THE FUNCTIONAL RESPONSE OF PLANTS TO TRAMPLING PRESSURE

VOLUME II OF II VOLUMES

Thesis presented by

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PART IV - FIELD STUDIES - PHOTOSYNTHESIS AND PRIMARY PRODUCTION

A. NET PHOTOSYNTHESIS BY C. VULGARIS

(a) Introduction

(i) The effect of water stress on the rate of net photosynthesis (F_n)

Trampling will ultimately affect plant productivity of a site. In a Calluna stand this may be a direct effect or one mediated through the effect water stress has upon physiological processes, one of which is photosynthesis.

The effects of water stress on net photosynthesis (F_n) may be classified into two groups (Hari & Luukkanen, 1973), those resulting from 'acute water stress', whereby a slight water deficit results in a decrease in F_n, which is completely and rapidly reversible, and secondly, the effects of 'physiological water stress' which develop slowly when water supply is limited and disappear slowly upon rewatering. There are three modes of action by which water stress may affect F_n (Slavik, 1965):

(1) stress resulting in deficits of sufficient magnitude to cause hydroactive stomatal closure thus increasing the stomatal resistance (r_s) to CO_2 uptake;

(2) direct effects of water stress on enzyme activity associated with photosynthesis, resulting from dehydration of the cytoplasm;

(3) decreased permeability of the cuticle, epidermis and cell membranes of internal cells, reducing the CO_2 supply to the chloroplasts.

These may be conveniently classified into stomatal and non-stomatal effects, the relative importance of each being controversial.

In considering the stomatal effects of water deficits on F_n, it is pertinent to consider the effects of water deficits on stomata.

Stalfelt (1955) identified a three phase response of stomata to increasing water deficits:

(1) the suboptimal water deficit at which stomatal opening and closing is controlled by forces outside the guard cells, which respond only passively. A fully turgid leaf will have turgid epidermal cells, which, by exerting a back pressure on the guard cells, prevent full stomatal opening;

(2) the optimal water deficit at which water loss from the epidermal cells has occurred faster than from the guard cells, the epidermal cells consequently do not inhibit the photoactive opening of stomata;

(3) the supraoptimal deficit at which the threshold to hydroactive closure occurs, in which the guard cells are actively involved. The guard cells lose water to the environment and neighbouring epidermal cells resulting in a reduction in guard cell turgor and hydroactive stomatal closure.

Recently a mechanism has been proposed by Raschke (1976) by which "... stomata resolve the dilemma of opposing priorities..." of maintaining adequate gaseous exchange for photosynthesis while simultaneously preventing excessive water loss. In essence the mechanism proposes that r_s is adjusted proportionally to the assimilatory activity of the mesophyll. While CO_2 depletion in the substomatal cavities results in stomatal opening, associated water stresses increase the concentration of abscisic acid (ABA) within the plant which increases cell permeability to solute loss thus resulting in stomatal closure. The more rapid the water deficit develops the greater the accumulation of ABA because of the greater transpirational flow, although the reduction in ψ sufficient to drive the transpiration stream does not result in stomatal closure until a threshold has been reached (-0.7 to -1.8MPa, Hsiao, 1973). Raschke's work (1976) with Vicia faba indicated that with a decrease in ψ, stomatal aperture gradually decreased although the stomata remained open at a ψ of -6.0MPa. This argued against a threshold effect although when the experimental epidermal strips remained attached to the mesophyll tissue, stomatal reaction was found to prevent ψ_1 from dropping below -0.7MPa. As the threshold ψ for stomatal closure was approached so ABA synthesis was induced overriding the opening effects of CO_2 depletion in Xanthium strumarium (Raschke, 1976). The system may therefore be capable of regulating the development of water deficits while simultaneously optimising photosynthesis, so maintaining efficient water use.

The transitory opening of stomata at an optimal water deficit is a widely observed effect. Meidner (1965), working with Phaseolus vulgaris, observed the three phase reaction of stomata to water loss reported by Stalfelt (1955). Similarly, Pieters & Zima (1975), on excising leaves of Populus euramericana, observed the 'Ivanoff' effect in which a sudden peak of transpiration was observed followed by a decrease in transpiration due to a rapid decrease in RWC. Whether this was a stomatal effect or one of releasing tension on the xylem water columns so enhancing water loss remains unclear.

A review of gaseous diffusion into and out of leaves was given by Meidner & Mansfield (1968) and Jarman (1974). Water diffuses through the leaf air spaces, through the stomatal pores and the boundary layer (with their associated resistances to diffusion, r_s and r_a) and into the atmosphere. CO_2 encounters similar resistances to diffusion on its inward passage but, in addition, encounters an internal resistance, r_m, composed of a liquid phase resistance and a biochemical resistance. These are composed of resistances to dissolution of CO_2 in the liquid of the cell walls, transport in solution to the chloroplast and the rate of carboxylation. In still air the major resistance to water loss is r_a and not until r_s approaches r_a in magnitude will stomatal control over water loss become effective. However, a reduction in r_a, by turbulent air movement, will increase the effectiveness of r_s on water loss

(Meidner & Mansfield, 1968). The situation is essentially comparable for CO_2 diffusion although the resistances will be of greater apparent magnitude because the diffusion coefficient is less ($0.249cm^2s^{-1}$ for water, $0.159cm^2s^{-1}$ for CO_2) (Meidner & Mansfield, 1968). However, the situation is further complicated by r_m. If r_m is the largest resistance encountered it will be rate limiting to F_n. Gaastra (1959) for example, found r_m to be one to four times greater than r_s in Brassica rapa, but the situation was changed when CO_2 rather than rate of diffusion became limiting. The effect of r_s on F_n is determined by the ratio $(r_m + r_a)/r_s$, so that when r_s equals $(r_m + r_a)$ it becomes effective in controlling F_n. Gaastra noted that if this ratio was unity, doubling r_s decreased F_n by a third but if the ratio was four, doubling r_s reduced F_n by one sixth. The third situation which may exist is if light is limiting. Under such circumstances the relationship between diffusion capacity and the rate of photochemical processes determines the effectiveness of r_s. For example, if r_m is low and diffusion is controlled by r_s, the photochemical rate will determine the rate of F_n when the diffusion rate is greater than the photochemical rate so that F_n will be unaffected by considerable changes in r_s. If r_m is controlling the rate of diffusion, however, variation in light intensity will have only a small effect on r_s and r_m will control F_n. Gaastra (1959) concluded that when r_m is low, r_s might be expected to control F_n, but when r_m is higher and CO_2 and light are limiting, r_s will have little influence on F_n. It will be apparent that the effect of r_s and r_m on F_n is dynamic and complex, depending upon physiological and environmental factors.

Even when stomata are closed, CO_2 may still enter the leaf. Zelitch (1971) noted that F_n may vary from $0 - 2.7 \times 10^{-5}mgCO_2\ dm^{-2}\ s^{-1}$ in leaves lacking stomata, to $1.1 \times 10^{-3} - 3.9 \times 10^{-3}mgCO_2\ dm^{-2}\ s^{-1}$ in leaves possessing stomata. The cuticular resistance (r_c) to CO_2

diffusion varies from 0.05 to 0.0125cm s^{-1} (Zelitch, 1971) and may result in F_n when the stomata are closed if r_s is greater than r_c. El-Sharkawy & Hesketh (1964) observed that CO_2 diffusion into the leaves of Sorghum vulgare and Helianthus annuus through non-stomatal areas probably resulted in positive values for F_n when the stomata were shut. Willis & Balasubramaniam (1968) considered that cuticular diffusion of CO_2 may bring a leaf above its CO_2 compensation point when the stomata are closed, although recycling of photorespiratory CO_2 must be taken into account. Troughton (1969) found that although cuticular transfer of CO_2 in Gossypium sp. was low (0.8×10^{-8}g cm^{-2} s^{-1}), uptake remained positive under constant illumination and short term water stress. This view was shared by Catsky (1965b) who considered that residual CO_2 uptake following stomatal closure, in Brassica oleracea, by cuticular absorption at low RWC's may have been responsible for a rate of F_n 25% of the maximal rate. However, the conclusion was based on independent estimates of the RWC at stomatal closure. Thus, in addition to the implication of a low cuticular resistance to CO_2 uptake, it is possible that the stomata were incompletely closed and therefore allowing the entry of CO_2 into the leaf. Indeed, the degree to which r_s increases upon stomatal closure raises doubts as to the validity of F_n based on cuticular CO_2 diffusion. Certain authors e.g. Holmgren, Jarvis & Jarvis (1965) considered that r_c is so large that its consideration as an effective diffusion pathway for CO_2 can be ignored.

Changes in the rate of photosynthesis have been associated with changes in r_s by many workers e.g. Brix (1962), Catsky (1965b), Barrs (1968), Puritch (1973), Pieters & Zima (1975). Oscillations in stomatal aperture have been observed to be paralleled by changes in CO_2 uptake e.g. Troughton (1969). Some workers e.g. Brilliant (1924) found that slight water deficits increased apparent assimilation rates, after which further water loss resulted in a decline in F_n. Larcher (1960), working with Quercus pubescens and Q. ilex, observed transpiration and F_n to reach a peak in the same range of RWC over which stomatal apertures were maximal, and in a later study observed maximum F_n in Olea europaea at a slight water deficit (Larcher, 1965). Similarly, Catsky (1965b) observed that maximal F_n in Brassica oleracea occurred at an RWC of 0.95. It might be postulated that a decrease in r_s associated with the optimal water deficit for stomatal opening may be responsible for increased F_n, but Brilliant (1924) removed the epidermis from the mesophyll being employed in the experiments, which suggested that stomatal movements were not responsible for the observed effects. Some workers have failed to observe the increase in F_n at a slight water deficit e.g. El-Sharkawy & Hesketh (1964).

In addition to the stimulatory effects of water deficits on F_n, a threshold response of F_n to water stress occurs. Increasing water stress may result in a progressive decrease in F_n (Hari & Luukkanen, 1973). Alternatively, a constant rate of F_n might be maintained until a threshold water stress is attained at which point F_n decreases with increasing water stress. This is often paralleled by a marked increase in r_s, which may occur at a particular ψ threshold (Hsiao, 1973). Observations on Zea mays revealed that F_n decreased below a threshold RWC of 0.92, which was attributed to an increase in r_s (Mederski et al., 1975). Puritch (1973), working with four Abies spp. observed a four phase response of F_n to water stress:

(1) an initial optimal rate, maintained constant over a range of low water stress;

(2) at a threshold ψ of -0.9 to -1.1MPa, a linear decrease in F_n of between 50% and 4% per unit decrease in ψ, depending upon species;

(3) a second threshold at which the rate of decrease was reduced;

(4) a specific threshold at which F_n became zero.

Puritch ascribed phase 2 to an increase in r_s, and while not eliminating the effects of increased r_m, considered that the parallel decline in transpiration implied that the reduction was linked to gaseous transfer.

Larcher (1960) considered that assimilation ceased before or simultaneously with the cessation of stomatal transpiration and that, when the stomata are nearly closed, assimilation is dependent upon stomatal pore width. Brix (1962) observed that in *Lycopersicon esculentum* a ψ of -1.4MPa was necessary to effect a reduction in F_n and that the correlation between F_n and transpiration implied that r_s was the controlling factor. Likewise, El-Sharkawy & Hesketh (1964) observed that F_n did not decrease in a number of species until the leaves were visibly wilted and inferred that an increase in r_s was responsible. Studies on *Pelargonium x hortorum* led Willis & Balasubramaniam (1968) to conclude that stomata have a closely regulating effect over transpiration and F_n. They concluded that water deficits leading to gradual stomatal closure restricted these two processes but Boyer (1976), in a re-examination of their data, found that r_s changed before F_n was affected. This suggests that relationships between r_s, transpiration and F_n require elucidation and implies that inferences regarding the effect of r_s on F_n from transpiration decline phenomena may not be valid.

Troughton (1969) observed a threshold RWC of 0.85 at which stomatal closure occurred in *Gossypium sp.*, which was associated with a decline in F_n. Similarly, Wardlaw (1969) noted that at an RWC of 0.9 in *Lolium temulentum* a reduction of 30% in F_n occurred which might have been attributable to increased r_s and Johnson *et al.*,(1974) concluded that the parallel reaction of transpiration and F_n to water stress suggested r_s as the major limiting factor, although they did not preclude the limiting effects of r_m on F_n. However, Pieters & Zima (1975), working with

Populus euramericana, observed that, following a transitory increase in transpiration, it decreased whilst F_n remained constant until an RWC of 0.64 to 0.78 was reached. r_s was found to have an effect only at light saturation since at light limiting values, the photochemical processes were rate limiting. Although there is considerable evidence suggesting that increased water deficits reduce F_n through hydroactive stomatal closure, evidence based on transpiration decline is speculative, especially when the evaporation sites within the leaf are considered. The epidermal pathway for water movement and its control over stomatal aperture (Meidner, 1975) implies that water loss and CO_2 uptake occur along different pathways and thus the effects of r_s on transpiration and photosynthesis may not be directly comparable. In addition, the hypothesis implies that r_s may be affected independently of any drying effects on the mesophyll tissue which might lead to an increase in r_m.

Certain observations have also indicated that F_n may be affected by water stress before any change in r_s occurs. Stocker (1960) found such an effect which he ascribed to decreased cytoplasmic hydration impairing CO_2 diffusion. Schulze et al. (1974) considered that stomatal control over F_n is of secondary importance. Slavik (1965) used the hepatic, Conocephalum conicum, to eliminate the possibility of stomatal control over F_n, and found that a decrease in RWC was paralleled by a decrease in F_n and π. This suggested that tissue hydration may have a direct effect on F_n. However, the linear correlation between F_n and π over the RWC range of 1.0 to 0.5 implied that the effect on F_n may be an osmotic one. Troughton's studies (1969) indicated that although r_m increased, F_n had decreased prior to any change in r_m and subsequent studies revealed that r_m in Gossypium hirsutum was constant down to an RWC of 0.56. Boyer (1970a) found no increase in r_m with decreasing ψ_l in Zea mays or Glycine max, Mederski et al. (1975) found no increase in r_m over the range of RWC employed in their experiments and Slatyer

(1973b) found that short periods of stress had no effect on the photosynthetic apparatus until the permanent wilting point was reached. Troughton & Slatyer (1969) noted, however, that "...the constant mesophyll resistance at all levels of water stress indicates that liquid phase diffusion of CO_2 is unaffected, but it is not evidence that the photochemical or biochemical reactions associated with CO_2 fixation are unaffected by water stress, unless these components contribute to r_m". Further investigations led Boyer (1971) to conclude that F_n may, under certain conditions, be limited by a reduction in photochemical activity rather than a reduction in the rate of CO_2 uptake.

The complexity of the situation is indicated by the studies of Redshaw & Meidner (1972) on Nicotiana tobaccum. They eliminated r_a and r_s by forcing air through the leaves and found that r_m (liquid phase resistance) doubled as RWC fell from 0.96 to 0.86. The absolute change in resistance was comparable with the change in gas phase resistance over the same range of RWC but since the gas phase resistance changed by a proportionately greater amount it represented a greater proportion of the total resistance to CO_2 diffusion, the proportion increasing as RWC decreased. However, although r_s would appear to be controlling CO_2 uptake, the linear relationship between r_s and r_m suggested, to Redshaw & Meidner, a situation in which r_s may not be directly responding to RWC but responding to r_m which is responding to RWC. They suggested that if r_m is not reducing CO_2 fixation, an apparent decrease in uptake may result from increased photorespiratory CO_2 output which is recycled into photosynthesis. However, the work of Meidner (1975) suggested that r_m may remain unaffected while r_s increases so that r_s, although possibly unrelated to bulk tissue RWC, may be responding directly to a component of leaf RWC, i.e. the epidermal pathway.

Longevity of water stress may also be a factor of some relevance in considering the relative contributions made by r_s and r_m to decreasing F_n with increasing water stress. Jones (1973) found that the initial decrease in F_n with water stress in Gossypium hirsutum could be related to stomatal closure. Prolonged stress, however, may affect intracellular processes, which may be of greater significance in the relationship between F_n and long term stresses. Boyer (1976) for example, noted that reduction in F_n was paralleled by a decrease in the activity of the Hill Reaction of wilted leaves, although severe desiccation may be required to damage certain processes (Santarius & Heber, 1967). It is apparent that non-stomatal effects on F_n with increasing water stress may occur but their effects must be balanced against stomatal effects. Boyer (1976) considered, for example, that at high light intensities carboxylation activities may not affect photosynthesis (providing carboxylation is not rate limited by the enzymes involved (cf. Wareing et al., 1968)), whereas, owing to the high flux of CO_2 from the atmosphere to the chloroplasts, changes in r_s will have an appreciable effect. In contrast, when light is limiting, chloroplast effects may outweigh the effects of r_s (cf. Gaastra, 1959).

Although Hsiao (1973) remarked that "...the finding of nonstomatal effects in some species should not be allowed to detract from the dominant influence that stomata normally have on CO_2 assimilation during water stress...", and although r_m may not be affected by deficits which might affect r_s (cf. Meidner, 1975), the absolute magnitude of r_m may determine the effectiveness of r_s (Gaastra, 1959). The independence of F_n on RWC and r_s in some species implies that r_s does not become effective in controlling F_n until it is greater than r_m (ignoring the effects of r_a), whereas, in species where changes in r_s and F_n are synchronous, r_m may be so low as to be ineffective in controlling F_n in comparison with r_s.

The above discussion is of relevance since in the desiccating plant the effect of stomatal control on transpiration may be important, providing CO_2 uptake is uninhibited and diffusion is not affected by an increase in r_m.

Gaastra (1959) noted that the concept that the rate of diffusion is determined by the sum of a series of resistances (i.e. $r_a + r_s$ for transpiration and $r_a + r_s + r_m$ for photosynthesis) implies that transpiration depends on stomatal opening to a greater extent than photosynthesis. Raschke (1976) quoted values for a hypothetical land plant (in which no resistance to CO_2 uptake is ~~pro~~ffered by the carboxylating mechanism and r_a is the only resistance to CO_2 diffusion) of twenty molecules of water lost per molecule of CO_2 assimilated at 20°C and 70% relative humidity but this increases to 430:1 at 50°C and 10% relative humidity. However, since the carboxylating system has a limited affinity for CO_2, the actual losses are between two and ten times greater than this (Raschke, 1976).

Although transpiration has not been considered in the present investigation, it may be relevant to consider the theoretical implications of stomatal closure on the conservation of water while a degree of assimilatory capacity is maintained. The effect of r_s upon transpiration has been extensively reviewed by Slatyer (1967), Cowan & Milthorpe (1968), Meidner & Mansfield (1968). Briefly, the effect of r_s is related to r_a. If r_a is large e.g. in still air, changes in r_s are ineffectual until r_s equals r_a. In moving air, r_a is comparatively small thereby allowing considerable stomatal control over transpiration. The cuticular resistance (r_c) may also affect r_s. Duniway & Durbin (1971) observed that fungal damage reduced r_c so that r_s would have to increase to a greater extent than normal to have a similar effect on water loss. This oversimplifies a more complex situation, for example, stomatal closure may raise leaf temperature, increase the water vapour concentration within the leaf and steepen the humidity gradient between the inside and the outside of the leaf. In addition, extrapolation of single leaf findings to the canopy situation may not validly represent the situation existing in the field (Slatyer, 1967). However, the effect of r_s on transpiration may be regarded as linear, (Raschke, 1976). By comparison, the effect of rate limiting factors other than CO_2 concentration, on CO_2 uptake results in the effect of stomatal movement on CO_2 uptake being less than that on transpiration. Raschke (1976) concluded that, in contrast to the linear relationship with transpiration, "...the sensitivity of CO_2 uptake to stomatal movements is inversely proportional to the square of stomatal conductance." In a study employing *Xanthium strumarium* Raschke found that at stomatal conductances greater than 0.5cm s^{-1}, an increase in r_s reduced water loss without having a significant effect on CO_2 uptake so that water loss per molecule of CO_2 assimilated was greatly reduced. At a conductance of 1.4cm s^{-1} the water loss to CO_2 assimilated was 300:1 while at a conductance of 0.1cm s^{-1} the ratio was 50:1. This re-emphasises the notion that changes in r_s inferred from transpiration data cannot be extrapolated to the effects r_s has on CO_2 uptake. Larcher (1960) noted that the shape of the assimilation curve in *Quercus pebescens* indicated the stage at which the stomata close while the transpiration curve did not. However, Willis & Balasubramanium (1968), using porometric methods of determining r_s, found that changes in r_s at low values of water stress, had substantial effects on transpiration and photosynthesis. Puritch (1973) observed that reduction in F_n resembled that of transpiration and occurred at similar stresses (i.e. -0.9 to -1.1MPa and -0.8 to -1.0MPa respectively) but he conceded that although F_n and transpiration decline occurred at similar stresses and may have been mediated by changes in r_s, the factors involved may not be identical in the two processes. Slatyer (1973b), working with Atriplex spongiosa and A. hastata observed a parallel decline in F_n and transpiration which he attributed to an increase in r_s. Similarly, Mederski et al. (1975), using stepwise changes in r_s whilst RWC remained constant, observed a parallel decrease in F_n and transpiration which was ascribed to increases in r_s, implying that both processes are regulated proportionately by stomatal resistance. These observations conflict with the differential response of F_n and transpiration to increased r_s observed by Raschke (1976) but Pieters & Zima (1975) also observed a decline in transpiration prior to a decline in F_n which suggested that an increase in r_s has a proportionately greater effect on transpiration than on F_n. Boyer (1976) considered that the majority of evidence suggested that F_n and transpiration are correlated, though possibly imperfectly in the early stages of desiccation, but this presupposes that for such a correlation to exist, F_n must be limited by diffusion of CO_2 through the stomata. Where this is not the case as when r_m is the limiting resistance, the effect of a change in r_s is likely to be greater on transpiration than on F_n. Meidner & Mansfield (1968) contended that when water is limiting, r_s will increase and reduce transpiration, and although F_n is also reduced, the decrease is probably less than that of transpiration. This obviously enables water to be conserved while CO_2 uptake remains relatively unimpaired. The 'transpiration ratio', or ratio of dry matter produced to water lost, indicates this advantage. Larcher (1960) considered that the greatest advantages require a wide stomatal aperture but not a maximal one, such that "...the water economy of assimilation may well reach its peak in the initial stage of stomatal closure rather than at the time when the stomata are most widely open, even though the yield of the process of assimilation proper does reach its absolute peak with maximum apertures". Larcher found, that for Quercus pubescens and Q. ilex, the most effective compromise between water loss and CO_2 uptake occurred at a water deficit 4-5% above the deficit required to induce hydroactive stomatal closure. In terms of Raschke's (1976) model of stomatal control, the balance between CO_2 depletion causing stomatal opening and possible ABA accumulation inducing stomatal closure may effectively protect the plant against severe desiccation in the presence of severe water deficits. However, under prolonged stresses, the inhibition of photosynthesis may also jeopardise survival. It is apparent, however, that the capacity to restrict water loss to a greater extent than photosynthesis, is of considerable advantage in species subject to drought.

(ii) Seasonal variation in F_n

Seasonal variation in F_n has been observed in a wide variety of evergreens (Kozlowski & Keller, 1966). Parker (1961) observed that CO_2 uptake in Chamaecyparis pisifera, Pinus sylvestris and P. palustris was at a maximum in November (for the November to June period), declining in December to an immeasurable rate by February. At the end of March, CO_2 uptake became positive, following a warm period, and coincided with the spring bud burst in native deciduous trees over the succeeding few weeks. The pattern was related to seasonal variation in cold hardiness. McGregor & Kramer (1963) found that CO_2 uptake increased from February to April in Pinus taeda and P. strobus, rising to a peak in midsummer, after which a decline to autumn and winter levels occurred. They found, however, that the unit of expression could alter the trend. For example, when expressed as rate per fascicle length instead of rate per seedling, the rate of F_n in P. taeda increased between February and March prior to the production of new needles and the maximum was attained four months earlier than previously. This led to the conclusion that as well as seasonal variation arising from changes in photosynthetic surface, a change in photosynthetic capacity occurred as a result of other factors. Relationships with chlorophyll content were not good enough to account for seasonal differences although rates per unit chlorophyll indicated maximum rates of F_n in May and a minimum in January. Although these findings conflict with those of Lange _et al_. (1974) regarding the basis of expression, McGregor & Kramer (1963) considered that partial reversible chloroplast disorganisation may be associated with low temperatures and could be responsible for the autumn-winter decrease in F_n whereas in spring the process was reversed leading to an increase in F_n before the new foliage appeared. Schulze _et al_. (1967) observed seasonal variation in F_n of _Pinus aristata_. In November the rate was 40% of that in the summer while by January CO_2 uptake was immeasurable. They pointed out that, unlike many conifers, this species does not exhibit a positive CO_2 uptake when temperatures rise above 0^oC. Some species exhibit a positive CO_2 uptake at subzero temperatures (Kozlowski & Keller, 1966; Ludlow & Jarvis, 1971).

Changes in the relationship between chlorophyll content and F_n may be responsible for seasonal variation in photosynthetic capacity (McGregor & Kramer, 1963). Bordeau (1959), working with _Picea excelsa_, _P. pungens_, _Pinus sylvestris_ and _Tsuga canadensis_, observed that decreases in photosynthetic efficiency were accompanied by decreases in chlorophyll content. When _P. sylvestris_ was moved to a warmer environment, an increase in F_n within two days was accompanied by an increase in chlorophyll content. Bordeau did not exclude the possibility of other factors affecting F_n and other work does not confirm his findings. Smillie (1962) observed that F_n declined faster than the decrease in leaf chlorophyll in _Pisum sativum_. Zelawski & Kucharska (1967) found that changes in F_n were not accompanied by changes in chlorophyll a and b in Pinus sylvestris and thus were unlikely to account for the winter depression in F_n. This suggests that factors other than chlorophyll content may be involved in seasonal variation in F_n but Hardwick et al. (1968) noted that, although variations in F_n of Perilla frutescens appeared to be independent of changes in chlorophyll content, this did not preclude the effect of chloroplast activity being affected by enzymic factors. They observed that decreases in photosynthesis were associated with decreases in RNA and protein content of the leaf and experimentation revealed that cessation of enzyme synthesis may have been responsible for photosynthetic decline with senescence. Smillie (1962) also observed that the decrease in photosynthetic capacity was paralleled by a decrease in photosynthetic enzymes e.g. ribulose diphosphate carboxylase, and Alderfer (1975) postulated that decreased carboxylate enzyme synthesis could account for the decrease in F_n with age in Glycine max. He also suggested that a general depression in enzyme synthesis may retard transport of CO_2 across membranes by carbonic anhydrase, effectively increasing r_m. Woolhouse (1968) found that a decrease in F_n of Perilla frutescens was associated with an increase in r_m but his evidence suggested carboxylation to be the limiting factor since 'fraction I' protein (containing carboxylation enzymes) was found to decrease at the same rate as photosynthetic activity. It will be apparent that ageing of photosynthetic tissue as it affects protein synthesis, may affect F_n during the course of the season. Grace & Woolhouse (1970) observed that new growth had a higher rate of F_n than previous season's growth in C. vulgaris.

McGregor & Kramer (1963) also postulated that seasonal changes in r_s and r_m may affect CO_2 uptake. Studies on Picea sitchensis by Ludlow & Jarvis (1971) indicated that r_m and r_s were large in young shoots (c. 0.0125cm. s^{-1} and 0.0714cm. s^{-1} respectively at 20 ± 0.5°C and

200 ± 20Wm^{-2}) but decreased with age. A minimum r_s (0.5cm s^{-1} at 20°C) was attained in July but then increased through autumn and winter to approximately 0.125cm s^{-1}, which was maintained through the following summer. Similar changes occurred in r_m and associated changes in F_n were observed. They postulated that as shoots elongate the rise in F_n may be related to the drop in r_m, which may be associated with increased enzyme activity or sinks for assimilates. Following shoot elongation they considered that further increase in F_n was associated with a greater decrease in r_s than in r_m but that increases in r_s and r_m were responsible for the decline in F_n, possibly due to reduced stomatal reactivity or blockage of the stomatal antechambers by wax accumulations.

Neilson et al. (1972), also working with P. sitchensis, found that changes in r_s and r_m were temperature related. Although r_s changed little over the temperature range 0-30°C, the reduction in F_n below 10°C was associated with a large increase in r_m. They observed that r_s was lowest in young shoots from June to October, rising through the winter, while long term adaptation to low temperature resulted in high values for r_s and r_m. Watts et al. (1976) found that r_s decreased and F_n increased as shoots elongated but that the independence of F_n on r_s implied that an increase in F_n was dependent on the maturation of the photosynthetic apparatus and probably the result of a decrease in r_m. One year old shoots exhibited lower rates of F_n possibly as a result of increased r_s resulting from changes in stomatal physiology and anatomy but the reduction was too large to be entirely explained by changes in r_s and was considered to reflect a reduction in photosynthetic capacity.

Stomatal reactivity may undergo seasonal variation and consequently affect F_n. Bordeau (1959) considered this unlikely but Zelawski & Kucharska (1967) obtained evidence indicating that changes in F_n were accompanied by changes in leaf water content and rate of water loss.

They considered that the rapid increase in F_n following transfer of the plant to warm conditions after being in the cold may have resulted from an improvement in the plants water balance leading to more efficient stomatal movement. Lange et al. (1974) considered that seasonal changes in the temperature optimum of photosynthesis might be mediated through changes in the temperature response of stomata since Schulze et al. (1973) had observed that r_s is dependent upon leaf temperature and the dependence varies during the season. A number of workers have suggested that low temperature may inhibit stomatal opening e.g. Loftfield (1921), Stalfelt (1962), Troughton (1969).

Anderson & McNaughton (1973) however, considered that low temperature reduction in growth did not result from an effect of water stress on photosynthesis or stomatal aperture (cf. Zelawski & Kucharska, 1967). They concluded that a critical RWC exists above which transpiration and photosynthesis are unaffected by RWC. Root chilling to 3°C in a number of species failed to lower the RWC to the critical point at which transpiration and photosynthesis were affected. This suggests that together with seasonal changes in stomatal reactivity, stomatal aperture as a function of water deficit may not effect a seasonal change in F_n capacity.

Cytokinins produced by the root may be important in the synthesis of carboxylating enzymes (Wareing et al., 1968), and Anderson & McNaughton (1973) suggested that low soil temperatures may inhibit synthesis and translocation, a view also expressed by Atkin & Barton (1973) who considered that the effect is a long term response of days or weeks. Thus, low temperature may have an indirect effect on photosynthesis via enzyme synthesis. In addition, Pallas & Box (1970) observed that r_s was lower in leaves treated with kinetin, thus a reduction in synthesis and translocation of root produced cytokinins at low soil temperatures may increase r_s and thereby reduce CO_2 uptake.

The significance of hormones in affecting CO_2 uptake has been noted by other workers. Spraying leaves of a number of species with indoleacetic acid (IAA) resulted in increased CO_2 uptake by 30-100% in the succeeding 30-60 minutes (Turner & Bidwell, 1965; Bidwell & Turner, 1966), and the breaking of bud dormancy which has been associated with an increase in CO_2 assimilation in adjacent leaves has been attributed to a hormone effect (Bidwell & Turner, 1966; Sweet & Wareing, 1966). Thorne & Koller (1974) also suggested that F_n may be related to hormonal levels influencing carboxylating enzymes but considered that sink demands for assimilates may also influence source leaf photosynthesis. The role of assimilates in controlling F_n is controversial (cf. Neales & Incoll, 1968; Humphries, 1969). Warren Wilson (1966) hypothesised that depression of respiration and new growth by low temperatures may result in assimilate accumulation to a level at which photosynthesis is depressed to a rate which balances assimilate utilization. A corollary of this is that as assimilates are utilized, when temperatures permit growth, F_n will increase as the concentration of assimilates in the leaf falls. Neales & Incoll (1968), in reviewing the evidence for assimilate control over photosynthesis considered the following possibilities:

(1) the physical obstruction of light transmission to the chloroplasts by starch grains, but this was considered unlikely;

(2) repressed enzyme activity by intermediate or end-product inhibition;

(3) an increase in r_s caused by the accumulation of sugars in the stomatal apparatus thus upsetting the turgidity potentials of guard and epidermal cells;

(4) an increase in r_m resulting from an increase in the CO_2 diffusion path through the cell as a result of starch grains interfering with diffusion.

Humphries (1969) considered that F_n may be reduced when plants lack sinks into which carbohydrates can be diverted, for example, following a period of stress, F_n may increase as a result of increased assimilate demand. Similarly, when low temperatures inhibit root growth a build up in assimilates in the source leaves may retard photosynthesis. Humphries concluded that the accumulation of assimilates in stems, leaves and roots appears to be a pre-requisite for photosynthetic depression. Fraser & Bidwell (1974) observed that as each leaf emerged, transient increases in F_n in adjacent leaves occurred, which led to the conclusion that F_n was regulated, in part, by the interaction between assimilate accumulation in the leaf, translocation of assimilate and assimilate demand. Thus, as a new sink developed, F_n in existing leaves altered to accommodate the change; however, new leaves eventually become self sufficient and consequently do not constitute long lasting sinks.

The effect of growth on F_n is not clear cut. McGregor & Kramer (1963) observed that an increase in F_n in Pinus spp. occurred just before the initiation of growth in spring but the causal connections remain obscure since the considerable food reserves present implied that initial spring growth was not dependent upon F_n. However, viewed from the perspective of Fraser & Bidwell (1974), it would appear that growth, in utilizing stored assimilates, would de-repress F_n by providing new sinks for assimilates. This is consistent with the regulatory role of hormones on F_n, the synthesis of auxins by buds which are translocated to the assimilatory areas (Bidwell & Turner, 1966) and the high level of auxin production by younger leaves (Sweet & Wareing, 1966). Gordon & Larson (1968) also found a strong interaction between vigorous terminal meristemmatic growth and increase in photosynthetic efficiency of older needles in Pinus resinosa which they ascribed to hormonal regulation and/or the provision of new assimilate sinks. The findings of Little &

Loach (1973) on Abies balsamea indicated that F_n may be independent of carbohydrate concentration. They found that starch levels and F_n both increased between April and June and concluded that carbohydrate content is determined by photosynthesis and that the increase in F_n which preceded budbreak implied that the provision of new assimilate sinks was not responsible. Thorne & Koller (1974) found that increased sucrose concentrations in Glycine max had little adverse effect on F_n but a reduction in starch concentration resulted in a substantial increase in F_n. They concluded that the effect of sucrose as a mass action regulator seems unsupported whilst the role of starch is questionable, rather the control of F_n would appear to result from a complex "source-sink-hormonal interaction". The role of assimilates in regulating F_n is therefore not unequivocally demonstrated.

The effect of environmental variables on the seasonal variation in photosynthetic capacity is complex because of the interaction between variables (Kozlowski & Keller, 1966). Lange et al. (1974) commented that the seasonal variation in the temperature relationship with F_n may reflect changes in photosynthetic and respiratory capacities of plants. Schulze et al. (1967) found that in Pinus aristata respiration rates remained high while F_n decreased and Lange et al. (1974) reported that acclimation to low temperature may be associated with increased respiration and reduced F_n. At high temperatures the reverse applied, suggesting that decreases in F_n may result from recycling of a higher respiratory CO_2 output when F_n is measured at a temperature greater than that to which it is acclimated. They failed to find any correlations between the temperature response of F_n, the maximum photosynthetic capacity and dark respiration, and concluded that "Further experimental evidence is necessary to learn more about the biochemical background of thermally induced photosynthetic modifications ..... a whole complex of different strategies, varying from species to species, might exist".

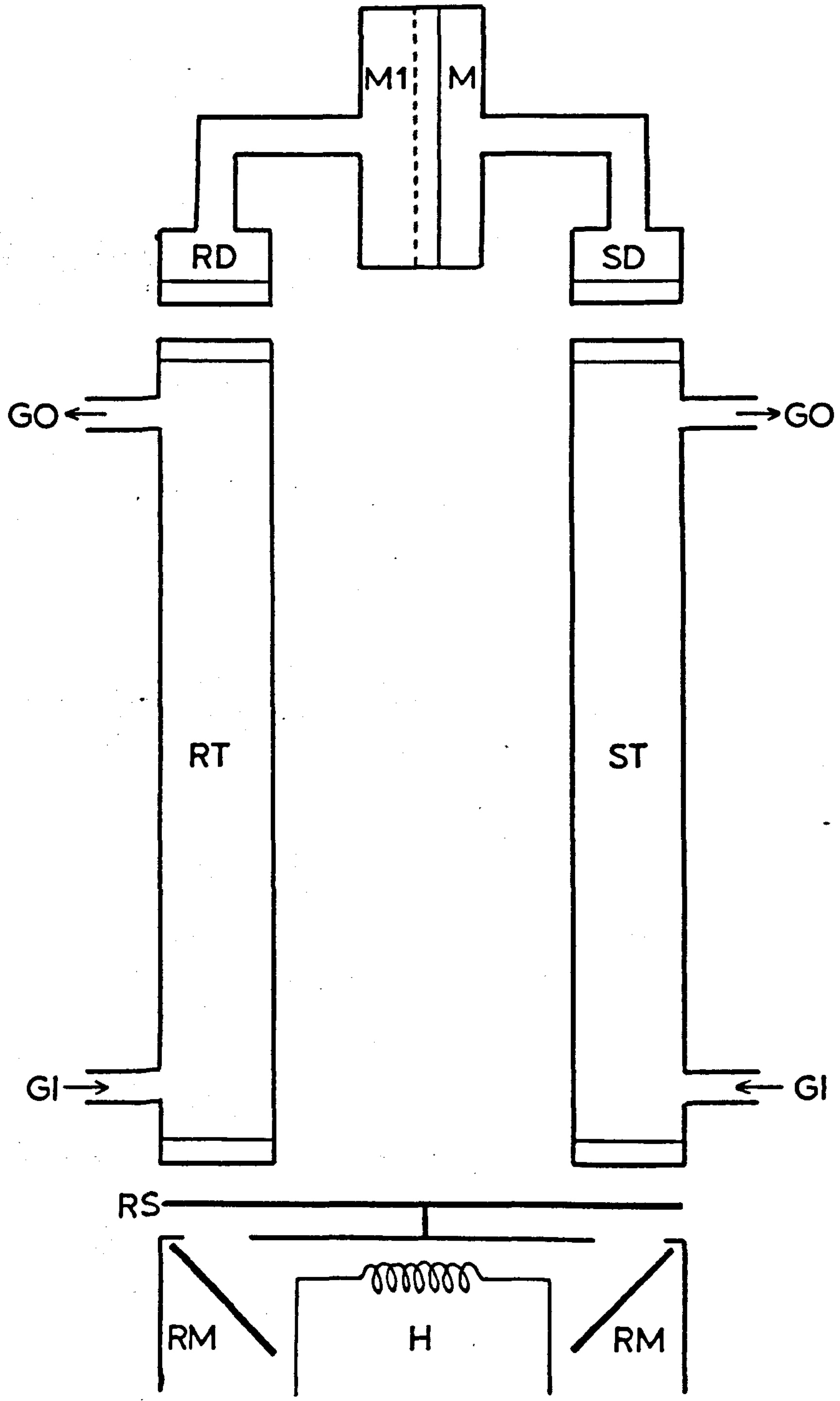
The seasonal variation in photosynthetic capacity is obviously complex and is probably ascribable to a number of mechanisms. The essential physiological processes involved are enzyme availability and activity, variation in r_s and r_m and the effects of source-sink and hormonal relationships. While exhibiting seasonal trends, such factors are also likely to be modified by environmental variables.

(b) Methodology associated with the measurement of CO_2 exchange by infra-red gas analysis techniques

(i) Principles of Infra-Red Gas Analysis

The measurement of carbon dioxide (CO_2) uptake and evolution provides a comparatively sensitive measurement of F_n and respiration, in that the absolute variation in CO_2 concentration arising from these processes represents a relatively large change in comparison to the CO_2 concentration of the air, e.g. consumption of $30\mu l\ l^{-1}$ (0.003% CO_2 by volume in air) is equivalent to a 10% reduction in the nominal atmospheric concentration of $300\mu l\ l^{-1}$ (0.03%). Gasometric measurements, which are both sensitive and accurate, make them a widely used method, one of the most commonly employed being that of infra-red gas analysis. The principle upon which infra-red gas analysis is based is the direct relationship existing between the molecular structure of a substance and its infra-red absorption spectrum, which is dependent upon the mass and number of atoms in the molecule, the molecular symmetry and inter-atomic bonding. The vibrational and vibrational/rotational spectra of molecules lie within the range 2.5 to 25μm (the intermediate infra-red region) so that by using infra-red gas analysis, gas mixtures may be selectively measured (Sestak et al., 1971). Gases made of two identical atoms e.g. oxygen, nitrogen, do not absorb in the infra-red in contrast to gases such as carbon dioxide

Figure 62 Diagram showing basic construction of the Grubb Parsons SB 2 differential Infra-Red Gas Analyser.



M&M1	metal diaphragms	GI	gas inlet
RD	reference detecting chamber	RT	reference tube
SD	sample detecting chamber	ST	sample tube
GO	gas outlet	RS	rotating shutter
		RM	reflecting mirror
		H	heater block

(CO_2) and water (H_2O), which facilitates the measurement of CO_2 without interference from oxygen and nitrogen. However, the interference of CO_2 and H_2O absorption spectra in the region 2.6 to 2.8μm means that if CO_2 is being measured, the gas must either be dried or the region of overlap filtered out (Sestak _et_ _al_., 1971). The portion of the spectrum used in measurements is determined by the optical material used in the analysis tubes; most commonly a 'window' of calcium fluoride is employed which transmits wavelengths up to 8μm, the range for CO_2 absorption being 4.2 to 4.5μm with a peak at 4.26μm (Grubb Parsons).

(ii) Principles of Detection

The principle of positive filtration, in which a selective radiation detector which is filled with the same gas as that being measured, is most commonly employed (Sestak _et_ _al_., 1971).

The Grubb Parsons SB2 type analyser employs detecting chambers which are optically in parallel, since this imparts high sensitivity, low response time and ease of adaptation to a variety of gases, although the reduced stability necessitates frequent recalibration. The description which follows applies to an infra-red gas analyser (IRGA), type SB2 (supplied by Sir Howard Grubb Parsons & Co. Ltd., Newcastle-upon-Tyne), and is based upon their operating manual.

At the base of the instrument (Figure 62) is a heater block (H) containing a nichrome metal heater, which, at a temperature of 600-800°C, glows dull red, thus providing a source of infra-red radiation. This is reflected, by mirrors (RM), into absorption tubes RT and ST, and passes to the detecting chambers RD and SD. A metal diaphragm (M), 5-10μm thick, partitions the detecting chambers, whilst a second plate (M1), positioned about 50μm from plate M, helps to form an electrical condenser between the two plates. Deformation of the diaphragm changes the electrical capacitance, which is inversely proportional to a change in voltage, thus, deformation results in an electrical voltage which is amplified to produce a millivolt output which is fed to a meter or recorded. Intermittent interruption of the radiation paths by the rotating shutter (RS) results in periodical pressure changes in the detector and simultaneous vibration of the diaphragms. This enables both absorption chambers to be connected by an acoustic filter, thereby maintaining a static pressure equilibrium between the two chambers. This, together with the use of an A.C. amplifier, which such an arrangement allows, confers greater stability upon the system than the use of a D.C. amplifier.

If the CO_2 concentration in analysis tubes RT and ST is identical, the amount of infra-red radiation arriving at RD and SD will be the same, producing an identical heating effect in both detecting chambers, so that no pressure differences arise between the chambers. If the CO_2 concentration in tube ST is less than that in tube RT, for example as a result of a plant having removed CO_2 during photosynthesis, less infra-red radiation will be absorbed and more will reach SD than RD. The heating effect will cause a greater expansion of gas in SD than in RD, resulting in a pressure differential deforming the diaphragm and changing the capacity of the condenser. The detector is filled with the same gas as that being analysed i.e. CO_2, since, of the wide spectrum emmitted by the heater, the gas will absorb only radiation in its own absorption bands. Acting as a selective detector it will not respond to radiation of other wavelengths, thus ensuring that measurement will not be affected by other gases in the sample tube absorbing infra-red radiation of other wavelengths.

The length of the analysis tubes is also of some significance and depends upon the gas being measured. The relationship between the concentration of the absorbing gas and the energy absorbed departs from linearity when 25% of the energy has been absorbed. When this limit has been passed it is necessary to reduce the volume of gas present absorbing the radiation, which may be achieved by reducing the cell length. The optimal path length for the measurement of CO_2, and used in the present instrument, is 30cm. The technical specifications are such that a minimum measuring range of 0 - 50µl 1^{-1} exists, with a reproducibility of 1% full scale deflection (f.s.d.) and a deviation stability of 1% f.s.d. per 24 hours.

To remove the error associated with water vapour concentration changes on CO_2 measurement, an interference filter has been fitted to remove radiation in the range 2.6 - 2.8µm. Use of such filters suppresses cross sensitivity between the absorption spectra of CO_2 and H_2O so that response to water vapour is not measurable. Without such filters a 1-2% by volume of water vapour in the gas mixture being analysed would result in a response in the region of 50 - 100µl 1^{-1} by volume of CO_2 equivalent. This would necessitate chemical drying of the gas which may decrease sensitivity and increase response time.

(iii) Gas handling circuitry

The components necessary to measure gaseous exchange rates by plants may be divided into four sections (Sestak et al., 1971):

(1) the Infra-Red Gas Analyser;

(2) the Assimilation Chamber, in which the plant or leaf under investigation is enclosed, so allowing changes in the CO_2 content of the enclosed volume of air as a result of the plant's metabolic activity, to be studied;

(3) the Gas handling system, which transports air from its source through the assimilation chamber and thence to the IRGA. It also transports

reference air to the analyser;

(4) the Air conditioning system, which predetermines the temperature, humidity, CO_2 and possibly O_2 levels in the gas entering the assimilation chamber. The most sophisticated conditioning systems have sensors analysing the sample air stream and automatically regulating its condition and composition, e.g. Koch, Lange & Schulze (1971).

The Assimilation chamber

The need to enclose a plant in a chamber to study its gaseous exchange may be regarded as a shortcoming of the gasometric method by imposing an unnatural environment on the plant. The objection is more serious in field situations where the internal environment may differ significantly from the environment experienced by the plant outside the chamber. Sophisticated techniques have been developed to overcome the problem, e.g. Koch *et al*. (1971) but in the laboratory situation not only is the problem less serious but often the effect of environmental variables on gaseous exchange is under consideration, and the chamber enables such variables to be easily manipulated. Plants used in laboratory investigations have frequently been raised under artificial conditions which may be accurately simulated using a chamber.

The design criteria upon which a chamber is constructed will, to a certain extent, depend upon the intended use of the chamber but certain basic requirements have been stipulated by a number of workers:

(1) the chamber should be able to accommodate the leaf conveniently and in a reproducible position in the chamber (Gaastra, 1959; Hardwick, Lumb & Woolhouse, 1966);

(2) the air must be regularly distributed over the cross section of the chamber (Gaastra, 1959);

(3) facilities should exist for adjusting the temperature to prevent overheating of the organ under investigation (Natr & Spidla, 1963);

(4) facilities should exist to accommodate instruments allowing measurement of chamber environment (Hardwick, Lumb & Woolhouse, 1966);

(5) insulation of the chamber from the external environment should be adequate to enable accurate control of the internal environment (Hardwick, Lumb & Woolhouse, 1966);

(6) the chamber should allow a source of radiation to impinge upon the leaf which may be accurately quantified from all directions "so that the leaf receives uniform, diffuse irradiance from all directions" (Sestak _et al_., 1971).

A full review of the principles involved in chamber design has been made by Sestak _et al_. (1971). Three chambers were constructed for use in the present investigation, referred to as slow, medium and fast response chambers.

(1) _Slow response chamber_ (Figure 63)

The design requirements of this chamber were to enable the measurement of whole plant photosynthesis under controlled conditions of temperature and humidity and to facilitate omnidirectional radiation from a unidirectional source under laboratory conditions.

Many studies have been made of whole shoot photosynthesis, e.g. Van Oorschot & Belksma (1961), Scott _et al_. (1970), Catsky & Ticha (1974) in which the aerial portion of the plant has been sealed within the chamber, leaving the root outside. With plants such as _Plantago lanceolata_, where no clearly defined internode exists, sealing round the stem of the plant is not possible. This factor, together with the kind

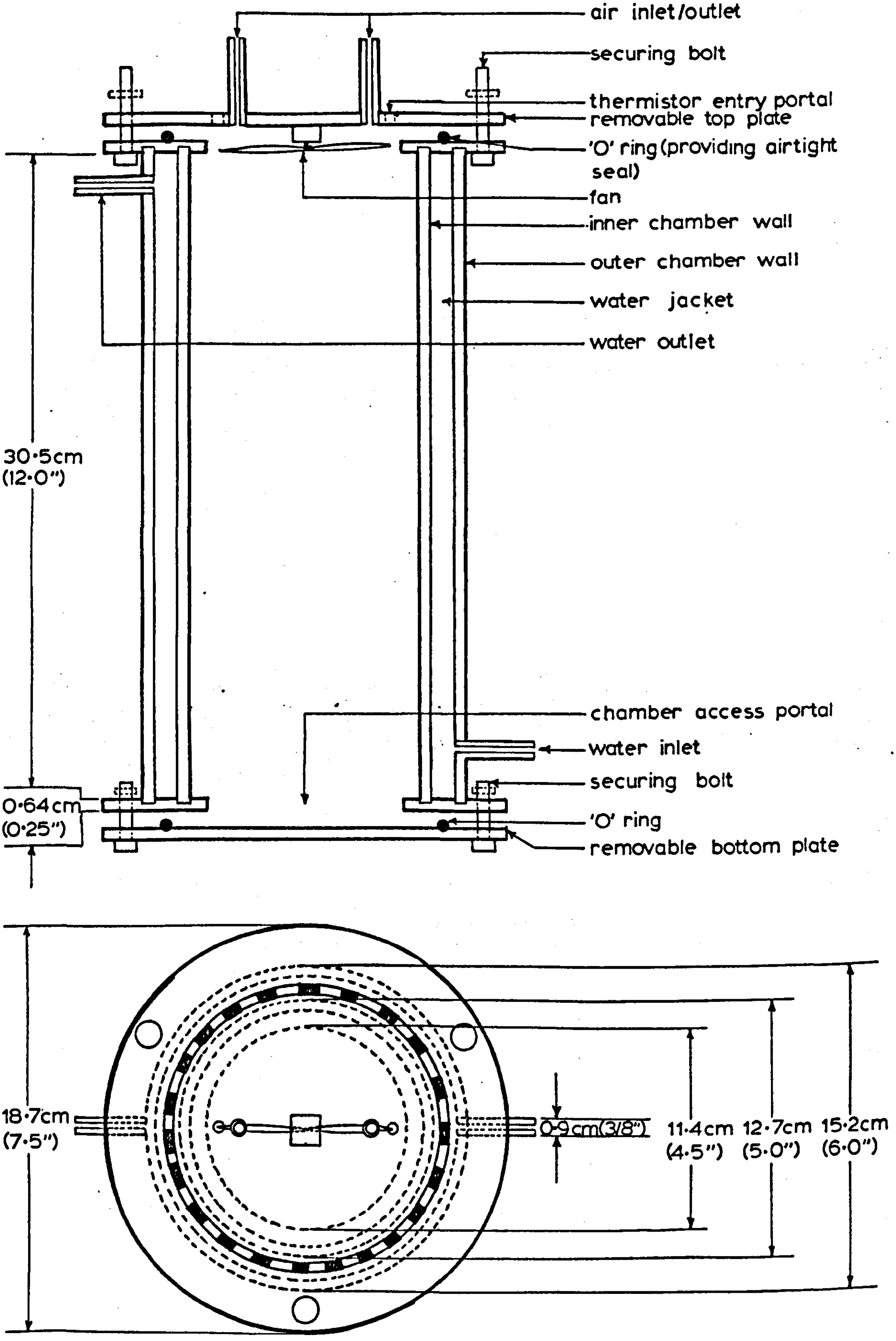


Figure 63 Diagram of 'slow response' (whole plant) assimilation chamber, showing basic construction. (Not drawn to scale.)

of damage resulting from the treatments applied, which for the most part precluded the use of individual leaves, necessitated the inclusion of the pot within the chamber. Initial attempts to restrict soil respiratory CO_2 output by sealing the soil surface with a mixture of vaseline and liquid paraffin were prohibitively expensive; consequently, following the determination of CO_2 exchange by the plant foliage, the shoot was harvested and the pot containing soil and root replaced in the chamber to determine evolution of CO_2 from the soil. An appropriate correction to the CO_2 exchange by the plant was made by assuming the chamber air CO_2 concentration was equivalent to the reference air CO_2 concentration plus the value for CO_2 evolution by the soil.

The chamber was constructed from acrylic plastic ('Perspex'), although difficulties of producing air and water tight joints were experienced, as noted by others, e.g. Jarvis & Slatyer (1966). Perspex has an absorption of less than 0.2% per centimeter thickness and a high, uniform transmittance of 0.92 over the wavelength range 400 - 800nm. (Sestak _et al_., 1971). Losses are likely to have been greater owing to the ease with which the material may be scratched, thus the photosynthetically active radiation impinging upon the leaf may have been significantly different from that experienced during growth, although for a given set of experiments, the conditions are likely to have been constant, thus enabling justifiable comparative investigations to be made.

To facilitate maintenance of a constant chamber temperature, the walls were constructed of two clear 'Perspex' tubes 30.5cm. long, with outside diameters of 15.2cm. and 12.7cm., thereby giving a water jacket running the length of the chamber of 0.9cm. in width. The transparent sides of the chamber also enabled reflected radiation, from the foil lined sides of the light housing, to be transmitted to the plant. The top and base plates of the chamber could be removed to allow positioning and access to

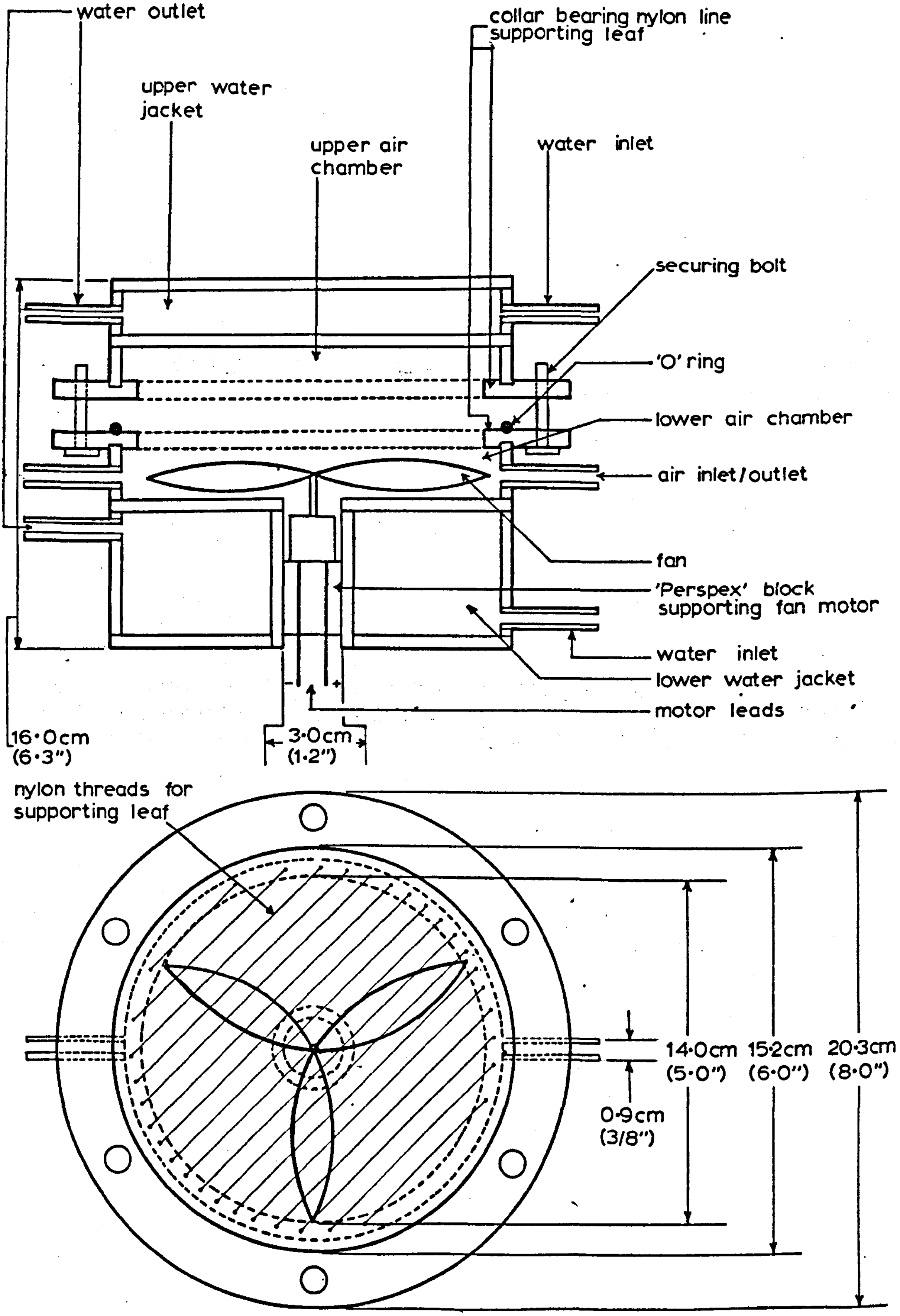


Figure 64 Diagram of 'medium response' (single leaf) assimilation chamber. (Not drawn to scale)

the plant. A 1.5V D.C. motor was attached to the top plate, driving a three bladed propellor to circulate the air within the chamber. The air inlet and outlet ports were, for technical reasons, mounted on the top plate. This could result in incomplete mixing of the chamber air, possibly leading to erroneous estimates of photosynthetic rates by producing a heterogeneous concentration of CO_2 with the chamber. Two additional entry portals were provided, on the top plate, to allow temperature sensors (thermistors) to be positioned in the chamber. The chamber was mounted on a moveable base forming an integral part of the light housing and thus ensuring repeatable positioning of the chamber under the lights. Owing to its relatively large volume, the time taken to achieve CO_2 exchange equilibrium following plant insertion was the longest of all chambers employed.

(2) Medium response chamber (Figure 64)

This chamber was modelled on that of Hardwick, Lumb & Woolhouse (1966), the construction being of 'Perspex' instead of metal and glass.

The chamber consisted of an upper and lower section which, on fitting together, produced an air tight chamber in which a leaf could be sealed. The vertical walls of the chamber were constructed from 0.32cm. thick 'Perspex' tube (15.2cm. outside diameter), and the horizontal surfaces from 0.64cm. thick 'Perspex' plate. Each section had a horizontal water cooled jacket to aid control of chamber temperature. Although 'Perspex' has a high transmittance of photosynthetically active radiation, the transmittance of thermal radiation is low (Avery, 1967), consequently, heat emanating from the plant either as re-radiation or as a result of metabolic activity will be absorbed by the chamber walls as will thermal radiation generated by the light source. This may result in the 'cuvette effect' (cf. Sestak *et al.*, 1971) in which leaf temperatures

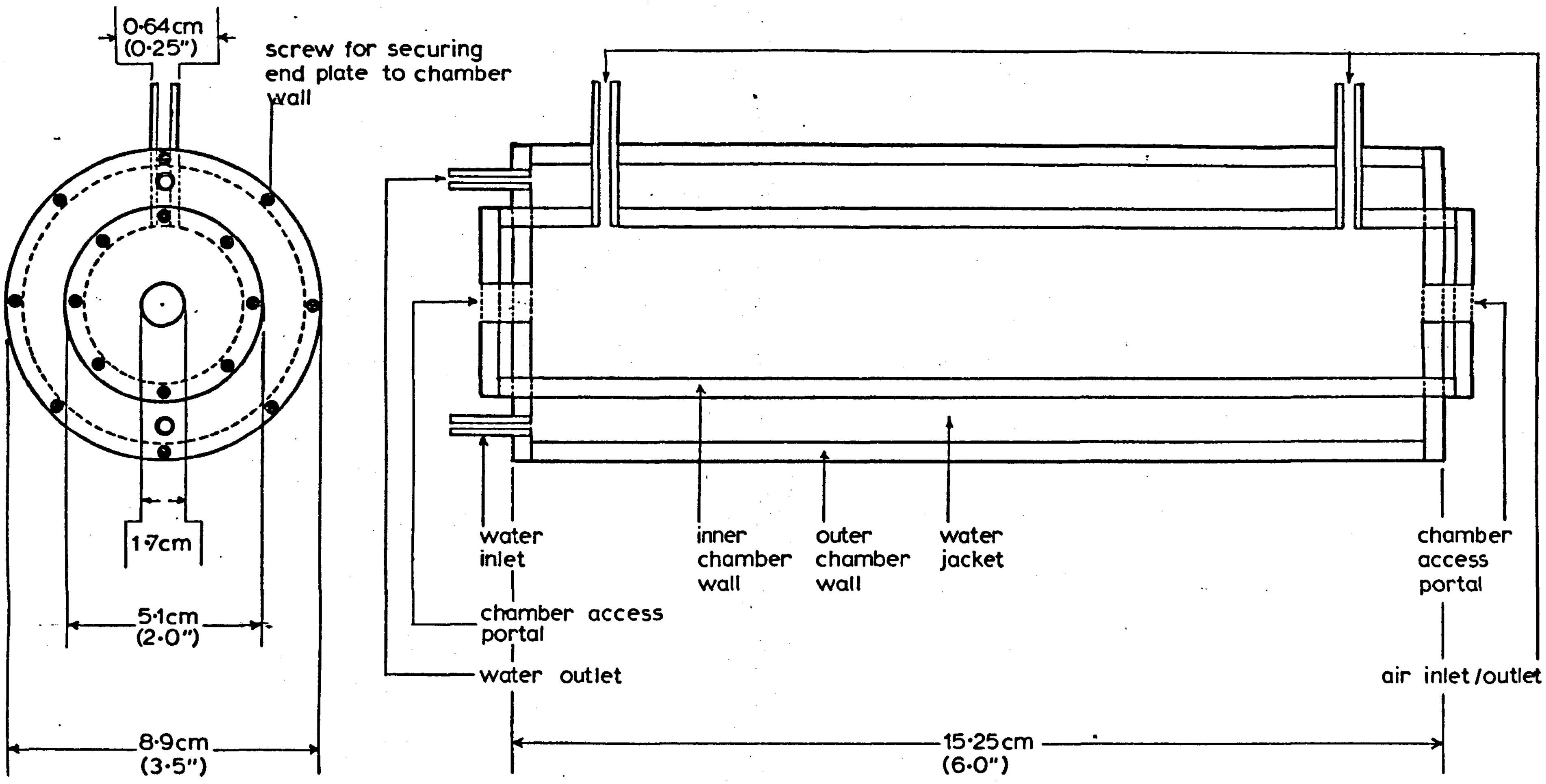


Figure 65 Diagram of 'fast response' (single leaf/shoot) assimilation chamber. (Not drawn to scale)

gradually rise. It may be alleviated by having an inner and outer wall for intercepting such thermal wavelengths and by running a coolant between the two walls so the heat will be conducted away from the chamber environment. The water inlet and outlet pipes, although opposite in the upper section, were diagonally disposed in the lower section to facilitate turbulent flow and increase the efficiency of the cooling mechanism.

A 1.5V D.C. motor was mounted on a tapered 'Perspex' plug fitting into the base of the lower water jacket. A three bladed fan mounted on the motor occupied the area immediately below the leaf and ensured turbulent mixing of the air within the chamber. The leaf was supported between two nylon thread meshes, mounted on the inner faces of the upper and lower sections, so keeping the leaf flat and preventing damage from the fan blade. A notch in the 'O'ring, which provided the air tight seal between the two sections, accommodated the leaf petiole which was sealed into place by vaseline.

(3) Fast response chamber (Figure 65)

The small volume of this chamber resulted in rapid equilibration times. It was designed specifically for use with shoots of Calluna vulgaris, and consisted essentially of a water jacketed clear 'Perspex' tube with air portals in the side to enable easy emplacement of the shoot. Unlike the chambers above, there was no fan to generate turbulent air flow, although the morphology of a C. vulgaris shoot is such as to inhibit laminar flow across the photosynthetic surfaces. The shoots could be placed entirely within the chamber or mounted in a tube to enable a water supply to be maintained to the shoot. Graminaceous leaves could be mounted in the chamber whilst remaining attached to the plant by sealing the entrance with a split rubber bung or foam rubber.

The tube ends were accurately machined to produce water and air tight seals when the end plates were bolted on. The curved surfaces of the chamber produced a problem of radiation reflection and light intensity may have been a limiting factor since the light compensation point of C. vulgaris at $20^{o}C$ lies between $62.8Wm^{-2}$ and $69.8Wm^{-2}$ (Grace & Woolhouse, 1970), whereas the irradiance of the shoot during the measurement of CO_2 uptake was c. $80Wm^{-2}$.

The Gas handling system (Figure 66)

Brown (1968) classified gas exchange systems into three major types. The reasons for adopting an 'open' system will be briefly discussed.

(1) Rejection of the 'closed' system. The 'closed' system is based upon the time taken for a plant to reduce the CO_2 concentration in the recirculating air from one level to another. However, since photosynthesis varies as a function of CO_2 concentration, the results obtained using such a system represent the integrated rates of photosynthesis occurring over a range of concentrations (Brown, 1968; Sestak et al., 1971). A second disadvantage of such a system is that since stomata may take up to one hour to respond to comparatively small changes in ambient CO_2 concentration, steady state stomatal apertures are difficult to obtain (Musgrave & Moss, 1961; Scott, Mendala & Rowley, 1970). Such a system is useful for studying CO_2 compensation points.

(2) Rejection of the 'semiclosed' system. This system is based upon maintaining the CO_2 concentration in the air stream constant and therefore demands accurate control over the volume of CO_2 added to the system. In the case of leaves and small plants which remove small amounts of CO_2 from the system this is difficult to regulate (cf. Koller & Samish, 1964) and demands precise recording flowmeters. It has the advantage of accurate regulation of the ambient CO_2 concentration in the air passing into the chamber.

(3) Adoption of the 'open' system. Such a system is open ended, in which air passes from an air source e.g. the atmosphere outside the laboratory, through the assimilation chamber and IRGA and is exhausted to the atmosphere. Although allowing considerable accuracy to be achieved, it requires accurate measurement of flow rate but, a feature of considerable advantage, is that the system does not have to be leak proof, especially if the air is pushed through. If air is sucked through the system CO_2 from the ambient air in the analysis room, which may be at a different concentration from that of the source air for the assimilation chamber, may be drawn into the system and introduce a source of error. However, by pushing air through the system a slight positive pressure results which prevents the inward diffusion of CO_2 whilst any slight leakage from the system will be of little consequence. The open system also operates close to atmospheric pressure so no concentration of CO_2 as a result of increased pressure in the system will result.

The gas handling system is designed to convey air from its source, through the air conditioning elements, through the assimilation chamber and ultimately to the IRGA. The major components of the system used in the present study were as follows:

(a) tubing: although metal or glass tubing is preferable to limit diffusion of CO_2 through tube walls (cf. Brown, 1968), thick walled nylon tubing was used in the present system for reasons of availability and flexibility. Diffusion of CO_2 through nylon is a possible source of error although thick walled tubing should minimize this (cf. Sestak *et al.*, 1971).

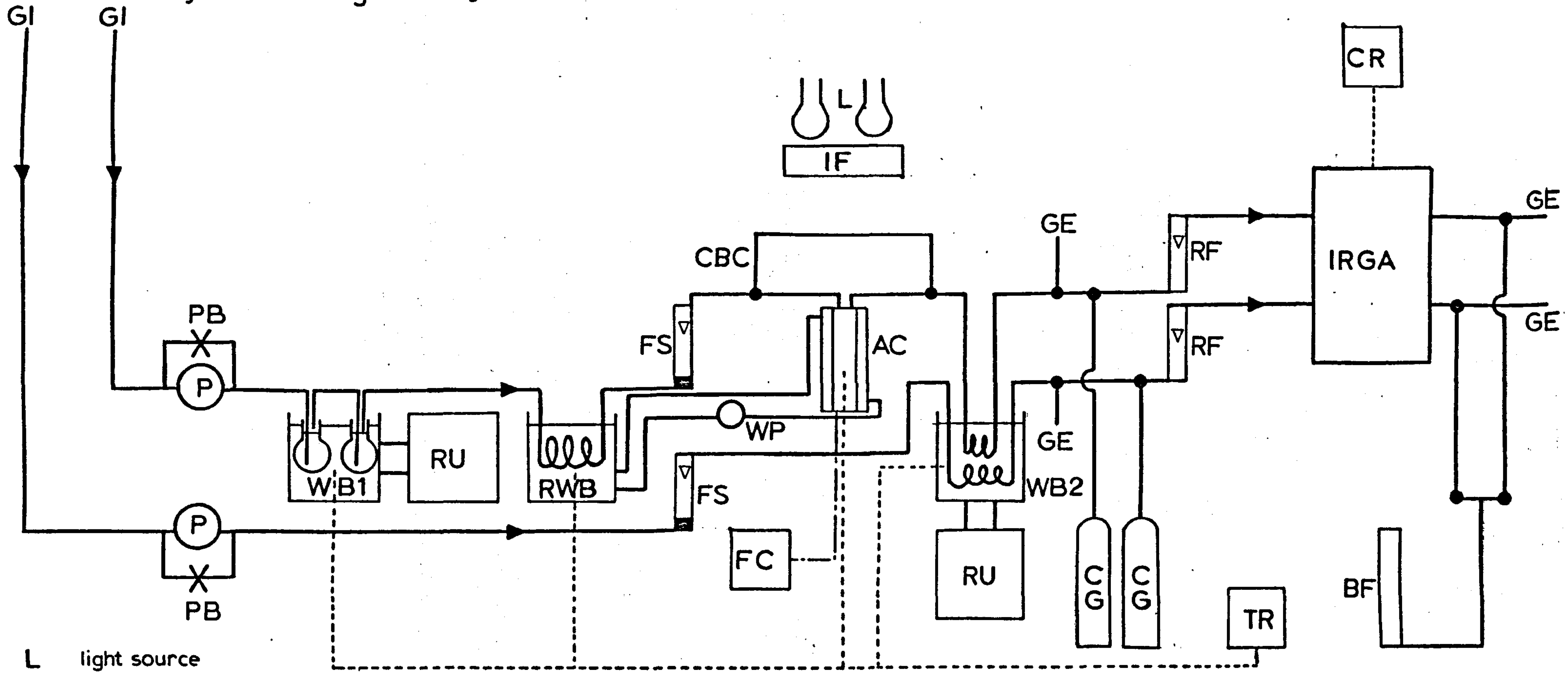
(b) pumps: two membrane pumps (Capex Mk II, Charles Austen Pumps Ltd., Sussex) were employed, one in the reference and one in the sample line. They were positioned between the air intake (mounted at a height of c. 20m. above ground level) and the beginning of the air conditioning system (Figure 66). The maximum rate of flow which could be generated was 5 l min^{-1}.

(c) flow regulators and flow meters: air flow rate was regulated using GAP flowstat regulators (G.A. Platon Ltd., Hants.). These were placed between the end of the air conditioning system and the assimilation chamber. Air flow was controlled by a needle valve attached to a flow meter (0-2 l min^{-1})so that despite a fluctuating inlet pressure as a result of the pump action, the output was constant. Two rotameters (Rotameter Manufacturing Co., Surrey) attached to the IRGA were used to monitor the flow rate of gas into the analyser. The 1:1 correspondence existing between the rotameters and the GAP flow meters resulted in the former being left fully open and flow rate being controlled entirely by the latter. The precision of the flow meters as indicators of the rate of air flow through the system was initially checked with the aid of a soap bubble flow meter attached to the IRGA exhaust portals (cf. Barr, 1934).

Since the IRGA is a pressure sensitive device, pressure regulators should ideally be placed at the entry of the gas into the IRGA to prevent pressure differentials developing between the air lines (Sestak et al., 1971). This is of less significance when air is exhausted to the atmosphere since the system runs virtually at atmospheric pressure. The lack of any appreciable drop in flow rate between the two sets of flow meters suggested leakages between these two points were negligible.

(d) drying columns: initially, gas entering the IRGA was dried after leaving the assimilation chamber. Two drying columns in each line were

Figure 66 Diagram of gas handling circuit used for the measurement of Carbon Dioxide exchange by Infra-red gas analysis.



L	light source
GI	gas inlet
GE	gas exhaust
P	membrane pump
PB	pump by-pass
WP	water pump
WB1	'dew point temperature' water bath
WB2	temperature equalisation water bath
RWB	refrigerated water bath
RU	refrigeration unit
BF	soap bubble flow meter
FS	'Flostat' flow regulator/meter
AC	assimilation chamber
CBC	chamber by-pass
IF	infra-red water filter
CG	calibration gas
RF	'Rotameter' flow meter
IRGA	Infra-red gas analyser
CR	chart recorder
TR	temperature recorder
FC	fan speed control

employed to give a combination of zinc chloride desiccant which has a high efficiency but low capacity for removing water vapour, and aluminium oxide which has a high capacity but low efficiency (Sestak _et al._, 1971). This was found to have an adverse effect on response time and, since the IRGA was fitted with optical filters for removing the absorption bands of water vapour, the drying columns were removed.

(e) _dust filters_: following the rotameters but preceeding the analysis tubes, dust filters were fitted to prevent dust entering the analysis tubes and introducing a source of error.

The Air conditioning system (Figure 66)

The features under control in most systems are temperature, humidity and CO_2 concentration, the object being to quantify and maintain constant or vary in a predefined manner, these parameters of the plant environment. A simple system was developed in the present study to control humidity and temperature. The CO_2 concentration of the air varied from 315-325μl l^{-1}. In terms of the comparative experiments performed, minor fluctuations in CO_2 concentration may have been relatively unimportant.

(a) _humidity control_: control of humidity in the sample air is comparatively complex since transpiration raises air humidity. This often necessitates drying the air before it passes into the IRGA to prevent condensation of water vapour in the gas lines or analysis tubes of the analyser, especially if these are running at a lower temperature than the chamber. Reduction of humidity prior to the air passing into the chamber may enhance transpiration, thus, to change the humidity from one predetermined level to another may require compensation in view of the contribution the plant is making toward the ambient humidity. This requires comparatively sophisticated sensing and regulatory apparatus. The system employed in the present study attempted to produce a constant and repeatable level of humidity in the air before it passed into the chamber.

Air was passed through two flasks containing distilled water immersed in a refrigerated water bath (WB 1) capable of producing temperatures down to 1-2°C. The bath temperature determined the dew point temperature of the air passing through the water, after which the air should have been saturated, i.e. have a relative humidity of 100%. The air was then passed through a second refrigerated water bath (RWB) running at a temperature to give the required chamber temperature. As the air warmed the ratio of the saturation vapour pressure at the lower temperature to that at the chamber air temperature determined the relative humidity of the air (Bierhuizen & Slatyer, 1964). By adjusting the temperature of the baths a range of humidities could be generated. This method has been employed by a number of workers e.g. Gaastra (1959), Bierhuizen & Slatyer (1964), Grace & Woolhouse (1970), Nevins & Loomis (1970), Samish & Pallas (1973), Beadle et al. (1974).

Although effective, the method was cumbersome, requiring at least two refrigerated water baths with accurate temperature control. Although it provided effective long term control, Jarvis & Slatyer (1966) rejected the method on the grounds that it takes a long period to establish new equilibrium vapour pressures and problems exist in obtaining low relative humidities.

In agreement with the findings of Grace & Woolhouse (1970), humidity control in practice was difficult and not sufficiently accurate to quantify transpirational losses by the measurement of relative humidity before and after the passage of the air through the chamber. Problems also arose in the condensation of water vapour at high humidities on 'cold spots' of the chamber walls and gas lines.

(b) temperature control: leaf temperature rather than air temperature is of primary importance to the photosynthetic process, but by regulating the exchange of heat between leaf and air, by controlling air temperature, attempts were made to control leaf temperature. Three methods exist by which air temperature may be controlled (Bjorkman & Holmgren, 1963; Samish & Pallas, 1973):

(1) control of the air temperature before the air enters the chamber;
(2) control of air temperature within the chamber;
(3) a combination of 1 and 2.

Option 3 was adopted in the present study. Air, after passing through the humidifier, passed through a coil of tubing lying in an accurately controlled water bath (RWB) (Grant Instruments Ltd., Cambridge). If a large difference existed between the desired chamber temperature and the ambient room temperature, the water bath temperature could be manually adjusted to compensate for heat loss or gain by air in its passage from the bath to the chamber. Stability of air temperature was enhanced by circulating the air over heat exchangers within the chamber, i.e. the chamber walls. The walls were temperature regulated by circulation of fluid from the water bath in which air temperature was raised after leaving the humidifier (RWB) (cf. Gaastra, 1959; Bierhuizen & Slatyer, 1964; Hardwick, Lumb & Woolhouse, 1966; Jarvis & Slatyer, 1966; Nevins & Loomis, 1970). The low heat transfer coefficient of 'Perspex' suggested that a water jacket alone would have probably been insufficient to provide accurate temperature control. Chamber air temperature was monitored using shielded thermistors connected to a nine channel portable recorder (Grant Instruments (Developments) Ltd., Toft, Cambridge) sampling each thermistor at 15 min. intervals. Stability was

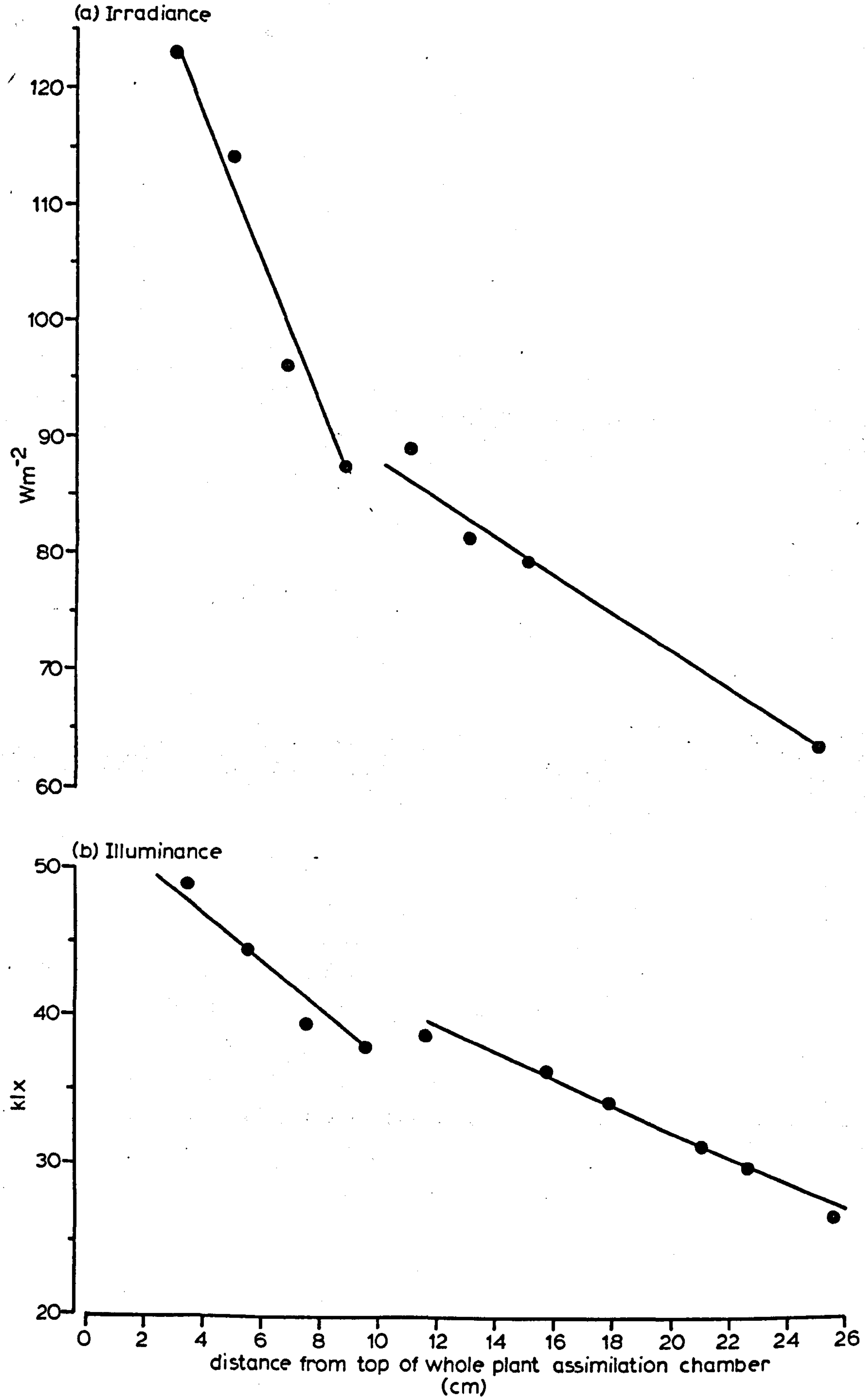


Figure 67 The Irradiance/Illuminance received by a leaf in relation to its position in the whole plant assimilation chamber.

to within ± 1C°, and, following the establishment of an equilibrium temperature, little drift occurred. Leaf temperature was not measured but was assumed to be proportional to the air temperature of the chamber.

(c) radiation source: this was provided by two 400W mercury vapour fluorescent lights (MBFR/U, Philips, Holland) mounted in a static light housing to give a definable energy input at leaf level. The infra-red components of the irradiation were removed by having a 5cm. thickness of circulating water interposed between the light source and the assimilation chamber (cf. Gaastra, 1959; VanOorschot & Belksma, 1961; Hardwick, Lumb & Woolhouse, 1966; Scott et al., 1970; Nevins & Loomis, 1970; Beadle et al., 1974). Gaastra (1959) observed that an 8cm. water filter reduced the infra-red components of a high pressure mercury vapour radiation source, resulting in only 6% of the radiation on the distal side of the filter being at wavelengths greater than 700nm.

The inner sides of the light housing were lined with aluminium foil to increase the lateral radiation intensity. Measurements of illuminance and irradiance impinging upon a leaf at a specified distance from the light source were made with a photometer (E.E.L., Halstead, Essex) and spectroradiometer (Instrumentation Specialities Co. Inc., USA) respectively. Determinations were made within the large chamber to compensate for absorbance by the chamber materials.

The illuminance and irradiance decrease rapidly with distance from the top of the plant assimilation chamber (Figure 67) but at a point between 9cm. and 12cm. from the chamber top an inflexion in the graphs occurs and the decline in radiation flux density decreases, probably as a result of the foil lining of the light housing. Although such a lining appears to have a beneficial effect in as much as the flux density at the bottom of the chamber is increased e.g. by c. 25% at a distance of

12cm. from the chamber top, it presents problems in defining the energy a plant receives within the chamber. It is also likely that, with such a substantial drop in flux density, light may become a limiting factor at lower levels within the chamber. The radiation flux at the midpoint of the chamber when allowance has been made for plant pot size is c. 38.5klx (≡ c. 87Wm^{-2}).

For single leaf studies, the flux density received by the leaf is more readily defined. The chamber was mounted on a platform which could be raised or lowered but throughout the study was maintained at a single height and at a constant flux density. However, for the shoots of C. vulgaris, as for the whole plant studies, the different planes of exposure and mutual shading of the leaves, make it difficult to accurately define the radiation received by the plant. Although the maximum radiation likely to be received by the shoot lies near the light compensation point of C. vulgaris (cf. Grace & Woolhouse, 1970), the photochemical efficiency of a shoot may be increased if radiation is reflected and diffused from all chamber surfaces (Sestak et al., 1971). By lining the light housing walls with reflective foil, the radiation balance may have been improved, although the predominant radiation supply was from above the plant.

Gaastra (1959) noted that photometric units e.g. lux, are not suitable for defining the light energy used in the photochemical processes of photosynthesis. Conversion factors have been given to convert such measurements into energy equivalents e.g. Bierhuizen & Slatyer (1964), Nevins & Loomis (1970). Alternatively, photometers such as silicon cells have been directly calibrated in energy units e.g. Hardwick, Lumb & Woolhouse (1966), or both irradiance and illuminance have been determined e.g. Catsky & Ticha (1974). Gaastra (1959) discussed the relationship between energy and photometric units for various light sources and gave a value of 3.48 x 10^{-7} W cm^{-2} per lux for a 400W mercury vapour fluorescent light source. The present system (800W light source) yielded 2.51 x 10 10^{-7} W cm^{-2} per lux. The light intensity of the present system is comparatively low compared to that used by other workers, e.g. 260 Wm^{-2} (Scott et al., 1970), 360 Wm^{-2} (Grace & Woolhouse, 1970), 675 Wm^{-2} (and an illuminance of 150 klx) (Catsky & Ticha, 1974). It is evident that the maximum of 123 Wm^{-2} and 49klx may be considerably improved and may have limited photosynthesis, especially if the plants had high light compensation points.

The IRGA was employed, throughout the study, in the differential mode in which the reference and sample air lines were compared simultaneously, so giving the direct difference in CO_2 concentration between the air streams. Using a range of bottled gases of CO_2 in nitrogen, (supplied by Rank - Hilger Watts Ltd., Kent), the IRGA was calibrated at the commencement of an experiment and at regular intervals over the duration of the experiment. Calibration drift was found to be negligible and the calibration was linear over the range of CO_2 concentrations employed.

(iv) Errors associated with Infra-Red Gas Analysis

There are a number of factors affecting the accuracy of a result obtained from infra-red gas analysis which have been discussed by Brown (1968) and Brown & Rosenberg (1968). These errors may be categorised as follows:

(1) errors associated with the mode of operation;

(2) calibration errors;

(3) errors resulting from fluctuating CO_2 concentrations in the reference air;

(4) errors from fluctuating CO_2 levels within the analyser housing;

(5) errors in determining mean air pressure;

(6) errors in determining flow rates;

(7) errors resulting from pump output, contamination and tubing;

(8) errors arising from pressure differentials between the gas carrying lines;

(9) errors resulting from the passage of air over the leaf.

When the IRGA is employed in the differential mode one value with one error is obtained representing CO_2 exchange by the plant, in contrast to the additive errors associated with the same value when reference and sample air are sequentially sampled. This error is associated primarily with analyser error. However, the precision of the result depends upon the precision of the calibration gases. The tolerance of the gases used in the present study was $\pm 5\%$. When absolute determinations are made the associated error is the same as that of the gases (Legg & Parkinson, 1968). When the IRGA was optically suppressed to give a full scale deflection of $100\mu l\ l^{-1}$, with the lower and upper limits defined by gases of $250\mu l\ l^{-1}$ and $350\mu l\ l^{-1}$ CO_2 concentrations the maximum error was $30\mu l\ l^{-1}$ (i.e. $237.5\mu l\ l^{-1}$ to $367.5\mu l\ l^{-1}$ for a gas of $\pm$ 5% tolerance), representing a 30% error. Legg & Parkinson (1968) argued that the use of bottled gases is inaccurate, and suggested alternative methods of calibration. Brown (1968) also noted that CO_2 concentrations of bottled gases may alter when the pressure of the gas in the bottle has fallen by one third of its original value.

Bottled gases may be employed to produce the reference gas. Alternatively, atmospheric sources may be employed as in the present study provided the intake is positioned away from possible changes in CO_2 concentration. Short term fluctuations can be eliminated by introducing mixing tanks into the air lines (Hesketh & Musgrave, 1962). Fluctuations in the CO_2 concentration of the air within the IRGA housing will affect the radiation reaching the detecting chamber, since part of the radiation pathway is through the air contained within the analyser. The CO_2 concentration within the analyser housing should be maintained constant e.g. by absorbing CO_2 chemically or flushing the analyser with nitrogen, especially during the course of a single experiment.

