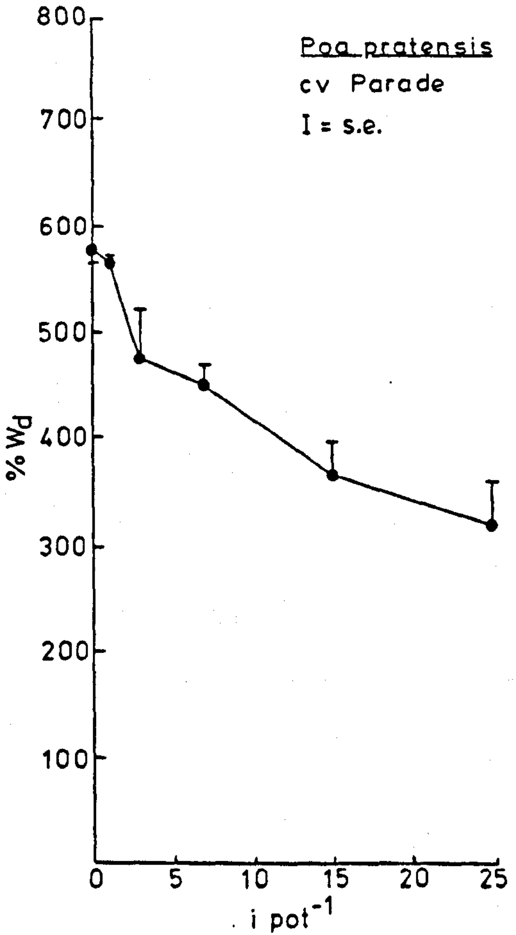


Fig. 72

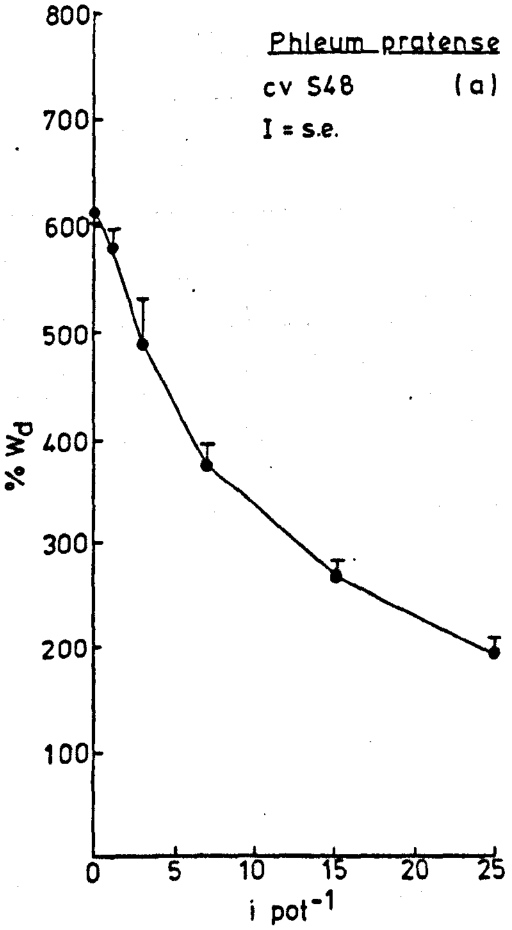


Fig. 73

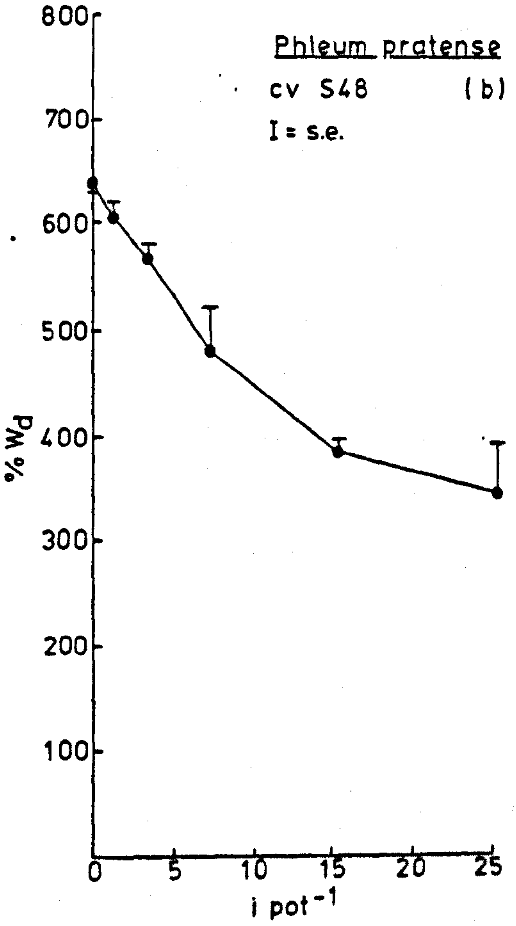


Fig. 74

Table 12 Summary of general linear model analysis of the intercept ("a") and gradient ("b") values from regressing $\%W_d$ on log (i pot^{-1}) for all species and cultivars.

Species	General Linear Model			
	Estimate of a	p	Estimate of b	p
Lolium perenne S23 (a)	761.0278	$p < 0.001$	-185.1182	$p < 0.001$
Lolium perenne S23 (b)	828.3292	$p < 0.001$	-170.0791	$p < 0.001$
Lolium perenne S23 (c)	804.7317	$p < 0.001$	-169.1378	$p < 0.001$
Lolium perenne Manhattan	798.5283	$p < 0.001$	-179.9129	$p < 0.001$
Lolium perenne Melle	801.1934	$p < 0.001$	-176.6063	$p < 0.001$
Agrostis tenuis Holfior	556.1668	$p < 0.001$	-117.0931	$p < 0.001$
Poa pratensis parade	596.3044	$p < 0.001$	-78.7892	$p < 0.001$
Phleum pratense S48 (a)	649.3616	$p < 0.001$	-136.1037	$p < 0.001$
Phleum pratense S48 (b)	663.5079	$p < 0.001$	-92.8776	$p < 0.001$

Table 13 Comparison between species and cultivars of intercept ("a") value from regressions of $\%W_d$ on log (i/p).

Lolium perenne S23 (a)	-								
Lolium perenne S23 (b)	$p < 0.05$	-							
Lolium perenne S23 (c)	n.s.	n.s.	-						
Lolium perenne Manhattan	n.s.	n.s.	n.s.	-					
Lolium perenne Melle	n.s.	n.s.	n.s.	n.s.	-				
Agrostis tenuis Holfior	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	-			
Poa pratensis Parade	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	n.s.	-		
Phleum pratense S48 (a)	$p < 0.01$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.05$	n.s.	-	
Phleum pratense S48 (b)	$p < 0.05$	$p < 0.01$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.01$	n.s.	n.s.	-
	Lolium perenne S23 (a)	*Lolium perenne* S23 (b)	*Lolium perenne* S23 (c)	*Lolium perenne* Manhattan	*Lolium perenne* Melle	*Agrostis tenuis* Holfior	*Poa pratensis* Parade	*Phleum pratense* S48 (a)	*Phleum pratense* S48 (b)

Table 14 Comparison between species and cultivars of gradient ("b") value from regressions of $\%W_d$ on log (i pot^{-1}).

Lolium perenne S23 (a)	-								
Lolium perenne S23 (b)	n.s.	-							
Lolium perenne S23 (c)	n.s.	n.s.	-						
Lolium perenne Manhattan	n.s.	n.s.	n.s.	-					
Lolium perenne Melle	n.s.	n.s.	n.s.	n.s.	-				
Agrostis tenuis Holfior	$p < 0.001$	$p < 0.01$	$p < 0.01$	$p < 0.001$	$p < 0.001$	-			
Poa pratensis Parade	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.05$	-		
Phleum pratense S48 (a)	$p < 0.05$	n.s.	n.s.	$p < 0.05$	$p < 0.05$	n.s.	$p < 0.01$	-	
Phleum pratense S48 (b)	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	n.s.	n.s.	$p < 0.05$	-
	Lolium perenne S23 (a)	*Lolium perenne* S23 (b)	*Lolium perenne* S23 (c)	*Lolium perenne* Manhattan	*Lolium perenne* Melle	*Agrostis tenuis* Holfior	*Poa pratensis* Parade	*Phleum pratense* S48 (a)	*Phleum pratense* S48 (b)

of simulated trampling). The other species are all highly significantly different ($p<0.001$) from all the Lolium perenne cultivars for both the intercept and the gradient. Within these other species, there seems to be only a little significant difference. Agrostis tenuis was significantly different ($p < 0.05$) from Poa pratensis only for the gradient and to Phleum pratense only for the intercept. Phleum pratense was significantly different from Poa pratensis only for the gradient value. These differences are summarised in Table 15.

c) Transpiration of an intact plant system when subjected to simulated trampling.

The response of different species of grass to simulated trampling is shown for transpiration rates averaged over the total 24 hours (Fig. 75), transpiration rates averaged over the first 3 hours (Fig. 76) and $\%W_d$ measured at 24 hours (Fig. 77) when the experiment was terminated.

There is a decrease in each of the above three measurements with increasing number of i pot^{-1} and this is so for every species. Analysis of variance showed that not only was there a significant effect of intensity of simulated trampling but also a significant effect of species for the three measurements. A summary of the analysis of variance tests is given in Table 16. Using the Tukey-Kramer method, all pairs of means were compared and a summary of the significant differences between means is given in Appendices 5, 6 and 7.

Transpiration rates averaged over 24 hours (see Fig. 75) were highly significant ($p<0.001$) for the effect of treatments, significant ($p<0.05$) for the effect of species and there was a significant treatment/species interaction ($p < 0.01$). Where there was no simulated trampling, Agrostis tenuis had the highest

Table 15 Summary of significant differences between species: regression of $\%W_d$ and i pot^{-1}.

(Line indicates no significant difference between species.)

	Lolium perenne	Agrostis tenuis	Poa pratensis	Phleum pratense
intercept a	798.16	556.17	596.30	656.44
gradient b	-176.06	-117.09	-78.79	-114.49

Fig. 75 Transpiration rates over 24 hours after simulated trampling at different intensities.

Fig. 76 Transpiration rates over first 3 hours after simulated trampling at different intensities.

Fig. 77 $\%W_d$ at 24 hours after simulated trampling at different intensities.

Fig. 78 Relative hydraulic conductance after different intensities of simulated trampling.

● *Lolium perenne* S23
■ *Agrostis tenuis* Highland
▲ *Poa pratensis* Primo
◆ *Phleum bertolonii* S50

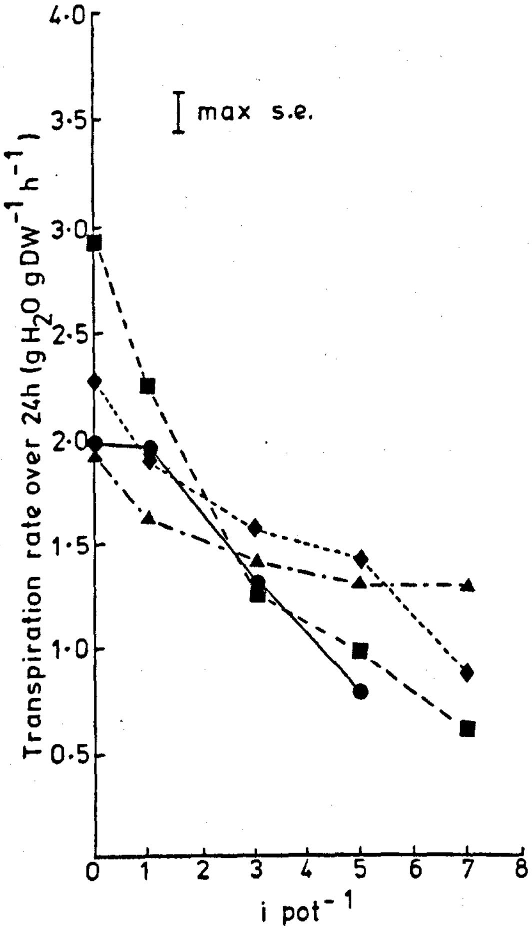


Fig. 75

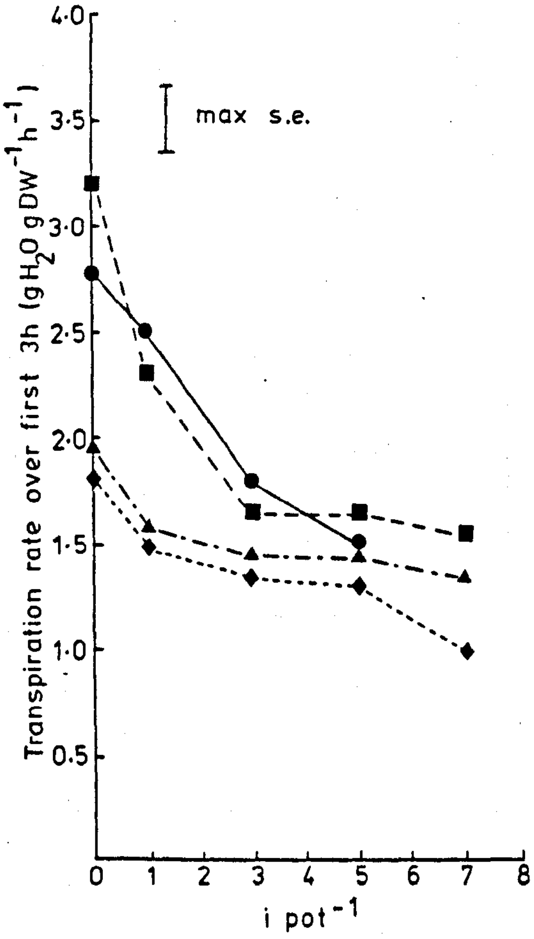


Fig. 76

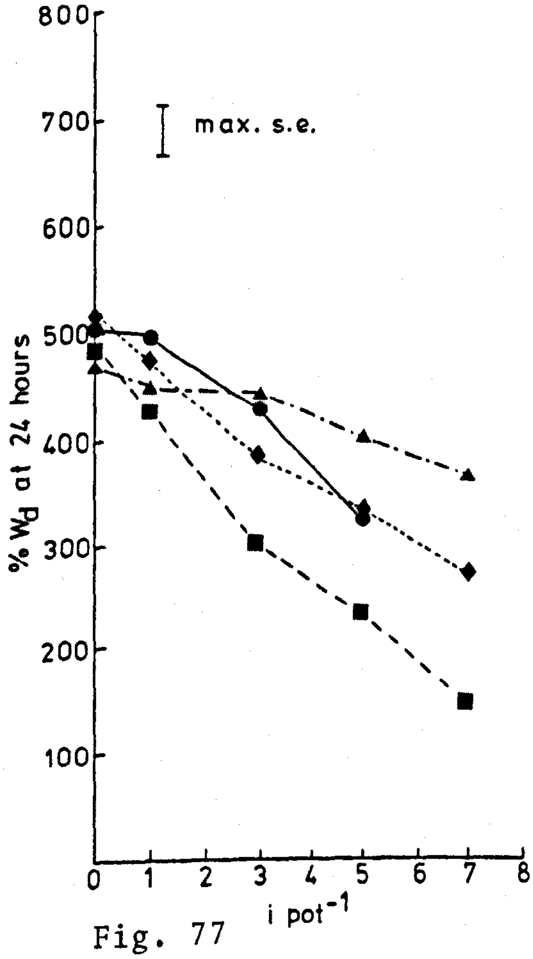


Fig. 77

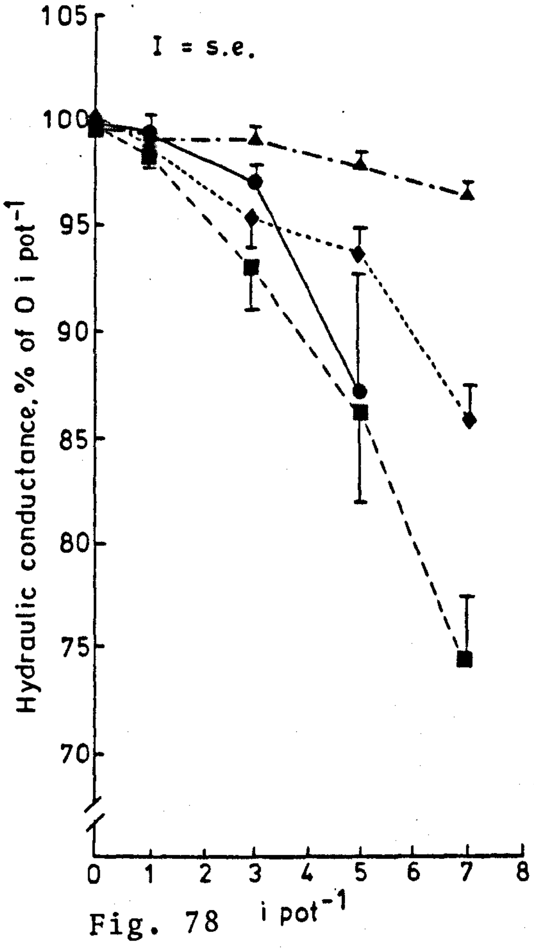


Fig. 78

Table 16 Summary of analysis of variance of simulated trampling treatments (0, 1, 3, 5 i pot^{-1}) and effect of different species (Lolium perenne S23, Agrostis tenuis Highland, Poa pratensis Primo and Phleum bertolonii S50).

	treatments	species	treatments/species interaction
Transpiration rate over 24 h	$p < 0.001$	$p < 0.05$	$p < 0.01$
Transpiration rate over first 3 h	$p < 0.001$	$p < 0.001$	$p < 0.05$
$\%W_d$ at 24 h	$p < 0.001$	$p < 0.001$	n.s.

transpiration rate which was significantly different to all but *Phleum bertolonii* (Fig. 75). *Poa pratensis* had the lowest rate, although there was no significant difference between *Phleum bertolonii*, *Lolium perenne* and *Poa pratensis*. However, addition of simulated trampling caused a dramatic change in relative transpiration rates. At 3 i pot^{-1} there is no significant difference between any of the species. For 5 and 7 i pot^{-1} *Agrostis tenuis* and *Lolium perenne* have the lowest rates and *Poa pratensis* has the highest rate. This difference in response is also seen from comparisons of treatment responses within each species (Fig. 75). For *Poa pratensis*, no treatment is significantly different. It appears that the transpiration rate of *Agrostis tenuis* is particularly sensitive to the effects of simulated trampling, responding by a decline in rate. *Lolium perenne* and *Phleum bertolonii* show a similar though not so sensitive response. *Poa pratensis* is relatively very insensitive to increasing intensity of simulated trampling.

Transpiration rates over the initial 3 hours (see Fig. 76) were highly significant ($p < 0.001$) for the effects of treatment and of species. The species/treatment interaction was also significant ($p < 0.05$). As for transpiration rate over the full 24 hour period, where there was no simulated trampling *Agrostis tenuis* had a significantly higher rate than *Poa pratensis* but also than *Phleum bertolonii* (Fig. 76). However, *Lolium perenne* had a significantly higher rate to *Poa pratensis* and *Phleum bertolonii*. Simulated trampling again caused a dramatic decrease in transpiration rate for *Agrostis tenuis* but also for *Lolium perenne* (Fig. 76). This contrasts again with *Poa pratensis* where there is a trivial decrease as seen in the absence of any significantly different pairs of treatment means. *Phleum bertolonii* has a similar response to that of *Poa pratensis*.

$\%W_d$ at 24 hours (see Fig. 77) was highly significant ($p < 0.001$) for both the effect of treatments and of species. Treatment/species interaction was not significant. With no simulated trampling, there was no significant differences between any of the species. Responses to simulated trampling were similar to the transpiration responses with *Agrostis tenuis* showing the greatest decline in $\%W_d$ (being significantly lower than the other species) and *Poa pratensis* showing the least decrease (Fig. 77). As before, most of the treatment means for *Agrostis tenuis* were significantly different from each other, yet all the treatment means for *Poa pratensis* were not significantly different (Fig. 77).

Comparing response of transpiration rate over 24 hours and (Fig. 75) transpiration rate over the first 3 hours (Fig. 76), there is a similar pattern of loss with increasing intensity of simulated trampling. For *Agrostis tenuis* in particular and *Lolium perenne* there is a substantial, mostly significant decrease in transpiration rate with increasing intensity of simulated trampling. *Poa pratensis* in particular and also *Phleum bertolonii* respond with a relatively small mostly insignificant decrease in transpiration rate with increasing intensity of simulated trampling (Figs. 75 and 76). It is of interest to note that the initial rates of transpiration (i.e. over the first 3 hours) are very similar to the daily rates of transpiration (i.e. averaged over the 24 hour period) for *Poa pratensis* for every treatment. However, the initial rates of transpiration for *Agrostis tenuis* and *Lolium perenne* are higher than the daily rate of transpiration.

$\%W_d$ at 24 hours (Fig. 77) also follows the same pattern of response to the transpiration rates by a general decrease with increasing intensity of simulated trampling. This would appear to be a paradox, especially considering that *Agrostis tenuis* which transpires the highest rate with no simulated trampling, transpires at the lowest rate with greatest intensity of simulated trampling (Fig. 75 and 76) and yet has the lowest $\%W_d$ at 24 hours with greatest intensity of simulated trampling (Fig. 77). In section b) it was shown that in an intact plant system, the greater the intensity of simulated trampling, the lower the $\%W_d$ and therefore the greater the loss of water from the leaves. Calculations were therefore made to explore a possible explanation for this paradox. Using the transpiration rates averaged over 24 hours and $\%W_d$ at 24 hours, estimates of the percentage conductance of water through the intact plant system, relative to that at 0 i pot^{1} were made, as follows:

% Relative Conductance

$$= \frac{[T_x.24.DW_x]-[[(\%W_{d(o)}/100).DW_x]-[FW_x-DW_x]]}{[T_x.24.DW_x]} \times 100\%$$

T_x = Transpiration rate (g H_2O g DW^{-1} h^{-1})
over 24 h for x i pot^{-1}

DW_x = Dry weight at 24 h for x i pot^{-1}

FW_x = Fresh weight at 24 h for x i pot^{-1}

$\%W_{d(o)}$ = $\%W_d$ for the mean value of 0 i pot^{-1}

First, estimates of the expected water content of the shoots were made by multiplying the mean $\%W_d$ for 0 i pot^{-1} by the DW for each replicate in every treatment. Next, the measured water content was subtracted from the estimated expected water content for each replicate in every treatment thus giving the amount of water lost as a consequence of the different simulated trampling treatments. This value will be referred to as "estimated water loss from the shoots". Using transpiration rates over 24 hours, the amount of water lost in transpiration over 24 hours was calculated. This will be referred to as "water lost in transpiration". By subtracting the "estimated water loss from the shoots" from the "water lost in transpiration" the amount of water conducted from the soil and transpired was estimated. Expressing this estimate as a percentage of the "water lost in transpiration" gave the % relative conductance.

The results are graphed in Fig. 78. For every species, there is a reduced conductance of water through the intact plant system, which is more pronounced, the greater the intensity of simulated trampling. Analysis of variance showed this treatment effect to be significant ($p < 0.001$). Appendix 8 gives all the significant differences between pairs of means, after comparisons using the Tukey-Kramer method (Sokal and Rohlf 1981). At 7 i pot^{-1} *Agrostis tenuis* has a significantly lower % relative conductance from *Phleum bertolonii* which also has a significantly lower % relative conductance from *Poa pratensis*. There is no significant difference between any of the treatment means for *Poa pratensis* again. In contrast, as before, there are various significant differences between the treatment means for *Agrostis tenuis*.

The changes in transpiration rate appear to be caused to some extent by changes in the water uptake through the whole plant system and the degree of effect varies according to species sensitivity.

Here, Agrostis tenuis appears to be most sensitive and Poa pratensis least sensitive to the effects of simulated trampling on the water relations of the plants.

d) Effects of humidity on the response of water content and transpiration rates of grass subjected to simulated trampling.

The graph in Fig. 79 compares the response of transpiration rate over 24 hours to simulated trampling of Lolium perenne S23 in 70% or 45% relative humidity. There is a very significant ($p < 0.001$) effect of humidity.

At 45% relative humidity there is a significantly higher rate of transpiration compared to that at 70%. This is the case at each intensity of simulated trampling (as well as the response of lower transpiration rate with increasing intensity of trampling as shown in section c)).

Fig. 80 similarly shows the response of transpiration rate over the first 3 hours. Again, the grass at 45% relative humidity had significantly higher rates.

The response to humidity of $\%W_d$ at 24 hours is shown in Fig. 81. Two-way analysis of variance showed no significant effect of humidity although the grass at 45% relative humidity had slightly lower values of $\%W_d$ at 24 hours for every treatment compared to the grass at 70% relative humidity.

Summaries of the significant differences between all pairs of means are given in Tables 17, 18 and 19 (using the Tukey-Kramer method).

The same % relative conductance calculations as described in section c) were made and graphed (Fig. 82). There is a lower value of relative conductance for 45% relative humidity compared to that for 70% relative humidity, although these were not significantly different. A summary is given of the test between all pairs of means,

Fig. 79 Transpiration rates over 24 hours at different relative humidities and different intensities of simulated trampling.

Fig. 80 Transpiration rates over first 3 hours at different relative humidities and different intensities of simulated trampling.

Fig. 81 $\%W_d$ after 24 hours at different relative humidities and different intensities of simulated trampling.

Fig. 82 Relative hydraulic conductance after different relative humidities and different intensities of simulated trampling.