A pressure difference of 10 Pa (= 0.1mb) between the analysis tubes has been found to represent a CO_2 difference of $0.05 \mu l\ l^{-1}$ (Saugier & Ripley, 1974). These workers maintained an absolute pressure in the analysis tubes of 196.03 to 490.33 Pa greater than atmospheric pressure, which they considered to have a negligible effect on the measurement of CO_2 differences. Pumping air through the circuitry will generate a pressure which should be evaluated for these reasons but Brown (1968) noted that in calculation procedures, mean local air pressure could be used as, in the open system, chamber pressure is unlikely to deviate greatly from this value. Any pressure differentials arising between the gas carrying lines may be corrected by a manometer connecting the two air lines by which pressure differentials may be equilibrated.

It is of importance that no leakage in the pump occurs as this may affect the CO_2 concentration in the gas circuit. This is especially important when the pump lies between the assimilation chamber and the IRGA. Condensation in the air lines may also affect the CO_2 concentration of the air flowing through the tubes, since water acts as a variable sink/source for CO_2 (Brown, 1968). Similarly, unless the IRGA is fitted with appropriate filters, water vapour in the air streams may result in erroneous measurements, necessitating drying of the gas before passing to the IRGA.

Air flow rate may also have a significant effect on CO_2 uptake.

Hesketh & Musgrave (1962) found a linear relationship between Net Assimilation Rate and wind speed over the range 0.13 to $0.2 m s^{-1}$, but at a speed of $0.08 m s^{-1}$ NAR had decreased to 75-90% of the rate at $0.16 m s^{-1}$. Although low flow rates may have an adverse effect on CO_2 uptake, flow rate must be sufficiently low to provide measurable CO_2 differentials. Koller & Samish (1964) argued that accuracy demands large differences in CO_2 concentration between incoming and outgoing air, for example, using an IRGA with an accuracy of $\pm 1 \mu l\ l^{-1}$, to reduce the error to $\pm$ 2% a CO_2 differential of $100 \mu l\ l^{-1}$ would be required. But the plant changes the CO_2 concentration of the air which may affect the plant response to CO_2 concentration, consequently differentials need to be reduced resulting in reduced accuracy. It is necessary to obtain a compromise situation but unless flow rate fluctuations can be shown not to influence the rate of photosynthesis, flow rate should be constant and precisely measured, since it plays an essential role in the calculation of CO_2 uptake. The error involved in measuring flow rate may be greater than that of determining CO_2 concentration and therefore may influence the precision of the result.

When photosynthesis of a single leaf is being considered, a CO_2 concentration gradient exists both perpendicularly to the leaf surface and in the direction of air flow. Gaastra (1959) described the effect and the corrections necessary to estimate the CO_2 concentration of the air at the midpoint of the leaf. Where CO_2 may be limiting, the effective CO_2 concentration over the leaf is the mean of the inlet and outlet concentrations. The situation is of some complexity and Gaastra (1959) concluded that rate of air supply and leaf position have a significant effect on the result obtained and may be the possible cause of conflicting data.

There are various minor errors such as zero and sensitivity drift of the IRGA, the accuracy with which the chart or meter may be read and the measurement of leaf area or leaf dry weight. It would appear that the sources of error may result in comparatively meaningless results, Hesketh & Musgrave (1962) for example, finding an error of 13%. However, the accuracy of a result, allowing comparability, may depend on short term stability of the system even if precision is not guaranteed.

(v) Calculation of Gaseous Exchange Rates:

Rates of CO_2 exchange are commonly expressed as mgCO_2 dm^{-2} (leaf area, one surface), h^{-1} or mgCO_2 g^{-1} (dry weight) h^{-1}. Larcher (1969) suggested micrograms (μg) may be used instead of milligrams (mg). In the present investigation, CO_2 exchange is expressed in μgCO_2 g^{-1} (dry weight) s^{-1}.

Gas exchange rates have been expressed in volumetric units e.g. Gaastra (1959):

$$F = \frac{J(C_o - C_e).10^{-2}}{A_1} \qquad (21)$$

where: C_o = CO_2 content of inlet air (%);

C_e = CO_2 content of outlet air (%);

J = rate of air supply (cm^3 s^{-1});

A_1 = leaf area (cm^2);

F = rate of photosynthesis (cm^3 CO_2 cm^{-2} s^{-1}).

More commonly they are expressed in terms of mass i.e. g cm^{-2} s^{-1} (Larcher, 1969). It is desirable to take into account temperature and pressure at the time of measurement i.e. (Sestak et al., 1971):

$$F_n = \frac{\Delta C}{A_1} \times \frac{J \times 44}{22{,}414} \times \frac{273}{T^o} \times \frac{P^{mb}}{1013.25} \; (\text{g cm}^{-2}\ \text{s}^{-1}) \qquad (22)$$

where: F_n = net photosynthesis (g cm^{-2} s^{-1});

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

A_1 = leaf area (cm^2);

ΔC = difference in CO_2 concentration of air stream before and after passing through the assimilation chamber measured at the same temperature and pressure ($\mu l\ l^{-1}$);

T^o = temperature (^oK) at which the flow meter is calibrated and at the time of observation;

P^{mb} = pressure (m.b.) at time of observation, the calibration of the flow meter having been corrected to 1013.25mb.

The function (44/22,414) converts units of volume (obtained from the IRGA) into units of mass. Since the gas laws are temperature and pressure dependent it is necessary to correct for deviations from STP (i.e. 273°K, 1013.25mb) by the function:

$$(273/T^o) \text{ x } (P^{mb}/1013.25) \qquad (23)$$

These functions are applicable if interference from water vapour is suppressed (Sestak *et al.*, 1971). If the water vapour pressures diverge greatly between the reference and sample air corrections must be made. If rotameters are employed further corrections must be employed to take into account the conditions under which the flow meters were calibrated (e.g. Baker & Musgrave, 1964). Equation 22 may be rewritten (Sestak *et al.*, 1971):

$$F_n = \frac{\Delta C}{A_1} \text{ x } \frac{J \text{ x } 44}{22,414} \text{ x } \frac{273}{T^o} \text{ x } \frac{P^{mb}}{1013.25} \text{ x} \sqrt{\frac{T^o}{T_1^o}} \text{ x} \sqrt{\frac{P_1^{mb}}{P^{mb}}} \qquad (24)$$

where: P^{mb} = pressure at time of observation (mb);

P_1^{mb} = pressure at which rotameter is calibrated (mb);

T^o = temperature of flow meter at time of observation (assumed to be the temperature of air flowing through it);

T_1^o = temperature at which the flow meter is calibrated.

This makes the assumption that the air within the chamber is being stirred. This latter equation (Equation 24) is applicable to open systems and has been used in the present study.

(c) Methods

The investigation considered the response of net photosynthesis (F_n) of healthy, undamaged shoots of C. vulgaris to induced water deficits, in an attempt to ascertain whether seasonal variation in the response occurred.

Current and previous season's shoots of C. vulgaris were collected from Cannock Chase, stood in water in a sealed polythene tube and stored in the dark at 3°C for 24 hours prior to utilization.[1] 24 hours before the determination of CO_2 uptake the bottles containing the shoots were placed in an incubator at 20 ± 1°C, to allow the shoots to become fully saturated. Two to three hours before use, the shoots were subjected to a light intensity of about 15klx to induce stomatal opening. Upon removal from the saturating chambers, the shoots were blotted dry, weighed on a 500mg. torsion balance, and mounted in a nylon capillary tube containing distilled water from a syringe to maintain a high shoot RWC while a stable rate of CO_2 uptake was attained for the shoot at maximum RWC (cf. Pieters & Zima, 1975). CO_2 uptake was determined using the infra-red gas analysis system described above (Section IVA, b) and the fast response chamber (Figure 65). Experiments were conducted at a temperature of 20 ± 1°C and relative humidity of 75% (nominal).[1] Following the attainment of a stable rate of CO_2 uptake, the shoot was

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[1] Sources of error associated with shoot storage and measurement of CO_2 uptake at a standard temperature are discussed in Appendix C.

removed from its water supply, weighed, and replaced in the light housing, at a level approximating that of the chamber but external to it so as to receive a comparable light intensity (c. 40klx), thereby reducing the RWC prior to the next CO_2 uptake determination. This process was repeated until measurable CO_2 uptake ceased. A set of determinations on a single shoot took between 2 and 3 hours. Following the final determination, the shoots were oven dried at 85°C for 48 hours, after which total shoot dry weight was determined to enable the calculation of shoot RWC. The shoot was then partitioned into photosynthetic tissues (i.e. green parts of the shoot) and non-photosynthetic stems, the former being weighed on an analytical balance. Rates of net photosynthesis have been expressed as $\mu g CO_2\ g^{-1}$ (dry weight green tissue) s^{-1}. The difficulty of determining leaf area in C. vulgaris necessitated the use of dry weight as a basis for expression (cf. Grace & Woolhouse, 1970).

Polynomial regression analysis (Appendix A 2(b)) was performed for each paired set of results, i.e. CO_2 uptake at a particular RWC, (the RWC being that of the shoot immediately after removal from the chamber and the CO_2 uptake the rate immediately prior to shoot removal). Each analysis consisted of the combined results of 12-15 replicate shoots for a particular sampling date. The number of determinations per shoot depended upon the rapidity with which each determination was made and the rate at which CO_2 exchange ceased to occur. From each regression (for November 1975 to July 1976) the following were calculated:

(1) the RWC at which the maximum rate of photosynthesis occurred (RWC optimum);

(2) the estimated rate of photosynthesis of shoots at the field RWC's existing for the particular sampling date, calculated from the

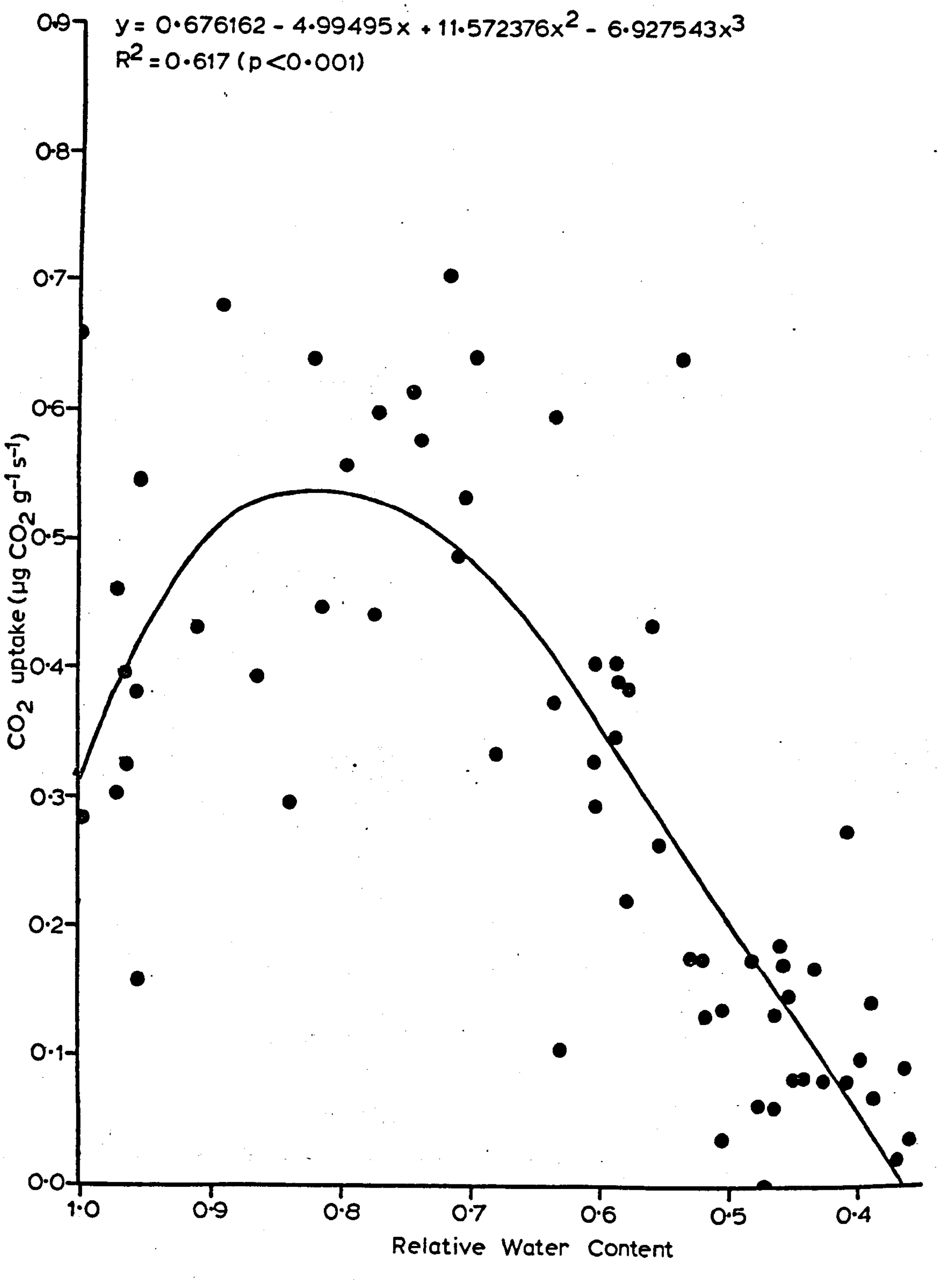

Figure 68 Relationship between shoot Relative Water Content and Carbon Dioxide uptake, C. vulgaris, (Cannock Chase, 10.12.75).

regression relationships[1] (F_n' 1, F_n' 3 and F_n' 6 for shoots treated 1, 3 and 6 months before sampling);

(3) the maximal rate of photosynthesis (F_n maximum).

(d) Results

(i) The effect of water stress on the rate of net photosynthesis of *C. vulgaris*

The monthly regression relationships between CO_2 uptake and RWC (Appendix 8 Table 7) account for a significant proportion of the variation in the data, ranging from 33.9% (19.11.75) to 70.4% (22.06.76). An example of such a relationship (Figure 68), in which a third order polynomial has been derived accounting for 61.7% of the variation in the data, indicates an optimal RWC of 0.82 at which F_n is maximal. At an RWC of 1.0, CO_2 uptake is 54% of the maximum rate attained and with increasing water stress below an RWC of 0.82 CO_2 uptake declines to zero at an RWC of 0.36. The F_n maximum obtained from the regression is 0.542μg g^{-1} s^{-1} but the highest individual rate recorded for the regression is 0.703μg g^{-1} s^{-1} which is 18.6% above the mean maximum, and occurs at a considerably lower RWC (i.e. 0.7155).

(ii) Seasonal variation in the maximum photosynthetic potential of *C. vulgaris* shoots (F_n maximum) (Figure 69)

Seasonal changes in F_n maximum indicate that, with the exception of the high value in January 1976 of 0.982μg g^{-1} s^{-1}, the mean rate of F_n

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[1] The estimated rate of photosynthesis for shoots in the field (F_n') assumes that all shoots are similar in their photosynthetic potential to those employed for experimental purposes. The value for F_n does not take into account any reduction in photosynthetic capacity arising from mechanical damage, and physiological changes arising from long term stresses resulting from the treatments applied.

Table 21 Multiple Regression Analyses relating seasonal variation in the maximum rate of net photosynthesis to Environmental and Physiological variables, *C. vulgaris* (Cannock Chase)

Step	Variable	Significance to enter (p)	Multiple R	Multiple R^2	R^2 Change	Simple R	Overall Significance (p)
(a) X_1	Stem Temperature (mean for 7 days prior to sampling)	n.s.	0.6037	0.3644	0.3644	0.6037	n.s.
X_2	Accumulated Temperature 7.2°C (7 days prior to sampling)	n.s.	0.7289	0.5313	0.1669	0.5739	n.s.
X_3	Vapour Pressure (0900h on day of sampling)	n.s.	0.7513	0.5644	0.0331	0.5690	n.s.
X_4	Daylength	n.s.	0.7535	0.5677	0.0033	0.4753	n.s.
(b) X_1	CRWC	< 0.01	0.8185	0.6699	0.6699	0.8185	< 0.01
X_2	Time to SCL	< 0.05	0.9155	0.8382	0.1683	0.5235	< 0.01
X_3	RWC - control	n.s.	0.9638	0.9288	0.0906	-0.2458	< 0.01
X_4	ψ_x - control	n.s.	0.9808	0.9619	0.0331	0.3398	< 0.01
X_5	browning - control	n.s.	0.9849	0.9702	0.0083	0.4195	< 0.05
X_6	PMC - control	n.s.	0.9928	0.9856	0.0154	0.5089	< 0.05
X_7	SCL	n.s.	0.9993	0.9886	0.0030	0.5690	n.s.
Regression equation:		$\hat{Y} = 0.3175 + 2.2909\, X_1$					< 0.01

(c)	X_1	CRWC	< 0.01	0.8185	0.6699	0.6699	0.8185	< 0.01
	X_2	Time to SCL	< 0.05	0.9155	0.8382	0.1683	0.5253	< 0.01
	X_3	RWC - control	n.s.	0.9638	0.9288	0.0906	-0.2458	< 0.01
	X_4	ψ_x - control	n.s.	0.9808	0.9619	0.0331	0.3398	< 0.01
	X_5	browning - control	n.s.	0.9849	0.9702	0.0083	0.4195	< 0.05
	X_6	PMC - control	n.s.	0.9928	0.9856	0.0154	0.5089	< 0.05
	X_7	Vapour Pressure	n.s.	0.9943	0.9986	0.0130	0.5690	n.s.
	Regression equation:		$\hat{Y} = -0.9261 + 2.1242\ X_1 + 0.6916\ X_2$					< 0.01

(a - environmental variables; b - physiological variables; c - combined variables)

($\hat{Y} = F_n$ max, ($\mu g\ g^{-1}\ s^{-1}$)

between November 1975 and April 1976 (excluding the January data) is relatively constant (i.e. 0.594 ± 0.015μg g^{-1} s^{-1}). In May 1976 there is a virtual doubling in rate from 0.583μg g^{-1} s^{-1} in April to 1.087μg g^{-1} s^{-1} in May. This is followed by a gradual decline in June and July to a value of 0.828μg g^{-1} s^{-1}. F_n maximum is significantly correlated with CRWC ($r = 0.8185$, $p < 0.01$) and RWC at stomatal closure (SCL) ($r = -0.6788$, $p < 0.05$), and shows trends with stem temperature over the 14 days prior to sampling ($r = 0.6037$, n.s.), accumulated temperature (base 7.2°C) over the same period ($r = 0.5739$, n.s.) and vapour pressure at the time of sampling ($r = 0.569$, n.s.).

Multiple Regression Analysis

Of the four environmental parameters entered into the analysis of variation in F_n maximum (Table 21a) none makes a significant contribution towards explaining the variance in the data, although together they account for 56.7% of the total variance.

When the physiological variables are entered (Table 21b) the total explained variance is 98.8%, but only CRWC and time to stomatal closure (SCL) make a significant contribution towards explaining variation in F_n maximum, accounting for 83.8% of the total variance, although all but the last variable entered (SCL) yield a significant regression equation (at $p = 0.05$). Combining environmental and physiological variables into a single analysis (Table 21c) does not affect the significant contribution made by CRWC and time to stomatal closure, and the computed F ratios are only sufficient to permit one environmental variable (vapour pressure on the date of sampling) to be entered into the analysis, although it makes a small and insignificant contribution to the regression.

The unexplained, and possibly spurious, nature of the contribution made by time to stomatal closure has led to the presentation of two regression equations in which variation in the data is described by CRWC

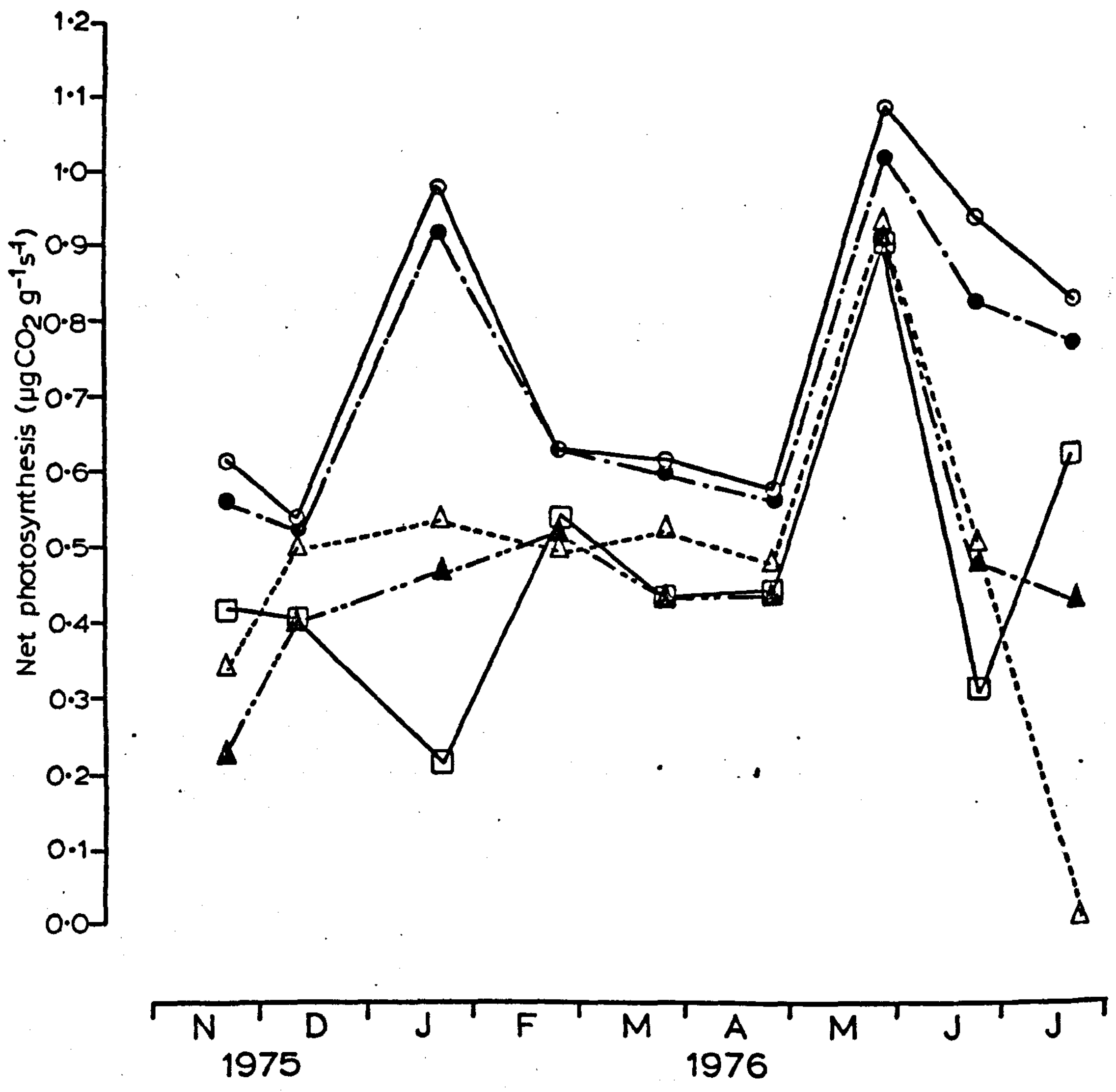


Figure 69 Seasonal variation in the rate of net photosynthesis of C. vulgaris shoots, (measured at 20±1°C, 75% relative humidity and c. 45 klx illuminance), and the calculated effect of field RWC's on net photosynthesis, (Cannock Chase).

○——○ maximum rate of net photosynthesis attained under standard measuring conditions.

●-·—·-● calculated rate of net photosynthesis for control shoot RWC

△-------△ calculated rate of net photosynthesis for the RWC, at time of sampling, of shoots treated 1 month previously.

▲··—··▲ calculated rate of net photosynthesis for the RWC, at time of sampling, of shoots treated 3 months previously.

□——□ calculated rate of net photosynthesis for the RWC, at time of sampling, of shoots treated 6 months previously.

alone (Table 21b) (Figure 73a) which accounts for 66.9% of the total variance and by CRWC plus time to stomatal closure, accounting for 83.8% of the total variance (Table 21c).

(iii) Seasonal variation in the calculated rate of F_n (F_n') indicating effects of the measured field RWC on potential F_n (Figure 69)

Seasonal variation in the control value (F_n' 0) closely parallels the variation in F_n maximum ($r = 0.991$, $p < 0.001$), but seasonal variation in the estimated rates of F_n of treated plants (F_n' 1, F_n' 3 and F_n' 6) is a little more erratic; only the variation in F_n' 3 is significantly correlated with F_n maximum ($r = 0.6786$, $p < 0.05$). Similarly, the inter-treatment correlations are only significant when F_n' 3 is compared with the remainder (i.e. $r = 0.7036$, $p < 0.05$; $r = 0.7277$, $p < 0.05$; $r = 0.726$, $p < 0.05$ for F_n' 0, F_n' 1 and F_n' 6 respectively). (Similar relationships exist for correlations between the RWC's of treatments ($r = 0.669$, $p < 0.05$ and $r = 0.695$, $p < 0.05$ for RWC 1 and RWC 6 respectively) although the control RWC is only significantly correlated with 1 month values ($r = 0.672$, $p < 0.05$)).

Although the respective treatments are responding independently, a general trend is evident in which the rates of F_n' rise in May from low winter values and then decline, although the January 1976 rise is not apparent.

To a certain degree, seasonal variations in F_n are paralleled by similar variations in RWC (Figure 71) but the correlation between RWC and F_n values, for control and trampled plots, is not significant. However, the May rise in F_n is paralleled by a rise in RWC of all treatments, the substantial decrease of F_n' 1 values in July 1976 is accompanied by a marked reduction in RWC 1 and similarly for F_n' 6 and RWC 6 in January 1976. Similarity in response might be expected since

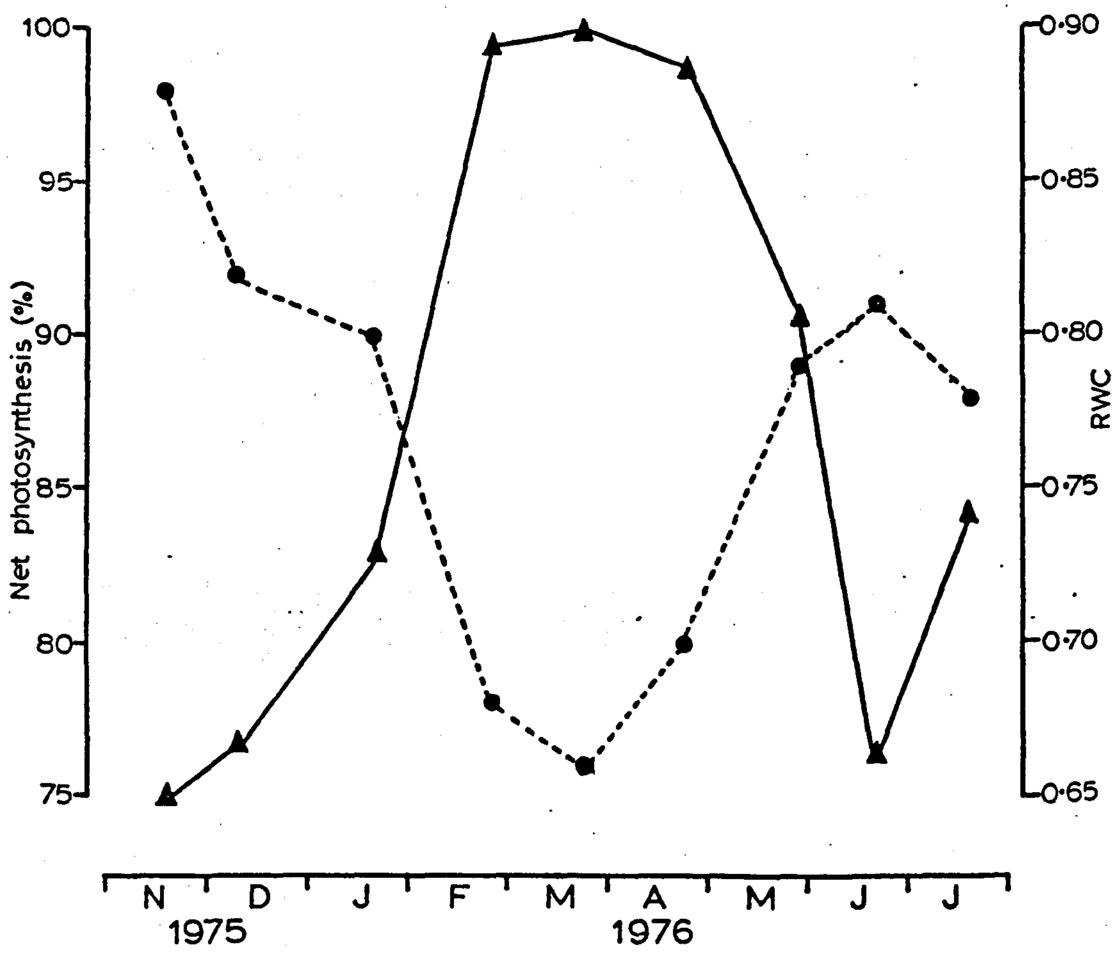


Figure 70 Seasonal variation in the rate of net photosynthesis at a constant RWC (0·65), expressed as a percentage of the maximum rate of net photosynthesis attainable for each sampling date, C. vulgaris, (Cannock Chase).

▲———▲ Percentage rate of net photosynthesis

●-------● Optimal RWC for maximum net photosynthesis

F_n' is directly derived from the RWC data but the absence of any significant correlations between RWC and F_n' over time implies that the relationship between the two parameters is not constant.

The regressions relating CO_2 uptake and RWC have not been statistically analysed to ascertain if they differ significantly, but a comparison of the rate of F_n (expressed as a percentage of the maximum attainable for a particular regression) at a constant RWC (0.65) (Figure 70) indicates that the effect of a given stress depends upon the optimal RWC for maximum CO_2 uptake. For example, the high RWC optimum in November 1975 is accompanied by the lowest percentage of the maximum F_n at a standard RWC, whilst the lowest optimum RWC, in March 1976, is accompanied by the highest percentage. The relationship between these two parameters is significant ($r = -0.942$, $p < 0.001$) and indicates that a particular deficit will have a different effect on F_n depending upon season, thus an RWC of 0.5757 in November 1975 will result in a 43.6% reduction in the rate of F_n whilst the same RWC in March 1976 will only reduce F_n by 7.8%.

Correlations between calculated rates of F_n (F_n') and environmental and physiological variables have not been made since they might be considered to be spurious and an artifact of relationships existing with F_n maximum from which they have been derived.

(iv) <u>RWC parameters associated with F_n measurements</u> (Figure 71)

It is noteable that the RWC of the control shoots from the field is closely correlated with the RWC optimum for F_n maximum ($r = 0.752$, $p < 0.05$). No such correlation exists with any other treatment ($r = 0.3394$, n.s.; $r = 0.4942$, n.s.; $r = 0.5368$, n.s. for 1, 3 and 6 month material respectively). This relationship is reflected in the observation that except for June 1976, the F_n of control shoots lies consistently above 90% of the maximum attainable (mean: 96.8 ± 1.3%)

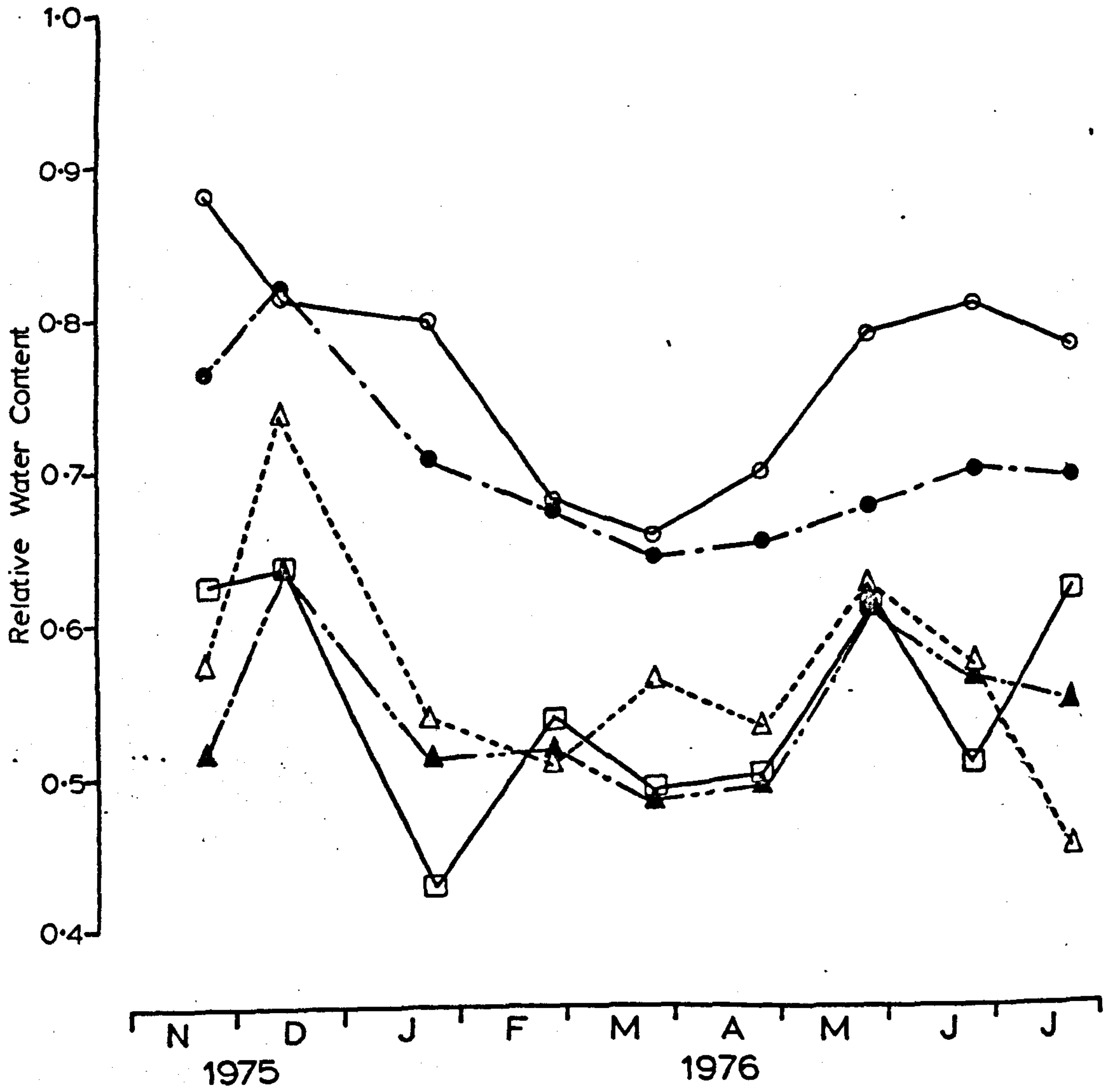


Figure 71 Seasonal variation in the optimum RWC for maximum net photosynthesis in shoots of C. vulgaris, and the effect of trampling on shoot RWC in the field, (Cannock Chase).

○———○ optimum RWC for maximum net photosynthesis.
△- - - - -△ RWC of shoots treated 1 month before sampling.
▲-·—·-▲ RWC of shoots treated 3 months before sampling.
□———□ RWC of shoots treated 6 months before sampling.
●-·—·-● RWC, control shoots.

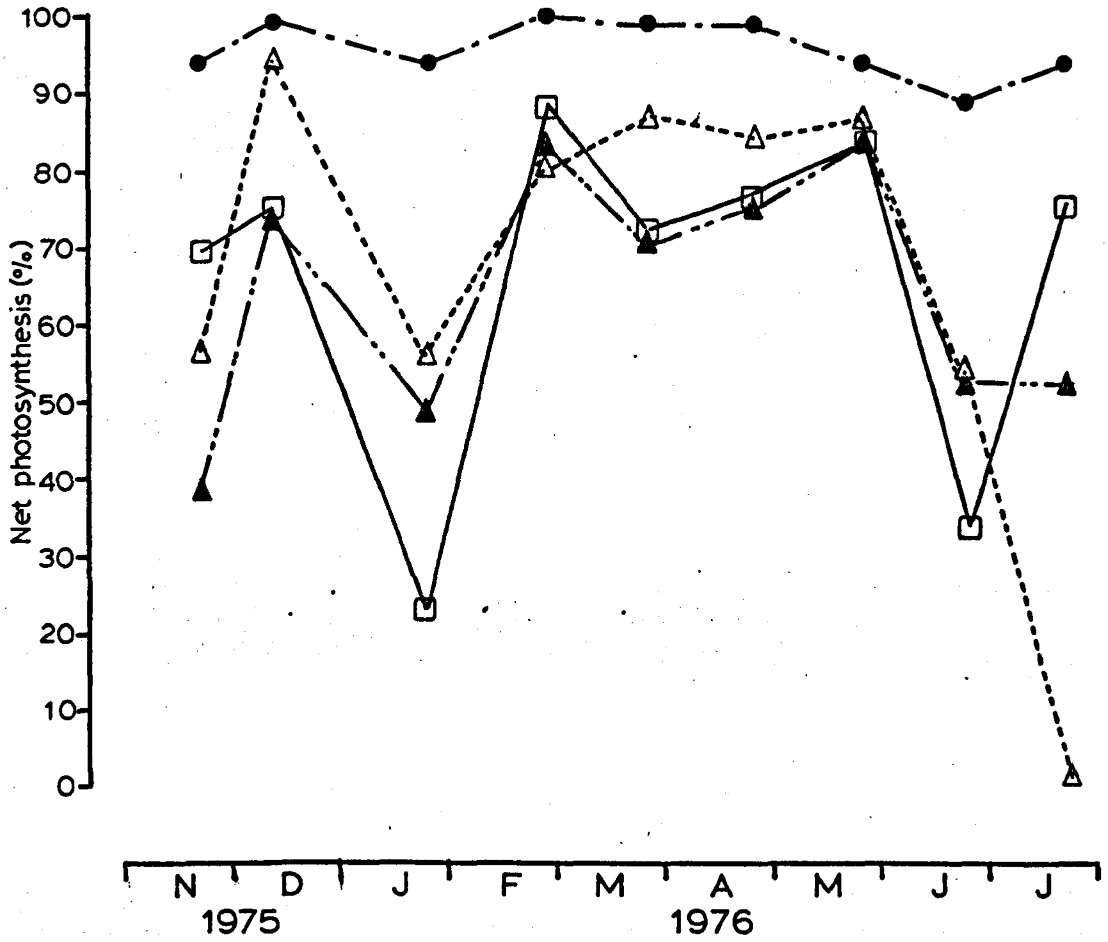


Figure 72 Seasonal variation in the calculated rate of net photosynthesis of trampled shoots of C. vulgaris, expressed as a percentage of the maximum rate attained, under standard conditions, at the time of sampling, (Cannock Chase).

●—·—·—● control shoots
△--------△ shoots treated 1 month before sampling
▲··—··—▲ shoots treated 3 months before sampling
□———□ shoots treated 6 months before sampling

Table 22 Relationship between the RWC at which CO_2 uptake ceases and the RWC at Stomatal Closure, *C. vulgaris* (Cannock Chase)

Date	RWC at which F_n = 0	SCI	SCL	F_n at SCI ($\mu g\ g^{-1}\ s^{-1}$)	F_n at SCL ($\mu g\ g^{-1}\ s^{-1}$)	F_n (SCI) as % of max F_n	F_n (SCL) as % of max F_n
19.11.75	0.42	0.5526	0.3970	0.2986	-0.0606	49.5	-10.1
10.12.75	0.34	0.5794	0.4466	0.3195	0.1365	58.9	25.2
21.01.76	0.35	0.4817	0.3726	0.3731	0.0585	38.0	5.9
25.02.76	0.30	0.5876	0.4042	0.5919	0.3012	94.6	48.2
24.03.76	0.34	0.5676	0.4298	0.5678	0.2947	92.1	47.8
24.04.76	0.30	0.5072	0.3600	0.4547	0.1717	78.0	29.5
27.05.76	0.35	0.5344	0.3258	0.7281	-0.1145	67.0	-10.6
22.06.76	0.38	0.5086	0.3489	0.3143	-0.0404	33.5	- 4.3
19.07.76	0.45	0.6260	0.4266	0.6475	-0.1213	78.2	-14.7

(column 2 - RWC calculated from regressions between RWC and F_n)

(column 5, 6 - F_n calculated from regressions between RWC and F_n)

SCI - Stomatal closure point, 'Intersection method'

SCL - Stomatal closure point, 'Lopushinsky method' F_n - net photosynthesis

(Figure 72), indicating that at the time of sampling the RWC existing is permitting near optimal CO_2 uptake. In contrast, the range of percentage F_n rates for treated shoots is large. Although the seasonal trends in F_n expressed as a percentage of the maximum, are similar for different treatments, with high values in December 1975 and February to May 1976 and low values in November 1975 and January and June 1976, the different treatments are not significantly correlated. In the former period, potential CO_2 uptake is reduced by an average of 15% through the effects of water deficits, in contrast to an average of 52.2% in the latter period.

The calculated RWC at which F_n becomes zero (Table 22) is not significantly correlated with either of the two RWC at stomatal closure parameters ($r = 0.3441$, n.s.; $r = 0.1654$, n.s. for SCI and SCL respectively). All of the RWC values at which F_n is zero lie below SCI, whilst four of the nine values lie below SCL.

The observations on RWC 1 indicated that 61% of the RWC values lay below SCI (Section IIIB, 2(ii)) implying that CO_2 uptake would be severely retarded. However, the RWC at SCI would be sufficient to permit CO_2 uptake at a rate varying from 33.5% to 94.6% of the maximum possible (Table 22). When SCL values are employed, four out of nine values yield negative CO_2 uptake rates indicating RWC's at stomatal closure lower than those at which F_n ceases. Positive rates of F_n occur at certain SCL values e.g. 25.02.76. at which, theoretically, the stomata should be shut.

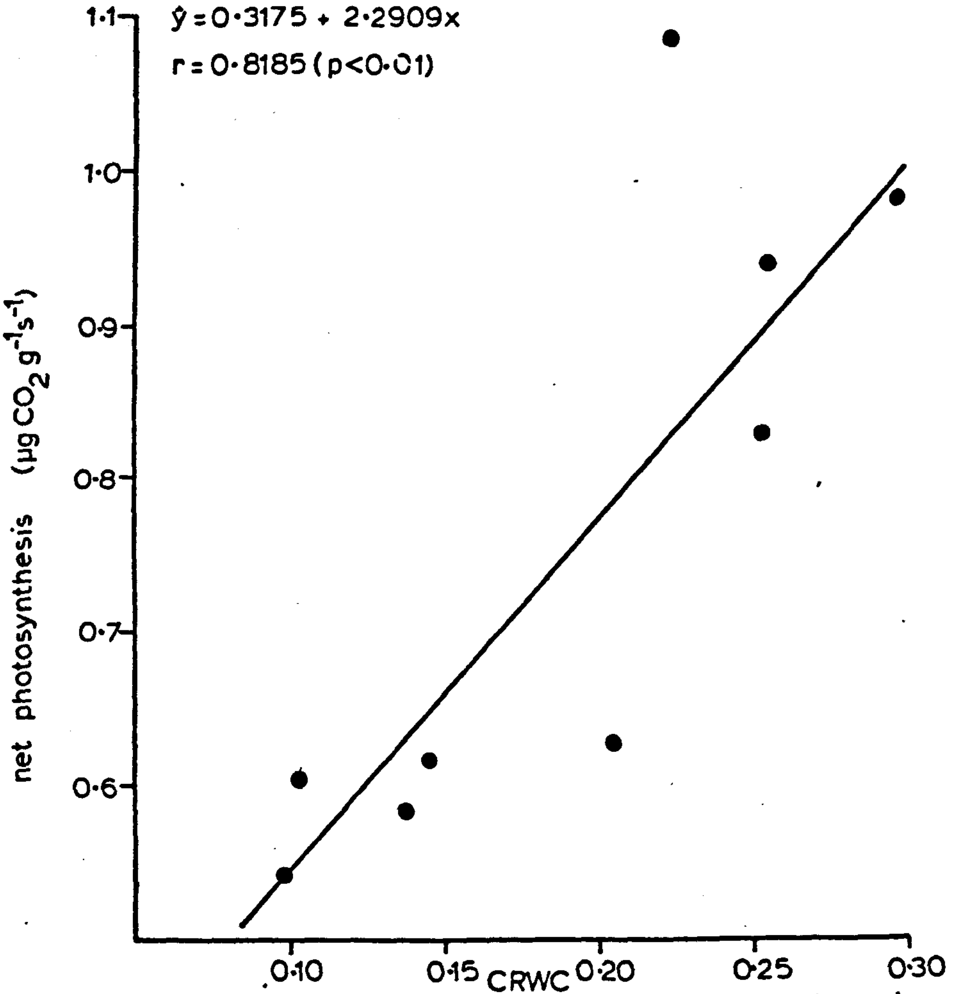
Owing to the nature of the measurements in which the RWC at stomatal closure is predicted, since the stomata were not directly observed, the dilemma is not easily resolved. It is not feasible to ascertain whether the RWC at which F_n is zero and the RWC at stomatal closure are significantly different. The magnitude of the difference with reference to SCI suggests a real difference, although the difference with reference to SCL is not so great and may be due to random variation.

Table 23 Multiple Regression Analyses relating seasonal variation in the optimal RWC for maximum net photosynthesis to Environmental and Physiological variables, *C. vulgaris* (Cannock Chase)

Step	Variable	Significance to enter (p)	Multiple R	Multiple R^2	R^2 change	Simple R	Overall Significance (p)
(a) X_1	Stem Temperature (mean for 7 days prior to sampling)	n.s.	0.2358	0.0559	0.0559	0.2358	n.s.
X_2	Daylength	n.s.	0.6595	0.4349	0.379	-0.2214	n.s.
X_3	Vapour Pressure	n.s.	0.9305	0.8659	0.431	0.0422	< 0.05
X_4	Rainfall (total, 7 days prior to sampling)	n.s.	0.9748	0.9503	0.0844	-0.0216	< 0.01
X_5	Accumulated Temperature 7.2°C (7 days prior to sampling)	n.s.	0.9764	0.9534	0.0031	0.2269	< 0.05
(b) X_1	RWC - control	< 0.05	0.7524	0.5661	0.5661	0.7524	< 0.05
X_2	SCL	n.s.	0.8781	0.7710	0.2049	-0.1271	< 0.05
X_3	ψ_x - control	n.s.	0.9103	0.8286	0.0576	-0.3000	< 0.05
X_4	CRWC	n.s.	0.9652	0.9317	0.1031	0.0061	< 0.05
X_5	browning - control	n.s.	0.9762	0.9530	0.0213	-0.3308	< 0.05

X_6	Time to SCL	n.s.	0.9988	0.9977	0.0447	-0.1768	< 0.01
X_7	PMC - control	n.s.	0.9995	0.9990	0.0013	-0.1251	n.s.
	Regression equation:	$\hat{Y} = 0.07606 + 0.98136\ X_1$					< 0.05
(c) X_1	RWC - control	< 0.05	0.7524	0.5661	0.5661	0.7524	< 0.05
X_2	SCL	n.s.	0.8781	0.7710	0.2049	-0.1271	< 0.05
X_3	Accumulated Temperature	n.s.	0.9192	0.8449	0.0739	0.2269	< 0.05
X_4	Rainfall	n.s.	0.9429	0.8892	0.0443	-0.0216	< 0.05
X_5	Daylength	n.s.	0.9695	0.9399	0.0507	-0.2214	< 0.05
X_6	Vapour Pressure	n.s.	0.9952	0.9903	0.0504	0.0422	< 0.05
	Regression equation:	$\hat{Y} = 0.07606 + 0.98136\ X_1$					< 0.05

(a - environmental variables; b - physiological variables; c - combined variables)



(a)Relationship between Desiccation Tolerance and maximum rate of net photosynthesis, <u>C.vulgaris</u>, (Cannock Chase).

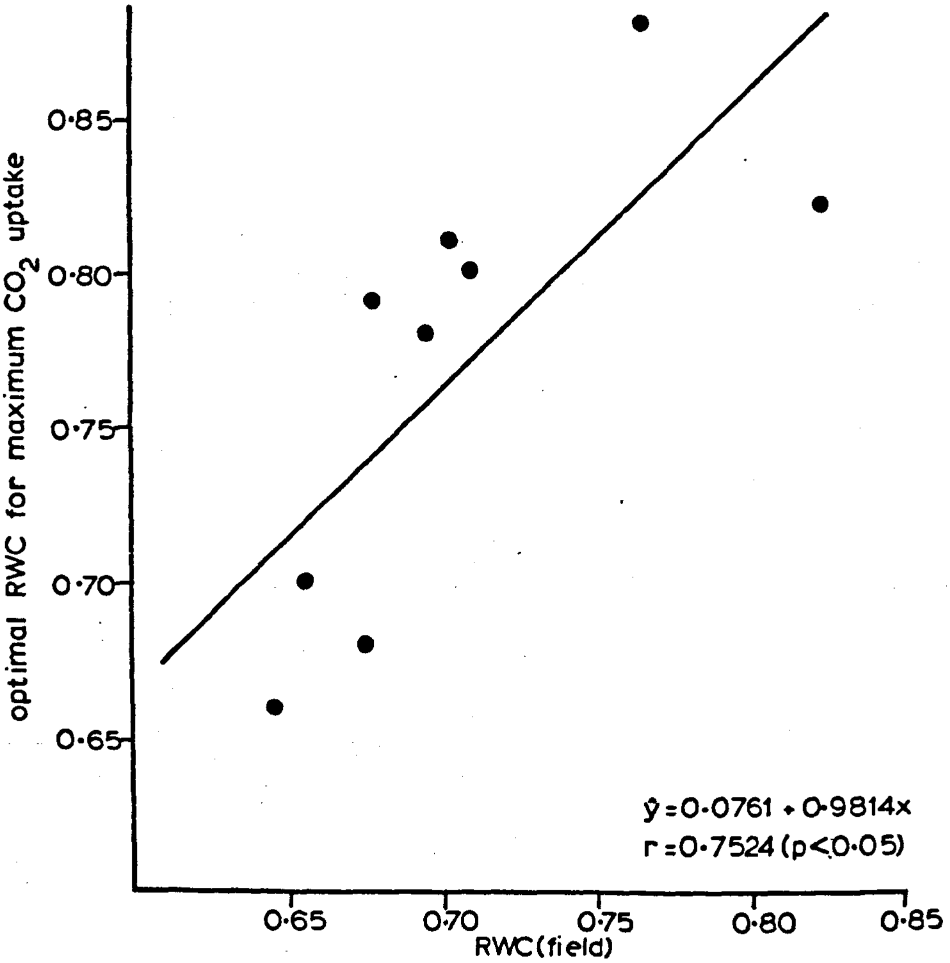


Figure 73 (b)Relationship between field RWC and optimal RWC for net photosynthesis, <u>C.vulgaris</u>, (Cannock Chase).

Multiple Regression Analysis

No measured environmental factor makes a significant contribution to the seasonal variation in RWC optimum, although together they account for 95.3% of the variation and yield a significant regression equation (Table 23a). The physiological variables account for 99.9% of the total variance, the 6 variables other than field RWC at the time of shoot sampling accounting for 43.3% of the variance. Only field RWC makes a significant contribution to the regression, accounting for 56.6% of the total variance (Table 23b). When the variables are combined into a single analysis it is of interest to note that variation in optimal RWC is described primarily by environmental parameters in contrast to the variation in maximal photosynthetic capacity which is explained primarily by physiological variables (Table 23c). The regression between field RWC and RWC optimum (Figure 73b) indicates that the optimal RWC for F_n lies just above the field RWC at the time of sampling.

(e) Discussion

(i) The effect of water stress on the rate of net photosynthesis of C. vulgaris

The type of response obtained with decreasing RWC is consistent with the observations of a number of workers, e.g. Brilliant (1924), Larcher (1960, 1965), Catsky (1965b), Mederski et al. (1975). It is possible that the effect results from hydropassive stomatal closure at high turgidities followed by the development of differential turgidities between guard and epidermal cells leading to stomatal opening, followed by hydroactive closure at higher water deficits (Stalfelt, 1955) which inhibits CO_2 uptake. From a review of the relative contributions made by r_s and r_m on CO_2 uptake, for r_s to be effective it must equal r_m, assuming r_a is small because of air turbulence. It is feasible that if high RWC's cause stomatal closure, thereby increasing r_s, a reduction in CO_2 uptake may occur. However, since appreciable CO_2 uptake occurs at an RWC of 1.0 it would appear that total closure has not occurred. The relationship between RWC and F_n also implies that stomatal control over CO_2 uptake is relative sensitive (cf. Troughton, 1969). An increase in r_m with increasing deficit is a possible cause for the decline in F_n but it seems unlikely in view of the observations by a number of workers on the independence of decreases in F_n while r_m remains unchanged e.g. Trougnton (1969), Troughton & Slatyer (1969),Puritch (1973), Slatyer (1973a,b), Mederski et al. (1975). It is also possible that an apparent decrease in CO_2 uptake results from increased photorespiratory CO_2 output as a result of elevated temperatures in water stressed leaves. Since r_s was not measured it is not possible to confirm the reasons for the decline in F_n with increasing water stress although it would appear that changes in r_s form the most logical explanation.

The observation that the optimal water deficit for maximising CO_2 uptake is closely correlated with the field RWC at the time of sampling is of some considerable interest. It does not appear to be an artifact of the general seasonal trends in water relations of C. vulgaris since it is not correlated with any environmental or physiological variables exhibiting seasonal trends. It would appear to represent a type of adaptation to the water deficit existing at the time of sampling whereby the plant is able to maximise its rate of CO_2 uptake by adjusting its water relations to minimise stomatal resistance to CO_2 uptake. The correlation may be spurious since the diurnal variation in RWC which may occur (Bannister, 1964b) implies that either F_n is submaximal except when the optimal water deficit for CO_2 uptake and field RWC coincide, or that the optimal water deficit for CO_2 uptake also varies diurnally.

The present data, in contrast to Raschke's studies (1976), imply that CO_2 uptake is sensitive to changes in r_s, assuming r_s to be sensitive to changes in RWC. The relationships discussed by Raschke would facilitate maximal CO_2 uptake over a range of water deficits which, although possibly affecting r_s and water loss, do not have an appreciable effect on F_n, thus invalidating the concept of an optimal water deficit for F_n. If transpiration were to exhibit a decline with decreasing RWC whilst F_n showed a transitory increase at a mild deficit this would imply that, at high RWC's, CO_2 uptake is independent of stomatal effects in contrast to transpiration. This would be of considerable advantage in plants under a constant water deficit since it would maximise CO_2 uptake while reducing water loss, i.e. an increase in the assimilation to transpiration ratio. This would not apply, however, if the increase in F_n results from a decrease in r_s since this would enhance water loss. The sensitivity of the transpiration decline measurements in the present study is not sufficient to determine the response of transpiration to high values of RWC. The argument is therefore unresolved but in a situation where field RWC is in part determined by existing environmental conditions, the ability to optimise CO_2 uptake at a deficit will maximise the assimilation potential.

The prediction of CO_2 uptake at an RWC at which stomatal closure should theoretically have occurred is something of a paradox. Catsky (1965b) also observed appreciable rates of photosynthesis at RWC's lower than RWC's at which stomatal closure should have ensued and ascribed it, possibly erroneously, to high cuticular diffusion. Although cuticular diffusion may account for positive CO_2 uptake (El-Sharkawy & Hesketh, 1964; Willis & Balasubramaniam, 1968; Troughton, 1969; Zelitch, 1971), the high CO_2 uptake rates observed in the present study appear unlikely to be due to cuticular diffusion alone, since it would imply that stomatal control over CO_2 uptake is relatively ineffective and that r_c was very low. This situation appears more improbable in view of the possibility of increased r_c with desiccation (Levitt, 1972) and consequently suggests that SCI is not a reliable indication of stomatal closure. The transpiration decline data suggest SCL may give a more realistic indication since at this RWC an inflexion in the curve occurs. However, as water deficits develop, the sensitivity of the guard cells to CO_2 increases, inducing stomatal closure and preventing the development of lethal deficits (Meidner & Mansfield, 1968). Thus, stomatal closure may occur restricting water loss but still facilitating CO_2 uptake owing to the differential effect of r_s on transpiration and F_n (Raschke, 1976). Even if CO_2 uptake is inhibited, photosynthesis may still occur by the use of recycled photorespiratory CO_2, providing the deficits are not damaging to the photosynthetic apparatus. A dynamic equilibrium may be established in which, upon stomatal closure, water loss is prevented and simultaneously the resistance to outward diffusion of respiratory CO_2 is increased so that respiratory CO_2 is retained (Puritch, 1973). Johnson _et al_. (1974) observed that F_n became zero at a ψ of -3.2MPa in _Hordeum vulgare_ and _Triticum aestivum_ which they attributed to stomatal closure, while substantial ribulose diphosphate carboxylase activity remained, thus the stress required to induce stomatal closure would appear to be less than that required to damage the photosynthetic apparatus (cf. Santarius & Heber, 1967; Slatyer, 1973b).

A possibility of incomplete stomatal closure may account for CO_2 uptake at RWC's at which the stomata should theoretically be closed, e.g. Lopushinsky (1969). The differential response of water vapour and CO_2 to r_s may account for the different responses observed at which CO_2 uptake continues whilst water loss is restricted to slight stomatal plus cuticular water loss. This could account for the inflexion in the transpiration decline curve but an inflexion might also be expected in the CO_2 uptake curve. Such an inflexion was not observed in the present study although Puritch (1973) observed a response where, at constant stomatal aperture, a gradual decrease in F_n occurred as a result of increasing r_m and damage to the photosynthetic apparatus due to desiccation. The absence of such a response in the present study makes it difficult to resolve the dilemma of the apparent differential effects of stomatal closure on transpiration and CO_2 uptake. Although increasing deficits reduce both, the critical question is whether water loss is retarded more than CO_2 uptake, since this would result in an improved water use efficiency. If stomatal closure does reduce F_n to zero, the conclusions made regarding stomatal control over RWC may be erroneous since a number of observations in the present study indicate that RWC's lie below SCI while the CO_2 uptake data indicate that, although water stress results in an appreciable decrease in CO_2 uptake, all the results nevertheless indicate a positive CO_2 uptake even at the highest water deficits occurring in damaged plants.

(ii) Seasonal variation in the maximum photosynthetic potential of *C. vulgaris* shoots (F_n maximum)

The maximum rates of F_n found in the present study are comparable to those obtained for *C. vulgaris* by Grace & Woolhouse (1970), although the seasonal pattern is different. This may result from the failure, in the present study, to partition shoots into young and old portions. Grace & Woolhouse (1970) observed a marked increase in F_n of current season's shoots to a maximum in August, followed by a decrease while previous season's growth exhibited a more gradual increase and did not attain CO_2 uptake rates comparable to those of current season's shoots. In the present study the increase in F_n occurred in May but it is evident that the wide age range of leaves on a particular shoot (Grace & Woolhouse, 1974) may modify the observed response. This situation may be confused by leaf abscission effects. Cormack & Gimingham (1964) noted that litter fall does not usually occur until the shoot is two to three years old and that a peak of short shoot fall occurs in autumn. Grace & Woolhouse (1970) stated that "...old shoots fall in great numbers when bud burst occurs". If old leaves are shed in autumn, prior to the initiation of measurements of CO_2 uptake in the present study, the age range of the remaining leaves may have been relatively constant from November 1975 to July 1976 with the exception of new growth and the results may indicate a real change in photosynthetic capacity of the assimilatory organs. If leaves were shed at the beginning of the growing season, i.e. March, April, apparent changes in photosynthetic capacity may reflect a change in age structure of the shoot, rather than a real change in the photosynthetic capacity of leaves. The increase in CO_2 uptake in May may result from younger tissues having an inherently higher rate of F_n rather than change in photosynthetic capacity of the older leaves. However, this may not account for the marked increase in F_n observed in January, 1976.

The magnitude of the increase in F_n in May, occurring at a time when new growth became apparent, suggests that a fundamental biochemical reorganisation may have occurred, possibly as a result of hormone synthesis by developing buds (Bidwell & Turner, 1966; Thorne & Koller, 1974), and the provision of new sinks for assimilates (Warren Wilson, 1966; Gordon & Larson, 1968; Neales & Incoll, 1968; Humphries, 1969; Fraser & Bidwell, 1974). The findings of a decline in F_n following the initial burst of growth is consistent with the observations of Little & Loach (1973) and may result from rapid self sufficiency of new shoots which pass from being sinks to sources of assimilate (Fraser & Bidwell, 1974). Alderfer (1975) suggested that younger leaves may release compounds during their development, inhibiting F_n in leaves lower on the stem. This may result in a preferential supply of metabolites involved in photosynthesis e.g. cytokinins, to younger leaves, which together with high auxin production of young tissue (Sweet & Wareing, 1966) may result in changes in shoot photosynthetic capacity, not through changes in leaf age structure but through metabolic inhibition of older leaves. Gordon & Larson (1968) noted that peak photosynthetic efficiency in new needles of Pinus resinosa coincided with increased export of photosynthates from new needles. As new tissues become efficient producers of assimilate an increase in assimilates may occur which reduces F_n (cf. Warren Wilson, 1966; Neales & Incoll, 1968; Humphries, 1969). The development of a non-photosynthetic sink e.g. reproductive structures, may lead to an increase in F_n (cf. Grace & Woolhouse, 1970), either as a result of hormonal stimulation or assimilate demand. The investigation period of the present study does not allow an evaluation of this response. Sampling following the May peak in F_n was not of sufficient duration to ascertain whether the increase is transitory or a genuine trend. The data of Grace & Woolhouse (1970) suggested F_n in winter is reduced which suggests the observed summer increase in F_n in the present study is a real effect.

C. vulgaris exhibits appreciable rates of F_n, when measured at a standard temperature (20°C), during the winter months, i.e. 0.5-0.7μg g^{-1} s^{-1}. The role of winter photosynthesis as a mechanism for building up reserves to initiate spring growth is controversial. McGregor & Kramer (1963) considered that food reserves in Pinus spp. were sufficient to facilitate initial spring growth without a dependence on increased F_n but Helms (1965) concluded that the capacity for winter F_n in Pseudotsuga menziesii made a significant contribution to food reserves accumulating prior to the spring flush of growth, and thus of importance to the food economy of the plant. Schulze et al. (1967) considered that Pinus aristata may be abnormal in showing a negative assimilation balance in winter to the extent that half the summer assimilate is required to survive a normal winter. C. vulgaris exhibits photosynthetic rates which are below those of many woody plants (cf. Sestak et al., 1971). Although possessing the capacity to photosynthesise at low temperature (Grace & Woolhouse, 1970), metabolic or mechanical disruption may impair not only the current season's productivity, but possibly the build up of reserves over winter, which may have repercussions on the initiation of spring growth, and the development of any resistances to environmental stresses dependent on assimilates (cf. Santarius, 1973).

Apical removal has been found to reduce F_n in Pinus radiata possibly due to sink removal and auxin production retardation (Sweet & Wareing, 1966). Grace & Woolhouse (1970) considered that, if hormonal regulation of F_n occurs, any mechanism influencing either hormonal production or distribution may alter the photosynthetic capacity of the plant. Wardlaw's observations (1969) suggested that water stress may not inhibit translocation. However, mechanical damage may retard transport of auxins synthesized in the root and shoot apices, thereby inhibiting F_n. Crushing, bruising and tearing of stem apices by trampling may therefore influence hormonal production and photosynthetic capacity.

It is apparent from the multiple regression analyses that one of the variables significantly related to F_n is desiccation tolerance (CRWC).[1] The relationship may not be directly causal but possibly arising from a relationship both may have with carbohydrate concentration. The role of

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[1] The higher the desiccation tolerance of the plant the lower the value for CRWC. This is associated with a decrease in the rate of F_n at low values of CRWC and higher rates of F_n at higher values of CRWC (i.e. a positive correlation coefficient) although desiccation tolerance per se and F_n may be regarded as negatively correlated.

sugars in the development of desiccation tolerance e.g. Santarius (1973), and the possible effect carbohydrates have upon photosynthesis e.g. Warren Wilson (1966), Neales & Incoll (1968) may account for the close relationship between the two variables. In this context the data for January 1976 are of especial interest since an apparently anomalous decrease in desiccation tolerance is accompanied by an increase in F_n, which also departs from the seasonal trend. Similarly, in May 1976 when possible bud break and vigorous growth may have resulted in a depletion of assimilate reserves, a similar relationship between desiccation tolerance and F_n occurs. However, in the absence of data concerning seasonal variation in carbohydrate levels within the shoot, the mechanistic explanation relating the two variables remains hypothetical. Grace & Woolhouse (1970) commented that F_n was generally correlated with sugar content in the leaves of C. vulgaris, although the relationship was somewhat imperfect. Consequently, if the two variables, F_n and desiccation tolerance, are related via carbohydrate concentrations, any damage impairing the photosynthetic capacity of the plant such as that resulting from trampling, might be expected to have repercussions on the development of desiccation tolerance, although the results of the present investigations into desiccation tolerance do not permit a definitive conclusion to be made regarding this hypothesis.

The relationship with time to stomatal closure (SCL) may be a reflection of stomatal reactivity and thus r_s (Section IIIA,2(d)). Blockage of stomatal pores e.g. Ludlow & Jarvis (1971), Watts et al. (1976) may increase r_s to CO_2 uptake in older leaves and may reduce the effectiveness of stomatal control over CO_2 uptake. In addition, the possible variation in efficiency of stomatal movements (cf. Loftfield, 1921; Stalfelt, 1962; Zelawski & Kucharska, 1967; Troughton, 1969) may result in higher values of r_s in the winter months, reducing the potential CO_2 uptake rate. This is difficult to reconcile with the positive correlations existing between the two parameters since longer times to stomatal closure might be expected to be associated with reduced stomatal efficiency. Wide open stomata, possibly indicative of a high efficiency, might take longer to close in view of the relationship between hydroactive closure and photoactive opening (Stalfelt, 1955) such that a greater bulk tissue water deficit may be required to induce closure. This is substantiated by the observation that one of the lowest RWC's at stomatal closure (SCL) was recorded in May 1976 when F_n is maximal. However, there is a likelihood that time to stomatal closure is an artifact of the RWC required to induce closure rather than an indication of stomatal reactivity.

A similar problem arises in providing a mechanistic explanation for the negative correlation between F_n and RWC at stomatal closure. The correlations may be spurious, reflecting a more generalised state of physiological activity of the plant. Changes in hormone activity at the onset of the growing season may enhance stomatal opening and decrease r_s to facilitate CO_2 uptake for new growth, although the dependence of new growth on current photosynthesis is controversial (e.g. McGregor & Kramer, 1963; Helms, 1965; Fraser & Bidwell, 1974). This implies a reduced desiccation avoidance capacity at a time when desiccation tolerance is low and may render the plant liable to damage.

Although r_s may be lower in younger tissues e.g. Ludlow & Jarvis (1971), Neilson *et al.* (1972), Watts *et al.* (1976), its significance in terms of CO_2 uptake may affect the meaning of the correlation between stomatal closure point parameters and F_n (Gaastra, 1959; Watts *et al.*, 1976). The present discussion has assumed a linear relationship between stomatal aperture and CO_2 uptake, although this may not be the case e.g. Raschke (1976). Similarly, it has been assumed that relationships between RWC and F_n indirectly reflect changes in stomatal resistance. It is apparent that, to substantiate any postulates made relating F_n to RWC through changes in stomatal efficiency and operation, an independent quantification of r_s is required to ascertain whether a seasonal variation in the relationship between r_s and RWC occurs which might modify the relationship between F_n and RWC. It is not possible, on the present evidence, to consider the relative importance of r_s in causing seasonal variation in CO_2 uptake. Anderson & McNaughton (1973) considered that seasonal change in F_n capacity was independent of seasonal change in stomatal aperture, while Grace & Woolhouse (1970) stated that their results argued against 'physical diffusional resistances' controlling F_n in C. vulgaris. The present evidence suggests that both biochemical and diffusional factors, as reflected in desiccation tolerance and stomatal closure point parameters, may influence seasonal variation in CO_2 uptake in C. vulgaris.

Although seasonal variation in CO_2 uptake appears to be independent of any environmental parameter measured, the trends with temperature may reflect the generalised progression of seasonal changes in plant and environmental parameters. Grace & Woolhouse (1970) suggested that temperature may affect F_n by the effect it has upon biochemical reactions per se and via the influence it has upon hormone synthesis and translocation which may affect photosynthesis. The apparent independence of F_n maximum on environmental variables suggests that the influence they have is through biochemical mechanisms controlling photosynthesis.

(iii) Seasonal variation in the calculated rate of F_n (F_n') of treated shoots

Seasonal variations in F_n' of damaged shoots are unrelated either to each other or to the control or maximal values. This is unexpected in as much as F_n' values are calculated from the rates of F_n of undamaged shoots but is indicative of seasonal changes in the relationship between F_n' and RWC, which requires further evaluation in terms of the factors which might affect it. Explanations of seasonal variations in F_n' are confused by problems of accounting for seasonal variation in RWC and the RWC/F_n relationship. Since the rates are calculated they may not be a true representation of seasonal variation in F_n in the field.

(iv) RWC parameters associated with F_n measurements

The optimal RWC for F_n maximum, although not significantly correlated with any measured environmental parameter, is more closely correlated with them than with physiological variables. Although field RWC is the main variable accounting for seasonal variation in optimal RWC for F_n, and which itself eluded explanation in terms of the factors considered in the investigation, it is pertinent to note that irrespective of the explanation for the correlation between the two parameters, field RWC is in part environmentally determined by the evaporative demand placed upon the plant's water content and water uptake by the plant, which are related to the environmental measurements of temperature, rainfall and vapour pressure, in addition to a number of other environmental factors not considered in the present study. Thus, since environmental parameters may have a more direct influence on the plant's RWC than on F_n, it is not unexpected that they play a more significant role in accounting for seasonal variation in the optimal RWC for F_n. An explanation accounting for the seasonal variation in this parameter in terms of a mechanism remains obscure but such variation is of considerable adaptive advantage if it occurs, in that at varying field moisture stresses, photosynthetic capacity is relatively unimpaired. This may be a reflection of Raschke's hypothesis (1976) in that the plant is maximising assimilation within the constraints of an available water supply.

The dynamic relationship between RWC and F_n indicates that the effects of water stress on CO_2 uptake vary, with possible implications for survival and productivity. The relationship between RWC and F_n suggests that when field deficits are likely to be severe, compensation occurs to maintain a high potential photosynthetic capacity. A corollary of this is that seasonal variation in the susceptibility of the photosynthetic system to desiccation may occur so that a particular stress has a more adverse effect at certain times of the year than at others. If survival of *C. vulgaris* following trampling is, in any respect, dependent upon the assimilatory capacity of the plant, it will, in part, be dependent upon the relationship between stress and F_n existing at the time of trampling. No relationship appears to exist between the damage indices used in the present study and the RWC/F_n relationship, but this may result from the use of inappropriate damage indices. This study has not, however, taken into account the effects of longevity of stress on photosynthetic capacity. Over long periods, structural damage and biochemical changes may reduce the assimilatory capacity of the plant to a greater extent than indicated by the effect of a short deficit on F_n, as used in the present study (cf. Jones, 1973). Recovery on rehydration may be dependent upon the capacity of the plant to synthesize the appropriate photosynthetic enzymes. Brix (1962) regarded recovery in *Pinus taeda* following water stress to be dependent upon the water absorbing capacity of the root system and that under conditions of stress, the youngest leaves died first. In contrast Catsky (1965b), working with *Brassica oleracea*, observed that young leaves maintained their photosynthetic activity longer than old leaves. The present study suggests older leaves may succumb to desiccation first and thus, the assimilatory capacity may be less adversely affected than if the young leaves were to die first, assuming, as Grace & Woolhouse (1970) suggest, younger leaves have a greater photosynthetic capacity.

Delay in stomatal activity following water stress may also retard CO_2 uptake following rewatering. Boyer (1971), for example, found that photosynthesis did not immediately return to control levels following rewatering leading to a recovery in ψ and chloroplast activity. Boyer (1976) suggested recovery of chloroplasts following rewatering is rapid whilst stomatal control may limit CO_2 uptake. Jones (1973), however, found the stomatal apparatus made a more rapid recovery 24 hours after rewatering, than intracellular transport resistances, carboxylation and carbonic anhydrase activity.

(f) Conclusions

A reduction in RWC below the optimal RWC for F_n reduces CO_2 uptake. It may be concluded that water stress *per se* retards photosynthetic carbon gain.

This response should be viewed in terms of the total response of the plant to water stress. In addition to the direct effect, leaf initiation and development may also be reduced. Desiccation to existing leaves may result in irreversible injury and death. These two factors will effectively reduce the leaf area index of a trampled stand of heather, consequently reducing its assimilatory capacity and therefore productivity. The effect of this will be aggravated by a reduction in photosynthetic capacity of the remaining viable tissue by the direct effect of water stress on CO_2 uptake. Although a certain degree of compensation for the effects of water stress on F_n may occur by a seasonal shift in the RWC/F_n relationship, regeneration of damaged parts might be impaired due to a reduction in assimilation potential. This is exacerbated by the observation that, in comparison to many plants, C. vulgaris has a relatively low photosynthetic capacity, irrespective of any stress placed upon the plant. Thus, its inability to survive in trampled habitats may, in part, be due to a low recuperative potential, as a result of low assimilation rates, which are reduced further by the effects of water stress on photosynthesis.

B I The Effect of Trampling on the Primary Productivity of C. vulgaris

(a) Introduction

The distribution of assimilates in C. vulgaris following their acquisition has been described and modelled by Grace & Woolhouse (1973b, 1974). The balance between wood and leaf utilization was found to be affected by season, for example, in August wood growth utilized twice as much assimilate as leaf growth but at the beginning and end of the growing season (i.e. June-July, September-October) the situation was reversed (Grace & Woolhouse, 1973b). These authors proposed a scheme by which meristematic activity, in terms of hormone production, which is temperature sensitive, stimulates the movement of assimilates from a source pool to a sink when the temperature exceeds 7.2°C, resulting in new leaf and, later, new wood growth. Distribution and utilization of assimilates may be regarded as a seasonal variable. Following a rise in temperature above a certain threshold, utilization is reactivated and a new compartment, for example leaves, is initiated, corresponding to the spring flush of growth (Grace & Woolhouse, 1974). The primary productivity calculated from their model was comparable to values obtained from harvesting techniques, but they realised that negative feedback mechanisms such as mutual shading of leaves, burial of stems and leaves by growth of other plants and a reduction in photosynthetic capacity as a result of leaf browning, may affect the accuracy of the model. Such an approach provides a link between the physiological aspects of production, and biomass and production estimates obtained by harvesting at the stand level, which have been reviewed by Gimingham (1972).

The concept of cyclicity, introduced by Watt (1955) in relation to C. vulgaris regeneration, has been discussed by a number of workers, e.g. Chapman (1967), Barclay-Estrup & Gimingham (1969), Barclay-Estrup (1970), Chapman et al. (1975a). Of the four phases, pioneer, building, mature, degenerate (cf. Barclay-Estrup & Gimingham, 1969; Barclay-Estrup, 1970), Chapman's studies (1967) in Dorset revealed that regeneration from seed was rare and consequently, a true pioneer phase following a fire was of limited applicability. Rather, the system enters the 'building phase' as a result of regeneration from rootstocks. Following burning there is an initially high rate of growth followed by a period in which biomass increases little if at all (Chapman et al., 1975a). Initial considerations led to the belief that this response was due to increased nutrient availability from the remains of the fire or utilization of rootstock food reserves, but further investigation revealed that the relationships between growth and mortality were primarily responsible. Litter production, which was inappreciable in younger material, occurred in the third and fourth growing seasons and balanced growth thus resulting in a static situation. The isolation of such a response led Chapman et al. (1975a) to suggest that the term 'post-burn' is a preferable alternative to 'pioneer' to distinguish a stand regenerating following a fire from a stand that has developed from seed. Such a response confuses the relationships between age and productivity, especially if it is unknown whether the stand has developed from seed or from regeneration following a fire.

The biomass and production of a stand at a given location will depend upon its stage in the cycle (Barclay-Estrup, 1970) unless a steady state has developed resulting in a stand of uneven age structure but stable productivity (Forrest, 1971). The stage in the life cycle will also influence the harvesting techniques employed, since structural changes resulting in a tussock type development in mature and degenerate stands increase the variance in the data which may lead to erroneous biomass estimates for the stand as a whole (Chapman et al., 1975a). This may be a factor of some relevance when stands of different ages are being compared.

Samples may be partitioned into a number of components (flowers, current season's green short shoots, current season's green short shoot increment, old green short shoots, brown short shoots, current season's long shoots, woody material) (Chapman *et al.*, 1975a). From such a partitioning it is possible to ascertain the current season's green production, but total production must take into account flower, wood and root production. By confining sampling to the end of the growing season the net production of new shoots for the current season may be estimated (Barclay-Estrup, 1970) but it is necessary to also account for litter production which, although adopting a seasonal pattern, occurs throughout the year (Cormack & Gimingham, 1964), and may have an appreciable effect on productivity estimates (Chapman *et al.*, 1975a). Such factors confuse comparisons of data of different workers.

Biomass estimates on a Scottish moor at an altitude of 107m. by Barclay-Estrup (1970) (Table 24) revealed that biomass, lowest in the pioneer phase, increased to a maximum in the mature phase and decreased in the degenerate phase. The productivity relationships however, were somewhat different, being nearly equal in the pioneer and degenerate phases but reaching a peak in the building phase, when biomass was the second largest,and then declining in the mature phase when biomass was largest. This data emphasised the variation in productivity estimates which may arise from age effects and suggests that a point at which steady state exists is the most useful basis for site comparisons (Chapman *et al.*, 1975a; Forrest, 1971). A further point of confusion arises from the dynamic contribution to the total biomass made by *C. vulgaris* as a result of the cyclical variation in species composition and contribution to stand biomass (Barclay-Estrup & Gimingham, 1969; Barclay-Estrup, 1970).

Stand	A	B		C		D		E	F	
Age	Bm	Bm	P_r	Bm	P_r	Bm	P_r	Bm	Bm	P_r
2		101						326		
3	507					224				
4						253	121			
5										
6		836		287.2	148.8	535	173	631.6		
7										
8	1099.7					623	221			
9				1507.6	442.4					
10								1115.2		
11										
12		1450				1114	288			
13										
14						1236	308			
15	(15yrs plus)		217							
16	2926.1					1588	318			
17		1740		1923.6	363.6					
18						1488	324	2217.6		
19			253						740	168
20										
21										
22										
23		1852								
24				1043.2	140.8	1819	282			
25		1840	278							

Table 24 Comparison of Biomass and Net Primary Productivity data for *Calluna vulgaris* stands from different sites in Britain

(adapted from Gimingham, 1972)

	Site	Altitude	Source of Data
A	Cairn O'Mount Kincardineshire	274m	Robertson & Davies, (1965)
B*(Bm)	Kerloch Kincardineshire	152m	Miller, (1972)
(P_r)	Kerloch Kincardineshire	150m	Miller & Miles, (1969)
(annual dry weight increment in new shoots (current year's growth))			
C*	Elsick Heath N.E. Scotland	107m	Barclay-Estrup, (1970)
D*	Dorset	60m	Chapman *et al.*, (1975a)
E	Dartmoor	425m	Chapman, (1967)
F	Sike Hill, Moor House, N.E.England	550m	Forrest, (1971)

(Bm : Biomass (g m^{-2}); P_r : Net Primary Productivity (g m^{-2} y^{-1}))

(* data for *Calluna vulgaris* only)

(data plotted with reference to mean age of stand)

The variation in biomass and primary productivity for C. vulgaris (where available) (Table 24) indicates the substantial variation between stands at different sites and of different ages. In contrast to the findings of Barclay-Estrup (1970), Chapman *et al*. (1975a) presented data indicating a levelling off in biomass with increasing age, peak biomass occurring in stands 38 years old (2225.0 g m^{-2}) while productivity increased from an average of 134.7 g m^{-2} y^{-1} over the first six years following regeneration to a peak of 323.1 g m^{-2} y^{-1} for 17-24 year old stands, thereafter declining to below 300 g m^{-2} y^{-1} in stands of 34 years in age and above. Thus, both pattern and age relationships were somewhat different to those of Barclay-Estrup's study (1970).

Chapman (1967) also stressed the importance of litter production in productivity studies on Calluna heathland. It was found to rise from a value of 42.0 g m^{-2} y^{-1} in a stand 9 years old to a relatively stable value of 200-270 g m^{-2} y^{-1} for stands 19-40 years old (Chapman *et al*., 1975a), so that about 43% of the total standing crop was being shed annually. This will have repercussions on biomass and productivity estimates since, in the mature phase of the cycle, dry matter accumulation rates decrease while litter production increases. This results in a reduction in biomass increment. In the degenerate phase, the marked drop in productivity combined with increasing litter production may result in a stabilisation or fall in biomass. This was illustrated by Chapman *et al*. (1975a) who presented evidence indicating that standing crop increment rose to a peak in stands 9-10 years old, followed by a marked decrease. When litter production exceeds primary production the rate of increment will be negative and the biomass will decrease. Thus, by taking into consideration death as well as growth rate a modification of the productivity age relationship will occur.

It will be apparent from Table 24 that although variation between sites exists, biomass data are relatively constant, e.g. the data for a 17 year old stand of C. vulgaris on Kerloch (1740 g m^{-2}) and Elsick Heath (1923.6 g m^{-2}) in N.E. Scotland compare favourably with the value (1588 g m^{-2}) for a Dorset heath, 16 years of age, despite variation in altitude and latitude. The data presented by Robertson & Davies (1965) for Cairn O'Mount, also in N.E. Scotland and of similar age, is substantially greater than the aforementioned estimates, despite being somewhat higher in altitude. The value for Cairn O'Mount represented the total above ground standing crop (i.e. C. vulgaris plus other species), and a similar value for Dorset, of 2008 g m^{-2}, reduces the discrepancy, although a substantial difference remains. Chapman (1967) ascribed this difference as possibly due to the greater litter accumulations on the Dorset heath. This may indicate an environmental effect on litter production and consequently biomass estimates. Cormack & Gimingham (1964) noted that long and short shoot fall, in addition to being affected by endogenous factors, was also affected by the environment. However, it also illustrates the problems encountered in comparing sites at which the proportional contribution made by C. vulgaris may vary (Barclay-Estrup & Gimingham, 1969; Barclay-Estrup, 1970).

In contrast to the cyclic situation discussed above, a system under steady state is one in which net input (i.e. the difference between total production and litter fall) is in equilibrium with output to standing dead material (Forrest, 1971). The net aerial productivity of C. vulgaris in Forrest's study of a blanket bog on Sike Hill in N.E. England, of 168 g m^{-2} y^{-1}, for a stand of Calluna biomass of 740 g m^{-2}, was partitioned into 130 g m^{-2} y^{-1} for green shoot productivity and 38 g m^{-2} y^{-1} for wood productivity. This was balanced by a litter productivity of 108 g m^{-2} y^{-1} and a standing dead productivity of 60 g m^{-2} y^{-1}, resulting in a stable biomass at the end of the season.

The period over which growth and litter production occur will obviously affect the result obtained, but Forrest noted that maximum litter fall was coincident with maximum growth rate of young shoots, possibly due to new growth mechanically dislodging dead material (cf. Watson et al., 1966), while a secondary peak of litter fall in winter may have been due to environmental conditions, notably wind, dislodging shoots. This emphasises that the time of year at which harvests are made may have an appreciable effect on biomass and productivity estimates.

It is of interest to note that Forrest (1971) considered the most productive section of the population to be in the age range 10-18 years. Comparisons between cyclical and steady state systems are difficult due to the uneven aged nature of the latter. Notwithstanding this criticism, Forrest (1971) considered that the total wood input (93 g m^{-2} y^{-1}), uncorrected for any losses, compared favourably with the average productivity of 120 g m^{-2} y^{-1} over the first 10 years of the Calluna cycle given by Chapman (1967), and concluded that, despite differences in biomass between Dorset and Sike Hill (1815 g m^{-2} for an 18 year old stand in Dorset and 740 g m^{-2} at Sike Hill), the productivities were not significantly different. Chapman et al. (1975a) made similar comparisons with Forrest's data and concluded that, despite biomass and productivity being twice that of Sike Hill at the Dorset site, "...when features such as green shoot, net aerial and litter production are compared on the basis of similar weights of above ground vegetation, they are very similar". This suggests that when making site comparisons, productivity data may be of considerably greater value than biomass estimates.

A further problem encountered in considering age/productivity relationships is that of the ratio of lignified to unlignified material. Robertson & Davies (1965) observed that, with increasing age, this ratio increased from 1:3 in 2-4 year old Calluna to 6:1 in 15 year old material.

Miller (1972) observed that the proportion of young shoots as a percentage of total above ground biomass fell from 81% to 21% as the stand aged from 2 to 12 years, whilst the proportion of stem weight increased from 12% to 62%. Miller's findings re-emphasised the differences that exist between cyclic and steady state systems, since unlike Forrest's observations (1971) on the static standing dead biomass, the proportion of dead material observed by Miller increased from 3% to 22% by a stand age of 6 years.

In the context of the present study, the productivity data of Miller & Miles (1969) is of especial relevance (Table 24). The productivity increased to a maximum of 278.0 g m^{-2} y^{-1} in stands age 23-27 years, declining slightly in older stands (236 g m^{-2} y^{-1}). Gimingham (1972) noted that the mean productivity (243.7 g m^{-2} y^{-1}) compared favourably with Barclay-Estrup's estimates (1970) for the nearby Elsick Heath site (273.9 g m^{-2} y^{-1}). The age relationships would appear to be substantially different however. For a 17 year old stand the data for Elsick Heath gave a value of 363.6 g m^{-2} y^{-1} in contrast to 217.0 g m^{-2} y^{-1} for a stand in the 13-17 year age group in the study by Miller & Miles (1969). A similar discrepency occurred for stands in the 23-27 year age group. Despite such variation, Chapman *et al.* (1975a) concluded that, with respect to data from different sites, variation in productivity estimates is small. Gimingham (1972) similarly considered that productivity estimates for different sites is generally similar, being of the order of 200-300 g m^{-2} y^{-1} for stands aged 6-20 years, and that variation in productivity in relation to altitude is likely to be small in comparison with site variability.

Notwithstanding such similarities in productivity, it is apparent that considerable variation exists, which, if not due to site environment, may be related to age structure of the stand. Barclay-Estrup (1970) observed variation in the performance of Calluna during its life cycle, a decrease in its vitality with age affecting not only the plant itself but associated vegetation. Age related changes in stand vitality might be expected to influence regeneration following damage to the stand. Studies on regeneration have focussed upon two main aspects of damage, that of burning and that resulting from grazing. Investigations into both types of damage response, involving the aerial portions of the plant, may involve a consideration of root biomass, of which there are few estimates. Chapman *et al*. (1975a), in a comparison of root biomass for different sites observed that although net aerial productivity may be similar, root biomass could vary considerably. Much depends upon site prehistory; for example, a burn may destroy the aerial vegetation while the roots remain relatively unaffected, so resulting in a disproportionate root to shoot ratio. Regeneration following damage may be heavily dependent on functional root biomass. Of particular relevance to the present investigation is damage following grazing since, in a number of respects, it reflects damage occurring as a result of trampling in as much as stem spices may be damaged or removed, the assimilatory surfaces may be reduced in area and the grazing animals may also damage the vegetation due to trampling effects. Watson *et al*. (1966) commented that "Heather which has been heavily grazed.....invariably turns brown in late winter". Although burning may have a more severe effect on a Calluna stand than grazing (Kayll & Gimingham, 1965; Miller & Miles, 1970), the latter may nevertheless modify both productivity and plant morphology.

Simulated grazing experiments have revealed that by clipping plants their morphology may be altered (Gimingham, 1949). Clipping to a height of 1.5cms. resulted in accelerated lateral shoot production which produced a procumbent spreading plant which eventually bore vertical shoots.

More severe clipping intensified the response, resulting in a tufted form due to the activity of the lower buds. Grant & Hunter (1966) observed a similar response in which 80% removal of the current season's growth in 2 out of 3 years resulted in a compact plant resembling that found in a young, vigorous stand. Clipping every year produced a cushion like morphology in which the current season's shoots were peripherally arranged. Clipping also increased the ratio of green to woody tissue in contrast to the decrease which occurs with age (Robertson & Davies, 1965), suggesting, to Grant & Hunter (1966), that clipping rejuvenates or maintains the juvenility of the plant. Opposed to this effect is the reduction in flowering vigour. Grant & Hunter noted this to be especially so in summer clipped plants where flowering initials were removed, but a seasonal response in productivity was also found to exist in which winter clipping had a less severe effect on productivity than summer clipping. In addition, the effects of frequent cutting suggested that the ultimate production of clipped plants may be greater than that of unclipped plants. Although realising that by not taking litter production into account, such a response may not be real, calculated litter production did not invalidate the proposition that the "long term effect of the intensity of clipping as practised....and certainly when administered during winter, is to stimulate productivity". (Grant & Hunter, 1966). The more severe effect of summer clipping was ascribed to an interruption in the summer growth period and a shorter period for recovery growth, but by removal of the shoot apical dominance, the number of meristemmatic regions in the plant might be expected to increase (Grant & Hunter, 1966) leading to an increase in assimilatory surfaces and thus productivity. In contrast, Miller & Miles (1970) found that material clipped in April 1965 and harvested in October 1965 exhibited a greater degree of regeneration than plots clipped in October

1964 and harvested in October 1965. This differential response was ascribed to damage from frost and infection over winter inhibiting regeneration the following spring for material clipped in 1964.

When apical buds were damaged over winter, the first buds to develop were the terminal buds on the short shoots just below the apex (Mohammed & Gimingham, 1970). Subsequent development was observed in the terminal buds of the previous season's long shoots, those of the short shoots below the flowering zone and the sprouting clusters on the bare stem where branches emerge. Mohammed & Gimingham noted that the development of such clusters is important in regeneration following burning or grazing. Grazing, by increasing the number of leading long shoots, increases the number of short shoots so increasing canopy density. In a series of clipping experiments, Mohammed & Gimingham (1970) found that light clipping stimulated the production of sprouting clusters, in contrast to the comparative absence of such clusters on control plants. More severe clipping resulted in the production of such clusters on the woody parts of the plant at a number of levels while total removal of the aerial portion of the plant resulted in such clusters being produced from a protected position at the stem bases. This latter response only occurred in young plants, for in plants older than 15 years, the number of sprouting clusters decreased with age, possibly due to the engulfment of deep seated buds in the stem by secondary thickening. From the work of Mohammed & Gimingham, two important points arise with respect to regeneration following damage, the ability to develop buds away from the site of injury and the decreasing capacity for sprout production with increasing age.

The variation in regeneration potential following damage may be related to the capacity for sprout production. Kayll & Gimingham (1965) found that stems older than 15 years were less able to regenerate following clipping or burning, the proportion regenerating falling from 67.5% in 12-13 year old plants to 17% in 23-25 year old plants. Miller & Miles (1970), in a study of simulated grazing on Calluna, found that regeneration was greatest in 6-8 year old plants, thereafter decreasing with increasing age, from a maximum of 41 g m^{-2} to 3 g m^{-2} in 34-37 year old plants. These biomass changes were paralleled by changes in the density of sprouting centres, being greatest in the 6-8 year old age groups in which rate of growth was also greatest (cf. Barclay-Estrup, 1970). Miller & Miles (1970) also observed changes in stem density, which was greatest in the 6-8 year old age group (1108 stems m^{-2}) and lowest in the 34-37 year age group (184 stems m^{-2}). Therefore, in addition to an apparently greater sprout density and rate of growth in the younger plants, the potential number of sprouting centres as determined by stem density, might also affect regeneration potential. Although Miller & Miles (1970) were unable to find any reason for a decrease in regenerative potential with increasing age, the relationship between bud development and secondary thickening (Mohammed & Gimingham, 1970) may account for such a response.

It is apparent that a number of factors may affect the regenerative capacity of C. vulgaris following trampling damage. Changes in stem density and productivity with age will affect the regenerative capacity of the stand and, although apical damage may increase the potential productivity of the stand, this is an age related phenomenon in which, above a threshold, bud development is mechanically impeded. Below this threshold the ability to produce sprouting centres, the higher rates of growth and the possible morphological adaptation which results in a procumbent form are characteristics which may enhance survival in the trampled situation. In this connection, the protection offered to deep seated buds in the stem is analogous to the advantage conferred by subsurface meristems of herbs and grasses in trampled habitats, (cf. Bates, 1935).

(b) Methodology

The production of a community may be divided into Gross Primary Production, which is the total assimilation of organic matter including any respiratory losses, and Net Primary Production (P_n) which is the organic material incorporated into a community less that lost in respiration. Both quantities are expressed in terms of weight per unit area, and may be time based[1] i.e. $g\ m^{-2}\ y^{-1}$ (Milner & Hughes, 1968). Production may be quantified by the following relationship:

$$P_n = (B_2 - B_1) + L + G \qquad (25)$$

in which: P_n = net production (unit dry weight per unit area) during the time interval t_1 to t_2;

B_1 = biomass at time t_1;

B_2 = biomass at time t_2;

L = loss by death or shedding over time t_1 to t_2;

G = loss by consumption e.g. by herbivores, over time t_1 to t_2.

P_n may be subdivided into Net Primary Aerial Production, which refers to production of stems, leaves, flowers and seeds, and Subaerial Production i.e. the roots.

Milner & Hughes (1968) suggested that the area under study should consist of a measurement area in which destructive sampling occurs, a sample area in which non-destructive measurements e.g. of microclimate, are made, and a buffer area of considerable width around the main sample

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[1] Production ($g\ m^{-2}$) expressed on a time basis ($g\ m^{-2}\ y^{-1}$) is referred to as Productivity (P_r).

area to prevent extraneous influences on the sampling areas.

The commonest estimate of production is that based on determining the biomass at the onset of the study and a second harvest after a specified time interval (i.e. the difference method), but 'intra interval' death, shedding and consumption (i.e. L and G) are often unaccounted for and lead to underestimates (Kelly et al., 1974). This makes the time of the second harvest of some significance in determining the biomass at t_2. For example, Kelly et al. (1974) employed three methods, a harvest at the peak biomass, a summation of species peak biomass contributions, and a summation of positive biomass increases, and found that significant differences in production estimates resulted from the use of different methods, the lowest estimate being from the first method and the highest from the second.

Gimingham & Miller (1968), recommending methods specifically for dwarf shrub communities, suggested a quadrat size of $0.25m^2$ with 20-25 replicates would be required if sampling took place twice a year, but that in older stands a larger quadrat ($0.5m^2$), with greater replication, may be required to overcome the greater variance resulting from pattern development in older plants. Van Dyne et al. (1963) discussed the effect of plot size on production estimates and concluded that a lower variance per unit area resulted if circular sampling areas were employed, and that larger plots gave more homogenous estimates than smaller plots. Variance should be minimal when a large number of small plots are employed (depending on the type of sward under investigation) but, if replication is limited, an increase in plot size will enhance accuracy (Milner & Hughes, 1968). Van Dyne et al. (1963) also noted that in a heterogenous herbage stand, species distribution may have a considerable influence on yield, e.g. scattered shrubs or clusters of large herbs may result in considerable replicate variation, but to obtain a comparable estimate in a homogeneous stand fewer plots would be required. Van Dyne et al. also found that as the ratio of plot perimeter to area increases, although the area remains constant, the yield estimate increases so that to help maintain homogeneity of variance a standard sampling shape and size should be employed in a particular investigation.

The actual method of harvesting raises certain problems in dwarf shrub communities. Gimingham & Miller (1968) suggested that secateurs enable a greater accuracy in harvesting thick stemmed plants. A further problem arises in such communities in the definition of 'ground surface', where peat or substantial litter accumulations exist. Gimingham & Miller suggested that under such circumstances a base level must be arbitrarily defined or related to a morphological characteristic of the plant. The slow rate of growth of woody shrubs such as Calluna vulgaris (Gimingham, 1972; Grime & Hunt, 1975) necessitates a long period between samples or high replication to detect differences in biomass over time (Gimingham & Miller, 1968).

As noted above, litter production is an important component in production measurements of C. vulgaris (Chapman, 1967; Chapman et al. 1975b). Although litter production shows certain seasonal peaks, it occurs throughout the year (Cormack & Gimingham, 1964) and these authors suggested a method by which litter which falls to the ground may be collected on stockinette to which it adheres. However, problems are encountered if stem density is high and the 'direct method' of Gimingham & Miller (1968), whereby new growth is directly assessed and from which litter production is negligible, provides an alternative method for making productivity estimates.

When harvesting, a certain, and often unknown proportion of material falls to the ground and remains uncollected. Van Dyne (1966) suggested that a vacuum cleaner may aid the collection of such material although allowance must be made for any soil material inadvertently collected in this way.

Finally, when large harvests are involved, a problem arises in dealing immediately with harvested material. This may be circumvented by subsampling after the total fresh weight per plot has been obtained. Subsample measurements may then be related to the total plot harvest. Edwards (1965) has shown that herbage may be kept in polythene bags for a number of hours without appreciable losses in moisture or dry matter, although chemical changes might occur. Consequently, following harvesting, samples should be dried as soon as possible. Alternatively, they may be frozen until required (Liddle & Greig-Smith, 1975b).

(c) Methods

Productivity estimates were made on even aged stands of C. vulgaris, subject to burning, on Kerloch, Kincardineshire (G.R. NO 710887) at an altitude of 300m on an easterly facing slope of slight angle (5^o-10^o slope).

(i) The effect of trampling intensity on Productivity by C. vulgaris

The main productivity studies were performed on a stand approximately 7 years old, consisting of a homogeneous cover of C. vulgaris, with erratic occurrences of Potentilla erecta and Erica cinerea.

Each of the three treatment blocks was laid out in a 4 x 4 Latin Square. Block 1 consisted of 2m x 1m plots, each of which was surrounded by a 1m wide buffer zone. Blocks 2 and 3 consisted of 2m x 0.5m plots surrounded by a 0.5m wide buffer zone. Surrounding each block was a buffer zone several meters in width and which served to accommodate the micrometeorological equipment and from which control plots were harvested at certain times during the duration of the experiment, (04.07.74; 05.09.74; 11.04.75). The treatment and harvesting dates are presented in Table 25, together with the trampling regime applied.

Table 25 Date of treatment and harvesting and trampling regime (p pl^{-1}) applied to *C. vulgaris* stands (Kerloch)

Block	Treatment Date (t_1)	Harvest Date (t_2)	(t_2-t_1)	Trampling Regime (p pl^{-1})	Harvested Quadrat Size
1	04.07.74.	09.07.75.	370	0,50,100,200	1m x 1m
2	11.04.75.	05.04.76.	360	0,100,200,300	1m x 0.5m
3	09.07.75.	05.04.76.	271	0,100,200,300	1m x 0.5m

Plots were harvested using an electromechanical hedge cutter (Tarpen Engineering Co., Leicestershire), driven by a portable electric generator (3kW) (Eisemann, Boschgruppe, Germany).

No assessment of litter production was undertaken, hence the productivity estimates indicate the minimum net aerial production.[1] Following harvesting, the total fresh weight of harvested material per plot was obtained, the material thoroughly mixed and three subsamples taken of approximately 100g in weight and deep frozen at -20°C for subsequent analysis. The remainder was dried at 85-95°C for 24 hours in a forced draught oven, reweighed and used to calculate total dry weight per square meter.

Two of the three subsamples per plot were partitioned into live and dead material. This was attended with the difficulty of determining the viability of woody stems. If such stems bore green, and apparently

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[1] Error from grazing (by *Lagopus scoticus* (Red Grouse), *Lepus timidus* (Mountain Hare) and possibly domesticated animals) may have occurred but fencing or caging of plots was considered impractical due to the comparatively large areas involved.

viable leaves, they were considered viable. Although not giving a precise value for living material on each plot, it gives an indication of the proportion of living material existing on the plot at the time of sampling.

The rate of production (i.e. the productivity) has been calculated from total live biomass existing at t_1 and total biomass at t_2 (minus the quantity of dead material (in g m^{-2}) predicted to be existing on the plots at t_1). In addition P_r (live) has been calculated from live material existing at t_1 and t_2, as an indicator of the potential productive capacity.

In a study in which accelerated death plays an important part, the absence of a mortality factor and litter production values is a serious limitation to the study. Although an estimate of dead standing crop may be obtained from the subsample data, this does not take into account material falling from the plant between treatment and harvest dates. Although Block 2 and 3 were harvested prior to the onset of the growing season, so giving an indication of the effects of trampling on the current season's productivity, this does not take into account the possible litter fall peak in winter.

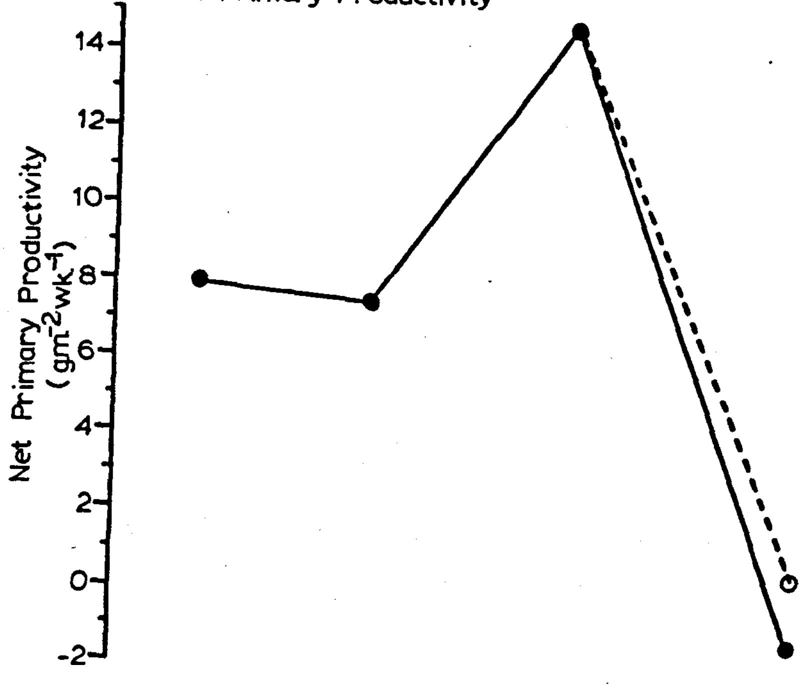
During trampling, material is broken from plants. This was collected immediately after the application of a treatment and the value for each plot subtracted from the mean control value for the stand at t_1, used in calculating the production over the period t_1 to t_2. This makes the assumption that the biomass for each block was homogeneous prior to the application of the treatment. P_n was calculated according to:

$$P_n = (B_2 - B_1) \qquad (26)$$

where B_1 = biomass at time t_1 minus (dead material at t_1 + material removed as a result of trampling at t_1);

B_2 = biomass at time t_2 minus dead material at time t_1.

(a) Net Primary Productivity



(b) Biomass

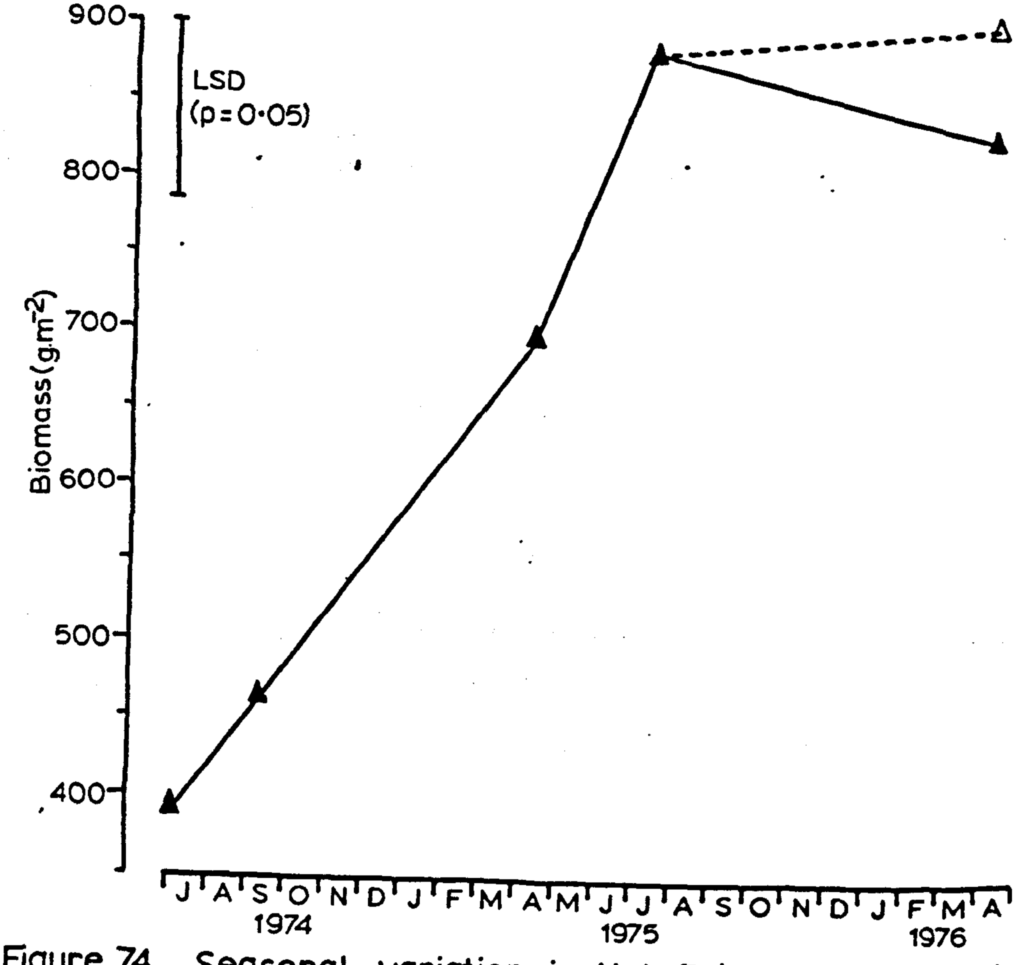


Figure 74 Seasonal variation in Net Primary Productivity and associated changes in Biomass of an even aged, building phase stand of C. vulgaris, (Kerloch).

(open symbols, data excluding second replicate block; see text).

(ii) The effect of trampling, at a single intensity, on the biomass of *C. vulgaris* stands of different ages

Plots (2m x 1m) of pioneer (3 years old) mature (7 years old) and degenerate (greater than 23 years old) stands of *C. vulgaris* were trampled at an intensity of 100p pl^{-1} on 04.07.74.. (Data for the building phase were extracted from c(i)). There were four replicates per phase. The plots were harvested on 05.04.76. together with 4 control plots from each phase. Plots 1m x 1m were harvested.

A similar trial was initiated on 09.07.75. in which four 2m x 0.5m plots of pioneer and four plots of building phase *Calluna* were trampled at an intensity of 100p pl^{-1}. These plots, together with control plots, were harvested on 05.04.76.. A harvesting plot size of 1m x 0.5m was employed. All material was dried at 85-95°C in a forced draught oven and weighed to determine biomass per square meter.

(d) Results

(i) Productivity of control stands of *C. vulgaris* in the Building phase (Figure 74)

There is a significant ($p < 0.001$) increase in the biomass of undamaged material over the period July 1974 to July 1975. Over the period July 1975 to April 1976 there is a slight decrease in biomass, although the decrease is not significant (Figure 74b). The productivity values (Figure 74a) have not been subject to analysis of variance since they have been calculated from the mean biomass values for the respective harvest dates. There is a slight decrease in productivity from the July to September 1974 period to the September 1974 to April 1975 period, but in the subsequent period April to July 1975 there is a marked increase in productivity to a maximum of 14.7g m^{-2} wk^{-1}. The 271 day period between

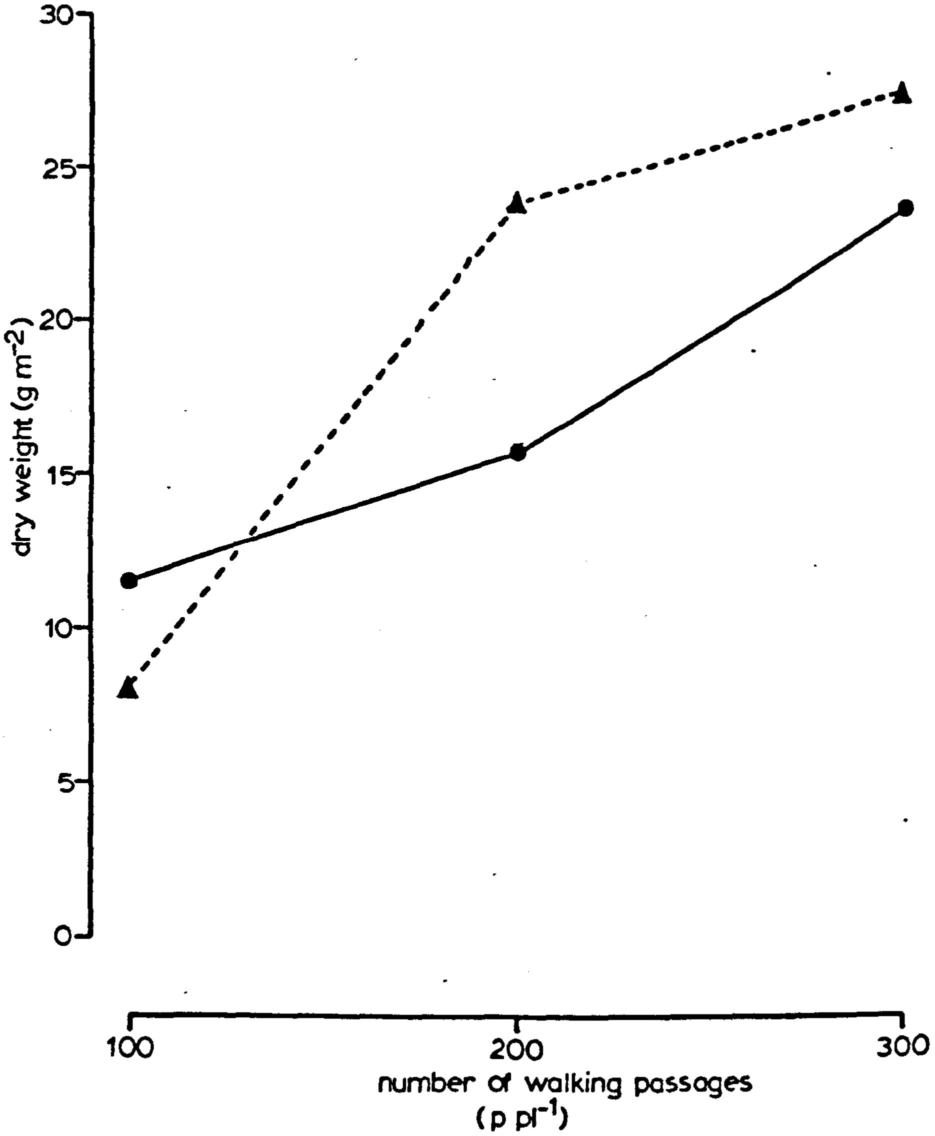


Figure 75 Relationship between trampling intensity and litter produced at the time of trampling an even aged stand of C.vulgaris, (building phase, Kerloch).

●——● block 2, treated 11.04.75

▲------▲ block 3, treated 09.07.75

July 1975 and April 1976 is accompanied by a marked decrease in productivity to a negative value indicative of a loss of material from the area which exceeds the photosynthetic input. This negative value is based upon the mean of eight replicate plots in contrast to the four replicates employed for previous sampling dates. Four of these plots were harvested from the upper portions of the slope on which the study area was located and four from the lower. However, even if the mean value for the four lower plots is employed, the resultant positive rate of production is very small, i.e. 0.4g m^{-2} wk^{-1} (open symbols, Figure 74). The period July 1975 to April 1976, giving rise to this low value for productivity, is longer than that employed in previous estimates over the period 1974-1976, including a considerable portion of the 'non-growing' period. This may account for an apparently erroneously low value for the rate of production although when compared with the 1974-1975 value for winter productivity, which included a smaller number of growing days, the 1975-1976 value is only 5.2% of that of the 1974-1975 period, which suggests site heterogeneity may be a factor of relevance in accounting for the low 1975-1976 winter productivity value.

The difference between years is re-emphasised when the annual production is considered. For the period July 1974 to July 1975 the primary productivity is 484.4g m^{-2} y^{-1} compared to 204.4g m^{-1} y^{-1} for the April 1975 to April 1976 period.

(ii) Litter produced on trampling a stand of *C. vulgaris* in the Building phase (Figure 75)

With increased trampling intensity there is a linear increase in litter produced ($r = 0.656$, $p < 0.05$; $r = 0.831$, $p < 0.05$ for Blocks 2 and 3 respectively). The increase on Block 2 is not significant but shows a similar trend to the significant ($p < 0.001$) increase on Block 3. An increase in trampling pressure from 100p pl^{-1} to 200p pl^{-1} results in a significant increase in litter on Block 3 and although litter production increases at higher pressures the increase is not significant, implying that immediate damage, involving stem breakage, attains a threshold value above which further trampling produces a proportionately lower increase in the quantity of litter. This might be expected since, with increasing intensity of use, there is a proportionate reduction in stems remaining to be broken, but the significant increase between 100p pl^{-1} and 200p pl^{-1} implies that litter production is not an all or nothing effect but is related to the intensity of trampling (as supported by the significant linear correlation coefficients). The values for Block 2 do not support the concept of a threshold effect but the relationship between trampling intensity and litter production does not yield any significant differences to either justify or invalidate the postulate.

The differences existing in litter production between Blocks 2 and 3 may reflect biomass differences between the blocks at the time of trampling i.e. Block 2 had a biomass 78.9% of that of Block 3. Thus, when the litter produced is represented as a percentage of the control plot biomass the proportions are 3.4% and 3.1% for 300p pl^{-1} for Block 2 and 3 respectively, and 2.3% and 2.7% for 200p pl^{-1}. This suggests that litter produced upon trampling is a biomass dependent variable in terms of absolute units, but that in terms of the proportion of litter produced, it may be a trampling dependent variable. The proportion of vegetation severed by trampling is comparatively small (3.4% at the maximum intensity employed) but because it results in a unidirectional source of error i.e. a decrease in biomass immediately after trampling which reduces the potential assimilatory capacity, it has been taken into consideration in calculating productivity.

(iii) The effect of trampling intensity on the Biomass of *C. vulgaris* in the Building phase (Figure 76)

Trampling results in a significant ($p < 0.01$) decrease in total and

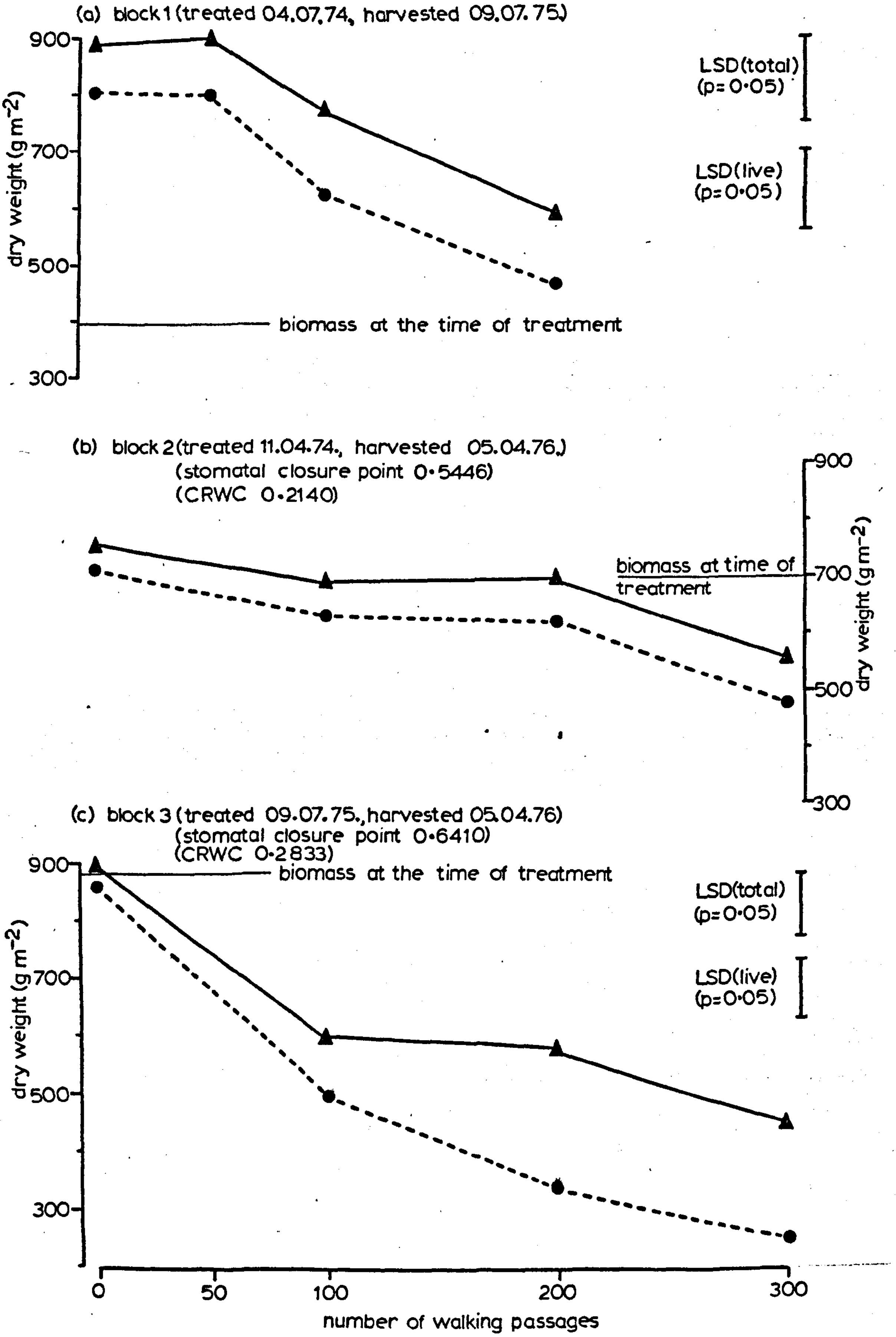


Figure 76 The effect of trampling intensity on the Biomass of a C. vulgaris stand, (building phase, Kerloch).

▲——▲ total biomass ●- - - -● live biomass live biomass of C. vulgaris on Blocks 1 and 3, and although a similar trend is found on Block 2, differences between treatments are slight and not significant.

The results of Block 1 indicate that a pressure of 50p pl^{-1} has no significant effect on biomass, the total biomass being slightly greater than that of the control. An intensity of 100p pl^{-1} results in a marked, but not significant, reduction in total biomass while a trampling pressure of 200p pl^{-1} is required before a significant reduction in biomass occurs. The live biomass shows a similar relationship with trampling intensity, although trampling at 100 p pl^{-1} and 200 p pl^{-1} results in a biomass which is significantly different from that of all other treatments (Figure 76a).

The results of Block 3, trampled at a similar time of year to that of Block 1, indicate that a pressure of 100 p pl^{-1} is associated with a significant reduction in total and live biomass but while further significant decreases occur at higher intensities of trampling, the relationship is curvilinear. There is also a differential response exhibited by total and live biomass for whereas 200p pl^{-1} has an inappreciable effect on total biomass compared to 100p pl^{-1}, the live biomass is significantly lower than that at a pressure of 100p pl^{-1} (Figure 76c).

The results of Block 2, however, trampled at the same intensities as Block 3 but in April 1975, indicate that although both total and live biomass decrease with increasing trampling intensity, the differential effect on total and live biomass apparent in Block 3 is not in evidence on Block 2 (Figure 76b). The decrease in biomass at a pressure of 300p pl^{-1} is also very slight compared to plots treated in July 1974 and 1975. Although the recovery period between treatment and harvest varied between the three blocks, which might have an effect on the biomass

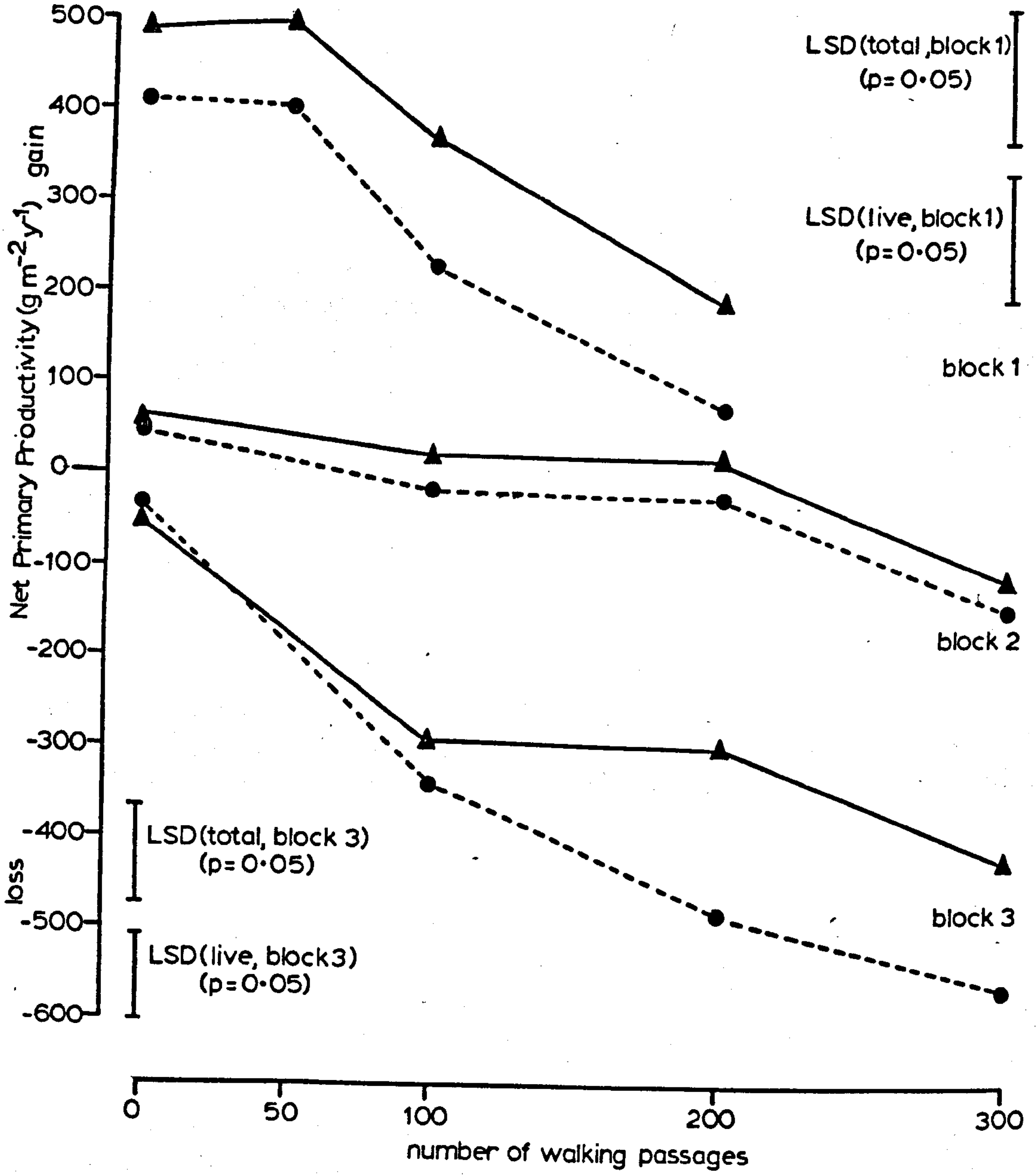


Figure 77 The effect of trampling intensity on net primary productivity of a C. vulgaris stand, (building phase, Kerloch).

▲——▲ total production
●- - - -● live production

of different treatments, significant treatment effects occur on Block 1 which has the longest recovery period. This suggests that time of treatment may influence the response obtained. This is supported by the significant ($p < 0.05$) time x treatment interactions obtained for total and live biomass when blocks 2 and 3 are compared. This implies that the effect of a particular trampling intensity on biomass is dependent upon the time of the year at which the treatment was applied.

It is also of note that, although differing in magnitude, the form of the response of total biomass to increasing trampling pressure is similar on Block 2 and 3, i.e. the effect of an increase in pressure from 100p pl^{-1} to 200p pl^{-1} is negligible on total biomass.

(iv) The effect of trampling intensity on the Net Primary Productivity of *C. vulgaris* stands in the Building phase (Figure 77)

(The absolute values for primary productivity should be regarded with a degree of caution owing to the possibly anomalous control values for material harvested on 05.04.76).

The values for Block 1 give a control productivity of 484.4g m^{-2} y^{-1}. A trampling intensity of 200p pl^{-1} results in a significant ($p < 0.01$) reduction in productivity to 191.3g m^{-2} y^{-1} (Figure 77), which is 39.5% of the control value. The productivity of live material existing at the final harvest at 200p pl^{-1} is more severely depressed to 72.9g m^{-2} y^{-1}, which is 17.8% of the control value (408.7g m^{-2} y^{-1}). Significant ($p < 0.001$) reductions in the productivity of Block 3 also occur but trampling has a small and non-significant effect on the productivity of Block 2. Were it not for the possibly dubious nature of the results, the relative productivities of the different treatments from different blocks might be of interest. The reduction in productivity on Blocks 1

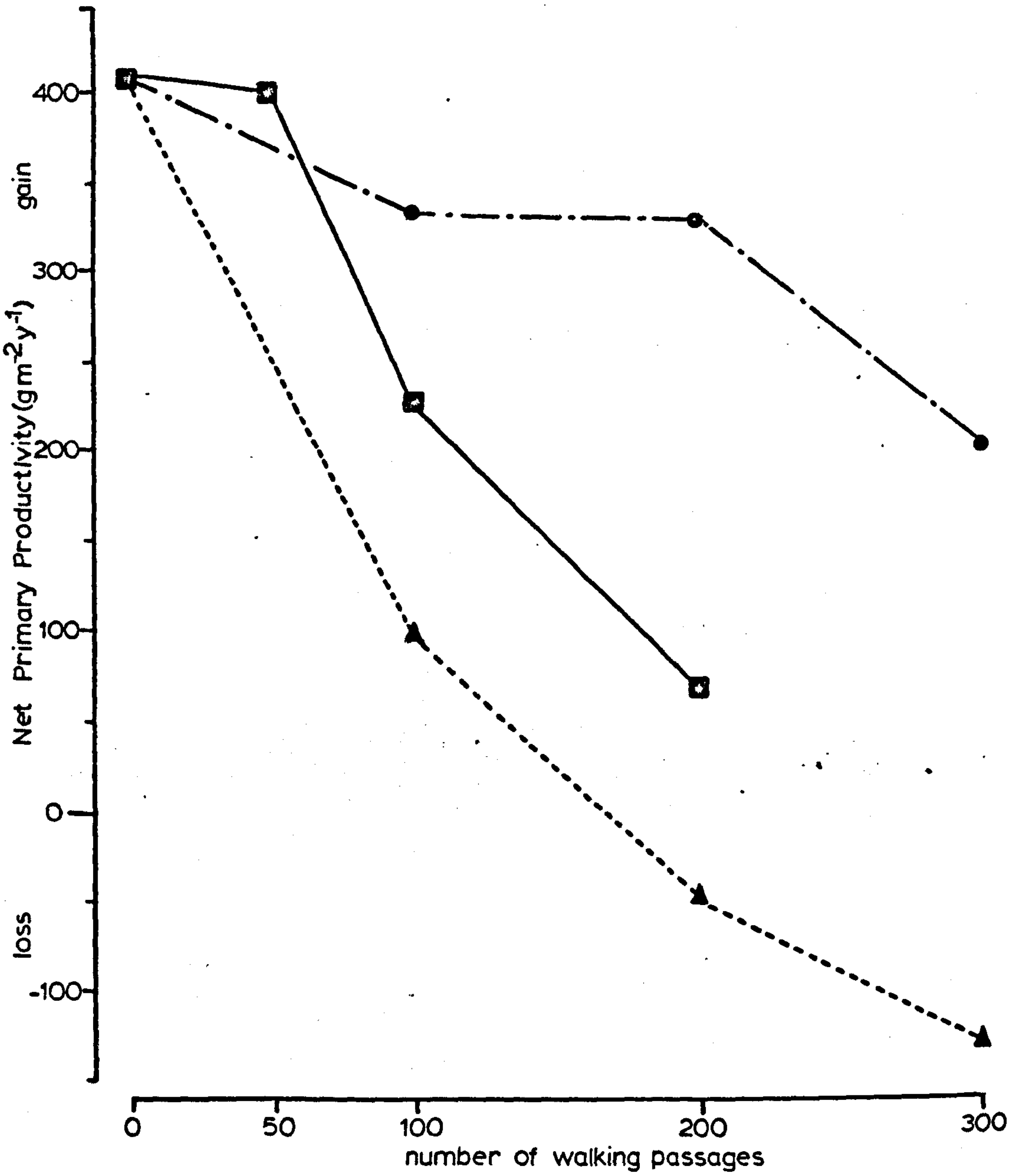


Figure 78 The relative effects of trampling intensity on the live net primary productivity of a C. vulgaris stand, (building phase, Kerloch).
(data for blocks 2 and 3 adjusted for comparability with block1)

■———■ block1, (treated 04.07.74., harvested 09.07.75.)
●-·-·-● block2, (treated 11.04.75., harvested 05.04.76.)
▲------▲ block3, (treated 09.07.75., harvested 05.04.76.)

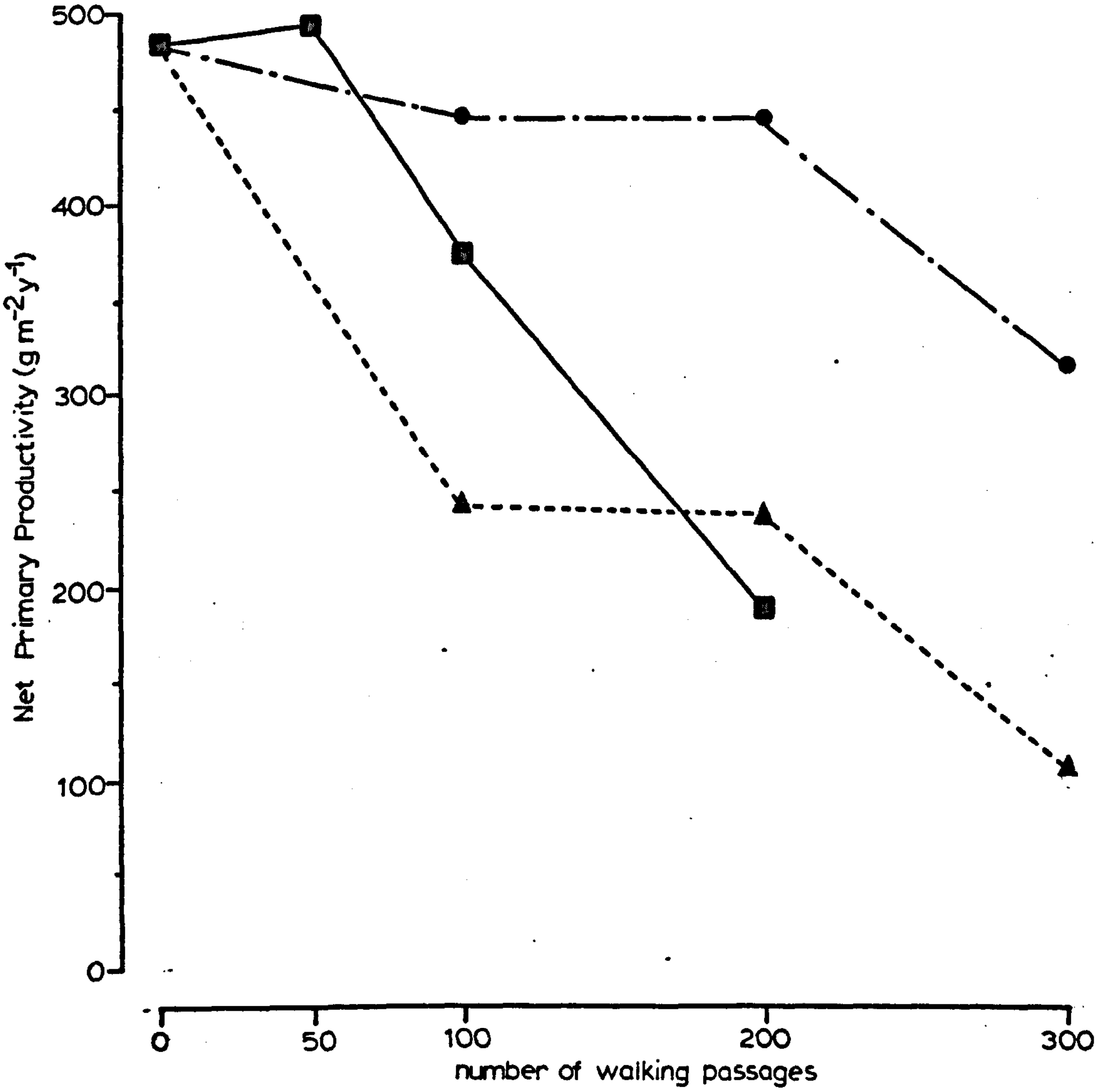


Figure 79 The relative effects of trampling intensity on the total net primary productivity of a <u>C.vulgaris</u> stand, (building phase, Kerloch).

(data for block 2 and 3 adjusted for comparability with block1)

■——■ block 1,(treated 04.07.74, harvested 09.07.75.)
●-·-·-● block 2,(treated 11.04.75., harvested 05.04.76.)
▲-----▲ block 3,(treated 09.07.75, harvested 05.04.76.)

and 3 at a trampling pressure of 200p pl^{-1} is comparable (293.1g m^{-2} y^{-1} and 248.1g m^{-2} y^{-1} respectively). Further comparisons are confused by the apparent negative productivities of the control plots of Block 3. If it is assumed that the productivity in 1975-1976 was comparable to that of 1974-1975 and the data adjusted accordingly[1](Figures 78 and 79) it is evident that the reduction in productivity by trampling on Block 1 and 3 is broadly similar. Despite comparatively high trampling pressures, productivity only becomes negative at an intensity of 200p pl^{-1} for live material on Block 3 (Figure 78). It otherwise remains positive for both live and total productivity for all treatments on all blocks (Figure 79). However, in real terms (Figure 77) a net loss of -425.2g m^{-2} y^{-1} at a trampling pressure of 300p pl^{-1} occurs. This is substantially greater than the loss from Block 2 at a similar pressure (-109.9g m^{-2} y^{-1}). However, although the control plots of Block 2 exhibit a slight positive productivity (52.9g m^{-2} y^{-1}), the comparatively low magnitude of this value suggests an error in productivity estimates.

Comparing real values for biomass at a trampling pressure of 200p pl^{-1} (Figure 76), a 49.0% increase occurs in biomass of Block 1 over the biomass existing at the time of trampling, compared to a 0.5% increase at the same pressure on Block 2 and a 34.1% decrease on Block 3. These values may not be totally unrealistic since visible damage at a trampling pressure of 200p pl^{-1} for Blocks 2 and 3 was 40.4% and 91.4%, indicating that the potential productivity of the former, although reduced compared to the control, is greater than the latter. Consequently, negative productivity estimates are not prohibited, although imply litter fall, plant decomposition and removal from the plots, but in the light of the values for Block 1, are questionable, especially in view of the control values.

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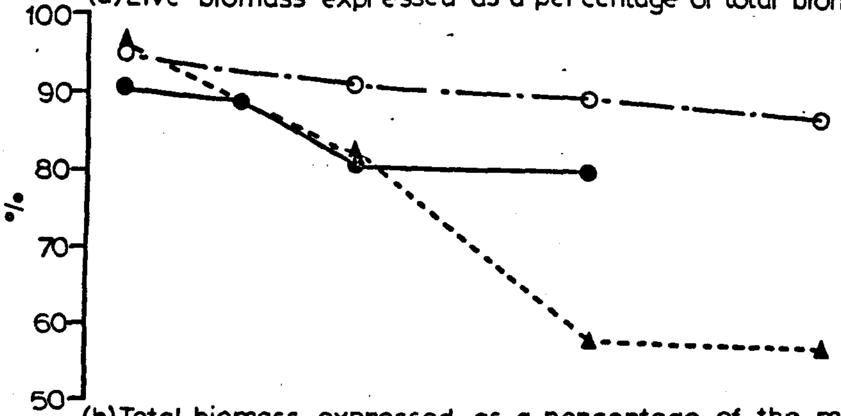
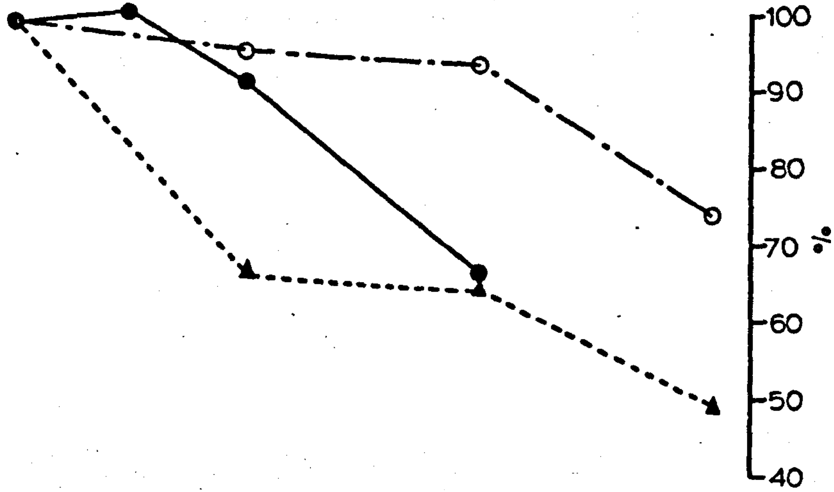
[1] data adjusted by the addition of the difference between control productivities of Block 1 and Block 2, and Block 1 and Block 3 to all treatments for the respective blocks 2 and 3.

(a) Live biomass expressed as a percentage of total biomass



(b) Total biomass expressed as a percentage of the mean control total biomass for each block



(c) Live biomass expressed as a percentage of the mean control live biomass for each block

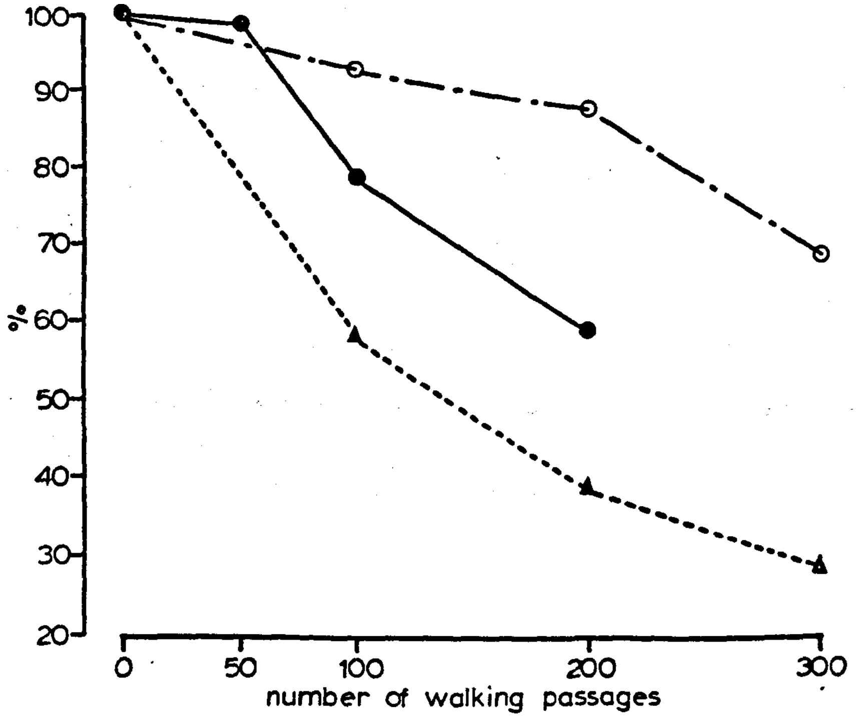


Figure 80 Biomass of C.vulgaris stands expressed as percentages to facilitate inter-block comparison of treatment effects, (Kerloch).

●———● block1, (treated 04.07.74, harvested 09.07.75.)

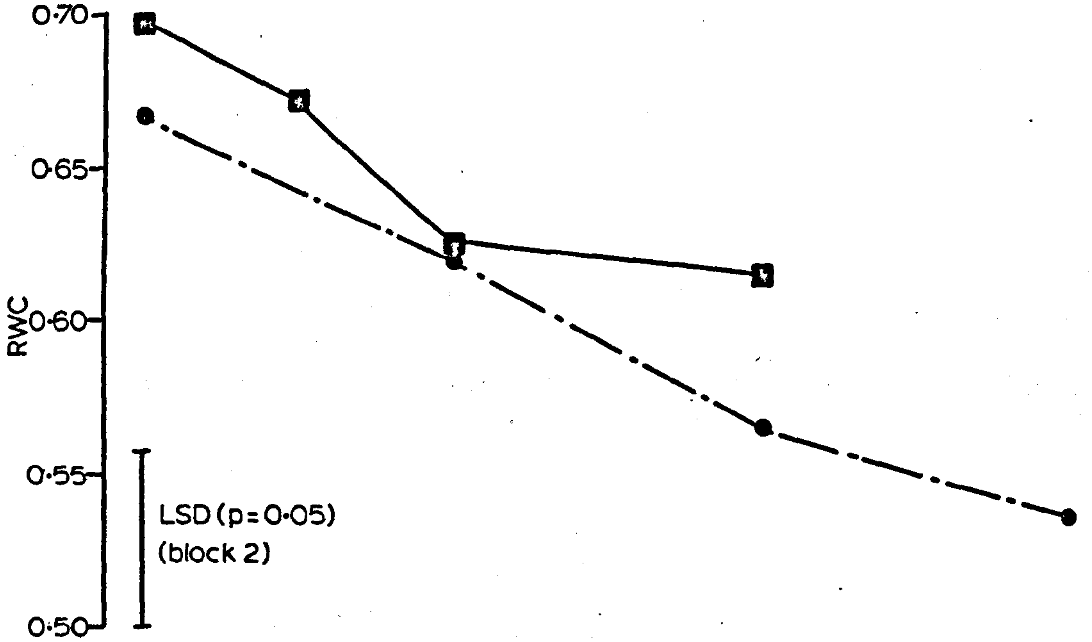
○-·—·-○ block2, (treated 11.04.75, harvested 05.04.76.)

▲------▲ block3, (treated 09.07.75, harvested 05.04.76.)

(v) The effect of trampling intensity on the biomass of *C. vulgaris* stands, represented in relative terms (percentages) for comparability (Figure 80)

The pattern of response (when treatment data are expressed as a percentage of the mean control value) is similar to that described above, a significant ($p < 0.01$) reduction in percentage total and live biomass occuring on Blocks 1 and 3 but no significant effect on Block 2 (Figure 80b, c). A pressure of 300p pl^{-1} reduces the percentage live material (on a dry weight basis) on Block 3 to 29.0% of the control value compared to 67.5% on Block 2. Such figures indicate a substantial reduction in potential assimilatory capacity of plants treated in July compared to April 1975, and is supported by the similar substantial reduction in live material occurring in stands treated in July 1974. These values represent the reduction in live material in relation to the control plot. In addition there is a percentage reduction in live material compared to total plot biomass (Figure 80a), which indicates the reduction in potential growing material compared to a plot totally composed of growing material. Since different trampling intensities have been employed, the blocks have not been compared statistically, but the percentage of live material existing on the control plots varies over a narrow range from 90.6% to 95.1%. The values at 100p pl^{-1} for Block 1 and 3 are also similar (80.3% and 82.1%) compared to Block 2 (90.9%). At a trampling intensity of 200p pl^{-1} a substantial difference exists between Block 1 and 3 (i.e. 79.2% and 57.1%) although both lie below the value for Block 2 (88.7%). These figures indicate that in absolute terms (Figure 80a) the potential productivity of the stand (with reference to a hypothetical plot with a 100% potential productivity) is substantially decreased by trampling, and that in relative terms (Figure 80c) the reduction is even more severe in comparison with the control plot. The data re-emphasise that the

(a) Shoot RWC



(b) Biomass (total)

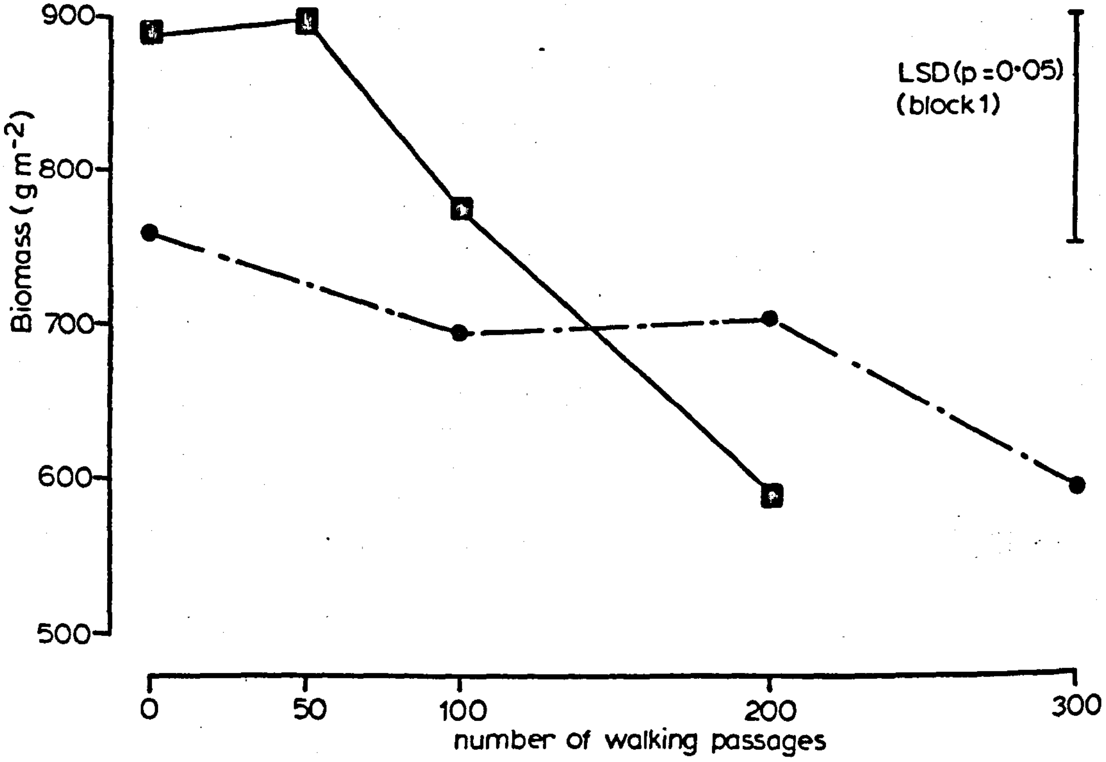


Figure 81 The effect of trampling intensity on shoot RWC and associated changes in stand biomass of C. vulgaris, (building phase, Kerloch).

■——■ block 1, (treated 04.07.74., harvested 09.07.75., RWC 04.09.74.)

●·—·—● block 2, (treated 11.04.75., harvested 05.04.76., RWC 13.07.75.)

effect is dependent upon the time of the year at which the treatment was applied (Block 2 versus Block 3) and possibly the longevity of the recovery period (Block 1 versus Block 3).

In absolute terms the reduction in productivity potential is translated into a greater real effect due to the decrease in absolute biomass as a result of trampling. For example, on Block 3, the live biomass is 4.9% lower than the total biomass for the control plots (857.6g m^{-2} compared with 901.2g m^{-2}). At a pressure of 200p pl^{-1}, the total biomass is substantially lower compared to the control, and the live component is only 57.1% of the total plot biomass (248.8g m^{-2} compared with 448.8g m^{-2}). Therefore, in addition to trampling affecting the percentage live material existing, the effect is compounded by the reduced total biomass existing so that the reduction in assimilatory capacity is greater than that implied by a consideration of the percentage proportion of live material existing on the plot at the time of sampling alone.

(vi) <u>The effect of trampling intensity on shoot RWC, and associated changes in stand biomass of *C. vulgaris*</u> (Figure 81)

The relationship between biomass and RWC indicates that, for Block 1, a decrease in RWC at 50p pl^{-1} is not accompanied by a trend for a decrease in biomass, but whereas a significant decrease ($p < 0.05$) in biomass is associated with further increases in trampling pressure, the RWC does not exhibit a similar significant decrease. In contrast, the data for Block 2 reveal a significant ($p < 0.05$) decrease in RWC with increased trampling pressure while biomass exhibits only a slight and non-significant decrease. There is no significant linear correlation between RWC and biomass for either block ($r = 0.215$; $r = 0.302$ for Blocks 1 and 2 respectively), thus, although there is a tendency for both RWC and biomass to decrease, they do not do so in linear proportions. This may be a reflection of the non-

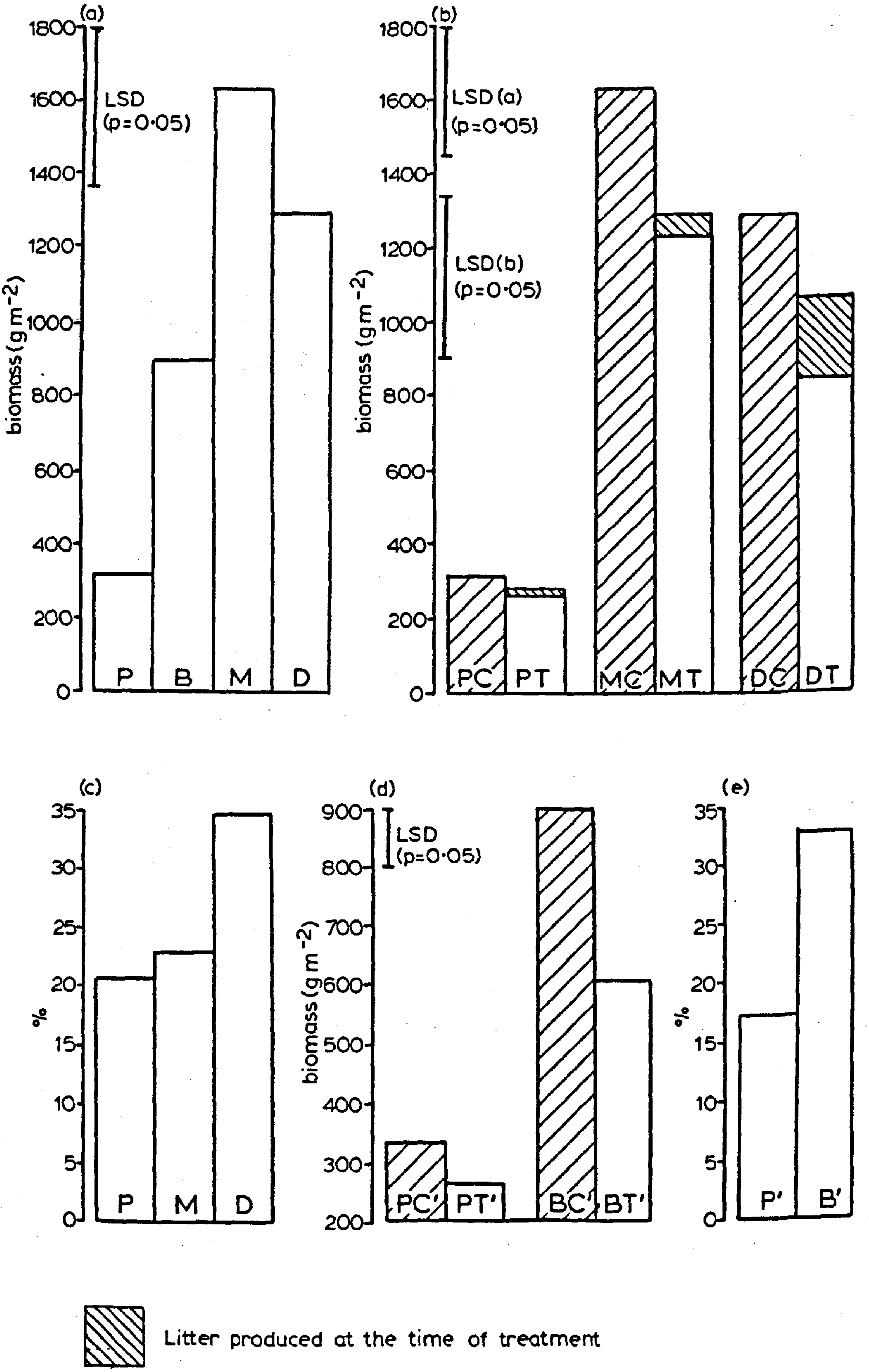

(a)
1800
1600
1400
1200
1000
800
600
400
200
0
biomass (g m $^{-2}$)
LSD (p=0·05)
P
B
M
D
(b)
1800
1600
1400
1200
1000
800
600
400
200
0
biomass (g m $^{-2}$)
LSD (a) (p=0·05)
LSD (b) (p=0·05)
PC
PT
MC
MT
DC
DT
(c)
35
30
25
20
15
10
5
0
%
P
M
D
(d)
900
800
700
600
500
400
300
200
biomass (g m $^{-2}$)
LSD (p=0·05)
PC'
PT'
BC'
BT'
(e)
35
30
25
20
15
10
5
0
%
P'
B'
Litter produced at the time of treatment

Figure 82 The effect of trampling (at an intensity of $100\ p\ pl^{-1}$) on the biomass of *C.vulgaris* stands in relation to the phase of the *Calluna* life cycle.

(a) Above ground biomass in relation to life cycle phase

P Pioneer phase

B Building phase

M Mature phase

D Degenerate phase

(b) The effect of trampling on total biomass

PC Pioneer phase - control

PT Pioneer phase - treated (04.07.74.)

MC Mature phase - control

MT Mature phase - treated (04.07.74.)

DC Degenerate phase - control

DT Degenerate phase - treated (04.07.74.)

(c) Percentage reduction in total biomass associated with trampling of *C.vulgaris* stands in relation to life cycle phase

(d) The relative effects of trampling on Pioneer & Building phases

PC' Pioneer phase - control

PT' Pioneer phase - treated (09.07.75.)

BC' Building phase - control

BT' Building phase - treated (09.07.75.)

(e) Percentage reduction in Pioneer & Building phase biomass associated with trampling of *C.vulgaris* stands at one intensity at one time

(All plots harvested 07.04.76.)

LSD(a) least significant difference between two means excluding litter component

LSD(b) least significant difference between two means including litter component produced at the time of treatment linear relationship existing between RWC and F_n (Section IVA, d(i)) which also changes seasonally, with the consequence that any influence RWC may have on biomass will be obscured by such changes together with changes in plant susceptibility to desiccation injury, death and stem severance.

(vii) The effect of trampling, at a single intensity, on the biomass of *C. vulgaris* stands of different ages (Figure 82)

The biomass (above ground) of control *C. vulgaris* stands exhibits a significant ($p < 0.001$) increase from pioneer to building phase to reach a maximum in the mature phase (Figure 82a). Thereafter, there is a decline in biomass of the degenerate phase, although the difference between the mature and degenerate phase biomass is not significant.

Trampling on stands of varying age is associated with a significant ($p < 0.001$) reduction in the biomass of mature and degenerate phases, and although the results for the pioneer phase trend in the same direction the difference is not significant, (Figure 82b). The difference is smallest in the pioneer phase (73.9 g m^{-2}), increasing in the mature phase (410.4 g m^{-2}) to a maximum in the degenerate phase (454.0g m^{-2}).[1] (Comparing the results of each phase individually, the pioneer phase also experiences a significant decrease in biomass associated with trampling.) The lack of significance may be due to the increasing variance for each phase (i.e. standard errors of ± 20.3g m^{-2}, 284.3g m^{-2} and 313.8g m^{-2} for pioneer, mature and degenerate phases) in which the within treatment variance for the older stands, in being greater than the treatment variance of younger stands, is obscuring real differences in younger phases. The situation revealed in Figure 82b, is modified when the litter produced at the time of trampling is added to the harvest data.

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[1] data for the equivalent building phase is missing due to plot destruction.

Although significant ($p < 0.001$) differences remain, such differences are due to biomass differences between the Calluna phases. Trampling is no longer associated with a significant reduction in stand biomass, although a trend for a lower biomass in trampled plots remains. Part of the trampling effect on stand biomass may therefore be associated with a decrease in biomass of the stand due to stems broken off at the time of trampling. The differences between control and trampled plots are reduced when the litter fraction is taken into consideration (i.e. 72.2g m^{-2}; 344.9g m^{-2} and 245.9g m^{-2} cf. above). The most marked reduction in biomass shifts from the degenerate to the mature phase, which results from differential litter production on the three phases, increasing from 1.7g m^{-2} in the pioneer phase to 65.5g m^{-2} in the mature phase and 208.2g m^{-2} in the degenerate phase ($p < 0.001$, L.S.D. 56.3g m^{-2}).

The percentage reduction in biomass associated with trampling (i.e. the reduction in biomass as a percentage of control biomass) (Figure 82c). indicates that trampling has a more severe effect on biomass as the age of the stand increases, although the differences are not significant.

The results of a similar trial performed on the pioneer and building phases (and harvested after 9 months) (Figure 82d) indicate a marked reduction in biomass of both phases on trampled plots, which is significant ($p < 0.01$) in the building phase. The reduction of 17.3% in the pioneer phase is comparable with the 20.6% reduction noted above for a pioneer phase, despite differences in the length of the recovery period (i.e. 9 months versus 21 months). The reduction in biomass of the building phase of 299.1g m^{-2} is considerable, representing a 33.1% decrease over the control value (Figure 82e).

Owing to the absence of certain data relating to the building phase it is not possible to ascertain whether the length of the recovery period influences the effect of trampling on stand biomass in relation to the phase of the life cycle of the stand being treated. The data for the pioneer phase suggests that no marked difference in the effect of trampling of biomass occurs in relation to the longevity of the recovery period.

(e) Discussion

(i) Productivity of control stands of *C. vulgaris* in the Building phase

The biomass data of July 1975 compare favourably with values for a 6 year old stand of *C. vulgaris* in the same area but 148m lower in altitude (Miller, 1972). Although the biomass data are at variance with those given by Barclay-Estrup (1970), the productivity values for the 1974-1975 period of the present investigation compare favourably with the productivity values of a 9 year old stand of *Calluna* in the building phase (Barclay-Estrup, 1970) but the values given by Chapman *et al*. (1975a) for an 8 year old stand in Dorset are approximately half those found in the present study.

As noted above, inter-site comparisons are confused by the management history of the stand. The present area is managed by burning and thus passes into a 'post-burn' rather than true pioneer phase (cf. Chapman *et al*., 1975a), consequently, biomass comparisons will be influenced by the regeneration rate from underground stems and rootstocks and thus not directly comparable with the area investigated by Barclay-Estrup (1970) which had not been burnt for approximately 100 years. However, while net productivity for the 1974-1975 period is in agreement with estimates of Barclay-Estrup (1970), it is at considerable variance with a slightly older stand in the same locality (Miller & Miles, 1969) although the 1975-1976 data, giving an annual productivity of 204.4g m^{-2} y^{-1} compares with the 217g m^{-2} y^{-1} given by Miller & Miles (1970), 221.0g m^{-2} y^{-1} for a similar aged stand given by Chapman _et_ _al_. (1975a), and 212.0g m^{-2} for yearly production given by Moss & Miller (1976) for a moorland site also in the Dee valley.

At an age of 5 years, the biomass of the stand in the present investigation is comparatively low, comparing more favourably with biomass data for the pioneer phase of Barclay-Estrup's (1970) study. However, the productivity over the 1974-1975 period is comparable with that of Barclay-Estrup's 'building' phase. This apparent differential response in terms of biomass and productivity may result from 'post burn' development in which, following an initial period of rapid growth, litter fall balances the rate of production resulting in a stabilisation of biomass over the following few years followed by an increase in biomass when productivity exceeds litter fall (cf. Chapman _et_ _al_., 1975a). The apparent reduction in productivity over the 1975-1976 period compared to that of the previous season, may result from a substantial production of litter counterbalancing the gross production. The situation is confused to the extent that although 12 month periods are under consideration the disposition in the seasonal cycle differs. The 1974-1975 period includes two parts of different growing seasons, separated by a winter period but the 1975-1976 period encompasses a single growing season i.e. that of 1975, and a winter period, in which the investigation was terminated prior to the onset of the 1976 growing season.

The data for the April 1975 to July 1975 period indicate maximal productivities in this part of the year, followed by a decrease over the remaining portion of the growing season. In comparing the 1974-1975 and 1975-1976 periods it is assumed that both include only one such period of high productivity. However, if the 1974-1975 period of investigation commenced prior to the spring flush of growth in 1974 but terminated after this period in 1975, an error would result in comparing it with the 1975-1976 data which included only one growing season.

A further anomaly in the data is the apparently high rate of production for the 1974-1975 winter period (September 1974 to April 1975) which may reflect growth between the beginning of September and the end of the 1974 growing season. Although the growing period has been defined as the period in which temperatures are in excess of 7.2°C, this may not be a valid parameter to employ. Possible acclimatisation to lower temperatures at higher altitudes (cf. Gimingham, 1972) may allow assimilate utilization for growth at temperatures lower than 7.2°C which, together with assimilate accumulation at temperatures too low to allow utilization, may result in an increase in biomass. However, these estimates do not take into account the role of litter production, the peak of which occurs in the September to April period (Cormack & Gimingham, 1964). Chapman _et_ _al_. (1975b) calculated that for a 9 year old stand of _Calluna_ in Dorset, the litter produced represented 5.4% of the above ground biomass and 18.2% of the above ground productivity. This increased to 13.6% and 64.8% respectively for a 15 year old stand. Although these may be above average values (Chapman _et_ _al_., 1975a), appreciable quantities of litter are produced on Kerloch. Cormack & Gimingham (1964) give a value of 16.16g m^{-2} y^{-1} for a mature stand on Kerloch at 152m in altitude (compared to 42.0g m^{-2} y^{-1} and 184.0g m^{-2} y^{-1} for stands quoted by Chapman _et_ _al_., 1975b). It is apparent that if litter production had been evaluated in the present study, the rate of primary production might be altered. It is conceivable that for the 1974-1975 period litter production over winter might have been inhibited. The late winter fall of flower capsules (Cormack & Gimingham, 1964) may have been prevented by snow cover since Gimingham (1972) noted that snow cover in February and March has been found to inhibit litter production.

Following the spring flush of growth between April and July 1975, further increase in biomass is very slight and may be negative, which implies a greater litter production than gross productivity over the period July 1975 to April 1976. This is a possibility if production following the spring flush of growth is channelled primarily into seed production, since the part of the annual productivity represented by seed capsules would be shed over winter, which, together with leaf litter production, may reduce the biomass below that existing in July. The net primary productivity for the stand from April 1975 to April 1976 is comparable to the data presented by Miller (1972) although considerably lower than the 1974-1975 estimates.

In addition to the effects of litter fall on annual net production, environmental conditions experienced over the period July 1975 to April 1976 may have limited the rate of production. Available water may have been limiting in August and October and although the total rainfall received in July was above average it fell in intense thunderstorms which may have affected its availability to the plant (cf. Section II (b)). Similarly, above average temperatures were experienced in July and August, which together with moisture unavailability may have accelerated litter production and retarded growth, so reducing net production.

Such explanations must remain tentative and heterogeneity of the biomass on the site is another, possibly more likely, reason for these anomalies in production rates.

The age related pattern of biomass in different phases is similar to that observed by Barclay-Estrup (1970), with the peak biomass occurring in the mature phase, but contrasts with the findings of Miller (1972) and Chapman _et al_. (1975a). The decrease in the biomass of the degenerate phase is consistent with the opening out of the centre of individual plants, leading to large spaces in the canopy (Barclay-Estrup & Gimingham, 1969). In terms of actual biomass present, the results are similar to those given by Miller (1972), for stands of approximately equivalent ages, for the 'building' and 'mature' phases but the relationship diverges for older 'degenerate' stands. Miller observed a slight but continued increase in biomass to a maximum at an age of 37 years compared to the decrease in biomass of degenerate stands observed in the present study. Thus, in contrast to the Gompertz type growth curves employed by Chapman _et al_. (1975a) to describe the data from a Dorset heath, the present data conform to the productivity relationships describing a 'nearly ideal cyclical process' (Barclay-Estrup, 1970). In view of Miller's observations (1972), the possibility remains that the data for the degenerate phase are atypical, resulting from the considerable sampling error which may occur when harvesting old stands of _C. vulgaris_ (Chapman _et al_., 1975a) such that the conclusion made regarding the expression of a cyclical process may be unjustified.

(ii) Litter produced on trampling a stand of _C. vulgaris_

The amount of litter produced would appear to be a function of the trampling intensity and plot biomass at the time of trampling while the proportion of material removed by a particular intensity of trampling remains relatively constant irrespective of plot biomass.

While the relationship between litter production and trampling intensity is linear in the building phase, litter production also increases with increasing age of the stand. These observations may be related to the degree of lignification of the stem which may determine the yield stress of the stem to an externally applied force. Although the absolute amount of litter produced will depend on the biomass of the stand, the proportion of the biomass that the litter represents would appear to increase with stand age. Possibly the younger stems are more pliable and less prone to snapping than older, thicker, more heavily lignified stems, consequently, when trampled they are elastically deformed and return to their original shape until a point of plastic deformation is reached and this no longer occurs, the stem remaining permanently distorted. In the present study it has not been possible to ascertain whether the yield stress of stems of varying ages is quantitatively different, which might account for the relationship between age and litter production. Similarly, it has not been feasible to determine whether, for an even aged stand, the relationship between litter production and trampling intensity is related to the increasing frequency with which a stem is trodden or whether it is due to variation in the yield stress of the stems composing the stand. The observation that the stem does not immediately break but may require a number of impacts to induce it to snap implies a certain degree of elasticity which may not be present in older, more brittle stems. However, opposed to this postulate are observations regarding the value of strengthening tissues in increasing tolerance to mechanical disruption. Cutter (1969) noted that mechanical agitation of plants for long periods resulted in an increase in wall thickness of collenchymatous tissue to aid mechanical support. Shearman et al. (1975c) stated that sclerenchyma fibres enable plants to withstand pressure from bending, stretching and compression without undue damage to softer, thin walled cells and considered that fibres may be of value in protecting plants against mechanical damage, such as that resulting from trampling. On the basis of leaf tensile strength and lignin content on a cross sectional area basis, correlations with the wear tolerance of a number of graminaceous species led them to conclude that the results indicated "... an excellent association between inter-species turf grass wear tolerance and the percent sclerenchyma and lignified tissue". The sensitivity of woody stemmed species noted by a number of workers e.g. Goldsmith et al. (1970), Bayfield (1971), Dale & Weaver (1974) suggests a point may be reached at which increased lignification becomes decreasingly advantageous as it renders the stem liable to an increasing possibility of breakage rather than bending, although in helping to maintain the rigidity of the stem and prevent damage from compression, lignification maybe of some benefit.

The severence of stems increases the susceptibility of woody stemmed plants to desiccation and infection (cf. Miller & Miles, 1970) which may be of greater significance in slow growing plants such as C. vulgaris than in rapidly growing herbs, owing to the lower capacity for repair and regeneration of damaged tissue. In species such as C. vulgaris this situation is accentuated by the observation that it is often the leafy, actively photosynthetic shoots which are severed so reducing the plant's photosynthetic potential.

Stem severence is considered by Bayfield (1971) to be one of the most serious effects of disturbance when C. vulgaris is trampled, but although the wear tolerance may be low, stem severance only reduces the biomass at the time of trampling by about 5%. However, this, together with subsequent tissue death resulting from crushing, tearing and bruising, which may develop over the months following trampling, will be compounded to reduce the assimilation capacity of the plant to a greater extent than is implied by the detachment of stems alone. A corollary of this observation is that in the assessment of 'recuperative potential' of the plant following trampling (Canaway, 1975a) a sufficient period must be allowed for all forms of damage to be manifested to enable an adequate assessment to be made.

(iii) The effect of trampling on the Biomass and Productivity of C. vulgaris

Trampling reduces the biomass of C. vulgaris due to detachment of stems, stripping of stems, and breakage and bruising of tissues which may result in shoot death and litter production (cf. Bayfield, 1971). The results of Block 1 suggest that light trampling pressures do not have a detrimental effect on biomass, which may be due to an ability to tolerate trampling to a certain degree before damage becomes apparent. Kellomaki (1973) observed that Vaccinium myrtillus and V. vitis-idaea had a higher trampling tolerance than that of herbs although lower than the tolerance of grasses, which suggests that woody stemmed plants may be able to withstand a certain amount of pressure without detrimental effect. The response observed, in which a threshold effect is apparent (Figure 76), contrasts with the lack of such an effect in grass and herb species observed by others e.g. Edmond (1958), Wagar (1964). However, the significant increase in visible damage of plants trampled at 50p pl^{-1} (Figure 58) suggests that the undamaged leaves are able to compensate in terms of the loss in assimilation capacity of damaged leaves or that the methods employed are not sufficiently sensitive to enable the effects of moderate desiccation injury on production to be ascertained. It may also be of some significance that no threshold effect was observed in the relationship between visible damage and trampling intensity. The paradox between apparent death, as evidenced by visible damage, and the lack of a significant effect on biomass is further illustrated by the results of Block 2 (Figures 58 and 76). This may result from the comparatively high tolerance of buds to desiccation damage prior to the onset of the growing season, (Iljin, 1957) i.e. in April when the block was treated, so that although the previous season's leaves were damaged, the buds were able to develop and photosynthesise normally. Since the younger tissue represents the major assimilatory capacity of the plant (Grace & Woolhouse, 1970), productivity in plants damaged before the onset of the growing season may be relatively unimpaired. Consequently, depending upon the degree of litter fall between treatment and harvest, the biomass may be relatively unaffected if little dead material has fallen from the shoot. However, this latter argument is not supported by the observation that the live material existing on the treated plots of Block 2 is comparable to the control values.

Damage to the apical buds by trampling may stimulate the development of buds further down the shoot which, as a consequence, may increase canopy density and possibly Leaf Area Index (cf. Mohammed & Gimingham, 1970) thus increasing the potential photosynthetic capacity of the stand, providing the water deficits induced as a result of trampling do not outweigh such possible increases. This may also result in an increase in the ratio of green to woody material (cf. Grant & Hunter, 1966) or green to dead material, possibly partly explaining the unimpaired ratio of living to dead material on Block 2. More severe damage may result in the development of sprouting clusters from buds protected by the stem (Grant & Hunter, 1966; Miller & Miles, 1970; Mohammed & Gimingham, 1970) and consequently increase the photosynthetic capacity of the plant, but the development of buds requires a favourable water economy to provide the forces for elongation (Hsiao, 1973).

The susceptibility of C. vulgaris to trampling induced water stresses introduces a time factor into the effect of trampling on biomass as implied by the significant time x treatment interactions in the present study. Plants trampled following the onset of the growing season and at a time when desiccation tolerance is comparatively low might incur injury to new growth and, even if the hormonal relationships of the shoot were shifted in a manner conducive to the development of undamaged, dormant buds, the shoot water relations may be unfavourable for their development. This might be expected to result in a reduction in biomass compared to the control and, if harvested prior to the onset of the following growing season, may, as a result of litter fall and a reduction in photosynthetic capacity of viable tissue as a result of water deficits, result in a biomass lower than that existing at the time of trampling, as indicated by the results of Block 3 (Figure 76). This suggests that in mid-growing season, when desiccation tolerance is low and when water deficits are likely to have a maximum impact on photosynthetic capacity, a trampled plant has a low 'recuperative potential' whereby the ability to repair and regenerate damaged tissues is slight or non-existent. This suggestion is in agreement with that of Grant & Hunter (1966) regarding the more severe effects of summer clipping on C. vulgaris due to the interruption of the summer growth period and short recovery period compared to winter clipped plants. The removal of floral buds or inhibition of their development may retard photosynthesis (cf. Grace & Woolhouse, 1970). Although the increase in photosynthesis associated with flowering may be associated with assimilates being employed primarily in the flowering process, excesses of assimilate over that required may be utilized elsewhere in the plant. Consequently, any reduction in flowering potential as a result of trampling may result in a comparative reduction in productivity compared to control plants. The difference between control and treated plot biomass will be exacerbated if the control shoots, at the time of harvesting, bear appreciable quantities of unshed flower capsules.

The response of Block 1 in which a slight increase in biomass occurred relative to that of the control plots, may be related to the period of growth prior to harvesting in the growing season following that in which the plot was treated. The re-establishment of a favourable water balance, as suggested by the observations of bud break on apparently dead shoots, may allow a certain degree of compensation in terms of biomass by trampled plants. The development of a denser canopy through development of sprouting clusters of buds on stems may permit a degree of over compensation resulting in a greater biomass of lightly trampled plots, although at higher trampling pressures, the degree of compensation would have to be large to recompense losses from leaf stripping, tissue death and stem severance. The possible development of an increased number of meristemmatic regions in trampled plants may also result in an elevation in net photosynthesis at bud break over that of the control due to the increased number of sinks for assimilates (cf. Neales & Incoll, 1968).

In comparison with plants trampled while in a state of considerable physiological activity, those trampled prior to the onset of the growing season are damaged less severely. The reduction in photosynthetic surfaces, as indicated by visible damage estimates (Section IIIC, 4(b)), is less marked and the tissues are also more tolerant of desiccation, so that the tissues are likely to retain a greater degree of physiological integrity than plants trampled when the tissues are in a greater state of physiological activity. There is no interruption in the growing season so that if a favourable water balance can be restored prior to the onset of growth, or new tissues preferentially supplied with water (cf. Catsky, 1962) growth may occur relatively unimpaired. The observation that productivity is relatively unimpaired on plots trampled in April is consistent with the observation by Grant & Hunter (1966) that winter clipping is less severe in its effects than summer clipping. Miller & Miles (1970) observed that heather clipped in April had a greater regenerative capacity than that clipped in October due to a greater number of sprouting centres and increased rate of growth after clipping. The material clipped in October was retarded in regenerating due to desiccation damage, frost injury and infection. The present study has not investigated the repercussions on plant survival for stands trampled in autumn but Miller & Miles' (1970) observations suggest that plants trampled in July may be more liable to winter injury owing to the shorter period for repair of trampling damage thereby allowing shoot desiccation and providing entry portals for infections compared to material trampled in April.

The differential response exhibited by stands of C. vulgaris to trampling as indicated by biomass, reinforces the tentative suggestion made regarding the seasonal changes in susceptibility of C. vulgaris to trampling and its ability to regenerate following damage.

The non-linearity of the response between trampling intensity and biomass at lower trampling pressures indicates a degree of trampling tolerance at the stand level, while the results of trampling at higher pressures indicate a saturation of the response, also observed by Allcock (1973) on grass turves, and suggested by the curvilinear relationship observed by Wagar (1964). It would appear that most of the observed damage occurs over a small range of trampling pressure, which may reflect the yield stress of the stems being trampled, above which an increase in pressure results in a disproportionate increase in damage. In this respect the threshold pressure reducing biomass by 50% is greater than the pressures applied to Blocks 1 and 2, although a pressure of 300p pl^{-1} reduced the total biomass of Block 3 by 50.2% and the live biomass by 70.9% compared to 25.9% and 32.5% respectively for Block 2. Although above the estimates producing a 50% reduction in biomass for similar sites in N.E. Scotland (Leney, 1974; cf. Liddle, 1975b), the results demonstrate the seasonal variation in the carrying capacity of the stand as a consequence of variations in susceptibility to damage and recuperative potential.