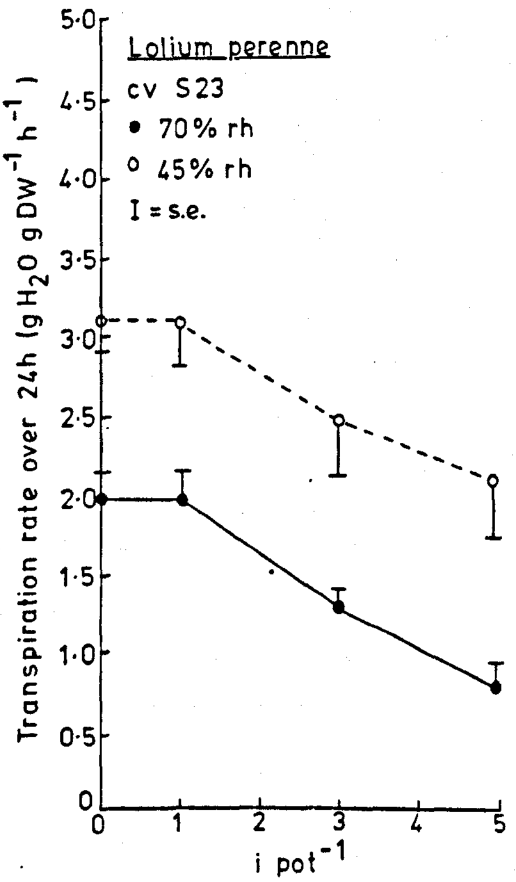


Fig. 79

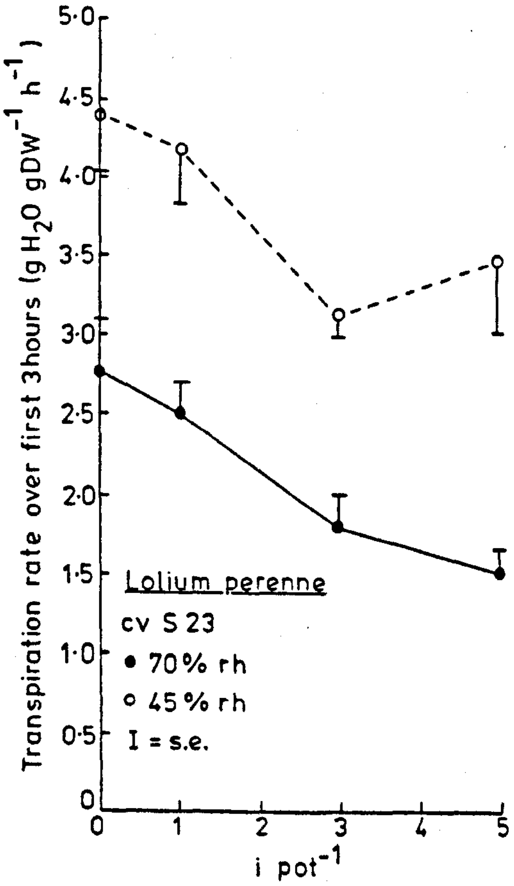


Fig. 80

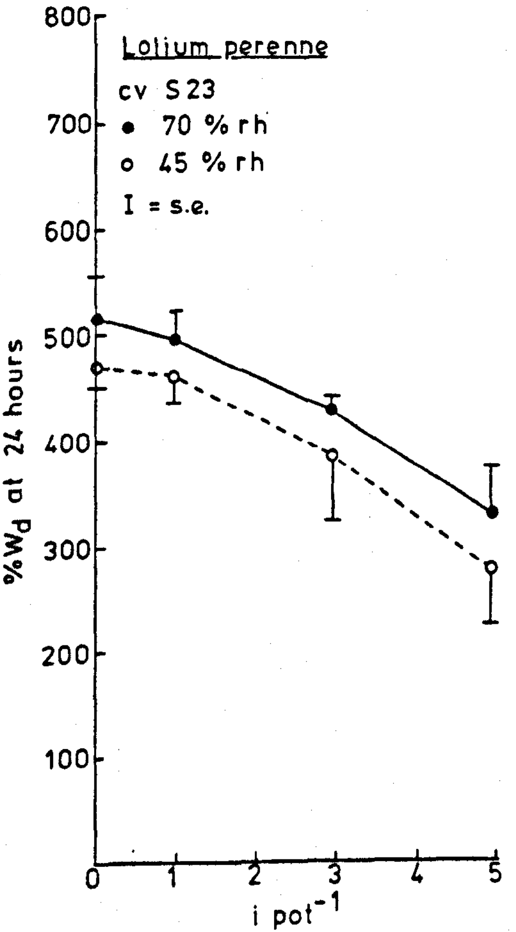


Fig. 81

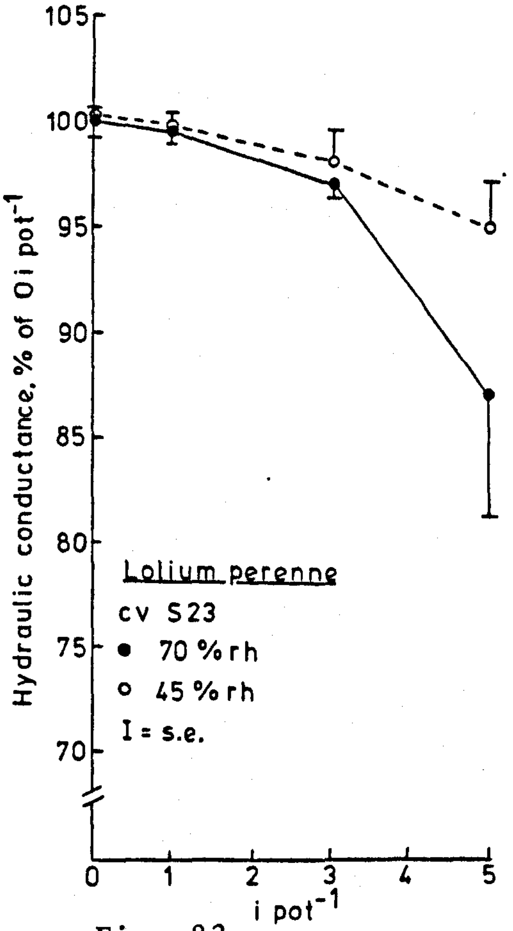


Fig. 82

Table 17 Summary of all significant differences for transpiration rates over 24 hours.

70% r.h.	0	-							
	1	n.s.	-						
	3	n.s.	n.s.	-					
	5	*	*	n.s.	-				
45% r.h.	0	*	*	*	*	-			
	1	n.s.	n.s.	*	*	n.s.	-		
	3	n.s.	n.s.	*	*	n.s.	n.s.	-	
	5	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	-
	i pot^{-1}	0	1	3	5	0	1	3	5
		70% r.h.				45% r.h.			

*	significantly different
n.s.	not significantly different

Table 18 Summary of all significant differences for transpiration rates over first 3 hours.

70% r.h.	0	-							
	1	n.s.	-						
	3	n.s.	n.s.	-					
	5	n.s.	n.s.	n.s.	-				
45% r.h.	0	*	*	*	*	-			
	1	*	*	*	*	n.s.	-		
	3	n.s.	n.s.	n.s.	*	n.s.	n.s.	-	
	5	n.s.	n.s.	*	*	n.s.	n.s.	n.s.	-
	i pot^{-1}	0	1	3	5	0	1	3	5
		70% r.h.				45% r.h.			

* significantly different

n.s. not significantly different

Table 19 Summary of all significant differences for $\%W_d$ at 24 hours.

70% r.h.	0	-							
	1	n.s.	-						
	3	n.s.	n.s.	-					
	5	*	n.s.	n.s.	-				
45% r.h.	0	n.s.	n.s.	n.s.	n.s.	-			
	1	n.s.	n.s.	n.s.	n.s.	n.s.	-		
	3	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-	
	5	*	*	n.s.	n.s.	*	*	n.s.	-
	i pot^{-1}	0	1	3	5	0	1	3	5
		70% r.h.				45% r.h.			

* significantly different

n.s. not significantly different

Table 20 <u>Summary of all significant differences for % relative conductance.</u>

70% r.h.	0	-							
	1	n.s.	-						
	3	n.s.	n.s.	-					
	5	*	*	n.s.	-				
45% r.h.	0	n.s.	n.s.	n.s.	*	-			
	1	n.s.	n.s.	n.s.	*	n.s.	-		
	3	n.s.	n.s.	n.s.	*	n.s.	n.s.	-	
	5	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-
	i pot^{-1}	0	1	3	5	0	1	3	5
		70% r.h.				45% r.h.			

* significantly different

n.s. not significantly different

using the Tukey-Kramer method in Table 20.

f) Scanning Electron Microscopy.

Various photographs were taken during the initial observations of *Lolium perenne* S23 and *Phleum pratense* S48. Plates 8, 19, 20, 21 and 22 are of healthy undamaged leaves from *Lolium perenne* S23. Plates 23 and 24 are of healthy undamaged leaves from *Phleum pratense* S48. These are typical of the two species with *Lolium perenne* S23 having deeper furrows compared to *Phleum pratense* S48. The epidermal cells appear to be turgid and have a fine covering of epicuticular wax crystals in both species (see Plates 25 and 26). It is interesting that there are some markings indicating loss of small amounts of the wax. This was a usual feature of untrampled grass and seems to be due to air movement causing a mild 'whip lash' like action which removes a narrow line of wax as suggested by Grace (1974) and shown by Thompson (1974).

The stomata are more clearly visible for *Phleum pratense* compared to *Lolium perenne*. This is a result both of the nature of the epidermal cells (shorter and wider for *Phleum pratense*) and the greater depth of the 'furrows' for *Lolium perenne*.

These preliminary observations gave no clear indications about the effect of simulated trampling on stomatal aperture, although samples of *Phleum pratense* taken immediately after a treatment of 1 i pot^{-1} had very wide open stomata (see Plates 27 and 28).

Damaged shoots from *Lolium perenne* that had received simulated trampling of 5 i pot^{-1} 5 weeks prior to sampling showed a remarkable contrast to healthy tissue as seen comparing Plates 19 and 20 with Plates 29 and 30. There appears to be complete loss of epicuticular waxes. There is evidently dehydration and shrinkage of the cells, especially of the ridges (Plate 29 compared to Plate 19). There is presence and germination of many fungal spores on the samples that

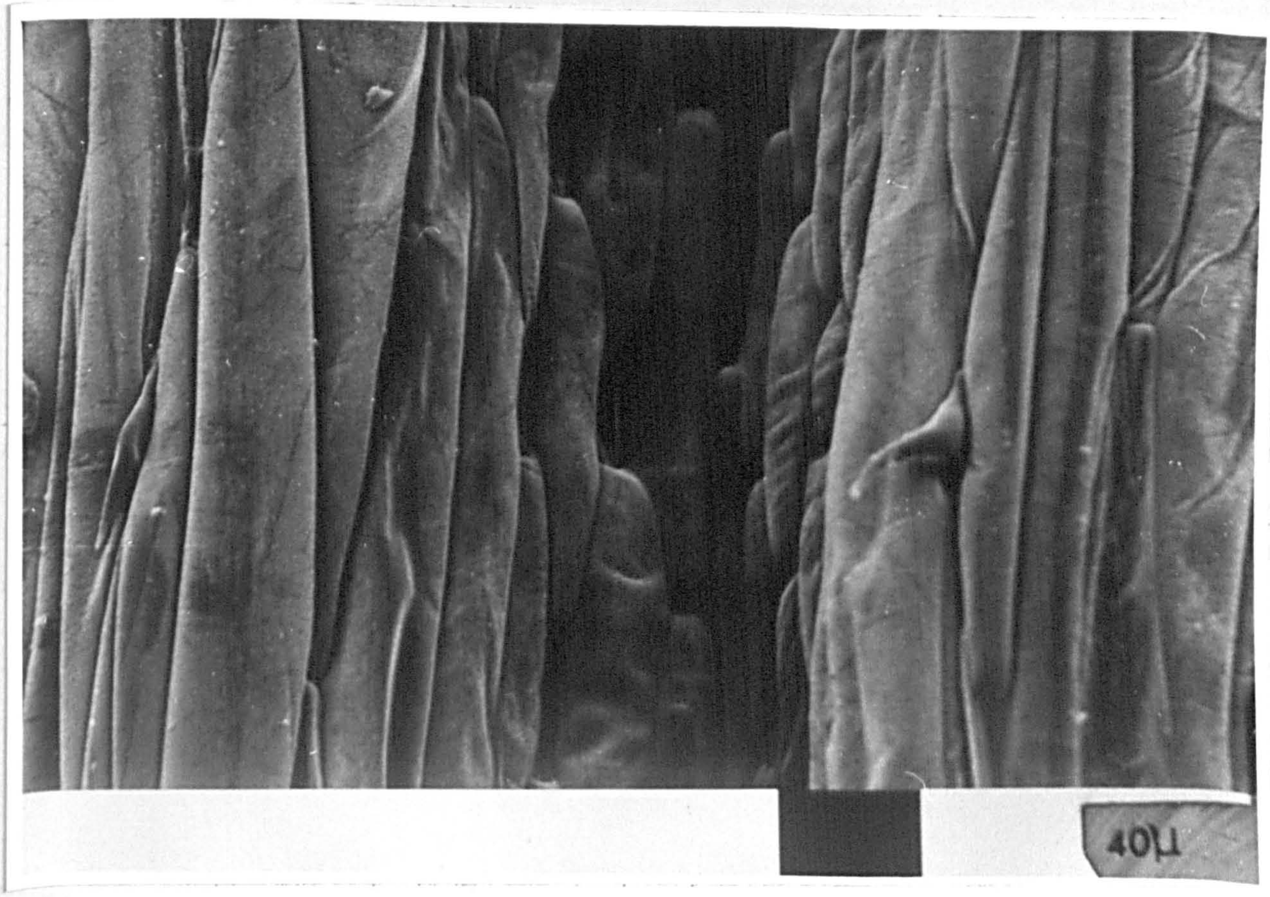


Plate 19 SEM observations:- Healthy undamaged adaxial leaf surface of Lolium perenne S23.

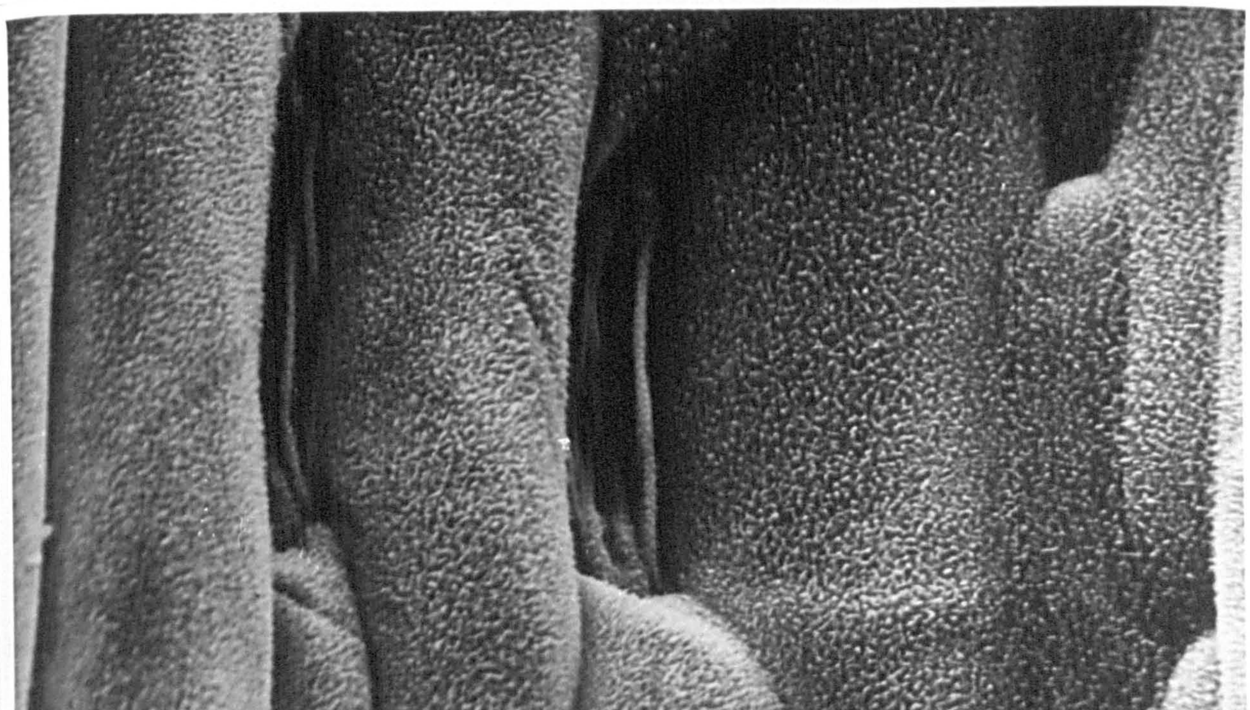


Plate 20 SEM observations:- Healthy undamaged adaxial leaf surface of Lolium perenne S23.

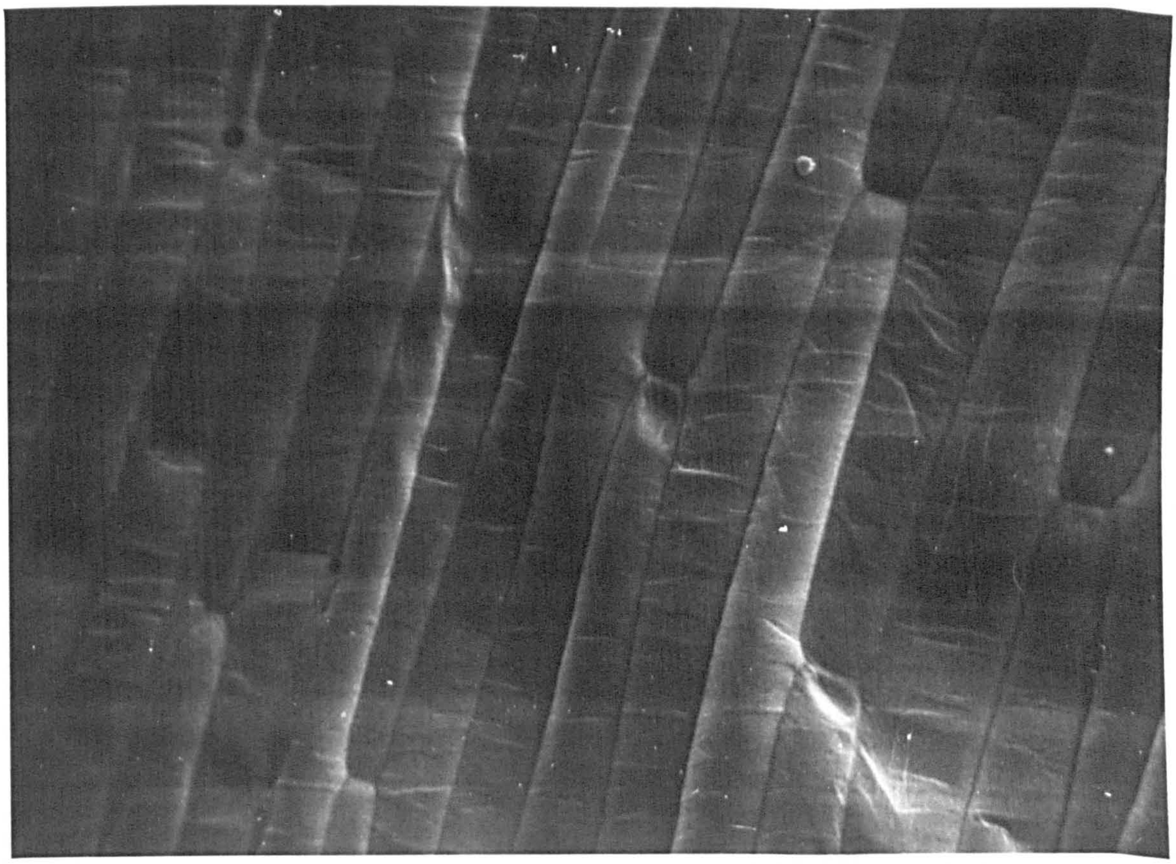


Plate 21 SEM observations:- Healthy undamaged abaxial leaf surface of Lolium perenne S23.

Plate 22 SEM observations:- Healthy undamaged abaxial leaf surface of Lolium perenne S23.

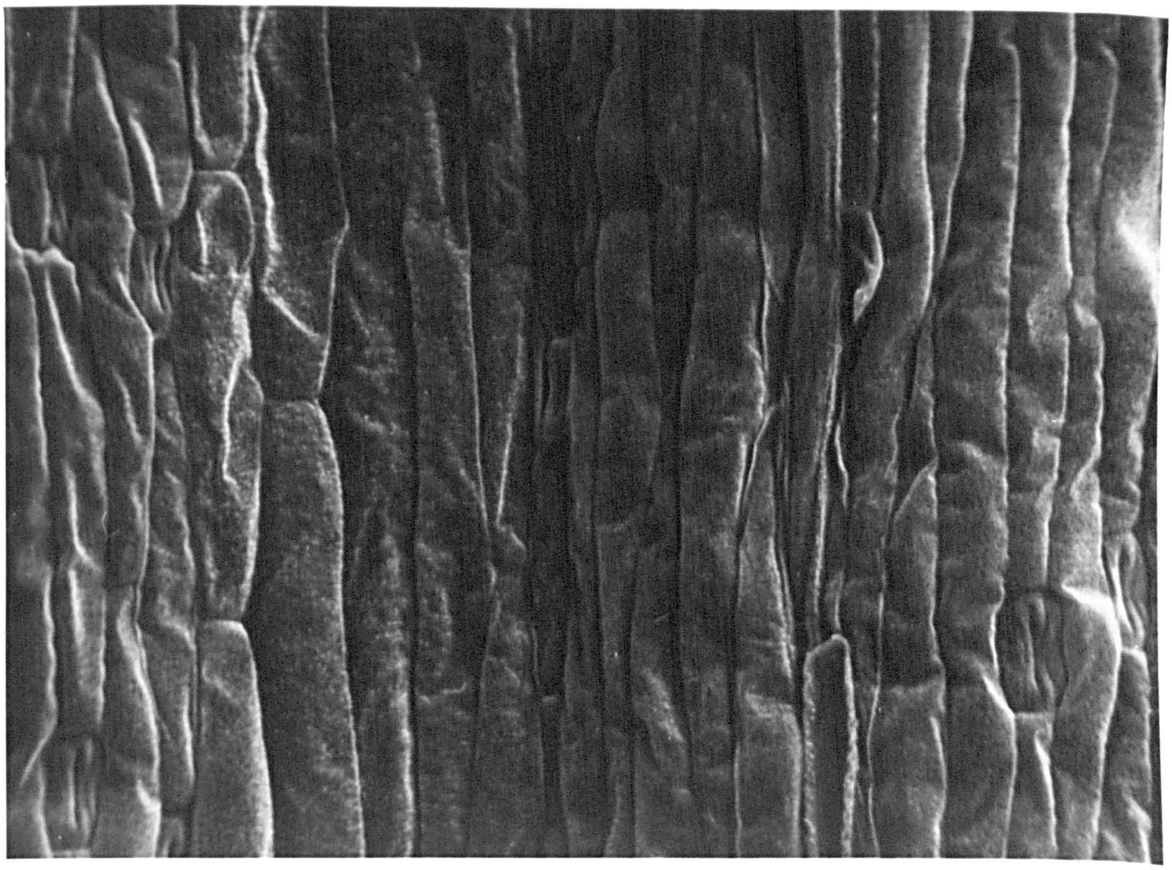


Plate 23 SEM observations:- Healthy undamaged adaxial leaf surface of Phleum pratense S48.

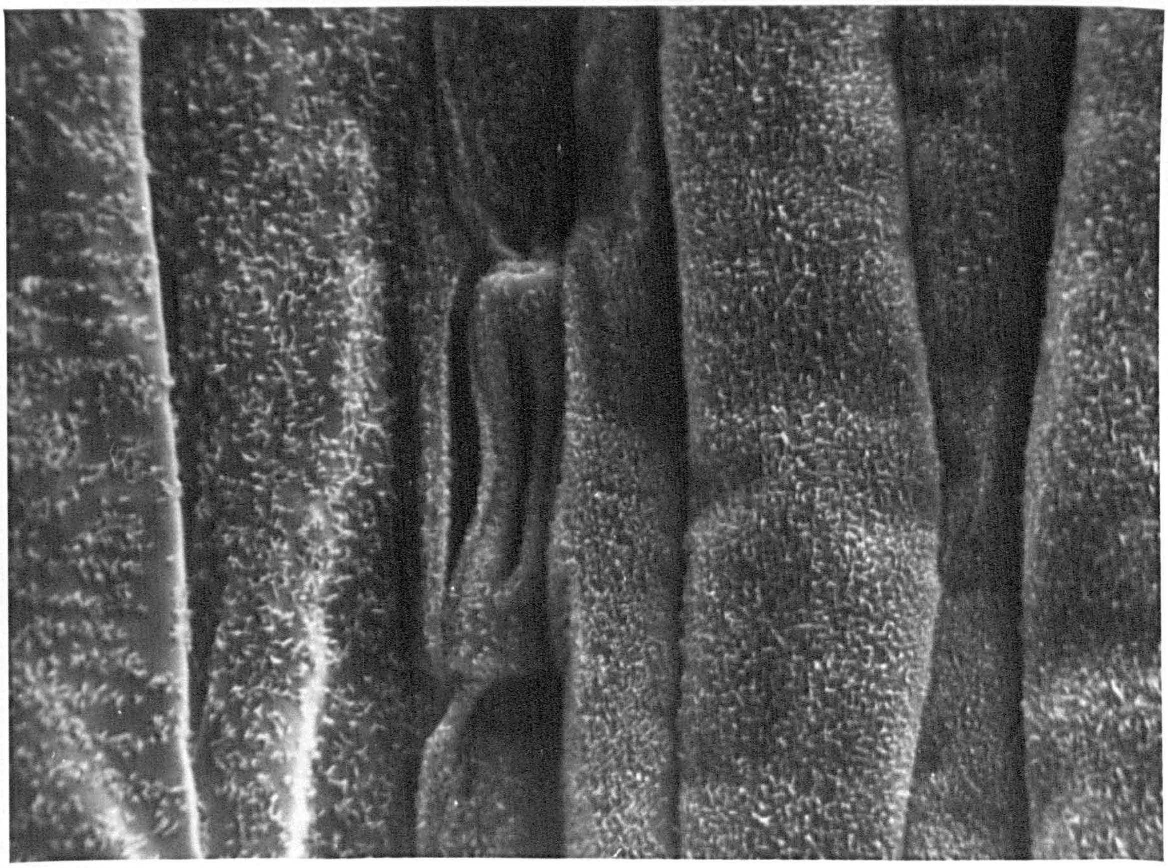


Plate 24 SEM observations:- Healthy undamaged adaxial leaf surface of Phleum pratense S48.