A comparatively minor effect compared to those discussed above may occur in a trampled stand. C. vulgaris is adapted, by virtue of its omnidirectional leaf orientation, to receiving light more efficiently from a diffuse light source (Grace & Woolhouse, 1970). Grace & Woolhouse (1973a) commented that "At all solar angles, leaf clusters show more efficient light distribution patterns than a continuous canopy", consequently, the opening up of a continuous canopy by trampling may enhance the potential photosynthetic capacity of the plant, by changing the canopy structure from a continuous to a clustered form, under light limiting conditions. Thus, more prostrate individuals may benefit from an improved radiation balance and shoots arising from stem bases may receive sufficient light to grow. Such an effect is likely to be of greatest significance at low trampling intensities where trampling opens up the canopy but does little damage to individuals. The effect is likely to be of short duration since the development of such clusters will eventually lead to mutual shading and light limitation of photosynthesis but while operational, will enhance the recuperative potential of the stand.

While biomass data give an instantaneous indication of the effect trampling is having on a specific stand, the primary productivity gives a more useful indication of vegetation vulnerability, especially in a species in which the stand may yield a considerable biomass but which is composed primarily of dead material. Burden & Randerson (1972) suggested that the trampling pressure at which the standing crop of a particular component of a sward becomes zero represents the 'trampling capacity' of that component, while Liddle (1975b) noted that evidence suggests a relationship between vulnerability of the vegetation and its primary productivity. The point at which net production becomes zero and biomass remains constant represents a situation of stability. Obviously, gross production must be positive to counterbalance losses due to death and other forms of destruction, and for a stable situation (i.e. $P_n = 0$) the gross production must increase with increasing damage resulting from increased trampling pressure. When net production becomes negative the stand, from the point of view of the species under consideration, has entered into degeneracy.

The present results do not permit these relationships to be fully explored since the net productivity of Block 1 does not reach zero over the range of intensities employed while the data for Block 2 and 3 may be erroneous. However, the pressure required to reduce net productivity by 50% on Block 1 is approximately half that required to reduce biomass by 50% i.e. approximately 170p pl^{-1} and 300p pl^{-1} respectively. The high levels of visible damage on Block 3 imply that at higher trampling pressures, negative productivity may occur so that the productivity of viable tissue is insufficient to compensate for litter loss, stem severance and leaf stripping, even though appreciable quantities of live material, in dry weight terms, remain at the highest pressures employed. There is a suggestion that visible damage and productivity may be related since the order of the blocks at a trampling pressure of 200p pl^{-1} is the same for both parameters. Such a relationship would not be unexpected in as much as an increase in visible damage reflects a reduction in potential photosynthetic capacity but the relationship is likely to be modified by a complex of factors, not least of which is water stress. Seasonal variations in the relationship between water stress and desiccation injury (Section IIIA, 1(d)) and water stress and net photosynthesis (Section IVA, d(iii)) makes it problematical to quantify the relationship between visible damage and productivity due to the multifactorial nature of the relationship.

(iv) The effect of trampling on the 'live' biomass of a *C. vulgaris* stand

The live material existing at any one instant gives an indication of the potential productivity of the stand since, although the biomass may be substantial, if none of it is living the productivity capacity of the stand would be zero. The generally low rate of growth of *C. vulgaris* may be regarded as a limitation to its recuperative potential, which, if expressed in growth terms, is liable to seasonal variation. Bayfield (1971) stated that "*Calluna* regrowth after trampling appeared to be extremely slow and the amount of live heather at the final examination ....represented a considerable reduction on that present before trampling..." (in terms of cover). This comment is endorsed by the findings of the present study.

The results of Block 2 indicate no significant reduction in potential productivity, as indicated by live biomass, as a result of trampling in contrast to the observations on Blocks 1 and 3. On Block 1 the lack of any reduction in live material at a pressure of 50p pl^{-1} compared to the control, is consistent with the lack of a reduction in biomass and production, but does not support the concept of an increase in green to dead material implied by visible damage estimates, which would require an increase in the percentage of live material or an increase in the efficiency of the remaining photosynthetic tissue accompanying a decrease in the percentage of live material. At higher trampling pressures the decrease in the percentage of live material is consistent with a decrease in productivity reflecting a decrease in the photosynthetic capacity of the stand. Although the decrease in percentage of live material levels out at higher trampling intensities, the decreases in total biomass imply a continued reduction in potential productivity. The greater percentage of live material at a trampling pressure of 200p pl^{-1} on Block 1 compared to Block 3 may reflect the contribution spring growth made toward the biomass on Block 1.

The percentage of live material on a dry weight basis is, however, inconsistent with the observations on visible damage. On block 3 at a pressure of 300p pl^{-1}, over 90% of the plot is visibly dead (Figure 58) but on a dry weight basis only 43.9% of the plot is dead. This may reflect a lack in the sensitivity of the methods employed. The point quadrat measurements of visible damage do not take into account the lower levels of the canopy, which may be the most productive when the upper levels have been injured by trampling. Similarly, the dry weight data do not separate productive leaves from stems. Stems may account for an appreciable proportion of the total dry weight of the plant e.g. up to 62% in a 12 year old stand (Miller, 1972). Consequently, since visible damage was based on leaves alone while the dry weight proportions included stems, the two estimates are not strictly comparable. Chapman *et al.* (1975a) calculated that a 20% loss in dry weight occurs when a green shoot turns brown owing to reabsorption of materials prior to shedding. If a large proportion of the stand is dead this may involve appreciable weights and may result in a significant alteration in the dry weight ratio of green to dead material not paralleled by a change in visual composition of the sward until dead material is shed.

(v) Relationships between productivity and water stress

In addition to the direct effects of mechanical damage on productivity, water stresses which occur in *C. vulgaris* when it is trampled may be expected to limit productivity, if not through a direct effect on photosynthesis possibly via an inhibition of leaf expansion (cf. Hsiao, 1973). Boyer (1968, 1970b) noted an appreciable reduction in leaf expansion of *Zea mays*, *Glycine max* and *Helianthus annus* at a comparatively slight water stress (a ψ of -0.4MPa) while prolonged desiccation might result in leaf senescence, death and loss of pre-existing photosynthetic surface (Runyon, 1934; Boyer, 1976).

The lack of a significant relationship between water stress and productivity possibly reflects a more complex situation existing between productivity and trampling than that involving water stress only. The development of a significant water deficit on Block 2, which may be translated into a larger stress in terms of ψ_x (see Section IIIB, 3(c)), is not associated with a significant reduction in yield. This may illustrate the dubious value of using a comparatively small number of replicates per plot for determining RWC and extrapolating these findings to yields on a plot basis. In addition, Hsiao (1973) cautioned the use of absolute values of ψ as indicative of water stress since adaptation by the plant to its growing environment could influence the level of ψ at which stress begins. Such changes imply that the effect of RWC on growth is variable, tissues in a physiologically active state being more sensitive to slight water deficits than dormant tissues,as suggested by the differential responses of Blocks 1 and 2.

Retardation of leaf development by stress may result in an ageing leaf population associated with which a decline in photosynthetic efficiency may occur (cf. Grace & Woolhouse, 1970). Zahner (1968) classified the response of leaf development to water stress into two types, that of species in which the whole season's leaf crop is present as overwintering buds and in which stress, at the time of primordial initiation may reduce the number of leaf primordia formed thus reducing leaf production the following season, and secondly, species capable of producing foliage throughout the growing season in which stress in the spring may reduce total leaf production. In species of the first type stress in the current season will not affect the number of leaves produced, only their rate of expansion and final size. Since C. vulgaris depends for the spring flush of growth on overwintering apical buds (Gimingham, 1972), if their initiation is limited by previous season's water deficits, the growth potential of the plant will be severely limited. If such buds are initiated prior to the onset of stress, desiccation occurring over winter may not affect the number of shoots produced although it may affect their development. This may account for the development of new growth on apparently dead shoots in spring (Section IIIC, 2) and may partly explain the differential response of the plant to summer and winter trampling in terms of biomass. Stress induced in summer may reduce current season's growth and primordial initiation which will become apparent in the succeeding growing season (i.e. Block 1). Plants trampled in winter (Block 2) however, after primordial initiation, may be affected by current water stress limitation on growth but the potential number of buds capable of development will not be reduced. Water stress also reduces wood production (Zahner, 1968) which, for a C. vulgaris, may have an appreciable effect on above ground biomass.

(vi) The effect of trampling, at a single intensity, on the biomass of C. vulgaris stands of different ages.

The pioneer phase would appear to be least affected by trampling whilst the degenerate phase is the most severely affected. In terms of immediate post treatment damage, the litter produced, as a percentage of the biomass 22 months later, rises from 0.5% in the pioneer phase to 3.9% in the mature and 16.0% in the degenerate phases. These are underestimates since, at the time of trampling, the biomass of the pioneer and mature plots may have been lower. The age related increase in litter production may be related to the increasing brittleness of stems with increasing age of the plant, which is most marked in the degenerate phase and is accompanied by the highest litter production, despite a lower biomass than that of the mature phase. This differential response results in a varying effect on potential productivity which varies with age anyway, being least in the degenerate phase and greatest in the building phase (Barclay-Estrup, 1970). Thus, as the stand ages, the proportion of young productive shoots declines (Robertson & Davies, 1965; Miller & Miles, 1970) together with the ability to regenerate. There is a reduction in the potential number of sprouting clusters as a result of decreased stem density and increased lignification, in addition to a decreased rate of growth of such centres (Kayl & Gimingham, 1965; Miller & Miles, 1970; Mohammed & Gimingham, 1970). Therefore, in addition to increasing damage with age, there is an inherent decrease in regenerative capacity. When litter is added to the biomass data, the mature phase would appear to be most severely affected. This may result from the appreciable productivity of the mature phase (Barclay-Estrup, 1970) whilst the regenerative capacity of the stand is reduced because of age (cf. Kayll & Gimingham, 1965; Mohammed & Gimingham, 1970) so that the ability to compensate for losses by the development of sprouting clusters of shoots is less than in younger phases, while the increment in the degenerate phase over the period between treatment and harvesting is less than that in the mature phase so the difference between the control and treated plot is smaller.

The maintenance of juvenility would appear to be conducive to recovery. Even if productivity is not directly stimulated the production of new shoots will maintain the juvenility of the stand so that its productivity will decline comparatively more slowly than that of the control. This may enhance its ability to tolerate trampling since control plots will pass into the less productive mature phase whilst the rejuvenated plants will be maintained at a relatively high rate of productivity. On the basis of present data it is not possible to ascertain whether differences in biomass resulting from trampling on *C. vulgaris* are eliminated over time but it is apparent that after 22 months the effects of trampling at one instant in time are still appreciable. This agrees with Leney's observations (1974) on the sensitivity of *C. vulgaris* to public pressure and the slow regrowth following trampling observed by Bayfield (1971).

The results indicate that the susceptibility and recuperative potential of C. vulgaris may be an age related phenomenon, the one increasing and the other decreasing with respect to a threshold age range over which the rate of production and the ability to produce new growth from deep seated buds decreases.

This may be of limited practical applicability since, as Leney (1974) observed, stands of C. vulgaris do not provide ideal recreational areas. This is especially so in mature and degenerate stands, but if the principles are applicable to a greater variety of woody stemmed species, those which present less of an obstacle to recreational pursuits may vary in the ability to withstand such pressures in accordance with their age and the time of the year as it affects their capacity to tolerate trampling pressures and repair and recover from trampling damage.

(f) Conclusions

Trampling on C. vulgaris has a detrimental effect on its productivity. The response is an integration of a number of responses by the plant to trampling e.g. the effect of water stress on photosynthesis and growth, the reduction in photosynthetic surface by desiccation injury and stem and leaf injury. There is an indication that a threshold trampling pressure exists below which the plant may be capable of tolerating trampling, and a higher threshold at which saturation of the response occurs. These threshold values may be influenced by the age of the stand involved, the longevity of the recovery period and the time of the year as it affects the physiological status of the plant. These factors combine to determine the durability of the stand to trampling, which is consequently a seasonal and age related variable. This implies that the carrying capacity of C. vulgaris is a dynamic quantity.

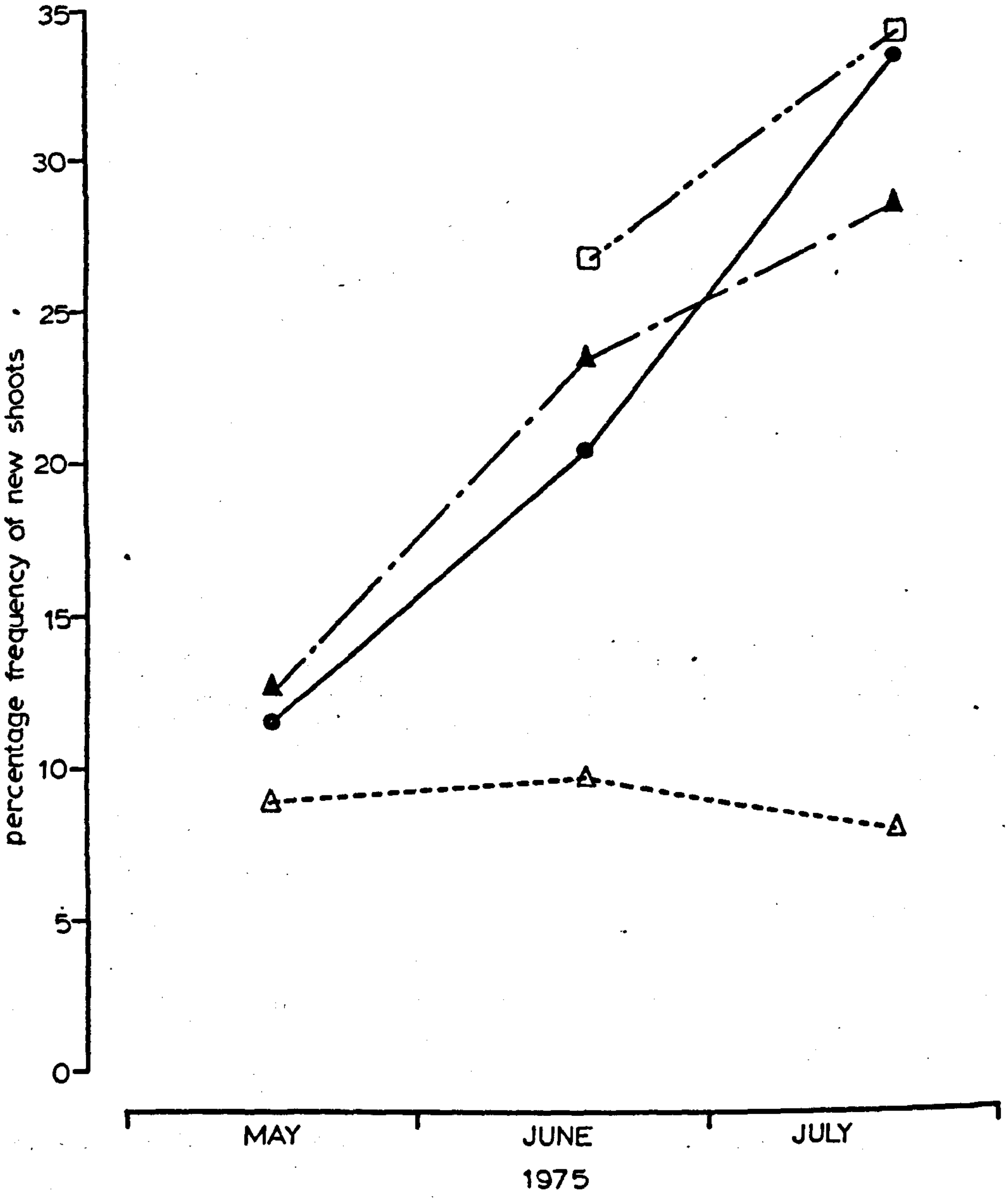


Figure 83 The effect of trampling (at an intensity of 100 p pl^{-1}) on the development of new shoots in a stand of C. vulgaris, (Cannock Chase)

●——● control shoots

△-----△ shoots treated 1 month before sampling

▲-·-·-▲ shoots treated 3 months before sampling

□-·-·-□ shoots treated 6 months before sampling

II THE DEVELOPMENT OF NEW GROWTH ON TRAMPLING-DAMAGED PLANTS OF C. VULGARIS

(a) Methods

Over the period May 1975 to July 1975, following the appearance of new growth on plants of *C. vulgaris* at the Cannock Chase site, the percentage of new growth on control and treated plots was recorded, using a point quadrat frame, in a 1m x 0.5m quadrat. Fifty hits per plot were obtained, with four replicate plots per treatment. New growth was identified by its paler green coloration in comparison with the previous season's growth, and is expressed as a percentage of the total number of vegetation hits.

(b) Results

Two way analysis of variance for control plots, plots treated 1 month and 3 months before sampling, indicates significant ($p < 0.05$) treatment and time effects and a significant ($p < 0.05$) time x treatment interaction. (A similar analysis, but including data for plots treated 6 months before sampling which was not available until June 1975, indicates similar significant ($p < 0.05$) treatment and time effects). For plots trampled prior to the onset of the growing season i.e. 3 and 6 month plots, percentage new growth is comparable to that on the control plots, all showing a substantial increase between May and July 1975 (or June and July 1975 for 6 month plots) (Figure 83). For plots treated in April, May and June 1975 the percentage new growth on the plots remains relatively constant (9.2 ± 0.6%), lying below that of the control plots, the difference becoming greater over time (i.e. time x treatment interaction) (Figure 83).

(c) Discussion

It would appear that plants damaged before the start of the growing season are relatively unaffected by trampling in terms of bud break. This is consistent with the effects desiccation has upon the shoot and subsequent bud development observed by Bannister (1964b) and Watson *et al.* (1966) and is consistent with the observations of Zahner (1968) regarding bud development prior to the onset of stress having little effect on the number of buds developing when the stress is alleviated. Thus, plants trampled over winter may not be affected in terms of subsequent bud break the following spring. Similarly, the present results suggest the buds are not damaged due to desiccation or frost injury or infection (cf. Miller & Miles, 1970).

Bud break in late April to early May results in approximately 11% new growth on the plot by mid-May on control plots compared to 9.2% on plots treated one month prior to sampling, suggesting that at this stage of the growing season trampling has not had a significant effect on bud break, which is consistent with the observation that plant apices and buds are resistant to desiccation (Iljin, 1957; Gates, 1968). The ability to break bud may also indicate that winter photosynthetic accumulations may not be vital to subsequent growth since plants treated during the winter 'dormant' period do not appear to be affected in their ability to break bud by a reduction in photosynthesis as a result of water stress (cf. Helms, 1965). The role of photosynthesis at the time of budbreak and in the immediate period after on subsequent growth remains unresolved. If photosynthesis is depressed in shoots from plots treated 3 and 6 months prior to treatment it would not appear to have an appreciable effect on the development of new growth. This is consistent with the suggestion of McGregor & Kramer (1963) that food reserves (accumulated prior to trampling in the present study) may be sufficient for new growth to be initiated so that it is not dependent on an increase in photosynthesis at the time of budbreak. Thus, for primordia initiated and for food reserves accumulated prior to the onset of stress, bud break may be unaffected by water stresses induced by trampling.

The effect of trampling on C. vulgaris following bud break is substantial since further new growth appears to be inhibited. This is consistent with the proposition that apices, although protoplasmically tolerant of stress are developmentally sensitive to it (Gates, 1968) and that young tissues are less tolerant of desiccation and more sensitive to water stress than older tissues. Further bud development and growth would appear to be inhibited by trampling in May and June 1975. This period coincides with a decrease in desiccation tolerance from a CRWC of 0.1406 in April 1975 to 0.2740 in July, 1975, so the plant is generally less tolerant of dehydration. Stresses induced over this period may result in greater metabolic disruption leading to greater injury. This, together with reduced cell division (Hsiao, 1973), possible assimilate impoverishment due to a reduction in photosynthesis failing to provide structural materials for growth, and insufficient turgor pressure to provide the mechanical force for elongation (Hsiao, 1973; Hsiao & Acevedo, 1974; Hsiao et al., 1976) may account for the reduction in new growth on plots trampled 1 month before sampling compared to the control. Trampling does not appear to reduce the potential for primordial development through mechanical damage since in May the percentage of new growth on trampled plots is not appreciably different from that on control plots, although which buds develop on the damaged plants requires experimental verification and this observation consequently does not exclude damage to the leading apex. Studies on clipping of C. vulgaris revealed that the plant has a reserve of dormant buds near the base of the stem (Gimingham, 1949) and has buds lying within the wood (Mohammed

& Gimingham, 1970) which may afford some protection against mechanical damage. Thus, following destruction of the aerial portion of the plant, new growth may occur from buds developing at ground level or at points on the stem below damaged areas, giving rise to sprouting clusters. The observation that removal of the leading apex results in the development of an increased number of apices (Grant & Hunter, 1966) may be responsible for the slight trend for the percentage new growth on 3 and 6 month plots to be greater than that of the control. There is also a possibility that the development of a procumbent morphology resulting from damage to the leading apex (Gimingham, 1949) may be advantageous to the plant in trampled situations since a procumbent stem might be able to withstand trampling more effectively than a vertical stem and, in the absence of sustained pressure, buds on the lower sides of stems may be protected from direct mechanical damage, e.g. tearing and bruising resulting from trampling, and therefore capable of giving rise to new growth.

(d) Conclusions

The results suggest that bud break and development is affected less in plants trampled prior to the onset of the growing season, than those trampled following bud break. This supports the observations that winter treading does not cause a significant reduction in production. The susceptibility of new growth to trampling may be mediated through the effects of water stress as it affects the ability of the plant to grow, both in terms of photosynthesis and cellular elongation. These observations are consistent with the apparently greater reduction in productivity of plants trampled in the growing season and the increased vulnerability of tissue to desiccation which occurs at the onset of the growing season.

Although regeneration may depend upon the potential number of viable buds remaining following mechanical damage, the response and thus the regenerative potential, may be related to the physiological state of the plant when trampled since it affects subsequent bud development and possibly primordial initiation. The response of <u>C. vulgaris</u> to trampling may be regarded as incorporating physiological, morphological and ontogenetical characteristics of the plant at the time of trampling and during its post-treatment recovery period.

PART V - CONTROLLED ENVIRONMENT STUDIES - THE EFFECT OF SIMULATED TRAMPLING ON HERBS AND GRASSES

General Introduction

In a recent review of the literature relevant to turfgrass wear, Canaway (1975a) made a distinction between 'wear tolerance' or the ability to withstand wear, and 'recuperative potential' i.e. the ability to recover from wear, which together define the 'durability' of the vegetation. The following investigations are primarily concerned with the mechanisms involved in the recuperative potential of a grass (Phleum bertolonii) and a monocotyledonous herb (Plantago lanceolata) and how such mechanisms may affect the durability of a species to trampling.

A review of the literature concerned with recreational ecology indicates that the response of nonwoody species to trampling has been more extensively studied than that of woody species. Some of this work has been done in an agricultural context e.g. Edmond (1958), some in a sports turf context (cf. Canaway, 1975a) and more recently studies have been performed in the context of recreational use (cf. Liddle, 1975a), although early studies into the effects of human trampling on vegetation exist e.g. Bates (1935).

Trampling bruises, crushes, tears and breaks plant tissue. Canaway (1975a) commented that "The characters thought to determine the degree of wear tolerance among different species and cultivars include anatomical and morphological features; physiological factors may also be important but experimental evidence is lacking". Shearman & Beard (1975a, b, c) attempted to relate the wear tolerance of seven grass species to physiological factors in addition to those of anatomy and morphology, whilst Leney (1974) observed that a plant's reaction to trampling may be dependent upon the morphology and physiology of the plant, with the consequence that certain species may be adapted to trampling in terms of these factors e.g. stems which bend rather than break, and the ability to rapidly replace damaged tissue. Leney noted that the capacity to take up nutrients rapidly following damage may also facilitate recovery, which consequently involves the root system and the effects trampling may have upon it.

The relative importance of root and shoot effects on a plant's response to trampling remains controversial, possibly due to differential responses exhibited by different species. Bates (1935) found that establishment of seedlings on a consolidated soil resulted in a reduction in size and weight of a number of species compared to their growth on a loose soil. Compaction following seedling establishment however, had little effect, reducing the size of shallow rooted species only, implying that compaction effects were confined to relatively shallow depths. This led Bates to conclude that soil consolidation was not responsible for the footpath flora, especially since the most adversely affected species in the experimental situation, *Poa pratensis*, was the most constant and abundant species on paths. However, it is not possible to infer that soil effects are unimportant in the response of plants to trampling, since Bates considered that soil puddling was one of the more important factors producing a footpath socies. His observations on wet soil revealed that perennial species could be crushed beneath the soil or squeezed out of the soil to lie on the surface where they were susceptible to other injurious factors.

That soil moisture content modifies a plant's response to treading has been observed by Edmond (1958), who considered that the immediate effects of direct injury to the plant should be distinguished from more persistent changes in soil condition and its associated affects on plants. Canaway (1975a) listed mechanical impedence to root growth, aeration, soil moisture and temperature, and the movement of water and nutrients as factors of significance in affecting plant response to soil. Although Edmond (1958) investigated the effects of sheep treading on pasture, with the consequence that the results may not be directly comparable to the effects of human trampling on vegetation (Liddle, 1975a) such treading effects may indicate the type of response given by the plant-soil complex to trampling. Edmond (1958) was of the opinion that the increase in soil bulk density attending sheep treading was probably insufficient to limit root growth, but the impedence of drainage and gaseous diffusion could have inhibitory effects. Although bulk density and soil impedence to root development are likely to have an effect on root development (Rosenberg & Willits, 1962; Wittsell & Hobbs, 1965; Liddle & Greig-Smith, 1975a), it is the density required to limit growth under a given set of conditions that is of significance. Liddle & Greig-Smith (1975a) postulated that the inhibition of gaseous diffusion by compaction may result in oxygen deficiencies which have an adverse effect on root respiration, in addition to which, an oxygen deficiency may retard organic decomposition leading to comparatively high root contents (percentage of total soil weight plus root dry weight per unit volume) in trampled areas. However, they also suggested that soil compaction may enhance summer plant growth since high root contents were observed on paths with a high percentage pore space, which is conducive to unimpeded gaseous diffusion. Gradwell (1960) observed that decreases in porosity of the soil as a result of treading did not occur until the soil dried out, which implies that a differential response by the plant to treading occurs depending on the conditions at the time of trampling. Edmond (1962) ascribed the reduction in yield due to treading on a dry soil as mediated through a direct effect i.e. bruising, tearing, on the aerial portions of the plant. However, the yields were more substantially reduced if treading occurred when the soil was wet. The response was not simple. The increase in yield of Trifolium repens suggested that the direct effect on leaves may have been less extensive, but the disruption and displacement of plants, burial of leaves and damage to roots, not apparent on dry soils, may have resulted in the more severe reduction in yield. This led Edmond (1966) to conclude that growth of Lolium perenne was reduced by puddling rather than compaction (cf. Bates, 1935). In contrast to these studies, Wood (1973) found that the survival of Lolium perenne and Poa pratensis in trampled situations was a function of soil compaction and that direct damage to the leaves was of secondary importance. However, increases in soil bulk density are not consistently deleterious to growth. Liddle & Greig-Smith (1975a) found that Festuca rubra, raised in a soil of bulk density 1.36g cm^{-3}, had a 45% higher fresh weight and 40% greater tiller number than when grown in a soil of bulk density 1.08g cm^{-3}, in addition to which, plants raised in the soil of higher bulk density had a greater ability to survive drought due to the increased availability of water. The differential effects of shoot damage and soil compaction on survival of F. rubra (Liddle & Greig-Smith, 1975b) indicated that an increase in soil bulk density resulted in a 37% increase in tiller number and 16% increase in fresh weight. Shoot damage however, although increasing tiller number, reduced fresh weight while both treatments applied together interacted to reduce fresh weight by 31% and result in a smaller increase in tiller number (19%). Liddle & Greig-Smith (1975b) suggested that the response of plants to increases in soil bulk density may be one of the factors explaining the field response of plants to trampling, in which peak biomass was observed at path margins, suggesting low trampling pressures stimulate productivity, possibly by increasing the availability of water to roots.

Leney (1974) investigated the response of a number of species to the differential effects of shoot damage and soil compaction. Plants with damaged shoots were usually smaller in stature and weight than control plants. Holcus lanatus and Plantago lanceolata were found to develop longer, less dense roots in response to shoot damage but soil compaction reduced both shoot and root size in contrast to the response exhibited by Calluna vulgaris and Ammophila arenaria, which were larger than untreated controls. Leney noted that where the roots benefit from compaction the whole plant may benefit, especially if the shoots are not damaged, whilst the stimulation of root growth in some species when the shoot is damaged may enhance survival. The complex reaction of plants to changes in soil bulk density is summarised by Rosenberg (1964) who stated that "Whether a given (soil bulk) density increment will hamper or improve plant growth depends .... upon whether the soil is looser than, at, or more compact than, the optimal density for the season and stage of growth of the crop growing in the soil". Leney (1974) made the observation that, in the trampling context, an equivalent amount of energy expended in damaging the shoot has a more severe effect than in compressing the roots and soil. Liddle & Greig-Smith's (1975b) comparative observations on vehicle and pedestrian tracks led them to similarly conclude that "the vegetation is more affected by the superficial wear and mechanical damage imposed by cars than by the soil bulk density and hardness changes associated with trampling and vehicles". This does not imply that appreciable changes in soil bulk density on recreational areas do not occur. Chappell et al. (1971) found an increase in this parameter of up to 49.5% in areas subjected to vehicular and trampling pressures and a 21.4% increase in areas subject to trampling pressures alone, while Liddle & Greig-Smith (1975a) found increases up to 15.3% in the path areas. Such changes may modify the species composition of an area, but following the establishment of a plant in such a soil environment, damage to its aerial portions is likely to be the main determinant in its survival, although soil compaction may reduce its vigour.

In an experimental consideration of plant response to trampling, the simplest situation which may be envisaged is the response of the plant to a vertical force delivered from above. Bayfield (1971) used such a technique to investigate the response of Phleum bertolonii (S 50 Timothy) to simulated trampling and found that pressures in excess of ten impacts per plant decreased tiller number and shoot and root dry weight. However, at lower pressures, many replicates had values for these parameters which exceeded those of the control, suggesting that low levels of pressure may stimulate productivity. Leney (1974) assessed the response of a number of species to a low level of simulated trampling (i.e. 5 drops of a tamp every fourteen days) over a period of a few months. In some species e.g. Achillea millefolium, a substantial reduction in biomass was observed, whilst in others, e.g. Koeleria cristata, biomass was increased relative to that of the control. Leney noted that the increase in yield was usually accompanied by an increase in tiller number (cf. Bayfield, 1971; Liddle & Greig-Smith, 1975b) and also made the observation that species which were morphologically similar exhibited different reactions to simulated trampling, for example, the yields of Agrostis stolonifera and Festuca rubra were increased while those of Agrostis tenuis and Festuca ovina were depressed.

Allcock (1973) employed a similar principle to investigate the response of turves to vertical forces, although the pressure was exerted by bringing the turf up into contact with the 'foot' rather than by using a falling tamp. All load applications were found to result in a substantial decrease in the productivity of all species in the turf.

Tamps have also been employed in field investigations e.g. Wagar (1964), Kellomaki (1973). The techniques employed have been reviewed by Canaway (1975a, b), who questioned their validity. Often the simulated technique has not been calibrated against real wear. Cieslinski & Wagar (1970) considered it unimportant but Kellomaki (1973) found that, despite certain discrepencies, the results obtained from simulated and real trampling were comparable. Canaway (1975a, b) also noted that the forces involved are often not quantified except in relative terms e.g. number of drops of a tamp, and considered that, although it is difficult to quantify the dynamic forces involved at the point of impact, such information would enable the effects of a treatment to be evaluated more closely with the effects exerted by a human foot. An additional disadvantage of the falling tamp is that the forces exerted are undimensional and do not simulate the horizontal forces exerted by a foot which may be up to c. 30% of the body weight (Harper _et_ _al_. 1961). Bayfield (1971) however, considered falling tamp methods to be of value in modelling the plant's reaction to trampling whilst Allcock (1973) suggested that the technique may be of use in evaluating the response of plants to individual factors. Leney (1974) however, was of the opinion that artificial trampling under controlled conditions gives little indication of the plant's survival ability under field conditions since laboratory investigations implied that species stimulated to grow by light pressure might have a greater survival potential in the field than species retarded by light simulated trampling. In reality however, certain species such as _Agrostis tenuis_ which were adversely affected in laboratory situations, were predominant in heavily pressurised areas.

The next approximation to an experimental reproduction of trampling effects is to walk on vegetation plots at controlled intensities and frequencies. Again it does not adequately represent the variation in pressure, in terms of frequency and intensity, a field site is likely to experience, nor the environmental factors existing at such a site which may influence a plant's survival under trampling pressure. Bates (1935), using such methods by sowing monocultures of various species and trampling the plots at known intensities, related species response to their morphology. Bayfield (1971) used similar techniques to study the reaction of a *Calluna-Trichophorum* heath to trampling. Leney (1974) simulated varying types of recreational activity e.g. running, walking, to assess their relative impacts on different types of vegetation, whilst Liddle (1973) employed walking and driving a vehicle at known rates across a dune system to ascertain the effects of such activities on plant species, for example, the leaf area of *Trifolium repens* was reduced by 57% after 400 passages of a vehicle along a track whilst 48 walking passages across a plot in winter reduced vegetation height by 15% in the subsequent summer. The persistence of recreational effects is a feature that has also been noted by Bayfield (1971) and Leney (1974) following the instantaneous application of a trampling treatment. On the basis of such simulations, Leney observed that 40 passages across a plot every 14 days over a period of 16 weeks had a less severe effect than 120-150 walking passages applied at one instant on an *Ammophiletum* in contrast to Bayfield's (1971) assumption that walking treatments staggered over a limited time period were additive and comparable to treatments applied on one occasion. This suggests that the recovery in the intertreatment periods in Bayfield's study was so slow as to be ineffective over the time period involved.

One of the most extensive studies on controlled rates of trampling has been made by Edmond (1958, 1962, 1963, 1964, 1966) into the effects of sheep treading on pasture. The generalised response was for a linear decrease in yield with increased treading intensity, associated with a decrease in tiller or node number (Edmond, 1958). He suggested that, on the basis that the rate of plant growth per unit area increases until there is maximal radiation interception, swards opened up by heavy treading may exhibit more rapid growth than control plots due to the absence of competition for light, but the lower yields on more heavily trodden plots was ascribed to lower species density and the inability of immature tillers to produce herbage in a quantity comparable to that of mature tillers. Edmond (1963, 1964) also observed seasonal differences in regrowth following treading, for example, treading on a moist soil was relatively more damaging in summer than in winter. The existence of a time x species x treading interaction made the situation somewhat complex. For example, Trifolium repens was more tolerant of treading in summer than in winter whilst Lolium perenne was more tolerant in winter. Similar seasonal effects have been noted by Holmes et al. (1976) who found that the susceptibility of vegetation to trampling was greater in late summer (September) than in early summer (July-August).

Edmond (1963, 1964) ascribed the generalised reduction in yield to a decrease in tiller number rather than a decrease in tiller vigour, and considered that in addition to morphological characteristics and the physical resilience of certain species to treading, "Active growth must reduce the intensity of the treading effect..." so that "...fast growing pasture overcomes treading damage most quickly".

However, investigations on monocultures and cultivated swards are unlikely to represent the response of semi-natural areas to trampling. Leney (1974) argued that such investigations may not reflect the response of a species when it is in competition with other species, and may not take into account intraspecific variation which may facilitate the survival of part of the population under conditions detrimental to the rest.

Due to the artificiality of such experimental investigations, the most appropriate quantification of the response of semi-natural vegetation to trampling is a consideration of sites used for recreational purposes. A number of studies have investigated cover response to trampling e.g. La Page (1967), Goldsmith et al. (1970), Willard & Marr (1970, 1971), Bayfield (1971), Chappell et al. (1971), Liddle & Greig-Smith (1975b). In addition, certain studies have considered biomass and productivity responses of plants to trampling. Burden & Randerson (1972) found that trampling reduced the biomass of cushion plants and 'straggling' plants e.g. Armeria maritima, Cerastium semidecandrum, whilst the biomass of rosette plants increased e.g. Plantago coronopus, but the reaction of grasses was more complex, the lowest biomass being observed at medium trampling pressures. Such changes in biomass associated with trampling pressures were explained as a result of differential resistances and competitive abilities of the species involved. Despite the tendency for certain vegetational components to be stimulated by trampling, vegetation is generally shorter and of lower biomass in trodden habitats (Leney, 1974; Liddle, 1975a; Liddle & Greig-Smith, 1975b). This is not a consistently observed effect. Kellomaki (1973) found that at low trampling pressures (1 passage per plot per week) both cover and biomass of the field layer of a woodland ecosystem were elevated above the control values, but where the vegetation is stunted it is evident that net productivity is reduced. Leney (1974) stated that this is reflected in a decrease in dry weight and height of individual tillers and fewer leaves per tiller. Although Edmond (1964) found contrasting evidence regarding yield per tiller and treading pressure, Leney's observations (1974) are consistent with those presented by Liddle (1973). Leney (1974) found that removal of recreational pressures resulted in a productivity greater than that of comparable control plots, an increase which was paralleled by cover response. Species composition changes, however, occurred at a much slower rate, which is consistent with Edmond's findings (1958) that species composition changes following treading were more persistent than changes in biomass. This two phase change which occurs at different rates emphasises Bayfield's (1971) assertion that when assessing the effects of trampling on a habitat, the ability to recover may be as important as the immediate signs of damage, since a plant with a high potential for regeneration may be capable of survival, even if completely defoliated, compared to a species not possessing this capacity (cf. Edmond, 1964). In a similar context, Cieslinski & Wagar (1970) considered that end of season 'stocking' (percent live vegetation) gave a more realistic indication of site durability than vegetation biomass, since decreases in weight could be compensated for more rapidly than a decrease in plant density (cf. Edmond, 1958).

Liddle & Greig-Smith (1975b) observed that maximum biomass in dune track areas occurred at track margins. This reflected a change in species composition, dicotyledonous species increasing at track margins where trampling pressures were less compared to the centre of tracks. That low trampling pressures may stimulate productivity is consistent with findings of simulated trampling experiments (Bayfield, 1971; Leney, 1974) but the field situation is confused by the differential susceptibilities and competitive abilities of different species to trampling. Liddle & Greig-Smith (1975b) suggested that light trampling pressure may reduce the height of monocotyledonous species in marginal areas, so reducing their shading effect on broad leaved species which then possess a competitive advantage, whilst in heavily trampled areas the protected apices and intercalary meristems of monocotyledons confer upon them a greater survival capacity than that of dicotyledons.

Growth would appear to be a factor of some relevance in assessing the durability of a particular species under trampling pressure.

Environmental factors, such as light, may influence the capacity of the habitat to withstand trampling through their effects on sward growth (Edmond, 1958; Canaway, 1975a; Liddle & Greig-Smith, 1975b). Bates (1935) suggested that the survival of Poa pratensis and Lolium perenne on footpaths over winter may be related to their 'evergreen' condition so that they are capable of growing when damage occurs, whilst Liddle & Greig-Smith (1975b) postulated that stimulated production may result from the "Response to damage by the dominant species or a more efficient utilization of resources by a change in species composition...". Liddle (1975a) considered that, on the basis of effects observed on defoliated plants, assimilates in undamaged tillers may be used to aid survival of grasses in trampled situations, whilst Canaway (1975a) suggested that cutting of plants may deplete reserves which might have been used for recovery growth following wear. Mowing (i.e. defoliation) may also reduce root growth which could influence wear tolerance (Canaway, 1975a). Leney (1974) also suggested that root development prior to the onset of the trampling stress may influence plant survival in a trampled habitat, the more developed the root the more beneficial the effect.

It is apparent that although morphological and anatomical characteristics such as position of apices and flexibility of leaves and stems, may be of paramount importance in determining the survival of a plant under trampled conditions, growth potential is a factor which may be of considerable significance, especially at trampling pressures exceeding the wear tolerance threshold. Rate of growth, the significance of stored reserves and the activity of the root system combine to determine such plant responses and will themselves be affected by environmental conditions other than the stress imposed by trampling.

Experimental simulation of Trampling

The forces applied to the floor by a walking foot are complex and not readily simulated (Harper et al., 1961), although to do so is technologically feasible (Canaway, 1975a). Harper et al. (1961) calculated the average forces involved in "straight walking" to be 1.48 kg cm^{-2} for the whole foot, although forces exerted by the heel were somewhat greater (12.0 kg cm^{-2}), and suggested that the vertical forces which an abrasion machine should exert should be between 1.41 kg cm^{-2} and 1.76 kg cm^{-2}, while exerting an horizontal force of 22.6 kg cm^{-2}. These values are greater than similar forces calculated by Goss & Roberts (1964) who gave a value of 0.564 kg cm^{-2} for a 81kg man walking, while Liddle (1975a) gave a value of 0.2kg cm^{-2} for the vertical force applied to the floor by a standing man.

A number of machines have been developed for simulating wear by human activity e.g. Goss & Roberts (1964), Shearman et al. (1974), Canaway (1975b) and a number of attempts have been made to simulate the vertical components of wear e.g. Wagar (1964), Bayfield (1971), Kellomaki (1973), Leney (1974), although the use of a falling tamp introduces a high degree of artificiality into studies. The quantification of the dynamic forces imposed by a falling object are difficult to assess, but may be appreciably greater than the static forces involved, e.g. 57.0kg cm^{-2} compared to a static force of 0.2kg cm^{-2} for a standing man (Liddle, 1975a). The simplest situation which may be envisaged is the vertical force applied by a static mass, in which the force is equivalent to the weight per unit area. The force applied by a falling tamp, however, is related to its mass and acceleration, and its deceleration on impact which is related to the resilience of the material onto which the tamp falls (Canaway, 1975b). When the tamp falls onto soil the resilience of the soil may change due to soil compaction with the

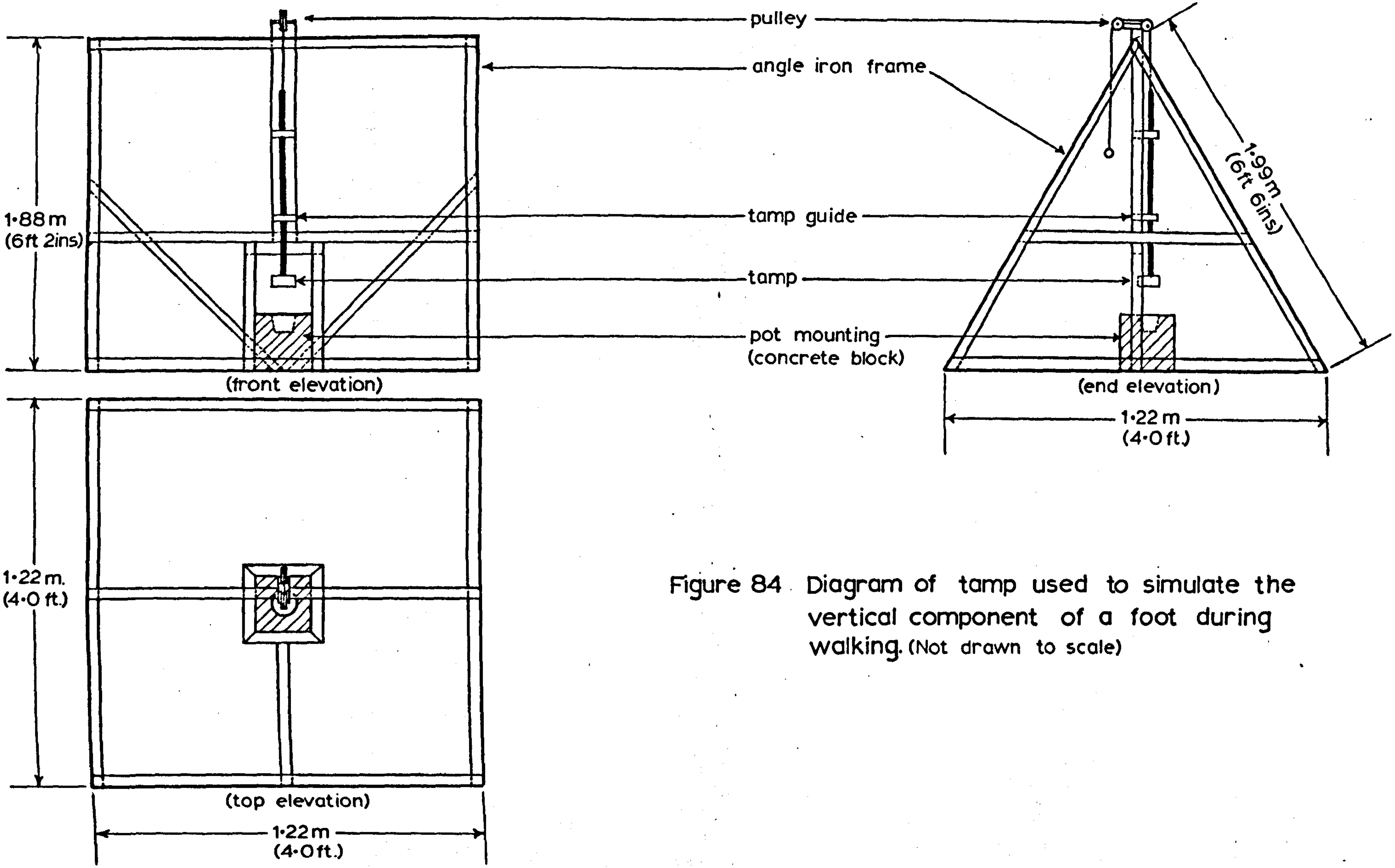


Figure 84 Diagram of tamp used to simulate the vertical component of a foot during walking. (Not drawn to scale)

consequence that the force applied also changes. As an alternative to quantifying the forces existing at the point of impact the potential energy input may be defined (Canaway, 1975b):

$$E_{pot} = mgh \text{ (joules)} \qquad (27)$$

where: E_{pot} = potential energy input

m = mass of object (kg)

h = height through which object falls (m)

g = acceleration due to gravity

Apparatus

The apparatus employed in the present study for simulating the vertical force applied to the floor by a foot consisted of an angle iron frame supporting a steel shaft which passed through two adjustable guides (Figure 84). The lower end of the shaft was threaded to take an interchangeable range of steel discs, the lower surfaces of which were clad with a rubber boot sole. The disc employed in the present investigation was 10cm in diameter (area: 78.5cm^2) of which 57.5cm^2 of the total area was in contact with a level surface. Disc and shaft weighed 2.64kg. The weight of the tamp could be increased, in six incremental stages of 0.68kg, by the addition of steel discs slipped onto the shaft and resting on the tamp. This could give a maximum weight of 6.72kg so that the static force generated could be varied from 0.05kg cm^{-2} to 0.12kg cm^{-2}.

The shaft guides were slightly larger than the shaft diameter to reduce frictional resistances to free fall of the tamp, but this resulted in a degree of imprecision in the point of impact of the tamp which, if not falling squarely, could lead to a proportion of the energy developed during the fall being dissipated upon the pot rim, which could lead to fracturing and shattering of the pot. To help alleviate this, the plastic pots were mounted in a concrete block which was accurately located beneath the tamp. The pot fitting was designed to support the sides and base of the pot to prevent breakage due to soil compression on impact.

Trials indicated that a tamp weight of 4.0kg (giving a static force of 0.07kg cm^{-2}) was the maximum that could be employed while minimizing pot damage. It was dropped from a constant height (27.5cm above the pot rim) yielding a potential energy input of 10.79J.

Discussion

The static force delivered by the present apparatus is comparable to that of Canaway's apparatus (1975b) which delivered a force of 0.014 - 0.271kg cm^{-2}. The forces exerted by a falling tamp are much greater. Wagar (1964) used a tamp delivering 8 foot pounds of energy per square foot whilst Bayfield (1971) cites a value of 1mkg of energy delivered by his apparatus. In terms of potential energy input these represent 10.85J and 9.82J respectively whilst Kellomaki (1973) dropped a 7kg weight from a height of 50cm., giving a potential energy input of 34.34J. However, the forces were dissipated over different areas which results in comparative values of 116.8J m^{-2} (Wagar, 1964), 1529.1J m^{-2} (Bayfield, 1971) and 137.4J m^{-2} (Kellomaki, 1973) compared to 1877.0J m^{-2} in the present investigation.

It is apparent that the potential energy inputs in the studies noted above are divergent, those used in greenhouse studies (Bayfield, 1971) being considerably greater than those employed in field studies (Wagar, 1964; Kellomaki, 1973). Such differences may result in extrapolation from laboratory to field situation being unjustified, although Leney (1974) stated that "It appears that the actual number of footfalls is more important than the pressure with which they are exerted".

The range of the number of impacts employed in the current investigation (2-40 impacts at one instant) are similar to those employed by other workers, e.g. Wagar (1964) used up to 40 drops of the tamp per week, Kellomaki (1973) employed a range of 1 to 64 drops of the tamp per week on natural vegetation, Bayfield (1971) used a range of 1 to 100 drops applied at one time to S 50 Timothy plants growing in pots under greenhouse conditions. The differences in forces employed and the frequency of application makes comparisons between the results of different workers of dubious value. However, Canaway (1975a) commented that even where the physical forces involved in simulations are unknown, the concurrence of results in cultivar evaluation implies the technique is valid despite severe limitations. The technique may be regarded as useful in process orientated approaches where individual plants response to trampling is being investigated, but it is of questionable validity in relating such findings to the field situation.

Plant material

Plant material used in the simulated trampling investigations described below i.e. Phleum bertolonii (Aberystwyth S 50 Timothy) and Plantago lanceolata (see Section I(c)) was raised from seed (supplied by A.G. Leighton Ltd., Shropshire). A standard procedure was adopted in which germination, under standard conditions appropriate to the experiment concerned, was followed by pricking out singly (unless otherwise stated) into 10cm diameter plastic pots containing John Innes No. 2 potting compost with added fertilizer. Following a minimum establishment period of 14 days, the plants were subjected to the experimental treatments discussed below.

A. Growth responses of Phleum bertolonii and Plantago lanceolata to simulated trampling pressure

(a) Introduction

Simulated trampling on non-woody species of plant under laboratory conditions results in two major effects, that of mechanical damage and defoliation on the aerial portion of the plant, and that of soil compression effects on root growth (see Section V B).

The effects of defoliation on growth in an agricultural context are possibly the most relevent to the present study. Such effects have often been considered with respect to the grain yield of a plant e.g. Walpole & Morgan (1974), Allison et al. (1975), Enyi (1975), Singh & Nair (1975a, b), generally reducing yield to an extent that increases with the severity of defoliation. Walpole & Morgan (1974) observed that although the relative importance of source supply and sink strength on grain production may change during the ontogeny of the plant, restriction in the rate of assimilate supply due to defoliation resulted in a reduction in grain weight rather than grain number. However, the stage of development at which defoliation occurs may have a significant effect on the response exhibited by the plant. Enyi (1975) for example, found the greatest reduction in seed yield of a number of legume crops occurred when they were defoliated in the early podding stage, but defoliation in the earlier stages of growth, when assimilates were produced primarily for stem and leaf growth, could have an indirect effect on seed yield by reducing stem growth and consequently the number of flowering nodes and pods per plant. Allison et al. (1975), working with Zea mays, found that although defoliation reduced grain number and yield, the plants could sustain a considerable degree of defoliation in the middle of the vegetative phase with an inappreciable decrease in grain yield since competition for assimilates only affected the 'sink' capacity of the grain in the immediate pre-flowering period. Singh & Nair (1975b) also found that defoliation at the ear initiation stage reduced the assimilatory surfaces to an extent sufficient to affect dry weight increment of the grain. However, the interaction between defoliation and stage of development could result in an increase in yield. Singh & Nair postulated that this may have been due to an increase in photosynthetic efficiency per unit area of leaf as the plant entered the 'grand period of growth' coincident with a rapid increase in photosynthetic surface. Allison _et al_. (1975) also suggested that defoliation, especially at higher plant densities, could enhance photosynthetic efficiency by reducing mutual shading effects of leaves.

Singh & Nair (1975a) also found a reduction in sucrose, reducing sugars and leaf protein with increased defoliation. Davidson & Milthorpe (1966a) noted that a number of studies suggested that growth following defoliation is accompanied by a decrease in the soluble carbohydrate content of roots and the remaining aerial portions of the plant (stubble). This led to an hypothesis implicating carbohydrate utilization in the production and development of new cells following defoliation, with the consequence that carbohydrate reserves were considered to control the rate of regrowth. Davidson & Milthorpe questioned the validity of this hypothesis and, using _Dactylis glomerata_, found that although defoliation reduced the rate of leaf appearance and area increase, it was not correlated with carbohydrate content when this was varied by defoliation, but was related to carbohydrate content if it was varied by dark treatments. Their results suggested that not all the soluble carbohydrate was available for leaf expansion but rather "... the carbohydrate of primary importance in developing the leaf surface is that present above the zone of expansion of expanding leaves (and possibly in roots of those grasses in which carbohydrate accumulates in roots)".

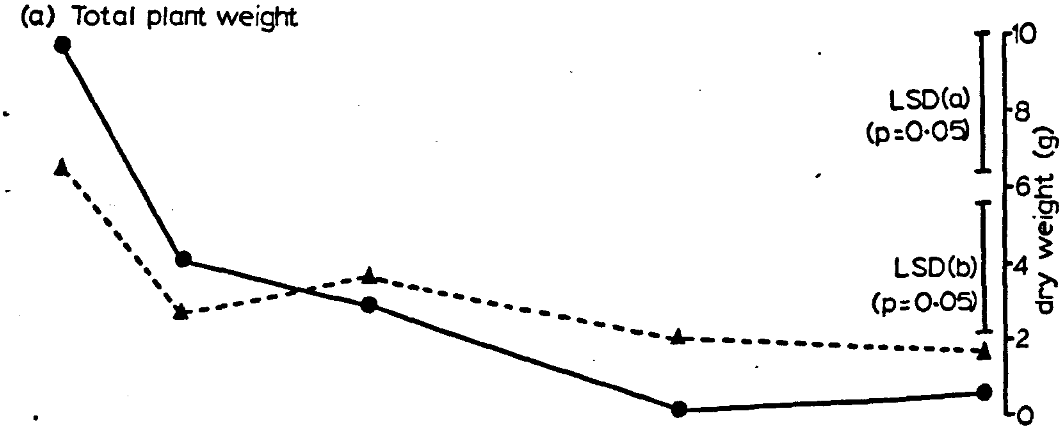
Defoliation may, in addition to depleting the substrate and photosynthetic supply of substrate, remove the reserve of cells upon which leaf expansion is dependent. Davidson & Milthorpe (1966a) found, in D. glomerata, that a reduction in photosynthetic area by removal of fully expanded leaves had little effect on expanding leaves, but a reduction in the photosynthetic area of expanding leaves had repercussions on their subsequent development, suggesting that photosynthesis by a developing leaf may play a significant role in its expansion. Although the carbohydrate balance of mature leaves may not be directly involved in regrowth following defoliation, Davidson & Milthorpe considered that it may influence regrowth through the supply of carbohydrate to the root, thereby facilitating nutrient uptake. In this context Davidson & Milthorpe (1966b) observed a decline in root respiration following defoliation which was accompanied by a virtual cessation in root extension. Their results suggested that rather than the root reserves contributing to regrowth of the shoot, continued respiration of the root was dependent upon a supply of carbohydrate from the stubble. However, although regrowth of defoliated leaves may be independent of reserves existing elsewhere in the plant, they suggested that reserves in older leaves may have a role in the "... development of new leaves, tiller buds and root growth". Davidson & Milthorpe (1966b) suggested that following the initial effects of carbohydrate on regrowth, the removal of a nutrient supply from mature leaves may retard mineral uptake due to reduced root metabolism, thus lowering the regrowth potential in terms of new tiller development. They concluded that "Although no claim can be made that carbohydrate reserves have a specific role in initiating regrowth, there seems no doubt that as part of the labile metabolic pool they, and other compounds, do contribute to new growth and respiration immediately after defoliation".

In contrast to Davidson & Milthorpe's (1966a, b) assertions that regrowth of defoliated tillers depends upon the carbohydrate content and photosynthesis of the expanding leaf, Smith (1974) considered that leaf area and carbohydrate reserves were important for regrowth of defoliated tillers of *Phleum pratense*. Jewiss & Powell (1965) had found that rate of regrowth following defoliation was initially related to carbohydrate reserves, and that it was sustained by assimilate production by the stubble. Smith (1974) found that leaf area was of greater significance than carbohydrate reserves following defoliation, but the two variables interacted so that "When the initial timothy tillers were low in carbohydrate, leaf area was needed, and when there was no leaf area, carbohydrate reserves were needed". A temperature effect was superimposed upon this relationship such that plants with low carbohydrate reserves and no leaf blades grew normally but with a reduced yield under a cool regime but in a warm regime "... barely stayed alive, had no inflorescences and produced very little dry matter". This suggests that under conditions of carbohydrate depletion, reduced photosynthetic capacity and conditions conducive to elevated respiration rates, growth and survival are adversely affected by defoliation.

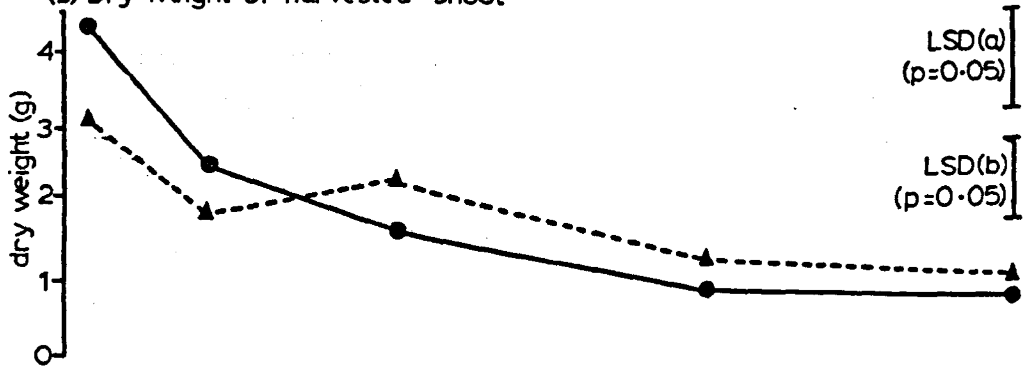
Ryle & Powell (1975) found that defoliation in *Hordeum vulgare* resulted in an increase in the export of assimilates from uncut leaves relative to comparable leaves on an undefoliated plant. As the expanding leaves developed as an assimilate source so the export from neighbouring leaves declined. Ryle & Powell considered that the leaves remaining after defoliation were capable of supporting all new growth, including that of stems and roots, although "...when all or nearly all photosynthetic surface is removed during a defoliation, regrowing leaves receive some energy substrate from a source or reserve already present in the plant". Although links between reserve levels and regrowth following defoliation have been advocated, e.g. Sullivan & Sprague (1943), Smith (1974), the results of Ryle & Powell's (1975) investigations suggested that removal of half the total leaf area was compensated for by a reallocation of current assimilates from roots and stems to leaf meristems. The reduction in root growth coincident with defoliation was considered to indicate such stress that it was unlikely the roots supplied carbohydrate for regrowth of the shoot. Marshall & Sagar (1965) were also of the opinion that root reserves of carbohydrate did not contribute to regrowth following defoliation of Lolium multiflorum.

Ryle & Powell (1975) argued that owing to the redistribution of assimilates in defoliated plants "... cut plants would show a disproportionately higher rate of leaf growth, compared with controls, and that in a short time this would express itself in a higher (relative) growth rate". They found that although whole plant relative growth rates were essentially identical for control and treated plants, partitioning of growth rates revealed a slightly higher shoot growth rate and a substantial reduction in root growth rate of defoliated plants. Ryle & Powell suggested that defoliated plants used their current assimilate with greater efficiency by channelling a larger proportion of it into new leaf growth which consequently resulted in a higher rate of increase in assimilate produced on successive days, although in absolute terms the control plants, because of a greater leaf area, grew more rapidly. However, they noted that the advantage possessed by control plants only exists when mutual shading does not result in competition for light since the "... advantage of a continually increasing photosynthetic surface is then lost and the efficiency of utilization of a finite amount of solar energy becomes paramount".

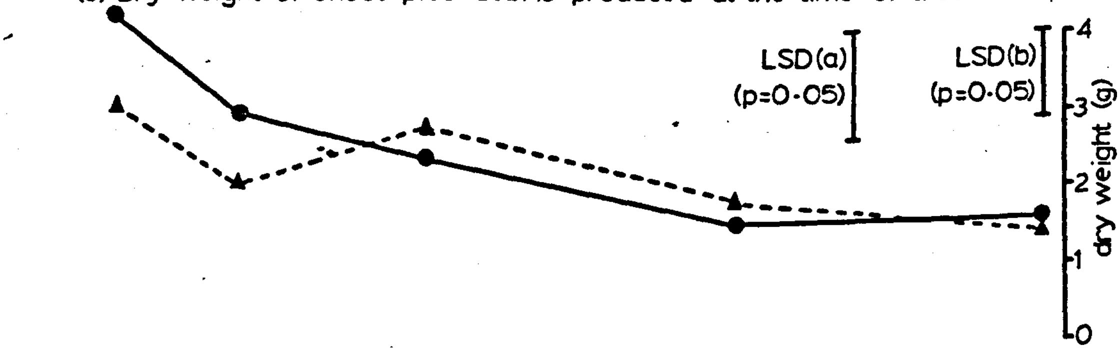
(a) Total plant weight



(b) Dry weight of harvested shoot



(c) Dry weight of shoot plus debris produced at the time of treatment



(d) Dry weight of plant root

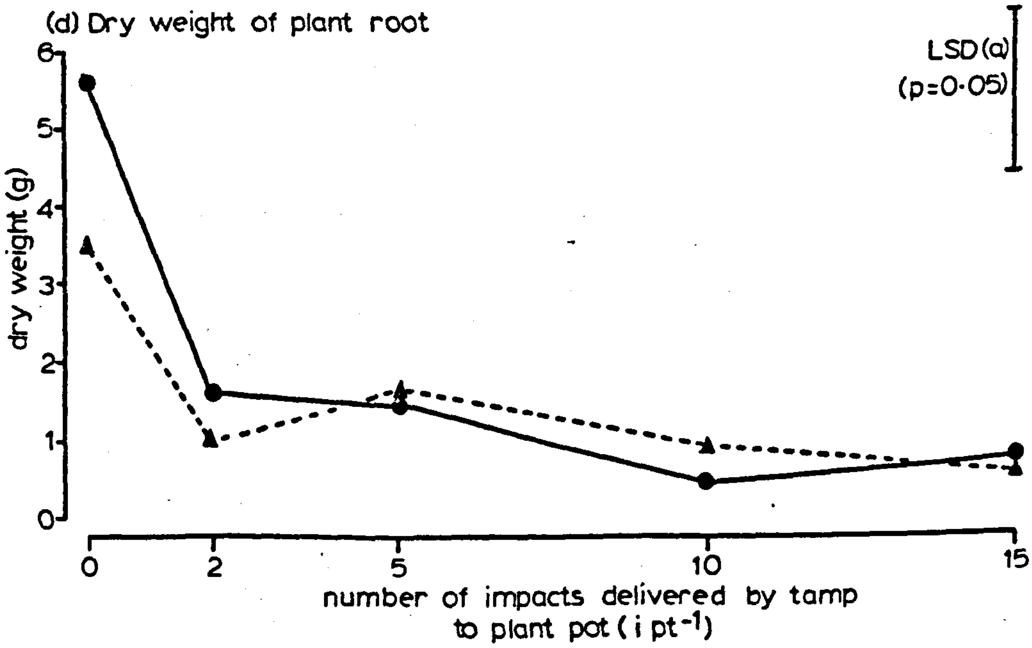


Figure 85 Effects of simulated trampling on dry weight of Plantago lanceolata (●) and Phleum bertolonii (▲).
(LSD a - P. lanceolata, LSD b - P. bertolonii)

(b) (i) The effect of simulated trampling on the biomass of Phleum bertolonii and Plantago lanceolata

(a) Methods

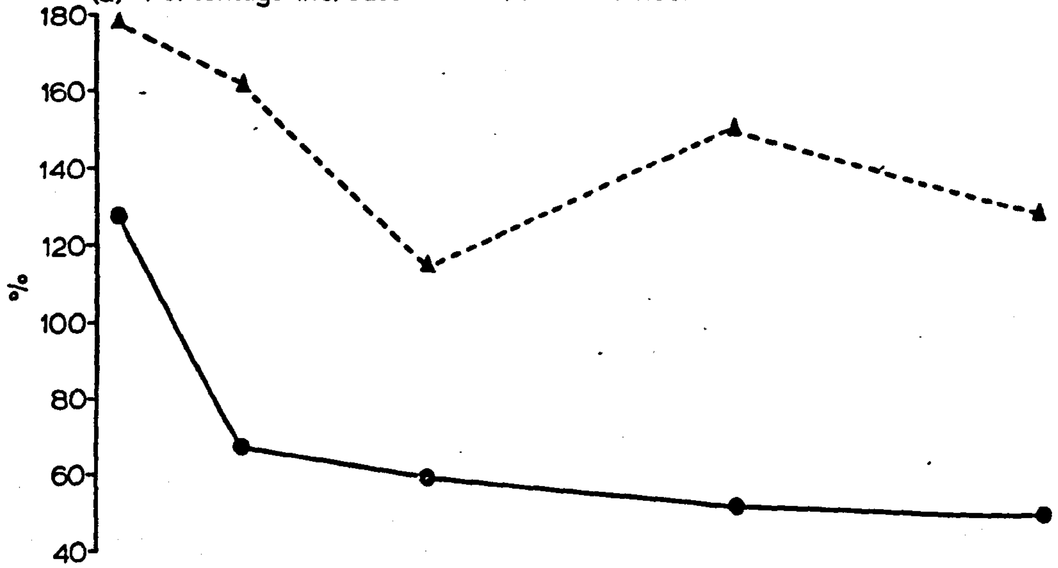
Individual 78-day old plants of Phleum bertolonii and Plantago lanceolata, grown in growth chambers (Fisons Scientific Instruments Ltd.) under standard conditions (20°C/15°C day/night temperature, 85 ± 10% relative humidity, 16h day at 13klx) (illuminance provided by 'Warm White' and 'Daylight' fluorescent tubes (Philips, Holland)) were subjected to five impact regimes of the tamp (0, 2, 5, 10 and 15 impacts per pot (i pt^{-1})) with five replicates per treatment. Following treatment, the plants were replaced in their respective growth chambers in a randomised layout, the two species being housed in separate chambers so that, in the event of a differential environment existing between chambers, inter-treatment effects would remain comparable within a species despite environmentally induced inter-species variation. Material severed from the plant due to the action of the tamp was washed and dried at 85°C (the 'debris' component).

Following a recovery period of 21 days, the plants were harvested, shoot and root portions being separated and dried at 85°C. Tiller number of P. bertolonii and leaf number of P. lanceolata were assessed immediately prior to harvesting.

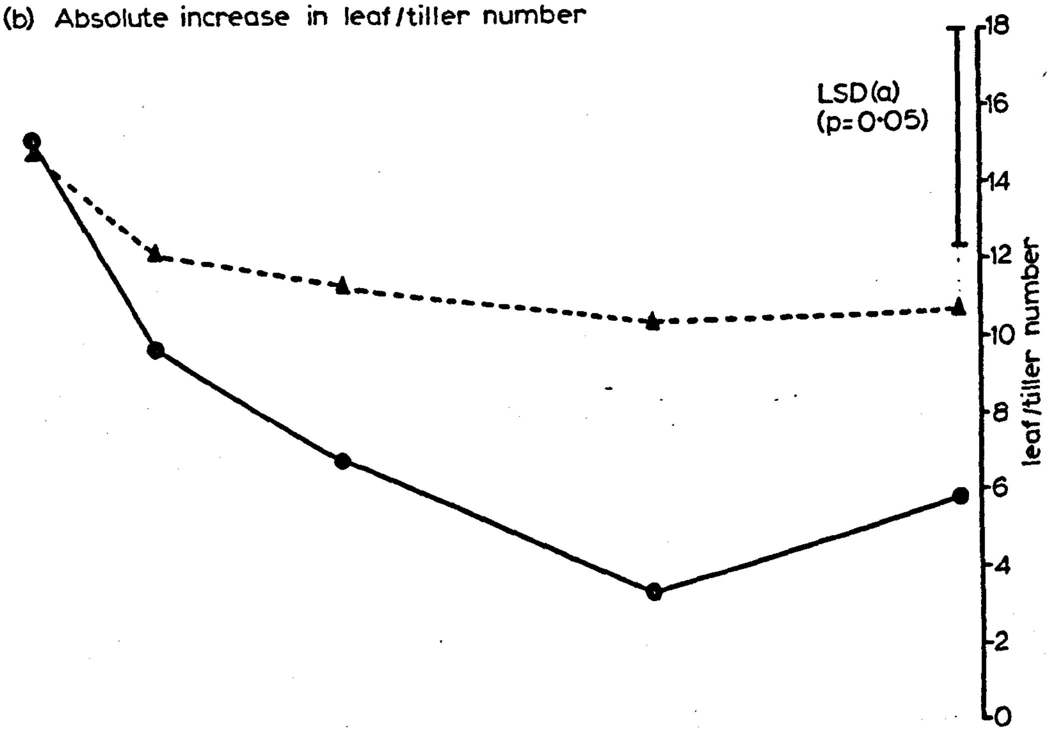
(b) Results

The trends in biomass at the time of harvesting are similar for both species (Figure 85). Shoot dry weight (Figure 85b) is significantly ($p < 0.001$) decreased by the lowest pressure employed (2i pt^{-1}). Further increases in trampling pressure lead to a further significant decrease in the shoot dry weight of P. lanceolata but not of P. bertolonii, although

(a) Percentage increase in leaf/tiller number



(b) Absolute increase in leaf/tiller number



(c) Weight per leaf/tiller

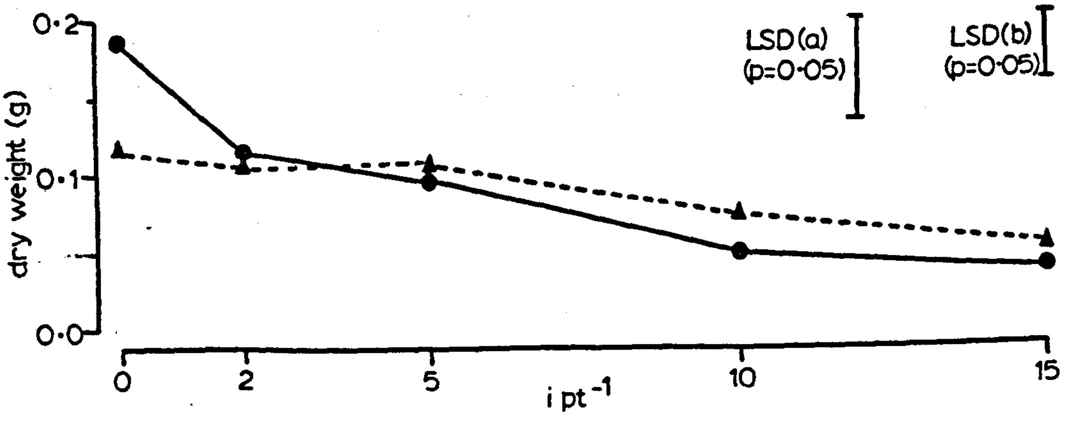


Figure 86 Effects of simulated trampling on leaf number of Plantago lanceolata (●) and tiller number of Phleum bertolonii (▲).

(LSDa - P. lanceolata, LSDb - P. bertolonii)

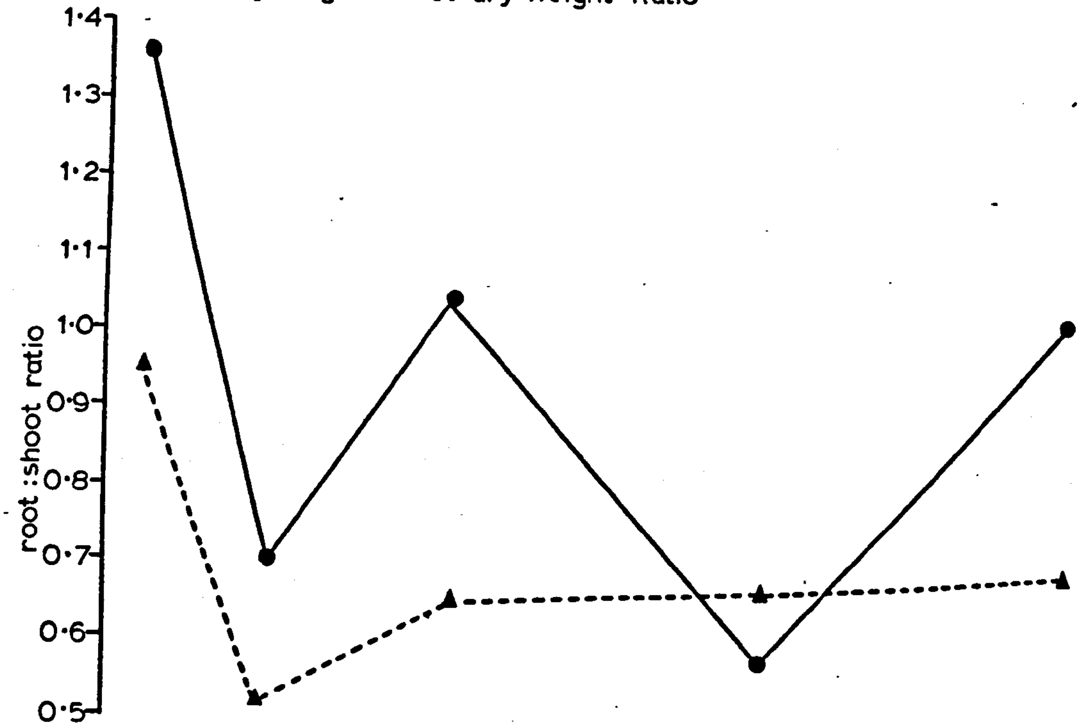
the trend for decreasing weight of the former is slight. The inclusion of the 'debris' component into shoot dry weight does not appreciably alter the relationship between pressure applied and dry weight, although the pressures required to produce a significant (at $p = 0.05$) reduction in dry weight are increased to 5i pt^{-1} for P. lanceolata and 10i pt^{-1} for P. bertolonii (Figure 85c).

Root weight is markedly depressed in both species but only significantly ($p < 0.001$) so in P. lanceolata. Following the initial decrease associated with a pressure of 2i pt^{-1}, there is no further marked change in root dry weight (Figure 85d).

Total plant weight exhibits similar relationships to those described above, the lowest pressure applied (2i pt^{-1}) resulting in a significant ($p < 0.05$) decrease in dry weight of both species, with little further decrease at higher pressures (Figure 85a).

Associated with changes in dry weight are decreases in leaf and tiller number (Figure 86b), which are marked and significant ($p < 0.01$) in P. lanceolata but less evident in P. bertolonii. The increase in leaf or tiller number of the control plants of the two species is approximately the same (5.1 leaves wk^{-1} and 5.06 tillers wk^{-1} for P. lanceolata and P. bertolonii respectively) while at the highest pressure employed leaf number increases by 1.9 leaves wk^{-1} for P. lanceolata and 3.6 tillers wk^{-1} for P. bertolonii. Changes in leaf or tiller number may be more meaningful if related to the number of leaves/tillers existing at the time of treatment. A 178.3% increase in tiller number of control plants of P. bertolonii compares with a 126.7% increase for plants receiving 15i pt^{-1}, while for P. lanceolata the percentage increase in leaf number decreases from 136.6% for control plants to 66.6% for plants receiving 15i pt^{-1} (Figure 86a). (The difference in response indicated by percentage increase and absolute increase in leaf/tiller number

(a) Root dry weight / Shoot dry weight Ratio



(b) Shoot moisture content ((fresh weight / dry weight) x 100)

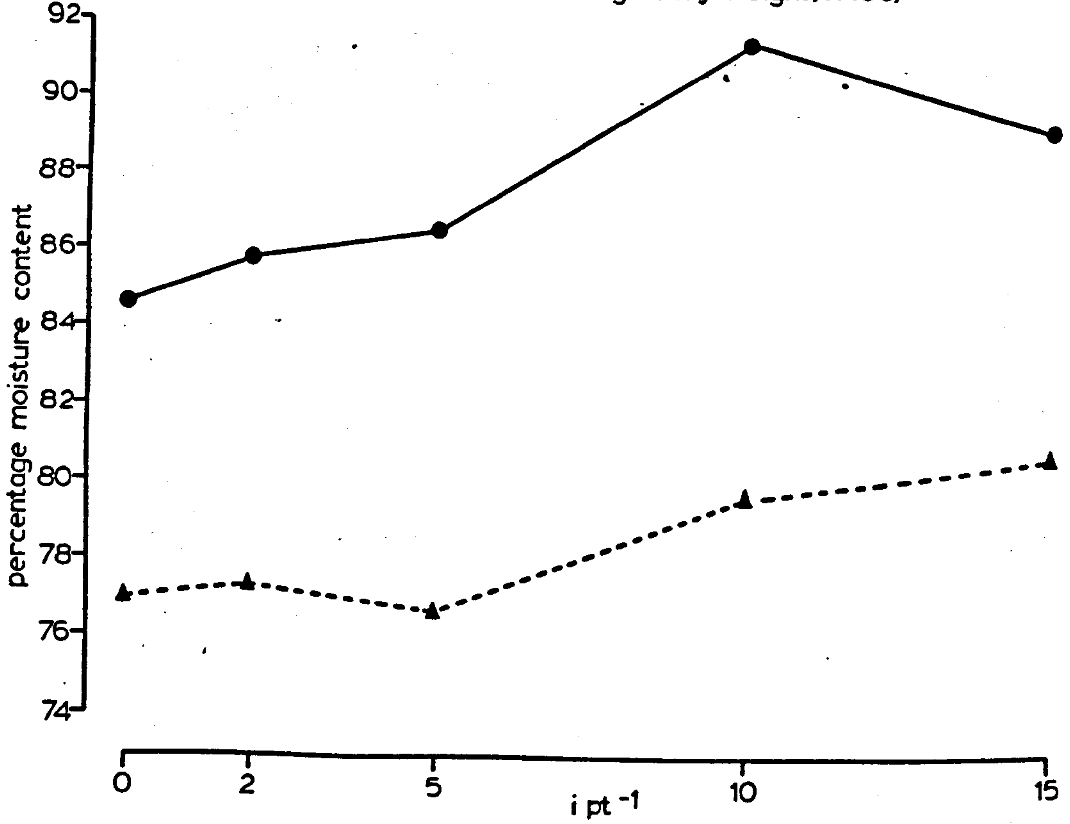


Figure 87 Effects of simulated trampling on root : shoot ratio and shoot percentage moisture content of Plantago lanceolata (●) and Phleum bertolonii (▲).

(Figure 86a, b) may reflect heterogeneity in the plants at the time of treatment. Thus, while leaf number may be more indicative of the potential photosynthetic surface development, treatment effects may be more accurately reflected by the percentage increases).

Although the two species are not directly comparable, because of possible differential recovery conditions, two way analysis of variance indicates significant ($p < 0.001$) species differences, in addition to treatment effects, in terms of leaf or tiller production (although leaves per tiller were not assessed). The range of increase over the treatments applied extends from 117.2% to 178.3% for P. bertolonii (i.e. 61.1%) and 40.9% to 136.6% for P. lanceolata (i.e. 95.7%) suggesting that simulated trampling has a more severe effect on leaf production by P. lanceolata than on tiller production by P. bertolonii.

An increase in trampling pressure also results in a significant ($p < 0.05$) decrease in dry weight per leaf or tiller in both species (Figure 86c). The effect does not become significant in P. bertolonii until a pressure of 10i pt^{-1}, yield per tiller not differing appreciably from the control value at lower pressures. In contrast, the response exhibited by P. lanceolata is markedly curvilinear, a pressure of 2i pt^{-1} causing the most marked drop in yield per leaf.

Root to shoot ratios show no significant association with the pressure applied, the pattern being erratic for P. lanceolata. The trends suggest that with increased pressure the shoot dry weight increases to a greater extent than the root dry weight (Figure 87a).

The percentage moisture content of the harvested shoot (Figure 87b) increases slightly with an increase in trampling pressure applied, the relationship being significantly linear for both species ($r = 0.596$, $p < 0.01$; $r = 0.517$, $p < 0.01$ for P. lanceolata and P. bertolonii respectively). However, the dependence of moisture content on environmental factors in addition to inherent species differences, makes inter-species comparisons futile.

(ii) The effect of simulated trampling on the Relative Growth Rate ($\bar{R}$) of Phleum bertolonii and Plantago lanceolata

A full analysis of Relative Growth Rate ($\bar{R}$) is given by Evans (1972). The concept is derived from a realisation that the rate at which a quantity changes is proportional to the pre-existing quantity, $\bar{R}$ expressing an "... integration of all the processes bringing about increase in the dry weight of the plant" (Evans, 1972). Evans defined $\bar{R}$ as the product of the Leaf Area Ratio (the leaf area per unit dry weight of the whole plant) and the Unit Leaf Rate (the rate of dry weight increase of the whole plant as a function of leaf area[1]) and noted that they vary with the ontogeny of the plant in addition to environmentally induced changes. $\bar{R}$ may be calculated according to (Evans, 1972):

$$\bar{R} = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1} \quad (\mathrm{g\ g^{-1}\ unit\ time^{-1}}) \qquad (28)$$

where : $\bar{R}$ = mean Relative Growth Rate over defined time interval (${t_1}$ to ${t_2}$):

W_1 = plant dry weight at time t_1;

W_2 = plant dry weight at time t_2.

Evans noted that in the measurement of $\bar{R}$ it is assumed that plants harvested to give W_2 were the same weight as plants giving W_1 at t_1. It is difficult to test the validity of this assumption since destructive harvests are involved but the estimate is likely to improve with increasing replication.

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[1] Unit Leaf Rate and Photosynthesis rate are not synomous since the former is dependent upon the overall metabolic balance of the plant.

Under standard conditions the population variability should be minimal although ontogenetic variation may introduce a source of heterogeneity. However, the treatments applied in the present investigation result in a variable decrease in plant biomass at t_1. To partially compensate for this it has been assumed that the initial population prior to treatment was homogeneous and the debris dry weight for each replicate has been subtracted from the mean dry weight for the hypothetical population at t_1, prior to the calculation of $\bar{R}$.

Evans (1972) also noted that homogeneity of variance in the population may be shortlived with the consequence that problems arise in analysis the variance of $\bar{R}$. This effect is possibly heightened by the unknown retardation imposed on plant development by the application of the treatment, resulting in substantial within treatment variance in addition to between treatment variance.

(a) Methods

Individual 70-day old plants of *P. bertolonii* and *P. lanceolata* (grown at 20°C/15°C day/night temperature, 85±10% relative humidity, 12h day at 13klx) were treated at 0, 5, 10, 20 and 40i pt^{-1}, with five replicates per treatment. After a recovery period of 14 days (conditions as above) plants were harvested and dried at 85°C. Debris produced at the time of treatment was washed and dried at 85°C. Five plants of each species were harvested at the time of treatment (t_1) to enable the calculation of $\bar{R}$.

(b) Results

The relationships between shoot and root dry weights and number of impacts per pot are similar to those presented above (Section V A, b(i)), the lowest pressure employed (5i pt^{-1}) having the most appreciable effect

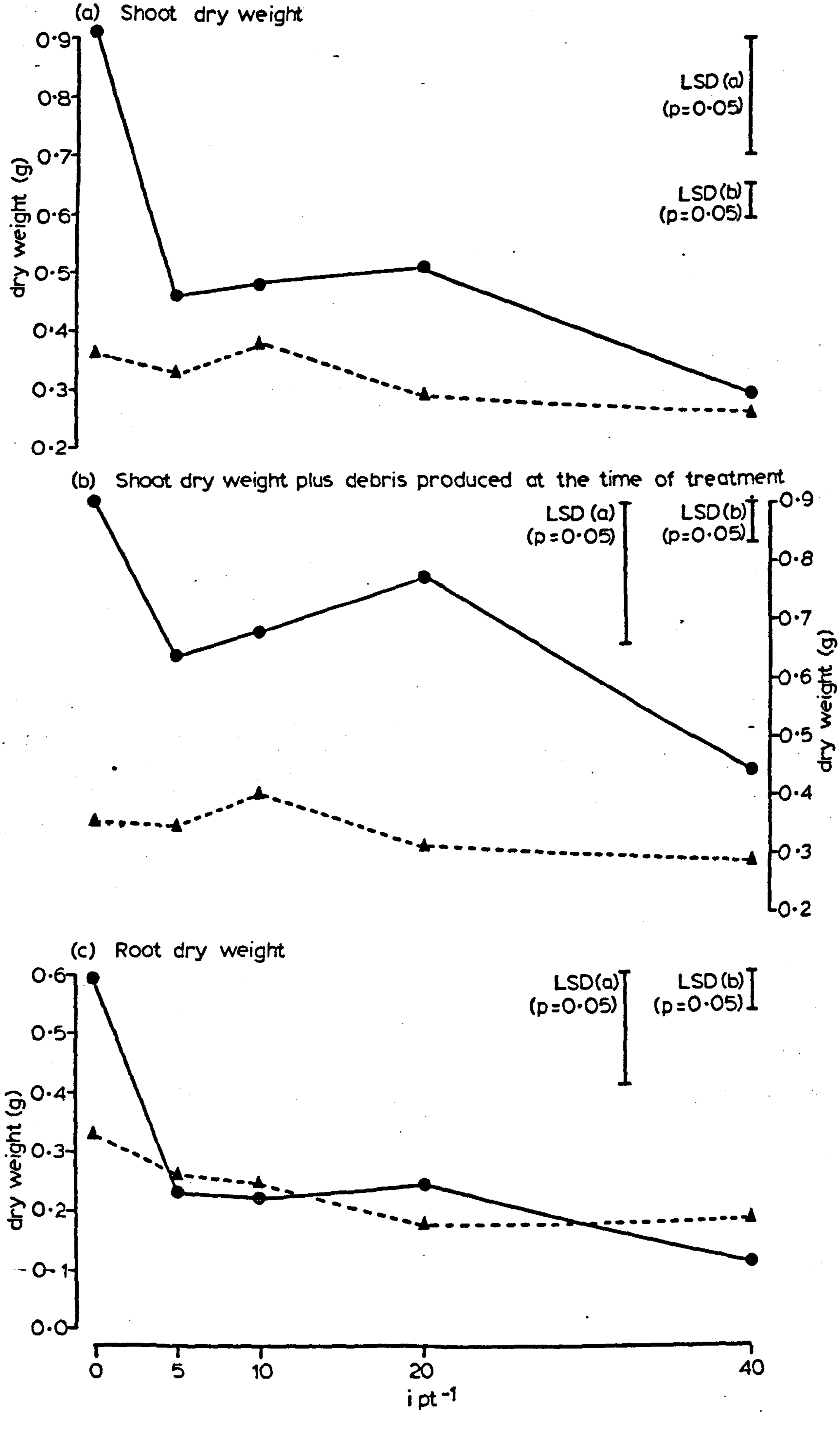
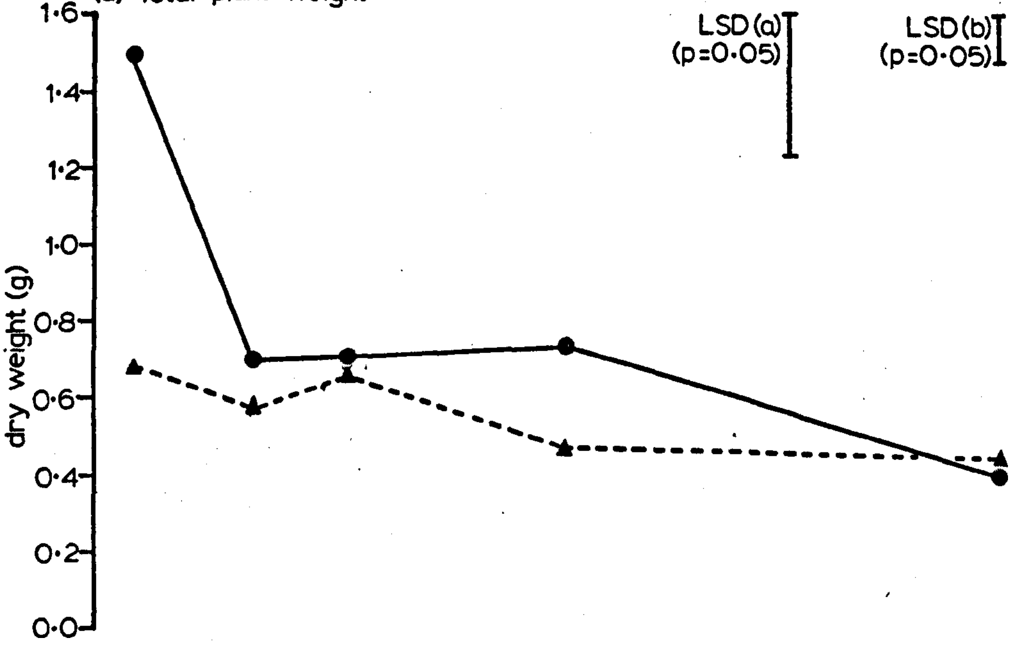


Figure 88 Effects of simulated trampling on the dry weight of Plantago lanceolata (●) and Phleum bertolonii (▲).
(LSD a - P. lanceolata, LSD b - P. bertolonii)


(a) Total plant weight

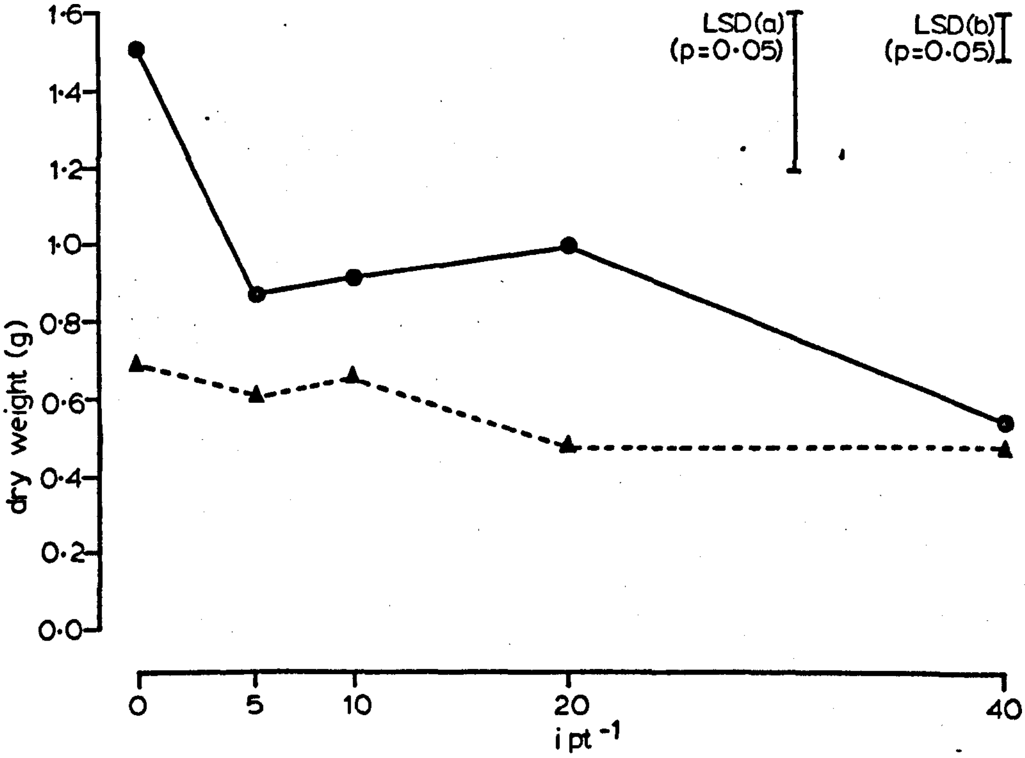

(b) Total plant weight plus debris produced at the time of treatment

Figure 89 Effects of simulated trampling on total plant weight of Plantago lanceolata (●) and Phleum bertolonii (▲)
(LSDa - P. lanceolata, LSDb - P. bertolonii)

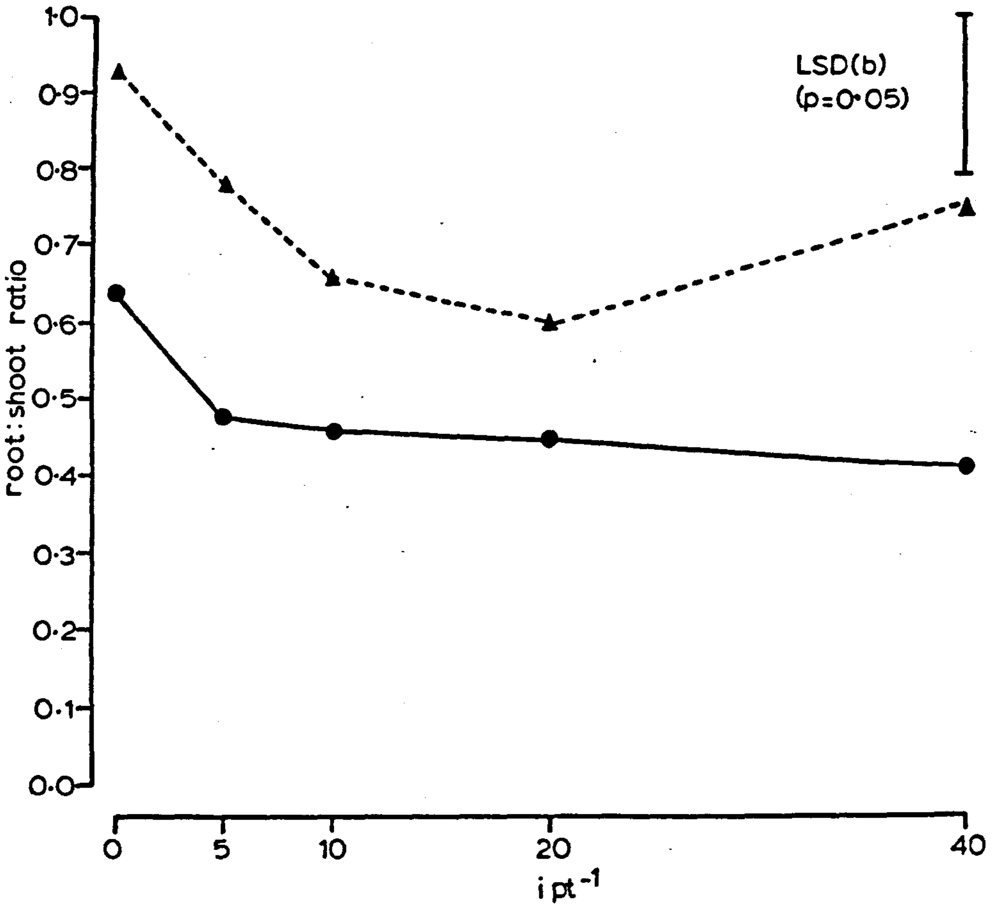


Figure 90 Effects of simulated trampling on the root:shoot dry weight ratio of <u>Plantago lanceolata</u> (●) and <u>Phleum bertolonii</u> (▲)

(LSDb - <u>P. bertolonii</u>)

on plant dry weight, further increases in pressure having a progressively less severe effect (Figure 88). (The younger plants employed and the shorter recovery period may contribute to the lower plant weights observed compared to those presented in Section V A, b(i)).

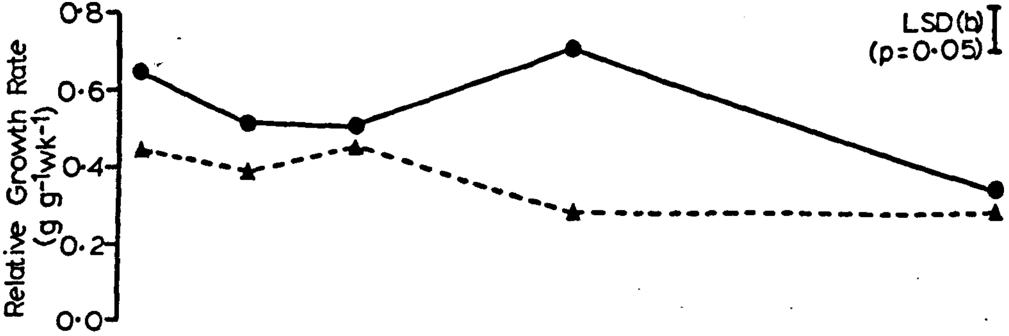
P. lanceolata appears to be affected to a greater extent than P. bertolonii. It is of interest to note that a pressure of 20i pt^{-1} results in a slight increase in dry weight compared to lower pressures for P. lanceolata (Figure 88a), which is appreciable when debris weights are included (Figure 88b). This results in only the dry weights of plants receiving 5i pt^{-1} and 40i pt^{-1} being significantly ($p < 0.05$) less than the control. This trend is less evident for root weight data, a pressure of 5 i pt^{-1} causing a significant ($p < 0.001$) reduction in root dry weight which thereafter remains relatively constant with increases in pressure (Figure 88c).

The data for P. bertolonii indicate that only the highest pressure employed (40i pt^{-1}) results in a significant ($p < 0.01$) reduction in shoot dry weight. A pressure of 10i pt^{-1} is associated with a biomass of the shoot that is greater than the control, though not significantly so (Figure 88b). The effect of trampling pressure on root weight is small but significant ($p < 0.001$) (Figure 88c).

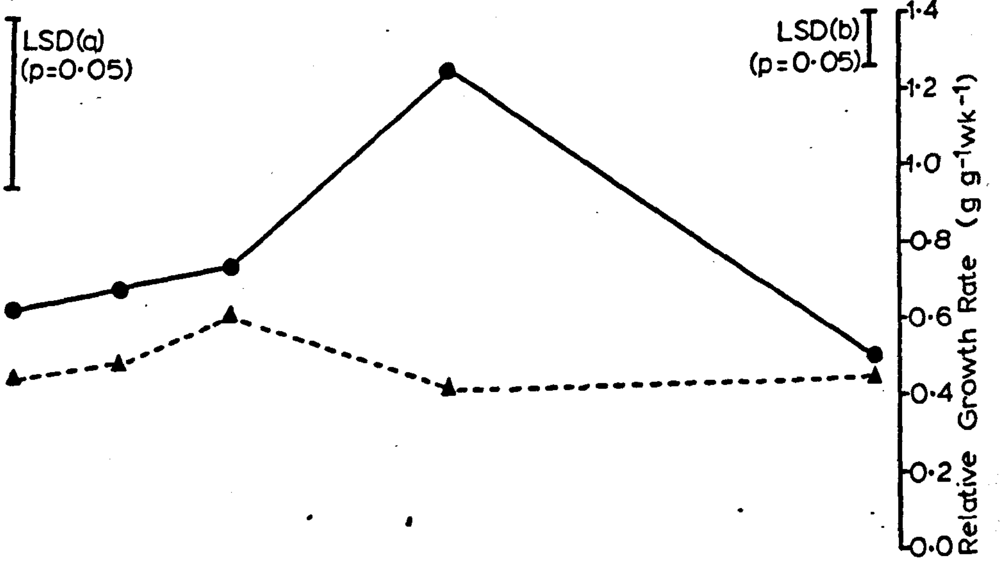
When the data are combined into total plant weight (Figure 89a), the stimulatory effects of trampling on both species are less apparent. A large and significant ($p < 0.001$) reduction in plant weight occurs at a pressure of 5i pt^{-1} for P. lanceolata, a further reduction occurring at a pressure of 40i pt^{-1}. Although P. bertolonii is affected significantly ($p < 0.01$) by simulated trampling pressures the effects are comparatively less pronounced.

The root to shoot ratios are more consistent than above (Section V A, b(i)) although the variation is only significant ($p < 0.05$) for

(a) Total plant Relative Growth Rate



(b) Shoot Relative Growth Rate



(c) Root Relative Growth Rate

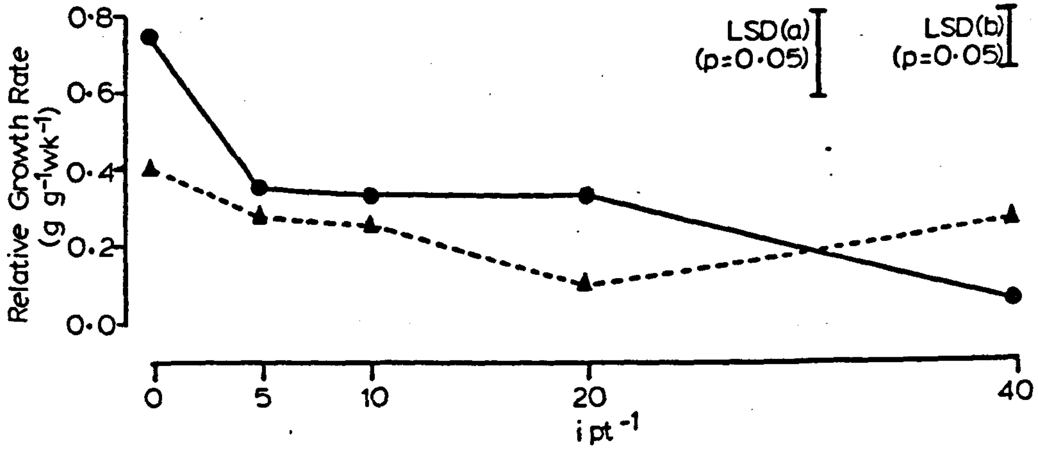


Figure 91 Effects of simulated trampling on the Relative Growth Rate of Plantago lanceolata (●) and Phleum bertolonii (▲).

(LSDa - P. lanceolata, LSDb - P. bertolonii)

P. bertolonii (Figure 90). In this species the lowest ratio occurs at a pressure of 20i pt^{-1} above which the ratio increases, but not significantly. The trend in P. lanceolata is for a decrease in root to shoot ratio which is most marked at a pressure of 5i pt^{-1}, higher pressures having little further effect on the ratio (Figure 90).

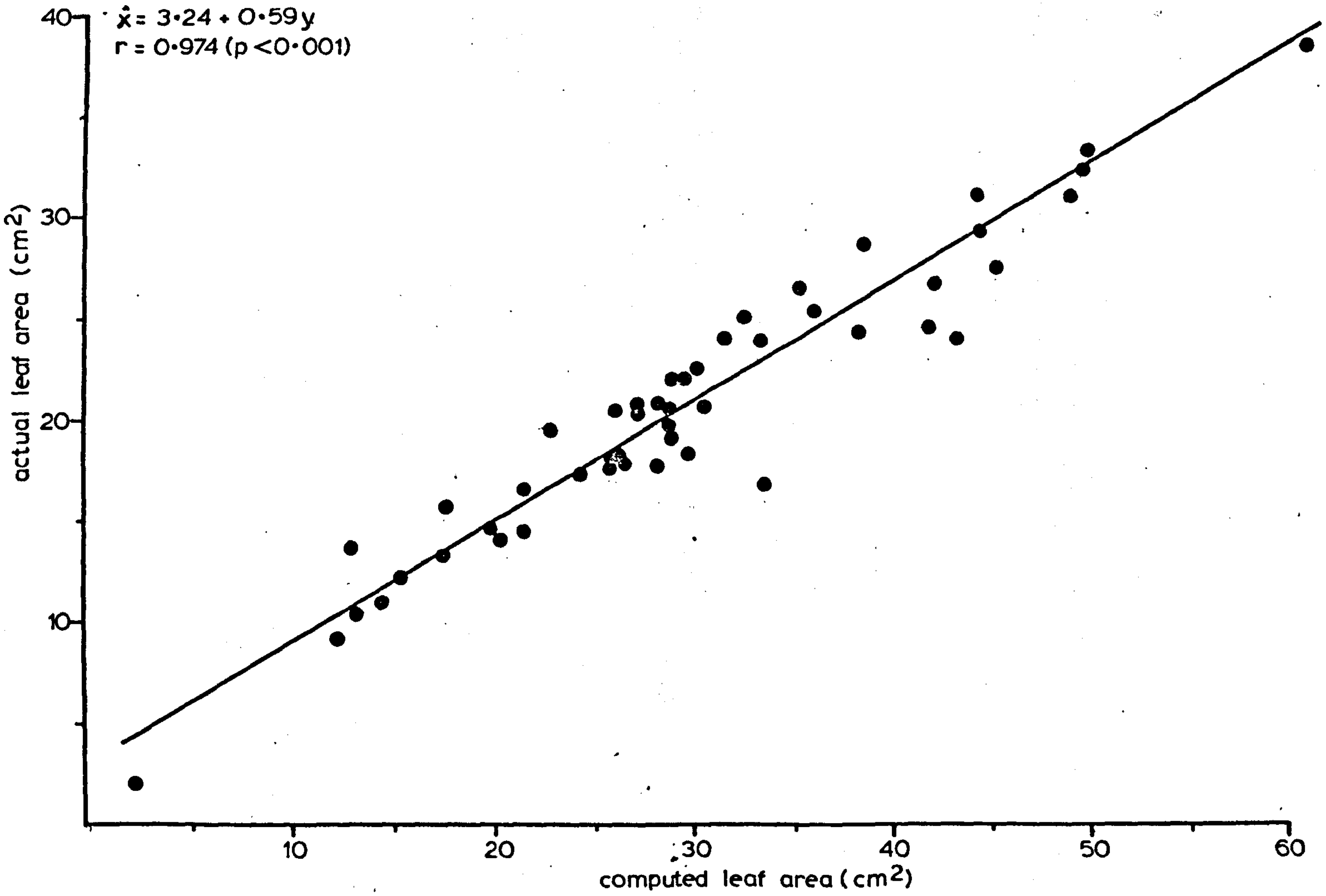
The control shoot $\bar{R}$'s for P. lanceolata and P. bertolonii (Figure 91b) are 0.646g g^{-1} wk^{-1} and 0.473g g^{-1} wk^{-1} respectively. There is a significant ($p < 0.05$) increase in shoot $\bar{R}$ to 1.268g g^{-1} wk^{-1} (at 20i pt^{-1}) and 0.638g g^{-1} wk^{-1} (at 10i pt^{-1}) for P. lanceolata and P. bertolonii respectively. At pressures greater than those stimulating shoot growth, $\bar{R}$ is depressed but is not significantly lower than that of the control. The stimulatory effect is not so apparent when total plant $\bar{R}$ is considered (Figure 91a). Although the trends are similar, simulated trampling does not have a significant effect on the $\bar{R}$ of P. lanceolata. The dampening of the stimulatory effect of simulated trampling on shoot growth may result from the significant ($p < 0.01$) decreases in root $\bar{R}$ at 10i pt^{-1} and 20i pt^{-1} for P. bertolonii and P. lanceolata, counteracting the increase in $\bar{R}$ of the shoots at these pressures. The root $\bar{R}$ of P. lanceolata decreases to a minimum of 0.034g g^{-1} wk^{-1} at a pressure of 40i pt^{-1}, and 0.103g g^{-1} wk^{-1} at a pressure of 20i pt^{-1} in P. bertolonii, compared to control values of 0.755g g^{-1} wk^{-1} and 0.409g g^{-1} wk^{-1}, representing a 74.8% and 95.5% reduction respectively (Figure 91c). In addition to significant ($p < 0.05$) species differences in $\bar{R}$ in which that of P. lanceolata is generally greater than the $\bar{R}$ of P. bertolonii, a significant ($p < 0.001$) species x treatment interaction suggests that the two species react differently to simulated trampling treatments. The results indicate that, while in terms of biomass a reduction occurs relative to the control as a result of simulated trampling pressures, in terms of growth rate, a stimulation in shoot growth occurs in both species which is accompanied by a decrease in root $\bar{R}$, although the quantitative relationships may have little practical meaning.

(iii) The effect of simulated trampling on the Leaf Area Increase of *Plantago lanceolata*

A quantity analogous to relative growth rate is the relative increase in leaf area, the Relative Leaf Growth Rate (RLGR).

The problems and assumptions regarding environmental and ontogenetic effects on $\bar{R}$ similarly apply to RLGR (Evans, 1972). The value of such a measurement lies in the assumption that the leaves are the primary photosynthetic organs. This may be a valid assumption in the case of *Plantago lanceolata* but does not take into account the photosynthetic capacity of other plant organs e.g. stems, of other species. Evans (1972) suggested that when considering the unit of photosynthetic production, the proportion of the production diverted into increasing the photosynthetic area of the plant is of some significance, especially in the way in which it is affected by age and environment. In the present context, age may affect the recovery of the plant but, in addition, the effects of mechanical damage and defoliation on the proportion of assimilates diverted into increasing photosynthetic surfaces may be of especial significance in aiding recovery. Thus, an 'index of growth in leafiness' (Evans, 1972), provided by RLGR, may be of relevance in considering the potential photosynthetic capacity of trampled plants. In addition, RLGR has the advantage over dry weight determinations in that sampling is not destructive, allowing repeated measurements of the same tissue over time.

Figure 92 Regression of computed leaf area on actual leaf area, <u>Plantago lanceolata</u>.



(a) Methods

Individual 55-day old plants of P. lanceolata (grown at 20 ± 1°C, 75 ± 10% relative humidity, 12h day at 13klx) received 0, 5, 10, 20 or 40i pt^{-1}, with five replicates per treatment. The plants were allowed to recover under conditions identical to those in which they were grown. Leaf area increase was followed, on the first leaf to emerge following treatment, over the five days following emergence.

Measurement of leaf area has been reviewed by Květ & Marshall (1971) and Evans (1972). The advantages of deriving leaf area from linear dimensions include the rapidity with which such measurements may be made and the minimum of interference to the plant, leaf length and breadth being most frequently used (Květ & Marshall, 1971). Simple relationships of the form

$$A = b \times LB \qquad (29)$$

where: A = area;

L = length of leaf;

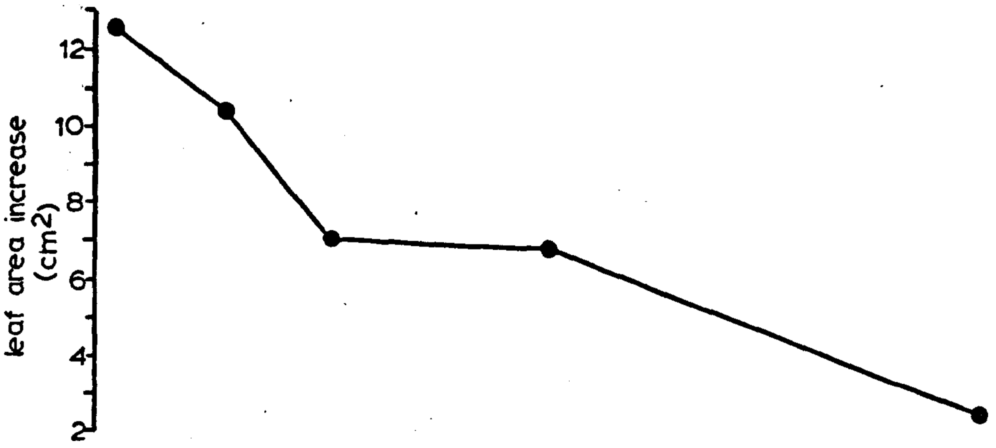
B = breadth of leaf;

b = empirical coefficient.

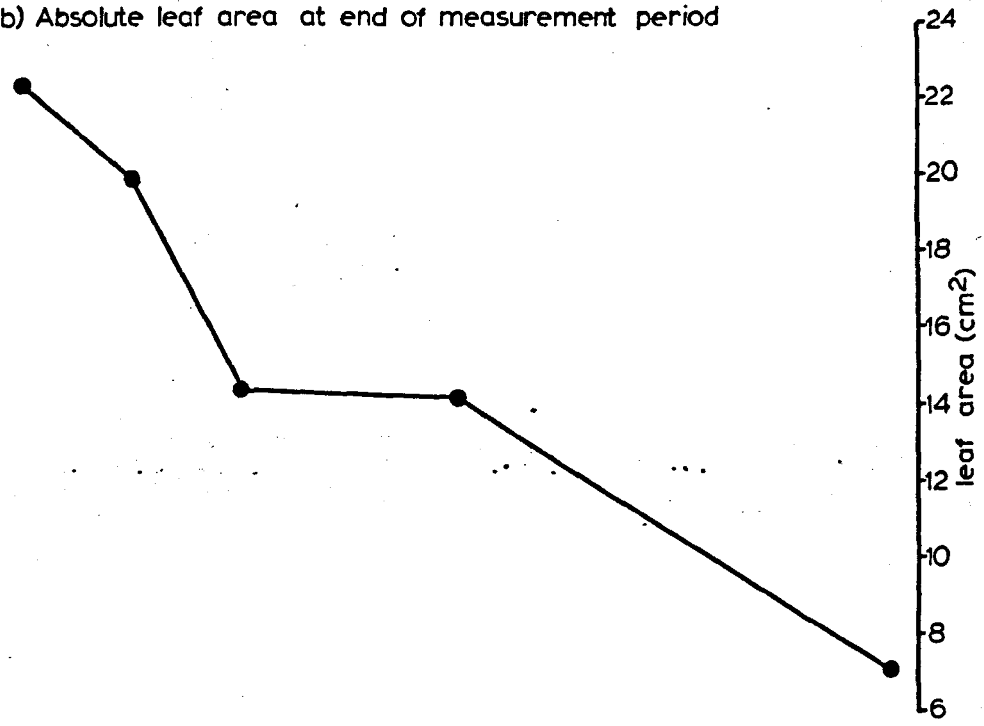
have been employed in addition to full regression equations. Květ & Marshall noted that leaf length alone is difficult to convert into leaf area, although in certain circumstances it is the only parameter which can be accurately measured e.g. in species with narrow leaves.

In the present study an empirical relationship was determined relating actual leaf area to computed leaf area determined from the product of leaf length multiplied by leaf breadth at the midpoint of the leaf. (Leaf length was arbitrarily defined as the point at which the leaf width exhibits a marked increase over the proximal part of the leaf structure, since in P. lanceolata the leaf blade is not clearly distinguishable from the petiole, to the leaf tip). Absolute leaf area

(a) Absolute increase in leaf area over measurement period



(b) Absolute leaf area at end of measurement period



(c) Relative increase in leaf area (Relative Leaf Growth Rate)

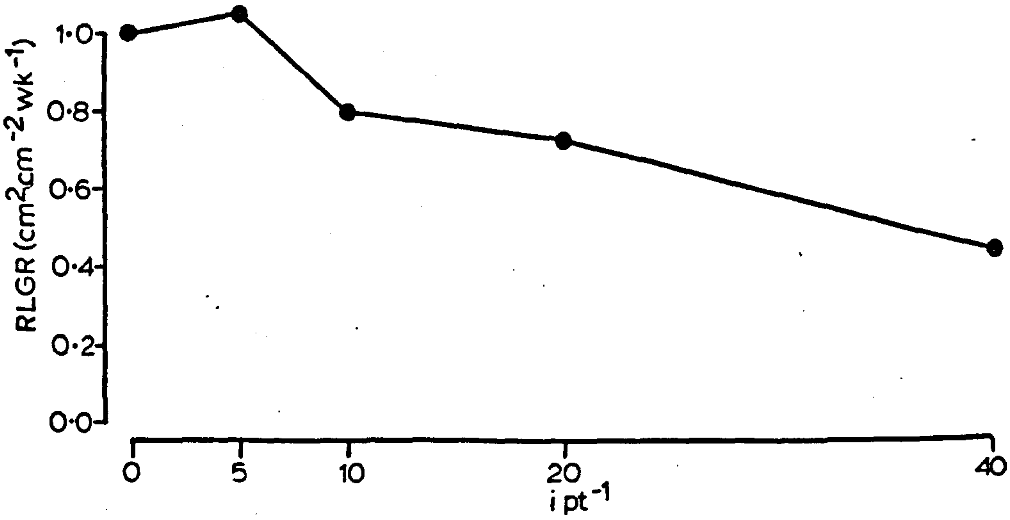


Figure 93 Effects of simulated trampling on leaf development of *Plantago lanceolata*, (measurements made on first leaf to emerge following application of the treatment).

was determined by planimetry. A significant linear correlation was found between the two measurements ($r = 0.974$, $p < 0.001$). The regression equation ($\hat{x} = 3.24 + 0.59y$, where y represents computed leaf area) was used to correct length x breadth measurements to actual leaf area (Figure 92).

Equation 28 was used to calculate RLGR ($cm^2\ cm^{-2}\ wk^{-1}$). All results have been subject to analysis of variance tests.

(b) Results

Although no significant treatment effects on leaf area increase are observed, certain trends are apparent (Figure 93). The increase in leaf area up to the fifth day after emergence is reduced by 92.1% from $12.7cm^2$ for control plants to $2.3cm^2$ for plants which received a pressure of $40i\ pt^{-1}$ (Figure 93a). In terms of absolute photosynthetic surface, this represents a reduction of 68.1% from $22.4cm^2$ for control plants to $7.2cm^2$ for plants receiving the highest pressure employed (Figure 93b).

The response in terms of RLGR (Figure 93c) suggests that the initial leaf areas at t_1 were not homogeneous throughout the treatments since although absolute leaf area is lower for plants receiving $5i\ pt^{-1}$, the RLGR is marginally greater than the control value. The general response, however, is for increasing pressure to be associated with an appreciable, although non-significant, decrease in RLGR from $1.0cm^2\ cm^{-2}\ wk^{-1}$ for controls to $0.4cm^2\ cm^{-2}\ wk^{-1}$ for plants receiving the highest pressure applied.

Daily measurements reveal a substantial, but non-significant, difference in leaf area ($4.8cm^2$) between the treatment extremes (0 and $40i\ pt^{-1}$) on day 1 of the measurements (Figure 94), thus the requirement of homogeneity of leaf size at zero time is not fulfilled. Significant ($p < 0.05$) treatment effects do not become evident until day 3 when

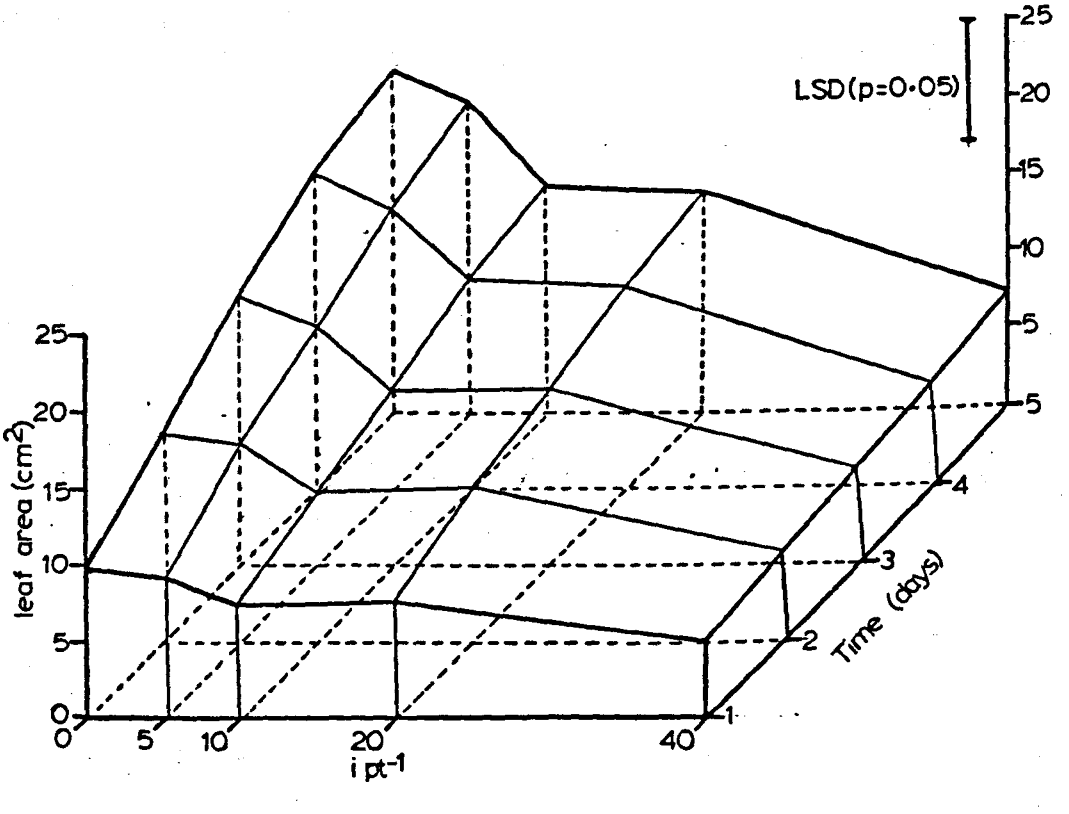


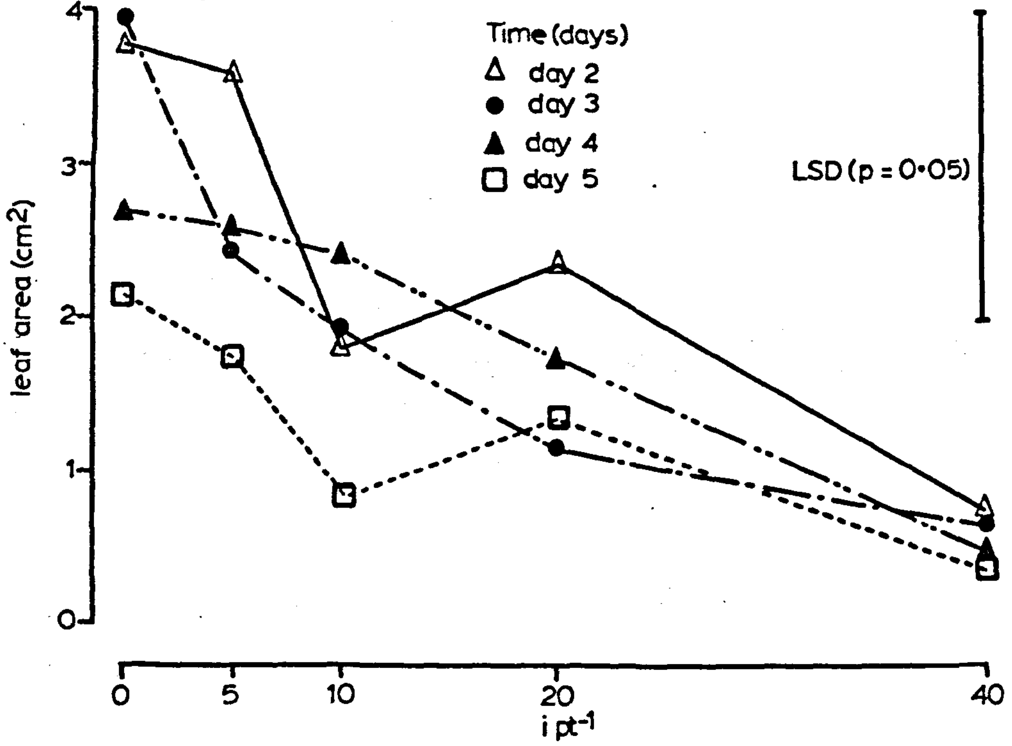
Figure 94 The effect of simulated trampling on leaf area increase, Plantago lanceolata.

(Measurements made on first leaf to emerge following application of the treatment.)

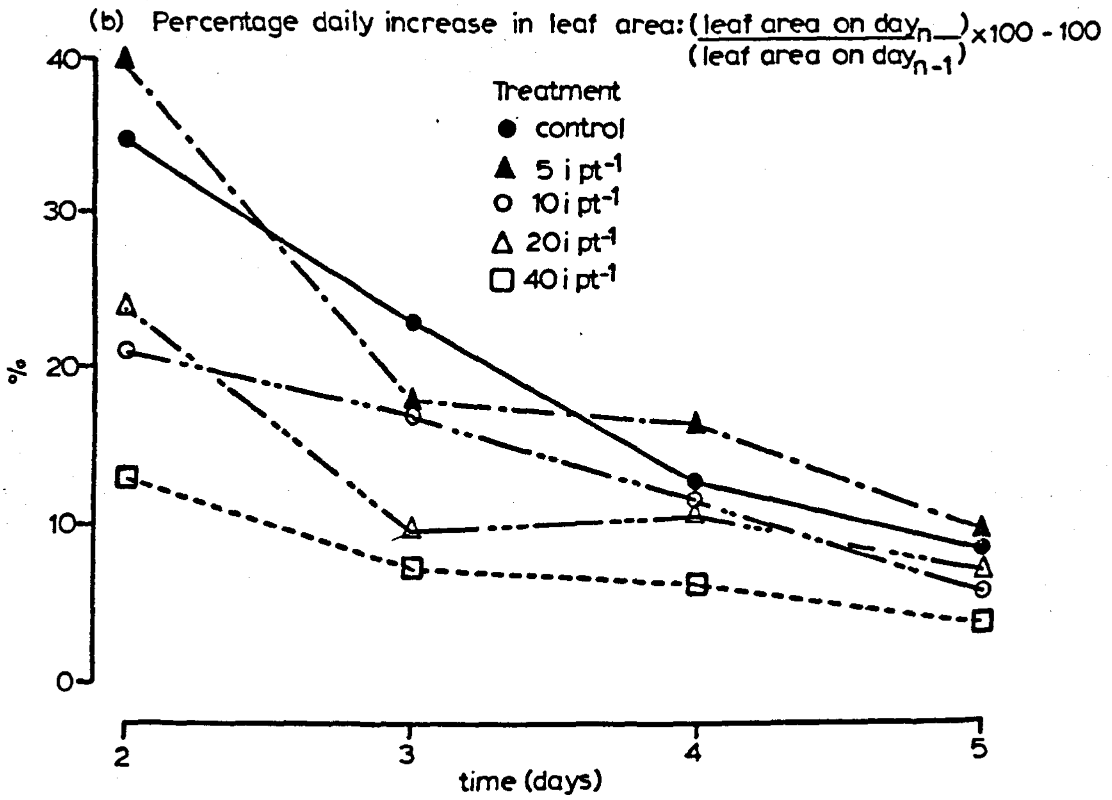
plants receiving 40i pt^{-1} have significantly lower leaf areas than control plants. Only the control plants and plants receiving 5i pt^{-1} exhibit a significant ($p < 0.05$) increase in leaf area over time (Figure 94), although all treatments,except that of 20i pt^{-1}, show a linear increase in leaf area over time ($r = 0.455$, $p < 0.05$; $r = 0.672$, $p < 0.001$; $r = 0.447$, $p < 0.05$; $r = 0.382$, n. s.; $r = 0.422$, $p < 0.05$ for 0, 5, 10, 20 and 40i pt^{-1} respectively). The decreasing slopes of the relationship between leaf area and time with increasing pressure (Figure 94) emphasises the greater leaf area increment of control plants over time compared to treated plants such that control leaves increase by an average of 230.3% in area over the five day measuring period compared to a 144.1% increase in plants treated at 40i pt^{-1}.

The highest daily increases in leaf area occur in control plants e.g. 3.9cm^2 (day 3), while the lowest occur in plants treated at 40i pt^{-1} e.g. 0.4cm^2 (day 5) (Figure 95a). Although significant ($p < 0.001$) treatment effects occur in the daily increases in leaf area, differences over time are not significant. However, whereas there is a progressive decrease in leaf area increment over time for plants treated at 40i pt^{-1}, the leaf area increases for the remaining treatments are somewhat erratic, no consistent pattern emerging. It is of interest to note that although for any particular day the leaf area increment is greatest in the control plants, decreasing from a maximum of 3.9cm^2 on day 3 to 2.2cm^2 on day 5, the leaf area increments on days 2 and 4 for plants receiving 20i pt^{-1} exceeds the lowest leaf area increase of the control plants, possibly redressing part of the imbalance existing between leaf area increases of control and treated plants.

As indicated by RLGR measurements, initial leaf area, which was heterogeneous, may affect absolute increases in leaf area. When such increases are expressed in percentage form (Figure 95b) plants receiving



(a) Daily increase in leaf area



(b) Percentage daily increase in leaf area: $\left(\frac{\text{leaf area on day}_n}{\text{leaf area on day}_{n-1}}\right) \times 100 - 100$

Figure 95 The effect of simulated trampling on leaf development of <u>Plantago lanceolata</u>, followed over time, (measurements made on first leaf to emerge following treatment).

5i pt^{-1} exhibit a slightly greater percentage increase in leaf area than control plants on day 2, although plants treated at 40i pt^{-1} have a consistently lower percentage increase in leaf area compared to other treatments. Percentage increase in leaf area also decreases over time, although this effect generally becomes less pronounced with increasing trampling pressure.

On days where the order of increase in leaf area is not synomous with increasing trampling pressures, an imbalance in the treatment effect occurs. For example, on day 2, 4 and 5 the percentage increase in leaf area of plants treated at 5i pt^{-1} is greater than that of other treatments (Figure 95b). This represents a comparatively greater gain in leaf area which if maintained would ultimately result in a larger absolute leaf area, but such an advantage is lost when the percentage increase in leaf area drops below that of other treatments. However, the percentage increase is a time dependent variable, decreasing, in all treatments, over time so that any compensatory effect does not become apparent in absolute leaf area, with the consequence that actual leaf area is consistently greater in control than in treated plants.

(iv) The effect of regular simulated trampling on the biomass and growth rate of *Plantago lanceolata*

(a) Methods

Individual 50-day old plants of *P. lanceolata* (grown at 20°C/15°C day/night temperature, 75 ± 10% relative humidity, 12h day at 13klx) were subject to the following treatments:

A - control (5);

B - 2i pt^{-1} applied once only (5);

C - 2i pt^{-1} applied every 14 days for 6 wks. (5);
D - 5i pt^{-1} applied every 14 days for 6 wks. (4).

(A fifth treatment, 5i pt^{-1} applied once only, failed due to a high mortality which also affected other treatments resulting in unequal replication (number of replicates in parentheses). Consequently, results were analysed using one way analysis of variance with unequal replication (Appendix A, 1(b)).

Five plants were harvested at the time of treatment to permit the calculation of $\bar{R}$ (Equation 28). Any material severed from the plant at each treatment date was washed and dried at 85°C. Material dying over the duration of the experiment was collected and oven dried. Following the fourth treatment application, a 14 day recovery period was allowed before plants were harvested and oven dried.

(b) Results

No significant treatment effects are observed in the dry weight of harvested green material although values from trampled plants are appreciably lower than that of the control. It is of note that treatments B and C are not appreciably different (Figure 96a). Similarly, the dry weight of dead material (representing death between the date of the final treatment application and that of harvesting) does not vary significantly between treatments (Figure 96b). Although the absolute values for dead material are similar, when these are expressed as a percentage of the respective treatment total dry weight, there is an appreciable increase in the proportion of dead material per plant from the control to plants receiving the highest pressure employed (A : 7.6%; B : 10.6%; C : 10.8%; D : 21.5%).

Flower production by the 106-day old plants complicates an analysis

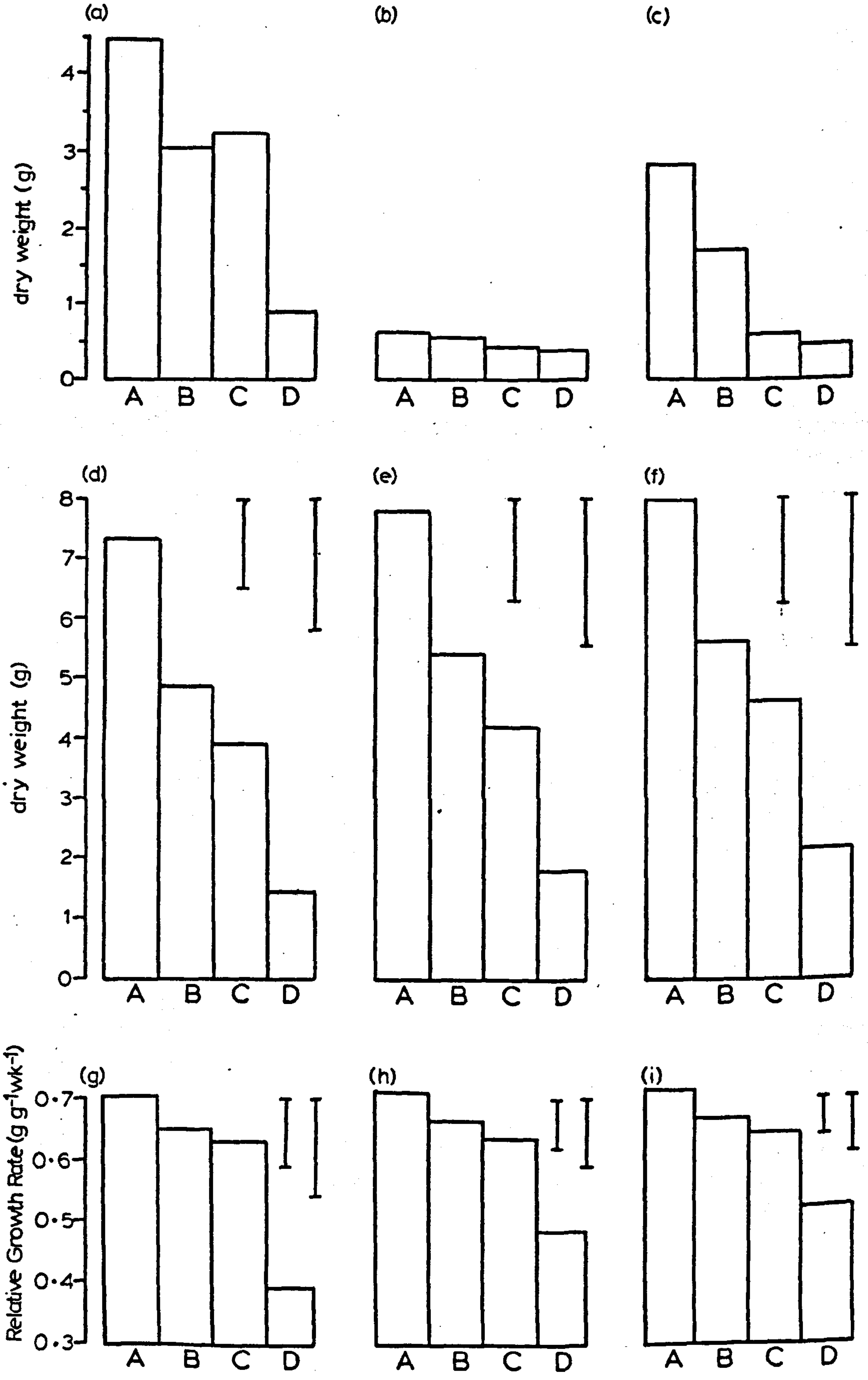

(a)
(b)
(c)
dry weight (g)
0
1
2
3
4
A
B
C
D
(d)
(e)
(f)
dry weight (g)
0
1
2
3
4
5
6
7
8
(g)
(h)
(i)
Relative Growth Rate (g g-1wk-1)
0·3
0·4
0·5
0·6
0·7

Figure 96 The effect of continuous simulated trampling on the growth of <u>Plantago lanceolata</u>.

Treatment

A	control
B	2 i pt^{-1}, applied once only.
C	2 i pt^{-1}, applied every 14 days; total 8 i pt^{-1}.
D	5 i pt^{-1}, applied every 14 days; total 20 i pt^{-1}.

(a) Dry weight of green material at time of harvest.
(b) Dry weight of dead material at time of harvest.
(c) Dry weight of flowers at time of harvest.
(d) Total dry weight of living fraction, (green material + flowers).
(e) Total dry weight at time of harvest, (green + dead + flowers).
(f) Total harvested dry weight plus debris produced as a result of the treatment and material dying over the duration of the experiment.
(g) Relative Growth Rate of living fraction.
(h) Relative Growth Rate of harvested plant.
(i) Relative Growth Rate of harvested plant plus debris produced as a result of the treatment and material dying over the duration of the experiment.

(bar lines indicate the Least Significant Difference between two means; the bar line to the left applies to comparisons between treatments A, B and C, the bar line to the right applies to comparisons between treatment D and any other treatment.)

of plant growth owing to possible effects on the rate of photosynthesis and assimilate utilization. The non-significant decrease in flower weight of trampled plants (Figure 96c) may have little meaning, being indicative of a possible adverse effect of trampling on flower number. The results suggest that in plants receiving regular simulated trampling (treatments C and D) flower initiation and growth occurs comparatively rapidly during the 14 day recovery period, resulting in a flower weight of approximately 30% of that of plants trampled at one time only (treatment B). Trampling applied at one time only would also appear to have an adverse effect on flower production despite a 56 day recovery period, flower weight being reduced to 61.3% of the control value.

All trampling treatments have a significant ($p < 0.001$) effect on the total dry weight of the living fraction of the plant (green material plus flowers) (Figure 96d). It is of interest that the same trampling pressure (2i pt^{-1}) applied regularly does not result in a significantly lower dry weight than when the pressure is applied once only. The effect of a higher pressure applied regularly is to result in a further significant lowering of plant dry weight. This pattern is maintained when dead material (Figure 96c) and debris produced over the duration of the experiment (Figure 96f) are included in the dry weight data.

No significant reduction in $\bar{R}$ occurs due to the lowest pressure applied (2i pt^{-1}) until material shed during the course of the experiment is taken into consideration (Figure 96, g-i), when treatment C becomes significantly ($p < 0.01$) lower than the control value. The $\bar{R}$ of treatment D is consistently significantly ($p < 0.01$) lower than all other treatments but whereas the $\bar{R}$ of treatments A, B and C are not appreciably altered by the inclusion of dead material and debris, the $\bar{R}$ of treatment D increases by 0.131g g^{-1} wk^{-1} by the inclusion of these fractions compared to 0.012 g g^{-1} wk^{-1} for control plants (Figure 96i). This suggests that severed material may make an appreciable difference to the calculated value of $\bar{R}$ for trampled plants. Since these values represent the mean relative growth rate over 56 days, an underestimate of the true rate of growth may arise for treatments C and D due to a reduction in plant size which occurs on each treatment date due to defoliation, so altering W_1 at t_1 for inter-treatment $\bar{R}$.

In cumulative terms, treatment C does not result in a significantly lower value for any dry weight or growth rate value than treatment B (i.e. 8i pt^{-1} and 2i pt^{-1} respectively). However, treatment D (20i pt^{-1}) is associated with a more marked, and generally significant, reduction in these parameters compared to treatments A, B and C.

(c) Discussion

In contrast to the findings of Bayfield (1971) and Leney (1974), there is no evidence of an increase in biomass of either Phleum bertolonii or Plantago lanceolata at low simulated trampling intensities, nor is an increase in leaf or tiller number apparent, which has been ascribed as a cause for increased size of trampled plants (Leney, 1974). The absence of such increases may be due to recovery periods of insufficient duration to permit possible stimulatory effects of low trampling pressures to become apparent, although Bayfield (1971) used a 21 day recovery period while Liddle & Greig-Smith (1975b) observed increased tillering of Festuca rubra 14 days after applying pressure. However, comparisons with the results of other workers may be confused by differential species responses to trampling (Leney, 1974), age of the plant at the time of trampling and growth conditions experienced by the plant following treatment.

Apart from the lack of stimulatory effects, the findings on the relationship between simulated trampling pressure and plant biomass are consistent with those of Bayfield (1971). The results presented in Section VA, b(i) suggest a threshold pressure exists above which further increases have an insignificant effect of plant biomass, indicative of a saturation of response. This occurs at a low pressure (2 - 5i pt^{-1}) possibly reflecting Edmond's observations (1958) that the first treading is the most damaging. In terms of growth potential, the results suggest a possible progressive reduction in photosynthetic surface to a point at which little viable photosynthetic tissue remains and regeneration is from basal meristems and buds. In the absence of any assimilation by remaining leaves on defoliated plants (cf. Davidson & Milthorpe, 1966a,b; Smith, 1974; Ryle & Powell, 1975), regeneration of totally defoliated plants may be retarded to a greater degree than their leaf bearing counterparts trampled at a lower pressure and less severely damaged. Following total defoliation, further pressure might result in damage to basal meristems and buds which, if protected by the soil (Bates, 1935), may require a greater pressure to produce a measurable effect, thus resulting in the observed curvilinear relationship. That regrowth following total defoliation at high levels of pressure is dependent on the development of new shoots was observed by Bayfield (1971) who found that recovery of S50 Timothy following total leaf destruction took the form of small new shoots after a period of seven days. Edmond (1958) also found that recovery and reappearance of damaged and buried tillers was rapid. The rate of reappearance may be of some significance for the continued survival of plants subject to regular trampling. The reduction in plant dry weight resulting from the application of simulated trampling pressure at one time implies that a recovery period of insufficient duration may result in a continual reduction in assimilatory surface, and thus potential photosynthetic capacity, leading to a degradation of the assimilatory mechanism in regularly trampled plants. If stored reserves are involved in regeneration (cf. Smith, 1974; Ryle & Powell, 1975) their eventual depletion could lead to an inability by the plant to regenerate. The results from plants regularly treated suggest that survival is not jeopardised by reserve depletion in the species used and at the intensity, frequency and duration of application employed, but the results imply that the recovery period is a function of the pressure applied and that recovery at high trampling pressures may take a comparatively long time.

The reduction in root biomass is also consistent with the findings of Bayfield (1971) and Leney (1974), although Leney noted that certain species responded to simulated trampling by developing larger roots. The root biomass data do not permit a distinction to be made between a comparative reduction in root dry weight, due to increased trampling pressure, resulting from root death or translocation of materials from root to shoot, (although no such processes were observed by Ryle & Powell (1975) in defoliated plants), or from a reduction in root growth rate due to increased mechanical impedence to penetration (cf. Rosenberg & Willits, 1962; Schuurman & de Boer, 1974) or a reduction in assimilate supply to the roots (Davidson & Milthorpe, 1966a; Ryle & Powell, 1975) because of a redirection of available assimilates for shoot regrowth.

The lower shoot weights of trampled plants may be due to a reduction in tiller or leaf number (cf. Leney, 1974). This is partly substantiated by the observation that, in _Phleum bertolonii_, no significant treatment effects on dry weight per tiller occur at low simulated trampling pressures implying the significant reduction in dry weight may be due to a decrease in tiller number. In _Plantago lanceolata_ the reduction in shoot weight associated with trampling may be due to a reduction in leaf number combined with a reduction in leaf weight.

An imbalance between root and shoot dry weight increments might be expected to be reflected in root:shoot ratios. The absence of a consistent relationship between root:shoot ratios and trampling pressure agrees with the observations of Leney (1974). Evans (1972) noted that "...the fraction of the increment of new dry matter going to the roots out of that added in a particular week can vary within wide limits ... and that this may be connected with the stage of ontogenetic drift". In the present study, changes in the growing environment, both of soil and aerial portions of the plant, might affect the ratio of root to shoot growth. Upon trampling, an immediate increase in the ratio occurs since shoot weight is decreased relative to that of root weight leading to an increase in the ratio. The observed tendency for the ratios to be lower in treated plants implies an imbalance in assimilate distribution relative to that of control plants, a greater proportion being channelled into shoot growth rather than root growth.

Data for $\bar{R}$ help elucidate the situation regarding the balance between shoot and root growth. Stimulation of shoot $\bar{R}$ at low simulated trampling pressures suggests that the stimulation in terms of biomass observed by Bayfield (1971) and Leney (1974) might be achieved by more rapid growth in trampled plants, while the concomitant reduction in root $\bar{R}$ supports the postulate that assimilates are being channelled preferentially into shoot growth following damage (cf. Davidson & Milthorpe, 1966a; Ryle & Powell, 1975). The negative interaction between shoot and root $\bar{R}$ results in no statistically significant treatment effects on total plant $\bar{R}$. A corollary of this observation is that a damaged plant may be aided in its recovery by an ability of the shoot to grow faster as a consequence of defoliation (cf. Ryle & Powell, 1975). Although the quantitative relationship between $\bar{R}$ and trampling pressure observed in the present study may have little real meaning, species differentials existing in terms of the stimulatory effect of trampling on $\bar{R}$ might affect their competitive ability in a trampled habitat and possibly their continued survival.

The differential response of shoots and roots to defoliation (Ryle & Powell, 1975) suggests that assimilate distribution may be a primary cause of elevated shoot $\bar{R}$, but since the decrease in root $\bar{R}$ is not in equilibrium with the increase in shoot $\bar{R}$, an assimilatory gain is implied which is sufficient to permit root growth while shoot growth is stimulated, suggesting enhanced photosynthetic activity. The situation is somewhat more complex than simple defoliation in which damage to the remaining tissue may be slight, thereby allowing photosynthesis and assimilate redeployment in regrowth (Davidson & Milthorpe, 1966a, b; Ryle & Powell, 1975). By contrast, tissues mechanically compressed but not severed may have a reduced photosynthetic capacity and reserve assimilates may play a significant role in repair of damage before defoliation losses can be compensated for by current assimilation. Davidson & Milthorpe (1966a) presented evidence suggesting that the degree of defoliation may have repercussions on recovery, for example, severance at the midpoint of the leaf may facilitate rapid regeneration due to the carbohydrate reserves existing in the remaining area above the zone of expansion. Liddle (1975a) noted that mobilisation of assimilates from undamaged tillers may aid the survival of grasses in trampled situations. The conflicting role of reserves and current photosynthesis in facilitating regrowth following defoliation (Davidson & Milthorpe, 1966a,b; Ryle & Powell, 1975) may reflect species differences. If such differences in the ability to reallocate assimilates exist, species possessing this capacity might have a greater regenerative potential in trampled situations than those unable to reallocate assimilates. The possible significance of reserves in regrowth (Smith, 1974) suggests that a high reserve level may be conducive to survival, especially if the photosynthetic potential is reduced. An extension of the hypothesis might consider the effects of environmental variables on carbohydrate gain and loss, and of seasonal variation in the balance between photosynthesis and respiration, which might affect the plant's capacity to repair and regenerate itself. However, the role of assimilates in bud growth and development is controversial, Jewiss & Lowry (1973) being of the opinion that assimilate supply alone is unlikely to repress the development of tiller buds and leaf primordia, although Ryle & Powell (1975) suggested that assimilate export may enhance growth of buds and development of meristems.

Defoliation may improve photosynthetic efficiency by permitting more efficient use of radiation (Allison et al., 1975; Ryle & Powell, 1975; Singh & Nair, 1975b). Liddle & Greig-Smith (1975b) also postulated that improved lateral lighting may result in an apparently stimulatory effect of trampling on biomass at path margins. The maintenance of an open community by trampling may be conducive to stimulating plant growth, thereby enhancing survival of certain species. These suggestions regarding elevated shoot growth are consistent with Leney's observations (1974) on elevated productivity of swards from which trampling pressures have been removed, although species composition changes must also be considered. Although the postulate is possibly oversimplistic, the capacity to rapidly replace lost and damaged foliage, and to build up reserves to aid regeneration following damage, may be conducive to plant survival in a trampled situation. This is partially counterbalanced by a retardation of root growth leading to a reduction in exploration of the soil environment with possible consequences for mineral uptake (Davidson & Milthorpe, 1966a, b; Ryle & Powell, 1975). Fertilizer applications may enhance ground cover response in trampled situations e.g. Wagar (1965), redressing a deficiency occurring in a root zone which is not expanding, but damage to the shoot may retard mineral uptake and reduce the efficiency of such measures. However, such effects require extensive further investigation.

The reduction in tiller number of Phleum bertolonii conflicts with observations on the response of grasses to simulated trampling pressure made by Bayfield (1971), Leney (1974) and Liddle & Greig-Smith (1975b). The response exhibited by Plantago lanceolata implies that a low trampling pressure results in a saturation of the response, above which the pressure applied must be comparatively greater to induce an effect, possibly due to protection of meristems by soil, although soil compaction effects may reduce potential leaf production. In addition to the effect on leaf number, simulated trampling also impairs leaf area development of newly emerging leaves of P. lanceolata. The RLGR declines with increase in the pressure applied and the decline over time in daily leaf area increment implies that the leaf area of treated plants might be expected to be less than that of control plants, which represents a reduction in potential photosynthetic capacity. These findings are consistent with observations on defoliated plants by Ryle & Powell (1975) who observed an increase in leaf area of $10.7\text{cm}^2\ \text{d}^{-1}$ in defoliated plants of Hordeum vulgare compared to $14.0\text{cm}^2\ \text{d}^{-1}$ for control plants. Allison et al. (1975) found that despite similar increases in leaf area of control and defoliated plants the absence of any compensation resulted in a substantial difference by the time of flowering.

In graminaceous species leaves tend to emerge at a late stage in expansion (Allison et al., 1975) and although those emerging from the leaf sheaths may still be expanding (Davidson & Milthorpe, 1966a) mature leaves once cut do not regrow (Ryle & Powell, 1975). Davidson & Milthorpe (1966a), however, observed that expansion of new leaves following defoliation was dependent upon older expanding leaves so that removal of leaf blades and meristems, and a decrease in substrate supply, could substantially retard leaf expansion. The findings of Davidson & Milthorpe (1966a) and Ryle & Powell (1975) suggested that photosynthetic potential may have an appreciable effect on leaf area development. Thus, reduction in leaf area as a consequence of trampling may depress assimilate production and limit the development of pre-existing and new leaves, as suggested by the present observations, thereby retarding the development of an assimilatory surface. Although there is some evidence of stimulated leaf area increases of trampled plants, the effect is not consistent and is insufficient to compensate for foliar losses arising from simulated trampling.

When the Leaf Area Index of a plant is less than unity radiation interception is less than maximal while at a value greater than unity mutual shading of leaves may reduce the photosynthetic efficiency of the plant. Edmond (1958) noted that rate of growth per unit area of ground increases until radiation interception is maximal while Ryle & Powell (1975) suggested that efficiency of utilization of radiation becomes more important than increase in photosynthetic surface when a closed canopy develops. Thus, although an increase in photosynthetic area may be advantageous to trampled plants, species differences in photosynthetic efficiency may affect the competitive advantage some species possess over others in trampled situations. The ability to undergo rapid leaf expansion following trampling damage, however, may facilitate photosynthetic gain, thereby enhancing replacement of damaged tissues.

The observation that regular application of a low intensity of trampling pressure to <u>Plantago lanceolata</u> does not have a more severe effect than a similar pressure applied at one time agrees with observations by Leney (1974) that trampling pressures applied over a period of time appeared less damaging than similar pressures applied once only. Conditions experienced by the plant during the period of recovery following damage is of significance in a seasonal context (cf. Liddle, 1975a; Holmes, 1976). Conditions not conducive to rapid growth may retard recovery and reduce the durability of the plant. Similarly, the duration of the recovery period may also affect the durability of a plant. The results of the current investigation suggest that the conditions and period between treatment applications were suitable to permit a simulated trampling pressure of 2i pt^{-1} to be sustained over the duration of the experiment without substantial detriment to the plant in contrast to the application of 5i pt^{-1}. In this context it would be of interest to examine the role of assimilate reserves in aiding recovery since plants under the sustained lower pressure may have had a capacity to accumulate reserves between treatment applications which faciliated rapid recovery follpwing damage in contrast to those plants receiving a higher sustained pressure.

The reduction in flower weight may reflect the allocation of assimilates to recovery growth of foliage instead of floral production, which is consistent with the reduction in grain yield associated with defoliation e.g. Walpole & Morgan (1974), Allison _et al._ (1975), Enyi (1975), Singh & Nair (1975a). For annual species this may endanger their perpetuation at a particular locality subject to trampling pressures, although the maintenance of an adequate photosynthetic surface may faciliate flower production when trampling pressure lessens (cf. Bates, 1935).

The observations on plant water content (Section VA, b(i)), although of limited value since they were not related to the saturated water content of the plant (cf. Section IIIB, 2(a)), may reflect changes in dry to fresh weight ratio of the foliage, due to young leaf tissue replacing that lost by defoliation effects resulting from trampling. Evans (1972) noted that at low total dry weight of tissue, a high proportion of cells may be meristemmatic and non-vacuolate. With vacuolation of cells and consequent leaf expansion a relative decrease in dry weight occurs until a steady state is achieved between expanding and meristemmatic tissue, ultimately followed by cessation of growth and changes such as cell wall thickening which result in a rise in percentage dry weight. This is an age related phenomenon, more mature leaves possessing cells with thicker walls, greater accumulations of metabolites and greater lignification. The increase in moisture content may indicate increasing juvenility of tissue from plants receiving higher simulated trampling pressures, possibly as a consequence of an imbalance between expanding and non-expanding tissue or the use of assimilates in regrowth rather than for storage purposes. However, Liddle & Greig-Smith (1975a) observed higher fresh weights of Festuca rubra from compacted soils which may reflect an enhanced ability of the plant to obtain water, although moisture availability should not have been limiting in the present study since soils were brought to field capacity twenty four hours prior to harvesting of the plants.

B. The effect of Soil Bulk Density increases on the growth of *Phleum bertolonii* and *Plantago lanceolata*

(a) Introduction

Conflicting evidence exists regarding the effect of soil compaction upon plant growth. A given degree of soil compaction may result in a retardation or an increase in plant growth, alternatively, an optimum degree of compaction may exist either side of which plant yield is reduced (Rosenberg & Willits, 1962). Gupta (1933), having observed that growth could be reduced in loose soils as well as in those which have been compacted, concluded that such a response is "...indicative of an optimum degree of packing of the soil particles when there is a balance between the adverse mechanical resistance of increasing density and beneficial effects of intimate contact".

The pore space of soil is important to allow root penetration and diffusion of gases and water in the soil. Due to the pore space, the true density of the soil solids (approximately 1.20g cm^{-3} to 2.65g cm^{-3}) is reduced to an apparent density of 1-2g cm^{-3} for most soils, depending upon composition, moisture content and degree of compaction (Milthorpe & Moorby, 1974). These authors stated that evidence exists indicating that roots are unable to penetrate pores with a diameter of less than 200μm or through a soil with a shear strength in excess of $2.9 \times 10^4 Nm^{-2}$, which is lower than for many bulked soils, but they concluded that few soils exist through which roots are unable to grow.

The penetration of a root through a soil depends upon its ability to displace the soil particles around it, thus, the effect of soil compaction on root growth is partly dependent upon soil structure. For example, the compaction of a coarse textured soil may still allow sufficient pore space for growth whilst conditions may improve to enhance plant growth (Liddle &

Greig-Smith, 1975a). However, the interaction of soil factors may result in such an improvement being offset by reduced oxygenation and water logging e.g. Edmond (1958). Rosenberg & Willits (1962) suggested that there is usually one principle factor affecting plant growth, for example, in a study of the response of Hordeum vulgare to soil compaction, they observed that compaction of a sandy soil resulted in increased growth, possibly related to a moisture factor, while on two other soils growth was reduced possibly due to the effects of reduced aeration and increased mechanical impedence to root penetration.

Wittsell & Hobbs (1965) noted that an increase in soil bulk density, from 1.2g cm^{-3} to 1.6g cm^{-3} reduced grain yield of Triticum aestivum (wheat) and Glycine max (sorghum) but for the former, subsurface compaction had the greatest effect on growth while the latter was more sensitive to surface compaction. This is similar to observations made by Bates (1935) regarding species differences in the response to depth of soil compaction which were ascribed to differences in plant rooting depth. Wittsell & Hobbs (1965) concluded that under normal field conditions, however, climatic factors were more likely to limit crop yields than soil compaction effects.

Variability in the response of plants to soil compaction was a feature noted by Gupta (1933). Experiments with Vicia faba revealed that in certain instances shoot growth was better in compacted soils and in others growth was better on loose soils. Gupta was also unable to detect a significant effect of soil compaction on leaf areas, which was interpreted as indicative of an equal conditioning of assimilation potential on soils of varying compaction. Gupta also found that on compacted soils, roots were superficial and shallow, possibly due to the increased mechanical impedence of compacted soil to root penetration, in contrast with deep roots from loose soils. However, the dry weight of roots from compacted soils was greater than that from loose soils which led Gupta to conclude that despite roots occupying a smaller soil volume on compacted soil, this volume was more extensively exploited.

Schuurman & de Boer (1974), working with Avena sativa (oat), found that soil compaction effects on shoot growth were not marked but that the transition from a loose to a dense soil was associated with a significant decrease in root weight, which was attributed to a decrease in the number of roots penetrating the denser soil combined with a reduction in root branching. They found that this effect did not impair water or mineral uptake implying that roots in the looser soil were of comparatively greater importance with the consequence that shoot growth per gram of root was better than on a uniformly loose textured soil.

That species differ in their response to soil compaction was a phenomenon noted by Leney (1974) who found a reduction in root size of some species with increased soil compaction e.g. Holcus lanatus, Plantago lanceolata but an increase in root and shoot size of other species e.g. Calluna vulgaris, Ammophila arenaria. Similarly, Liddle & Greig-Smith (1975b) found that soil compaction could increase tiller number and fresh weight of Festuca rubra.

Taylor et al. (1966) stated that "Provided no other growth factor becomes limiting, a specific change in soil strength will cause a specific response of underground portions of a plant..." while Gupta (1933) commented that the greater energy required to overcome the mechanical impedence to root penetration in a compacted soil is reflected in a smaller root system. Taylor et al. (1966) similarly considered that "For seedlings to grow, their hydrostatic presssure (turgor) must be sufficient to overcome internal restraints imposed by cell structures plus mechanical restraints imposed by the surrounding media". They found, in experiments with Gossypium hirsutum, that an increase in soil strength resulted in a curvilinear reduction in root penetration to zero at a soil strength of $2.5 \times 10^6 N\ m^{-2}$. The cellular aspects of the response of cells to compressive stresses have been considered by Barley (1962). Working with Zea mays, he found that increasing pressure applied to newly emergent radicals resulted in a proportional decrease in growth and a reduction in the size of the root apical meristem. In contrast, mature tissue responded to an increase in pressure by an interruption in growth, which in some cases failed to resume, and where growth recommenced it did so at a substantially reduced rate. Compression of the whole plant was found to have a more severe effect on plant growth than compression of the roots alone. Barley viewed these responses by the plant to soil compression in terms of an internal accommodation of the applied stress by changes in turgor pressure. The effects of soil compression on plant growth were observed to interact with soil oxygen deficits in which, independently, neither factor significantly reduced dry weight but combined resulted in a substantial reduction. Barley considered this effect in terms of a mechanical disruption of cells within the root impeding the supply of oxygen to the root tip. The rate at which the rate of gaseous diffusion recovers determines the speed at which root regrowth resumes, possibly accounting for the differential response observed between mature and new roots to pressure.

Although the root normally lies in a channel formed by the growing tips, from Barley's analysis it is evident that an increase in soil compaction following the development of the root may hinder subsequent growth, not solely due to an increased mechanical resistance to root penetration, but also as a result of internal mechanical disruption of plant tissues. In addition, since root growth is dependent upon internal osmotic relationships (Barley, 1962), a depletion in osmotically active substrates, due to a reduction in carbohydrate supply from the shoot because of defoliation resulting from trampling, may retard further root growth.

The response of plant growth to soil compaction may be regarded as complex, dependent upon the optimal soil bulk density for a given plant at a given stage of its development (Rosenberg, 1964).

(b) (i) Growth of seedlings of *Plantago lanceolata* on compacted soils

(a) Methods

John Innes No. 2 potting compost was compressed in 10cm. diameter plastic pots, with the aid of the trampling tamp, to a constant volume but varying weight, to give a range of soil bulk densities (1.0g cm^{-3}, 1.1g cm^{-3}, 1.3g cm^{-3}, 1.4g cm^{-3}, 1.46g cm^{-3}) with five replicates per treatment. Soil bulk density was calculated from oven dry weight over soil volume. (At lower soil bulk densities, settling of the soil on watering may have altered the theoretical bulk densities obtained). Following compaction, fifty seeds of *P. lanceolata* were sown on the soil surface of each pot and allowed to germinate at 20 ± 1°C, 90 ± 10% relative humidity, and a 12h day at 13klx.

In the absence of significant treatment effects on the percentage germination and the rate of germination, times were measured from the sowing date, there being no marked increase in the number of germinated seeds after day 5, by which time an average of 54% of the seeds in each pot had germinated.

Ten plants per pot were randomly selected and the first true leaf to emerge was tagged and its elongation followed from day 12 to day 42 by periodic measurements of its length.

Although a significant relationship was found to exist between leaf length and leaf area for mature leaves ($r = 0.831$, $p < 0.001$) this

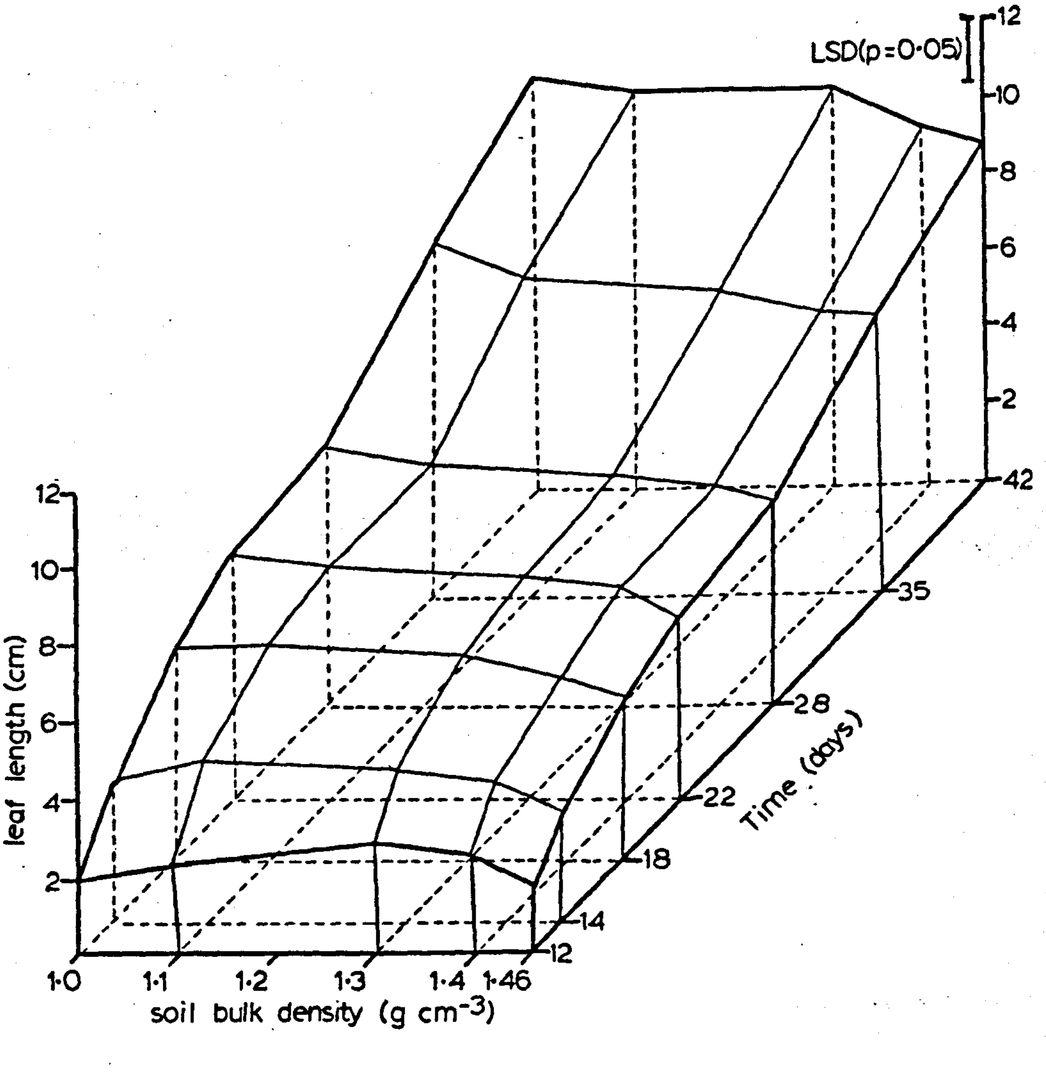


Figure 97 The effect of soil bulk density on the elongation of leaves of Plantago lanceolata.

relationship did not hold for leaves during the early stages of their development. In view of the error attached to the measurement of leaf breadth on small leaves remaining attached to the plant, measurements were restricted to leaf length and not converted to leaf area.

42 days after sowing, plant material was harvested. Shoot and root length were measured prior to oven drying at 85°C. The parameters for the ten plants per pot have been averaged to give a mean value for each replicate pot in the experiment which was subsequently used for analysis of variance tests.

(b) Results

There are significant ($p < 0.001$) soil bulk density effects on leaf elongation and significant ($p < 0.001$) increases in length of leaves from all soil bulk density treatments over time. Soil bulk density effects on leaf length do not become apparent until day 22, when leaf lengths of plants growing in soils at the extremes of the bulk density gradient (i.e. 1.0g cm^{-3} and 1.46g cm^{-3}) differ significantly. After day 22 leaf lengths of plants growing in soils of a bulk density of 1.0g cm^{-3} are consistently greater, and leaf lengths from soils of 1.46g cm^{-3} bulk density consistently lower, than leaf lengths from plants growing in soils of intermediate bulk density (Figure 97).

A reduction in the rate of elongation is apparent over the 18 to 28 day period, no significant increases occurring, but this is followed by significant increases in leaf length of all treatments over the 28-35 day and 35-42 day periods. Except for the two lower soil bulk density treatments, the absence of significant increases over time in the initial periods of measurement (12-14 days, 14-18 days) may reflect the shorter intervals between measurements. The significant ($p < 0.05$) increases in leaf length between day 12 and day 14 at soil bulk densities of 1.0g cm^{-3}

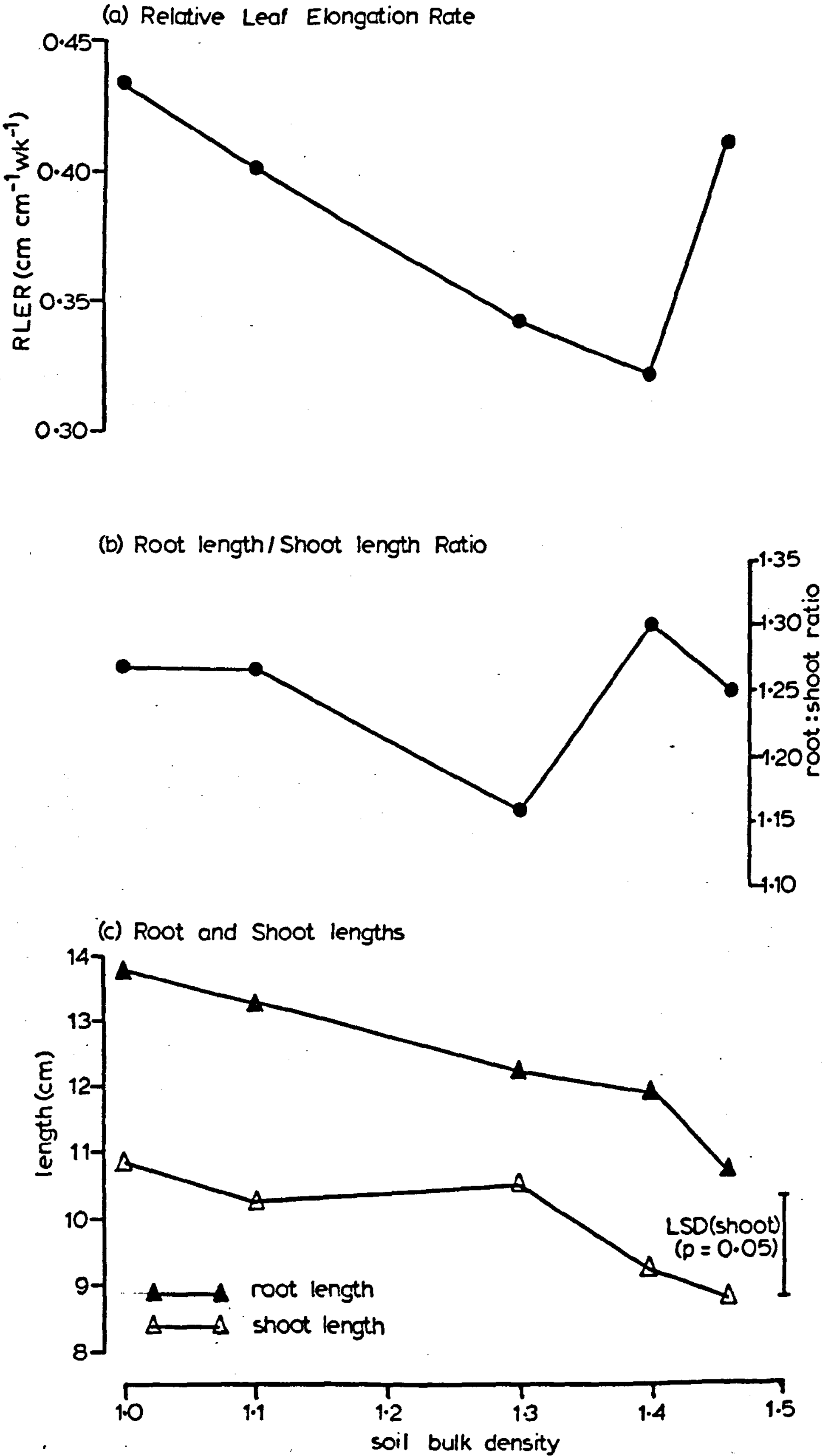
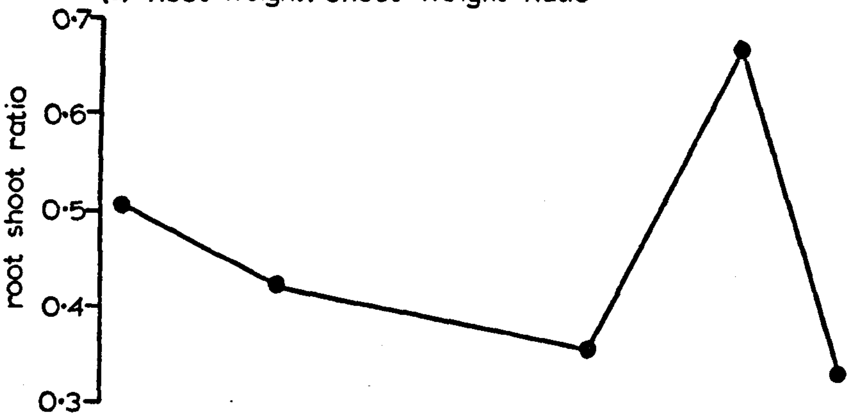


Figure 98 The effect of soil bulk density on root and shoot length of Plantago lanceolata.

and 1.1g cm^{-3} reflect leaf elongation rates of 0.83cm d^{-1} and 0.98cm d^{-1} respectively, which decrease to 0.24cm d^{-1} and 0.29cm d^{-1} for the period between day 35 and day 42. These values compare with 0.56cm d^{-1} and 0.24cm d^{-1} for the same time periods for plants growing in soils of 1.46g cm^{-3} bulk density. The average leaf elongation rates of P. lanceolata (Figure 98a) are not significantly affected by soil bulk density although a marked downward trend exists with increasing soil bulk density except for plants growing in a soil bulk density of 1.46g cm^{-3} where the rate of leaf elongation is comparable to that of plants growing in soil of the lowest bulk density employed. The final leaf length of plants from a soil bulk density of 1.46g cm^{-3} is the least of all treatments (Figure 98c) despite a trend for a comparatively high leaf elongation rate. This may reflect a shorter leaf length at the commencement of the measurements (day 12) (Figure 97). In contrast, despite an initially greater leaf length of plants from a soil of bulk density 1.4g cm^{-3}, the final leaf length is significantly lower than that of plants from the lowest soil bulk density used, reflecting lower elongation rates (Figures 97; 98a,c).

Root lengths, although tending to show a relative decrease with increasing soil bulk density, are not significantly affected by soil bulk density variation (Figure 98c). Similarly, no significant trends are apparent in the relationship between root/shoot length and soil bulk density (Figure 98b). The lowest value,at a bulk density of 1.3g cm^{-3}, reflects a relatively greater increase in shoot length over root length compared to other treatments while at higher soil bulk densities root length would appear to be increasing disproportionately to shoot length. A similar response is observed in root/shoot weight ratios (Figure 99a). Although no significant differences occur, the value at a bulk density of 1.4g cm^{-3} departs, appreciably, from the general downward trend in

(a) Root weight / Shoot weight Ratio



(b) Root and Shoot weights

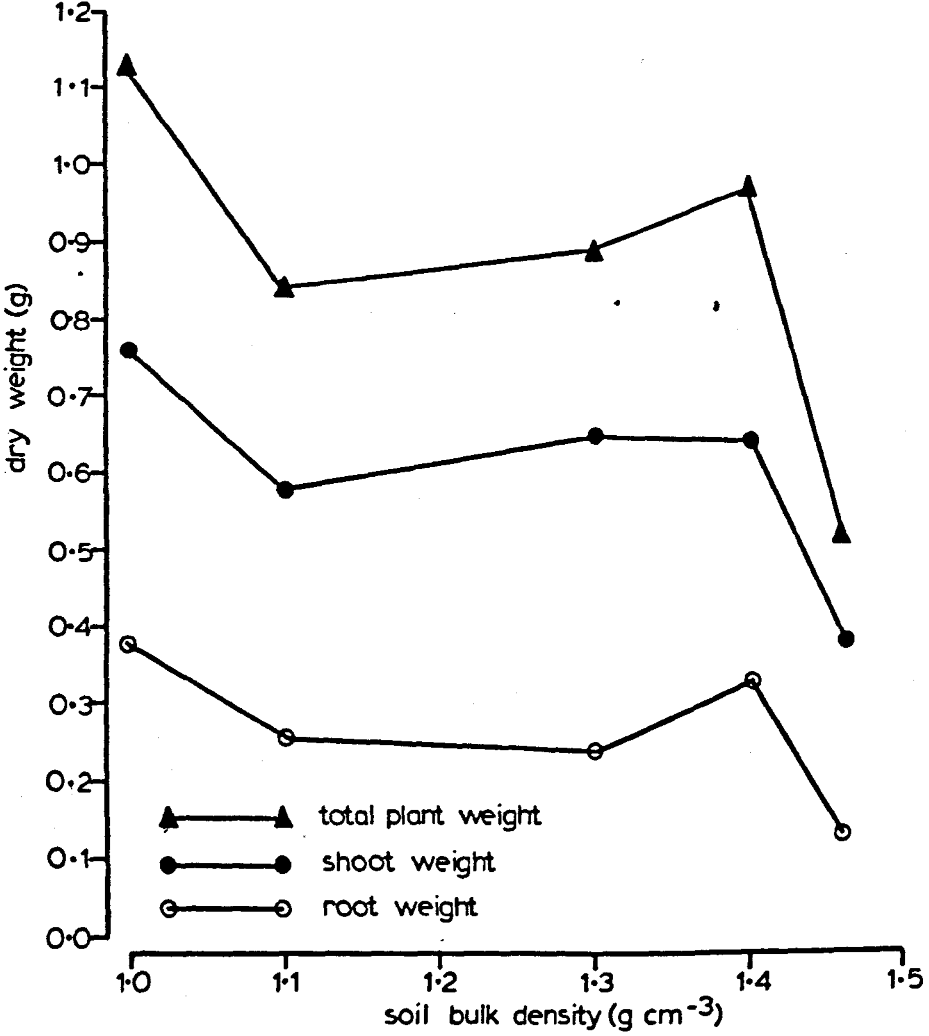


Figure 99 The effect of soil bulk density on root and shoot weight of Plantago lanceolata.

root/shoot ratio with increasing soil bulk density. This trends indicates that root weight increase is depressed to a greater extent than shoot weight, although both are depressed to some degree by an increase in soil bulk density (Figure 99b). Although no significant differences occur, the most marked effect on plant weight occurs with the increase in soil bulk density from 1.4g cm^{-3} to 1.46g cm^{-3}.