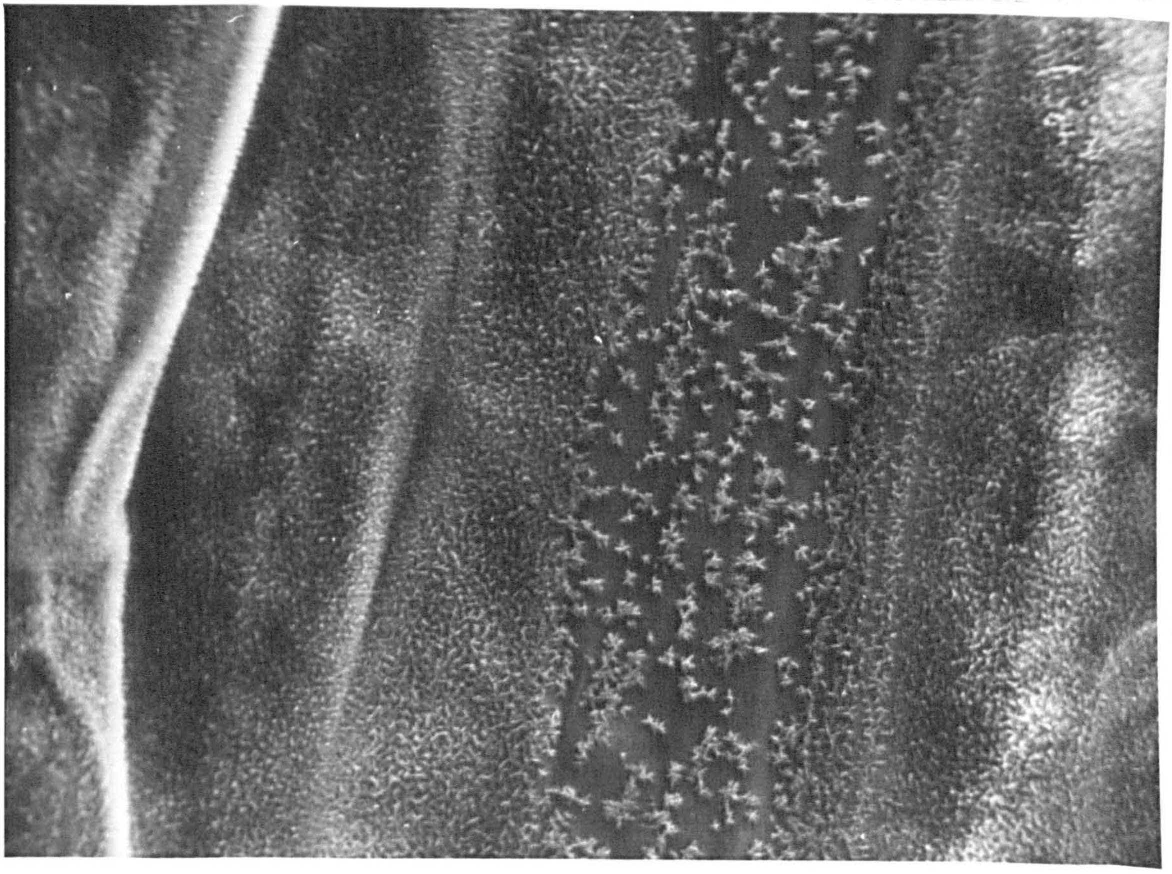


Plate 25 SEM observations:- Adaxial leaf surface of Lolium perenne S23 showing turgid cells and epicuticular wax crystals.

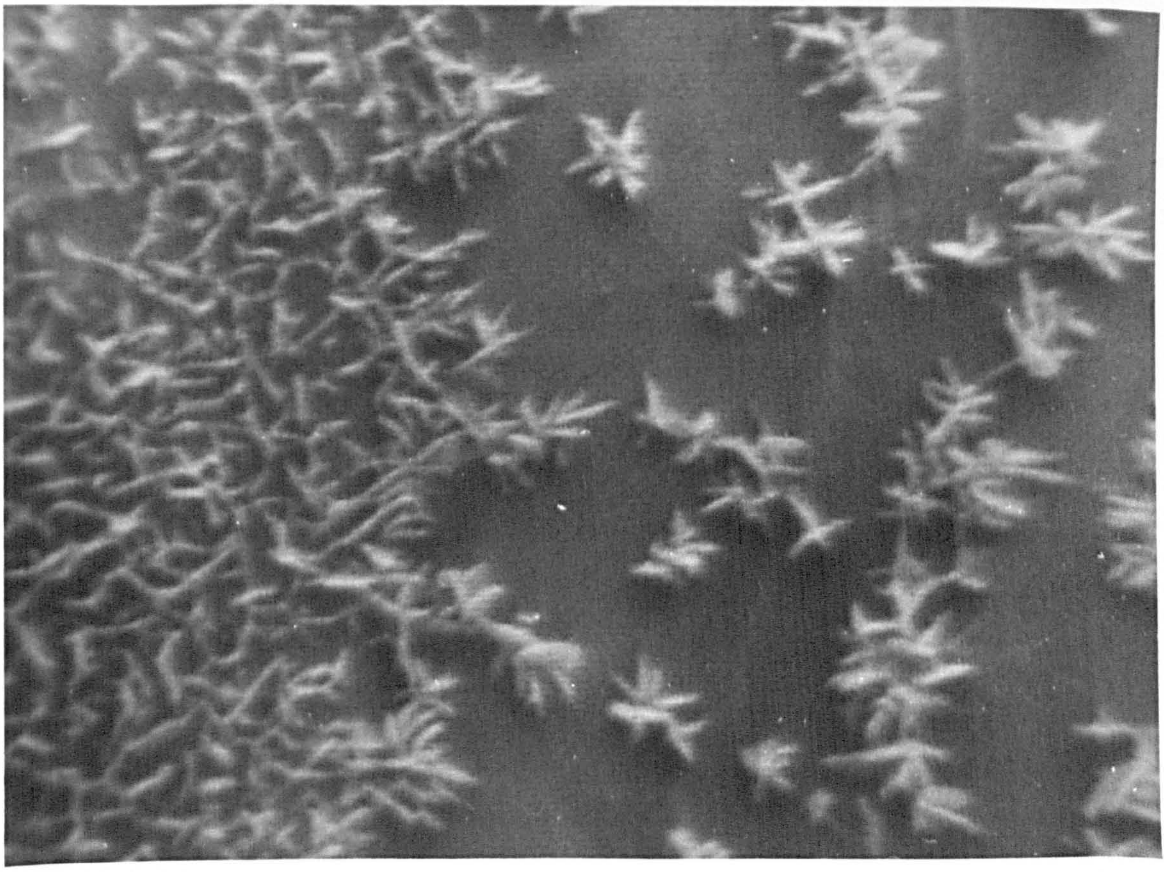


Plate 26 SEM observations:- Adaxial leaf surface of Lolium perenne S23 showing epicuticular wax crystals.

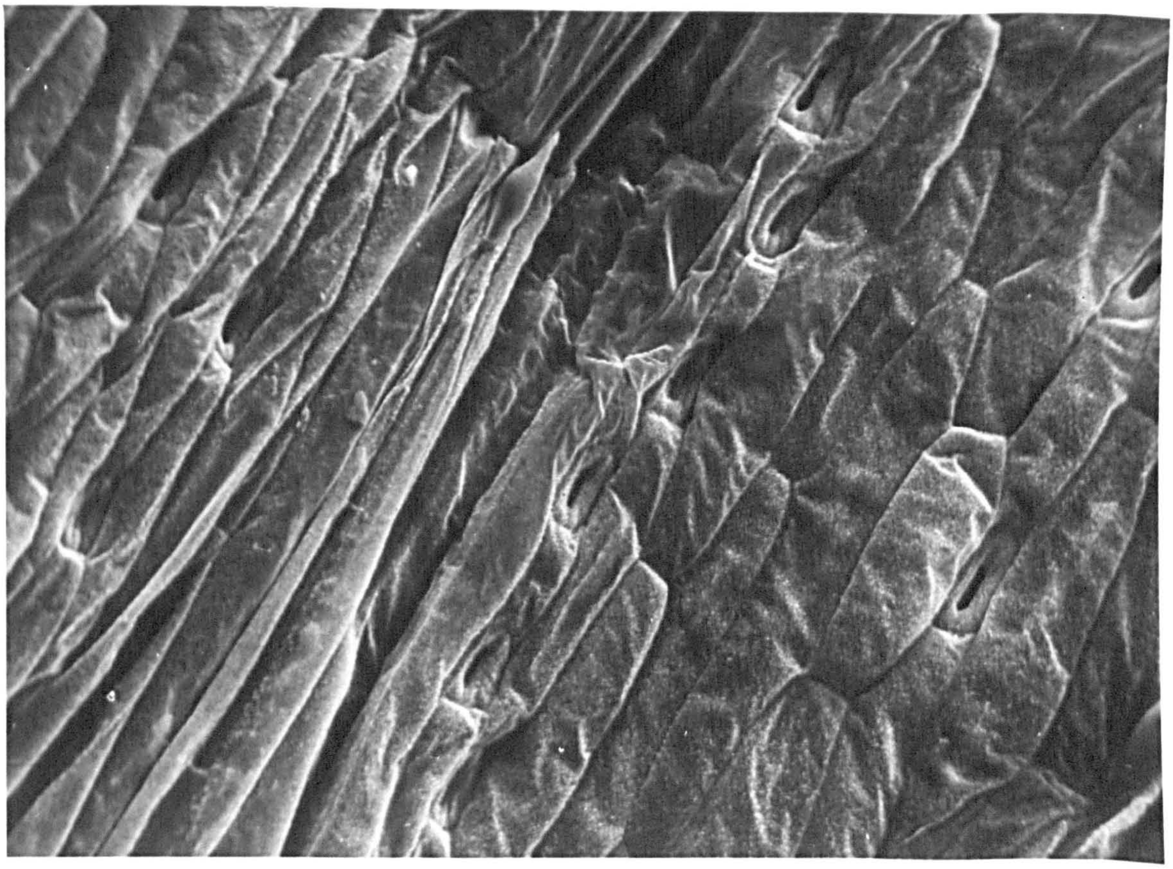


Plate 27 SEM observations:- Wide open adaxial stomata of Phleum pratense S48 immediately after one impact of the tamp.

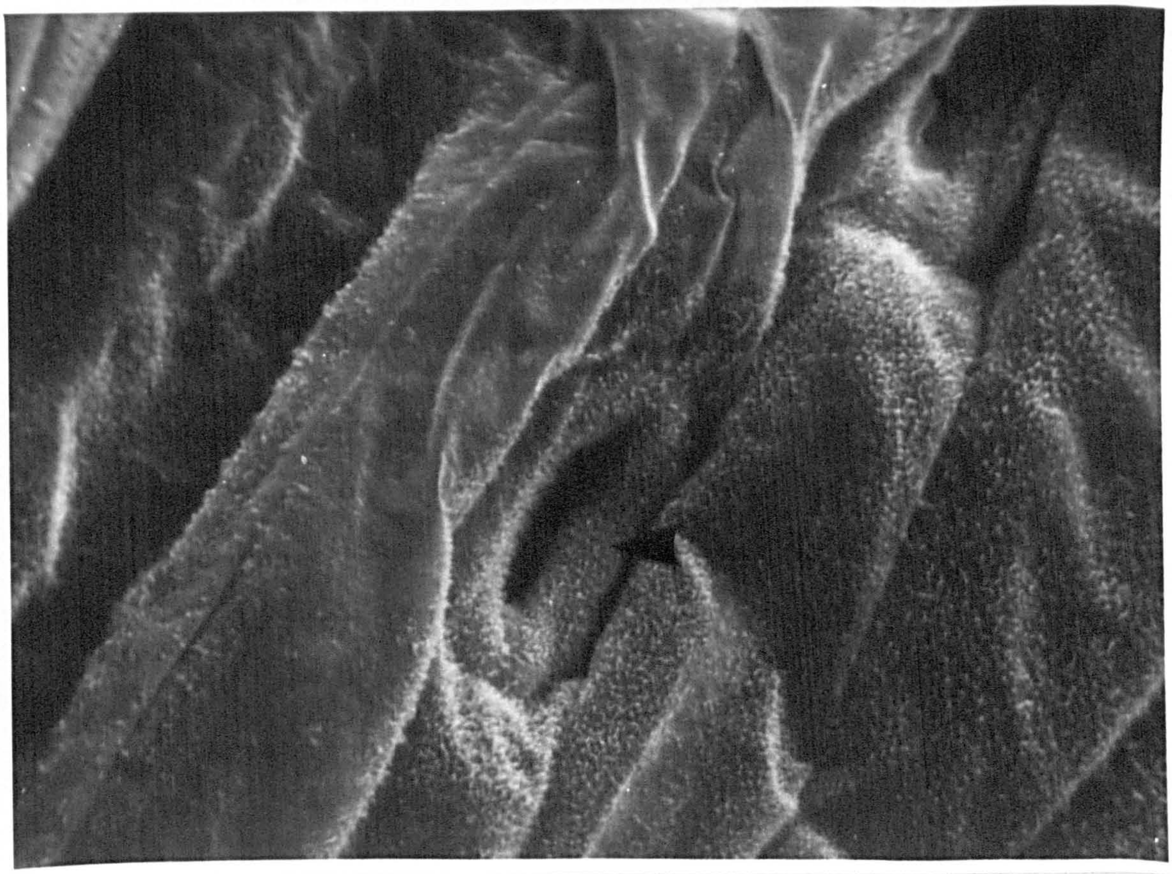


Plate 28 SEM observations:- Wide open adaxial stoma of Phleum pratense S48 immediately after one impact of the tamp.

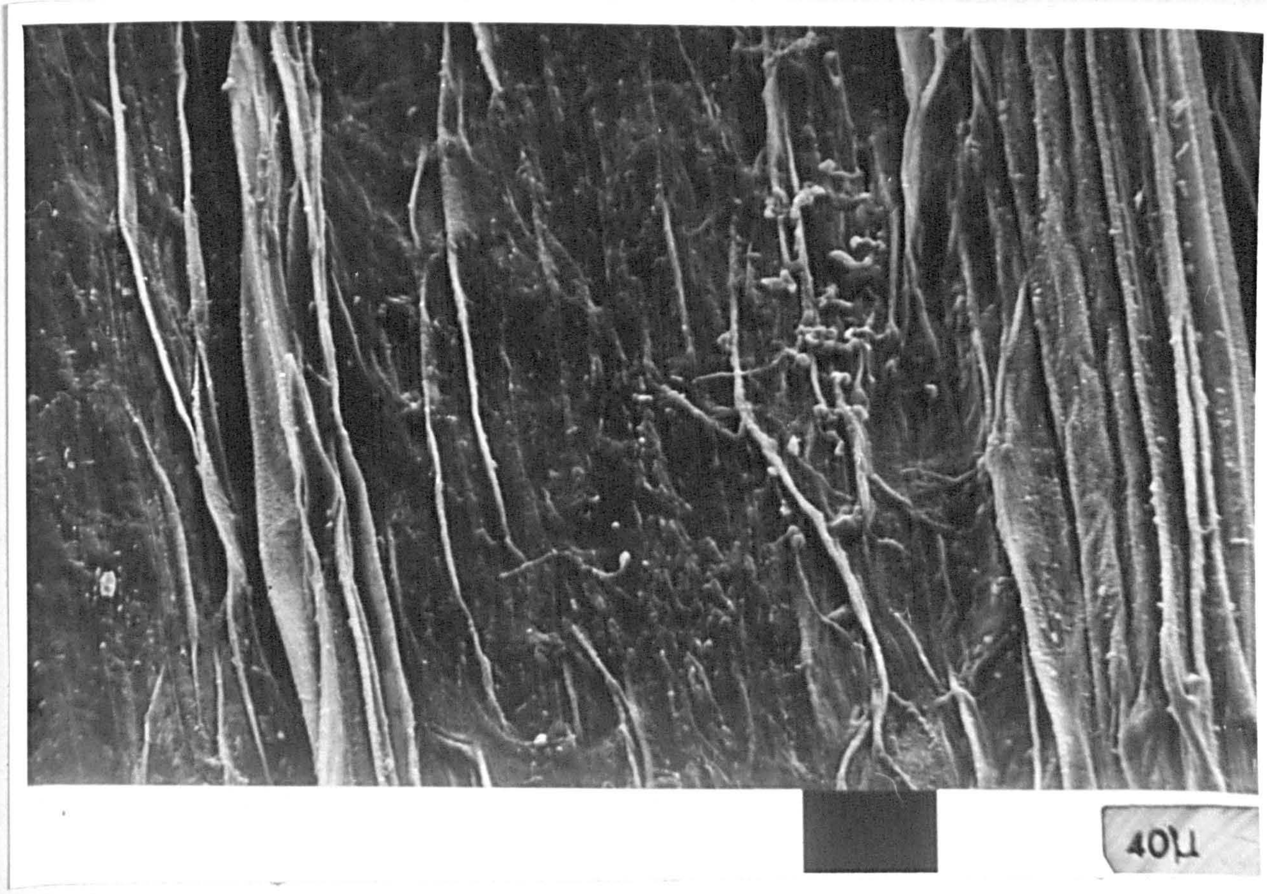


Plate 29 SEM observations:- Damaged adaxial leaf surface of Lolium perenne S23, 5 weeks after five impacts of the tamp.

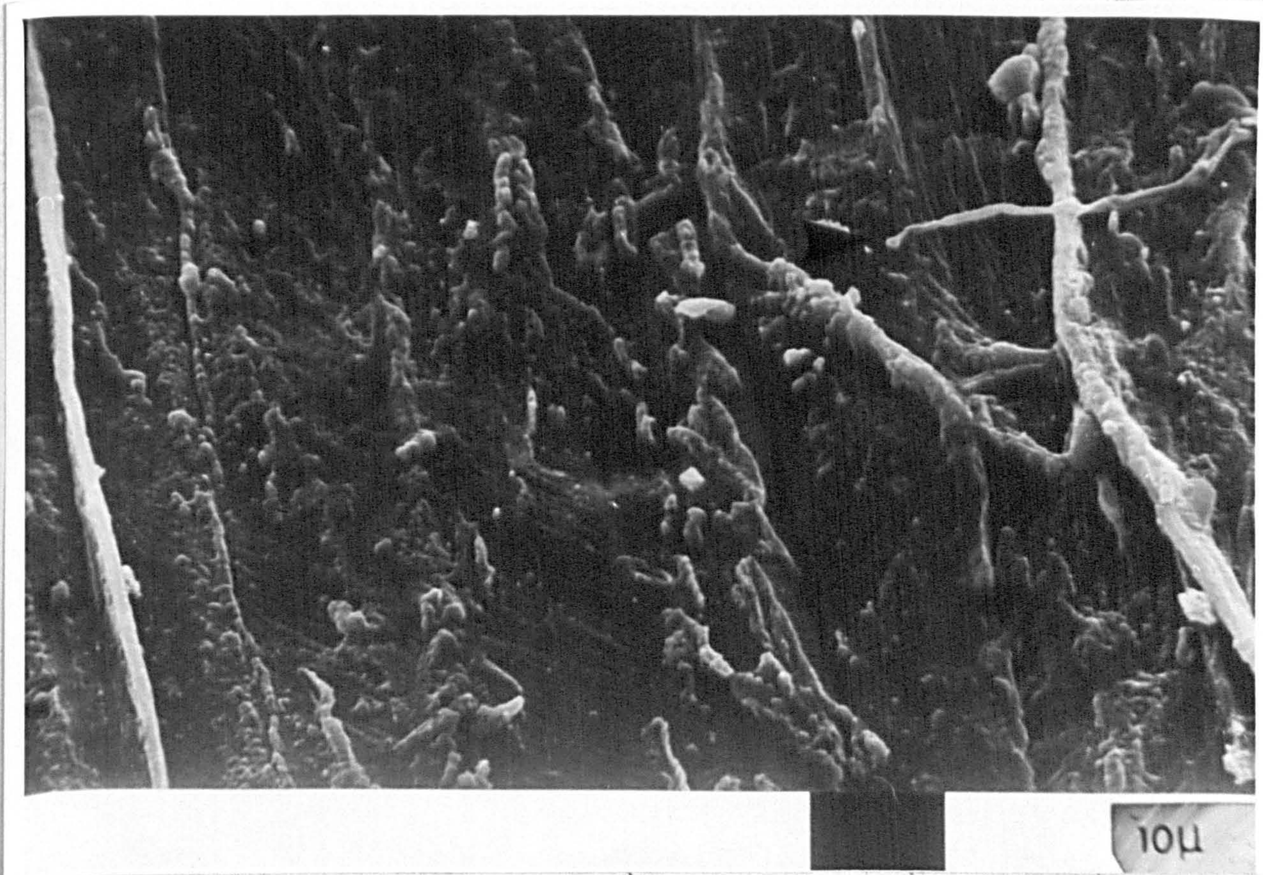


Plate 30 SEM observations:- Damaged adaxial leaf surface of Lolium perenne S23, 5 weeks after five impacts of the tamp noting germinated fungal hyphae.

had received simulated trampling.

The use of Lolium perenne samples prepared without the nitrogen fixation and accelerated freeze drying processes was unsatisfactory. Samples of untreated grass such as the typical one shown in Plate 31 appeared to have dehydrated considerably. This would make evaluation of the stomatal response to simulated trampling impossible. This method was therefore rejected in favour of the full fixation and freeze drying method.

Results of the stomatal response to simulated trampling in respect of time were rather indecisive. It appears that under the standard growth conditions with no simulated trampling, the stomata are mostly "partially open". With damage by simulated trampling the parts of the leaves and other whole leaves, apparently not directly affected, remained "partially open" over the period of time monitored. In the immediate area around damaged parts, conclusions were difficult to draw due to the presence of debris which could possibly consist of lumps of wax and fragments of damaged cells as well as soil particles (see Plate 17). However, most seemed to be partially open at every time investigated. Additionally, immediately after simulated trampling some stomata were open very wide (see Plate 32); at 1 and 1.5 hours no stomata were "open" and some were "closed"; and at 7 hours some stomata were very widely open. A noteable observation, as seen in Plate 32 is that adjacent stomata could be responding very differently.

Overall, results from this experiment were not clearly conclusive and many further examinations of samples seems imperative to ascertain what responses, if any, are made by stomata when simulated trampling damage is sustained. However, it does seem clear that epicuticular waxes are abraded by simulated trampling; there is dehydration of cells; in the long term, there is fungal

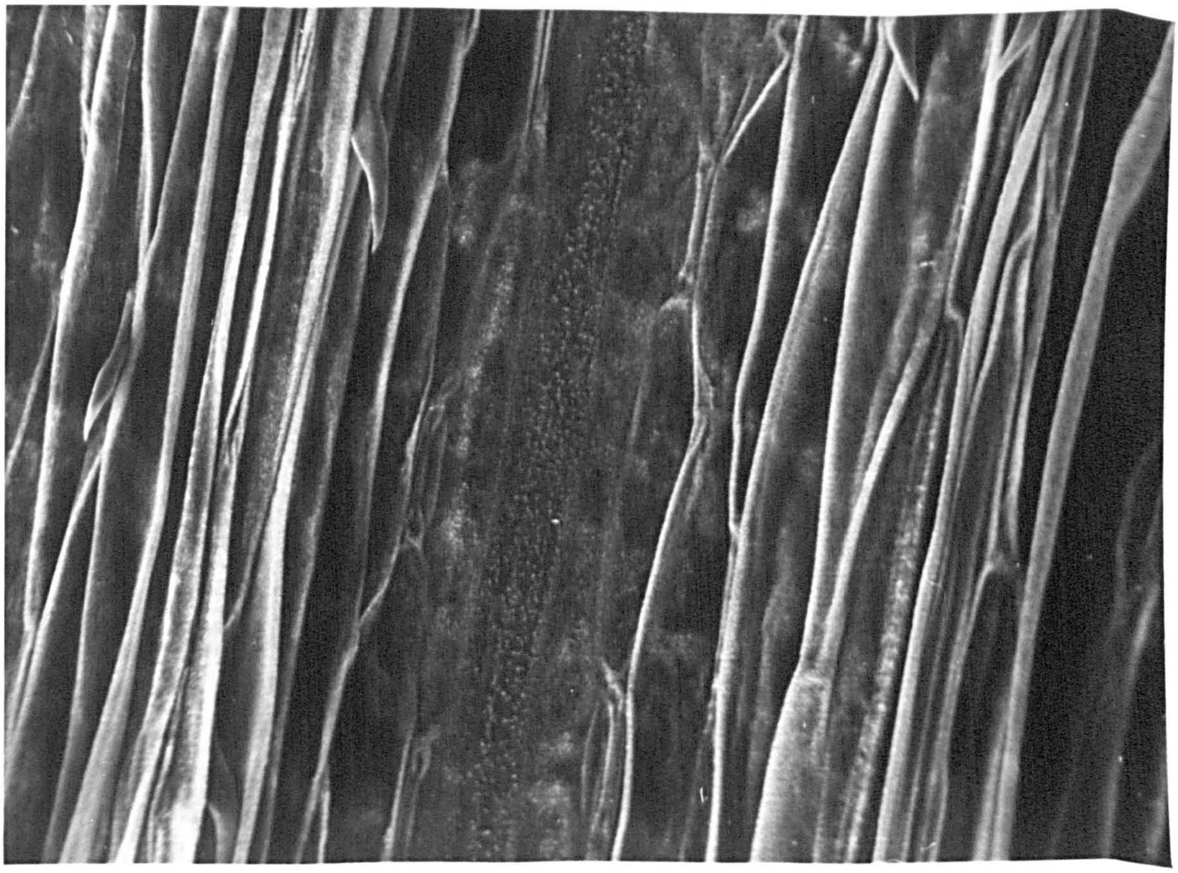


Plate 31 SEM observations:- Dehydrated adaxial leaf surface of Lolium perenne S23 following preparation omitting nitrogen fixation and accelerated freeze drying.

Plate 32 SEM observations:- Adaxial stomata of Lolium perenne S23 immediately after three impacts, noting a wide open stoma and a closed stoma.

attack where tissue has been damaged by simulated trampling.

Samples of grass blades taken from field plots (Chapter 6) and prepared for and observed in the SEM, were largely selected from the 100 passage plots. The time interval since the last trampling treatment was not known because the plots had been trampled once every week over the previous months so the damage could have been sustained at any time. However, it was considered that these samples may be comparable with those of the controlled environment samples of Lolium perenne taken 5 weeks after simulated trampling treatment (see Plates 29 and 30). In the trampled field samples (Chapter 6) there was evidence of disruption and loss of epicuticular waxes (see Plate 33). In places there was much abiotic material present. Fungal spores were in evidence, some of which had germinated and were developing hyphae networks (see Plate 34). Other parts had relatively undamaged waxes (see Plate 35) and usually the stomata were closed, although a few were partially open (see Plate 36).

These observations are similar to those found in controlled environment Lolium perenne that had received 5 i pot^{-1} 5 weeks prior to sampling. It may therefore be concluded that waxes are disrupted, dehydration of epidermal cells occurs and fungal activity is encouraged by both simulated trampling in controlled environment studies and by actual human trampling in the field.