(ii) The effects of an increase in soil bulk density on *Plantago lanceolata* following plant establishment in uncompacted soil

(a) Methods

Seedlings, 28 days old, of *P. lanceolata* were transplanted, singly, into 10cm. diameter plastic pots containing John Innes No. 2 potting compost with added fertilizer and allowed to establish for 14 days (at 20 ± 1°C, 75 ± 10% relative humidity, 12 h day at 13klx). The 42-day old plants were subjected to increases in soil bulk density by compacting the soil surrounding the plant with the aid of a tamp.

The tamp (Figure 100) consisted of a 1.25cm. thick circular steel plate in two sections held together by lugs. The centre of the plate was drilled to produce a 1.9cm. diameter hole through which the base of the plant fitted. Three steel rods, which slotted into the lower plate, were mounted on the top plate, thereby providing a cage into which the foliage fitted. This enabled a vertically applied force from above to be transmitted to the soil while minimizing damage to the shoot. The cage was supported within a removeable steel cylinder mounted onto the concrete block of the tamping apparatus (cf. Figure 84). A range of bulk densities could be achieved by compacting the soil within the pots to varying volumes which together with oven dry weight enabled soil bulk

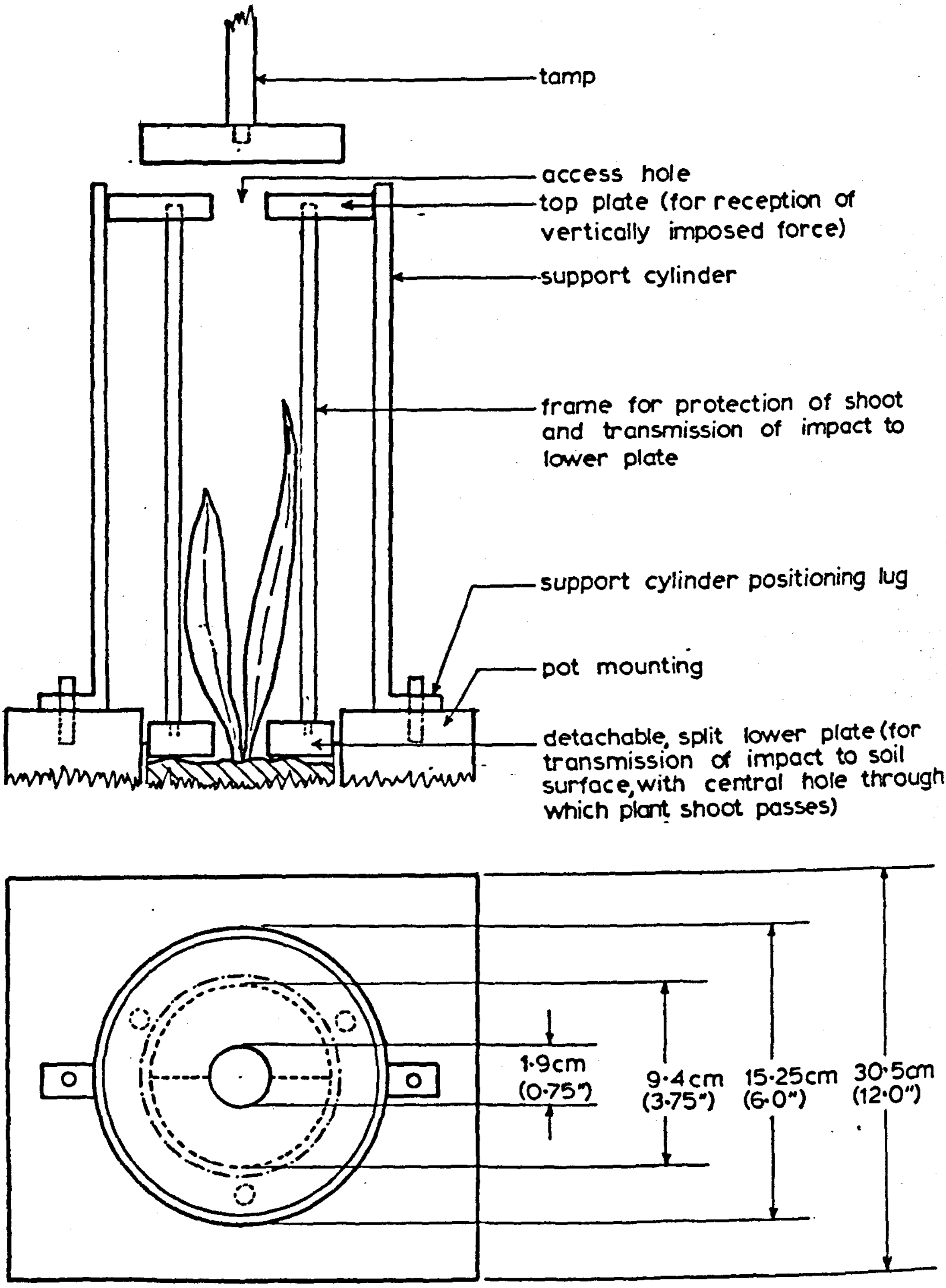


Figure 100 Diagram of apparatus used to compact the soil around an established plant whilst preventing damage to the shoot. (Not drawn to scale).

density to be calculated.

Four bulk densities were produced (0.893g cm^{-3}; 1.024g cm^{-3}; 1.149g cm^{-3}; 1.276g cm^{-3})[1] with ten replicates per treatment, all significantly ($p < 0.001$) different (LSD at $p < 0.001 = 0.019\text{g cm}^{-3}$).

Prior to increasing soil bulk density, a young leaf, approximately the same dimensions for each replicate, was labelled and length and breadth measurements made 6, 13 and 20 days following the increase in soil bulk density. The first leaf to emerge on each plant, following the increases in soil bulk density, was also labelled and length and breadth measurements made 1, 3, 5, 7 and 9 days after emergence. Leaf areas were calculated with the aid of the regression equation $\hat{y} = 3.24 + 0.59\ x$, where x is the leaf area calculated from length x breadth measurements (see Figure 92).

Twenty one days after the artificial increases in soil bulk density, shoots and roots were harvested and dried at 85°C. Relative Growth Rate ($\bar{R}$) was calculated in conjunction with ten plants harvested at the time of treatment.

(b) Results

Following treatment, the pre-treatment emerged leaves do not exhibit a significant percentage increase in leaf area[2] over the 14 day measurement period, although an appreciable increase occurs (up to 27.7%). The within treatment variation may be obscuring the time effect, in which the leaves of some replicates ceased to grow, possibly due to the effect of the treatment, whilst others were relatively unaffected by the increases

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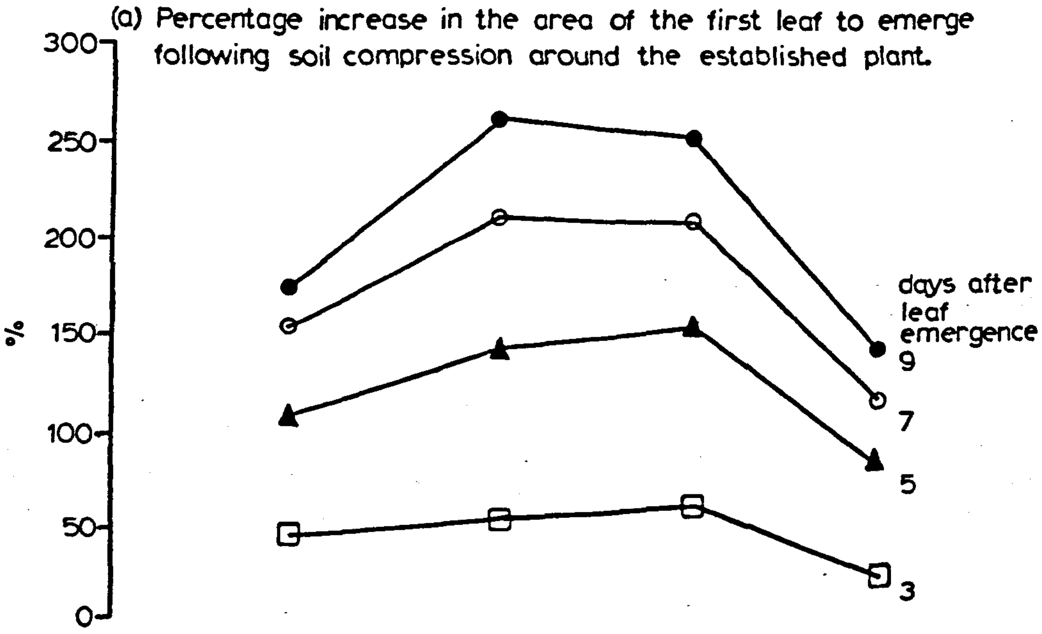
[1] A soil bulk density of 0.893g cm^{-3} represents that in which the plants became established, i.e. no increase in soil bulk density occurred.

[2] The increase in leaf area expressed as a percentage of the leaf area at time of treatment.


(a) Percentage increase in the area of the first leaf to emerge following soil compression around the established plant.

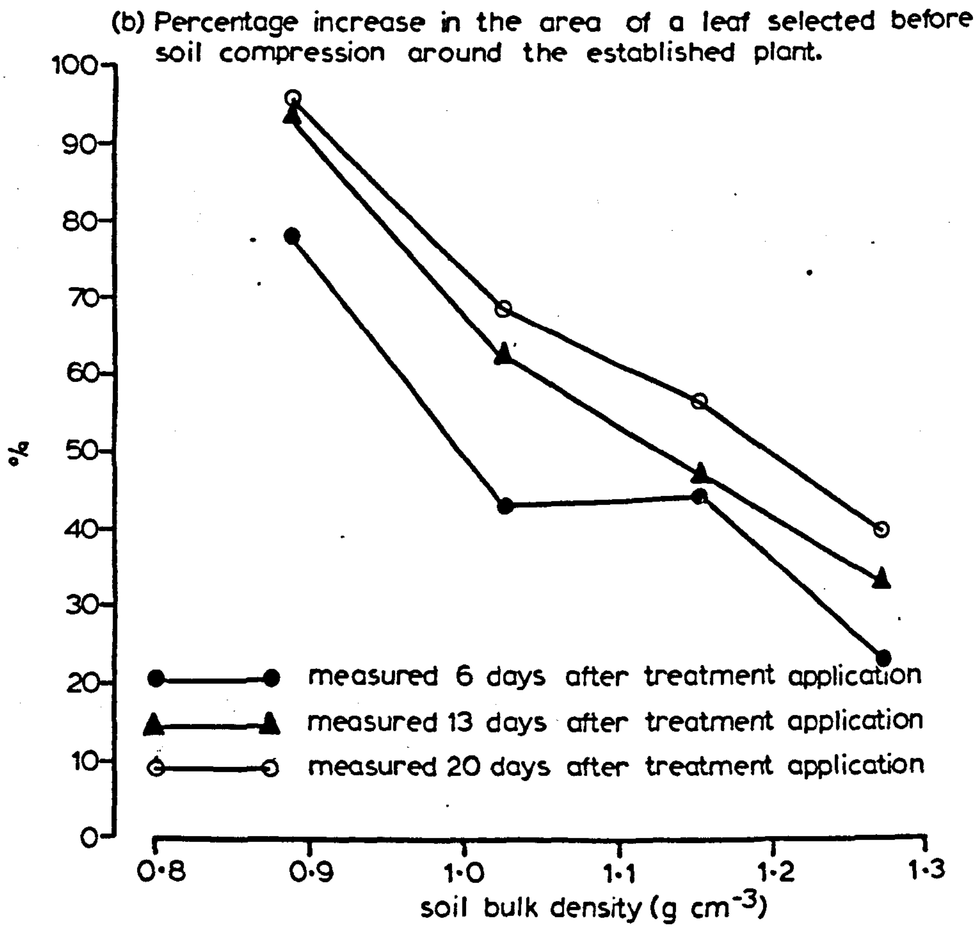
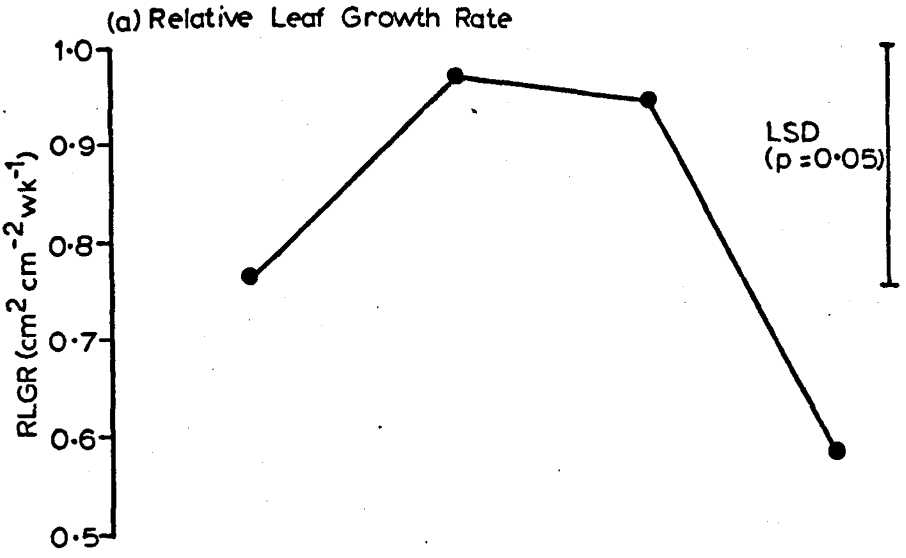
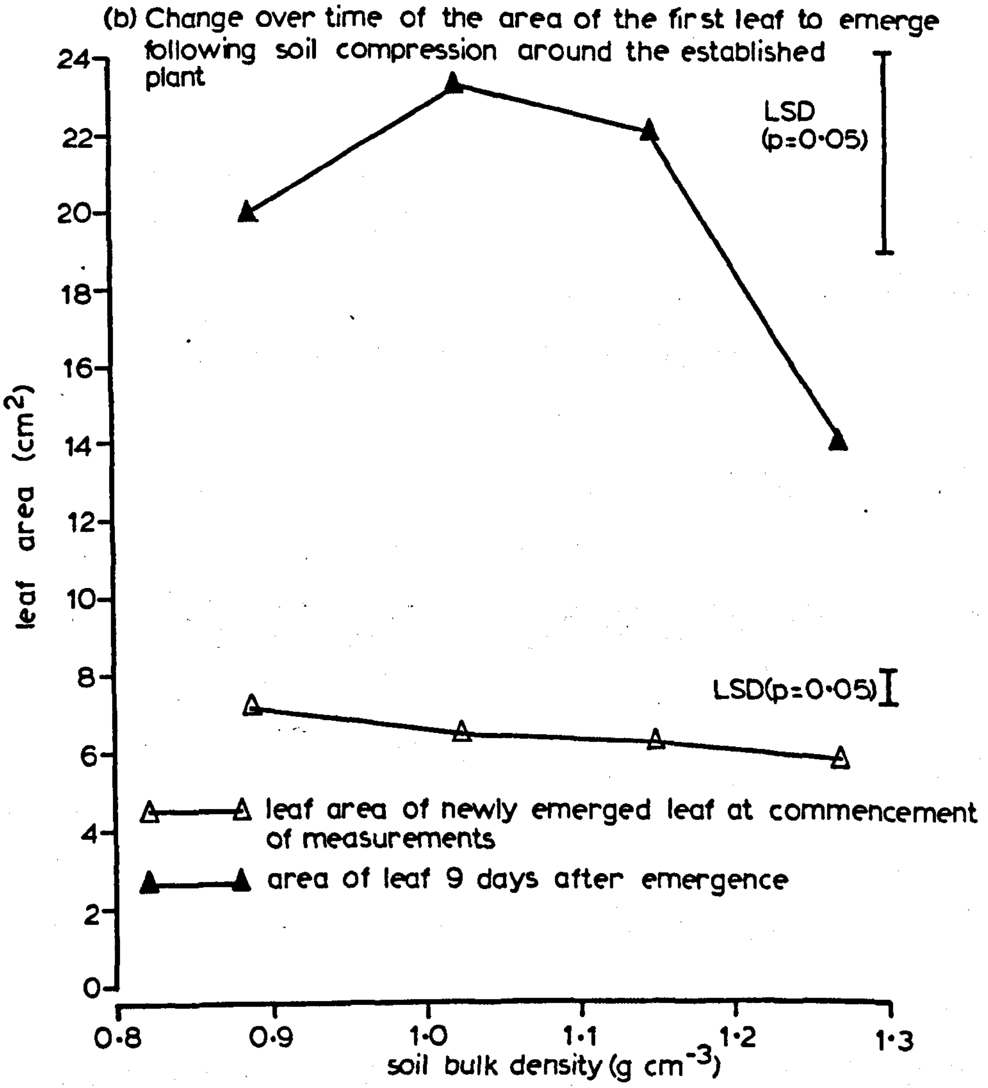

(b) Percentage increase in the area of a leaf selected before soil compression around the established plant.

Figure 101 The effect of increasing soil bulk density, by compressing the soil around the established plant, on the percentage increase in leaf area of pre- and post-treatment emergent leaves of *Plantago lanceolata*.



(a) Relative Leaf Growth Rate



(b) Change over time of the area of the first leaf to emerge following soil compression around the established plant

Figure 102 The effect of increasing soil bulk density, by compressing the soil around the established plant, on increase in leaf area of the first leaf to emerge following treatment of <u>Plantago lanceolata</u>

in soil bulk density. Significant ($p < 0.001$) treatment effects occur, a greater retardation in percentage increase in leaf area being associated with higher increases in soil bulk density (Figure 101b).

Post treatment emergent leaves increase by a maximum of 262.1% over the 9 days following emergence, from $6.4cm^2$ to $23.3cm^2$, at a soil bulk density of $1.024g\ cm^{-3}$. There is a significant ($p < 0.001$) percentage increase in leaf area over time for all soil bulk density treatments, although the increases become progressively less over time, being smallest between day 7 and day 9. The increase in leaf area is retarded to a greater extent at soil bulk densities of $0.893g\ cm^{-3}$ and $1.276g\ cm^{-3}$ than at the intermediate soil bulk densities, resulting in a significant ($p < 0.001$) treatment effect on percentage increase of leaf area. The relationship between percentage leaf area increase and soil bulk density is markedly curvilinear, unlike the linear trend observed in the leaf area increases of pre-treatment emerged leaves. This trend becomes more marked over time although no significant time x treatment interaction is observed (Figure 101a).

On an absolute basis, variations in soil bulk density have a significant ($p < 0.01$) effect on mean Relative Leaf Growth Rate, the rate of leaf area increase at soil bulk densities of $1.024g\ cm^{-3}$ and $1.149g\ cm^{-3}$ being significantly greater than leaf area increases at $1.276g\ cm^{-3}$ (Figure 102a). These differences in RLGR are reflected in significant ($p < 0.01$) differences in leaf area of plants growing in soils of varying bulk density on day 9 (Figure 102b). Although a significant ($p < 0.05$) difference in leaf area existed between plants grown at the two soil bulk density extremes at zero time, the linear downward trend with increasing soil bulk density is translated into a curvilinear relationship over time (Figure 102b).

In contrast to the leaf area relationships with soil bulk density,

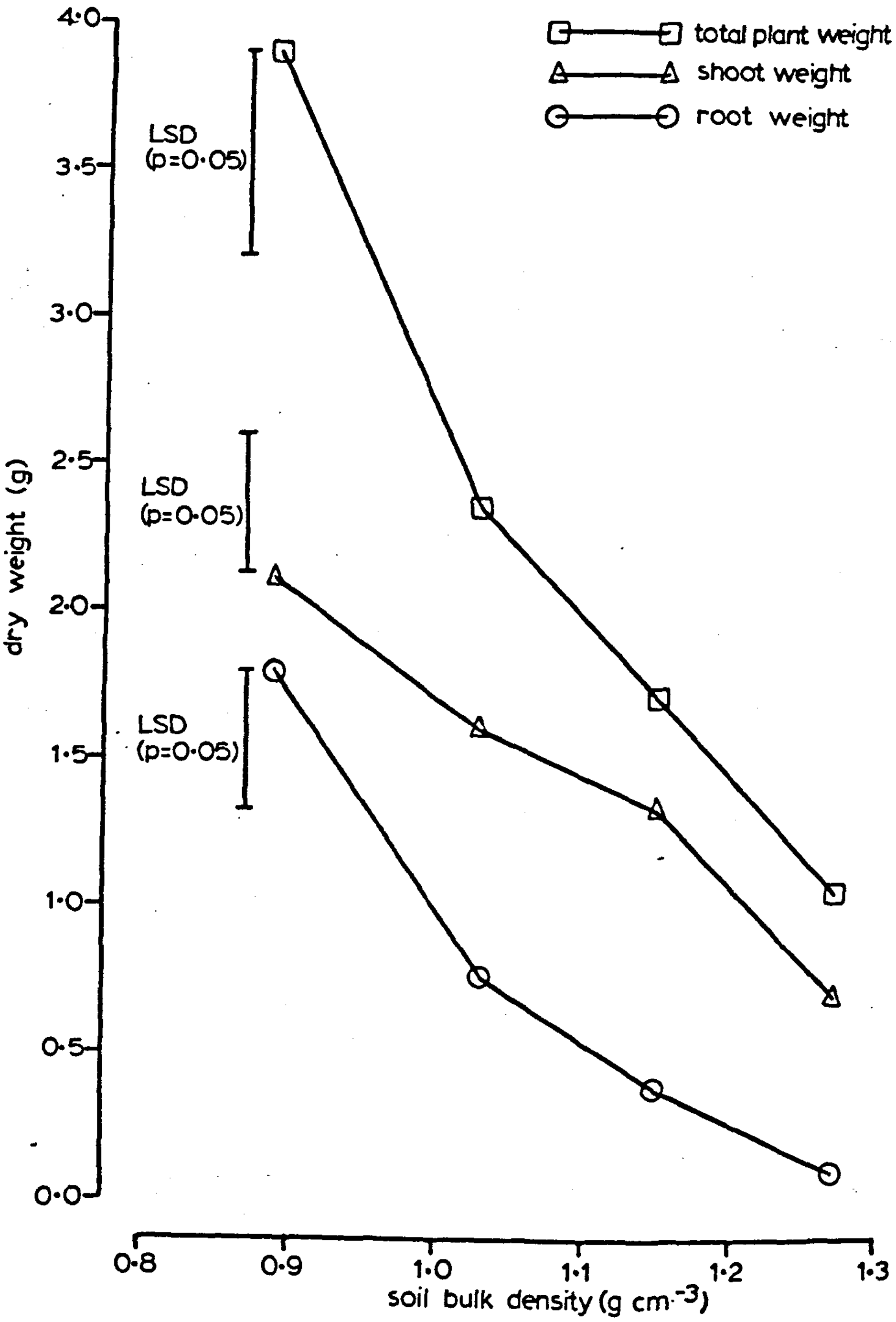
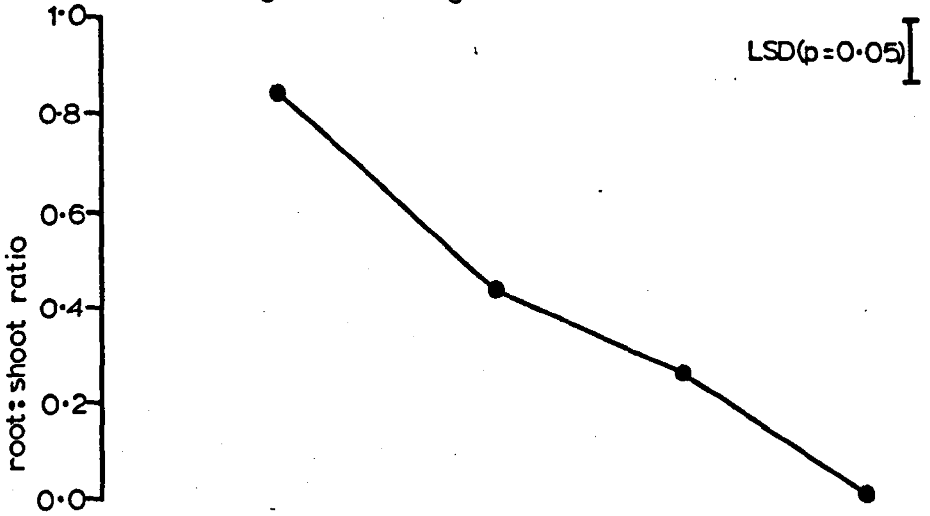


Figure 103 The effect of soil compression, subsequent to plant establishment in a loose soil, on the dry weight of *Plantago lanceolata*.


(a) Root weight/Shoot weight Ratio

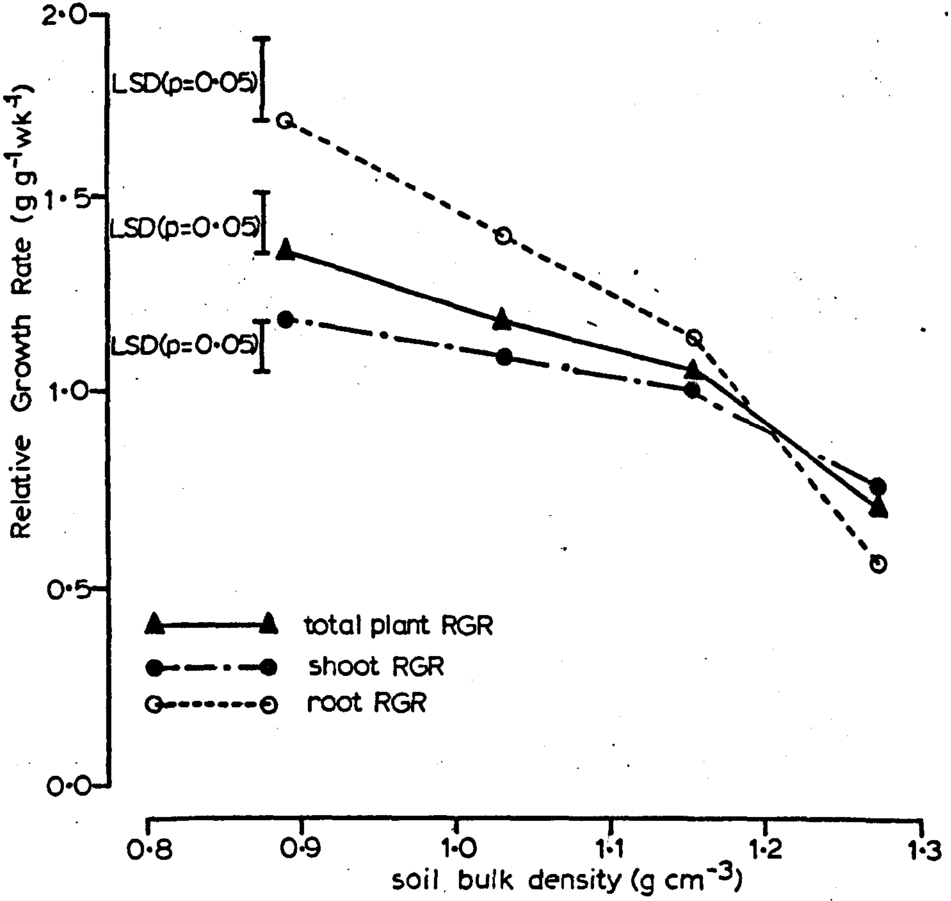

(b) Relative Growth Rate

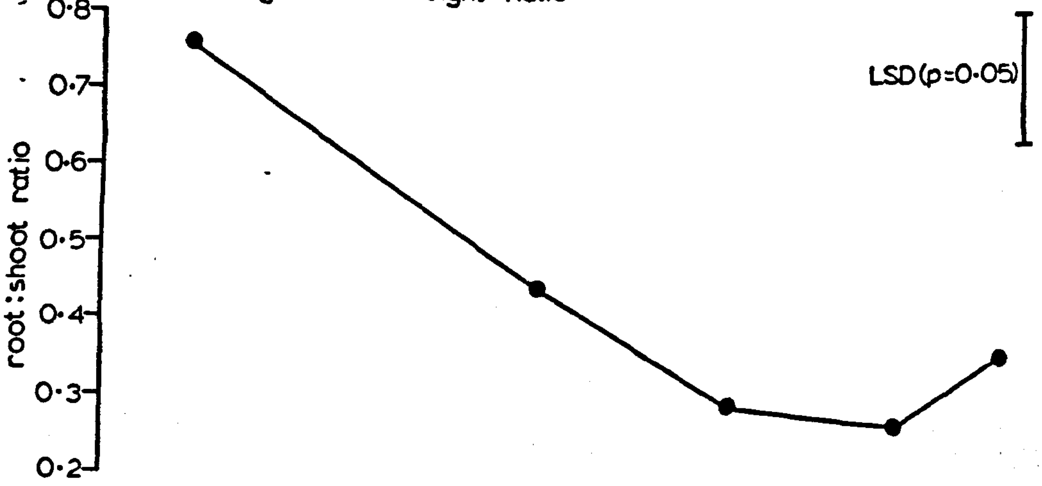
Figure 104 The effect of soil compression, subsequent to plant establishment in a loose soil, on growth of _Plantago lanceolata_.

plant weight parameters exhibit significant negative correlations with soil bulk density ($r = -0.770$; $r = -0.710$; $r = -0.767$; all $p < 0.001$ for total, shoot and root weight respectively) (Figure 103). An increase in soil bulk density results in a significant ($p < 0.001$) decrease in plant weight. The disproportionate effect of increases in soil bulk density on root and shoot growth is reflected in the significant ($p < 0.001$) reduction in root:shoot ratio (Figure 104a) in which, with an increase in soil bulk density, root growth is retarded to a greater extent than shoot growth. This is further illustrated in the changing relationship between shoot and root $\bar{R}$ (Figure 104b). Shoot, root and total plant values for $\bar{R}$ are significantly ($p < 0.001$) lowered by increases in soil bulk density, the differential effects on shoot and root growth being illustrated by the convergence of the relationships between soil bulk density and $\bar{R}$. Root $\bar{R}$ decreases by 67.1% over the range of bulk densities employed, from 1.701g g^{-1} wk^{-1} to 0.560g g^{-1} wk^{-1}, compared to a reduction in shoot $\bar{R}$ of 36.3%, from 1.197g g^{-1} wk^{-1} to 0.763g g^{-1} wk^{-1}. The root:shoot ratios indicate that root biomass is consistently less than shoot biomass over the range of soil bulk densities employed.

(iii) The effects of an increase in soil bulk density on *Phleum bertolonii* following plant establishment in uncompacted soil

(a) Methods

Seedlings, 28 days old, of *P. bertolonii*, were transplanted, singly, into 10cm. diameter plastic pots containing John Innes No. 2 potting compost with added fertilizer, and allowed to establish for 14 days (at $20 \pm 1^{o}C$, $75 \pm 10\%$ relative humidity, 12h day at 13klx). Soil bulk density was increased, from a base value of 0.733g cm^{-3}, to give the


(a) Root weight/Shoot weight Ratio

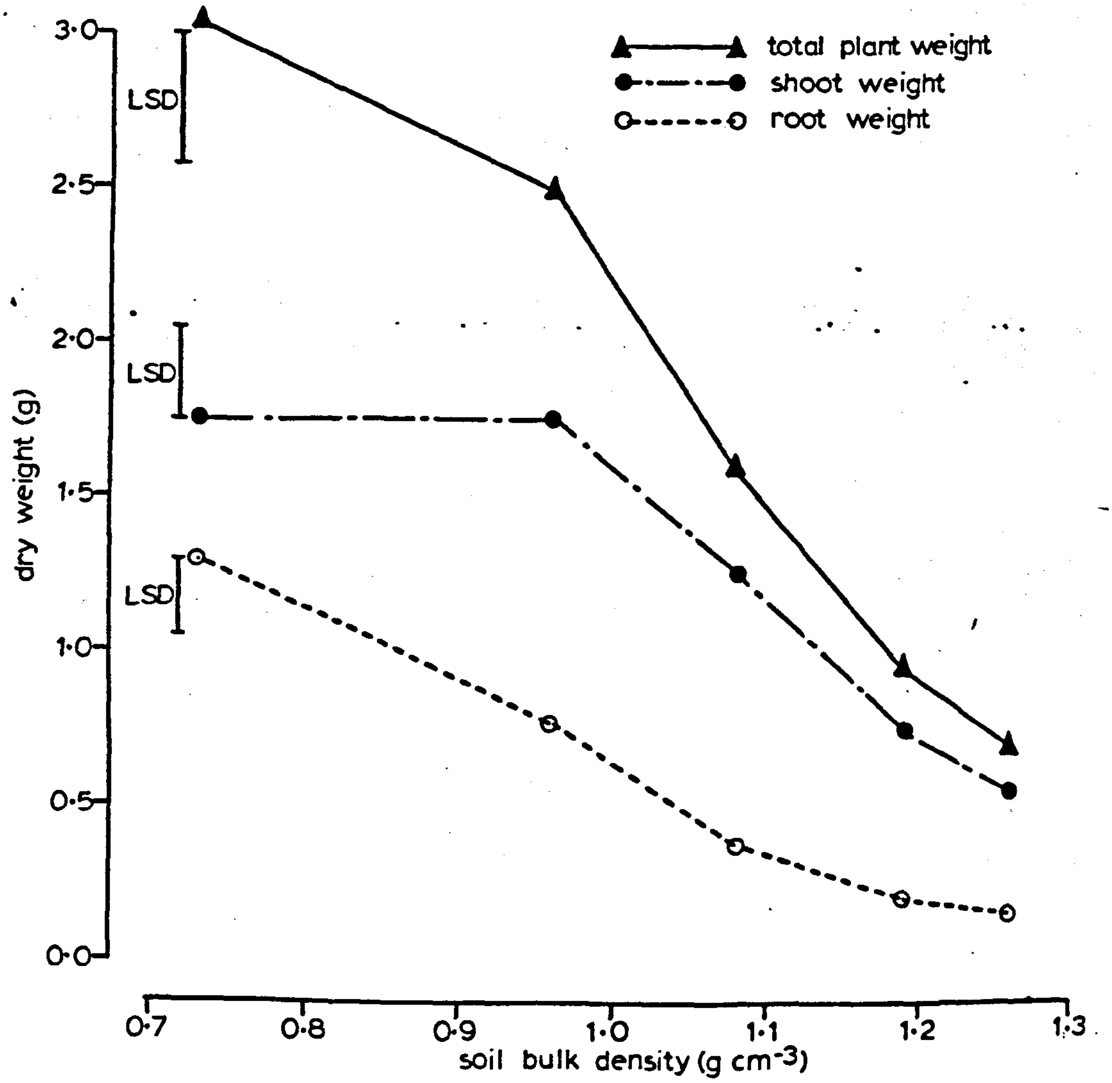

(b) Dry weight of shoot and root

Figure 105 The effect of soil compression, subsequent to plant establishment in a loose soil, on the dry weight of Phleum bertolonii.

following treatments with ten replicates per treatment, 0.733g cm^{-3}, 0.961g cm^{-3}; 1.083g cm^{-3}; 1.189g cm^{-3}; 1.259g cm^{-3} (all significantly different at $p < 0.001$, LSD = 0.017g cm^{-3}).

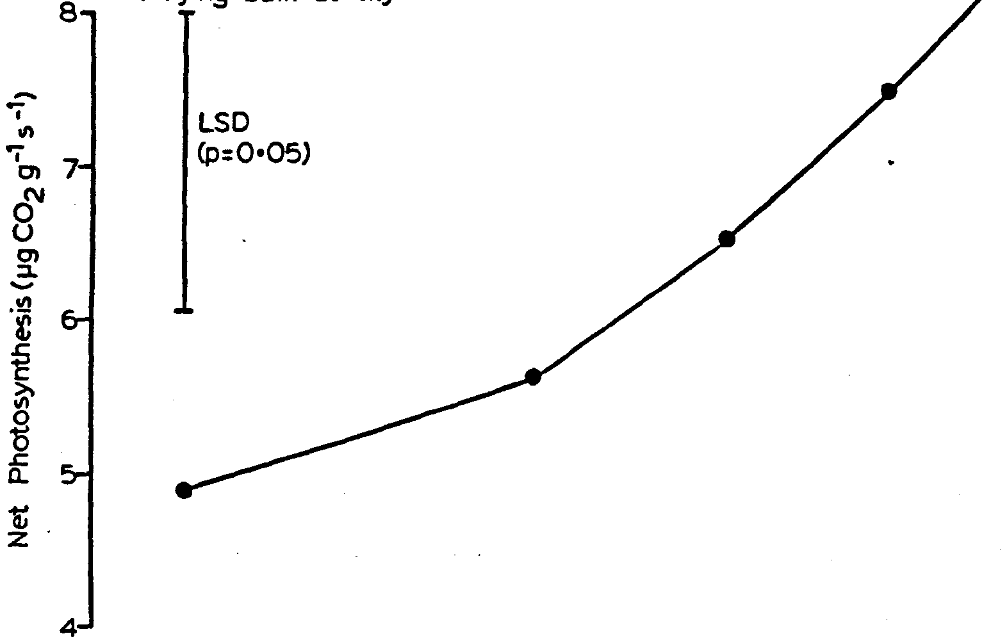
21 days after soil bulk densities had been increased CO_2 uptake was determined using the whole plant chamber (see Section IVA, (b)), and the plants harvested and oven dried at 85°C.

(b) Results

An increase in soil bulk density is associated with a significant ($p < 0.001$) decrease in shoot, root and total plant weight (Figure 105b). A differential response between shoot and root is observed. An increase in soil bulk density, from 0.733g cm^{-3} to 0.961g cm^{-3} results in a significant decrease in root weight but shoot weight is unaffected. However, an increase in soil bulk density from 1.083g cm^{-3} to 1.189g cm^{-3} results in the reverse situation occurring. This differential response by roots and shoots to increases in bulk density is illustrated by the significant ($p < 0.001$) reduction in root:shoot ratio (Figure 105a). The slight, but non-significant increase in root:shoot ratio with an increase in soil bulk density, from 1.189g cm^{-3} to 1.259g cm^{-3}, reflects the decreasing effect of soil bulk density increases on root weight while shoot weight continues to decrease with an increase in soil bulk density.

The relative growth rate data (Figure 106b) indicate that increases in soil bulk density do not begin to markedly affect shoot, root and total plant $\bar{R}$ until the soil bulk density rises above 0.961g cm^{-3}. Further increases are associated with significant ($p < 0.001$) reductions in $\bar{R}$. The differential effect on shoot and root growth is indicated by convergence of the graphs, root $\bar{R}$ decreasing by 39.5% whilst shoot $\bar{R}$ decreases by 29.1% over the range of soil bulk densities employed. However, the situation, with respect to individual treatment effects, is

(a) Carbon dioxide uptake of *P. bertolonii* grown in soils of varying bulk density



(b) Relative Growth Rate

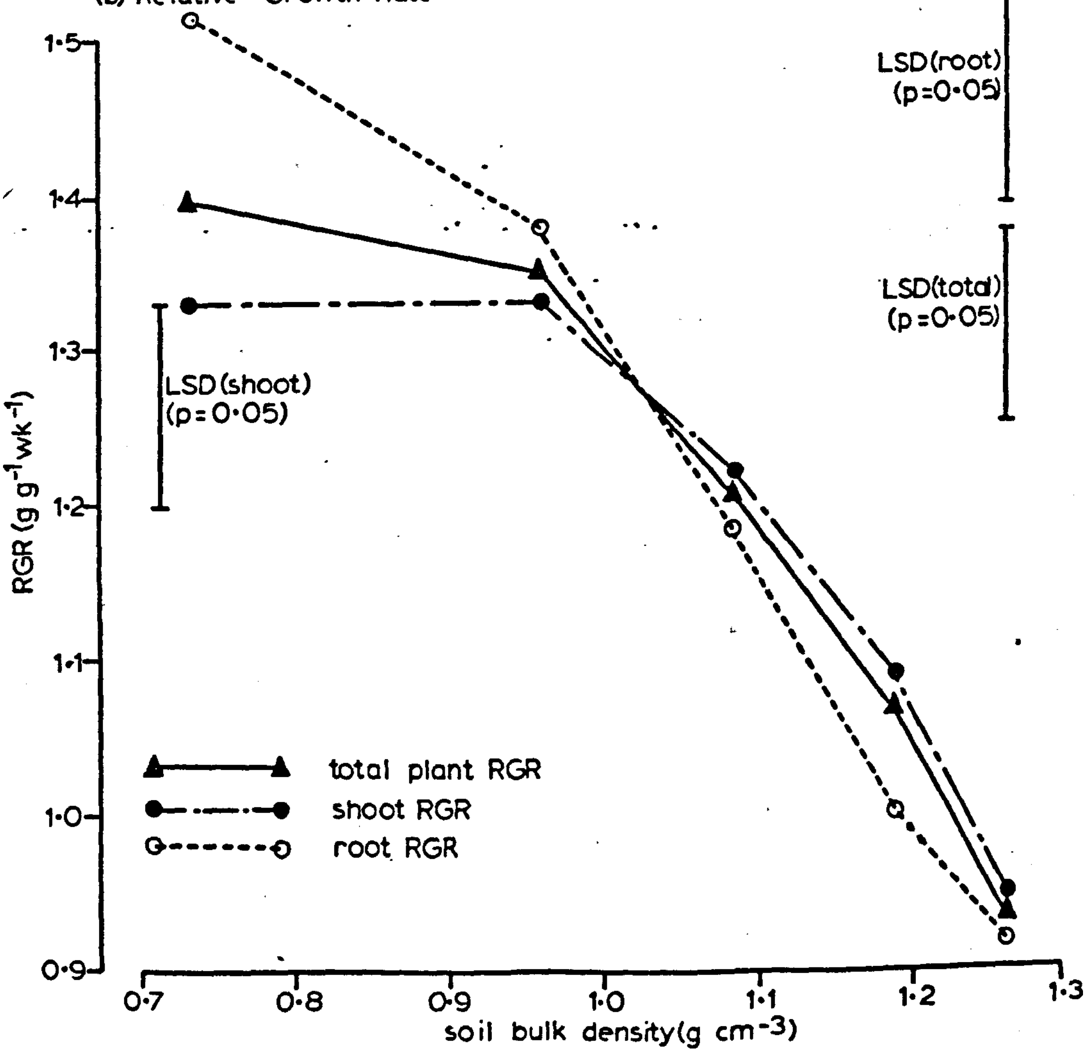


Figure 106 The effect of soil compression, subsequent to plant establishment in a loose soil, on Net Photosynthesis and growth of *Phleum bertolonii*

confused by unequal increases in soil bulk density between treatments.

Conflicting with the observations on $\bar{R}$, the rate of CO_2 uptake (Figure 106a) exhibits a significant ($p < 0.05$) increase with increase in soil bulk density, the relationship being linear ($r = 0.478$, $p < 0.01$). CO_2 uptake is negatively correlated with root weight ($r = -0.537$, $p < 0.001$; $\hat{y} = 7.77 - 2.13x$) and shoot weight ($r = -0.537$, $p < 0.001$; $\hat{y} = 9.13 - 2.14x$). However, care must be exercised in interpreting the increase in view of the changes in leaf area:leaf weight relationship (see Section VC, b(vi)), implying that the expression on a dry weight basis may be liable to error.

(iv) The relative effects of increases in soil bulk density on the Relative Growth Rate ($\bar{R}$) of Phleum bertolonii and Plantago lanceolata
(cf. Plates 5a and 5b)

The responses of the two species, in terms of $\bar{R}$, to increases in soil bulk density would appear to be similar (Table 26). The data are obtained from the experiments described in Section VB, b(ii) and (iii) in which, although performed at different times, plant age and growing conditions were nominally similar. The effect of an increase in soil bulk density from 1.0g cm^{-3} to 1.2g cm^{-3} results in an estimated reduction in $\bar{R}$ of 0.305g g^{-1} wk^{-1} for P. lanceolata compared to 0.174g g^{-1} wk^{-1} for P. bertolonii, suggesting that the increase may have a more severe effect on the growth of the former.

It is also of interest to observe that, at a soil bulk density of 1.0g cm^{-3}, the estimated root $\bar{R}$ is greater than the estimated shoot $\bar{R}$ for both species but an increase in bulk density has a more severe effect on shoot growth than root growth of P. bertolonii while for P. lanceolata, the effect is more severe on root growth than shoot growth.

Plate 5a. The effect of increasing soil bulk density on the growth of Plantago lanceolata following plant establishment in an unconsolidated soil. (Soil bulk density increases from left to right).

Table 26 Regression relationships between soil bulk density and $\bar{R}$ of *P. bertolonii* and *P. lanceolata*

Total plant $\bar{R}$	Regression Equation	r	y at X=1.0	y at X=1.2
P. bertolonii	$\hat{y} = 2.74 - 1.53\ X$	-0.812	1.21	0.91
P. lanceolata	$\hat{y} = 2.10 - 0.87\ X$	-0.752	1.23	1.06
Shoot $\bar{R}$				
P. bertolonii	$\hat{y} = 2.19 - 1.07\ X$	-0.715	1.12	0.90
P. lanceolata	$\hat{y} = 1.94 - 0.72\ X$	-0.660	1.22	0.87
Root $\bar{R}$				
P. bertolonii	$\hat{y} = 4.28 - 2.83\ X$	-0.864	1.45	0.88
P. lanceolata	$\hat{y} = 2.44 - 1.18\ X$	-0.834	1.26	1.02

(all values of r significant at $p < 0.001$)

(X = soil bulk density (g cm^{-3}); $\hat{y} = \bar{R}$ (g g^{-1} wk^{-1}))

(c) Discussion

The absence of a significant effect of soil compaction on the emergence of seedlings of *P. lanceolata* is at variance with findings of Blom (1976) who observed higher percentage emergence of *P. lanceolata* seedlings on looser soils. Similarly, the absence of a significant effect on the rate of germination conflicts with Blom's findings (1976) which indicated that the shortest time between sowing and emergence was on compacted soils, possibly due to improved moisture availability on soils of low moisture level. However, Blom suggested that mechanical resistance, rather than soil moisture was the most important unfavourable factor affecting the emergence of *P. lanceolata* in field trials.

Despite the lack of significant soil bulk density effects on seedling

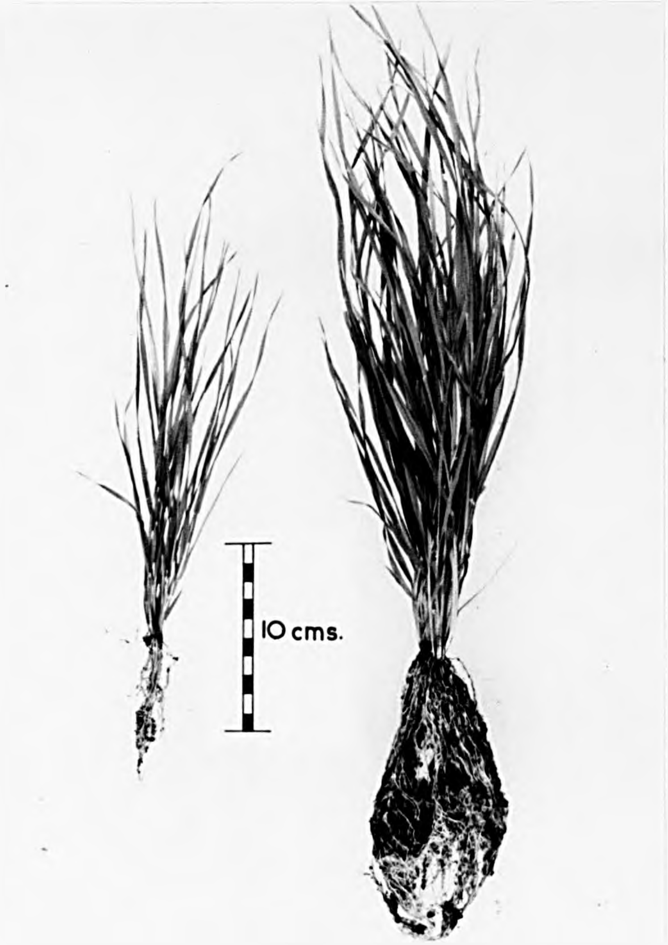


Plate 5b. The response of shoot and root of Phleum bertolonii to increases in soil bulk density following plant establishment in an unconsolidated soil. (The smaller of the plants grew in a soil of final bulk density of 1.259g cm^{-3}. The larger of the plants was obtained from a soil of bulk density 0.733g cm^{-3}).

emergence, subsequent growth and development is affected, appreciably, by soil bulk density variation. The main addition to dry weight in a plant is due to photosynthetic carbon gain. The photosynthetic capacity of a plant may be increased by leaf expansion which often exhibits a sigmoidal relationship with time (Evans, 1972). Assuming leaf length gives some indication of leaf area increase, the curvilinear relationship between increase in leaf length and time suggests that as the leaf approaches maturity, so less assimilate is channelled into expansion of the existing leaf but maybe channelled into expansion of new leaves and root growth (cf. Ryle & Powell, 1975).

Increasing soil bulk density has an adverse effect on leaf elongation. The anomalous rate change between day 22 and day 28 after sowing may be due to a change in the environmental conditions within the growth chamber due to recurrent mechanical failure. The effect of increasing soil bulk density on growth appears to be progressive, no critical value at which a marked effect occurs being apparent. This implies that at higher soil bulk densities, the potential photosynthetic capacity of the plant, as reflected in its ability to intercept light, is reduced. However, this is not a uniform response since, although the Relative Leaf Elongation Rate exhibits a general decrease with increase in soil bulk density, at the highest soil bulk density employed, the RLER is greater than that of all plants except those from the lowest soil bulk density. With reference to the shoot weight data, this observation may reflect a channelling of resources into the expansion of existing leaves instead of the initiation of new leaves. Although the RLER may be comparatively high, the leaf length (and possibly area) are still lower than at lower soil bulk densities and a relationship may exist between the photosynthetic capacity of the plant and the production of new leaves. As Evans (1972) noted, however, the partitioning of assimilates into new leaf area, existing leaves and the rest of the plant is a complex phenomenon depending upon the 'correlation mechanism' of the plant, which is incompletely understood.

For plants emerging and developing on soils of varying bulk density, the effects on plant dry weight are in agreement with the findings of Rosenberg & Willits (1962) and Wittsell & Hobbs (1965) but the variability in response agrees with Gupta's findings (1933) and may be a reflection of an adaptation by the plant to the soil environment in which it is growing. The stunted nature of the roots is in agreement with observations by Gupta (1933), Bates (1935), and Schuurman & de Boer (1974). The parallel decreases in root and shoot parameters, as reflected in non-significant root:shoot ratio differences, suggests that retarded root growth may limit shoot growth, although it is difficult to separate the causal effects of one portion of the plant upon another. Barley's observations (1962) suggested that resistance to root growth may have repercussions upon the shoot. Decrease in shoot growth in compacted soils has been widely observed e.g. Bates (1935), Rosenberg & Willits (1962), Wittsell & Hobbs (1965), Leney (1974) and although Gupta (1933) found that shoot dry weight was not significantly affected by compaction, there was a tendency for shoot dry weight to decrease with increased compaction. A similar tendency, together with a lack of significant effects, is observed in the present study. A decrease in root:shoot ratio indicates that root growth per unit dry weight of shoot decreases with increased soil bulk density, except for the value at a soil bulk density of 1.40g cm^{-3} at which the rate of shoot growth would appear to be substantially retarded compared to root growth, although the lack of significance suggests that it may be certain individual plants which are responding anomalously. The lack of significant effects implies that plants establishing on compacted soil may be compensating for increased mechanical impedence to growth. Barley (1962) suggested that plants may adjust their cellular osmotic relationships to overcome the increased mechanical impedence to root penetration. The greater energy requirements of roots in compacted soils to enable such resistances to be overcome may result in stunted growth of the whole plant as observed in the present study. This may reflect a situation in which the allocation of available resources results in a greater utilization, in terms of structural growth, in the shoot compared to the greater requirements to overcome the mechanical resistance to growth in the soil (cf. Taylor et al., 1966). The general stunting of the plant however, might be expected to limit assimilate production which would have repercussions on the growth of both shoot and root. The trend for greater root growth at lower soil bulk densities may indicate a more extensive exploitation of the soil but not a necessarily more intensive exploitation (cf. Gupta, 1933). Alternatively it may represent an expanding sink for excess assimilates (Humphries, 1969).

The effect of root growth on shoot growth may be mediated through hormonal effects on photosynthesis. Schuurman & de Boer (1974) noted that root number and branching are limited by increases in soil bulk density. This may limit production of root produced cytokinins, which are implicated in photosynthesis (cf. Wareing et al., 1968), which in turn may limit assimilate production and consequently plant growth, although such a postulate requires experimental investigation.

With reference to trampled habitats, associated increases in soil bulk density (Chappell et al., 1971; Liddle & Greig-Smith, 1975a) may reduce plant vigour in the establishment phase by limiting the exploitation of soil resources and possibly aerial productivity and the accumulation of reserves. This may have repercussions on plant survival following trampling. However, this response will depend upon species responses to soil compaction and the depth to which compaction occurs so that shallow soil compaction may have a minor effect on growth of deep rooted species which may therefore possess a certain competitive advantage in situations where the soil is compacted (cf. Bates, 1935).

The effect of soil compaction on plants following their establishment in loose soil varies according to their rooting depth. Bates (1935) found trampling-induced soil compaction had little effect on plant growth except of shallow rooted species, the zone of compaction extending to a depth of three to five centimetres, whilst Liddle & Greig-Smith (1975a) found that the most resistant soil layer of a trampled site was in the second centimetre of soil from the surface. In the present investigation, although a vertical gradient in soil bulk density may exist, the total rooting zone is likely to be subjected to a degree of compaction, although the applicability of such findings to the field situation is contentious.

The absence of a significant increase in leaf area of pre-treatment emerged leaves of *P. lanceolata* may reflect a situation in which the leaves had entered the mature phase in their ontogeny in which further increases in leaf area are negligible (cf. Evans, 1972), but increases in soil bulk density would appear to have an adverse effect on leaf growth. A mechanistic explanation of such a response is difficult to propose owing to the different response exhibited by post-treatment emergent leaves. It is conceivable that mechanical disruption of root tissues leads to a reduction in growth (Barley, 1962) which may affect root metabolism and limit the production of metabolites involved in photosynthesis leading to a reduction in assimilation (cf. Wareing *et al.*, 1968). The mechanical disruption of root tissues may also impede mineral, and possibly water uptake, the latter limiting leaf elongation due to insufficient turgor pressure to generate the necessary expansive forces (cf. Hsiao, 1973). The degree of damage and rate of recovery may depend upon the compressive forces generated by soil compaction and may be reflected in the negative relationship existing between leaf area increase and increase in soil bulk density. Higher degrees of compaction may cause greater damage to the root which retards the rate of recovery in proportion to the degree of damage. However, increases in soil bulk density appear to have a stimulatory effect on the leaf area of post-treatment emergent leaves. This may reflect the benefit of a more intimate contact between soil and root (cf. Gupta, 1933) although this is not supported by the dry weight data. The increasingly curvilinear relationship over time suggests that assimilates may be channelled into expansion of existing leaves rather than an increase in leaf number. This would benefit the plant by increasing the Leaf Area Index so increasing the efficiency of light interception, but only to a point at which mutual shading of leaves does not result in a diminishing return from further leaf area increase.

The discrepency in response between pre- and post-treatment leaves may arise from the fact that the latter are dated from the date of emergence and consequently no account of the time taken to repair root damage resulting from soil compaction is made. The results may therefore represent leaf growth following repair of damage and new root growth following the increase in soil bulk density. Increase in soil compaction may result in a more intimate contact between root and soil which may enhance mineral and water uptake and enhance leaf growth (cf. Liddle & Greig-Smith, 1975a). However, the adverse effects of the highest soil bulk density on RLGR of P. lanceolata may be due to a retardation of root development in accordance with the suggestions of Barley (1962) in which the plant is unable to accommodate an increase in soil compaction by an adjustment of cellular osmotic relationships, leading to a reduction in soil exploration, a possible decrease in physiological activity and a concomitant limitation on growth.

The observations regarding decreased root weight of P. lanceolata with increased soil bulk density are consistent with the findings of

Leney (1974). It is not possible from the results of the present study to discriminate between the effects of mechanical impedence, aeration effects or moisture availability. In terms of the physical resistance to root growth, the latter may be reduced by the increased energy demands needed to overcome such resistances (cf. Taylor et al., 1966). The direct compressional effects of soil on root meristems may also lead to a reduction in potential root growth (cf. Barley, 1962). Damage to subsurface meristems which give rise to leaves may reduce the potential for leaf production. This may result in assimilates being deployed into pre-existing leaves or those capable of further development,while at lower soil bulk densities available assimilates may be channelled into production of new leaves, ultimately resulting in an increase in yield. The possible disproportionate utilization of resources is reflected in the decrease in root:shoot ratio with increasing soil bulk density, where the absence of a physical resistance to shoot growth may result in a greater utilization of resources into structural components of the shoot in contrast to assimilate utilization in the root. In this context it is of interest that Schuurman & de Boer (1974) observed that where root development was retarded shoot growth was enhanced. The present results suggest a similar situation may exist at higher soil bulk densities where a differential effect on shoot and root $\bar{R}$ is observed. However, reduction in root growth may ultimately be expected to reduce total plant growth when soil mineral reserves become exhausted.

The relationships discussed above with reference to P. lanceolata are similar to those existing between plant growth and soil bulk density for P. bertolonii. There is a tendency for the former species to be more adversely affected than the latter by an equivalent increase in soil bulk density which implies that species may differ in their response to soil compaction. This is a phenomenon observed by others e.g. Bates (1935), Rosenberg & Willits (1962), Leney (1974). Although differences in rooting depth may result in such differences (Bates, 1935) in the present investigation soil compaction is likely to have occurred throughout the rooting zone. Species difference, if real, may result from differences in cellular osmotic relationships (cf. Barley, 1962) or may result from a physical effect. Species with finer roots may be less severely affected by a given degree of compaction in a soil of given structure since the roots may be less severely damaged, or the reduction in soil pore space may be insufficient to retard root growth to the same extent as for thicker roots. Such postulates require experimental investigation but may be a factor of significance in differential species survival in trampled habitats when related to soil exploration and exploitation.

The results relating CO_2 uptake to increases in soil bulk density whilst yield is reduced, may be erroneous. The explanation for such a paradox remains obscure but an increase in dry weight per unit area as a result of assimilate accumulation in the leaf (cf. Ryle & Powell, 1975) may lead to an error in the unit of expression which may not occur if the results were to be expressed on a leaf area or chlorophyll content basis. Compaction may retard the physiological ageing of the plant while adjustments to the new soil environment are made so that lower rates of photosynthesis at lower soil bulk densities may be associated with physiologically older plants, since a decrease in potential photosynthetic capacity is related to ageing (Hodgkinson, 1974; Ryle & Powell, 1975). The paradox between yield and CO_2 uptake may result from a channelling of assimilates into overcoming the increasing resistance to root penetration associated with increased soil compaction. In the absence of data relating to the distribution and utilization of assimilates, such postulates remain tentative.

C. The effect of simulated trampling on Carbon Dioxide (CO_2) exchange by *Phleum bertolonii* and *Plantago lanceolata*

(a) Introduction

The role of assimilates in plant regrowth following defoliation may have repercussive effects on assimilate production by the plant. The relationship between assimilates and net photosynthesis (F_n) is controversial. An early postulate by Boussingault (1868) proposed "... that the accumulation of assimilates in an illuminated leaf may be responsible for a reduction in the net photosynthesis of that leaf". Evidence for and against the hypothesis has been reviewed by Neales & Incoll (1968) and Humphries (1969). Conflicting results from the manipulation of assimilate source, sink and transport do not resolve the question of the photosynthetic response to assimilate demand and supply.

Sink removal is an effective method of producing assimilate accumulations at source. Nosberger & Humphries (1965) found that removal of tubers from *Solanum tuberosum* was associated with an increase in sugar, starch and protein nitrogen content of stems and leaves and was attended by a significant reduction in Net Assimilation Rate (NAR) (a quantity dependent on but not synonomous with F_n). Assimilates destined for tubers were redirected into leaf and lateral stem growth. Sink removal may therefore result in a redistribution of assimilate and the production of alternative sinks (Neales & Incoll, 1968). Humphries (1969) considered that excess carbohydrate could be channelled into root growth and stated that "The root system of an intact plant can probably accept all carbohydrate the shoot can supply. The root system is an efficient carbohydrate sink because it is potentially of unlimited growth and has many meristems, whereas shoot meristems are limited in number and their production under internal control". This suggests that carbohydrates may be produced without accumulating in the leaves and possibly affecting F_n. Humphries (1963) and Humphries & Thorne (1964) concluded that NAR was positively correlated with root size. Working with leaves of Phaseolus vulgaris, roots were induced at the base of the petiole and an increase in root growth rate was found to be associated with an increased NAR. They suggested that the rate of assimilate translocation was of fundamental importance since restricted root growth could lead to an accumulation of assimilates in the leaf without affecting NAR but root severance could retard CO_2 uptake which did not increase until regeneration began. Maggs (1964), however, found that root removal from Malus sylvestris led to an increase in NAR. Neales & Incoll (1968) considered that Humphries' work did not prove that root size and NAR were invariably correlated. Thorne & Evans (1964) manipulated grafts of two varieties of Beta vulgaris in an attempt to elucidate the effects of sink size on F_n. Spinach beet possesses a lower root storage capacity and NAR than sugar beet. Grafts of sugar beet tops on spinach beet roots resulted in a reduction in NAR whilst the reverse resulted in an increase in NAR. They suggested that lower assimilate demand by spinach beet root restricted assimilate translocation from leaves leading to a reduction in F_n.

In addition to subsurface sink alterations, aerial sinks have been manipulated. King et al. (1967), working with Triticum aestivum removed the ear, which is the main sink for flag leaf assimilates, and found a reduction in F_n of 40-50% within twenty four hours, a significant proportion of assimilates remaining within the flag leaf. They found that by substituting alternative sinks e.g. roots, young tillers, which were normally supplied by other leaves which were removed, F_n of the flag leaf could be restored to normality. King et al. suggested, on the basis of a literature survey, a number of alternatives to account for the effect. Evidence existed that an increase in assimilate concentration in the leaf could directly repress F_n, e.g. Thorne & Evans (1964), Nosberger & Humphries (1965). Alvim (1960) and Togoni _et al._ (1967) suggested that growth rate of assimilate sinks could be affected by growth substances which may have repercussions on assimilation, utilization, translocation and ultimately F_n. Booth _et al._ (1962), working with _Solanum andigena_ and _Pisum sativum_ found that the exogenous application of Indolyl-3-acetic acid (IAA) stimulated movement of substances toward the point of application, possibly through an indirect effect on stimulated active uptake and accumulation in the tissues to which it was applied. Davies & Wareing (1965) however, concluded that auxin effects on assimilate movement may be independent of growth effects. Another possibility is that auxin production, for example by a bud, may be transported to assimilate sources leading to a stimulation in F_n and translocation of assimilates to the organ to facilitate growth as a response to increased demand for assimilates (Turner & Bidwell, 1965). Bidwell & Turner (1966) found that spraying IAA onto leaves could result in a stimulation of F_n analogous to the response of F_n to bud break in a variety of species. Sweet & Wareing (1966) advanced a similar hypothesis to account for the reduction in CO_2 uptake when shoot apices of _Pinus radiata_ were removed. They postulated that not only may this result from a reduction in the sink capacity for assimilates but also a reduction of auxin levels within the plant.

Auxins may have a direct effect on F_n. The role of root produced cytokinins in protein synthesis (cf. Letham, 1967) may result in a reduction in F_n when root size is reduced artificially (Wareing _et al._, 1968). King _et al._ (1967) however, considered that auxin mediated effects were unlikely in their investigation and that the observed response of F_n was due to the direct effect of sink size and assimilate build up at source.

Neales & Incoll (1968) found evidence that removal or destruction of the phloem could lead to an increase in soluble carbohydrates in the leaf and a decrease in assimilation rate. For example, the findings of one worker showed that breaking of the leaf midrib of Saccharum officinarum reduced translocation by 34-38% and reduced F_n by 30% in the area above the break although leaf water content remained constant, indicating the effect was not due to water stress. A causal relationship between assimilate translocation and F_n is not necessarily implied however. Little & Loach (1973) found that cooling, to 2^oC, of the base of a branch of Abies balsamea inhibited assimilate translocation but did not affect F_n. They also found that ringing of the bark increased foliar concentrations of starch (but not sugar) and although a slight decrease in F_n occurred, it was attributed to water stress effects rather than starch accumulation effects. On the basis of observations in which carbohydrate content was found to be positively correlated with F_n and a decrease in starch and sugar concentrations was not accompanied by an increase in F_n, they concluded that F_n is not restricted by assimilate accumulations under normal growing conditions.

Source manipulation investigations fall into two main categories, those in which the plant is left intact and defoliation investigations. Neales & Incoll (1968) questioned the usefulness of the latter in relating assimilate distribution to F_n since associated imbalances in hormonal, assimilate and nutritional distributions may make one cause/one effect explanations facile. In view of this criticism, observations on undamaged arctic plants are of interest since it was suggested that "Low temperatures depress respiration and new growth so that assimilates are utilized only slowly. As a result assimilates (especially sugars) accumulate, and their concentration in the leaves rises to a level at which they depress assimilation to a rate that for a daily or longer period, roughly balances the rate of utilization of assimilates" (Warren Wilson, 1966). Thorne & Koller (1974), using Glycine max, adopted shading techniques and found that starch concentration decreased while sucrose concentration and CO_2 uptake both increased. They also observed that mesophyll resistance (r_m) of the source leaf was lower together with higher phosphate concentrations, ribulose-1-5-diphosphate carboxylase activity and translocation rate in the source leaf of shaded plants compared to the control. The data suggested that assimilate demand had a significant influence on photosynthesis, carbohydrate formation and export and that control plants were photosynthesising at less than full capacity, an observation consistent with Maggs' (1964) statement that "...both leaves and roots normally operate at levels well below the maxima of which they are capable". Parallel increases in sucrose levels and CO_2 uptake implied that soluble carbohydrate accumulations were not limiting F_n. Thorne & Koller (1974) suggested that the responses to shading may involve complex source-sink-hormonal interactions.

Defoliation studies are of immediate relevance to the present investigation, since they simulate part of the multifactorial response of plants to trampling. Maggs (1964, 1965) found that partial defoliation of Malus sylvestris resulted in an increase in NAR. Sweet & Wareing's (1966) investigations on Pinus radiata revealed that defoliation resulted in an increase in F_n and a higher relative growth rate, still evident eight weeks after defoliation. Although Ryle & Powell (1975) found a slight stimulation in shoot relative growth rate of defoliated plants this was ascribed to redistribution of assimilates rather than an elevation in F_n. If leaves operate at submaximal levels (Maggs, 1964), retention of an active 'sink' i.e. shoot apices, whilst the 'source' is reduced might be expected to result in the remaining source leaves supplying a relatively larger sink capacity, the demands of which might be met by an increase in F_n (Sweet & Wareing, 1966; Neales & Incoll, 1968). Sweet & Wareing (1966) postulated that an increase in the relative growth rate of defoliated plants may be perpetuated by an increasing assimilate sink combined with the more prolific auxin production of younger leaves leading to an increase in F_n.

Wareing _et al._ (1968) studied defoliation effects on _Phaseolus vulgaris_, _Salix acutifolia_, _S. viminalis_ and _Zea mays_. Three days after partially defoliating _P. vulgaris_ an increase in ribulose-1-5-diphosphate carboxylase (RuDP carboxylase) activity occurred. This was associated with an increase in total protein content suggesting that observed increases in F_n may have been associated with increased enzyme synthesis. Investigations on _Z. mays_ revealed that root removal inhibited the increase in F_n, at saturating light intensities, following defoliation but the inhibitory effect could be alleviated by spraying the plants with 6 furfuryl-aminopurine (kinetin) which suggested that cytokinins produced by the roots were affecting F_n via enzyme synthesis. They postulated that partial defoliation of the plant reduced the competition for cytokinins resulting in an increase in endogenous cytokinin levels in the remaining leaves, leading to an increase in enzyme synthesis and F_n. However, such an effect must be considered in the light of a reduction in root growth and respiration which occurs upon defoliation (Davidson & Milthorpe, 1966a; Ryle & Powell, 1975), which may reduce mineral uptake and the production of root metabolites, leading to a retardation of F_n. This may account for the observation by Davidson & Milthorpe (1966a) that F_n per unit leaf area remained constant following partial defoliation of _Dactylis glomerata_ while Ryle & Powell (1975) found a 5-10% decrease in F_n one to three hours after defoliation of _Hordeum vulgare_.

Observations by Wareing et al. (1968) suggested that in addition to source-sink relationships, a hormone mediated effect on r_m may occur via a decrease in the carboxylation resistance. Gifford & Marshall (1973) however, found that following defoliation of Lolium multiflorum, a change in r_s rather than r_m occurred while Hodgkinson (1974) attributed changes in F_n of Medicago sativa to intracellular changes in resistances in agreement with the observations of Thorne & Koller (1974) on Glycine max. Hodgkinson (1974) considered that differences in response may reflect species or age differences. The increase in F_n occurring a few days after defoliation was attributed, by Hodgkinson, to a decrease in the resistance to CO_2 transfer between the intercellular spaces and the carboxylation sites, resulting in a rejuvenation of the remaining leaves. Gifford & Marshall (1973) similarly considered that defoliation prevents the decline in F_n with age. Woolhouse (1968), working with Perilla frutescens, attributed the decline in F_n with age to an increase in r_m, since the 'fraction I' protein content (containing the carboxylation enzymes) declined with age. Hodgkinson (1974) argued that defoliation decreased r_m, the lowered value of which could be maintained by continual removal of young leaves, so reducing competition with older leaves for auxins and minerals. The effects of a decrease in photosynthetic surface on carbohydrate production may therefore be buffered by an increase in F_n.

Substantial evidence exists that in addition to the effects of assimilate demand on F_n, a more subtle metabolic mechanism may be involved in which hormonal balance is implicated. However, Neales & Incoll (1968) concluded that although assimilation manipulation appears to affect assimilate production, there is no proof that the two are causally related.

General Method

Following the recovery of plants of Phleum bertolonii and Plantago lanceolata subjected to simulated trampling pressures, CO_2 exchange rates of remaining tissue were analysed by infra-red gas analysis (Section IVA, (b)). Chamber air was conditioned to simulate the conditions experienced by the plant during recovery in controlled environment growth chambers, although the light intensity employed was greater than that experienced by the plants in the growth chamber.

Certain problems were experienced in attempting to measure CO_2 exchange of 'trampled' plants. Measurement of the area of damaged leaves was difficult and the enclosure of a single, mutilated leaf into an assimilation chamber presented practical difficulties. Consequently, preference has been given to expressing CO_2 uptake on a dry weight basis and measuring the CO_2 exchange of the entire shoot. This necessitated separate measurement of soil respiratory CO_2 output and presented problems in ensuring adequate mixing of air within the chamber. The CO_2 exchange data may therefore be attended by a considerable error which may interact with the treatment applied.

(b) (i) The effect of simulated trampling on Net Photosynthesis and Dark Respiration Rates of Plantago lanceolata

(a) Methods

Individual, 28-day old, plants of P. lanceolata (grown at 20 ± 1°C, 75 ± 10% relative humidity, 12h day at 13klx) were treated at 0, 5, 20 and 40i pt^{-1}, with eight replicates per treatment. Following recovery periods of 7 and 14 days, CO_2 exchange rates were measured, on four replicates for each date, at 20 ± 1°C, 75 ± 10% relative humidity and

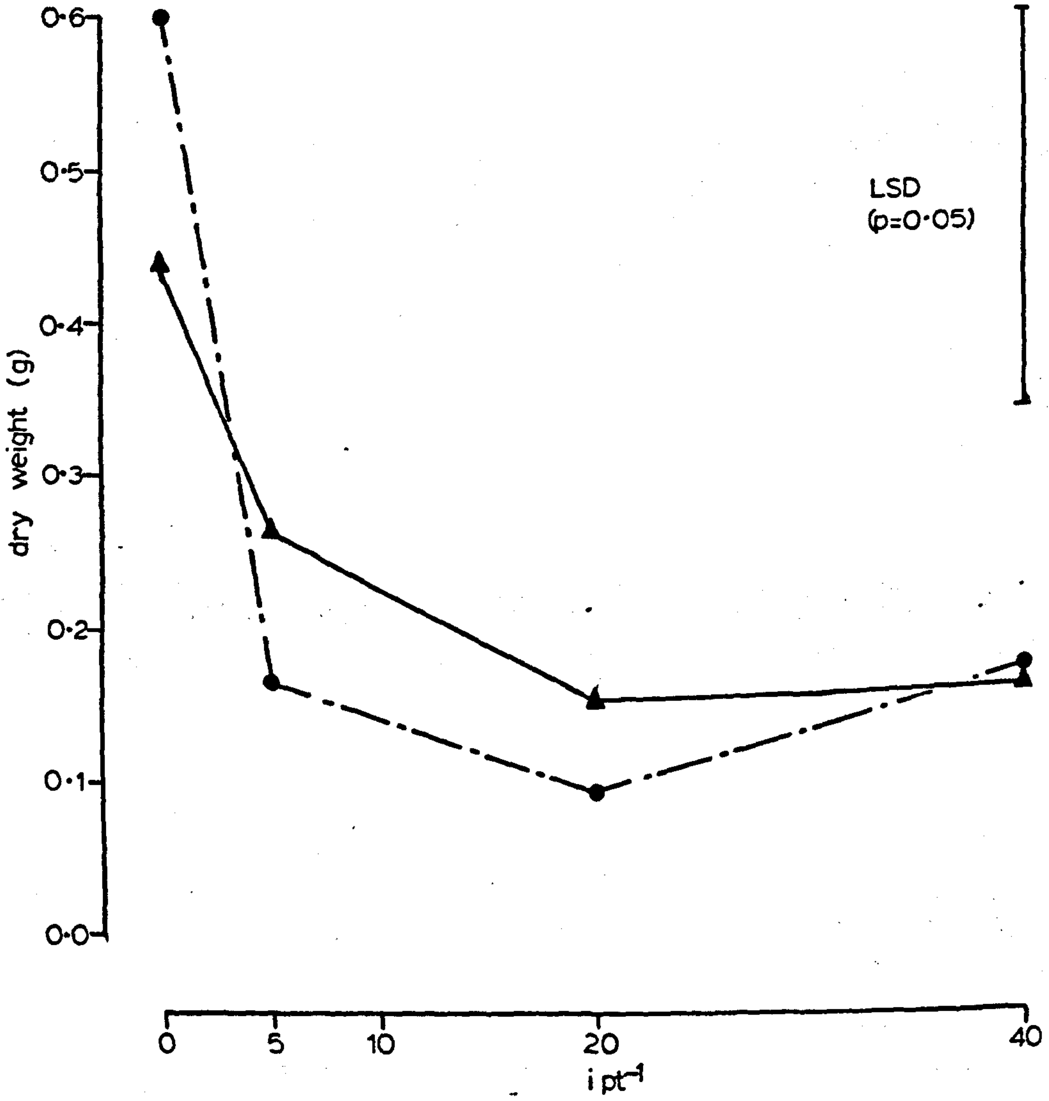


Figure 107 The effect of simulated trampling on the dry weight of *Plantago lanceolata*.

▲——▲ dry weight of plant 7 days after application of treatment

●-·—·-● dry weight of plant 14 days after application of treatment

45-49klx illuminance.

(b) Results

There are significant ($p < 0.001$) treatment effects on plant dry weight but no significant increase in dry weight over time (Figure 107). After 7 days the dry weights of plants treated at pressures of 20 and 40i pt^{-1} are significantly lower than the control value but by 14 days after treatment application all treated plants have a dry weight significantly less than the control. There is a trend for the dry weight of control plants to increase over the day 7 to day 14 period, giving an $\bar{R}$ value of 0.313g g^{-1} wk^{-1} (cf. 0.646g g^{-1} wk^{-1} for control plants of P. lanceolata; Section VA, b(ii)) but treated plants tend to decrease in dry weight over the same period, although the decline is not significant.

There is a significant ($p < 0.05$) increase above the control value in CO_2 output associated with a trampling pressure of 5i pt^{-1} and although dark respiration rates at higher treatment pressures are higher than the control 7 days after treatment application, the increase is not significant (Figure 108a). 14 days after the plants have been treated, respiration rates of treated plants are still greater than that of the control but not significantly so.

No significant treatment effects on CO_2 uptake are observed although there is a tendency for treated plants to possess higher CO_2 uptake rates than control plants, the effect being more evident on day 7 than on day 14 (Figure 108b). CO_2 uptake rates lie consistently above CO_2 output rates. After 7 days the difference between the two parameters is 6.0μg g^{-1} s^{-1} for control plants, declining to 5.1μg g^{-1} s^{-1} by day 14, both in favour of photosynthetic carbon gain. On day 7 the maximum difference between CO_2 uptake and output is found in plants treated at

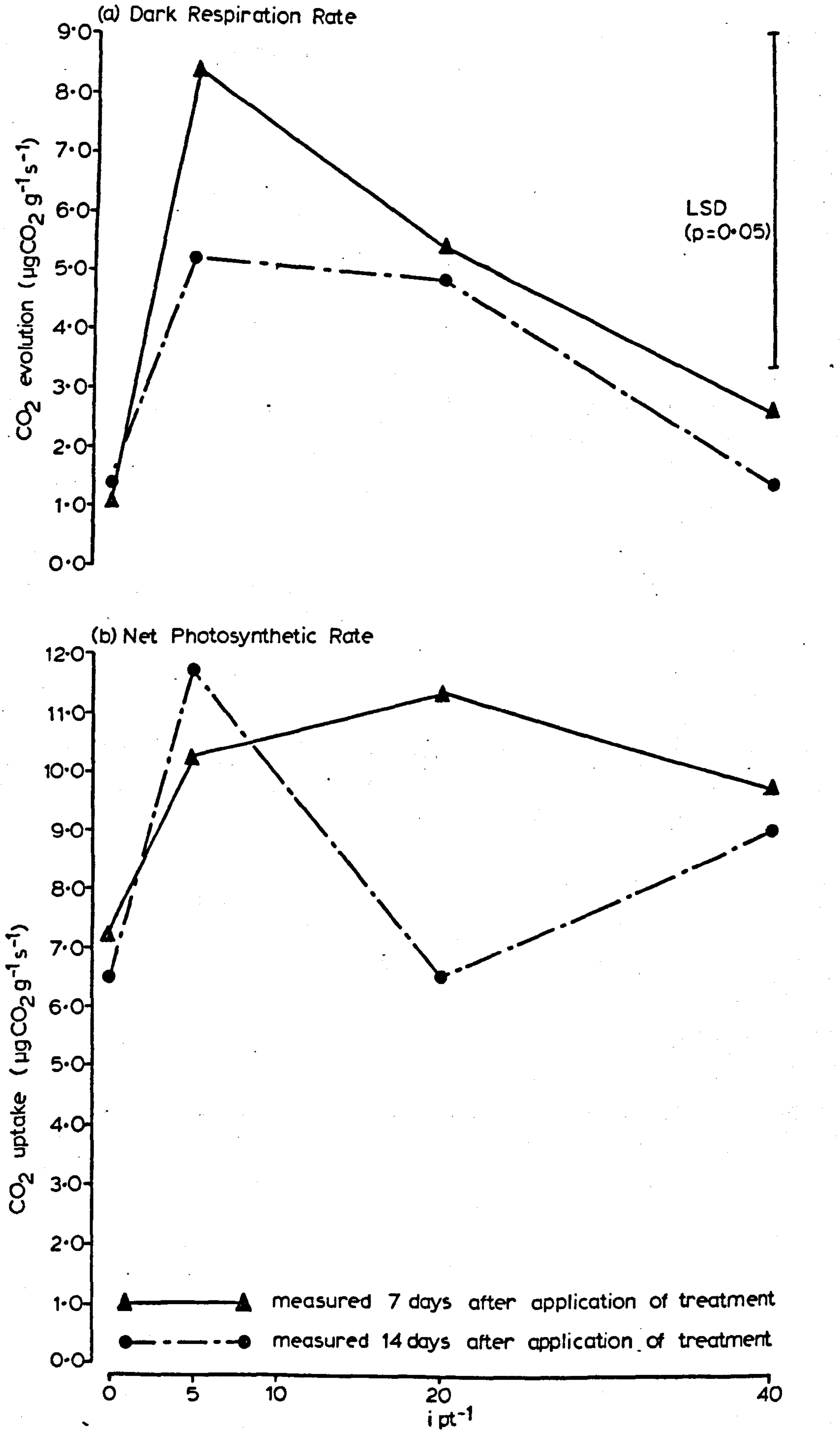


Figure 108 The effect of simulated trampling on Carbon Dioxide exchange (whole plant) of Plantago lanceolata.

40i pt^{-1} (7.1μg g^{-1} s^{-1}) while the minimum (1.8μg g^{-1} s^{-1}) occurs in plants treated at 5i pt^{-1}. By day 14 net photosynthesis and dark respiration rates have generally declined. The smallest difference between CO_2 uptake and output (1.7μg g^{-1} s^{-1}) is found in plants treated at 20i pt^{-1}, due primarily to elevated respiration rates, while plants treated at 40i pt^{-1} continue to exhibit the largest difference between CO_2 gain and loss. With reference to the control value, simulated trampling pressure results in an imbalance in CO_2 exchange amounting to a 33% increase in favour of CO_2 gain at a pressure of 40i pt^{-1} and a 83% decrease at a pressure of 5i pt^{-1}.

The greatest increase in respiration rate occurs in plants from a simulated trampling treatment (5 i pt^{-1}) with the greatest proportion of shoot tissue remaining after treatment, the decrease in respiration rate at higher levels of pressure being associated with increasing severity of debris production.

(ii) The effect of simulated trampling, at one intensity applied at one time, on the CO_2 uptake of *Plantago lanceolata*

(a) Methods

Individual 70-day old plants of *P. lanceolata* (grown at 20 ± 1°C, 75 ± 10% relative humidity, 12h day at 13klx) were subject to 5i pt^{-1}. CO_2 uptake was measured on single leaves, 1, 5 and 7 days after the application of the treatment, at 20 ± 1°C, 75 ± 10% relative humidity and 45klx. Three replicates for treated plants and two replicates for control plants were analysed on each date. Following the measurement of CO_2 uptake for each leaf, leaf RWC was determined by cutting three 8mm. discs from each leaf, weighing, and floating on distilled water in the dark at 20 ± 1°C for 4 hours, reweighing and drying at 85°C (cf. Section IIIB, 2(b)).

The light simulated trampling pressures employed resulted in a sufficient number of intact leaves remaining, following the application of the treatment, for whole leaves to be utilized in measurements.

(b) Results

Two way analysis of variance (for unequal replication) indicates no significant time or treatment effects on CO_2 uptake of *P. lanceolata*. There is a tendency for CO_2 uptake to increase over time in control plants and decrease in treated plants while RWC's decrease in both control and treated plants (Table 27).

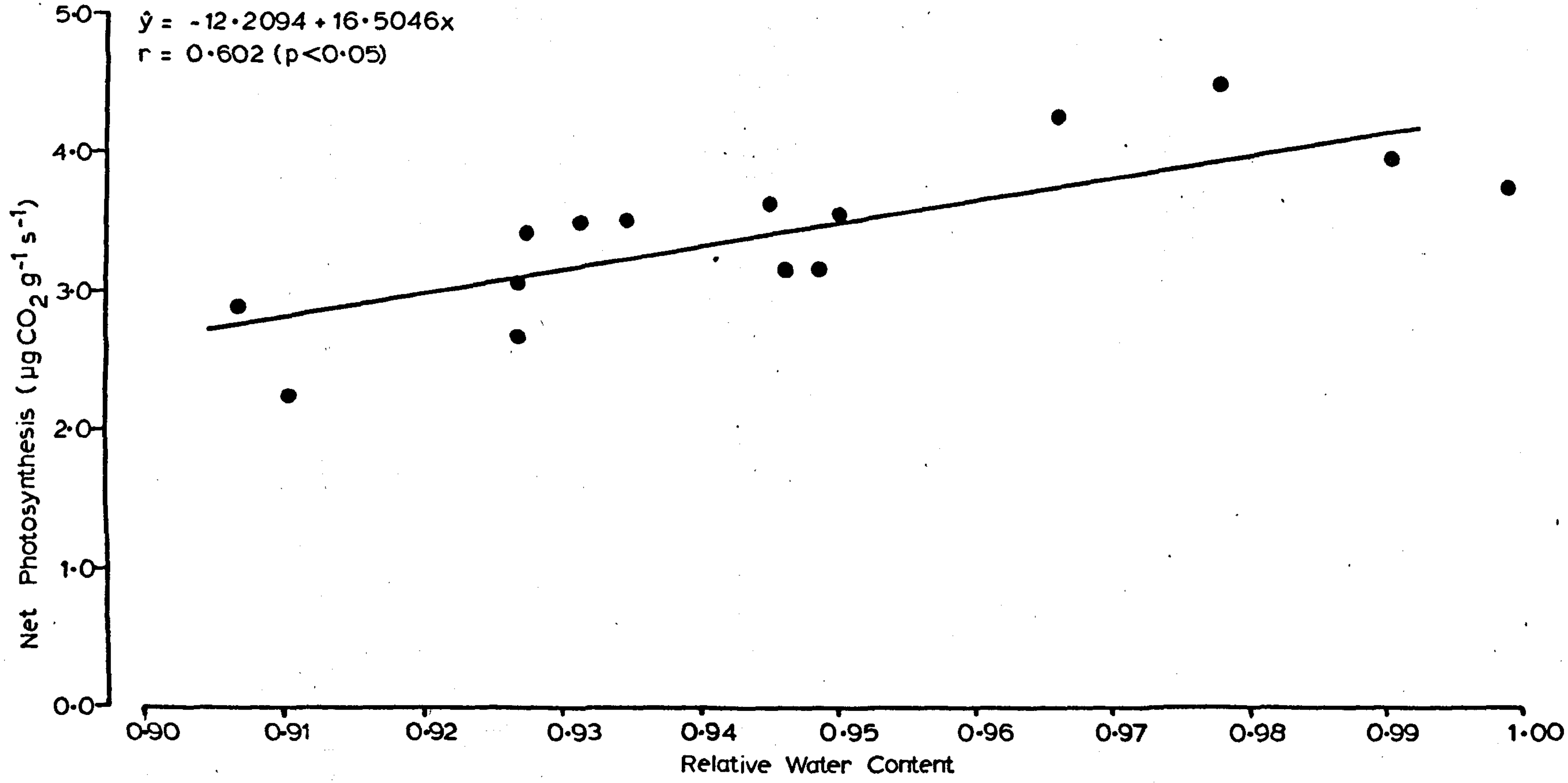
Table 27 The effect of a single, simulated trampling pressure, applied at one time, on CO_2 uptake and Relative Water Content of *P. lanceolata*

Days after treatment	1	5	7	Mean
Control - CO_2 uptake	3.84	4.06	4.23	4.04
Control - RWC	0.9947	0.9618	0.9547	0.9704
Treated - CO_2 uptake	3.35	3.15	2.82	3.10
Treated - RWC	0.9352	0.9230	0.9293	0.9292

(CO_2 uptake rate in $\mu g\ g^{-1}\ s^{-1}$)

In the absence of significant time effects, the data have been pooled, and one way analysis of variance with unequal replication performed (Appendix A, 1(b)). The mean rate of CO_2 uptake by treated plants is significantly ($p < 0.01$) lower than that of control plants.

Figure 109 The relationship between leaf Relative Water Content and Net Photosynthesis in *Plantago lanceolata*.



A significant ($p < 0.05$) linear regression is obtained between CO_2 uptake and RWC, a decrease in RWC being positively correlated with a decrease in CO_2 uptake (Figure 109). No improvement in fit is obtained by including quadratic or cubic terms into the regression (cf. Appendix A, 2(b)).

(iii) The effect of simulated trampling intensity on CO_2 uptake by Plantago lanceolata, 7 and 21 days after treatment

Following observations that CO_2 uptake may be stimulated by simulated trampling, experimental techniques were refined to ascertain whether the response is consistent (cf. Section VC, b(i)).

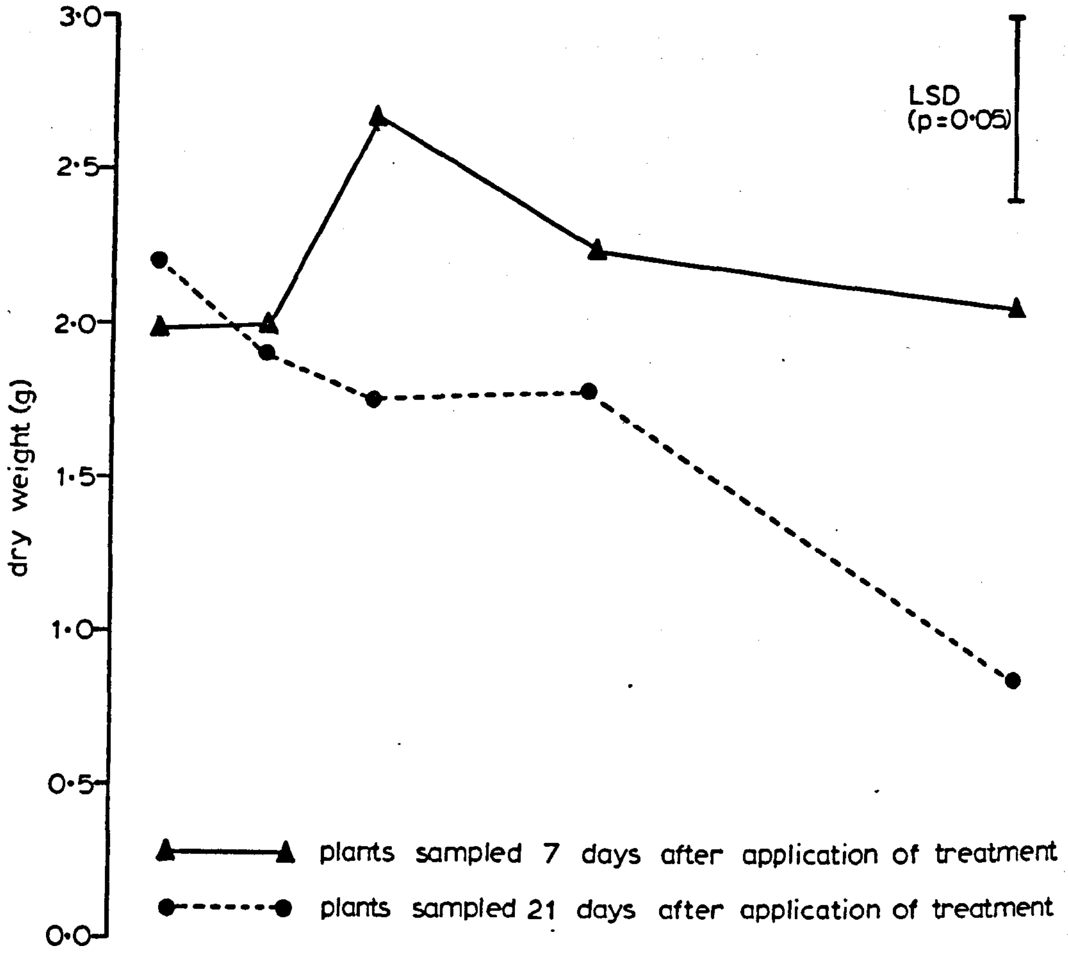
(a) Methods

Individual, 50-day old plants of P. lanceolata (grown at 20 ± 1°C, 75 ± 10% relative humidity, 12h day at 13klx) were subjected to a range of trampling intensities, 0, 5, 10, 20 and 40i pt^{-1}, with ten replicates per treatment. Seven days after the treatment had been applied, CO_2 uptake, of five replicates per treatment, was measured, the remainder being assessed for CO_2 uptake twenty one days after the treatment had been applied. After measurement of CO_2 uptake, the shoot was harvested and separated, subjectively, into parts appearing capable of photosynthesis, i.e. living and green, and those parts which appeared incapable of photosynthesis i.e. dead material, senescing tissue which had lost its green coloration.

(b) Results

Simulated trampling results in a significant ($p < 0.001$) reduction in

(a) Total shoot weight



(b) Percentage of the Total shoot dry weight involved in Net Photosynthesis measurements

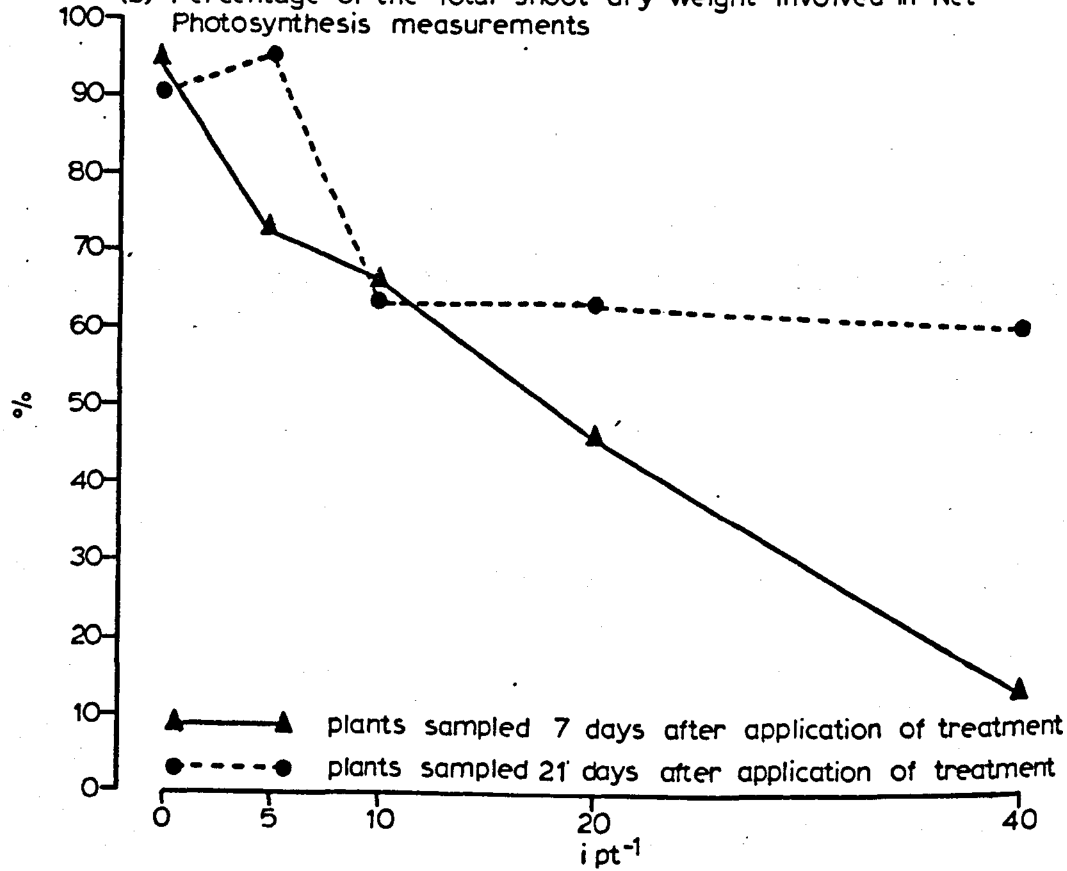


Figure 110 The effect of simulated trampling on dry weight parameters of Plantago lanceolata, employed in Photosynthesis investigations.

the dry weight of green (living) tissue but no significant increase occurs over time. There is a slight tendency for all treatments, except that of 10i pt^{-1}, to have a higher dry weight of green tissue after 21 days than 7 days after treatment (Figure 111a). The absence of a significant increase in dry weight may result from death and decomposition of damaged leaves over the 14 days following the measurements on day 7. Alternatively, it may imply a lower growth rate resulting from different environments existing in the growth chambers which, although nominally identical, may have affected growth of plants measured at different times.

The phenomenon of death and decomposition is re-emphasised by total plant weight data (Figure 110a). Significant ($p < 0.05$) treatment effects, not apparent on day 7, become marked by day 21 and give rise to significant ($p < 0.001$) time effects in which total plant dry weight at a pressure of 40i pt^{-1} is significantly lower on day 21 than on day 7 in comparison with the control value. It is of some interest to observe that on day 7 at a pressure of 10i pt^{-1}, total dry weight is significantly greater than the control value. However, although death and decomposition may account for lower dry weights on day 21 compared to values on day 7, the lack of a significant increase in the dry weight of control plants strengthens the possibility of different environments existing in the growth chambers.

The proportion of plant capable of photosynthesis varies with treatment and over time (Figure 110b). The significant ($p < 0.05$) time x treatment interaction indicates the effect of the treatment varies over time. The general response is for the trampling treatment to result in a significant ($p < 0.05$) reduction in the percentage of the plant capable of photosynthesis. This reduction may be partly compensated for by an increase in CO_2 uptake (Figure 111b). Significant ($p < 0.05$) time, treatment and time x treatment interaction effects are present. On day

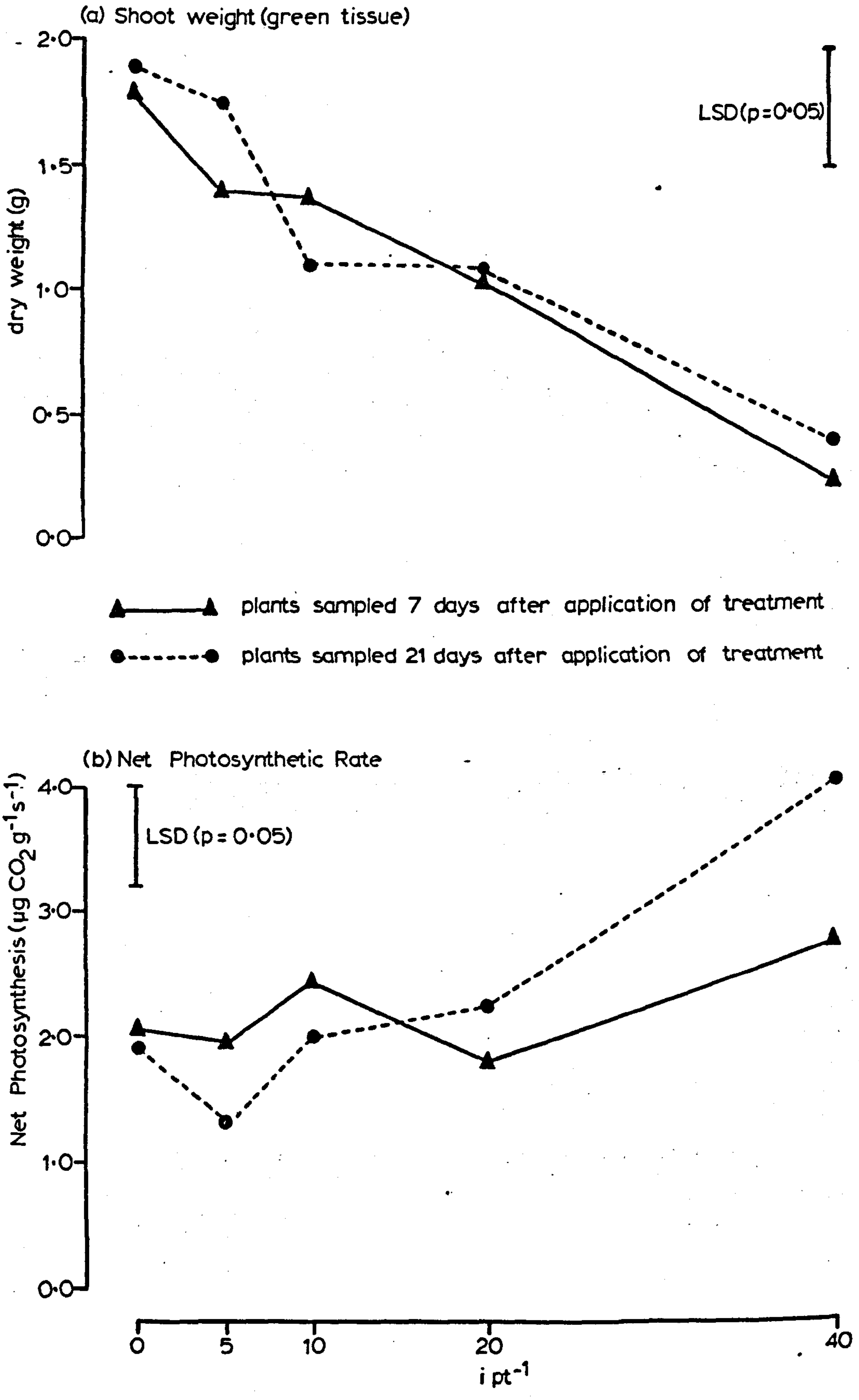


Figure 111 The effect of simulated trampling on the Net Photosynthesis of Plantago lanceolata.

7, no significant change in CO_2 uptake with reference to the control value occurs, although uptake, at pressures of 10i pt^{-1} and 40i pt^{-1}, is slightly greater than the control value. By day 21 treatment effects are more marked, there being a significant increase in CO_2 uptake by plants receiving a pressure of 40i pt^{-1}, which is also significantly greater than the CO_2 uptake by plants on day 7 receiving a similar pressure.

(iv) The effect of simulated trampling intensity on CO_2 uptake by *Phleum bertolonii*

(a) Methods

Individual, 50-day old, plants of *P. bertolonii* (grown at 20 ± 1°C, 75 ± 10% relative humidity, 12h day at 13klx) were subjected to 0, 5, 10, 20 and 40i pt^{-1} of the tamp, with ten replicates per treatment. Following a 21 day recovery period, CO_2 uptake was measured on a portion of the shoot enclosed in the medium response assimilation chamber. After measurement of CO_2 uptake, plants were harvested and dried at 85°C.

(b) Results

There is a significant ($p < 0.001$) decrease in root and shoot weight associated with an increase in simulated trampling pressure (Figure 112a). The response is curvilinear, saturation of root response occurring at 10i pt^{-1}, above which no further significant decrease in root weight occurs, while a saturation of shoot response occurs at 20i pt^{-1}. The root:shoot ratio, which is consistently less than unity, exhibits a significant ($p < 0.001$) decrease, over the control value, at 5i pt^{-1} above which no further significant change occurs (Figure 112b).

A significantly ($p < 0.001$) higher CO_2 uptake occurs in plants treated at 10, 20 and 40i pt^{-1} compared to the control value (Figure 112c). At

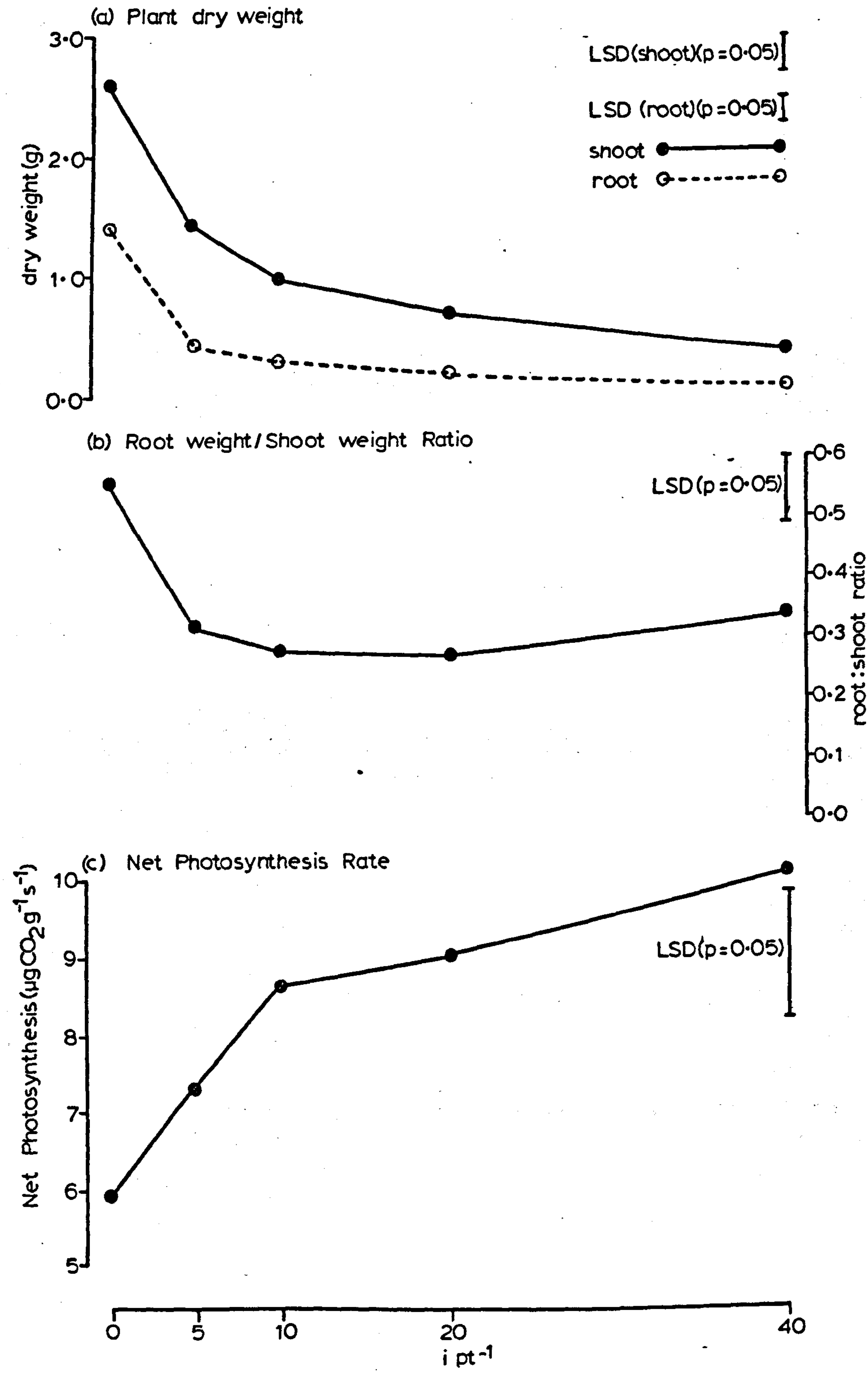


Figure 112 The effect of simulated trampling on the Net Photosynthesis of *Phleum bertolonii*.

its maximum, the CO_2 uptake rate is 69.2% higher in treated plants than in the control plants. Above a pressure of 10i pt^{-1}, although an upward trend in CO_2 uptake rates exists, further increases are not significant.

It is of interest to note that a significant negative correlation exists between root weight and CO_2 uptake ($r = -0.488$, $p < 0.001$). The regression coefficients ($\hat{y} = 9.17 - 2.30x$) approximate to those found for a similar relationship between root weight and CO_2 uptake in P. bertolonii (Section VB, b(iii), ($\hat{y} = 7.27 - 2.13x$)). At a root weight of 1.0g, the predicted CO_2 uptake is 6.87μg g^{-1} s^{-1} and 5.55μg g^{-1} s^{-1} respectively. The relative comparability of values suggests that root size may exert an influence on CO_2 uptake rate, although the effect may be modified by defoliation of the shoot in the former instance but not the latter.

(v) The response of Plantago lanceolata to the exogenous application of 6 furfuryl-aminopurine (kinetin)

(a) Methods (from Mitchell & Livingstone, 1968)

21.5mg of 6 furfuryl-aminopurine (molecular weight 215.2) were dissolved in 1 litre of distilled water giving a 10^{-4}M solution which was serially diluted to give 10^{-5}, 10^{-6} and 10^{-8}M solutions. Since the compound is not readily soluble in water its dispersion was aided by the use of a cosolvent with strong surfactant properties to aid penetration of the compound into the leaf i.e. Tween 80. The surfactant was dissolved to give a final concentration of 0.05% by volume in the final solutions. Dissolution of the kinetin was aided by slight warming (not above 50°C) and continuous stirring for 24 hours.

Leaves of uniform size were selected from three-month old plants and the measurement of an area defined by a vaseline boundary was made.

0.015ml cm^{-2} of hormone solution was applied to one leaf from each of five replicate plants per treatment. There were five treatments, control (treated with a 0.05% solution of Tween 80); 10^{-4}M; 10^{-5}M; 10^{-6}M; 10^{-8}M. Each solution was applied daily for six days.

CO_2 uptake of each leaf was determined prior to the application of the kinetin solutions. On the day following the final hormone application, CO_2 uptake of treated leaves was measured.

(b) Results

No significant differences in CO_2 uptake before the application of kinetin solutions (t_o) are apparent. On day 7 after the first application of kinetin solution, all treatments, except that of the 10^{-6}M concentration, have a lower CO_2 uptake than at t_o, although the decrease is not significant (Table 28).

Table 28 The effect of the concentration of kinetin in solution on CO_2 uptake by leaves of *P. lanceolata*

Kinetin concentration	Control	10^{-4}M	10^{-5}M	10^{-6}M	10^{-8}M
CO_2 uptake rate (t_o)	3.15	3.44	3.87	3.52	3.08
CO_2 uptake rate (day 7)	2.86	2.89	3.76	3.97	2.81
Percentage change	-9.4	-16.1	-2.7	12.8	-8.8

(CO_2 uptake expressed in μg dm^{-2} s^{-1})

The CO_2 uptake of leaves receiving a 10^{-6}M concentration of kinetin is significantly greater than all treatments except that receiving a 10^{-5}M concentration ($p < 0.05$, L.S.D. = 0.97μg dm^{-2} s^{-1}).

On a percentage change basis, and when the naturally occurring

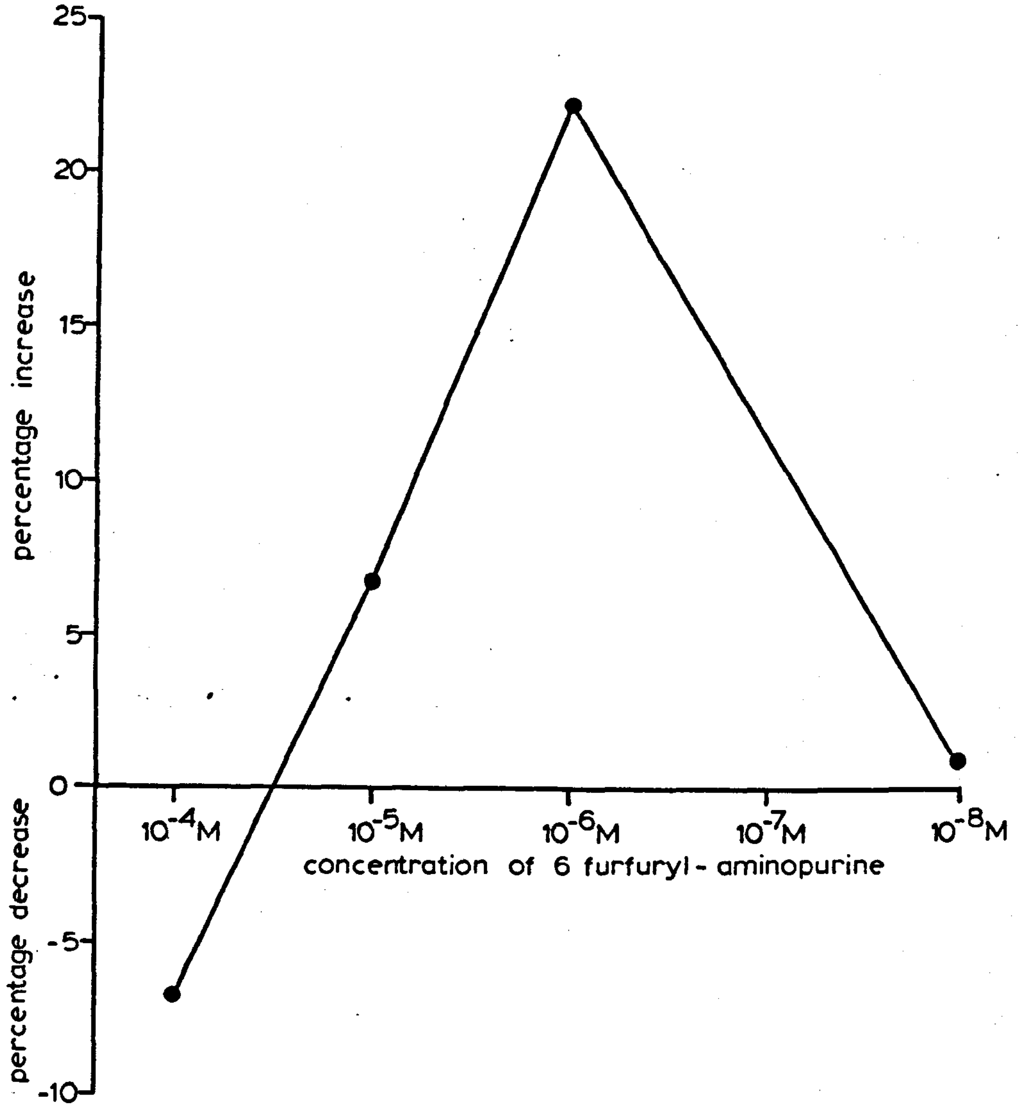


Figure 113 Percentage change in Carbon Dioxide uptake by <u>Plantago lanceolata</u> related to the concentration of kinetin (6 furfuryl-aminopurine) applied to the leaves.

decrease in CO_2 uptake of the control is taken into consideration, significant ($p < 0.01$) effects of kinetin concentration of CO_2 uptake are apparent, re-emphasising that a solution of 10^{-6}M concentration has the greatest stimulatory effect on CO_2 uptake while a 10^{-4}M solution appears to have an adverse effect on CO_2 uptake, reducing it to a greater degree than the natural decrease in CO_2 uptake apparent in the control (Figure 113).

(vi) The effect of the exogenous application of 6 furfuryl-aminopurine on the response of *Phleum bertolonii* to regular simulated trampling

(a) Methods

50-day old plants of *P. bertolonii* were subjected to the following treatments, with four replicates per treatment:

A - control, sprayed with a 0.05% v/v solution of Tween 80;

B - control, sprayed with 10^{-6}M solution of 6 furfuryl-aminopurine;

C - 2i pt^{-1}, applied at beginning of experiment, sprayed with 0.05% v/v solution of Tween 80;

D - 2i pt^{-1}, applied at beginning of experiment, sprayed with 10^{-6}M solution of 6 furfuryl-aminopurine;

E - 2i pt^{-1} wk^{-1}, applied for five weeks, sprayed with 0.05% v/v solution of Tween 80;

F - 2i pt^{-1} wk^{-1}, applied for five weeks, sprayed with 10^{-6}M solution of 6 furfuryl-aminopurine.

48 hours after the application of a trampling pressure, all plants were sprayed with the appropriate solution until the solution was running off the leaves (approximately 10ml. per plant). Growth and recovery conditions of 20 ± 1°C, 75 ± 10% relative humidity and 12h day

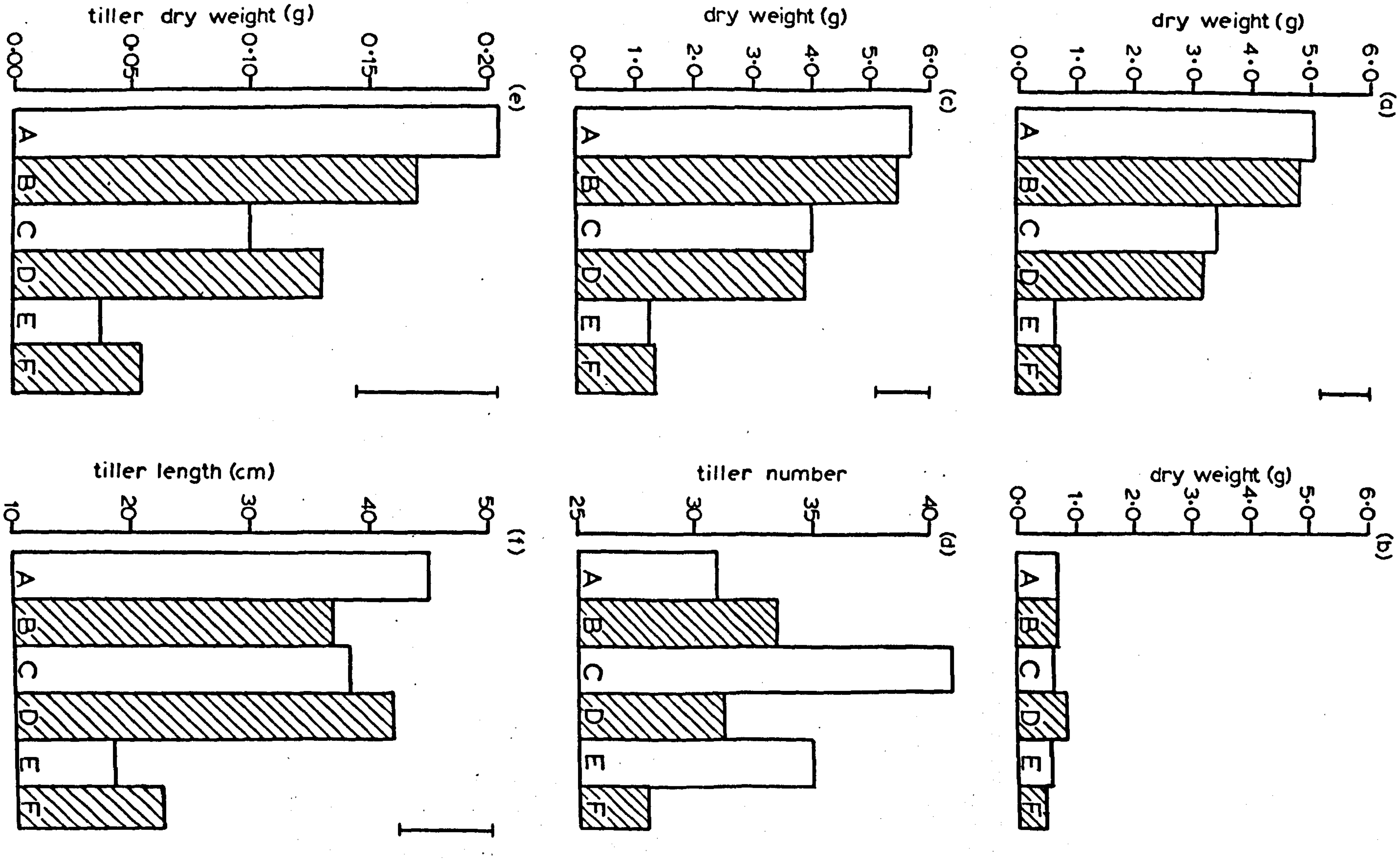

(a)
dry weight (g)
0·0
1·0
2·0
3·0
4·0
5·0
6·0
(b)
dry weight (g)
(c)
dry weight (g)
(d)
tiller number
25
30
35
40
(e)
tiller dry weight (g)
0·00
0·05
0·10
0·15
0·20
(f)
tiller length (cm)
10
20
30
40
50
A
B
C
D
E
F

Figure 114 The effect of the application of 6 furfurylaminopurine to the leaves of <u>Phleum bertolonii</u>, subjected to regular simulated trampling.

Treatment

A	control,
B	control, sprayed
C	2 i pt^{-1}, applied at begining of experiment
D	2 i pt^{-1}, applied at begining of experiment, sprayed
E	2 i pt^{-1}, applied every 7 days (total 10 i pt^{-1})
F	2 i pt^{-1}, applied every 7 days (total 10 i pt^{-1}), sprayed

(sprayed plants received a solution of 6 furfurylaminopurine(10^{-6}M) containing Tween 80(0·05% v/v), remaining treatments sprayed with distilled water containing Tween 80 (0 05%v/v))

(a) Dry weight of green material at time of harvest

(b) Dry weight of dead material at time of harvest

(c) Total harvested dry weight (shoot) plus debris produced as a result of simulated trampling and material dying over the duration of the experiment

(d) Tiller number

(e) Average tiller weight

(f) Average tiller length

(bar lines indicate LSD (p = 0·05) between two means) at 10-15klx were employed. Fourteen days after the final treatment application the CO_2 uptake of each replicate was measured followed by harvesting of shoot material. CO_2 uptake was measured, at $20 \pm 1^{o}C$, $75 \pm 10\%$ relative humidity and 45klx, on the third leaf to emerge, on one tiller per replicate, using the fast response assimilation chamber.

Tiller length was determined on ten randomly selected tillers per plant, and leaf weight/leaf area data extracted from leaves employed for CO_2 uptake measurements.

A parallel set of plants, receiving the same treatments, were assessed for CO_2 uptake, at 4 hours, 2 days, 4 days, 6 days and 8 days after the final spraying treatments, on the third emergent leaf.

(b) Results

There is a significant ($p < 0.001$) reduction in the dry weight of green (living) material associated with both trampling treatments (i.e. instantaneously and regularly applied) although the effect is more severe in plants treated regularly (Figure 114a). (cf. Section VA, b(iv) where regular simulated trampling, at 2i pt^{-1}, did not have a more severe effect on Plantago lanceolata than an identical pressure applied at one time). No significant effect of hormone application is observed. This pattern of response is not changed by including dead material and debris produced as a result of the trampling treatment, the differences noted above remaining highly significant ($p < 0.001$) (Figure 114c).

The standing crop of dead material (Figure 114b) is similar in all treatments but as the total dry weight decreases so the proportion of the plant which is dead increases from 12.0% in treatment A to a maximum of 45.3% for treatment E (Figure 115a).

No significant differences in number of tillers per plant are

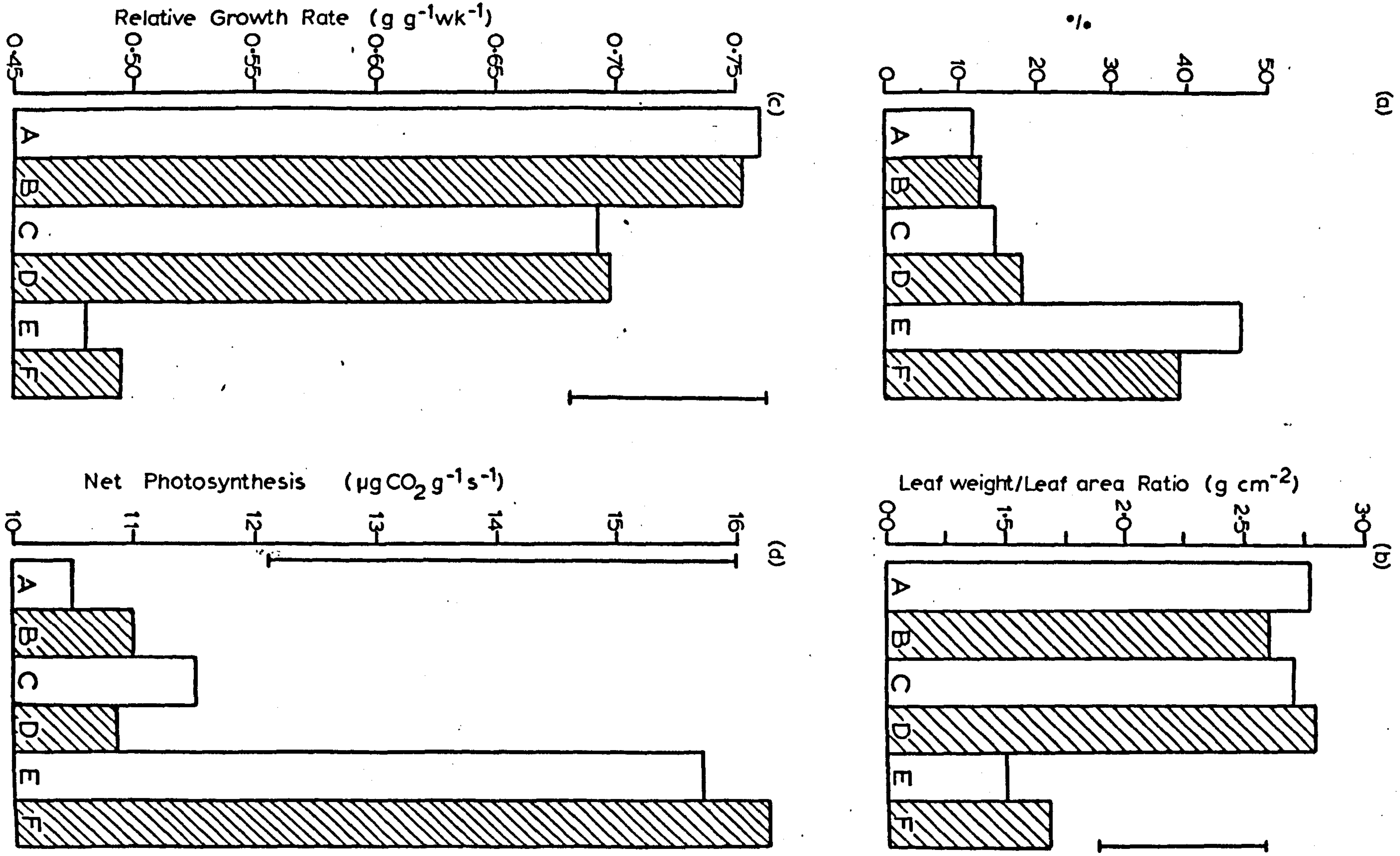

(a)
°/•
0
10
20
30
40
50
A
B
C
D
E
F
(b)
Leaf weight/Leaf area Ratio (g cm^{-2})
0·0
1·5
2·0
2·5
3·0
A
B
C
D
E
F
(c)
Relative Growth Rate (g g^{-1}wk^{-1})
0·45
0·50
0·55
0·60
0·65
0·70
0·75
A
B
C
D
E
F
(d)
Net Photosynthesis (µg CO$_2$ g^{-1} s^{-1})
10
11
12
13
14
15
16
A
B
C
D
E
F

Figure 115 The effect of the application of 6 furfurylaminopurine to the leaves of <u>Phleum bertolonii</u>, subjected to regular simulated trampling, on growth and Net Photosynthesis.

(Treatments A - F see Figure 114)
(bar lines indicate LSD ($p = 0{\cdot}05$) between means)

(a) Dry weight of dead material expressed as a percentage of total harvested dry weight
(b) Leaf weight / Leaf area Ratio
(c) Relative Growth Rate
(d) Net Photosynthetic Rate

observed although for trampled plants which were not sprayed[1] there is a tendency for tiller number to be greater than that of the control, although the increase is less in plants trampled regularly. There is also a trend for sprayed plants to have a lower tiller number than their unsprayed counterparts, except for plants not subjected to simulated trampling pressures (treatment A and B). Treatments D and F have tiller numbers which are 76.2% and 80.0% lower than treatments C and E while treatment B possess 8% more tillers than treatment A (Figure 114d).

Significant ($p < 0.001$) differences in dry weight per tiller (Figure 114e) reveal that plants subject to simulated trampling have lower dry weights per tiller than the control, the most severe reduction being observed in regularly treated plants. The application of 6 furfuryl-aminopurine does not result in any appreciable change in this relationship although treatments B and D no longer differ significantly. Sprayed control plants tend to have a lower dry weight per tiller compared to their unsprayed counterparts. This is a reflection of tiller number, implying that more energy is diverted into increasing tiller number in unsprayed plants compared to sprayed plants where an increase in tiller weight occurs.

Tiller length is also significantly ($p < 0.001$) reduced but only by regular simulated trampling (Figure 114f). There is a tendency, except in control plants, for tiller length of sprayed plants to be greater than tiller length of unsprayed plants.

The leaf weight to leaf area ratio (Figure 115b) exhibits a significant ($p < 0.05$) decrease in treatments E and F, indicating that dry weight per unit leaf area is lower in plants regularly trampled compared to control plants and those trampled only at the beginning of the experiment. Again, no significant effects of spraying are observed.

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[1] 'sprayed' refers throughout to plants sprayed with a 10^{-6}M solution of 6 furfuryl-aminopurine.

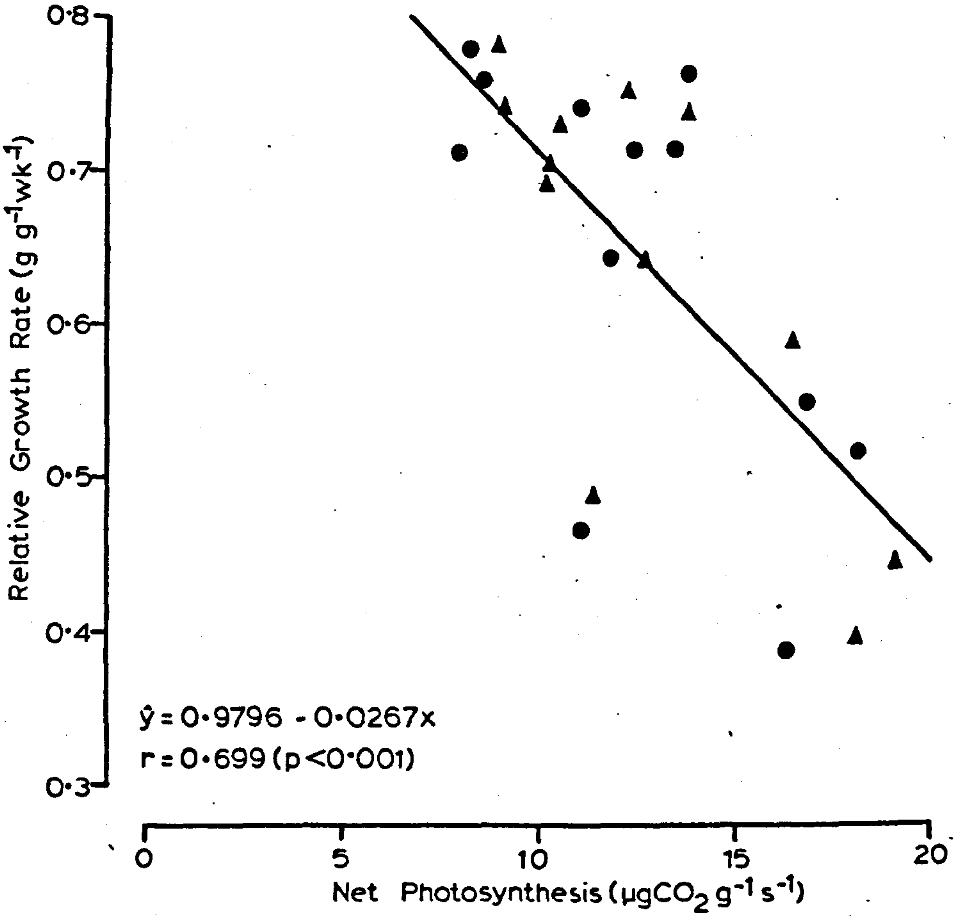


Figure 116 The relationship between Net Photosynthesis and Relative Growth Rate in _Phleum bertolonii_.

- ● measurements on plants sprayed with distilled water containing Tween 80 (0·05% v/v concentration)
- ▲ measurements on plants sprayed with a solution of 6 furfuryl - aminopurine (10^{-6}M), containing Tween 80 (0·05% v/v concentration)

The pattern of $\bar{R}$ values (Figure 115c) is similar to that for dry weight data. However, although the $\bar{R}$ of treatments C and D is lower than that of the controls, the reduction is only significant ($p < 0.001$) for treatments E and F. The $\bar{R}$ values presented represent the average weekly dry matter increment when the reduction in dry weight at time zero, as a result of defoliation due to the trampling treatment, has been allowed for. Although allowing for debris produced over the duration of the experiment due to death and severance, the calculation does not take into account the reduction in plant size, due to trampling induced defoliation, occurring each week in treatments E and F. Consequently, the $\bar{R}$ value presented may be an underestimate of the true $\bar{R}$ (cf. Section VA, b(iv)). The observations contrast with the findings for Plantago lanceolata where regular trampling did not have a more adverse effect on $\bar{R}$ than the same pressure applied only once (Section VA, b(iv)). Spraying plants with 6 furfuryl-aminopurine has no significant effect on $\bar{R}$.

In contrast with the observations on $\bar{R}$, simulated trampling is associated with a significant ($p < 0.05$) increase in CO_2 uptake of treatments E and F (Figure 115d). No significant effect or consistent trend produced by spraying on CO_2 uptake by leaves of Phleum bertolonii is evident. The high rates of CO_2 uptake by plants in treatments E and F may be due to error introduced through the significantly lower leaf weight:leaf area ratios since leaf weight affects the calculation of net photosynthesis rates but may not reflect, accurately, the photosynthetic potential of the tissue.

The CO_2 uptake results do not take into account dark respiration losses which will be integrated into values of $\bar{R}$, thus reducing the amount of carbon converted into a dry weight increment. There is a highly significant negative correlation between $\bar{R}$ and CO_2 uptake

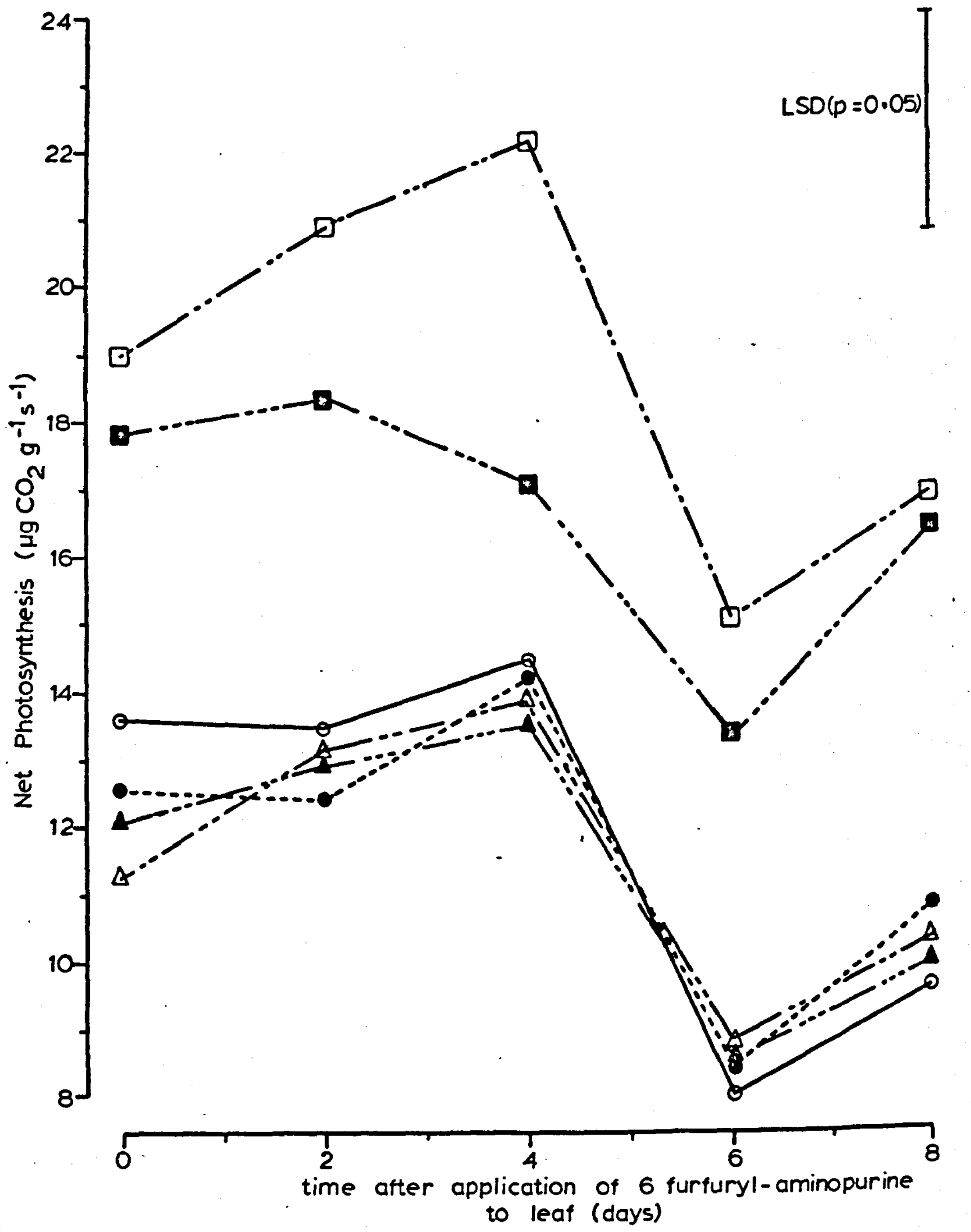


Figure 117 Change, over time, in the rate of Net Photosynthesis of leaves, sprayed with a solution of 6 furfuryl - aminopurine, of <u>Phleum bertolonii</u> subjected to regular simulated trampling.

○————○ control

●- - - - - - ● control, sprayed

△— ·—— ··—△ 2 i pt^{-1}, applied at begining of experiment

▲···——···—▲ 2 i pt^{-1}, applied at begining of experiment, sprayed

□—— ·——··—□ 2 i pt^{-1}, applied every 7 days (total 10 i pt^{-1})

■- ···——···■ 2 i pt^{-1}, applied every 7 days (total 10 i pt^{-1}), sprayed

(Figure 116).

The relationship between CO_2 uptake over time and the application of 6 furfuryl-aminopurine (Figure 117) reveals that treatments A to D are never significantly different while CO_2 uptake in treatments E and F is consistently significantly ($p < 0.001$) greater than remaining treatments (except on day 4). No significant effect of spraying is discernable except on day 4 when the CO_2 uptake by treatment F is lower than that of treatment E. Changes over time are erratic. There is a tendency for an increase in CO_2 uptake of all treatments, except F, up to day 4 although the increase is only significant ($p < 0.05$) in treatment E. The marked and significant decrease in all treatments on day 6 may be due to a systems error since it departs markedly from the general trend of change over time.

(c) Discussion

An analysis of simulated trampling effects on CO_2 uptake by *Plantago lanceolata* may be confused by the effects of age on F_n. Hodgkinson (1974) noted that certain evidence suggests that F_n increases to a maximum attained at the completion of leaf expansion while studies on herbaceous dicotyledonous species indicated that maximum F_n occurs when leaves are 20-60% of their final size. Wareing *et al.* (1968) maintained a constant ratio of young to mature leaves in their defoliation experiments so that changes in F_n were not attributable to changes in the age structure of the plant. In the present study, trampling may result in a change in leaf age structure so that elevation of CO_2 uptake rates may result from a higher proportion of younger leaves rather than a physiological change in existing leaves.

The comparatively low observed $\bar{R}$ of control plants (Section VC, b(i)) may be a reflection of plant age. The value, considerably lower than the $\bar{R}$ value of 1.4g g^{-1} wk^{-1} given by Grime & Hunt (1975), may also reflect less than optimal growth conditions but it has been observed that $\bar{R}$ exhibits a curvilinear relationship with time (Evans, 1972). The possible juvenility of the plants has implications for assessing defoliation effects on CO_2 uptake. Hodgkinson (1974) noted two types of response, that in which defoliation of young leaves delays the decline of F_n with age and, secondly, the defoliation of mature or senescing leaves which results in a significant increase of F_n. Although there is a tendency in the present study for elevated rates of photosynthesis to be observed in damaged plants, the effect is not consistently statistically significant. This may reflect variation in leaf age, after treatment, of the plants concerned. The ratio of young leaves to mature leaves may determine the overall plant response, as reflected by CO_2 uptake measurements, and is an unquantified variable in the study. The problem of variation in response was also noted by Leney (1974) who suggested that "... variability in the effect (of trampling) on roots and shoots has been caused by the different reaction of the individual plants rather than inaccurate treatment", resulting in non-significance due to biological variation. Leney considered that statistical non-significance suggests that population response may not reflect the success of individuals in response to trampling. Some individuals may therefore exhibit a positive response to trampling which may facilitate their survival.

The lack of a consistent response is illustrated when the results presented in Section VC, b(i) & (iii) are compared. It is not possible to attach any importance to the quantitative relationships existing between simulated trampling intensity and CO_2 uptake. The decrease in F_n at 5i pt^{-1} relative to the control value may be due to a water stress effect. The results presented in Section V C, b(ii) indicate that plants subject to low simulated trampling pressures may develop water deficits which could have repercussions on CO_2 uptake (cf. Section IVA, a(i)). The significantly lower RWC's of treated plants may be causally associated with the significant reduction in CO_2 uptake. The reduction in RWC may result from a tearing and mechanical abrasion of the epidermal cuticle, enhancing water loss (cf. Hall & Jones, 1971; Grace, 1974). Internal damage as a result of crushing may also occur restricting water supply to the leaf. The phloem cells may also be damaged, inhibiting transport and leading to an accumulation of assimilates at the site of production which may have repercussions on photosynthesis (cf. Neales & Incoll, 1968; Little & Loach, 1973). Damage to the xylem may also inhibit the transport of root metabolites and minerals to the leaves, indirectly leading to a reduction in photosynthesis by inhibiting enzyme synthesis. The severity of such effects may be an age related phenomenon in which larger, less pliable leaves of older plants are more susceptible to snapping than young leaves. Differences in the capacity of a leaf to bear a load and bend without breaking may also result in differential species responses to trampling both in terms of their tolerance to withstand trampling pressure (Shearman & Beard, 1975c) and in their capacity to maintain an undamaged internal structure thereby enhancing recovery of tissues which are damaged.

However, an age effect may account for a progressive increase in CO_2 uptake by plants subjected to increasing simulated trampling pressure. Older plants may undergo a greater rejuvenation than younger plants at similar degrees of defoliation (Hodgkinson, 1974) whilst the decrease in F_n associated with increasing age (Hodgkinson, 1974; Ryle & Powell, 1975) may result in control plants having lower rates of F_n as a result of a generally older leaf age structure in comparison with treated plants. The increasing severity of defoliation with increased simulated trampling pressure may alter the plant's leaf age structure in favour of younger leaves which may lead to an increase in whole plant photosynthesis. The trend for increased rates of F_n in trampled plants is consistent with observations by a number of workers on the effects of defoliation on F_n e.g. Maggs (1964, 1965), Sweet & Wareing (1966), Wareing et al. (1968), Gifford & Marshall (1973), Hodgkinson (1974). Following severe defoliation the remaining leaves, and possibly newly emerged leaves, may be at a favourable advantage with respect to supply of cytokinins from the root, leading to an increased capacity for photosynthesis. The lack of a consistent response may be due to differences in plant age and development. Although the differences are not significant, observations on plants treated at a comparatively young age (Section VC, b(i)) indicate a reduction in the stimulatory effect over time. In relation to the hypothesis advanced regarding the role of root produced cytokinins on F_n, the channelling of assimilates into shoot growth at the expense of the root following defoliation may lead to a reduction in root growth and respiration (Davidson & Milthorpe, 1966a, b; Ryle & Powell, 1975) which, together with the effects of soil bulk density increases (Section VB), may retard root metabolism, leading to an increasing competition for cytokinins over time and a consequent reduction in photosynthetic capacity. However, with older plants (Section VC, b(iii)) which may possess a greater functional root biomass, although root growth may be retarded, competition effects for root produced cytokinins may not develop in defoliated plants until a longer period has elapsed during which reduction in shoot size has been compensated for. It has not been feasible to statistically compare the response of plants of different ages but the results may be a reflection of Leney's (1974) suggestion that plant age may be an important factor in a plant's response to trampling and that root size may affect plant survival under trampled conditions.

The observed increases in respiration rate of trampled plants (Section VC, b(i)) may be due to injury and repair of damage. Variation over time might, in part, be due to death of severely damaged tissue or completed repair of injury. The decrease in respiration rate after fourteen days compared to the findings seven days after treatment, is most marked in plants which received the lowest simulated trampling pressure (5i pt^{-1}) which might induce greater injury than defoliation effects compared with higher simulated pressures. Abscission of damaged foliage may alter the equilibrium between injured material, with high respiration rates, and material less severely damaged and possessing lower respiration rates. As defoliation becomes more severe, with increasing trampling pressure, the proportion of damaged material remaining on the plant declines, resulting in a higher relative proportion of new growth, possibly with respiration rates lower than those of injured tissue.

The net diurnal gain of carbon may be of some significance to the recuperative potential of the plant. The data indicate that the net gain (i.e. the difference between net photosynthesis and dark respiration) is greatest in plants treated at 40i pt^{-1} compared to the just positive gain on a daily basis (12h day) at pressures of 5i pt^{-1} and 20i pt^{-1} (Section VC, b(i)). Such an effect might be expected to result in a relatively higher dry weight increment per unit time compared to control plants, especially in plants where the differences are greatest. Sweet & Wareing (1966) observed that elevated rates of F_n of defoliated plants were accompanied by higher relative growth rates. The perpetuation of such a situation could lead to an increase in biomass to values greater than that of the control as found by other workers in the trampling context e.g. Bayfield (1971), Leney (1974), although this might depend upon the maintenance of a comparatively greater proportion of young tissue to outweigh the effects of leaf ageing on photosynthesis (cf. Hodgkinson, 1974) and the circumvention of assimilate accumulations in source leaves which may have depressing effects on F_n (Neales & Incoll, 1968). Stimulated relative growth rates, of trampled plants, observed in the present study (Section VA, b(ii)), are consistent with the above suggestions regarding the balance between photosynthesis and respiration on dry weight increment. However, for an increase in biomass above that of control plants to become apparent, growth would have to compensate for defoliation effects, the effects of soil bulk density on plant growth and higher respiration rates associated with injury. The provision of new and more numerous assimilate sinks may, however, be conducive to maintaining elevated rates of photosynthesis (Sweet & Wareing, 1966). Such an effect may be produced by increased tillering observed to occur on trampled plants by some workers e.g. Bayfield (1971), Leney (1974), Liddle & Greig-Smith (1975b), although no evidence exists in the present study for a significant increase in leaf or tiller number of plants subjected to simulated trampling pressures.

Although photosynthesis might be expected to affect plant dry weight, the effect is not apparent in the current study. There is a tendency for shoot dry weight to decrease over time (Figures 107 and 110) which if real, and not the result of differential recovery environments, may illustrate the suggestion of Bayfield (1971) that sufficient time should be allowed for dieback to occur and recovery to commence. The importance of dead material is reflected in the lack of a significant treatment effect on total dry weight but a significant decrease in green tissue with increasing simulated trampling pressure 7 days after recovery, reported in Section VC, b(iii). Over time leaf death and decay will be counterbalanced by regrowth so that although total dry weight decreases, the absolute dry weight of green material remains constant. Despite a decline in the proportion of the plant potentially involved in photosynthesis, a mechanism whereby the potential photosynthetic capacity may be increased at the physiological level, may contribute towards counterbalancing the decrease in photosynthetic surface resulting from trampling.

The observed increase of F_n in Phleum bertolonii following simulated trampling (Figure 112) is in agreement with the responses described above for Plantago lanceolata. Although no increase in tillering is observed this does not preclude the possibility of a replacement of older damaged tillers by young tillers which have a higher photosynthetic capacity. It has also been observed that F_n may increase in actively tillering plants (Chatterton et al., 1972). The negative correlation found to exist between root size and F_n of Phleum bertolonii conflicts with the postulate that root metabolism and mineral uptake may limit F_n, unless these factors were operating in excess of shoot requirements. This argues against the findings of Wareing et al. (1968) and also conflicts with the positive correlation between F_n and root size observed by Humphries (1963) and Humphries & Thorne (1964). The situation is obviously one of complexity since Maggs (1964) observed an increase in NAR with root pruning, but the discrepencies may result from complex source-sink-hormonal relationships which are affected by the relative sizes of the various plant components. Humphries (1963) and Humphries & Thorne (1964) postulated that translocation rate rather than root size is of primary importance, whereby a restriction on root growth reduces assimilate translocation which inhibits F_n. In such an instance, sink size is indirectly correlated with F_n. When an alternative sink is provided i.e. repair and regrowth following defoliation, assimilate redistribution may retard root growth e.g. Maggs (1964, 1965), Davidson

& Milthorpe (1966a, b), Ryle & Powell (1975) but not reduce assimilate translocation. Thus, although root growth is retarded, the distribution of assimilate to the nearest utilizing region (Maggs, 1965; Ryle & Powell, 1975) may not inhibit F_n. Consequently, the negative correlation between root size and F_n may be a reflection of assimilate redistribution and elevated demand by regenerating assimilate sinks. This postulate, however, must be considered in the context of a similar negative correlation found when increased soil bulk density restricts root growth (Section VB, b(iii)). Thus, in addition to assimilate redistribution to the aerial portions of the plant, assimilates channelled to the root may be required to overcome the increased mechanical resistance to growth resulting from an increase in soil compaction with the consequence that despite a higher rate of F_n, fewer assimilates are available for incorporation into the structural framework of the plant.

The observation that root growth of P. bertolonii is retarded relative to that of shoot growth while F_n is elevated implies that root metabolism i.e. hormone production and mineral uptake may not be limiting photosynthesis. However, the root biomass of the control plants may not represent the root biomass required to support the shoot, but the root biomass resulting from excess assimilate production by the shoot which is translocated to the root (Humphries, 1969). Despite a reduction in root growth of trampled plants, the functional root available may remain capable of providing the shoot with root metabolites, although this argues against the competition effects described by Wareing et al. (1968). It is also necessary to consider the effects of auxin production by the aerial sinks on F_n. Sweet & Wareing (1966) suggested that auxin production by the shoot apex and young leaves may stimulate F_n whilst a number of workers have observed a stimulatory effect on F_n of exogenously applied hormones e.g. Alvim (1960), Booth et al. (1962), Davies & Wareing

(1965), Turner & Bidwell (1965), Bidwell & Turner (1966), Togoni *et al.* (1967). F_n may therefore be affected directly by hormones other than those produced by the root with the consequence that root development may not limit photosynthetic carbon gain.

The effect of exogenously applied hormones on plant F_n

The effects and action of kinetin-like compounds has been reviewed by Katsumi (1963), Letham (1967) and Burrows (1975). Evidence suggests that cytokinins are involved in nearly every aspect of plant growth, maintaining the integrity of cellular membranes, stimulating chlorophyll formation and promoting RNA, protein, lipid and starch synthesis (Letham, 1967).

The results of applying kinetin (6 furfuryl-aminopurine) to the leaves of *Plantago lanceolata* indicate that the decline in F_n over time may be retarded by the application of kinetin which is consistent with the suggestion that cytokinins delay senescence and promote protein synthesis (Letham, 1967). The application of a relatively high concentration of kinetin (10^{-4}M) appears more detrimental to CO_2 uptake than lower concentrations, including no application, while the only treatment in which CO_2 uptake was higher than prior to the hormone applications was that receiving a 10^{-6}M solution. Wareing *et al.* (1968) found that a c. 10^{-4}M solution of kinetin applied to leaves of *Zea mays* resulted in an increase in F_n which they considered consistent with the postulate that cytokinins enhance enzyme synthesis, including that of carboxylating enzymes, which, in ageing plants, decreases the biochemical component of r_m (cf. Woolhouse, 1968).

Although kinetin application may promote F_n by enhancing enzyme synthesis this may not be the sole explanation of the observed response. Livnè & Vaadia (1965) observed that kinetin applied to the leaf bases of

Hordeum vulgare (barley) was accompanied by an increase in transpiration rate due to a reduction in r_s. Meidner (1967) found that a 3×10^{-6}M solution of kinetin applied to mature leaves of the same species resulted in an increase in CO_2 uptake. This was associated with a reduction in the substomatal concentration of CO_2 which consequently led to a reduction in r_s but in addition, a direct effect on the stomatal mechanism was observed. Pallas & Box (1970) also observed a direct effect of kinetin on stomatal aperture. They suggested that a change in the osmotic potential differentials existing between guard and subsidiary cells led to a relative increase in the turgidity of the guard cells leading to an increase in stomatal aperture. Meidner (1967) had contested that direct stomatal effects were sufficient to influence CO_2 uptake, although the role of r_s in controlling CO_2 uptake at low resistances is controversial (cf. e.g. Gaastra, 1959; Raschke, 1976). A direct effect of kinetin on r_s is consistent with observations by Gifford & Marshall (1973) regarding the increase in CO_2 uptake via a reduced r_s in defoliated plants. The effect of cytokinins on r_m however, is also consistent with Hodgkinson's (1974) findings on the association between CO_2 uptake and decreased r_m in defoliated plants.

It is possible that by stimulating the photosynthetic capacity of trampled plants, the recuperative potential may be enhanced although such a beneficial effect would have to be considered in relation to the possible effects of increased water loss which might result from a hormone induced decrease in r_s, which might have adverse consequences on trampled plants by inducing water stress.

The effects of regular simulated trampling on *Phleum bertolonii* are partially consistent with similar effects observed on *Plantago lanceolata* although the further significant decreases occurring in *P. bertolonii* in response to regular trampling compared to a similar pressure applied at one time contrasts with the response exhibited by P. lanceolata (Section VA, b(iv)). The difference in response may indicate differences in species response but probably reflects the shorter recovery period employed with P. bertolonii. The application of a 10^{-6}M solution of kinetin to P. bertolonii following treatment has no significant effect on any of the measured parameters, possibly due to an inappropriate concentration. Penetration of the solution into the leaves may have been ineffectual despite the use of a surfactant to decrease surface tension and aid penetration of the solution. Green & Bukovac (1974) stated that 'Tween 20' was the least effective surfactant from a range investigated to observe the effect of surfactants on foliar absorption. They concluded that stomatal penetration by foliar sprays applied containing the commonly employed surfactants is probably minimal. It is therefore possible that spraying of kinetin solution onto the leaves of P. bertolonii was not accompanied by infiltration of the solution into the leaf, although this is at variance with observations on Plantago lanceolata and the spraying techniques employed by Wareing et al. (1968).

There is a possibility that spraying modifies the strategy adopted for assimilate distribution. Although the effects are not statistically significant, there is a tendency for unsprayed trampled plants to possess a higher number of tillers than the controls, which is consistent with observations by Bayfield (1971), Leney (1974) and Liddle & Greig-Smith (1975b). The reduction in tiller weight of these plants compared to control and sprayed plants, suggests that available assimilates are being employed in the more numerous production of tillers which may eventually increase the photosynthetic capacity of the plant by an increase in photosynthetic surface. The opposite trends for sprayed plants suggest that the hormone, at the concentration applied, may inhibit tillering while continued photosynthesis results in an increase in assimilate accumulation in the leaves. The synergistic effects of hormones (Letham, 1967) makes the isolation of the response by the plant in terms of the application of one hormone less than satisfactory. Davies & Wareing (1965) found that kinetin applied to Pisum sativum and Populus robusta stimulated cell to cell transport of ^{32}P but had little effect on phloem transport, while IAA and kinetin applied simultaneously stimulated transport throughout the plant. It is possible that an upset in the endogenous hormonal balance may retard assimilate transport in the plant reducing assimilate availability for new tiller development. A reduction in the potential for new sink development may lead to assimilate accumulation and an increase in dry weight of source leaves (cf. Ryle & Powell, 1975) which could lead to a reduction in photosynthetic capacity (Neales & Incoll, 1968).

The observation that regular treatment has a more detrimental effect on plant growth than instantaneous treatment may indicate a recovery period between treatments of insufficient duration to enable the plant to withstand the regular application of the pressure employed, despite a possible increase in CO_2 uptake. The paradox between $\bar{R}$ values and CO_2 uptake rate is difficult to reconcile with suggestions made regarding stimulated shoot growth rates following defoliation resulting from mobilisation of assimilates and an elevated CO_2 uptake in trampled plants. The situation may arise from the role reserve assimilates and current photosynthetic production have in repairing damage rather than producing new growth. The lower biomass of plants treated at one time compared to controls may result from an elevated respiration rate and the inability to compensate for defoliation losses. In regularly treated plants there may be an increased tendency for respiration rates to be maintained at a consistently high level and, despite an elevation in CO_2

uptake rate, the inability to compensate, over the time period allowed, for a consistent loss in foliage from defoliation and death. That CO_2 uptake in regularly treated plants is significantly greater than that of control plants or those treated once only, accords with the findings that continuous defoliation maintains high CO_2 uptake (Gifford & Marshall, 1973). These authors observed that continual trimming maintained a low r_s and prevented an increase in 'residual resistance' with age. Hodgkinson (1974) stated that "continual removal of new shoots would ensure an excellent supply of nutrients and growth substances to residual leaves and permits a steady build up of enzyme levels and improves membrane permeability in these leaves", thereby enhancing CO_2 uptake. Such a physiological explanation may account for the observed effects in the present experiment but the significant effect of simulated trampling on the leaf weight:leaf area ratio may account for the effect. Expression on a dry weight basis may not accurately reflect the efficiency of the photosynthetic apparatus which may be represented, more realistically, by leaf area or chlorophyll content, since an increase in assimilate concentration of the leaves, for example in control leaves, while chlorophyll content or light intercepting capacity of the leaf remains constant, would result in an apparent decrease in CO_2 uptake on a dry weight basis although the true photosynthetic efficiency of the plant may remain unchanged.

The higher rates of CO_2 uptake in damaged plants may also illustrate an age related photosynthetic response in which the increasing juvenility of plants from control to regularly treated plants may result in higher rates of F_n.

The leaf weight:leaf area ratio data may also illustrate the depletion or lower accumulations of assimilates in regenerating tissues. The significantly lower ratio in regularly treated plants is in agreement with a situation in which there is a consistent demand on assimilates for regrowth of damaged plants, and juvenility of leaves in which maturity has not resulted in an increase in assimilate in the leaf (cf. Ryle & Powell, 1975). The slight tendency for sprayed plants which have been subject to simulated trampling to possess higher leaf weight: leaf area ratios is consistent with the possibility of higher assimilate accumulations leading to higher yields per tiller than for plants which have not been sprayed. A tendency for elevated assimilate accumulations in plants liable to be trampled may be advantageous in facilitating continued survival. Reserve carbohydrates are those carbohydrates "... which vary in amount and are alternatively accumulated and utilized by the plant in its growth cycle or when disturbed by changes in its environment", (Sullivan & Sprague, 1943). The shortage of carbohydrate following defoliation may be overcome by increased photosynthetic efficiency since it is unlikely that root reserves make a significant contribution to regrowth following defoliation, rather the plant is integrated in a manner enabling undamaged shoots to aid damaged shoots by assimilate export (Marshall & Sagar, 1965; Ryle & Powell, 1975). An increase in photosynthetic efficiency may be regarded as enabling the effects of environmental hazards to be buffered by the plant, "... the subsequent reduction in yield being far less than would be expected" (Marshall & Sagar, 1968). In a grazing context, Hodgkinson (1974) considered that the enhancement of carbohydrate supply from undamaged leaves may buffer the effects of a reduction in photosynthetic area. A similar effect may occur in trampled situations and species differences in ability to compensate for assimilate reserve losses and a reduction in photosynthetic capacity by a loss of leaf area may be one of a number of factors determining the success of an individual plant or species in a trampled habitat.

D. The effect of trampling intensity, applied at a constant frequency, on a grassland sward

(a) Introduction

The findings presented above (Section V A-C) add support to Edmond's (1964) conclusion that "Active growth must reduce the intensity of the treading effect...". This does not conflict with his observation regarding a consistent reduction in yield with treading (Edmond, 1958, 1962, 1963, 1964) but the reaction of a plant community to trampling is confused by changes in species composition, species competition and species differences in response to trampling (Burden & Randerson, 1972; Leney, 1974; Liddle & Greig-Smith, 1975b) which may result in an increase in biomass relative to that of untrampled vegetation. Kellomaki (1973) observed differential trampling tolerances of various vegetation components in which grasses and dwarf shrubs were found to possess a higher tolerance to trampling than herbs. Although light trampling pressures would eventually result in a total destruction of the vegetation, he found that biomass could be increased by light trampling pressures (1 and 4 passages per plot per week (p pl^{-1} wk^{-1})).

Leney (1974) found that removal of public pressure could result in an increase in biomass at a rate greater than that of untrodden vegetation, implying a more rapid growth rate. Investigations revealed that biomass could be reduced by a reduction in the number of plants per unit area (cf. Edmond, 1958) or a reduction in the size of individual plants (cf. Liddle, 1975a). Edmond (1964) found that although yield per tiller could be reduced by heavy treading, a reduction in sward yield was primarily due to a reduction in tiller number.

The relationship between primary productivity and trampling has been integrated into a theoretical model by Liddle (1975b) in which an increase in productivity is related to an increased tolerance to trampling. Goldsmith (1974) had observed that trampling initially stimulates productivity but as trampling pressure increases productivity is reduced. Liddle (1975b) identified three factors affecting wear tolerance, namely, plant structure, potential for regrowth and plant environment, the latter two factors being incorporated in primary productivity measurements.

Liddle considered that if the relationship between primary productivity and trampling tolerance is valid it may enable the trampling capacity of different habitats to be predicted but he noted that "The causality of the relationship may depend on the speed of growth between periods of wear". Grime & Hunt (1975) observed that species with a low maximum relative growth rate (R_{max}) had a low frequency on paths, the frequency increasing with an increase in R_{max}, which led them to conclude that "slow growing species are ... infrequent in habitats such as paths in which the vegetation experiences a high intensity of damage or disturbance". They suggested that species with low values of R_{max} are not adapted to rapid establishment on bare areas or the rapid replacement of foliage following defoliation. Grime & Hunt associated high values of R_{max} with potentially productive situations in which species possessing tall stature and a high capacity for lateral spread of root and shoot are at an advantage with respect to efficient light interception, mineral and water uptake and 'space' utilization. Such factors, except tall stature (cf. Liddle & Greig-Smith, 1975b) are advantageous in trampled habitats although Grime & Hunt (1975) noted that the high respiration rates associated with species possessing high R_{max} values may be disadvantageous in habitats subject to environmental extremes. However, rapid growth and completion of life cycle associated with species of high R_{max} (Grime & Hunt, 1975) may be advantageous in perpetuating the existence of species in trampled situations (cf. Bates, 1935). In

Plate 6. The effect of weekly trampling, at a range of intensities from 0 to 40 passages per plot per week, over a twelve week period on the development of a grassland sward. Trampled areas may be identified by the shorter vegetation and absence of inflorescences. (Botanic Garden, Keele, Staffs., 29.07.75).

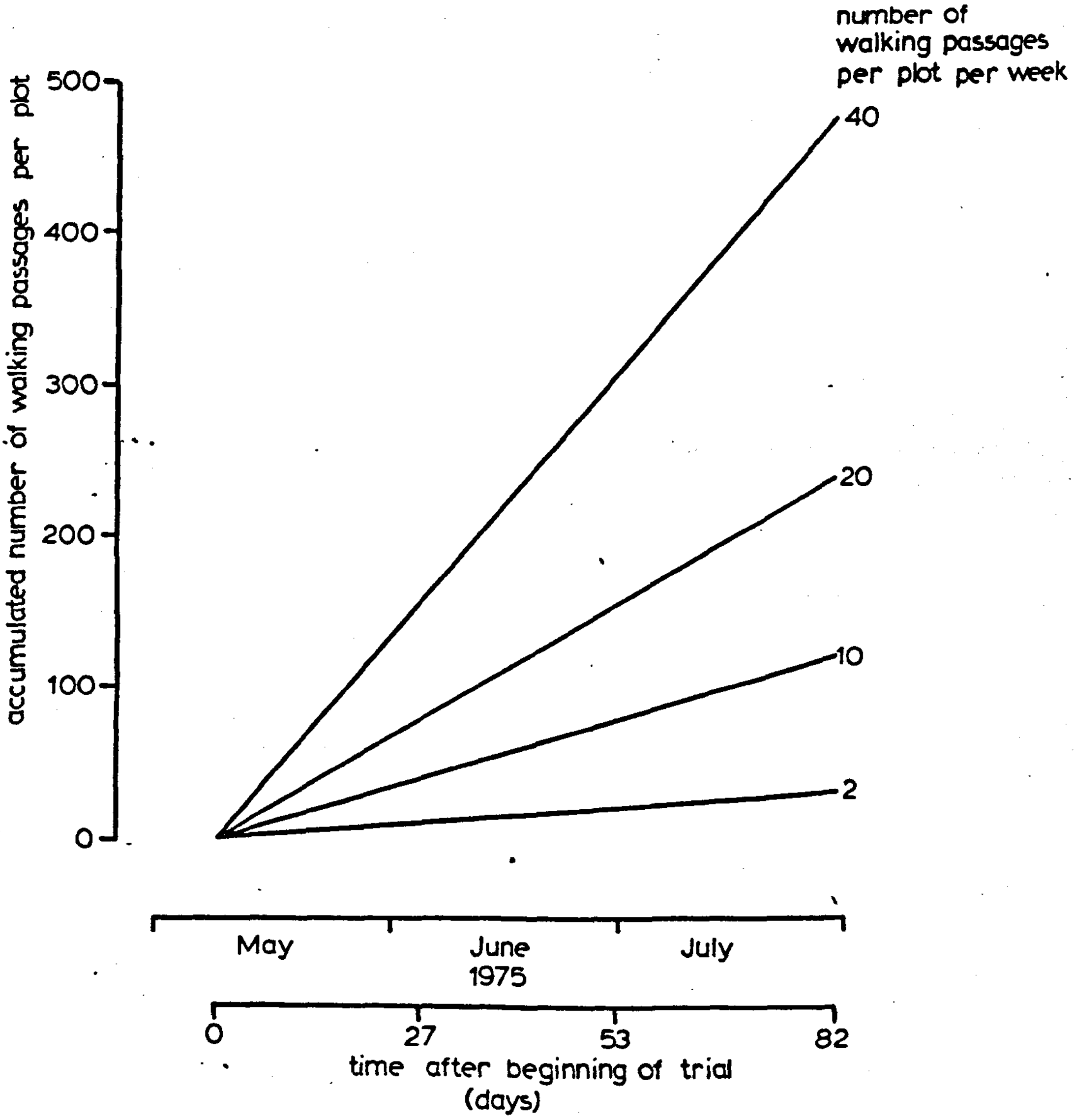
contrast, Grime & Hunt (1975) suggested that species with low R_{max} values make small demands upon the environment, are possibly suited to survive periods when little growth is possible and deploy assimilates into the accumulation of reserves rather than new structure. Thus, if stress limits growth, the low respiration rate and extended life span confers a competitive advantage on species which might otherwise have a low competitive ability. Under trampled conditions, low respiration rates may prevent starvation effects occurring but may also inhibit recovery, whilst the accumulation of carbohydrate reserves may have considerable advantages in regrowth following defoliation (Marshall & Sagar, 1965, 1968; Ryle & Powell, 1975). It would appear, however, that growth rate may be an important determinant of plant survival under trampled conditions.

(b) Methods

An intensively managed grassland sward, subject to regular mowing, was used to ascertain sward response to controlled levels of trampling pressure. The predominant species were graminaceous; *Anthoxanthum odoratum*, *Festuca rubra*, *Lolium perenne*, *Poa annua*, *P. trivialis*, with less frequent occurrences of *Achillea millefolium*, *Bellis perenne*, *Cardamine pratense*, *Plantago spp.*, *Ranunculus spp.*, *Rumex acetosa*, *Trifolium repens*.

Three blocks (14.0m x 4.5m) were established, separated by a 2-3m. wide buffer area. Each block was divided into a 5 x 5 Latin Square of 2m. x 0.5m. plots. The five treatments applied to each block were 0, 2, 10, 20 and 40 walking passages per plot per week (p pl^{-1} wk^{-1}). Treatment application commenced in May (08.05.75), prior to which the sward had been mown once in the 1975 growing season. Each block was

Figure 118 The relationship between weekly applied trampling intensity and accumulated trampling intensity over a 12 week period.



harvested seven days after the final treatment application for a specific block (i.e. 04.06.75; 02.07.75; 29.07.75). A 1m x 0.5m area was harvested from each plot. The total fresh weight of harvested material from each plot was obtained and three subsamples, of approximately 100g fresh weight, taken and accurately weighed. Two subsamples were immediately dried at 85°C and used to determine total harvested dry weight for each plot. The third subsample was frozen at -20°C and subsequently partitioned into green ('live') and dead material from which their percentage contribution to the dry weight of each plot could be calculated.

On 08.05.75, six $0.5m^2$ plots were harvested from the buffer zone surrounding the experimental area to enable the calculation of Relative Growth Rates ($\bar{R}$) (Equation 28).

(c) Results

The interpretation of the response of a grass sward to regular trampling is confused by changes over time in sward growth rate, due to ontogenetic and environmental effects, and the effect of cumulative trampling totals which confuse inter-block comparisons. The relationship between weekly trampling intensity and cumulative trampling total over time (Figure 118) may be responsible for the time x treatment interactions occurring in subsequent statistical analyses since the four basic trampling treatments are translated into twelve cumulative trampling totals.

Both significant ($p < 0.001$) treatment and significant ($p < 0.01$) time effects are evident on the total biomass of a grass sward subjected to trampling pressure (Figure 119). There is a linear increase in biomass over time of the control plots which becomes more curvilinear with increasing trampling pressure with the consequence that by day 82[1] the

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[1] time (in days) measured from beginning of trial.

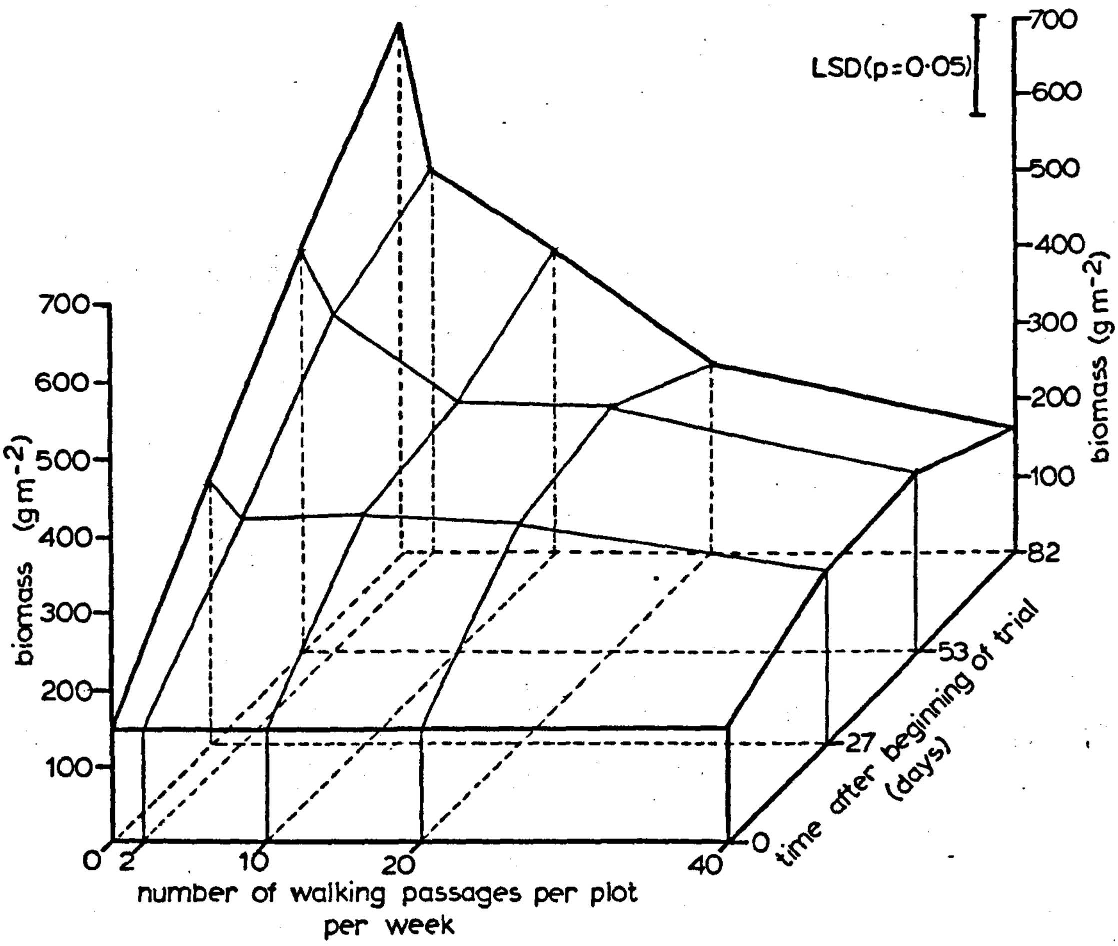


Figure 119 The effect of a range of trampling intensities, applied at weekly intervals over a 12 week period, on the total biomass of a grass sward.

highest weekly pressures employed (20 and 40p pl^{-1} wk^{-1}) result in a total biomass that is less than that existing on day 27 and in the most extreme case is approaching the biomass existing at day 0, (i.e. 154.7g m^{-2} and 149.8g m^{-2} for plots receiving 40p pl^{-1} wk^{-1} on day 82 and day 0 respectively). Due to variation in the cumulative trampling totals applied over time treatment effects become more evident over time, the effect of higher weekly trampling pressures being most apparent on day 82 and least evident on day 27. The data suggest that although higher trampling pressures have a greater absolute effect on reducing total biomass, in relative terms the lowest pressure applied (2p pl^{-1} wk^{-1}) has the most severe effect on total biomass, further increases in pressure having a relatively less detrimental effect, i.e. a curvilinear response. The composite analysis of the data tends to obscure statistically significant effects which are apparent when the data for each harvest are analysed separately (by analysis of variance for Latin Square arrangements, Appendix A, 1(c)). For each harvest date significant ($p < 0.01$) treatment effects occur but whereas on day 27 a trampling pressure of 20p pl^{-1} wk^{-1} (cumulative total: 80p pl^{-1}) is required before a significant reduction in biomass occurs, on days 53 and 82 a pressure of 2p pl^{-1} wk^{-1} (cumulative total 16p pl^{-1} and 24p pl^{-1} respectively) results in a significant reduction in total plant yield. For the duration of the trial under consideration, a critical trampling threshold, which is a function of the cumulative trampling total and the time over which the total is accrued, of between 10 and 20p pl^{-1} wk^{-1} exists. Pressures below this threshold are associated with an increase in biomass over that existing at the previous harvest while above this value biomass is severely depressed, or decreased with reference to the previous harvest. For example, an increase in biomass of 11.9g m^{-2} between day 27 and 53 at a pressure of 40p pl^{-1} wk^{-1} (cumulative total: 320p pl^{-1}) represents a

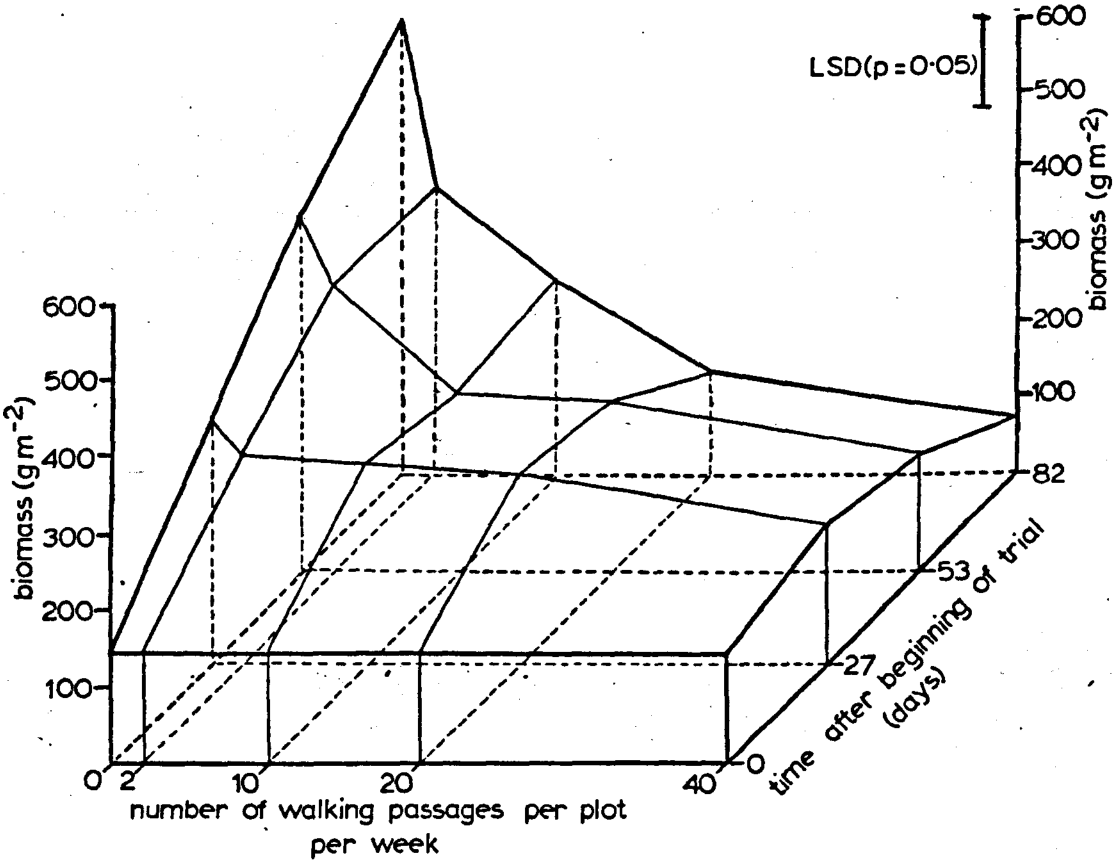


Figure 120 The effect of a range of trampling intensities, applied at weekly intervals over a 12 week period, on the biomass of green (live) material of a grass sward.