Discussion

The similar response curves for decreasing RWC and $\%W_d$ of excised shoots (section a) (i)) indicated that in the circumstances as discussed in section a) (ii), $\%W_d$ was a reasonable substitute for RWC when measurement of water content of grass blades subjected to simulated trampling was undertaken. There was a significant decrease in $\%W_d$ with increasing intensity of simulated trampling. A rapid decrease in particular, over the initial 1.5 to 2 hours was recorded

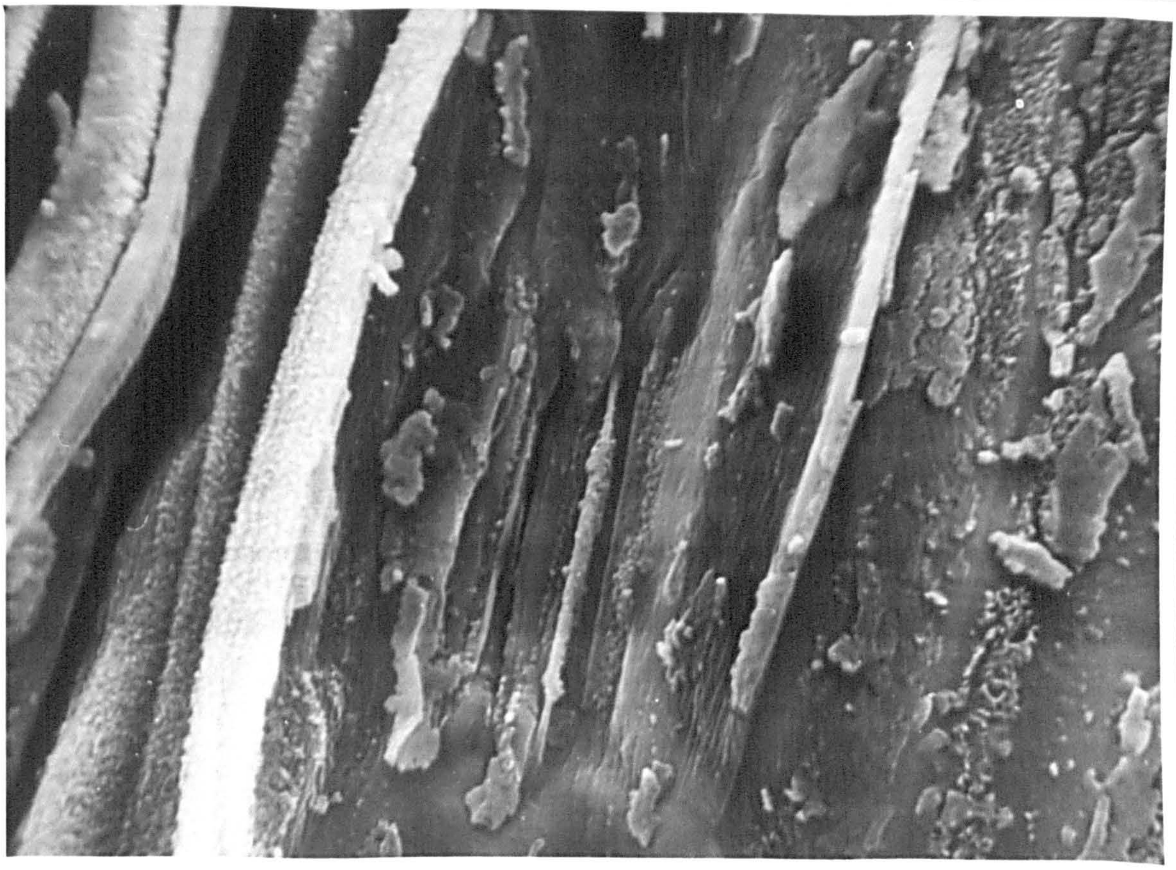


Plate 33 SEM observations:- Adaxial leaf surface of Lolium perenne S23 from field plots receiving 100 passages per week, showing loss and disruption of epicuticular waxes and much abiotic material.

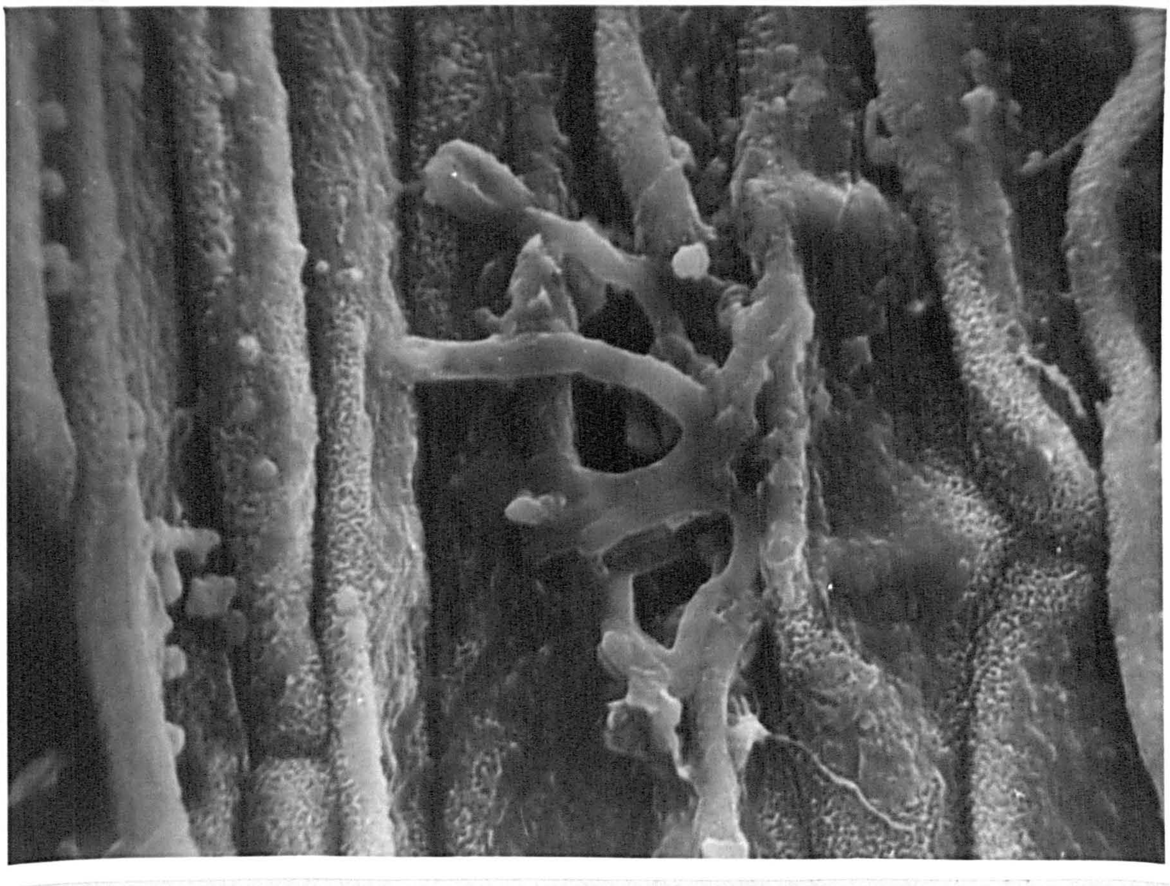


Plate 34 SEM observations:- Adaxial leaf surface of Lolium perenne S23 from field plots receiving 100 passages per week showing development of attacking fungal hyphae.

Plate 35 SEM observations:- Adaxial leaf surface of Lolium perenne S23 from field plots receiving 100 passages per week showing relatively undamaged waxes.

Plate 36 SEM observations:- Partially open stoma on adaxial leaf surface of Lolium perenne S23 from field plots receiving 100 passages per week.

for all treatments and the greater the intensity of simulated trampling, the greater was the decrease over this period of time. Grace (1974) noted a decreased water content in Festuca arundinacea subjected to wind stress and this was later seen in work by Grace and Russell (1982) and Pitcairn and Grace (1982).

Estimating stomatal closure points by calculation of phases during water loss was pioneered by Hygen (1951, 1953) and modified by Jarvis and Jarvis (1963a,b) and Bannister (1964, 1976) but is based on RWC. Stomatal closure in untreated Lolium perenne S23 (section a) (i)) was at around 70% RWC which compared to that of Calluna vulgaris (Bannister 1964). Grace (1974) found stomatal closure point in Festuca arundinacea at 83% RWC. After simulated trampling (section a) (ii)), estimates of stomatal closure could only be based on $\%W_d$ but in light of the similar response of RWC and $\%W_d$ in section a) (i), it was deemed to be acceptable. Surprisingly, the higher the intensity of simulated trampling, the lower the $\%W_d$ at stomatal closure point yet the timing of stomatal closure was similar for the different treatments. Grace (1974) estimated stomatal resistance of various grasses subjected to wind stress and found higher stomatal transpiration rates compared to that of control plants for Festuca arundinacea, Dactylis glomerata, Festuca pratensis, Lolium perenne and Lolium multiflorum. The stomatal closure points were at lower leaf water contents for wind treated compared to control plants (for example at 68% compared with 83%, respectively for Festuca arundinacea) but as in the present study, stomatal closure point occurred at similar time for all treatments.

Pasternak and Wilson (1974) compared rapid and slow loss of water in sorghum and found that when water loss was rapid, stomatal closure was at much lower leaf water contents to that of leaves subjected to slow water loss. The mechanism behind this, however, was not suggested. Willis et al. (1963) noted substantial stomatal opening in leaves of non-water stressed plants on leaf excision, causing increased transpiration rate for a short period of time before subsequent stomatal closure. Similar suggestions of this phenomena (called the Iwanoff effect after Iwanoff (1928)) had been recorded as early as Darwin and Pertz (1911). Stalfelt (1955) thought this may be due to changes in epidermal compared to guard cell turgor. As pointed out by Grace (1974), however, such a phenomenon was only temporary, lasting up to 50 minutes (Willis et al. 1963). The responses observed in the present study and by Grace (1974) were over several hours so further explanations are required.

Grace (1974) suggested that puncturing of subsidiary cells and other epidermal cells may cause less pressure to be exerted on the guard cells hence the stomata open wide. Also, the leaf water deficit required for stomatal closure would therefore be extremely high, as observed. Grace (1974) therefore concluded that wind causes leaf buffetting which results in an increased transpiration rate due to decreased stomatal and cuticular resistance. In the present study, cuticular transpiration rates (estimated after stomatal closure) were also found to be greater in grass treated with simulated trampling as was the case for wind-treated compared to untreated grass (Grace 1974).

Evidence to support the hypothesis of Grace (1974) and also to explain the increased cuticular transpiration rates was supplied by Thompson (1974, 1975). Scanning electron micrographs of Festuca arundinacea which had been exposed to wind showed removal of epicuticular waxes and also puncturing of epidermal cells, thus backing up the hypothesis of Grace (1974). Other SEM observations, such as Wilson (1984) on Acer pseudoplatanus have recorded considerable damage to leaves from leaf buffetting by wind. Russell and Grace (1978a) investigated the effect of wind and drought on Lolium perenne. They found, however, little evidence of damage resulting from collisions between neighbouring leaves in contrast to the observations for Festuca arundinacea (Grace 1974, Thompson 1974). No SEM observations or transpiration rate investigations were however made. Scanning electron micrographs in the present study, of grass that had been subjected to simulated trampling, showed remarkable similarity to those of wind treated grass (Thompson 1974, 1975). Loss of epicuticular wax crystals and dehydration of epidermal cells were frequent observations. So although Russell and Grace (1978a) doubted the significance of wind in damaging the epicuticular waxes and epidermal cells in Lolium perenne in contrast to the damage observed with Festuca arundinacea (Grace 1974, Thompson 1974), there is a very obvious effect with simulated trampling.

It would have been significant if conclusions about stomatal condition at various times after simulated trampling treatments were forthcoming but no clear reliable data was achieved. Investigation of stomatal aperture using diffusive resistance porometry was abandoned due to practical problems. Results from SEM observations failed to conclusively demonstrate the effect of simulated trampling on stomatal aperture. The various reasons include practical difficulties of observing stomata occurring at such positions along the surface that visually, conclusions were problematic. The presence of much debris, often over the stomata, again made conclusions difficult to draw. The actual process of excision and immersion in liquid nitrogen may have also influenced the stomata. Although there was a very small time lapse between excision and rapid freezing, the actual process of excision may have affected the stomata as observed by Stalfelt (1955) and Raschke (1970), thus giving erroneous observations. It would appear that further work would be very valuable in order to establish the pattern of stomatal response to such injury. It would seem, however, that simulated trampling causes disruption of the epicuticular waxes of grass blades and perhaps damage to the epidermial cell walls which leads to dehydration of the affected parts because of a resulting reduced stomatal and cuticular resistance to water loss.

Water loss of excised shoots with simulated trampling (section a) (ii)) indicated that the critical water contents when stomatal closure occurred were usually between 1.5 and 2 hours after excision. Experiments in section b) were therefore carried out measuring $\%W_d$ 2 hours after trampling treatment when grass plants were left intact. A range of species and cultivars were chosen to see if a differential response was present which may therefore indicate a reason for the observed differences in shoot yield response in Chapter 3. Each species and cultivar was treated with the full range of simulated trampling treatments. The greater the intensity of simulated trampling, the lower was the $\%W_d$ 2 hours after treatment. This was also true 24 hours following simulated trampling treatment (section c)). A log relationship was evident. This was similar to the curvilinear (log) relationship recorded between injury index and number of impacts in Chapter 3 (section a) page 44). An experiment relating injury index and $\%W_d$ at 2 hours (see Appendix 9) gave a significant correlation of $\%W_d$ with injury index for Lolium perenne S23.

The order of greatest to least reduction in $\%W_d$ with increasing number of impacts was Lolium perenne (no significant difference between cultivars), Phleum pratense S48, Agrostis tenuis Holfior and least of all, Poa pratensis Parade. There were differences between species for $\%W_d$ at 0 impacts but this was unlikely to contribute to the observed differences in response to increasing intensity of simulated trampling. The ranking from results of %W_d at 2 hours after treatment did not seem to correspond to rankings using shoot yield responses and injury index responses recorded in Chapter 3.

The results of section a) (ii) and section b) indicated that simulated trampling caused a greater decrease in water content from the shoots which was related to the intensity of treatment. This implied that the transpiration rate of grass subjected to simulated trampling was increased, especially as this had been observed in the response of plants to wind stress by Grace (1974), Grace and Russell (1982), Pitcairn and Grace (1982), Fluckiger et al. (1978), where there was much evidence of cuticular injury mediating an increase in transpiration (Thompson 1974, 1975). The observed decline in transpiration rates with increasing intensity of trampling, demonstrated in section c) was therefore not expected. This decrease was found to be significant not only over the first 3 hours but also over a 24 hour period after simulated trampling.

Despite the general concept in the literature that high wind leads to greater transpiration rates (Daubenmire 1959, Bidwell 1979), various work (Monteith 1965a, Grace 1977, Dixon and Grace 1984) has shown this view to be questionable. Dixon and Grace (1984) found a decrease in transpiration rate with increasing wind speed and using the Penman-Monteith equation (Monteith 1965a) predicted a range of responses of water loss to wind speed at a variety of light energy, saturation vapour pressure deficits and stomatal resistances. Only at very low levels of light energy was there an increase in transpiration with increase in wind speed. Dixon and Grace (1984) however, did not include factors such as the effects of leaf epidermal cell damage in their calculations, although in experimental trials there was some suggestion of a reduction in leaf surface resistance in Quercus robur after 5 days wind treatment. Dixon and

Grace (1984) concluded that leaf surfaces differ in their vulnerability to damage on a microscale, both as a result of cuticular differences and leaf posture.

Significant species/treatment interactions were obtained for transpiration rates after simulated trampling in section c). *Agrostis tenuis* Highland proved to be particularly sensitive to the effects of simulated trampling changing from having the highest rate of transpiration without treatment to the lowest rate with treatment. *Lolium perenne* S23 was also sensitive. *Phleum bertolonii* S50 showed an intermediate response while *Poa pratensis* Primo was least affected, with a relatively small reduction in transpiration rate with trampling. This response seemed to have some similarity to the results of shoot yield and injury index (Chapter 3). Further experiments including all species and cultivars would be required before any correlations were feasible.

The paradox of reduced shoot water content yet reduced transpiration rate was investigated by estimating the % relative conductance as described in section c). For each species used there was greater reduction in % relative conductance the greater the intensity of simulated trampling, but also differences in response between species. *Agrostis tenuis* Highland had greatest decrease, then *Lolium perenne* S23, *Phleum bertolonii* S50 and with smallest decrease was *Poa pratensis* Primo. This reduction in % relative conductance represents a reduced conductance of water through the intact plant system. Pitcairn and Grace (1984) concluded that wind resistant and wind susceptible ecotypes of *Molinia caerulea* displayed differences in transpiration rate and water potential with wind stress because of a differential effect on the hydraulic pathway from soil to leaf surface. Water potential measurements of grass subjected to simulated trampling were attempted (section g)) but practical difficulties prevented any results being obtained. Radin and Boyer (1982) found the reduced growth of nitrogen deficient plants to be due to inadequate turgor for cell elongation (Hsaio 1973) which was due to a low daytime water potential in expanding cells while transpiration was occurring. This was explained by decreased root hydraulic conductivity. They concluded that anatomical, ultrastructural or biochemical features may cause differences in hydraulic conductivity. The resistance to water movement across a root is believed to be largely in cell membranes and there are suggestions that hydraulic conductivity may be related to membrane fluidity (Markhart et al. 1979a,b, Markhart et al. 1980). Despite incomplete understanding of the mechanism, however, reduced hydraulic conductivity may well explain the phenomenon of reduction of leaf water content yet reduced transpiration rate at 3 and 24 hours after simulated trampling. Pitcairn and Grace (1984) suggested that there may be breaking of the water continuum either by cavitation within the xylem vessels or by breakage at the root-to-soil interface. This would cause substantially decreased hydraulic conductivity. Damage to xylem vessels of shoots with trampling may well cause a decreased hydraulic conductivity. Indirect effects of soil compaction may have initiated greater root resistance to water uptake in view of work by Goss and Scott-Russell (1980). There may also be an effect on hydraulic ocnductivity by hormonal changes in the roots and/or shoots (Markhart 1979b).

The apparent effect of reduced hydraulic conductivity was further suggested in section d) where the effect of humidity on transpiration rate and %W_d was examined. The lower humidity caused higher transpiration rates as observed by Turner et al. (1984) but the decline in rate with increasing intensity of simulated trampling was evident. There was a lower %W_d at the lower relative humidity but again this declined with increasing intensity of simulated trampling. These results would seem to accomodate the observed reduced hydraulic conductivity with simulated trampling but also the effect of vapour pressure deficit. At the higher vapour pressure deficit there would be a greater gradient in water potential, which therefore causes greater water loss, hence a higher transpiration rate. The % relative conductance, however, is reduced with simulated trampling which indicates an impaired hydraulic conductivity. A lower shoot $\%W_d$ therefore occurs.

From these observations on some aspects of water relations of grass, it would appear that simulated trampling causes an immediate reduction in leaf water content which is related to the intensity of treatment. This water loss appears to be a result of reduced cuticular resistance because of disruption of epicuticular waxes and damage to epidermal cells and also a possible effect of stomatal opening in response to rapid turgor changes of surrounding cells. The time scale of this rapid water loss appears to be a matter of two hours or less. It is also observed that the transpiration rate is reduced before 3 hours after simulated trampling. The reduced leaf water content at a reduced transpiration rate would appear to be due to a reduced hydraulic conductivity which may be caused by a variety of factors.

CHAPTER 6

Field Studies: Response of Lolium perenne S23 in the field to simulated trampling and real trampling.

Introduction

In their trampling stress studies, Bayfield (1971a), Leney (1974) and Smith (1978) investigated both the effects of simulated trampling in the laboratory and the effects of real trampling in the field. Both Leney (1974) and Smith (1978) emphasized that there are limitations to the conclusions made from laboratory studies of plant response to simulated trampling stress. This is because simulated trampling does not reproduce all the forces involved in trampling (Canaway 1975a) and also the modifying effects of the environment, soil and surrounding plant community, all of which are variable, may exert a variety of other stresses.

It was therefore necessary, as in the work of Bayfield (1971a), Leney (1974) and Smith (1978), to relate the laboratory experiments of chapters 3, 4 and 5 in the present study to some comparative investigations in the field. This was achieved firstly by investigating the physiological responses to simulated trampling of pots of grass grown out in the field then tested in the laboratory. Secondly, the effects of real trampling on field plots of *Lolium perenne* S23 receiving management similar to that of a typical football pitch were examined.

a) Effects of simulated trampling on pots of Lolium perenne S23 grown in the field.

Introduction and Methods

One of the criticisms of laboratory studies (Leney 1974) is that plants are subjected to an unnatural environment, particularly in respect of absence of factors such as wind and natural environmental fluctuations.

To examine if this had a major influence on the plant response to simulated trampling, pots of Lolium perenne S23 were germinated and grown to the 4 week stage in the greenhouse as described in Chapter 2 (page 18) so they attained a viable size. The pots of grass were then sunk into holes in the turf by the field trial plot (section b)) so the rim of the pot was flush with the soil surface. The pots of grass were thus exposed to normal environmental conditions for a period of 4 weeks. These conditions included all the meteorological variables and shoot competition from the surrounding turf. Only root competition was excluded because of enclosing in a pot to facilitate the later experiments. In parallel with the field plots (section b)) the area with the embedded pots of grass was mown weekly to 3 cm, hence giving a weekly cutting treatment as for a football pitch. At the 8 week stage, the pots were lifted and transferred to the growth cabinets (conditions as described in chapter 4, page 74) to acclimatize to the laboratory conditions for 5 days. The following investigations were then carried out. Pre-method comments in the previous chapters apply in each of the following investigations.

(i) Injury to shoots of outdoor pots of grass with simulated trampling.

The procedure of Chapter 3 section a) (page 41) using the method described in Chapter 2 (page 39) was carried out for 0, 3, 7 and 20 i pot^{-1} and 5 replicates. Values of I were obtained and results were analysed using analysis of variance and regression analysis.

(ii) Shoot yield of outdoor pots of grass with simulated trampling.

The procedure of Chapter 3 section b) (page 42) was followed, similarly using the pots of grass from section (i). Dry weights of weekly cuttings to 3 cm were obtained (pots of grass housed in the greenhouse subsequent to treatment) and the values for the 3rd week after treatment were particularly noted. Results were analysed using analysis of variance and regression analysis. The relationship between shoot yield and I was examined (as for Chapter 3 section b)).

(iii) CO_2 flux of outdoor pots of grass with simulated trampling.

The same procedures as described in Chapter 4 section b) (i) (page 78) were followed to measure and evaluate dark and light whole pot CO_2 flux after treatments of 0, 1, 3 and 5 i pot^{-1} with 3 replicates per treatment. Readings were taken at 30 minutes after treatment (30 minutes in the assimilation chamber) as before and were analysed using analysis of variance.

Further, the methods of Chapter 4 section d) (page 81) were followed to continuously monitor whole pot CO_2 flux of a pot of grass before and after 15 i pot^{-1} at illuminance >45 klux.

(iv) $\%W_d$ of outdoor pots of grass with simulated trampling.

$\%W_d$ of pots of grass was found at 2 hours after simulated trampling treatment as described in Chapter 5 section b) (page 104). Treatments were 0, 3, 7 and 20 i pot^{-1} with 5 replicates. Results were analysed using analysis of variance and the relationship of $\%W_d$ with I and shoot yield was examined using regression analysis.

Results

(i) Injury to shoots of outdoor pots of grass with simulated trampling.

Results are given in Fig. 83. There was a highly significant ($p<0.001$) increase in I with increase in impacts and regression analysis showed a significant ($p<0.001$) linear relationship of increasing I with increasing i pot^{-1}.

Fig. 83 Injury of outdoor pots of grass with simulated trampling.

Fig. 84 Shoot yield of outdoor pots of grass with simulated trampling: weekly cuttings 3 weeks after treatment.

Fig. 85 Whole pot CO_2 flux (root/soil + shoots) of outdoor pots of grass with simulated trampling:

○ in light at >45 klux
● in dark conditions

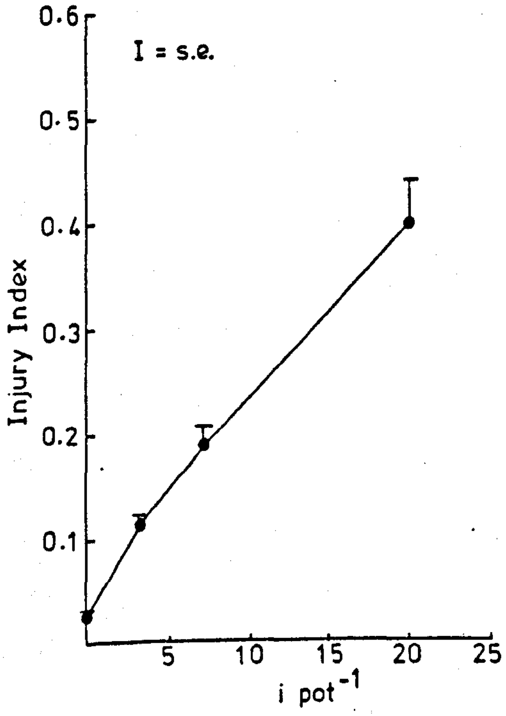


Fig. 83

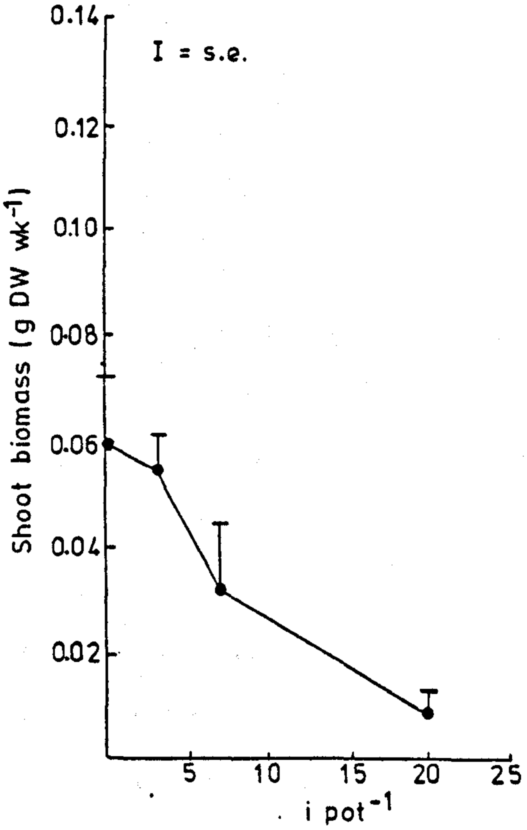


Fig. 84

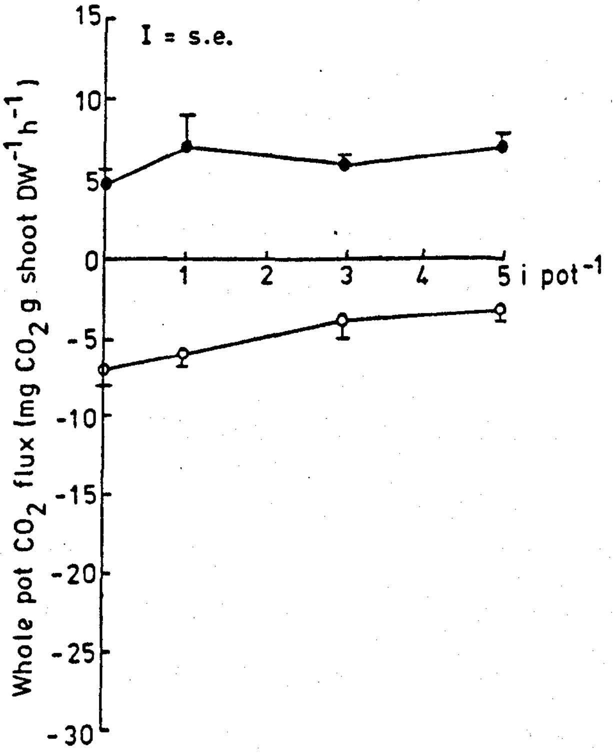


Fig. 85

(ii) Shoot yield of outdoor pots of grass with simulated trampling.

Results are shown in Fig. 84. There was a significant reduction ($p<0.01$) in shoot yield (i.e. 3rd weekly cutting after treatment) with increasing intensity of trampling. The correlation of decrease in shoot yield with increase in I was highly significant ($p<0.001$).

(iii) CO_2 flux of outdoor pots of grass with simulated trampling.

Figs. 85 and 86 show the results. There was a significant reduction ($p<0.05$) in light whole pot CO_2 flux with increasing intensity of trampling but no significant effect of simulated trampling treatment on dark whole pot CO_2 flux (Fig. 85).

When a pot of grass was continuously monitored (Fig. 86), pre-treatment light whole pot CO_2 flux was comparable with that for grass receiving 0 i pot^{-1} (Fig. 85). Immediately after simulated trampling there was an immediate reduction in light whole pot CO_2 flux (Fig. 86) which then gradually increased, but at 33 hours, when the experiment was terminated, it had not reached the pre-treatment rate.

(iv) $\%W_d$ of outdoor pots of grass with simulated trampling.

Fig. 87 graphs the response. There was a significant reduction ($p<0.001$) in $\%W_d$ with increasing number of i pot^{-1}. The correlations of $\%W_d$ with I and also with shoot yield were both highly significant ($p<0.001$).

b) Effects of real trampling on a field plot of Lolium perenne S23.

Introduction

Relating results from the unnatural laboratory set-up to the field has been attempted in other pieces of research using simulated trampling such as Bayfield (1971a), Leney (1974) and Smith (1978).

Leney (1974) found that simulated trampling laboratory tests with greenhouse grown monoculture populations (of 15 different grass species) did not necessarily reflect the resistance of the species to

Fig. 86 Continuous whole pot CO_2 flux monitoring of one pot of grass, before and after 15 impacts in light >45 klux.

Fig. 87 $\%W_d$ at 2 hours after simulated trampling of outdoor pots of grass with simulated trampling.

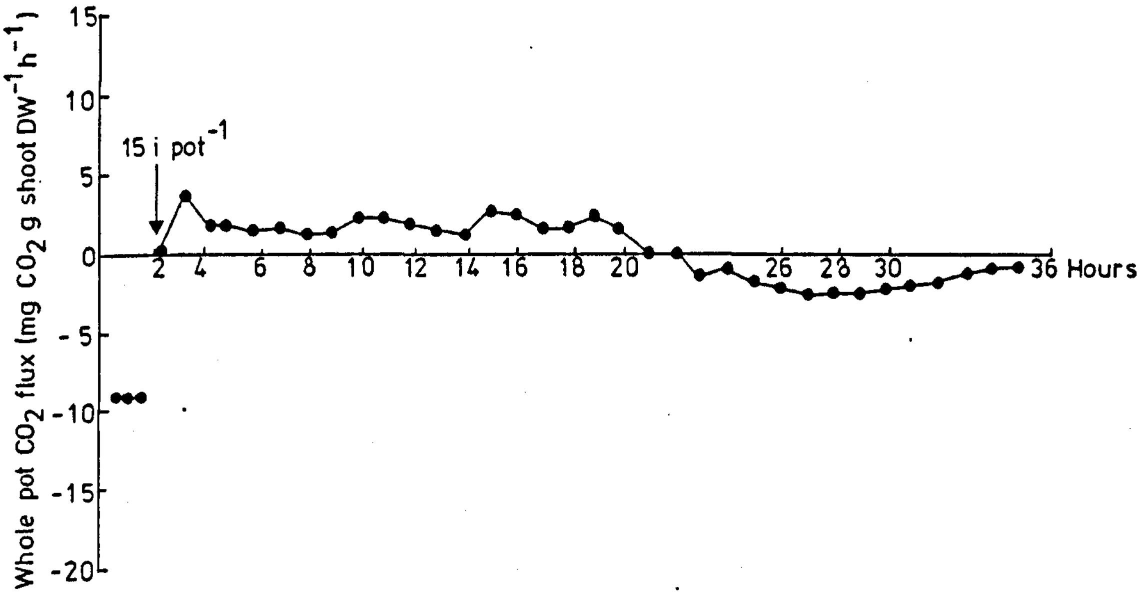

Whole pot CO$_2$ flux (mg CO$_2$ g shoot DW^{-1}h^{-1})
15
10
5
0
- 5
-10
-15
-20
15 i pot^{-1}
2
4
6
8
10
12
14
16
18
20
26
28
30
36
Hours

Fig. 86

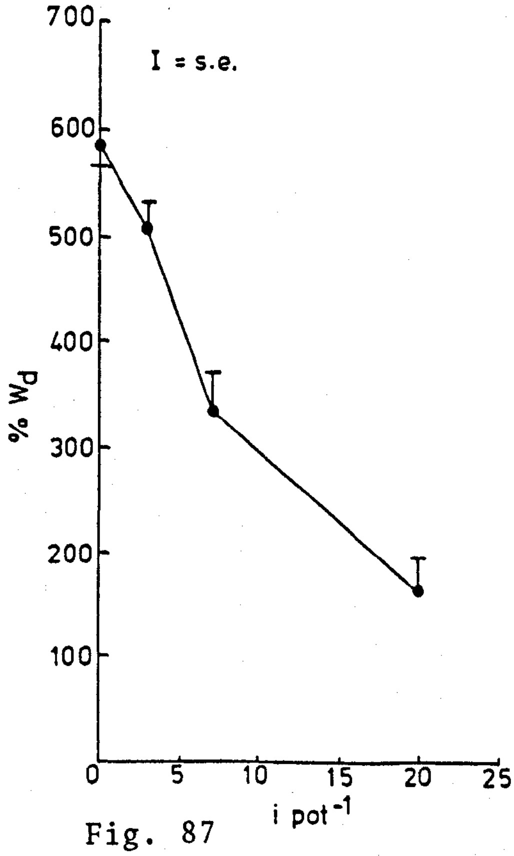

700
600
500
400
300
200
100
% W$_d$
I = s.e.
0
5
10
15
20
25
i pot^{-1}

Fig. 87

trampling in the field.

Modifying factors include the environmental variation and stresses and also the difference between simulated trampling and real trampling. It was therefore appropriate to compare laboratory studies of shoot yield, CO_2 flux and water relations with field studies of these factors. To this end, a field plot was established and used.

Ideally, all species and cultivars listed in Table 1 would have been tested in both monoculture sward plots and also in typical sports turf mixture plots but as time was limited a plot of the standard grass Lolium perenne S23 (see Chapter 2 page 19) only was investigated.

Field Plot establishment, management and application of real trampling.

In September 1979 at the Botanic Gardens, Keele, Staffs (O.S. SJ/813449), a flat unshaded area of previously regularly mown grassland was selected. An area 7 m x 4 m was marked out and treated with a glyphosate weedkiller, "Tumbleweed". A buffer zone 1 m wide around the plot was treated with "Asulam" to kill and prevent any regrowth which may otherwise invade the monoculture plot.

After 3 weeks when all vegetation appeared dead, the plot was raked to remove the dead material, surface rotovated and remnant plant material raked up. The surface was levelled and the plot was sown by hand with seed of Lolium perenne S23 at a rate of 32.4 g m^{-2}. Germination was observed 9 days later and the grass had reached about 5 cm height 16 days after sowing.

The plot was then left over winter so that the sward could become more established. At the beginning of April 1980, after the onset of dry sunny weather the plot was rolled with a 152.4 kg roller, raked to loosen the surface and remove dead material, then watered. The plot received its first mowing 2 days later, to a height of 4 cm and cuttings were removed. Areas of poor grass density were seeded and any surface depressions were corrected by addition of soil. 4 weeks later, the sward was mown to 5 cm and thereafter, fortnightly until the end of June, resulting in an even, compact turf (Plate 37). During this period the plot was fertilised once (by hand) at a rate of 25 g m^{-2} using a standard sports turf fertiliser (8:12:8, N:P:K). At the end of June 1980, the plot was considered ready for trampling treatments to commence. It was divided into 8 strips of width 0.5 m and length 7 m. The plots were mown weekly thereafter, to 3 cm, strip by strip with the cuttings collected. Weekly trampling treatments were applied the day following mowing. Earlier preliminary trials of trampling on the adjacent grassland had indicated a suitable range of treatment intensity. Trampling treatments were 0, 10, 30 or 100 "passages" where 1 "passage" consisted of walking up and down the length of the strip keeping to the right and hence the whole area of the strip was trampled rather than just the central portion. With a weight of 50.80 kg and size 4 walking boots (with vibram soles giving a contact area of 120 cm^2), the estimated force when standing was 0.212 kg cm^{-2}, hence during "trampling" the static force would reach 0.423 kg cm^{-2} and the dynamic forces would exceed this. Treatments were randomly allocated to the strips and the treatments were replicated once, giving 2 blocks.

Weekly mowing and trampling continued to mid October (Plate 38). Trampling was continued over winter, thus simulating a typical winter sports field, but only on half of the length of the strips (Plate 39). Weekly mowing was resumed at the end of March 1981 and continued along with weekly trampling until the field experiments and measurements were terminated in July 1981 (Plate 40).

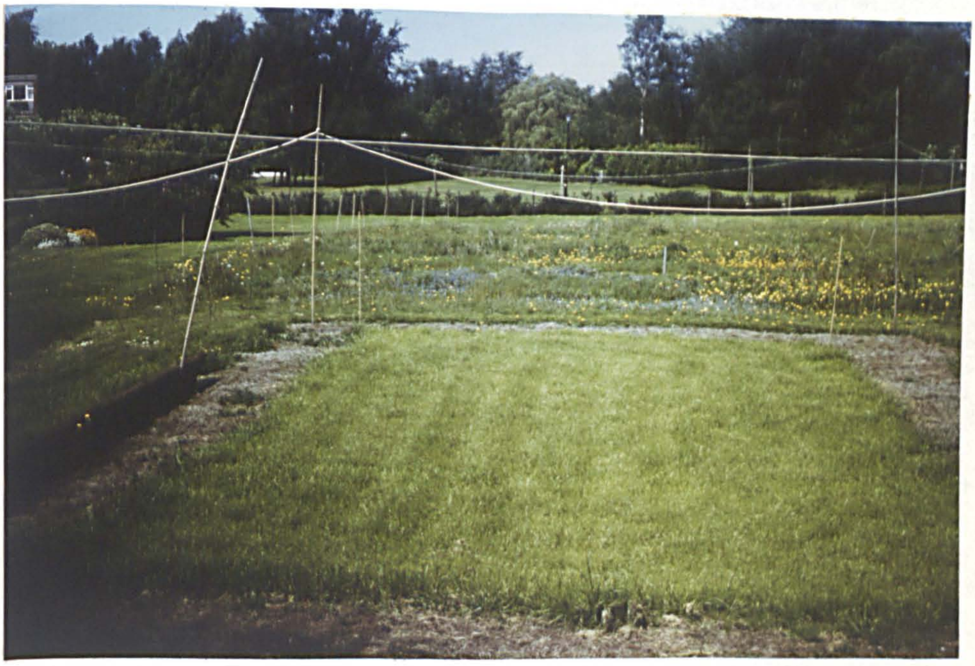


Plate 37 Field plots: end of June 1980 - ready to commence treatment.

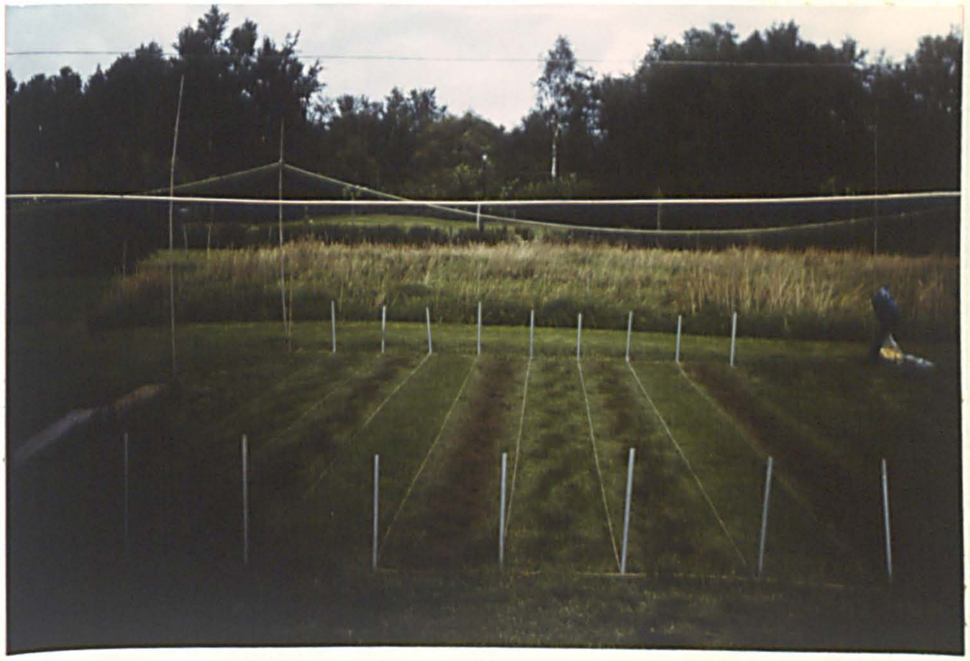


Plate 38 Field plots: mid October 1980 - effects of approximately 3 months of trampling treatment.

Plate 39 Field plots: late October 1980 - effects of trampling treatments continued on one half only.

Plate 40 Field plots: June 1981 - termination of trampling treatments.

Field Plot experiments and measurements.

In order to discover if the results from the laboratory studies in Chapters 3, 4 and 5 may apply to real trampling in the field, as many similar experiments as possible were carried out. Measurement of injury, shoot yield, CO_2 flux and $\%W_d$ were all attempted as follows:

(i) Injury of grass with real trampling.

A week was chosen when the weather was dry (April 1981) because from experience, trampling in wet conditions led to much soil contamination of the grass blades.

After the plots had been trampled as usual, a quadrat was used to randomly select a 10 x 10 cm square in each strip. The shoots were excised and half the sample was placed in a boiling tube and covered with parafilm. 40 ml of double glass distilled water was added on return to the laboratory (within 20 minutes) and the rest of the method for measurement of injury using electrolyte release as described in Chapter 2 (page 39) was followed. For each strip, there were 5 replicates. Values of I were obtained, graphed and analysed using analysis of variance. Problems of very limited grass on the 100 passage strips were partly overcome by sampling from the edges.

Samples of grass blades damaged by the trampling treatments were also collected from the 100 passage strips and photographed for comparison with results of Appendix 3.

(ii) Shoot yield of grass with real trampling.

The weekly mowings from each strip were collected. Fresh weights and dry weights after drying overnight at 95°C were obtained. Results were graphed and analysed using analysis of variance.

During the period July to October 1980, general observations of differences in ground cover (Plates 38 and 39) suggested that measurement of ground cover was very relevant. Point quadrat readings were therefore taken in October 1980. Each strip was sampled 13 times and each sample consisted of 16 points. The number of points that contacted vegetation was expressed as a proportion of the total 16 points. After arc sin transformation of the data, analysis of variance was carried out.

(iii) CO_2 flux of grass with real trampling.

Parkinson (1981) presented a method of measuring field soil respiration rate by taking sequential samples from a specially designed chamber placed over the soil. The CO_2 flux was obtained from regression analysis of calculated values incorporating the series of readings, time of readings, chamber volume, sample volume and area of soil surface. The theory and equations for the regression are presented in Parkinson (1981). Some details have, however, proved to be unreliable.

The principles of the method, nevertheless, seemed applicable to measurement of field net photosynthesis which usually involves the use of complex, controlled-environment field systems such as those of Koch et al. (1971) or Balding et al. (1973).

In theory, where there is net photosynthesis, there is a reduction of CO_2 concentration in the chamber. Where there is net respiration, there is an increase in CO_2 concentration in the chamber. Periodical sampling of the air in the chamber to obtain the CO_2 concentration and calculation of the rate of change of CO_2 concentration should give the CO_2 flux of the sward.

The experimental procedure was as follows. Trampling treatments were applied to the plots as usual. At between 0.5 to 1 hour after treatment, readings commenced. A brass ring (see Plate 41) was pressed into the ground to form a sealable surface for the chamber (the position of the ring chosen at random along the plot). The area inside the ring was 78.54 cm^2. The chamber consisted of a perspex

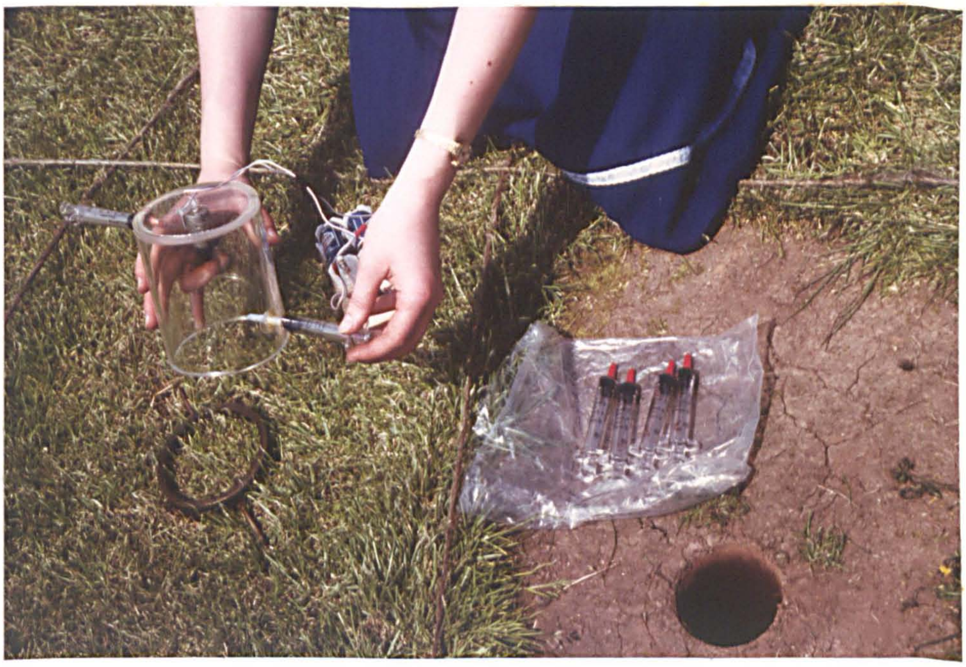


Plate 41 Field plots: Apparatus used to measure sward CO_2 flux.

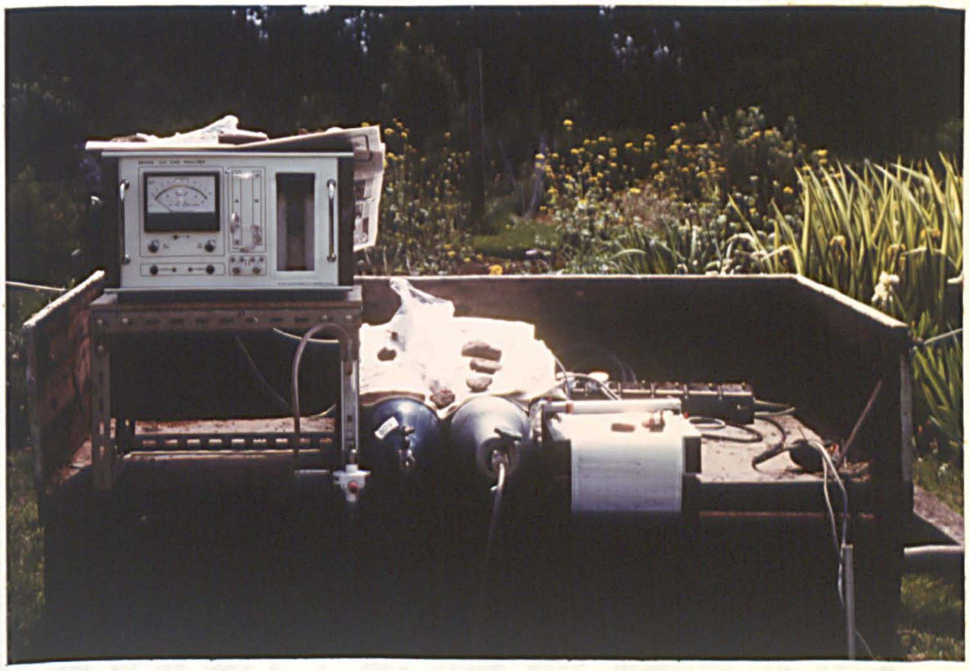


Plate 42 Field plots: Portable IRGA set up for analysis of samples.

cylinder of inner radius 5 cm with a perspex disc sealing one end. The other end was left open for placing on the brass ring. The chamber is shown in Plate 41. The total volume inside the chamber and brass ring (allowing for 1 cm penetration of the brass ring into the soil) was 1.077 l. A battery operated fan at the top of the chamber ensured mixing of air inside the chamber (although in trials it proved to be unnecessary). Two hypodermic syringe heads were sealed into the chamber walls, one at the top of the chamber for input of air as described below, and one lower down at the other side of the chamber for taking samples (see Plate 41). Firstly equilibrium of air inside and outside the chamber was ensured. The chamber was then rapidly fitted onto the brass ring (petroleum jelly facilitated a good seal) and immediately, a sample of air was taken using a Gillette surgical plastic 5 ml syringe (Plate 41). At the same instant, 5 ml of ambient air was injected into the chamber at the other point to prevent any pressure changes within the chamber (otherwise there may have been drawing in of soil CO_2 into the chamber giving an erroneous rate). The sampling procedure was repeated at 30, 60, 90 and 120 seconds, hence the chamber was closed over the sample area for only 2 minutes. A portable IRGA system was set up by the field plot so the air samples could be analysed as soon as possible for CO_2 concentration (Plate 42).

Each 5 ml sample was injected into the CO_2-free air stream (produced by the IRGA) as it entered the sample tube, based on the method of Clegg _et al._ (1978). CO_2 concentration was obtained in ppm which was converted to mg CO_2 l^{-1}. All plots were measured for sward CO_2 flux with 3 replicates. Some measurements were made of soil CO_2 flux after excision of the shoots. The full experiment was attempted on 7 occasions of different weather conditions.

(iv) $\%W_d$ of grass with real trampling.

As both fresh weights and dry weights were obtained for the weekly cuttings, $\%W_d$ could be calculated. These values were however 6 days after treatment in contrast to $\%W_d$ calculated at 2 hours after treatment in Chapter 5 section b). Results were analysed using analysis of variance.

(v) Soil compaction with real trampling.

At the termination of the field plot experiments in June 1981, a penetrometer (Wykeham Farrance Engineering Ltd.) was used to estimate soil compaction in kg load after the different treatments.

On each strip, 10 measurements were made at random. After conversion from the calibration graph, results were analysed using analysis of variance. Regression analysis of soil compaction and ground cover was carried out.

Results

(i) Injury of grass with real trampling

There was no significant difference between values of I from 1, 10, 30 and 100 passage strips (Fig. 88).

(ii) Shoot yield of grass with real trampling

Results of shoot yield are given in Fig. 89. There was no significant difference between treatments.

Results of ground cover are shown in Fig. 90. There was a highly significant reduction ($p<0.001$) in cover with increasing intensity of real trampling.

(iii) CO_2 flux of grass with real trampling

Results were not as easily obtained as had been indicated by the theory of the method (Parkinson 1981). Only in about half of the measurements was there a clear sequence of increasing or decreasing values of CO_2 concentration. Rather than using regression of the sequence of values on time to give CO_2 flux, a simpler method of

Fig. 88 Injury index of a Lolium perenne S23 sward with real trampling.

Fig. 89 Shoot yield of a Lolium perenne S23 sward with real trampling.

Fig. 90 Proportionate cover of a Lolium perenne S23 sward with real trampling.

Fig. 91 Soil compaction of a Lolium perenne S23 sward with real trampling.

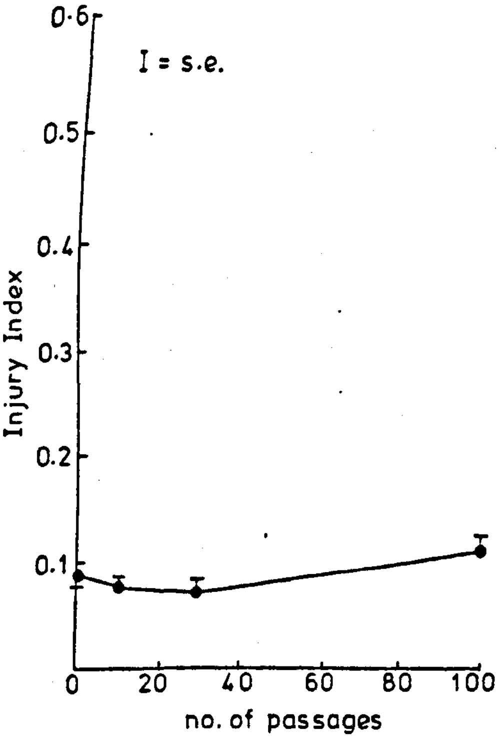


Fig. 88

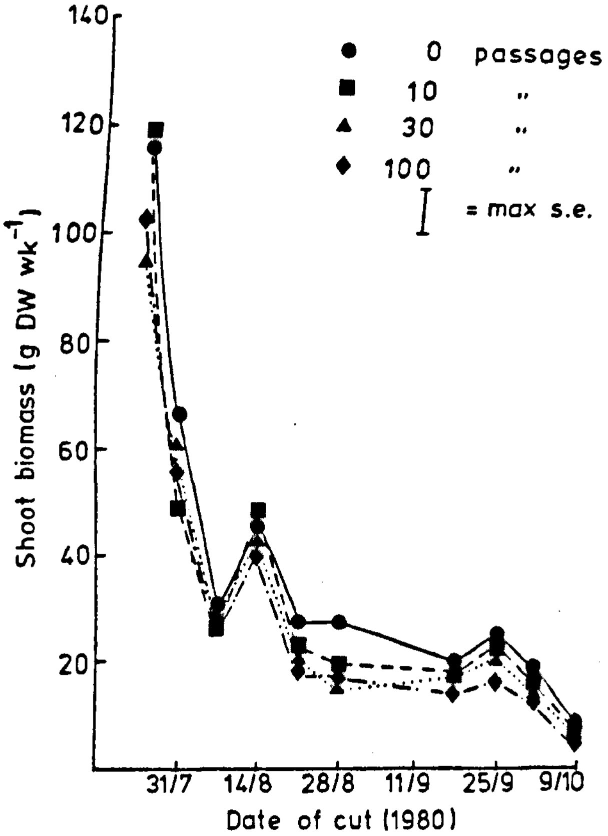


Fig. 89

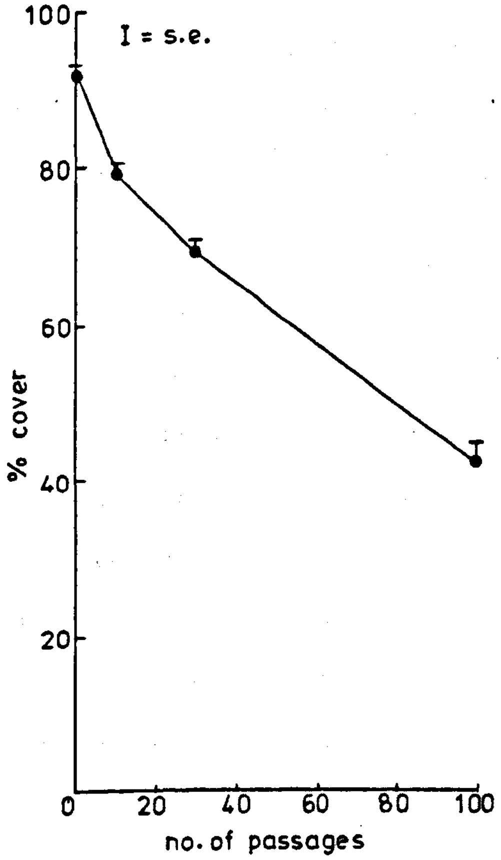


Fig. 90

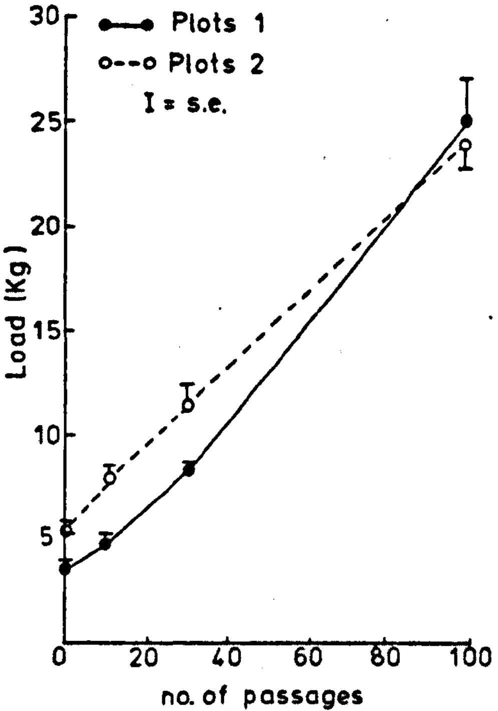


Fig. 91

calculating CO_2 flux was used. The difference between initial and last readings were calculated giving the change in CO_2 concentration in mg CO_2 l^{-1} over 2 minutes for an area of 78.54 cm^2. This was corrected for the value of the chamber giving the change in CO_2 concentration as an absolute quantity (mg CO_2). This absolute quantity was then expressed in terms of area and time, giving the CO_2 flux in mg CO_2 m^{-2} h^{-1}.

A typical set of results are given in Table 21. During these particular measurements, there had been constant sunshine at an illuminance of 60 klux and no cloud interruption. Response is rather variable with a mixture of positive and negative CO_2 flux for 1, 10 and 30 passages per plot. For 100 passages, however, there was only positive CO_2 flux results which in 3 cases were of similar value to estimates by Parkinson (1981) of soil and root respiration of a barley field (785 mg CO_2 m^{-2} h^{-1}). These results were obtained during early August 1980 which was 4 weeks after commencement of trampling treatments. Cuttings collected from the previous days mowing were greatest from the 100 passages plots although differences between treatments were not significant. Objective measurements of ground cover did not commence until early October 1980 (when average measurements of 0.94, 0.81, 0.71, 0.44 were obtained for 0, 10, 30, 100 passages respectively). At this 4 week stage using subjective assessment, reduction of ground cover was not obvious.

Further results of sward CO_2 flux are given in Table 22. These were taken at the termination of the field plot investigation (June 1981) hence it was possible to harvest the shoot material and therefore relate CO_2 flux to shoot biomass and also to obtain root/soil CO_2 flux measurements. This was only possible for the 0 and 10 passages plots (as little biomass was present for 30 and 100 passage plots as is obvious in Plate 41). Sward CO_2 flux values were

Table 21 CO_2 flux of field plots after real trampling.

		CO_2 flux (mg CO_2 m^{-2} h^{-1})		
Passages per plot	Replicate	Block 1	Block 2	Mean and standard error of blocks and replicates combined
0	i	+157.63	-42.03	$\bar{x}$ = -54.67 s.e. = 57.43
	ii	-189.15	-231.19	
	iii	+8.08	-31.35	
10	i	-514.11	+209.36	$\bar{x}$ = -164.50 s.e. = 111.08
	ii	-335.47	-63.05	
	iii	+42.03	-325.77	
30	i	+83.26	+230.38	$\bar{x}$ = +92.29 s.e. = 48.11
	ii	-42.03	+167.33	
	iii	-52.54	+167.33	
100	i	+660.42	+419.54	$\bar{x}$ = +518.96 s.e. = 211.86
	ii	+93.77	+388.01	
	iii	+860.09	+691.95	

Table 22 CO_2 flux of field plots after real trampling.

Passages per plot	Replicate	mg CO_2 m^{-2} h^{-1}			mg CO_2 g live shoot DW^{-1} h^{-1}
		Complete sward	soil/root only	net photosynthesis	net photosynthesis
0	i	-415.50	+1042.44	-1457.94	-16.24
	ii	-753.58	+627.99	-1381.57	-20.67
10	i †	-328.41	+2715.17	-3043.58	-25.43
	ii †	-748.64	+386.47	-1135.11	-11.86

† Mean of 2 measurements.

all negative at rates varying from -232 to -754 mg CO_2 m^{-2} h^{-1}. Root/soil respiration rates varied from +377 to +2759 mg CO_2 m^{-2} h^{-1}.

As seen in Table 22 there is considerable variation of soil/root respiration. This was most likely due to very variable horizontal root distribution (Dickinson 1980). At least 12 samples have been found necessary for below ground biomass measurements (Dickinson 1980, Dickinson and Polwart 1982). Such variable root/soil respiration rates would therefore influence the CO_2 flux rates and this is obvious from results in Tables 21 and 22.

It was possible to calculate net photosynthesis rates in Table 22 and express the results in terms of mg CO_2 g live shoot DW^{-1} h^{-1}. These results compared well with values recorded in Chapter 4.

By taking mean sward CO_2 flux rates, a clearer picture was obtained. Allowing for root/soil respiration there appears to be net photosynthesis after 0 and 10 passages, a reduced net photosynthesis after 30 passages and minimal net photosynthesis after 100 passages. Despite some probable reductions in amount of biomass for 100 passage plots (and perhaps the 30 passages plots) therefore giving reduced rates compared to rates for 0 or 10 passage plots, the indications are that 100 passages seriously reduced the net photosynthesis of the grass (and may have increased root/soil respiration). This was found to be the case in Chapter 4 where increasing intensity of trampling caused greater reductions in net photosynthesis after treatment.

Firm conclusions cannot be made without considerably more experimental work (and perhaps a modified method), particularly including measurements of root/soil respiration (hence requiring a different field plot design to that of the present study), but the indications were that in the field as with laboratory experiments, trampling caused a reduction in shoot photosynthesis.

(iv) $\%W_d$ of grass with real trampling.

$\%W_d$ of cuttings to 3 cm, 6 days after trampling showed no significant difference between treatments.

(v) Soil compaction of grass with real trampling.

Soil compaction was highly significantly increased ($p<0.001$) with increasing intensity of trampling at termination of the field measurements in June 1981 (Fig. 91).

Correlation of soil compaction with ground cover was highly significant ($p<0.001$), cover decreasing with increasing soil compaction.

Discussion

The results of the outdoor pot experiments with simulated trampling (sections a) (i), (ii), (iii), (iv) above) confirm the results obtained in the greenhouse pot experiments with simulated trampling (Chapter 3 a) page 44, Chapter 3 b) page 45, Chapter 4 b) page 84 and d) page 86, and Chapter 5 b) page 113, respectively). This was despite the relatively small number of pots of grass that could be grown outdoors and hence a restricted number of treatments that could be applied in each experiment. The range was more or less covered however in all but section (iii) (CO_2 flux). Exposure to a natural environment for half of the growth period would therefore seem to have little influence on the physiological response of grass to simulated trampling compared to that of only greenhouse grown grass.

The results of the field plot experiments with real trampling (section b) (i), (ii), (iii), (iv), (v) above) however show some apparent discrepancies compared to results of related sections in chapters 3, 4, 5 and 6 a).

There was no significant difference in injury index, I between the different field plot treatments, yet I increased significantly with increasing number of impacts under simulated trampling. This may be because the grass sampled from the field plots included leaf blades of a variety of age and therefore of varied electrolyte permeability (as initial experiments had shown a higher I for old leaves). For all but the untrampled plots, there would also be the effects of previous weeks trampling damage. It is interesting that there is a slightly higher value of I for the untrampled plots. This may be due to the presence of a greater proportion of mature and dead leaf matter in the sward as found by Smith (1978) in his experimental field studies. I is highest for 100 passages per plot, although it would be expected to be significant in view of results in Chapter 3 a) (i) and 6 a) (i). The lower than expected value may be explained by the presence of very little plant material (Plate 45) so samples were taken from the very edge where the full trampling treatment was perhaps not received.

Realistic comparisons of I would seem to require sampling immediately after the first trampling treatment. In view of this and also the possible problems encountered with soil contamination of leaves in wetter field conditions, the use of I in the field situation appears to be restricted but in suitable experiments may prove to be a useful tool in estimating how much shoots are being damaged by wear.

The photographed grass blades damaged by real trampling (Plates 43 and 44) show great similarity to the photographed grass blades damaged by simulated trampling (Plates 50 to 59 in Appendix 3). This suggests that injuries from real trampling and simulated trampling are of very similar nature and the observations of both confirm the findings of Shearman and Beard (1975a) and general comments on the nature of trampling injury (e.g. Bayfield 1971a, Speight 1973, Beard 1973, Canaway 1975a).

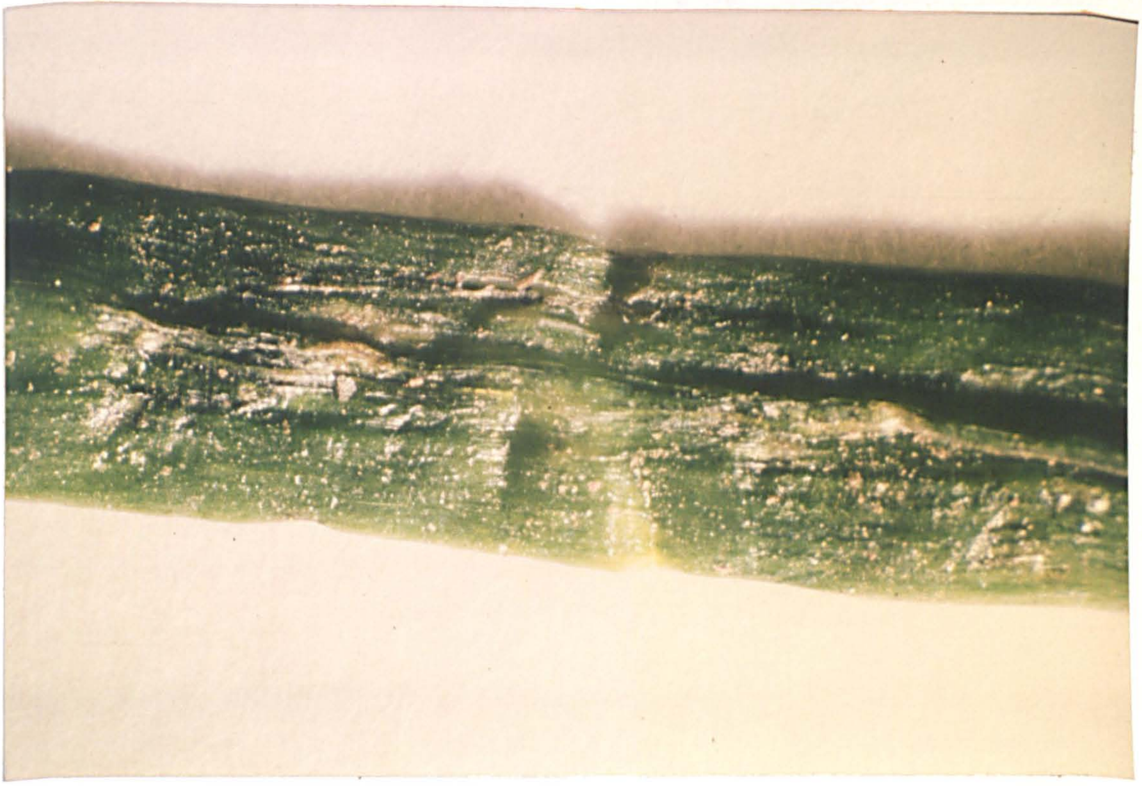


Plate 43 Field plots: damaged grass blade from 100 passage plots

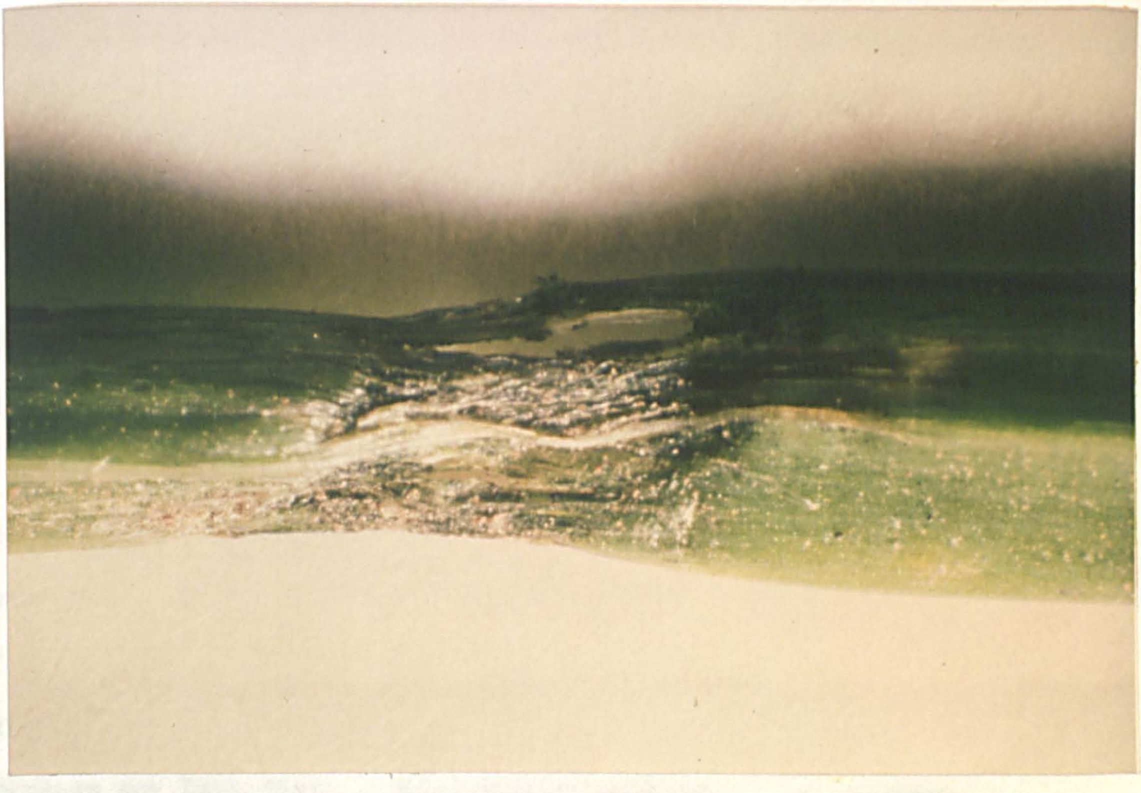


Plate 44 Field plots: damaged grass blade from 100 passages plots.

The lack of any difference in shoot yield between the different treatments of real trampling in the field contradicts the laboratory simulated trampling results where shoot yield was very significantly reduced by increasing number of impacts on Lolium perenne S23 (Chapter 3 b)). It is even more surprising, given that the 100 passages field plots had very significantly reduced ground cover. The reasons for this are not entirely clear but there may have been considerable contribution to the 100 passages plot mowings from the plot edges where growth was perhaps greater due to no or little trampling and reduced competition for light and nutrients. There have been observations (Bayfield 1971a, Leney 1974) that a light intensity of trampling may cause greater growth than on untrampled areas and this is a further possibility in explaining this situation although there were no indications of increased growth on the lightly trampled plots (10 passages) in the present study. Smith (1978) similarly, found no stimulatory effect of light trampling pressure on biomass in the field.

In most comparative studies on real wear in the field (Bayfield 1971a, Kellomaki 1973, Leney 1974, Smith 1978), the field plots consisted of unmown mixed species swards and the shoot response was measured in terms of dry weight of above ground material. A general reduction was noted with trampling although this was complicated by changes in species composition (Bates 1935, Leney 1974, Smith 1978).

Although Leney (1974) commented that "reduction of standing crop in response to trampling occurs before there is an appreciable change in cover value", it is suggested that weekly cuttings in the present study may be somewhat misleading and that values of total above ground biomass may have given a more realistic value of the effects of real trampling on grass growth. Smith (1978), however, concluded that the use of biomass estimates as an indicator of sward tolerance to trampling is limited because a high proportion may be dead. Canaway (1981a) recorded large amounts of dead material present in relation to the canopy of mown swards of grass of different turf species grown in monoculture and calculated that about 75% of the above ground biomass was dead material. Live and dead material should have been taken into account to show a significant decrease in live shoot biomass with increased trampling (Smith 1978). The required method however, in this investigation, was a regular trampling treatment to simulate wear on a football pitch so harvesting to ground level was not desirable.

The sports turf context of the present study also brings other considerations such as the different growth form encouraged for sports turf varieties of grass. Cultivars exhibiting a more prostrate morphology are generally sought to enable less frequent mowing (NERC 1977). Although Lolium perenne S23 is not noted for its prostrate form, general observations were that the shoots were fairly prostrate (see Plates 46, 47, 48). This factor may have affected the quantity of grass removed in weekly cutting treatments in the field, whereas in the laboratory experiments, the varying prostrate forms of the different species and cultivars had no effect on results. This was because laboratory weekly cuttings to 3 cm involved first gathering all the shoots together gently into a vertical stand so the shoot yield included prostrate leaves as well as vertical leaves. In the field situation, however, use of a mower to cut the grass would have removed only vertical shoots and this may have given an unrepresentative value of shoot yield. Indeed, Canaway (1981a) noted that Lolium perenne S23 grown as a monoculture sward and exposed to artificial sports wear (Canaway 1976a) consistently showed high wear tolerance, yet low biomass.

These investigations by Canaway appear to be the most relevant

Plate 45 Field plots: June 1981 - 100 passages plot.

Plate 46 Field plots: June 1981 - 30 passages plot.

Plate 47 Field plots: June 1981 - 10 passages plot.

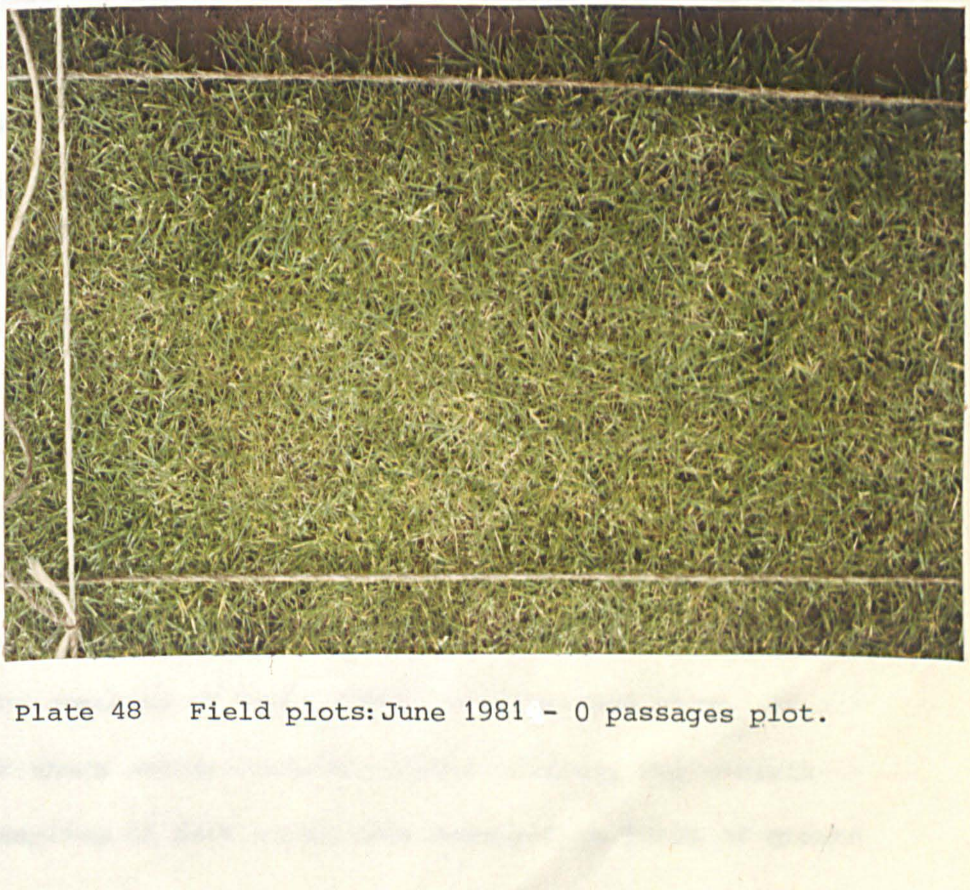


Plate 48 Field plots: June 1981 - 0 passages plot.

studies in relation to the present research. Canaway (1976a) developed a differential slip wear machine to reproduce sports wear and was then able to compare real and artificial wear using football pitches (which were utilised as usual) with adjacent experimental plots receiving artificial wear (Canaway 1976b, 1981b). He concluded that real wear reduced ground cover and the greatest reduction was where the wear was greatest. He also found a significant reduction in ground cover with increasing simulated wear (1976b) and over a period of time comparable effects of real and simulated wear were recorded for ground cover (1981b) at corresponding levels of wear. Canaway (1981a) found a positive correlation of biomass with ground cover but when biomass was separated into live, dead and shoot base fractions, none of the individual fractions correlated with ground cover.

In the present study, ground cover was significantly reduced with increasing number of passages and this was subjectively confirmed (Plates 45, 46, 47 and 48). Laboratory studies in Chapter 3 c) (page 46) included analysis of the effects of simulated trampling on the shoot bases and in all but Lolium perenne S23, a significant decrease was recorded in response to increasing number of impacts. This shoot base fraction would seem to correspond to the fraction left after mowing in field trials, and hence relate to the ground cover. It may therefore be suggested that for regularly cut/mown grass, simulated laboratory wear and real wear studies on experimental plots and real wear on sports pitches all cause a decrease in ground cover and the decrease is greater, the greater the intensity of wear.

Despite the comments of Leney (1974) as mentioned above, it would seem that where swards receive regular cutting, the overall response to trampling is most accurately measured in terms of ground cover, especially as point quadrats such as that of Laycock and Canaway (1980) have been developed for use on mown turf. Total biomass gives reasonably accurate indications of response but biomass of weekly mowings may not give representative results.

The measurements of field CO_2 flux were a little disappointing especially as many trials and full experiments were carried out. The main problem was the inconsistency of the sequential readings. This would seem to be due to considerable variation in soil/root CO_2 flux (Dickinson 1980). Where soil/root respiration readings were taken there was usually a consistently increasing sequence of readings, as demonstrated by Parkinson (1981). The soil/root respiration rates were also comparable with Parkinson's values for a barley field.

The method is based on that of Parkinson (1981) who developed the technique to measure soil respiration in the field. No indication of how the technique may be adapted to measure net photosynthesis was given. It would seem however, that the method has potential for this purpose but that more work to modify and test the technique for measurement of field sward photosynthesis is required. Factors such as length of time between sequential readings may need to be extended for example, and many more measurements would seem to be required to overcome the soil/root respiration horizontal fluctuations in rate across the plots. The time limits for the present study however, prevented further work on this method.

Although only cautious conclusions can be made, it would seem that at one hour after 100 passages across the plots, net photosynthesis was considerably reduced and this would agree with simulated trampling laboratory studies (Chapter 4, Chapter 6 a) (iii)) where increasing simulated trampling caused a slight increase in dark respiration and a significant decrease in net photosynthesis such that at the higher intensities of treatment, there was net respiration.

Results of %W_d at 6 days after treatment showed no significant difference and similar results were obtained by Smith (pers. comm.). It would appear that by this time after treatment, the decrease in %W_d observed 2 hours after laboratory simulated trampling treatments (Chapter 5 b) page 113) is no longer found because damaged tissue will have died back (Shearman and Beard 1975a, Appendix 3) and the grass cuttings will consist largely of new growth. In order to carry out experiments for comparison with those of Chapter 5 b), it would have been necessary to leave the grass sward for 6 days after mowing and then apply real trampling with subsequent sampling of grass cuttings 2 hours later. This was not possible because of the overall sports turf management regime required. Ideally separate plots designed to receive real trampling once (6 days after mowing) and then cut at 3 cm to find %W_d of shoots 2 hours after treatment, were needed, but limited time of the present study prevented further field investigations.

Of significance in this respect, however may be the SEM observations of blades of grass taken from the 100 passages plots, (described in Chapter 5 section e)). There were many similarities of these compared to SEM observations of laboratory grown grass that had received simulated trampling, especially epicuticular wax disruption and fungal activity. This implies that similar water loss observed in laboratory experiments may be the case in the field.

Soil compaction was significantly increased the greater the number of passages. Similar results have been recorded by Canaway (1976b, 1981a,b) in both real and simulated wear of sports turf. Soil compaction has also been widely and well documented as a result of trampling in the amenity grassland context (e.g. Bates 1935, Speight 1973, Liddle 1975a,b, Canaway 1975a, Crawford and Liddle

1977, Blom 1979). In the agricultural context, soil compaction has been a cause of reduced yield (Rosenberg 1964, Cannell 1977). The contribution that soil compaction makes to the overall response of a plant however, is to some extent dependent on many other factors such as soil type, moisture content of soil, soil fertility and the plant species (Rosenberg 1964, El-Karouri & Gooderham 1977). In some instances the soil compaction effect of trampling has conferred an advantage rather than a disadvantage (Rosenberg 1964, Leney 1974, Blom 1979).

Experiments by Leney (1974) indicated different responses according to species. Some such as *Holcus lanatus*, *Plantago lanceolata*, *Trifolium campestre* showed depressed shoot growth in response to soil compaction while others such as *Ammophila* and *Calluna* showed increased plant growth with soil compaction. Carrow (1980) investigated the response of 3 turfgrass species to compaction. All showed a decline in percent turf cover which was still in evidence 8 months after the treatments. Differentials in response were recorded and Carrow ranked the species tolerance to compaction with *Lolium perenne* Pennfine equal to *Poa pratensis* Baron and greater than *Festuca arundinacea* Kentucky 31.

In the present study, laboratory experiments (Chapter 3 d) page 47) showed a significant reduction in shoot yield with soil compaction and also a significant reduction in shoot yield with shoot injury only. This suggests that for the field plots in this study, the significantly increased soil compaction with increasing number of passages contributed to the significant decrease in ground cover. Indeed, there was a significant ($p<0.001$) correlation of ground cover with soil compaction.

Further studies would seem to be necessary to elucidate the soil compaction response of different species and cultivars in particular conditions. Work on the effects of mechanical impedance (Goss 1977, Goss and Scott Russell 1980) indicated how extensive an effect soil compaction may have and the extent of the response. The physiological aspects, particularly (which appear to be largely unresearched), may be extremely important in understanding the overall response of a plant to trampling stress.

CHAPTER 7

Discussion

In the 1977 NERC report it was clear that study of the fundamental reasons for differential durability of plant species and cultivars when subjected to wear was a priority for research. Canaway (1981a) suggested that these differences in wear tolerance and recuperative potential were likely to be due to "extremely complex and subtle physiological factors related to the plant response to damage and to the ability to store and mobilise reserves for repair and regrowth", and that there was "much scope for further work in a number of areas before the subject is completely understood".

This study has attempted to clarify some of these areas, particularly the neglected subject of the physiological responses to trampling. The response of plant shoot yield, CO_2 exchange and water relations to trampling has therefore been investigated.

In Chapter 2, the index of injury was developed to measure the cellular damage using an adapted electrolyte release method. It correlated significantly with the intensity of simulated trampling treatment (Chapter 3), thus indicating it was a relevant measurement of plant injury. Further, differences between species and cultivars were obtained which generally related to the wear tolerance of the species/cultivar in both the present study and other studies (e.g. Canaway 1981a, 1983). Injury index was also highly correlated with shoot yield. It would therefore seem to be a very useful measurement of plant injury and may have further application in indicating response to levels of trampling stress in different conditions and situations or even of indicating the relative wear tolerance of cultivars such as in new cultivar trials.

Shoot yield response in this study was much as expected. There was reduced biomass with increasing intensity of simulated trampling. This has been recorded by others such as Wagar (1964), Bayfield (1971a), Kellomaki (1973), Canaway (1978, 1981a, 1983) and Smith (1978). Except for a few anomalous cultivars, shoot yield was related to cultivar wear tolerance (Canaway 1981a, 1983). The relationships were generally curvilinear as similarly reported by Bayfield (1971a) and Smith (1978). Decreased yield has also been found to be a feature of other stresses as discussed in Chapter 1.

Response of CO_2 flux to trampling was recorded particularly over the initial few hours. It was evident that photosynthesis rates were reduced. This had been observed in wind stressed grasses (Grace and Thompson 1973). Dark respiration rates were slightly increased. More detailed examination revealed the response over time which consisted of an immediate rapid reduction in photosynthesis rates to below the compensation point followed by a gradual recovery although it was not recorded whether pre-treatment rates were eventually attained. Rate of photosynthesis correlated significantly with injury index, suggesting the rate is related to the amount of injury. No comparison of different species or cultivars was obtained and this prevented comparison of CO_2 flux response with observed wear tolerance or recuperative potential.

Water relations of trampled grass was affected in various ways. Stomatal closure was at a lower water content and cuticular transpiration was higher in trampled grass (Chapter 5). Similar observations followed wind stress (Grace 1974). In Chapter 5, water content of shoots rapidly reduced and was lower the greater the intensity of trampling. A differential effect of species was recorded. The water content was reduced most in the least wear tolerant species tested. Transpiration rates were reduced by trampling stress which along with the observed reduction in leaf water content suggested that leaf hydraulic conductivity was reduced in trampled grass. Calculations confirmed this (Chapter 5). Suggestions of effects of wind stress on hydraulic conductivity have also been made (Pitcairn and Grace 1984). Water content correlated significantly with injury index suggesting the reduced water content is related to the amount of injury. SEM studies indicated disruption of epicuticular wax which may contribute to the observed higher cuticular transpiration rates. Similar observations and conclusions were obtained for wind stressed plants by Thompson (1974,1975) and Grace (1974).

Correlations of CO_2 flux and water content are given in Appendix 10. Results were significant from data where the whole pot of grass received treatment and also from other data where shoots only were treated. This indicated the importance of direct shoot injury in the photosynthetic and water content response of grass to trampling, at least in the short term.

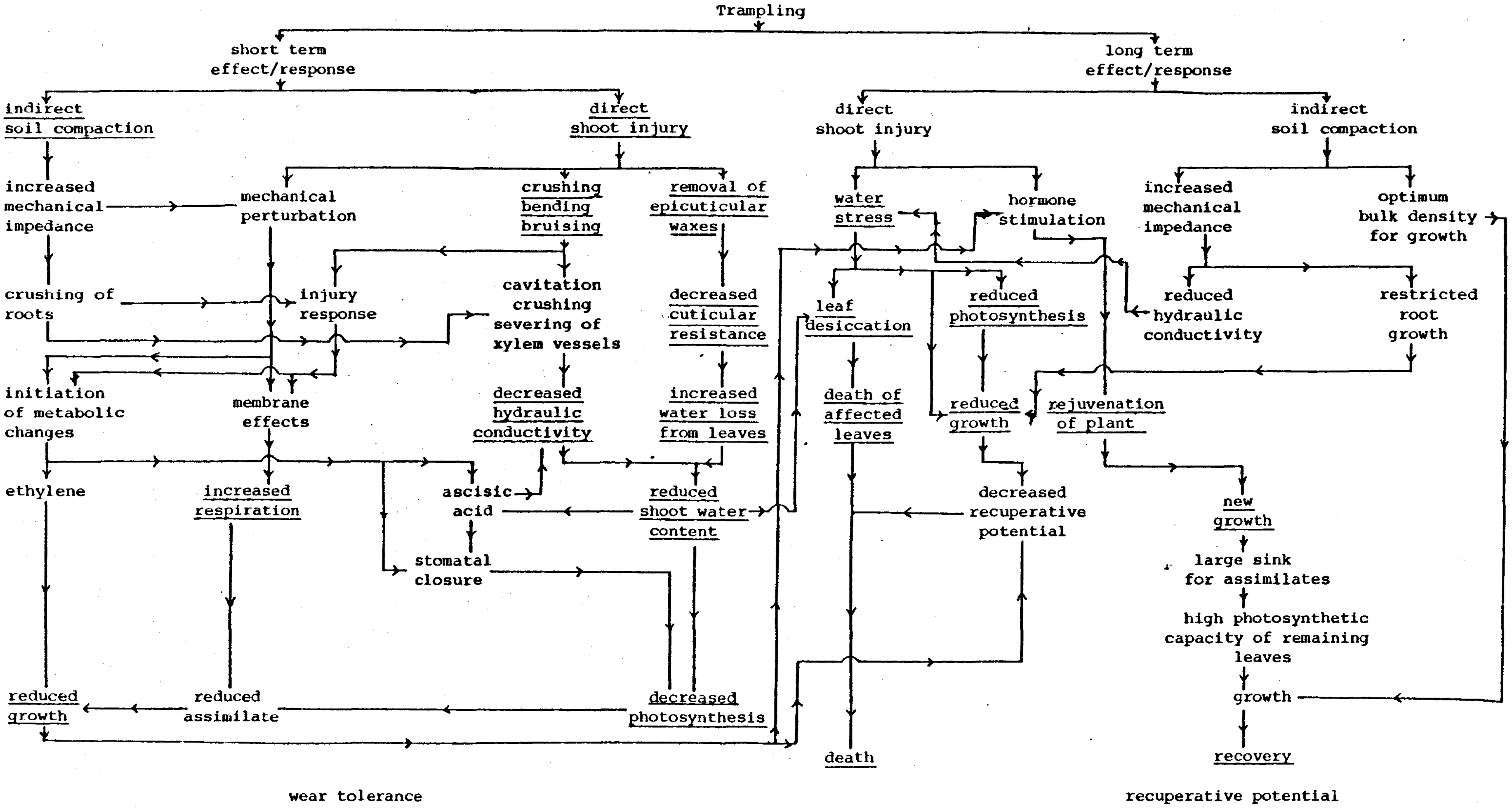
Field investigations (Chapter 6) confirmed the observations in the laboratory and hence suggested that the laboratory experiment results may apply to the real situation.

Other observations included evidence that injured parts of grass blades desiccate and die over several days after simulated trampling (Appendix 3) which had been similarly recorded by Shearman and Beard (1975a) when grass was subjected to wear.

From all these results, a pattern of short term response of grass to trampling has emerged and the main features are schematically presented in Fig. 92 along with the long term consequences which include results of work such as Bayfield (1971a) and Smith (1978).

In the short term, trampling causes direct shoot injury (Chapter 3) and indirect soil compaction (Chapters 3 and 6). Direct shoot

Fig. 92 Schematic representation of inter-relationships between observed and hypothetical processes involved in plant response to trampling. (Observed effects underlined).



injury consists of removal of epicuticular waxes (Chapter 5), crushing, bending, bruising of shoots (Chapters 2 and 3) and it is also suggested that mechanical perturbation (Jaffe and Biro 1979) may be an effect as shoots will not necessarily all receive injury but may be touched or shaken by trampling. Removal of epicuticular waxes may cause decreased cuticular resistance and therefore the increased water loss from leaves when stomata are closed (Chapter 5). This was suggested by Grace (1974) in the case of wind stress. An estimate of crushing, bending and bruising of the shoots is given by the injury index (Chapters 2 and 3). Changes in hydraulic conductivity (Chapter 5) may be due to crushing and severing of the xylem vessels and/or cavitation in the xylem. Reduced water content yet reduced rates of transpiration may be explained by this reduced hydraulic conductivity (Chapter 5). An injury respose may also result from the damage as suggested in the work on wounding by, for example, Macnicol (1976). The results of wounding are not clear but metabolic changes are likely (Macnicol 1976). These may have an effect on membranes (Jaffe 1980) and cause reduced hydraulic conductivity (Radin and Boyer 1982). Such water stress is likely to influence the CO_2 flux (Lawlor 1972a) causing reduced photosynthesis as recorded (Chapter 4).

The suggested mechanical perturbation may add to the effects of the recorded crushing bending and bruising of shoots as it appears to cause metabolic changes (Jaffe 1980). These include increased ethylene synthesis which results in reduced growth (Jaffe and Biro 1979), and abscisic acid production which has been shown in response to other stresses (e.g. Riken et al. 1976). It is suggested by the work of Markhart et al. (1979b) that abscisic acid may reduce the hydraulic conductivity and hence also contribute to the observed reduced shoot water content (Chapter 5). Evidence that abscisic acid reduces root hydraulic conductivity is however controversial

(Bradford and Hsiao 1982). It is well established that abscisic acid is produced during water stress and that it causes stomatal closure (Willmer 1983). Stomatal closure will reduce the rate of photosynthesis (Gaastra 1959).

Metabolic changes following mechanical perturbation may also cause the observed increased respiration (Chapter 4) as noted by Audus (1935). The result of decreased photosynthesis (Chapter 4) is to reduce the plant levels of assimilate which may cause reduced growth (Chapter 3). Increased respiration (Chapter 4) would similarly reduce assimilate levels and may reduce growth (Chapter 3).

The short term response of indirect soil compaction (Chapters 3 and 6) may cause mechanical impedance (Goss 1977, Goss and Scott Russell 1980). At extremes it may cause crushing of roots and hence involve the injury response, finally effecting reduced growth and also crushing and severing of xylem vessels, hence decreased hydraulic conductivity and the subsequent events of water deficit. At lower levels, there may be an effect similar to that of mechanical perturbation.

These short term responses contribute to the wear tolerance of a plant. According to the plant susceptibility and response (which may relate to anatomical and morphological features (Shearman and Beard 1975a)), effects may be severe or mild. The eventual plant response will also depend however on long term aspects as even if there is shoot death, some plants may have effective regrowth mechanisms to replace damaged tissue and overcome the injury (Chapter 3).

The current study concentrated mainly on short term responses but some long term ones were identified. The long term response may also be divided into direct and indirect effects. Direct shoot injury caused water stress (Chapter 5) which may have brought about the reduced photosynthesis (Chapter 4) as indicated by Lawlor (1972a) and Grace (1974). Where there was severe damage, relevant areas were osberved to desiccate (Chapter 5) and then die (Appendix 3). This had similarly been observed by Shearman and Beard (1975a). Reduced photosynthesis (Chapter 4) may cause reduced assimilates and therefore limit growth (Chapter 3). Reduced growth (Chapter 3) may also result from water stress (Hsaio 1973, Hsaio and Acevedo 1974). The decreased growth suggests decreased recuperative potential which may result in plant death as observed in some cultivars and species (Chapter 3, Bates 1935). It is also suggested that hormones are produced as a result of direct injury which stimulate new growth and rejuvenation of the plant such as that observed by Hodgekinson (1974). This may lead to new growth, hence large sinks for assimilates. Photosynthesis in surviving leaves is therefore not limited by build up of assimilates at the source (Neales and Incoll 1968). High photosynthetic capacity may result. This was observed in <u>Phleum</u> <u>bertolonii</u> by Smith (1978), 3 weeks after simulated trampling. The photosynthetic rate was higher the higher the intensity of simulated trampling. In the suggested model, this will lead to growth and there will be recovery from the effects of trampling stress.

Indirect soil compaction may cause either an increase in bulk density to an optimum for plant growth as found by Blom (1979). In this case plant growth will be promoted. Alternatively the increase in bulk density may cause increased mechanical impedance of the roots which may result in restricted root growth (Goss 1977, Blom 1979) which may cause reduced plant growth and hence decreased recuperative potential. This may lead to plant death. There may also be a reduced hydraulic conductivity as a result of possible mechanical perturbation effects (Goss and Scott Russell 1980, Jaffe 1980) leading to plant water stress and hence the problems of desiccation and reduced photosynthesis as already discussed.

These long term responses appear to contribute to the recuperative potential. According to the plant response to trampling stress, recuperative potential may be low or high and therefore dictate whether there is recovery or death of a plant.

Evidently the proposed mechanism is complex and many of the components mentioned require further research as information is scant or is absent. The model consists of two main responses to trampling stress in the short term. There is an effect of water deficit and an effect of injury/mechanical stimulation. Both of these refer to research in other areas of plant response to stress. The water stress aspect is seen in response of plants to frost and water and wind stress as reviewed in Chapter 1. Most relevant was the work on wind stress where similar events of cuticular disruption have been recorded (Grace and Thompson 1973, Grace 1974, Thompson 1974, 1975, Grace 1977). The effects of mechanical stimulation were also suggested as being responsible for the observed effect in other studies of wind stress (Russell and Grace 1978a,b, 1979a,b). Further research on both these aspects of trampling seem to be needed to confirm observations and to measure certain aspects that have been neglected. Such aspects in the present study include stomatal diffusive resistance and leaf water potential which were unsuccessfully attempted. More detailed work is also needed comparing the photosynthetic response of the different species and cultivars to trampling. This may indicate further the response (and mechanism) of susceptible species/cultivars compared to resistant species/cultivars. The species comparisons in the water relations studies were limited. More experiments using the full range of species/cultivars would appear to be necessary to further evaluate the role of water relations in response to trampling. This may help indicate if the major effect is plant water deficit. If so, the more susceptible species and cultivars would be expected to experience more rapid and severe water deficits following treatment compared to more resistant ones.

Research into the subject of mechanical perturbation is also necessary, first to establish if such a response is present in grasses but also to examine the consequences on membranes as this may have a major effect on hydraulic conductivity (Markhart _et al._ 1979b) and hence the plant response. There is no apparent work on the metabolic change and hormone aspect of trampling stress. Such information may give much greater insight into the mechanisms behind plant response to trampling, particularly of recuperative potential.

The short term and long term effects of indirect soil compaction have much scope for futher research as work has been very restricted. Whether trampling causes root damage would seem to be a priority and the effects of mechanical impedance particularly require further investigation.

In conclusion, this study proposes an electrolyte release method for determining differences in species/cultivar wear tolerance and has identified various physiological responses of turfgrass species to trampling stress. Most of these responses have been short term which relate to plant wear tolerance. Long term responses have also been indicated and in a model, observed and theoretical relationships between different factors have been presented. Similarities with other stresses have been noted. In particular, the response to wind stress has many similar features. Further areas of research have been suggested which would help to clarify the series of events and factors involved in the response of plants to trampling.

APPENDIX 1

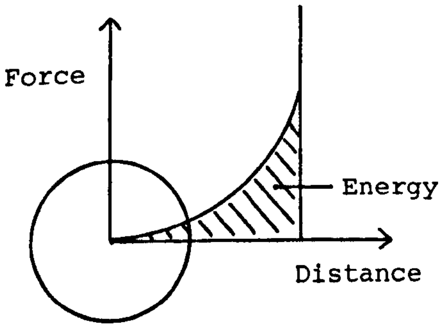
The potential energy of a falling tamp can be calculated easily using the equation

$E_{pot} = m.g.h.$ Where m = mass (kg)

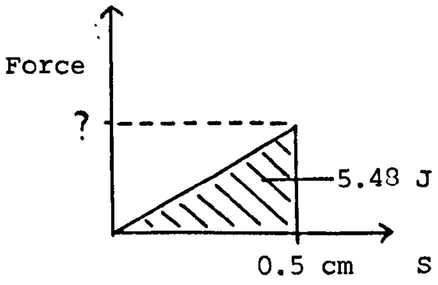
g = acceleration due to gravity (9.81 m s^{-2})

h = height (m)

The actual force applied to the turf by the falling tamp is more difficult to calculate. The energy imparted to the turf (that is, the potential energy which is known, having been calculated) is equal to the force actually applied (which is unknown) multiplied by the distance moved against the medium (that is, the distance the turf is depressed), but this is difficult to calculate as the relationship between the force applied and distance moved against the medium depends on unknown factors such as the resilience of the soil and turf. In practice there is probably an exponential relationship between force and distance because it will be easy at first to compress the soil and turf but increasing force will be required for increasing compression (i).



(i)



(ii)

As the total energy used is known (5.48 J in this case) and from many observations that the distance moved was small, an approximation that the force-distance relationship is linear, can be used. This estimate gives a triangle (ii) and since

$A = 0.5\ bh$ Where A = area of a triangle

b = length of base of a triangle

h = length of height of a triangle

the force applied to the turf can be approximately calculated. An estimate of 0.5 cm turf depression was made. This gives a force of 2192 N. As the area of the tamp head was 44.2 cm, this gives a pressure of 49.59×10^4 N m^{-2} (5.05 kg cm^{-2}). This is about one third of the average maximum heel pressure applied on a hard flat surface as calculated from the data of Harper _et al._ (1961).

APPENDIX 2

In order to make the simulated trampling treatments more realistic, feasible ways of introducing horizontal forces to supplement the vertical force imparted by the falling tamp were investigated: Canaway (pers. comm.) suggested 1) rotating the tamp as it fell or alternatively 2) rotating the pot of grass as the tamp hit it. A further possibility was 3) applying a torque force separately.

1) The forces involved in dropping a rotated tamp are very difficult to quantify due to friction in the screw effecting the rotation.

2) Equally problematic is achieving the perfect timing of turning a pot of grass at the exact moment of impact of the tamp.

3) Applying a torque separately also presented many practical difficulties. In the static situation, it was calculated that the tamp head needed a torque of less than 0.6 Nm to move it. Most commercial torques only measure far above this range and the few available that measure down to this type of value tend to be very inaccurate at such low forces anyway. In a dynamic situation, the forces are not known at all.

4) Information provided by a force platform to give an accurate force/time graph might be used to apply a torque at exactly the right time to give a realistic simulated trampling treatment. Such a system was not available.

APPENDIX 3

Pots of grass were grown as described in the general methods. At the 8 week stage they were transfered to constant environment growth cabinets set at conditions of 20°C/70% r.h. for a 16 hour photoperiod and 13°C/90% r.h. for an 8 hour dark period.

Treatments of 0, 1, 5 impacts per pot were given and the pots of grass were replaced in the growth cabinets. Leaves were selected for examples of very severe damage or moderately severe damage and were marked using loosely tied pieces of cotton of different colours. Photographs were then taken of the chosen leaves at 0, 2, 18, 24, 90 and 168 hours after treatment.

Typical results are given in Plates 49 to 59. It is apparent that the damaged areas of the leaves desiccate and then gradually lose chlorophyll until at 7 days after treatment the damaged parts of leaves have died.

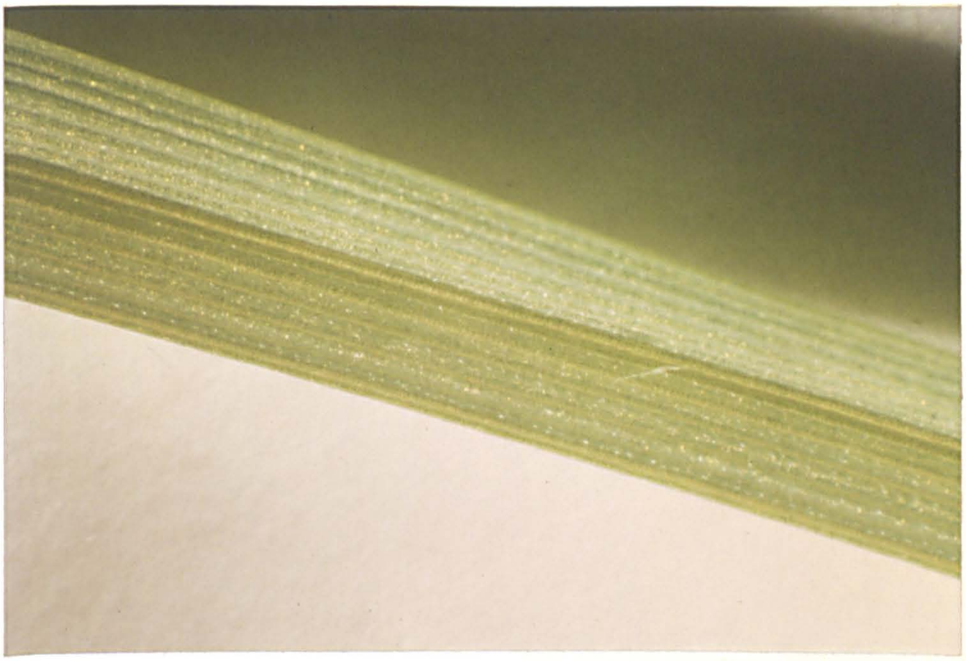


Plate 49 Undamaged leaf of Lolium perenne S23

Plates 50 - 54

Moderately damaged leaf of Lolium perenne S23 at certain times after simulated trampling:

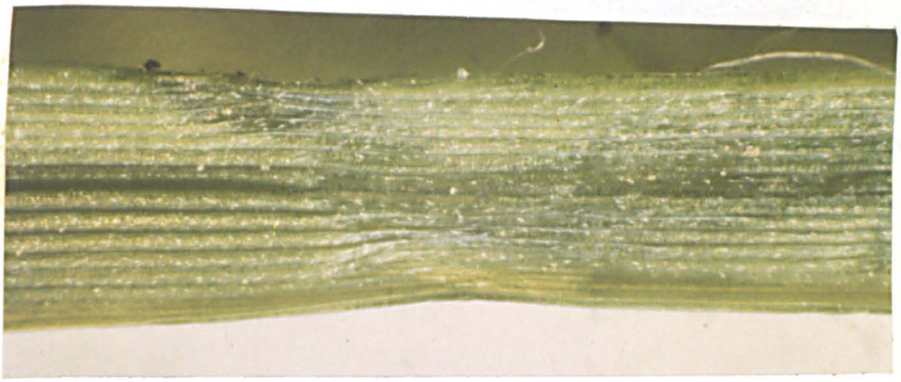
2 hours (Plate 50)

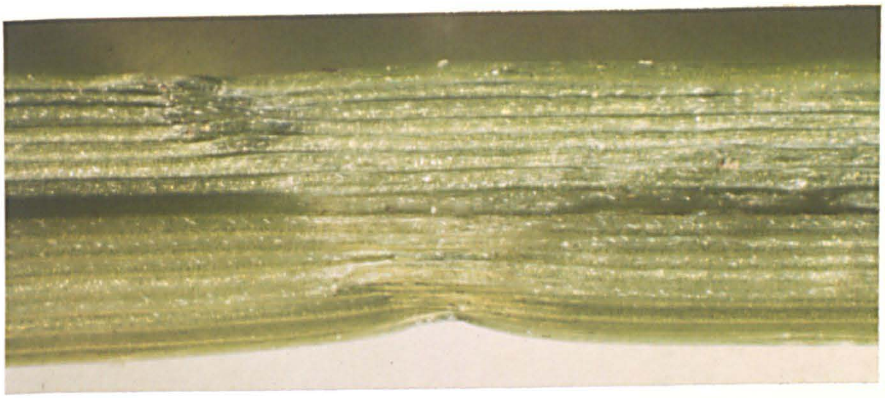
18 hours (Plate 51)

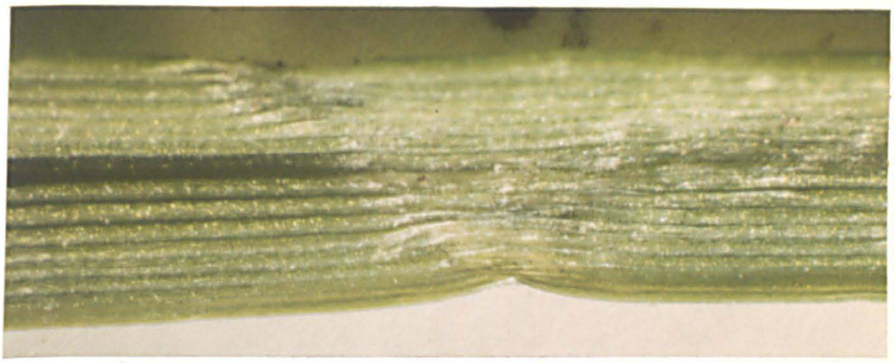
24 hours (Plate 52)

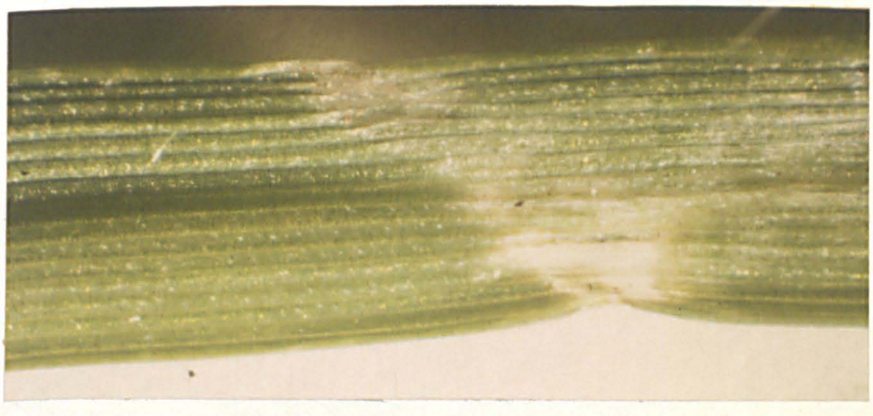
90 hours (Plate 53)

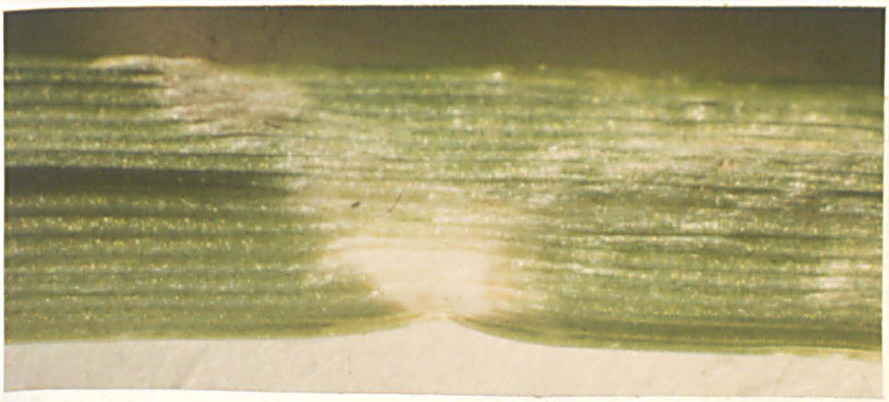
168 hours (Plate 54)







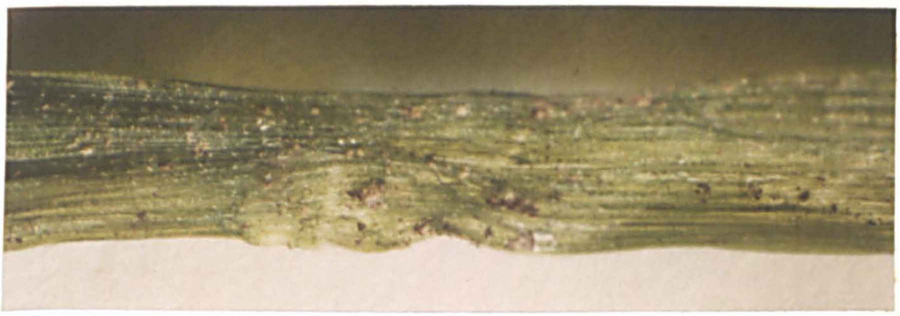




Plates 55 - 59

Severely damaged leaf of Lolium perenne S23 at certain times after simulated trampling:

2 hours (Plate 55)
18 hours (Plate 56)
24 hours (Plate 57)
90 hours (Plate 58)
168 hours (Plate 59)



APPENDIX 4

Correlation of leaf dry weight with leaf surface area in Lolium perenne S23.

Method

7 pots of grass were chosen at random from 50 pots that had been housed in the growth cabinets for 5 days at the usual conditions described in chapter 4. All shoots of each pot of grass were excised at the usual 3 cm level. For 1 pot of grass, all shoots were used. For the other 6 pots, the excised shoots were subsampled so that 10 leaves were randomly taken. For each of the 7 pots of grass, the sampled leaves were sorted into two categories: older leaves that had a cut end from previous cutting treatments, or younger leaves with the apex narrowing to a point. For the older leaves, leaf area was calculated using

$A = L \times B \text{ cm}^2$

where A = leaf area (one surface only) (cm^2)

L = leaf length (cm)

B = leaf breadth halfway along the leaf segment (cm)

For the younger leaves, leaf area was calculated using the formula of Kemp (1960):-

$A = 0.905 \times L \times B \text{ cm}^2$

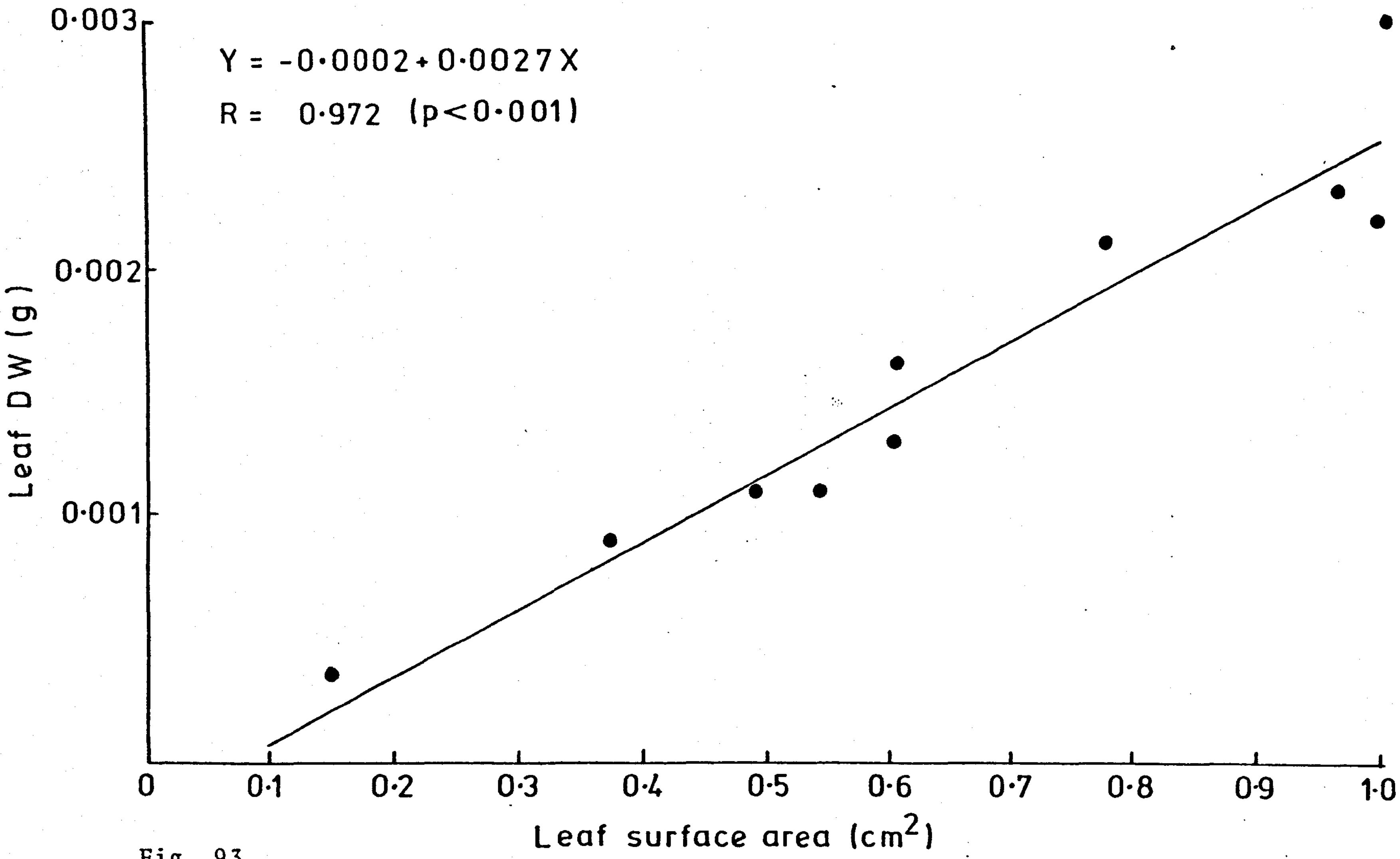
Leaves were individually dried overnight at 95°C and then weighed to obtain dry weights.

Regression analysis of dry weight on leaf area was carried out.

Results

All regression analyses gave very highly significant ($p<0.001$) regressions of leaf dry weight on leaf surface area. A typical set of results is shown in Fig. 93.

Fig. 93 Regression of leaf dry weight on leaf surface area for *Lolium perenne* S23.



$Y = -0{\cdot}0002 + 0{\cdot}0027X$

$R = 0{\cdot}972 \quad (p < 0{\cdot}001)$

Fig. 93

Conclusion

Leaf dry weight correlates very significantly with leaf surface area and may be a useful alternative to measurements of leaf surface area for *Lolium perenne*.

Appendix 5 <u>Summary of all significant differences for transpiration rate over 24 hours.</u>

(* = significant difference: $p < 0.05$
n.s. = no significant difference).

Lolium perenne	0	–																		
	1	n.s.	–																	
	3	n.s.	n.s.	–																
	5	*	*	n.s.	–															
Agrostis tenuis	0	*	*	*	*	–														
	1	n.s.	n.s.	*	*	n.s.	–													
	3	*	n.s.	n.s	n.s.	*	*	–												
	5	*	*	n.s.	n.s.	*	*	n.s.	–											
	7	*	*	n.s.	n.s.	*	*	n.s.	n.s.	–										
Poa Pratensis	0	n.s.	*	n.s.	*	*	n.s.	n.s.	*	*	–									
	1	n.s.	n.s.	n.s.	*	*	n.s.	n.s.	n.s.	*	n.s.	–								
	3	n.s.	n.s.	n.s.	n.s.	*	*	n.s.	n.s.	*	n.s.	n.s.	–							
	5	n.s.	n.s.	n.s.	n.s.	*	*	n.s.	n.s.	*	n.s.	n.s.	n.s.	–						
	7	n.s.	n.s.	n.s.	n.s.	*	*	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	–					
Phleum bertolonii	0	n.s.	n.s.	*	*	n.s.	n.s.	*	*	*	n.s.	n.s.	*	*	*	–				
	1	n.s.	n.s.	n.s	*	*	n.s.	n.s	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	–			
	3	n.s.	n.s.	n.s.	*	*	*	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	–		
	5	n.s.	n.s.	n.s.	n.s.	*	*	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	–	
	7	*	*	n.s.	n.s.	*	*	n.s.	n.s.	n.s.	*	*	n.s.	n.s	n.s.	*	*	*	n.s.	–
	i pot^{-1}	0	1	3	5	0	1	3	5	7	0	1	3	5	7	0	1	3	5	7
		Lolium perenne				Agrostis tenuis					Poa pratensis					Phleum bertolonii				

Appendix 6 Summary of all significant differences for transpiration rate over first 3 hours.

Lolium perenne	0	–																		
	1	n.s.	–																	
	3	*	n.s.	–																
	5	*	*	n.s.	–															
Agrostis tenuis	0	n.s.	n.s.	*	*	–														
	1	n.s.	n.s.	n.s.	*	*	–													
	3	*	*	n.s.	n.s.	*	n.s.	–												
	5	*	*	n.s.	n.s.	*	n.s.	n.s.	–											
	7	*	*	n.s.	n.s.	*	*	n.s.	n.s.	–										
Poa Pratensis	0	*	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	–									
	1	*	*	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	–								
	3	*	*	n.s.	n.s.	*	*	n.s.	n.s.	*	n.s.	n.s.	–							
	5	*	*	n.s.	n.s.	*	*	n.s.	n.s.	*	n.s.	n.s.	n.s.	–						
	7	*	*	n.s.	n.s.	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	–					
Phleum bertolonii	0	*	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	–				
	1	*	*	n.s.	n.s.	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	–			
	3	*	*	n.s.	n.s.	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	–		
	5	*	*	n.s.	n.s.	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	–	
	7	*	*	*	n.s.	*	*	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	–
	i pot^{-1}	0	1	3	5	0	1	3	5	7	0	1	3	5	7	0	1	3	5	7
		Lolium perenne				Agrostis tenuis					Poa pratensis					Phleum bertolonii				

(* = significant difference p < 0.05

n.s. = no significant difference)

Appendix 7 Summary of all significant differences for %W_d at 24 hours.

(* = significant difference $p < 0.05$
n.s. = no significant difference)

Lolium perenne	0	–																		
	1	n.s.	–																	
	3	n.s.	n.s.	–																
	5	*	*	n.s.	–															
Agrostis tenuis	0	n.s.	n.s.	n.s.	n.s.	–														
	1	n.s.	n.s.	n.s.	n.s.	n.s.	–													
	3	*	*	*	n.s.	*	*	–												
	5	*	*	*	n.s.	*	*	n.s.	–											
	7	*	*	*	*	*	*	*	n.s.											
Poa Pratensis	0	n.s.	n.s.	n.s.	*	n.s.	n.s.	*	*	*	–									
	1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*	*	n.s.	–								
	3	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*	*	n.s.	n.s.	–							
	5	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*	n.s.	n.s.	n.s.	–						
	7	*	*	n.s.	n.s.	*	n.s.	n.s.	*	*	n.s.	n.s.	n.s.	n.s.	–					
Phleum bertolonii	0	n.s.	n.s.	n.s.	*	n.s.	n.s.	*	*	*	n.s.	n.s.	n.s.	*	*	–				
	1	n.s.	n.s.	n.s.	*	n.s.	n.s.	*	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	–			
	3	*	*	n.s.	n.s.	*	n.s.	n.s.	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	–		
	5	*	*	n.s.	n.s.	*	n.s.	n.s.	n.s.	*	*	*	*	n.s.	n.s.	*	*	n.s.	–	
	7	*	*	*	n.s.	*	*	n.s.	n.s.	*	*	*	*	*	n.s.	*	*	*	n.s.	–
	i pot^{-1}	0	1	3	5	0	1	3	5	7	0	1	3	5	7	0	1	3	5	7
		Lolium perenne				Agrostis tenuis					Poa pratensis					Phleum bertolonii				

Appendix 8 Summary of all significant differences for % relative conductance.

Lolium perenne	0	-																		
	1	n.s.	-																	
	3	n.s.	n.s.	-																
	5	*	*	n.s.	-															
Agrostis tenuis	0	n.s.	n.s.	n.s.	*	-														
	1	n.s.	n.s.	n.s.	*	n.s.	-													
	3	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-												
	5	*	*	*	n.s.	*	*	n.s.	-											
	7	*	*	*	*	*	*	*	*	-										
Poa pratensis	0	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	*	*	-									
	1	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	*	*	n.s.	-								
	3	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	*	*	n.s.	n.s.	-							
	5	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	*	*	n.s.	n.s.	n.s.	-						
	7	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*	n.s.	n.s.	n.s.	n.s.	-					
Phleum bertolonii	0	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	-				
	1	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-			
	3	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-		
	5	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-	
	7	*	*	*	n.s.	*	*	n.s.	n.s.	*	*	*	*	*	*	*	*	*	n.s.	-
	i pot^{-1}	0	1	3	5	0	1	3	5	7	0	1	3	5	7	0	1	3	5	7
		Lolium perenne				Agrostis tenuis					Poa pratensis					Phleum bertolonii				

(* = significant difference $p < 0.05$

n.s. = no significant difference)

APPENDIX 9

Relationship between leaf water content and injury index in Lolium perenne S23.

In Chapter 3, a reduction in shoot yield with increasing intensity of simulated trampling was demonstrated and this was correlated with an increasing value of injury index, I. A reduction in light whole pot CO_2 flux was also correlated with increase in I in Chapter 4. To see if there was similarly a relationship between $\%W_d$ and I, the following experiment was carried out.

Methods

Pots of Lolium perenne S23 were grown to the 8 week stage and were transferred to growth cabinets at the usual conditions as described in Chapter 2 (page 18). After 5 days, treatments of 0, 1, 3 or 5 i pot^{-1} were applied. Immediately after treatment shoots were either used for measurement of I as described in Chapter 2) (page 39) or for measurement of $\%W_d$ as described in Chapter 5 section b) (page 113). There were 6 replicates. Results for $\%W_d$ and I within each treatment were randomly paired and correlation analysis was carried out.

Results

There was a significant correlation ($p<0.01$) between $\%W_d$ and I. As shown in Fig. 94 increase in I was related to decrease in $\%W_d$.

Fig. 94 Regression of $\%W_d$ on injury index.

Fig. 95 Regression of light and dark whole pot CO_2 flux on $\%W_d$ with simulated trampling.

Fig. 96 Regression of light and dark whole pot CO_2 flux on $\%W_d$ with simulated trampling of shoots only.

○ light conditions

● dark conditions

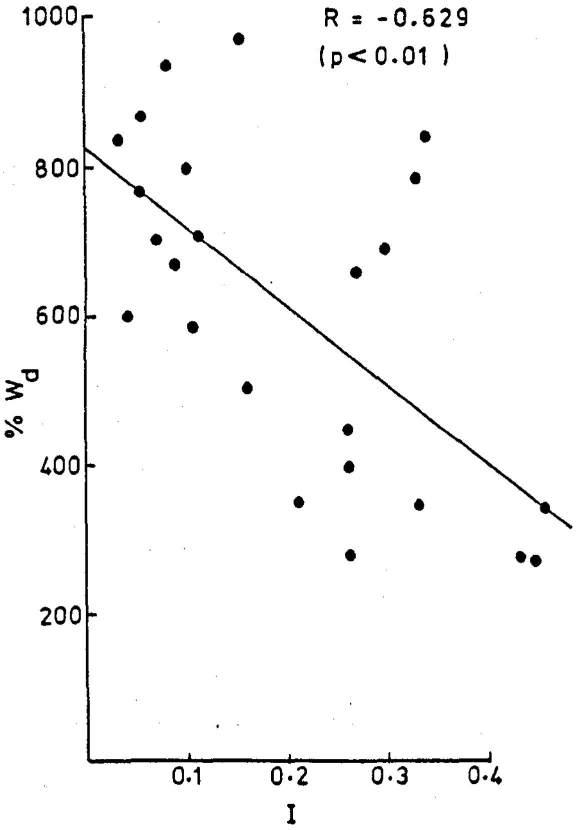


Fig. 94

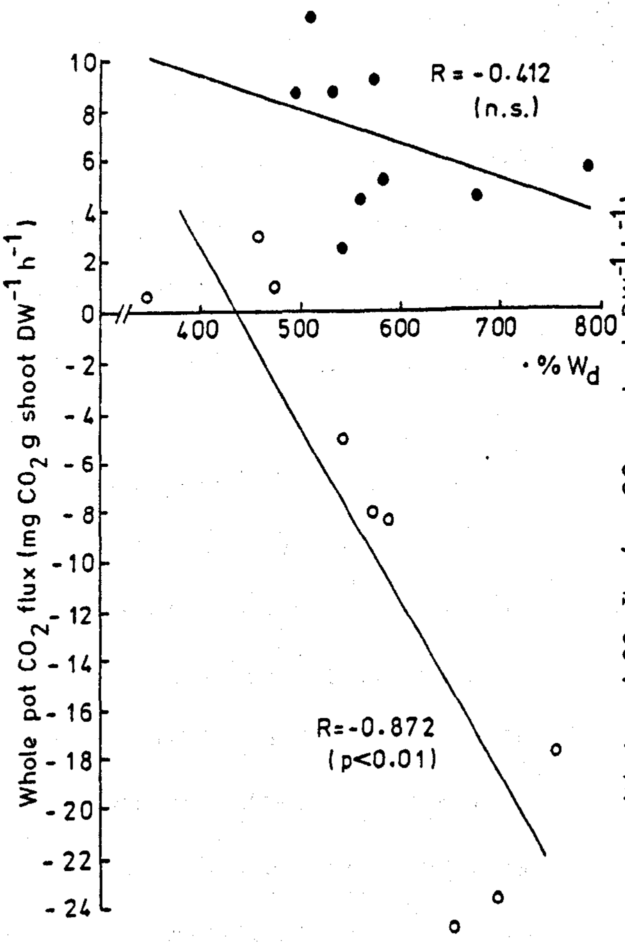


Fig. 95

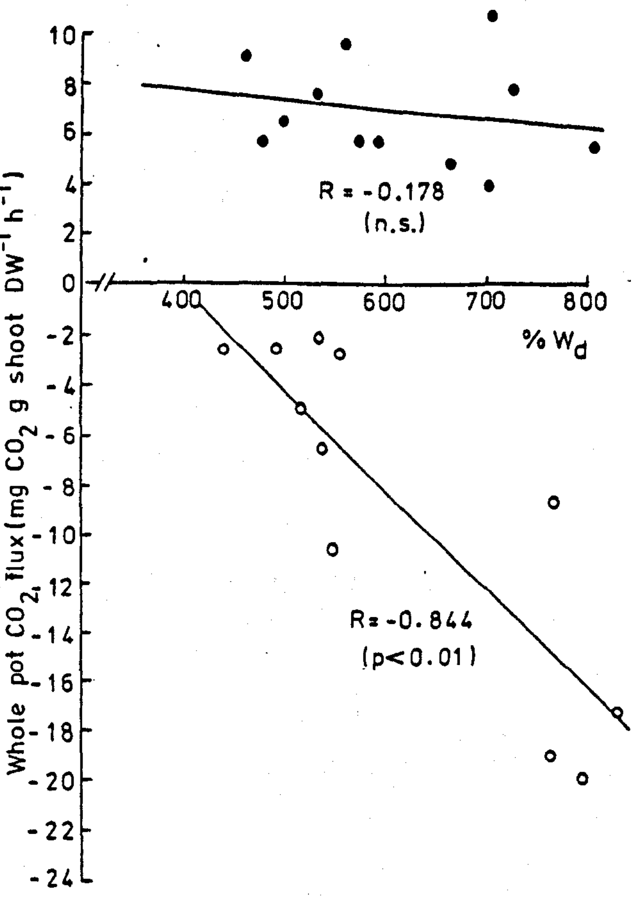


Fig. 96

APPENDIX 10

Relationship between whole pot CO_2 flux and $\%W_d$ with simulated trampling.

(i) Simulated trampling of whole pots of grass

Method

The procedure described in Chapter 4 section b) (i) (page 78) was carried out to measure light or dark whole pot CO_2 flux, 30 minutes after 0, 2 or 7 i pot^{-1} with 3 replicates for each treatment. When shoots were subsequently excised for dry weight determination, fresh weights were immediately recorded so that $\%W_d$ could be determined as for Chapter 5 section b) (page 104) but at 30 minutes after simulated trampling treatment.

Regression and correlation analysis of light whole pot CO_2 flux at 30 minutes with $\%W_d$ at 30 minutes and of dark whole pot CO_2 flux at 30 minutes with $\%W_d$ at 30 minutes was carried out.

Results

Results and regression equation line are given in Fig. 95. There was a significant ($p<0.01$) relationship between light CO_2 flux and $\%W_d$ but not between dark CO_2 flux and $\%W_d$ when pots of grass were subjected to different intensities of simulated trampling.

(ii) Simulated trampling of shoots only

Method

In the experiment described in Chapter 4 section b) (iii) (page 79) fresh weights were also immediately taken when shoots were excised following CO_2 flux measurement at 30 minutes after treatment of 0, 1, 3 or 5 i pot^{-1} to the shoots only. Calculation of $\%W_d$ was therefore possible as described in Chapter 5 section b) (page 104), but at 30 minutes after treatment.

Regression and correlation analysis of light whole pot CO_2 flux at 30 minutes on $\%W_d$ at 30 minutes and of dark whole pot CO_2 flux at

30 minutes on $\%W_d$ at 30 minutes was carried out.

Results

Results and regression equation line are given in Fig. 95. There was a significant ($p<0.01$) relationship between light CO_2 flux and $\%W_d$ but not between dark CO_2 flux and $\%W_d$ when shoots only were subjected to different intensities of simulated trampling.

Conclusion of (i) and (ii)

Dark whole pot CO_2 flux, following simulated trampling treatments applied to whole pots of grass and applied to the shoots only, was not significantly related to $\%W_d$.

Light whole pot CO_2 flux, following simulated trampling treatments applied to whole pots of grass and applied to the shoots only, was significantly related to $\%W_d$. The lower the $\%W_d$, the lower the rate of photosynthesis.

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