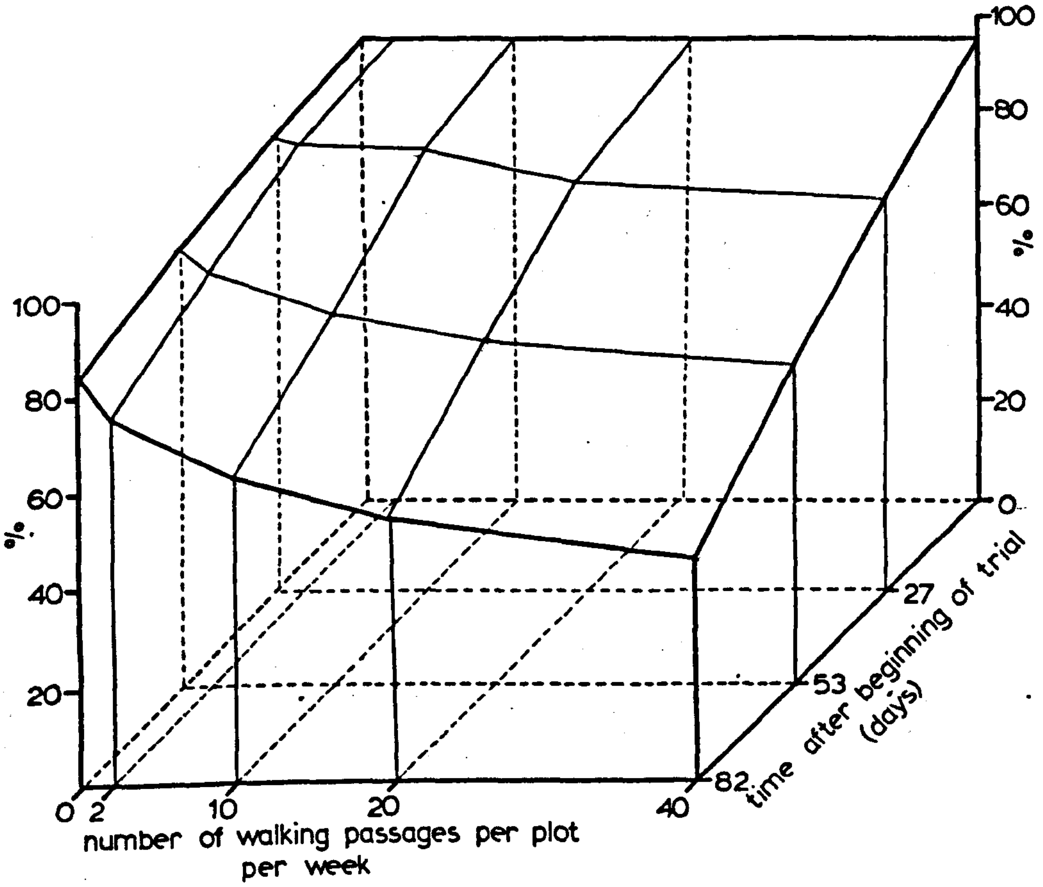


Figure 121 The effect of a range of trampling intensities, applied at weekly intervals over a 12 week period, on the proportion of green (living) material existing in a grass sward, (expressed as the dry weight of green material as a percentage of the total biomass dry weight for each plot).

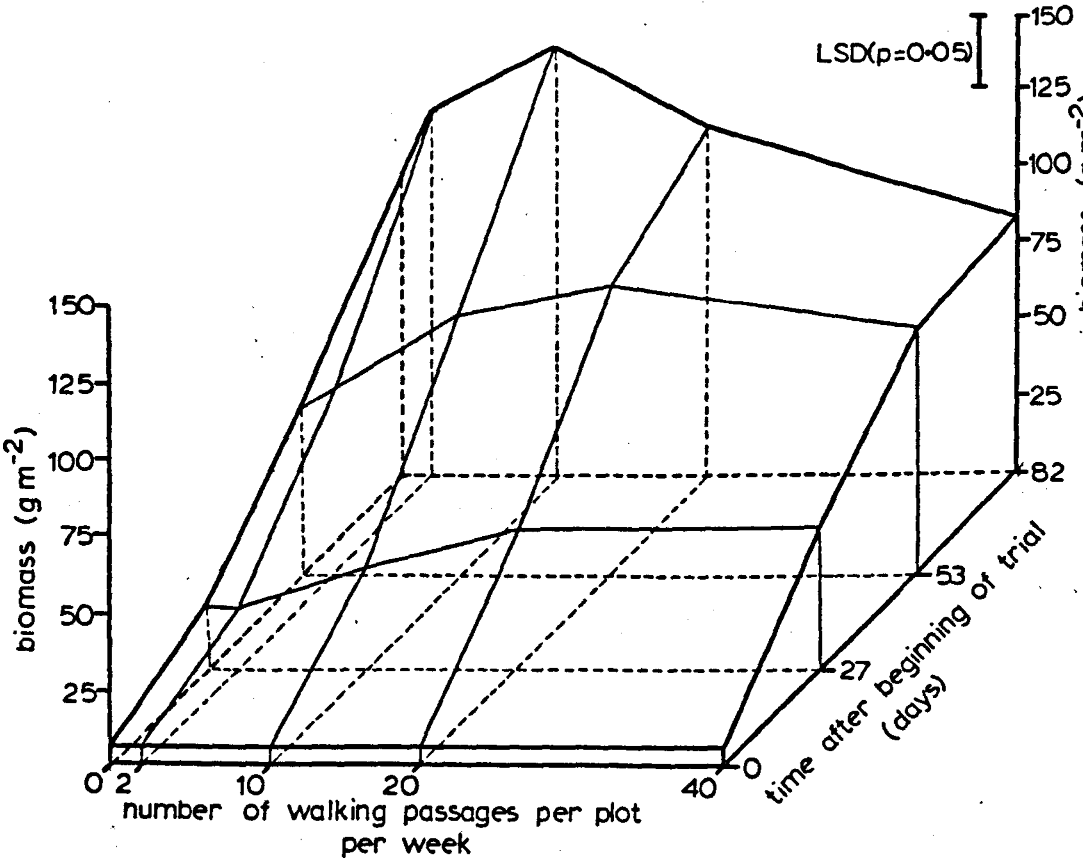


Figure 122 The effect of a range of trampling intensities, applied at weekly intervals over a 12 week period, on the biomass of dead material of a grass sward.

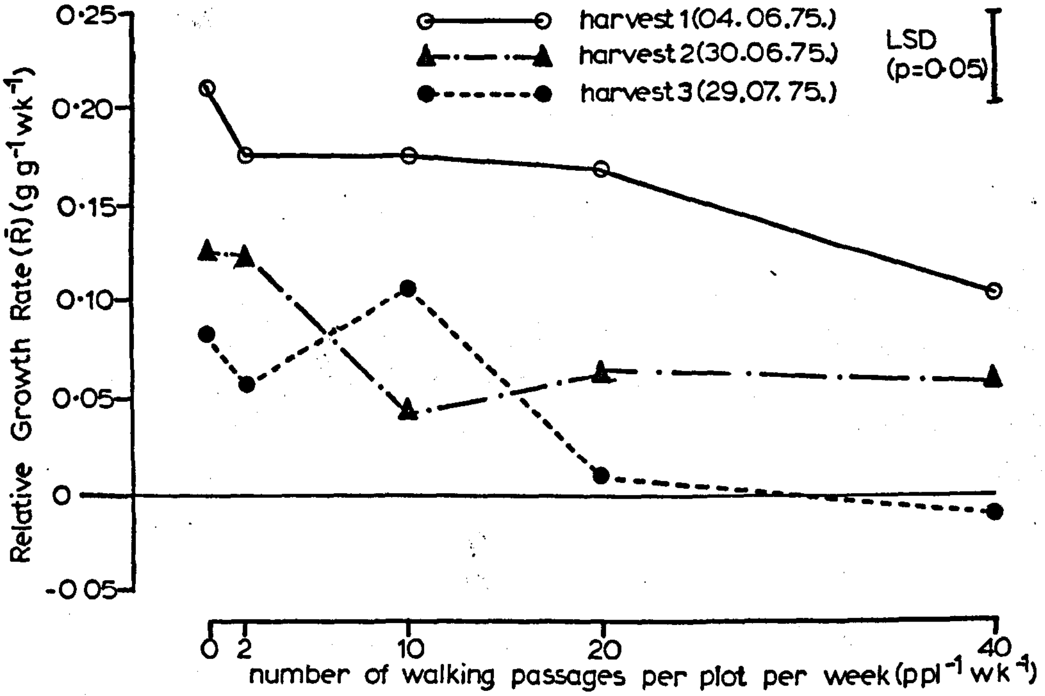
93.4% reduction compared to the increase on the control plots of 181.8g m^{-2}. Between day 53 and day 82 the yield, at a pressure of 20p pl^{-1} wk^{-1} (cumulative total: 240p pl^{-1}) is reduced by 75.7g m^{-2} compared to the biomass existing on day 53 in contrast with a further increase on control plots of 173.3g m^{-2}.

The continued assimilatory potential of the sward is dependent upon the amount of living material, capable of photosynthesizing, existing at a given moment in time. Significant ($p < 0.001$) time and treatment effects on green biomass are apparent but the situation is again confused by a significant ($p < 0.001$) time x treatment interaction. The pattern of response (Figure 120) resembles that described for the response in terms of total biomass. The green biomass on day 82 is lower, on plots receiving 10, 20 and 40p pl^{-1} wk^{-1}, than that existing on day 27. The green biomass on plots trampled at 40p pl^{-1} wk^{-1} is 56.3% of the control value on day 27. By day 82 this has decreased to 11.7% of the control value and is 51.2% lower than the green biomass existing on day 0.

The proportion of green material existing on the plot is significantly ($p < 0.001$) affected by treatment and time, the decrease becoming increasingly greater with increased pressure over time (Figure 121). The decrease in the proportion of green material on control plots of 11.6% over the measurement period reflects 'natural' death of material during the growing season. This compares with a reduction of 50.4% over the three month treatment period for plots trampled at the highest pressure employed.

The effect of trampling on dead biomass is slightly more complex due to the reduction in total biomass with increased trampling pressure over time. Significant ($p < 0.001$) time and treatment effects occur in which the dead biomass of all treatments increases over time, but whereas an increase in trampling pressure is associated with an increase in dead

(a) Change in $\bar{R}_t$ related to trampling pressure



(b) Change in $\bar{R}_t$ related to duration of trampling

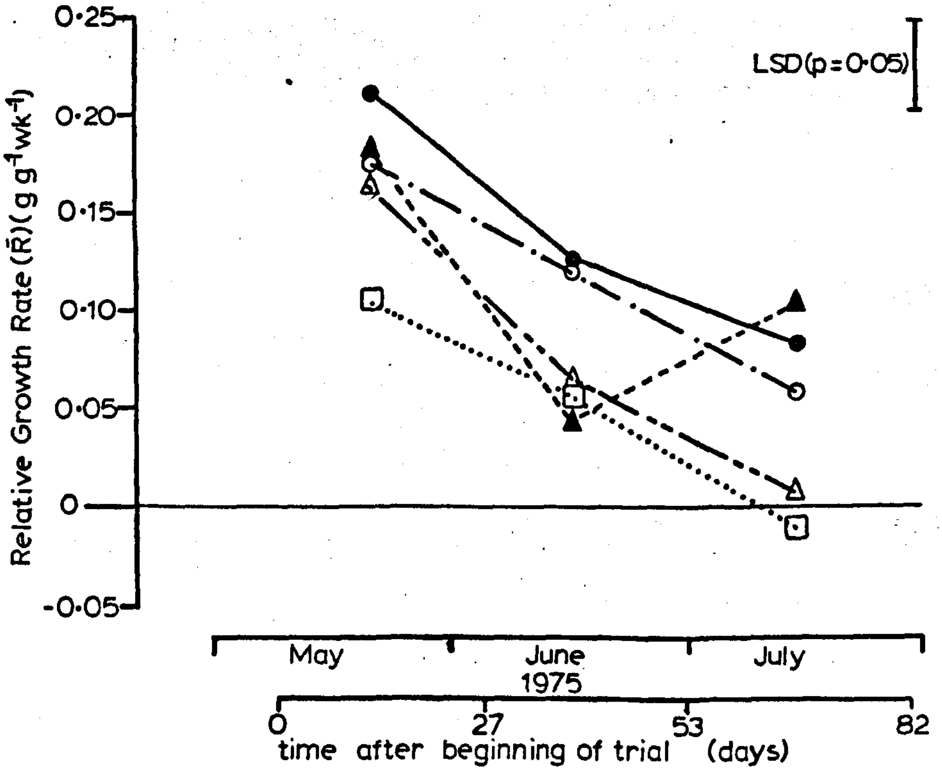
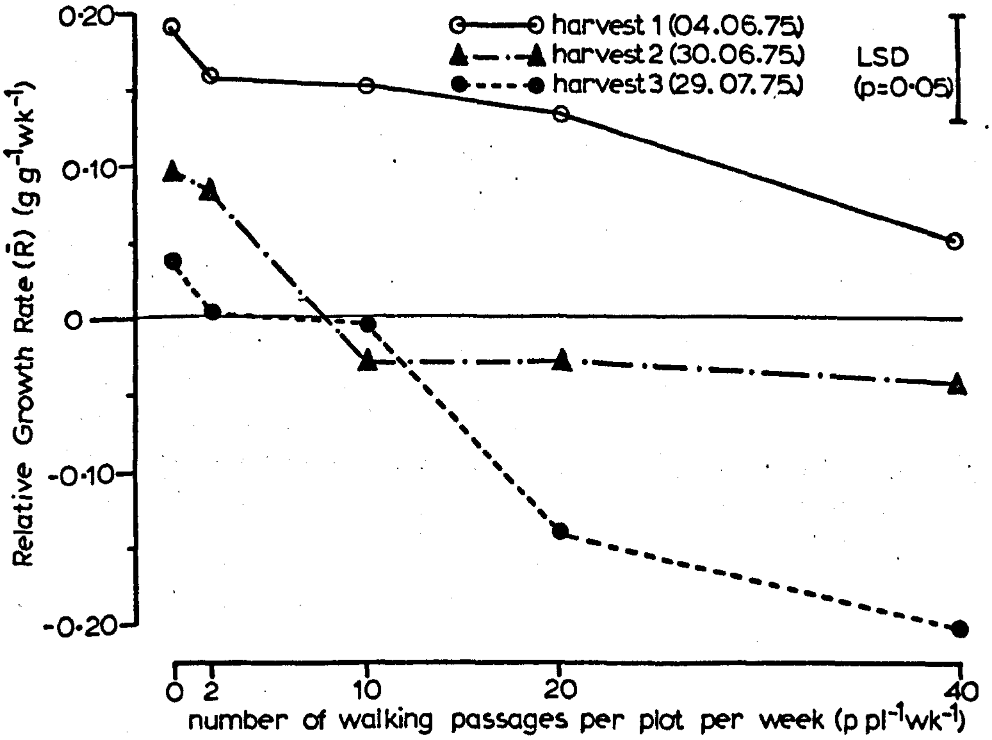


Figure 123 The effect of a range of trampling intensities, applied at weekly intervals over a 12 week period, on the Relative Growth Rate ($\bar{R}_t$) (total) of a grass sward.

●——● 0 $p\ pl^{-1} wk^{-1}$ ▲- - -▲ 10 $p\ pl^{-1} wk^{-1}$ ⊡·······⊡ 40 $p\ pl^{-1} wk^{-1}$

○-·—·-○ 2 $p\ pl^{-1} wk^{-1}$ △-·—-△ 20 $p\ pl^{-1} wk^{-1}$

(a) Change in $\bar{R}_l$ related to trampling pressure



(b) Change in $\bar{R}_l$ related to duration of trampling

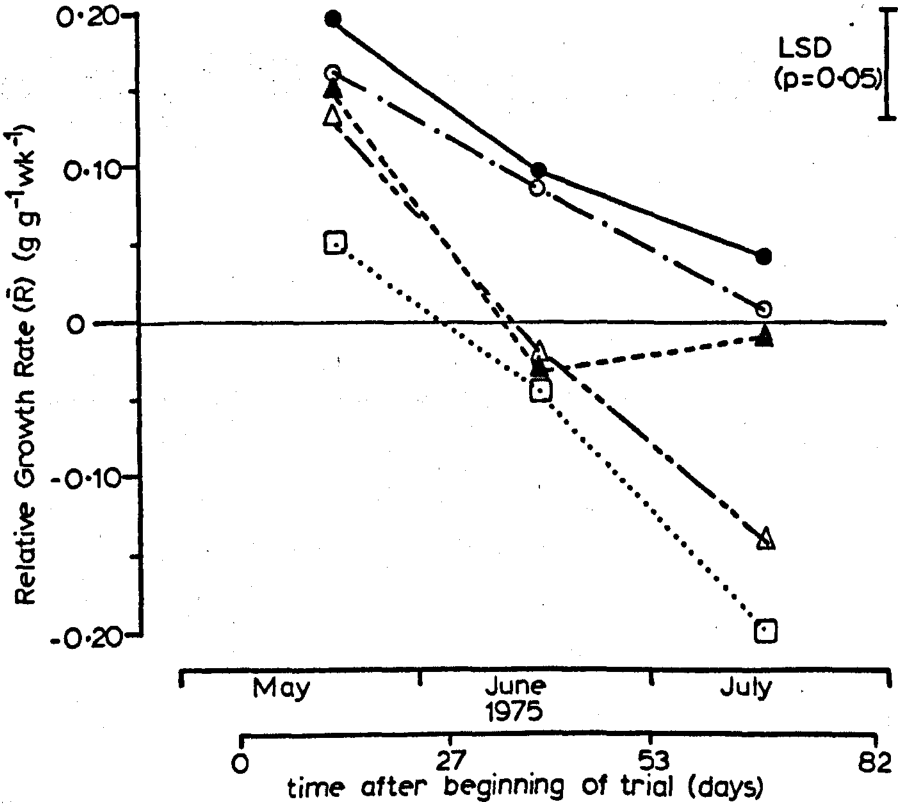


Figure 124 The effect of a range of trampling intensities, applied at weekly intervals over a 12 week period, on the Relative Growth Rate ($\bar{R}_l$) of green material of a grass sward.

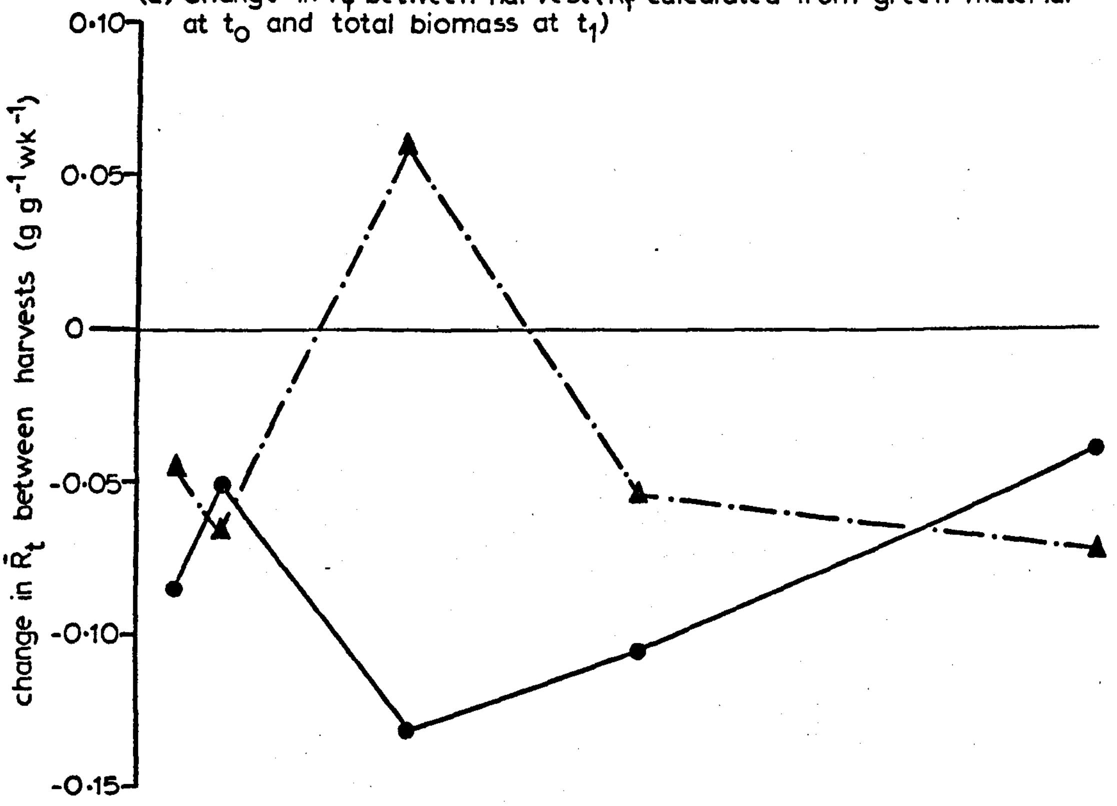
● 0 p pl^{-1}wk^{-1} ▲ 10p pl^{-1}wk^{-1} ⊡ 40p pl^{-1}wk^{-1}
○ 2 p pl^{-1}wk^{-1} △ 20p pl^{-1}wk^{-1}

material on day 27, by day 82 the relationship is markedly curvilinear in which the peak dead biomass is observed at a pressure of 10p pl^{-1} wk^{-1} while at a pressure of 40p pl^{-1} wk^{-1} the absolute dead biomass is lower than that of the control (Figure 122).

The maintenance of the sward is dependent upon growth rate. Total $\bar{R}$ ($\bar{R}_t$) has been calculated for the period between harvests and is based upon the live material existing at t_o and total biomass at t_1 (where t_o and t_1 represent successive harvest dates). $\bar{R}_t$ is significantly ($p < 0.01$) affected by trampling (Figure 123a) and a significant ($p < 0.001$) reduction occurs over time (Figure 123b). The $\bar{R}_t$ of control plots declines by 60.5% over the period of the trial which may reflect an ontogenetic drift in growth rate and a diversion of energy into the flowering process. The most severe effect of trampling on $\bar{R}_t$ over time is observed on plots receiving 20p pl^{-1} wk^{-1} where a reduction of 94.1% occurs over the same period, while the $\bar{R}_t$ of plots receiving a pressure of 40p pl^{-1} wk^{-1} is reduced to a negative value indicating that loss from the system exceeds inputs. The effect of trampling on $\bar{R}_t$ is not consistent. Although a general decline in $\bar{R}_t$ is associated with increasing trampling pressure over the period day 0 to day 27, for the period day 53 to day 82 the $\bar{R}_t$ of plots trampled at 10p pl^{-1} wk^{-1} is greater, although not significantly so, than the control $\bar{R}_t$ but for the period day 27 to day 53 the $\bar{R}_t$ for the same treatment is the lowest observed (Figure 123b).

The $\bar{R}$ of green material ($\bar{R}_1$) (based on green material at t_1) is affected, significantly ($p < 0.01$), in a similar manner to that of $\bar{R}_t$ by trampling and time (Figure 124). The decline of 76.8% over time for control plots is more marked while the $\bar{R}_1$ of plots trampled at 40p pl^{-1} wk^{-1} declines by 481.1% over the value for the time period day 0 to day 27. For the period day 0 to day 27 a pressure of 20p pl^{-1} wk^{-1} is required to

(a) Change in $\bar{R}_t$ between harvest ($\bar{R}_t$ calculated from green material at t_0 and total biomass at t_1)



(b) Change in $\bar{R}_l$ between harvest ($\bar{R}_l$ calculated from green material at t_0 and green material at t_1)

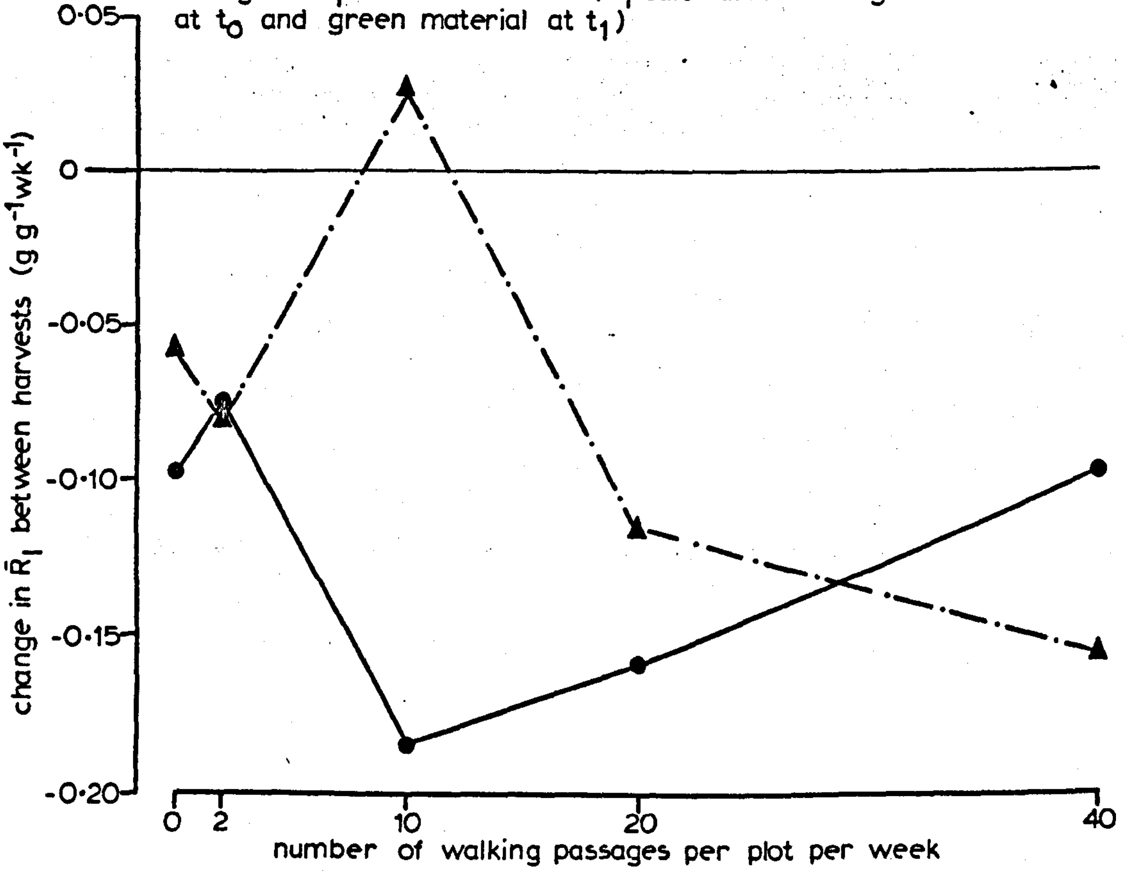


Figure 125 Change in Relative Growth Rate ($\bar{R}$) over time of a grass sward, associated with the weekly application of a range of trampling pressures.

●——● change in $\bar{R}$ between harvest 1 (04.06.75.) & harvest 2 (30.06.75.)

▲-·—·-▲ change in $\bar{R}$ between harvest 2 (30.06.75.) & harvest 3 (29.07.75.)

significantly reduce $\bar{R}_l$, a similar pressure being required for the period day 53 to day 82, but for the intervening period (day 27 to day 53) a pressure of 10p pl^{-1} wk^{-1} significantly reduces $\bar{R}_l$. It is not until the harvest on day 53 that negative values of $\bar{R}_l$ become apparent, all trampling pressures of 10p pl^{-1} wk^{-1} and over resulting in negative $\bar{R}_l$, a situation maintained over the subsequent period despite the apparent compensation, over the period day 53 to day 82, of plots trampled at 10p pl^{-1} wk^{-1}. It is of note that while a trampling pressure of 2p pl^{-1} wk^{-1} is associated with a significant decrease in total and green biomass, it does not significantly reduce $\bar{R}_t$ or $\bar{R}_l$. This emphasises the different perspective which may be obtained on plant response to trampling by a consideration of the growth rate.

The change in $\bar{R}$ between harvests indicates that decreases are generally of greater magnitude for trampled vegetation (Figure 125). Assuming the decrease of control plots represents the basal rate of change, the decline in $\bar{R}_t$ between day 0 to day 27 and day 27 to day 53 is greater than the basal rate on plots trampled at 10 and 20p pl^{-1} wk^{-1} while the decrease on plots trampled at 2 and 40p pl^{-1} wk^{-1} is less than the basal rate (Figure 125a). For the decline in $\bar{R}_t$ between day 27 to day 53 and day 53 to day 82 all trampling pressures, except that of 10p pl^{-1} wk^{-1}, result in a decrease greater than the basal rate. Only at a pressure of 10p pl^{-1} wk^{-1} is a stimulation in $\bar{R}_t$ observed resulting in an increase in $\bar{R}_t$ rather than a retardation in the relative decline of $\bar{R}_t$. Generally the decrease in $\bar{R}_t$ is greater in the former period than the latter. No consistent trend between the decline in $\bar{R}_t$ and trampling intensity emerges although for the first period there is a tendency for the magnitude of the decrease to become less with increasing pressure which is not evident for the second period.

The relationships described for $\bar{R}_t$ are broadly applicable for the decline in $\bar{R}_l$ although the magnitude of the decline of $\bar{R}_l$ is greater,

Table 29 The calculated trampling pressure (p pl^{-1} wk^{-1}) reducing Biomass and Relative Growth Rate ($\bar{R}$) of a grassland sward to 50% of the control value

Harvest (day)	Regression equation	Significance (p)	R^2	Trampling pressure required to reduce parameter by 50% (p pl^{-1} wk^{-1})
Total Biomass				
1 (day 27)	$324.26 - 2.35 X$	< 0.001	0.540	> 40
2 (day 53)	$515.87 - 34.72 X + 1.81 X^2 - 0.03 X^3$	< 0.001	0.742	39
3 (day 82)	$629.19 - 27.89 X + 0.40 X^2$	< 0.001	0.610	15
Green Biomass				
1 (day 27)	$300.11 - 3.01 X$	< 0.001	0.711	> 40
2 (day 53)	$468.27 - 41.03 X + 2.07 X^2 - 0.03 X^3$	< 0.001	0.839	35
3 (day 82)	$520.53 - 30.38 X + 0.48 X^2$	< 0.001	0.613	10
$\bar{R}_t$				
1 (day 27)	$0.199 - 0.002 X$	< 0.001	0.558	> 40
2 (day 53)	$0.103 - 0.001 X$	< 0.05	0.162	36
3 (day 82)	$0.081 - 0.002 X$	< 0.05	0.190	20

$\bar{R}_1$				
1 (day 27)	$0.199 - 0.002\ X$	< 0.001	0.750	> 40
2 (day 53)	$0.093 - 0.009\ X + 0.0002\ X^3$	< 0.05	0.597	6
3 (day 82)	$0.046 - 0.006\ X$	< 0.001	0.653	5

(X = trampling pressure (p pl^{-1} wk^{-1}))

indicating the more severe effects of trampling on the growth of green (live) and potentially productive material.

The ultimate aim of the present methodology is an attempt to predict the trampling pressures required to generate a given change in biomass or growth rate. It will be apparent that the derivation of relationships between trampling pressure and growth or yield are confused by change over time and change in cumulative trampling totals, indicating that a refinement in methodology is required.

Polynomial regression analysis (Appendix A, 2(b)) between trampling pressure and biomass or $\bar{R}$ yields regression equations from which the trampling pressure required to reduce the parameter by 50% for each harvest have been calculated (Table 29). Four weeks of trampling at the highest pressure does not reduce biomass or $\bar{R}$ (total and live) by 50% compared to the control value. After eight weeks of trampling, the pressures required to produce a 50% reduction in all but $\bar{R}_1$ are in excess of 35p pl^{-1} wk^{-1}. Trampling sustained for twelve weeks has a more severe effect on biomass and $\bar{R}$, a pressure of 20p pl^{-1} wk^{-1} reducing total biomass by 50%. In contrast, after eight weeks of trampling $\bar{R}_1$ is halved by 6p pl^{-1} wk^{-1} while after twelve weeks a similar pressure (5p pl^{-1} wk^{-1}) is still required to halve $\bar{R}_1$.

(d) Discussion

The response of a plant community to 'real' trampling may reflect plant response to trampling pressure more accurately than laboratory simulations (cf. Leney, 1974). However, it is attended by a complex interaction of factors resulting from changes in species composition of communities in disequilibrium with the pressures exerted upon them, the effects of differential competitive abilities of individuals and species

Plate 7. A section of the block shown in plate 6 in which the heaviest trampling pressure applied (40 passages per plot per week) is in the foreground with a gradient of trampling pressure (see text) extending to the control plot at the far end of the block. Grasses in flower are abundant on the untrampled areas while vegetation on trodden areas is comparatively short. (Botanic Garden, Keele, Staffs., 29.07.75).

to trampling stress (cf. Burden & Randerson, 1972; Leney, 1974; Liddle & Greig-Smith, 1975b) and from reactions of the plant to changes in the soil and aerial environment. The findings of this field trial are consequently of little value.

The trial indicates that yield and trampling intensity are curvilinearly related, agreeing with the observations of Wagar (1964) and Kellomaki (1973). There is no evidence of a stimulatory effect of light trampling pressure on biomass. The results imply a reduction in net productivity whilst a reduction in biomass to a value less than that before trampling began suggests severe defoliation and death effects in addition to the effects on net productivity. Net productivity may be affected by defoliation, death, a reduction in tiller density (Edmond, 1958; Leney, 1974), a reduction in tiller productivity as a consequence of tiller immaturity (Edmond, 1964) or a reduction in leaf size, all of which reduce the potential photosynthetic capacity of the sward.

The balance between productivity, trampling pressure and period over which the pressure is sustained may be employed to define the trampling 'carrying capacity' for a particular sward, although the degree of stability desired is a managerial rather than ecological decision. The suggestion by Burden & Randerson (1972) that the 'trampling capacity' of a component of a sward is the trampling pressure at which 'standing crop' becomes zero may be redefined 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. For comparative purposes a trampling pressure resulting in a 50% reduction in growth rate has been employed (LD_{50}). The results indicate that the LD_{50} is a dynamic quantity which is a function of the growth rate of the sward and the duration and intensity of trampling. Consequently, an definition of 'carrying capacity' should take into account variations in growth rate during the period of wear.

The decline in $\bar{R}$ over time and with increased trampling pressure implies that the 'recuperative potential' of the sward declines over time and with increased pressure. Laboratory investigations (Section VA; Bayfield, 1971; Leney, 1974) have indicated that the growth rate of individual plants of some species e.g. Phleum bertolonii, Plantago lanceolata, may be stimulated by a low degree of trampling which might lead to a lower reduction in yield, as a consequence of trampling, than might be expected (cf. Marshall & Sagar, 1968). The evidence of the field trial is insufficient to verify this postulate, although there is some evidence suggesting that a certain degree of compensation, in terms of growth rate, may be occurring on trampled vegetation. Changes in growth rate illustrate Liddle's (1975b) postulate that the relationship between trampling and productivity may be dependent upon the growth rate between periods of wear. Changes in growth rate, resulting from ontogenetic, environmental or physiological effects, may be accommodated by a change in the trampling pressure applied to the sward or changes in the longevity of the recovery period. It is therefore suggested that the carrying capacity of a vegetation sward is a dynamic quantity.

The differential effects of trampling pressure on the reduction in growth rate over time, in which, at certain pressures, the decline may be retarded, agrees with Leney's (1974) observations of elevated productivity on swards from which trampling pressure is removed, and the stimulation of productivity at low trampling pressures noted by Goldsmith (1974). The extent to which this is due to a physiological adaptation to defoliation or a change in species composition of the sward with associated variations in growth rate remains obscure.

The use of biomass estimates as an indicator of sward tolerance to trampling is limited. Leney (1974) observed that trampling on an

Ammophiletum reduced live material but dead material remained on the plot. The results of the present field trial indicate that despite a considerable plot biomass, a high proportion may be dead. 'Standing dead' material, harvested as part of the total plot biomass, may result in an erroneous prediction of the trampling capacity of the sward if used in biomass/trampling pressure relationships since it would not reflect the capacity of the sward for continued productivity upon which survival depends. This is indicated by the discrepancy between the LD_{50} values for $\bar{R}_t$ and $\bar{R}_1$. The reduction in live material with increasing trampling pressure and over time implies a constant deterioration in the potential productive capacity of the sward. This situation is confused by the possible increases in photosynthetic efficiency resulting from trampling such as more efficient light utilization or changes in the physiological efficiency of photosynthesis. The decline in $\bar{R}$ is not unexpected however, since the possible continual reduction in assimilate reserves used in regrowth might be expected to limit growth.

E. General Conclusions

Laboratory simulations of the vertical component of trampling pressure result in a reduction in plant biomass, tiller and leaf production and leaf area increase of Plantago lanceolata and Phleum bertolonii. There is a possibility that, under certain conditions, the relative growth rate may be stimulated. This response may be associated with observed tendencies for elevated rates of net photosynthesis and respiration. The difference between these CO_2 exchange rates may result in a photosynthetic carbon gain greater than that of undamaged plants, facilitating more rapid growth in Plantago lanceolata, but the negative relationship between CO_2 uptake and $\bar{R}$ in Phleum bertolonii does not support the postulate.

If the stimulation in net photosynthesis is a 'real' phenomenon it would represent a physiological adaptation towards increasing the potential photosynthetic capacity of the plant thereby partially offsetting the limitations placed upon the photosynthetic capacity of the plant due to defoliation effects. However, the situation is made complex by possible rejuvenation of the plant resulting from defoliation, which results in plants effectively of different physiological ages being compared on a treatment basis.

In addition to the effects of trampling on the shoot, trampling also has repercussions on root growth, generally retarding it possibly due to soil compaction effects. There is no evidence that soil compaction improves yield, although a stimulation in leaf area increase may occur which would enhance the photosynthetic capacity of the plant. Growth of the plant is retarded to a greater extent in plants subject to soil compaction following plant establishment than for plants establishing on compacted soil, possibly due to damage to the root when the soil, containing the root, is consolidated. Such an effect may reduce the potential for recovery following damage from trampling. Since root growth is limited by soil bulk density increases, root development prior to trampling may modify the plants response to trampling pressure. The investigation on Plantago lanceolata and Phleum bertolonii suggests that the capacity to withstand trampling pressure may be related to plant age, stage of development and conditions and duration of the recovery period which determine plant response to defoliation, mechanical damage to tissues, soil structure changes and modification of the aerial environment. Species differences in response to these factors may affect species composition and survival in trampled situations.

There is no evidence that the findings of laboratory experiments may be extrapolated to the field situation, which is in agreement with Leney's (1974) comments on the value of simulated trampling experiments. The findings of the field trial suggest that growth rate may be a factor of some significance in determining plant response to trampling, although it is evident that a considerable refinement of methodology is required to produce an adequate model which describes sward response to trampling pressure and which can take into account species composition changes, environmental effects and their associated effects, over time, on growth. It is suggested however, that Liddle's (1975b) comment that "Factors such as the morphology of species...frequency and season of trampling, soil wetness, and soil type will all have a considerable influence on the vulnerability of vegetation to trampling..." may be amplified to include physiological factors which are integrated into the growth response of the plant to trampling.

PART VI - GENERAL DISCUSSION

The main features of the response of woody stemmed and herb/grass species to trampling pressures are schematically presented in Figures 126 and 127[1]. In the presence of high levels of trampling pressure, the main response exhibited by *Calluna vulgaris* i.e. a woody stemmed species, is one of tolerance of the trampling pressure applied. In contrast, although tolerance may also be a factor affecting the durability of herbs and grasses to trampling pressures, the ability to recover i.e. the recuperative potential, is of considerable relevance to the survival and perpetuation of an individual in a trampled habitat. The difference in response may partially arise from the appreciable differences in rates of assimilation[2] and growth possessed by the two classes of plant, consequently, rapid recovery following damage, conferred upon herbs and grasses by comparatively higher growth rates, is not a feasible alternative to tolerance for a woody stemmed plant. In addition to an apparently low recuperative potential possessed by woody stemmed plants, their tolerance of trampling pressures is not exceptional, despite possessing comparatively incompressible stem tissues which might be expected to confer a mechanical advantage on the plant compared to softer stemmed species.

The inability of woody stemmed plants to tolerate trampling is related to the formation of water stresses within the plant, in addition to direct mechanical damage, in the form of stem breakage, leaf stripping of stems and bruising and tearing of tissues, following trampling. The water stresses which develop within the trampled plant are dependent upon the

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[1] solid boxes indicate observed functions or consequences based upon observation, discontinuous boxes represent hypothetical intermediates.

[2] Rates of F_n: *Calluna vulgaris* - 0.6 to 1.1μg g^{-1} s^{-1}; *Plantago lanceolata* - 2.8 to 7.2μg g^{-1} s^{-1}, *Phleum bertolonii* - 5.9μg g^{-1} s^{-1}.

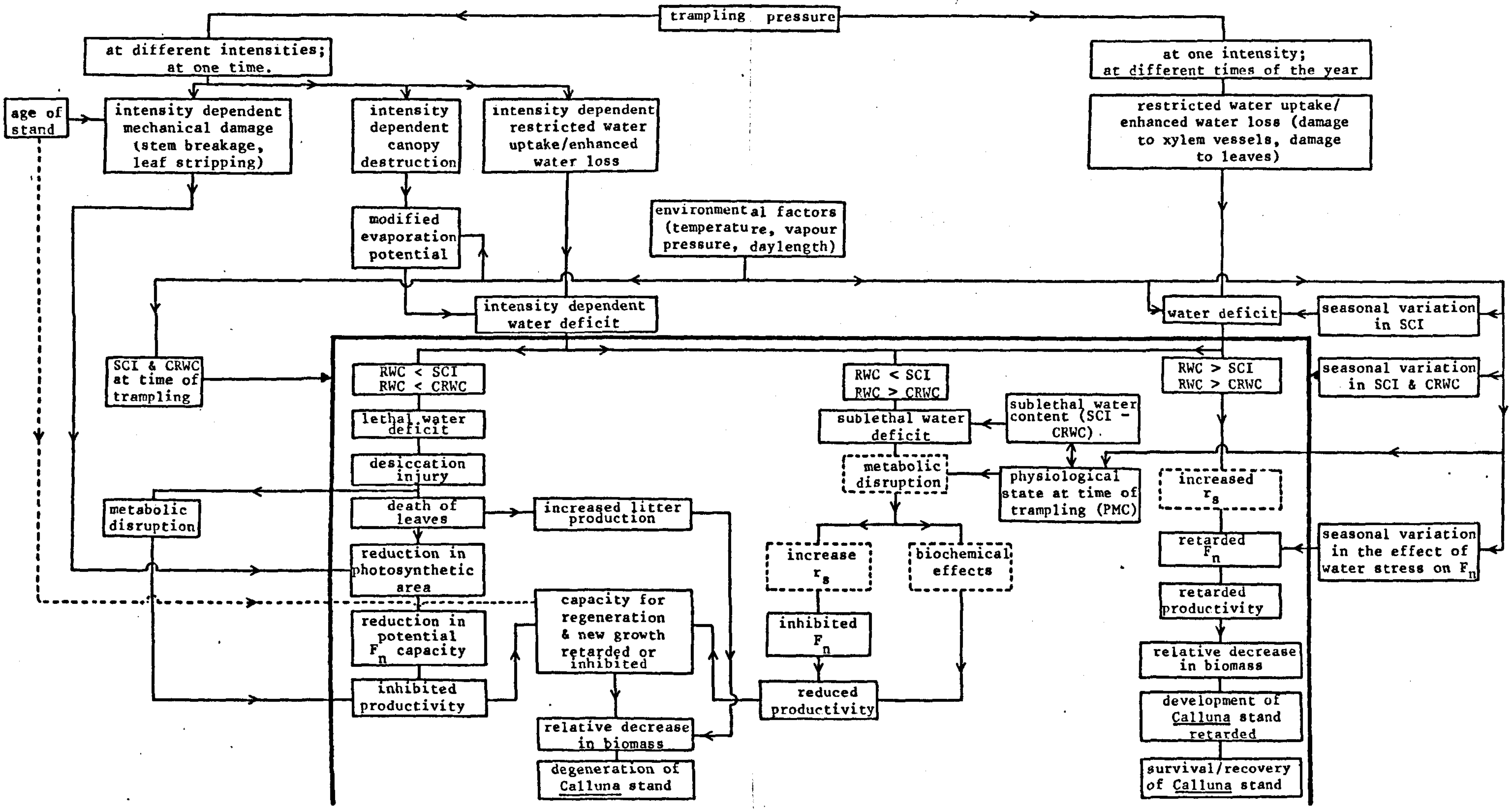

Figure 126. Summary of inter-relationships between functional factors affecting the tolerance of Calluna vulgaris to trampling pressure.

intensity of pressure applied, and may be related to the degree of internal damage restricting water uptake, and enhanced water loss arising from damage to the epidermal cells of the leaves. The rapidity with which water stresses develop within the plant is partly dependent upon the evaporation potential of the atmosphere. Intensity dependent canopy modification, arising from trampling, will alter the boundary layer resistance to water loss, generally resulting in conditions more conducive to water loss from the plant. Superimposed upon the physical factors leading to a water deficit in the trampled plant is the physiological state of the plant at the time the damage is inflicted. Since trampling induces water deficits when moisture availability is not limited, the response of the plant to desiccation is a factor of influence in determining the response of the plant to trampling. This response has been quantified in terms of the plant's ability to avoid desiccation (SCI) and tolerate desiccation (CRWC), both factors being subject to seasonal variation which is influenced by environmental variables and the ontogenetic development of the plant over the season. The 'intensity dependent' and 'seasonal' effects have been considered in relative isolation in the present study but in reality they will interact to determine plant response to trampling pressure. The consequences of the relationship between shoot RWC and the physiological factors mentioned above are presented within the solid line of Figure 126. The responses have been considered primarily in terms of the photosynthetic process and its effect on plant production. (The effect of water stress on other metabolic factors and the protoplasm, although important, have not been experimentally investigated in the present study). The effect of water stresses on physiological processes will ultimately have repercussions upon plant growth, as manifested in productivity estimates. It has been assumed that the relationships between water stress and the physiological parameters investigated for individual shoots may reflect the behaviour of the plant at the stand level. However, the response of an individual shoot may not be uniform, and the three possibilities presented on Figure 126 may exist on one shoot. In terms of desiccation injury this may be observed on shoots bearing brown (dead) and green (living) tissues simultaneously, although the extent to which the living tissues are capable of functioning metabolically has not been assessed. With decreasing severity of the water deficit (i.e. from left to right within the solid line) the detrimental effects on productivity decline. The most severe effects occur when lethal water deficits occur, a reduction in leaf area causing a direct reduction in the potential F_n capacity. Where tissues are not killed, the postulated effect is an increase in the physical resistance to CO_2 uptake resulting from stomatal closure. If the water deficit is of sufficient magnitude to cause total stomatal closure, CO_2 uptake will be inhibited and productivity reduced accordingly in relation to the longevity of the water stress, although the tissues may be capable of metabolic activity depending upon the severity of the stress. The extent to which metabolic activity is disrupted will be dependent upon the sensitivity of the tissues to water stress, and consequently will vary depending upon the environment and state of plant development, and therefore physiological activity, of the plant. In contrast, the least severe effect postulated, where water stress does not result in total stomatal closure, is one in which an increased resistance to gaseous transfer will retard but not inhibit photosynthesis. The extent to which this occurs is a seasonal variable, although in terms of productivity, at temperatures high enough to permit growth the assimilatory mechanism is most sensitive to water stress compared to times of the year when the plant is dormant. Although the severity of a given water stress on plant productivity may vary seasonally, and the magnitude of the water stress at a given trampling pressure may vary, the generalised response by the plant to the stress is for a decrease in primary productivity. Compared to untrampled vegetation this will manifest itself in a lower biomass, an effect which is aggravated by the consequences of direct mechanical damage. The capacity possessed by the plant to replace or repair damaged tissues has not been experimentally investigated but it may be inferred that a reduction in the assimilation capacity might reduce the 'recuperative potential' of the plant. Thus, in a manner analogous to the age related degeneration of the Calluna stand, the development of the community may be retarded or inhibited and the stand degenerate. This generalised response may be translated into one of greater heterogeneity at the 'individual plant' level, where individual plants and even parts of the plant may be reacting differentially to the applied stress, resulting in a variable and individualistic response by the plant to trampling pressure. This may be manifested by the success of certain individuals and the failure to survive of others in trampled habitats which may be extrapolated to encompass species differentials in response to trampling pressure.

Since the effects of trampling induced water stresses on the plant vary seasonally in relation to the variation in certain physiological parameters, it may be inferred that the trampling pressure required to produce a specified effect is also dynamic. The precision to which plant response to trampling may be predicted depends, in part, upon the precision with which the physiological status of the plant may be defined. Although this facilitates a quantification of the durability, and thus the carrying capacity, of the vegetation in terms of trampling pressure, the effect of plant microclimate on damage development following the imposition of the trampling stress reduces the precision with which plant response to trampling may be predicted prior to the stress being applied.

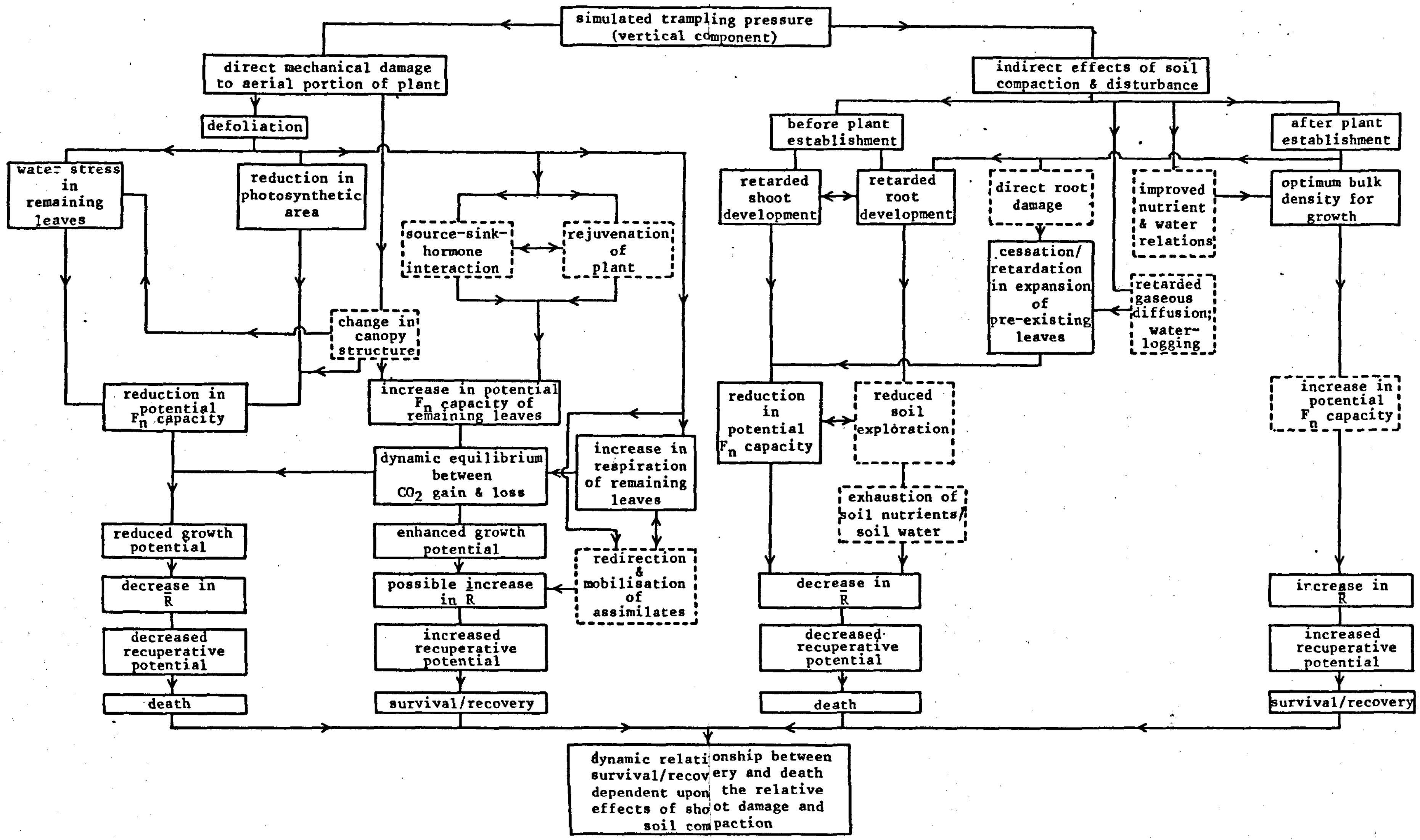

Figure 127. Possible consequences of simulated trampling pressure on plant development: observed and hypothetical inter-relationships suggested by the responses of <u>Plantago lanceolata</u> and <u>Phleum bertolonii</u> under controlled environment conditions

Thus, while plant response may be described with reasonable accuracy in terms of a number of plant and environmental variables, the inability to predict the degree of damage ensuing from a given level of use limits the extent to which the tolerance of the plant may be predicted and carrying capacity specifications defined accordingly. In generalised terms however, the tolerance of Calluna vulgaris to trampling pressure would appear to be greatest when the plant is physiologically inactive i.e. dormant, although the extent to which this observation arises from a delayed manifestation of symptoms arising from trampling damage remains unquantified.

The herb/grass type of response, exhibited by Phleum bertolonii and Plantago lanceolata (Figure 127), indicates some of the factors of relevance to their durability in trampled situations. The species chosen are known to be relatively durable to trampling pressures, but the study was limited to a consideration of only the vertical forces involved in trampling. There are a number of hypothetical intermediates presented in the scheme which have an interactive or multiple effect, sometimes in opposing directions. For example, a change in canopy structure may improve radiation interception for remaining leaves but it may also lower the boundary layer resistance facilitating water loss from the plant so aggravating a water stress situation. Simultaneously it may enhance CO_2 uptake, or impair CO_2 uptake if the leaves are pressed into the substrate. The observed increase in F_n in damaged plants may result from an interaction between rejuvenation of the plant and a source-sink-hormone relationships but the interdependence of these effects is unquantified. The equilibrium between CO_2 gain and loss is also of some importance to plant survival. A shift in favour of increased respiratory losses, despite elevated rates of photosynthesis, will reduce the growth potential of the plant and endanger its survival while a shift in favour of carbon gain will place more assimilates at the disposal of the plant for repair and regeneration of damaged tissues and replacement of lost foliage. In this context the assimilate reserves of the plant may be of significance in affecting plant survival in trampled situations.

Although root response to trampling pressure has been divorced from shoot response, in reality this is a false dichotomy. An interaction between the root and shoot is expected since roots provide metabolites necessary for assimilation and shoot growth while shoots provide assimilates for root growth and consequently further soil exploration and exploitation. Retarded root development will therefore have consequences on shoot growth whilst a reduction in assimilation capacity, whether by defoliation or increasing competition for root produced metabolites, will retard root growth. Although soil consolidation may physically restrain root growth, under certain circumstances nutrient and water relations may be improved, increasing the growth potential of the plant. Opposed to this beneficial effect of a more intimate contact between root and soil, are the problems of oxygen deficiency and water logging resulting from a decrease in soil porosity and impeded drainage in compacted soils. In addition, compaction of soil following plant establishment may mechanically damage root tissues, ultimately retarding plant growth.

The extent to which shoot and root effects interact in a trampled situation is dependent upon the degree to which soil bulk density changes affect plant development and the degree to which existing root resources are in excess of shoot requirements. Thus, defoliation will reduce competition for root produced metabolites, enhancing the assimilation capacity of remaining leaves, while the diversion of assimilates into regeneration of the foliage may impair root growth but not detrimentally so if the root, prior to damage occurring, was acting as a reservoir for excess assimilates. The degree to which soil bulk density changes affect plant development depends upon the effect the trampling pressure involved has upon the soil bulk density of the root zone of the species involved.

The level of shoot damage is obviously an intensity dependent phenomenon, and as such the capacity to tolerate trampling pressure is a factor of importance. However, at high levels of pressure the capacity to replace damaged foliage becomes increasingly important to enable continued survival.

The relationship between indirect and direct effects is one of complexity. Root and shoot effects may be compounded to enhance plant growth and facilitate survival, or to retard plant growth and endanger survival. When the effects interact the effect on the plant will be dependent upon which factor is of greatest significance to the plant at the time of damage. For example, soil compaction may enhance nutrient uptake, thereby offsetting the effect of reduced soil exploration. This may facilitate plant growth and counteract the effects of a reduction in photosynthetic area resulting from defoliation, by reducing competition for nutrients and enhancing the assimilatory capacity. However, total defoliation may result in plant death despite an improved soil bulk density for growth.

The study has concentrated upon intensity dependent effects but trampling frequency, which has not been extensively investigated, may also have repercussions on the capacity of the plant to survive. Continued damage and defoliation will ultimately exhaust the capacity of the plant to recover. The extent to which this capacity is affected is dependent upon the longevity of the recovery period which permits sufficient regrowth to occur to cushion the plant against repeated damage. The duration of the recovery period is a dynamic quantity which is dependent upon the plant's rate of growth. In a manner analogous to that described for the tolerance of <u>Calluna vulgaris</u> to trampling, the recuperative potential of Phleum bertolonii and Plantago lanceolata is variable, dependent upon the stage of plant development, its physiological activity and plant environment. However, whereas the tolerance of C. vulgaris would appear to be maximal at times of minimum physiological activity, the capacity of herbs and grasses to survive in trampled situations is dependent upon a level of physiological activity capable of replacing and repairing lost and damaged foliage.

The species employed in this study indicate the type of responses plants make in response to trampling pressure. It is not intended to imply that they are the only strategies adopted by plants in trampled situations. Species differences in response to trampling pressures may determine the competitive abilities and success of individuals and species in trampled habitats. While the extent to which the findings may be extrapolated to the field situation is unknown, physiological function, in addition to anatomical and morphological factors, would appear to have a significant effect on the response of plants to trampling pressure.

APPENDIX A STATISTICAL METHODS

(1) Analysis of Variance:

(a) One way analysis of variance with equal replication:

Analysis of variance may be employed to partition variation in the data into that associated with definable sources of variability, e.g. the effects of a range of treatments on a population, and that due to random effects affecting the total population. The analysis therefore permits treatment effects to be discriminated from random effects (cf. Snedecor, 1946).

From an experiment with n number of treatments and k number of replicates within each treatment, the variance may be analysed as follows, (Snedecor, 1946):

Example: The effect of simulated trampling on the dry weight of *Plantago lanceolata*, following a 14 day recovery period

Treatment	1	2	3	4	5
	1.755 1.428 1.767 1.061	0.888 0.885 0.530 0.489	0.647 0.701 1.004 0.455	0.799 0.663 0.494 1.033	0.603 0.140 0.385 0.476
Σx_n	6.011	2.792	2.807	2.989	1.604
$\bar{x}_n$	1.503	0.698	0.702	0.747	0.401

(i) Correction factor: $\frac{(\Sigma(\Sigma x_n))^2}{nk}$

$$= \frac{(16.203)^2}{20}$$

$$= 13.127$$

(ii) Total sum of squares: $\Sigma(x^2)$ - correction factor

$$(1.755^2 + 1.428^2 + 1.767^2 \ldots + 0.476^2)$$

$$= 16.734 - 13.127$$

$$= 3.604$$

(iii) Between treatment sum of squares: $\frac{\Sigma(\Sigma x_n)^2}{k}$ - correction factor

$$\frac{(6.011)^2 + (2.792)^2 \ldots\ldots + (1.604)^2}{4}$$

$$= 15.829 - 13.127$$

$$= 2.702$$

(iv) Within treatment sum of squares: (ii - iii)

$$= 3.604 - 2.702$$

$$= 0.902$$

Analysis of variance table:

Source	Degrees of freedom	Sum of squares	Mean square
between treatments	4	2.702	0.676
residual	15	0.902	0.060
Total	19	3.604	

Snedecor's 'F' ratio may be calculated to determine if treatment variation is due to random effects alone:

$$\text{Variance (F) ratio} = \frac{\text{Between treatment mean square}}{\text{Residual (within treatment) mean square}}$$

$$= \frac{0.676}{0.060}$$

$$= 11.267$$

The F values (from tables)[1] for 4 and 15 degrees of freedom are:

$p = 0.05$ $F = 3.06$

$p = 0.01$ $F = 4.89$

$p = 0.001$ $F = 8.25$

---

[1] e.g. Fisher & Yates (1963).

The significance limit used in the present study is 5% (i.e. $p = 0.05$). For F values lower than the F value at the 5% level from the tables, the null hypothesis, i.e. the samples have been drawn from the same population and the treatment applied does not have a significant effect on the sample, may not be rejected, For F values lying above the F value from the table, the null hypothesis may be rejected. In the present example the computed F ratio is greater than the F value at the 0.1% level of probability. It may be concluded that highly significant differences exist between the treatment means and that the chance of such differences occurring randomly is less than one in one thousand.

To test whether any two means are significantly different, the Least Significant Difference (LSD) may be calculated (Snedecor, 1946):

LSD = t (from the tables) x standard error (s.e.) of the difference between any two treatment means

$$\text{s.e.} = \sqrt{\frac{2 \times \text{residual mean square}}{k}}$$

$$= \sqrt{\frac{2 \times 0.060}{4}}$$

$$= 0.173$$

t at $p = 0.05 = 2.131$ (14 degrees of freedom)

$$\text{LSD} = 2.131 \times 0.173$$

$$= 0.369$$

Any two treatment means differing by a value greater than 0.369 may be considered significantly different at the 5% level of probability.

(b) One way analysis of variance between treatments with unequal replication (Snedecor, 1946):

Example: The effect of continuous simulated trampling on the dry weight of *P. lanceolata*

Treatment	1	2	3	4
	7.475	3.255	3.765	2.720
	7.400	5.585	5.465	0.130
	7.255	4.690	3.790	
	8.100	6.015	3.960	
	5.905	4.695	2.400	
Σx_n	36.135	24.240	19.380	2.85
$\bar{x}_n$	7.227	4.848	3.876	1.425

(i) Correction factor: $\frac{(\Sigma(\Sigma x_n))^2}{nk}$

$$= \frac{(82.605)^2}{17}$$

$$= 401.387$$

(ii) Total sum of squares: $\Sigma(x^2)$ - correction factor

$$= 473.019 - 401.387$$

$$= 71.632$$

(iii) Between treatment sum of squares:

$$\left(\frac{(\Sigma x_1)^2}{k_1} + \frac{(\Sigma x_2)^2}{k_2} + \frac{(\Sigma x_3)^2}{k_3} + \ldots\ldots \frac{(\Sigma x_n)^2}{k_n} \right) - \text{correction factor}$$

(where k_n = number of replicates in each treatment)

$$= \frac{1305.738}{5} + \frac{587.578}{5} + \frac{375.584}{5} + \frac{8.123}{2}$$

$$= (261.148 + 117.516 + 75.117 + 4.061) - 401.387$$

$$= 56.455$$

(iv) Within treatment sum of squares: (ii - iii)

71.632 - 56.455

= 15.177

Analysis of variance table:

Source	Degrees of freedom	Sum of squares	Mean square
between treatments	3	56.455	18.818
residual	13	15.177	1.167
Total	16	71.632	

$$\text{F ratio} = \frac{18.818}{1.167}$$

$$= 16.125$$

F ratio at p = 0.001 with 3 and 13 degrees of freedom = 10.21

The null hypothesis may be rejected and it may be concluded that highly significant differences ($p < 0.001$) exist between treatments.

LSD = t x s.e.,

where s.e. $= \sqrt{\left(\frac{1}{k_1} + \frac{1}{k_n}\right) \times \text{residual mean square}}$

$$= \sqrt{\frac{1}{5} + \frac{1}{5} \times 1.167}$$

$$= 0.683 \times 2.160 \text{ (t)}$$

$$= 1.475$$

or $\sqrt{\frac{1}{5} + \frac{1}{2} \times 1.167}$

$$= 0.904 \times 2.160$$

$$= 1.952$$

To compare any two treatment means, the number of replicates in each treatment must be taken into consideration and an LSD calculated for the specific comparison. In the present example, means of treatment 1 and 2 are significantly different, the LSD being 1.475 and the difference between the two means 2.379. The difference between treatments 3 and 4 is 2.451 but since the number of replicates are different, i.e. 5 and 2, the second of the two LSD's must be used, i.e. 1.952. It will be observed that the difference is significant at the 5% level.

(c) <u>One way analysis of variance for a Latin Square design</u> (Snedecor, 1946):

The Latin Square experimental design is of use in permitting the effect of factors other than those of the treatment to be taken into account, although not necessarily explained, in subsequent statistical analyses. Replicate distribution is dictated by the superimposition of two blocks at right angles, giving a set of 'rows' and a set of 'columns'. Since the arrangement is a 'square' there will be k number of rows and k number of columns, giving k^2 number of plots. The design dictates that each treatment occurs in each row once and in each column once, consequently, the number of replicates per treatment is equal to the number of treatments in the experiment.

<u>Example</u>: The effect of Trampling on the ψ_x of <u>Calluna vulgaris</u> (Cannock Chase, 21.05.75.)

Plot layout (data in MPa):

	A -1.15	B -0.325	C -0.250	D -0.100	TT -1.825
	B -0.200	A -0.950	D -0.150	C -0.350	-1.650
	C -0.175	D -0.175	A -0.900	B -0.425	-1.675
	D -0.250	C -0.525	B -0.400	A -0.900	-2.075
TT	-1.775	-1.975	-1.700	-1.775	

A - control

B - 50 p pl^{-1}

C - 100 p pl^{-1}

D - 200 p pl^{-1}

TT - row/column total

Treatment data:

	A	B	C	D
	-1.150	-0.325	-0.250	-0.100
	-0.950	-0.200	-0.350	-0.150
	-0.900	-0.425	-0.175	-0.175
	-0.900	-0.400	-0.525	-0.250
Σx_n	-3.900	-1.350	-1.300	-0.675
$\bar{x}_n$	-0.975	-0.338	-0.325	-0.169

(i) Correction factor: $\frac{(\Sigma(\Sigma x_n))^2}{k^2}$

$$= \frac{(7.225)^2}{16}$$

$$= 3.263$$

(ii) Total sum of squares: $\Sigma(x^2)$ - correction factor

$$4.948 - 3.263$$

$$= 1.685$$

(iii) Row sum of squares: $\frac{\Sigma(\text{row totals})^2}{k}$ - correction factor

$$\frac{(1.825)^2 + (1.560)^2 + (1.675)^2 + (2.075)^2}{4}$$

$$= \frac{13.165}{4} - 3.263$$

$$= 0.028$$

(iv) Column sum of squares: $\frac{\Sigma(\text{column totals})^2}{k}$ - correction factor

$$\frac{(1.775)^2 + (1.975)^2 + (1.700)^2 + (1.775)^2}{4}$$

$$= \frac{13.093}{4} - 3.263$$

$$= 0.010$$

(v) Treatment sum of squares: $\frac{\Sigma(\text{treatment totals})^2}{k}$ - correction factor

$$\frac{(3.9)^2 + (1.35)^2 + (1.3)^2 + (0.675)^2}{4}$$

$$= \frac{19.178}{4} - 3.263$$

$$= 1.531$$

(vi) Residual: (ii) - (iii + iv + v)

$$1.685 - (0.028 + 0.010 + 1.531)$$

$$= 0.116$$

Analysis of variance table:

Source	Degrees of freedom	Sum of squares	Mean Square	F ratio
rows	3	0.028	0.009	0.474
columns	3	0.010	0.003	0.158
treatments	3	1.531	0.510	26.842
residual	6	0.116	0.019	
total	15	1.685		

The F ratio is calculated for row, column and treatment effects by dividing the respective mean square by the residual mean square. The F value, from tables, at 3 and 6 degrees of freedom at $p = 0.05$ is 4.76, and at $p = 0.001$ is 23.7. Neither row nor column F ratios are significant, any difference in row or column means being due to random effects. The F ratio for the treatment effects is highly significant, ($p < 0.001$), and it may be concluded that the treatment has a significant effect on the population.

The LSD may be calculated as above, in this example it is 0.237, therefore, any two treatment means differing by a value in excess of this are significantly different at the 5% level.

Table A1 Data for two-way analysis of variance for the effect of trampling on the RWC of *C. vulgaris* over time

Trampling treatment time (h)	replicate	control	trampled	Σ row	$\bar{x}$ row
0	1	0.7808	0.6930		
	2	0.7728	0.7169		
	3	0.6573	0.6267		
	4	0.7251	0.7008		
	Σx_1	2.9360	2.7374	5.6734	0.7092
	$\bar{x}_1$	0.7340	0.6844		
1	1	0.7095	0.6037		
	2	0.7382	0.6385		
	3	0.7130	0.6397		
	4	0.7049	0.6316		
	Σx_2	2.8656	2.5135	5.3791	0.6724
	$\bar{x}_2$	0.7164	0.6284		
2	1	0.6861	0.5914		
	2	0.6946	0.5369		
	3	0.6179	0.5482		
	4	0.6916	0.5676		
	Σx_3	2.6902	2.2441	4.9343	0.6168
	$\bar{x}_3$	0.6726	0.5610		
3	1	0.6993	0.5526		
	2	0.6928	0.5452		
	3	0.6872	0.5781		
	4	0.7046	0.6070		
	Σx_4	2.7839	2.2829	5.0668	0.6334
	$\bar{x}_4$	0.6960	0.5707		
5	1	0.7080	0.5466		
	2	0.7008	0.5634		
	3	0.7053	0.5455		
	4	0.7061	0.6304		
	Σx_5	2.8202	2.2859	5.1061	0.6383
	$\bar{x}_5$	0.7051	0.5715		

/...

Table A1 Data for two-way analysis of variance for the effect of trampling on the RWC of *C. vulgaris* over time (cont'd)

Trampling treatment time (h)	replicate	control	trampled	Σ row	$\bar{x}$ row
24	1	0.7187	0.5638		
	2	0.6633	0.5189		
	3	0.6907	0.5680		
	4	0.7155	0.5721		
	Σx_6	2.7882	2.2228	5.0110	0.6264
	$\bar{x}_6$	0.6971	0.5557		
48	1	0.7148	0.4748		
	2	0.6409	0.5270		
	3	0.6725	0.5736		
	4	0.6682	0.5339		
	Σx_7	2.6964	2.1093	4.8057	0.6007
	$\bar{x}_7$	0.6741	0.5273		
72	1	0.7079	0.5924		
	2	0.6988	0.5511		
	3	0.6701	0.5175		
	4	0.6866	0.5957		
	Σx_8	2.7634	2.2567	5.0201	0.6275
	$\bar{x}_8$	0.6909	0.5642		
	Σ column	22.3439	18.6526	40.9965	GRAND TOTAL
	$\bar{x}$ column	0.6982	0.5829		

(d) Two way analysis of variance, with equal numbers of replicates in each cell (Snedecor, 1946):

Experiments may be designed in which two treatments are applied to a population, for example, the effect of soil type and the effect of nutrient application on plant growth. Two way analysis of variance procedures may be employed to determine whether each treatment has a significant effect on the measured parameter.

Example: The effect of trampling, at one instant in time, at an intensity of 100 p pl^{-1}, on the RWC of *C. vulgaris*, over time, (Kerloch, 04.04.76. to 08.04.76.)

This experiment was performed to determine the rate at which water deficits develop after the plant has been trampled.

The variables may be divided into:

Variable 1 - control and trampled plants

Variable 2 - 8 time periods

Each treatment in variable 1 is composed of 4 replicate plots, the value for each replicate being RWC of 4 shoots sampled from each replicate plot.

Data: (Table A1)

(i) Correction factor: $\frac{(\Sigma(\Sigma x_N))^2}{N}$ (where N = total number of entries in data table)

$$\frac{(40.9965)^2}{64}$$

$$= 26.261$$

(ii) Total sum of squares: $((x_1^2) + (x_2^2) + (x_3^2)....+ (x_N^2))$ - correction factor

$$(0.7808)^2 + (0.7728)^2 + (0.6573)^2...+(0.5957)^2$$

$$= 26.60063 - 26.26114$$

$$= 0.33949$$

(iii) Cell sum of squares: $\frac{(\Sigma x_1)^2 + (\Sigma x_2)^2 \ldots + (\Sigma x_N)^2}{k}$ - correction factor

$$\frac{(2.9360)^2 + (2.8656)^2 \ldots + (2.2567)^2}{4}$$

$$= \frac{106.22}{4}$$

$$= 26.555 - 26.26114$$

$$= 0.29386$$

(iv) Within cell sum of squares (residual): (ii - iii)

$$0.33949 - 0.29386$$

$$= 0.04563$$

(v) Time treatment (variable 2) sum of squares (rows):

$$\left(\frac{(\Sigma \text{row}_1)^2 + (\Sigma \text{row}_2)^2 \ldots + (\Sigma \text{row}_{n_1})^2}{n_1 k}\right) - \text{correction factor}$$

(where n_1 is the number of treatments in the row)

$$\frac{(5.6734)^2 + (5.3791)^2 \ldots + (5.0201)^2}{8} - 26.26114$$

$$= \frac{210.62}{8} - 26.26114$$

$$= 26.3275 - 26.26114$$

$$= 0.06636$$

(vi) Trample treatment (variable 1) sum of squares (columns):

$$\frac{(\Sigma \text{column}_1)^2 + (\Sigma \text{column}_2)^2 \ldots (\Sigma \text{column}_{n_2})^2}{n_2 k} - \text{correction factor}$$

(where n_2 is the number of treatments in the column)

$$\frac{(22.3439)^2 + (18.6526)^2}{32} - 26.26114$$

$$= 26.47404 - 26.26114$$

$$= 0.2129$$

(vii) Time x treatment interaction: (iii) - (v + vi)

0.29386 - (0.06636 + 0.2129)

= 0.0146

Degrees of freedom:

Total	N-1	63
residual	$n_1n_2(k-1)$	48
row	n_2-1	7
column	n_1-1	1
interaction		7

Analysis of variance table:

Source	Degrees of freedom	Sum of squares	Mean Square	F ratio
Time(var.2)	7	0.06636	0.00948	9.979
Trampling(var.1)	1	0.21290	0.21290	224.105
Interaction	7	0.01460	0.00209	2.200
Residual	48	0.04563	0.00095	
Total	63	0.33949		

The F ratios may be computed in the usual manner and compared with the F ratio for the appropriate degrees of freedom from the tables. The F ratio at 7 and 48 degrees of freedom is 4.33 at $p = 0.001$, the computed F ratio for Time exceeds this, consequently it may be concluded that significant time effects exist. Similarly, the F ratio for trampling effects is greater than that from the tables, and it may therefore be concluded that trampling has a highly significant effect on plant RWC. These main effects may be considered independently when the interaction is not significant. The test of significance does not involve any assumptions regarding the effects of interaction, and may be regarded as valid for testing main treatment effects (Lindquist, 1953). The method employed in the present study has been to test main effects against the residual mean square. This may oversimplify a more complex situation. Rao (1952) stated that if the interaction is not significant, the main effects should be tested against the residual or the interaction mean square, whichever is the greater. In so doing, an interaction effect characteristic of only a section of the table may be taken into account but Lindquist (1953) regarded tests of interaction applied to a part of the table when the overall interaction is nonsignificant as of dubious value.

The interpretation of 'interaction' effects is complex and fully discussed by Snedecor (1946) and Lindquist (1953). In the present example the F ratio for the interaction is not significant. If the interaction were significant the effect of one set of treatments could not be alluded to without reference to the second set of treatments, i.e. the two main effects may not be considered independently. As a consequence of this, no generalised statement regarding the main effects may be made (Rao, 1952) but this does not preclude testing for significant differences between the means of variable 1 at a specified level of variable 2 or vice-versa, i.e. comparisons may be made between rows or between columns but not diagonally.

The LSD may be computed in the usual fashion, using the residual mean square, where the number of replicates in each cell is used;

$$\begin{aligned} \text{LSD} &= 2.010 \times \sqrt{\frac{0.00095 \times 2}{4}} \\ &= 0.0438 \end{aligned}$$

Any two cell means which differ by an amount greater than this may be considered significantly different at the 5% level.

Similarly, the LSD between overall treatment means may be computed using the residual mean square and the number of individuals in each row or column;

$$\text{LSD} = 2.010 \times \sqrt{\frac{0.00095 \times 2}{8}}$$

$$= 0.0309 \text{ (row means)}$$

$$\text{LSD} = 2.010 \times \sqrt{\frac{0.00095 \times 2}{32}}$$

$$= 0.0155 \text{ (column means)}$$

The methods used in the present study have assumed a homogeneous interaction effect, although this may not be the case (cf. Snedecor, 1946).

It is of note that percentages may be expected to yield a valid test in analysis of variance when the values range between 20% and 80% (Snedecor, 1946). When this condition has not been fulfilled, the data have been subject to angular transformation.

Table A2 Data for correlation and regression analysis on rainfall (mm) measured at Penkridge (x) and at Cannock Chase (y)

	x	y	x^2	y^2	xy
1	2.0	5.76	4.0	33.178	11.52
2	12.8	10.39	163.84	107.952	132.99
3	44.6	56.6	1989.16	3203.56	2524.36
4	2.9	6.9	8.41	47.61	20.01
5	26.2	31.8	686.44	1011.24	833.16
6	0.4	1.9	0.16	3.61	0.76
7	52.7	42.8	2777.29	1831.84	2255.56
8	41.5	65.5	1722.25	4290.25	2718.25
9	49.0	61.3	2401.00	3757.69	3003.70
10	34.7	46.9	1204.09	2199.61	1627.43
11	47.8	59.4	2284.84	3528.36	2839.32
12	50.6	50.1	2560.36	2510.01	2535.06
13	46.4	50.8	2152.96	2580.64	2357.12
14	20.9	31.6	436.81	998.56	660.44
15	0.0	2.1	0.0	4.41	0.0
16	43.1	42.4	1857.61	1797.76	1827.44
17	58.2	43.3	3387.24	1874.89	2520.06
18	15.3	23.6	234.09	556.96	361.08
Total	549.1	633.15	23870.55	30338.129	26228.26
$\bar{x}$	30.5	35.2			

(2) Correlation and Regression analysis:

(a) Linear correlation and regression analysis (Snedecor, 1946):

The correlation coefficient (r) varies between -1 and +1, i.e. perfect negative to perfect positive correlation. The value depends upon the closeness of the relationship between variable x and variable y, i.e. if the increase from x_1 to x_2 is paralleled by a proportional increase from y_1 to y_2 and the increase is maintained constant to x_n and y_n a perfect positive correlation would result, (i.e. +1). If an increase in x is accompanied by a decrease in y a negative correlation results. The coefficient r is a measure of the covariation in the data, i.e. the degree to which they keep in step as they change.

The two variables may be numerically related by the use of regression analysis in which the value of the dependent variable y may be described or predicted when x is known. The regression line given by the equation is the best fit line, in which the positive and negative deviations from the line are equal. Unless the regression passes through the origin two regression coefficients exist, (a) defining the point at which the regression intercepts the y axis when x is zero, and (b) giving the increase in y for each increment in x, i.e. the slope of the regression line. An equation of the form $y = a + bx$ is produced.

Example: The correlation between rainfall measured at Penkridge meteorological station (independent variable x) and that measured on Cannock Chase (dependent variable y)

Data: (Table A2)

(i) Sum of squares of deviations from sample mean x ($\Sigma d\ x^2$):

$$\Sigma(x - \bar{x})^2$$

$$= \Sigma x^2 - \frac{(\Sigma x)^2}{n} \quad \text{(where n is number of pairs of data)}$$

$$= 23870.55 - \frac{(549.1)^2}{18} = 7119.95$$

(ii) Sum of squares of deviations from sample mean y ($\Sigma d\ y^2$):

$$= 30338.129 - \frac{(633.15)^2}{18}$$

$$= 8067.078$$

(iii) Sum of products of deviations in x and y (covariance) ($\Sigma d_x\ d_y$):

$$\Sigma xy - \frac{(\Sigma x \Sigma y)}{n}$$

$$= 26228.262 - \frac{(549.1 \text{ x } 633.15)}{18}$$

$$= 6913.67$$

(iv) Correlation coefficient (r):

$$\frac{\Sigma d_x d_y}{\sqrt{(\Sigma dx^2 . \Sigma dy^2)}}$$

$$= \frac{6913.67}{\sqrt{7119.95 \text{ x } 8067.078}}$$

$$r = 0.913$$

(v) Regression coefficients:

regression of y on x: $b = \frac{\Sigma d_x d_y}{\Sigma dx^2}$

$$= \frac{6913.67}{7119.95}$$

$$= 0.97103$$

regression equation (y) $= \bar{y} + b(x - \bar{x})$

$$= 35.175 + (0.97103)(x - 30.505)$$

$$= 35.175 + 0.97103x - 29.62127$$

$$\underline{\hat{y} = 5.554 + 0.971x}$$

regression of x on y: $b = \frac{\Sigma d_x d_y}{\Sigma dy^2}$

$$= \frac{6913.67}{8067.078}$$

$$= 0.85702$$

$$\begin{aligned}\text{regression equation } (x) &= \bar{x} + b(y - \bar{y}) \\ &= 30.505 + (0.85702)(y - 35.175) \\ &= 30.505 + 0.85702y - 30.1456 \\ \underline{\hat{x}} &\underline{= 0.3594 + 0.857y}\end{aligned}$$

The value of r at (n - 2) degrees of freedom (16) from the tables is 0.708 at $p = 0.001$; the computed value of r exceeds this and it may be concluded that a highly significant correlation exists between the two variables. The value of y may be predicted from the equation $\hat{y} = 5.554 + 0.971x$ for any value of x within the limits of the data.

Snedecor (1946) made a distinction between correlation analysis, which assesses the relationship between two variables which may not be consequentially related, and regression analysis in which one variable is dependent upon the other. The coefficient r does, however, give an indication of the accuracy with which y may be calculated from x. The square of r gives the percentage variation accounted for by the regression. r may also be employed to indicate the significance of a particular regression but a significant value of r does not prove causality since the variables x and y may be related by a third variable, z.

Unless r is unity, the regression of x on y is different from the regression of y on x.

(b) Curvilinear regression analysis:

Fitting a curve to a scatter of points is of use for describing one variable in terms of another, or for smoothing out irregulatities in data, the simplest form of curve being that of a straight line. Snedecor (1946) stated that the fitting of curves by eye should be avoided. In the present investigation, polynomial regression analysis has been performed to permit the objective estimation of y where curvilinear relationships are prevalent.

Table A3 Polynomial regression analysis for net rate of photosynthesis regressed on RWC, *C. vulgaris* (Cannock Chase, 27.06.76.)

1st degree polynomial:

multiple R^2 = 0.251
multiple R = 0.501
d.f.1 = 1
d.f.2 = 41
F for analysis of variance on R = 13.755
B weight = 0.98299
intercept constant = -0.223713

2nd degree polynomial:

multiple R^2 = 0.478
multiple R = 0.691
d.f.1 = 2
d.f.2 = 40
F for analysis of variance on R = 18.315
B weight = 12.13536, -7.453474
intercept constant = -4.207908

3rd degree polynomial:

multiple R^2 = 0.489
multiple R = 0.699
d.f.1 = 3
d.f.2 = 39
F for analysis of variance on R = 12.421
B weights = -9.258805, 22.642209, -13.74652
intercept constant = 0.732827

Table A3 Polynomial regression analysis for net rate of photosynthesis regressed on RWC, *C. vulgaris* (Cannock Chase, 27.06.76.) (cont'd)

Analysis of variance for polynomials:

1st degree:

reduction due to linear fit = 0.2512 d.f. 1
residual sum of squares = 0.7488 d.f. 41
residual mean square = 0.0183
F for linear fit = 13.755

2nd degree:

reduction due to general quadratic fit = 0.4780 d.f. 2
reduction mean square = 0.2390
residual sum of squares = 0.5220 d.f. 40
residual mean square = 0.0131
F for quadratic fit = 18.315
reduction due to quadratic term alone = 0.2268 d.f. 1
F for quadratic term alone = 17.381

3rd degree:

reduction due to general cubic fit = 0.4886 d.f. 3
reduction mean square = 0.1629
residual sum of squares = 0.5114 d.f. 39
residual mean square = 0.0131
F for general cubic fit = 12.421
reduction due to cubic term alone = 0.0106 d.f. 1
F for cubic term alone = 0.808

A programme from the University of Manchester Regional Computing Center Applied Statistics Library (volume 2) has been employed; an example of the print out is given in Table A3.

The generalised form of a polynomial regression equation is $y = a + bx + cx^2$. From the analysis of variance for the polynomials it will be observed that the F ratio for the linear fit (13.755 with 1 and 41 degrees of freedom) is highly significant, (F at 1 and 41 degrees of freedom = 12.550 at $p = 0.001$). A linear fit accounts for a significant proportion of the variation in the data (i.e. 25.12%).

The F ratio for the quadratic term alone (17.381) is also highly significant, (F at 1 and 40 degrees of freedom = 12.61 at $p = 0.001$). A further 22.68% of the variation in the data is accounted for by the quadratic term, bringing the total 'explained' variation to 47.8%.

The F ratio for the cubic term is not significant and the reduction in the sum of squares is only 1.06%, consequently, it may be concluded that the quadratic term makes a significant contribution to the regression but the cubic term does not. This yields a regression equation where $\hat{y} = -4.207908 + 12.1353x - 7.453474x^2$.

The multiple R^2 describes the total variance accounted for by each order of the regression. The first term in the equation defines the point at which the curve intersects the y axis when x is zero. The subsequent terms describe the form of the curve. The degree of correlation is indicated by multiple R, the significance of which may be determined from tables (Snedecor, 1946).

The regression coefficients are given to the maximum number of decimal places calculated as considerable error may ensue from the use of 'rounded up' figures.

Table A4 Multiple regression analysis for browning (treated minus control values) of *C. vulgaris* subjected to 100 walking passages per plot (Cannock Chase, 1974-1976)

Dependent variable: browning

Independent variables entered into analysis:
desiccation tolerance
percent moisture content
sublethal water content (difference between RWC at stomatal closure (Intersection method) and CRWC for desiccation tolerance)

Step 1

Variable entered: desiccation tolerance

Multiple R = 0.571

R^2 = 0.326

Analysis of variance:

Source	d.f.	Sum of squares	Mean square	F
regression	1	1227.61636	1227.61636	7.739
residual	16	2538.08761	158.63048	

Variables in the equation:

	B weight	F	Significance
desiccation tolerance	128.98429	7.739	0.05
constant	12.267271	1.618	n.s.

Step 2

Variable entered: percent moisture content

Multiple R = 0.741

R^2 = 0.549

Table A4 Multiple regression analysis for browning (treated minus control values) of *C. vulgaris* subjected to 100 walking passages per plot (Cannock Chase, 1974-1976) (cont'd 1)

Analysis of variance:

Source	d.f.	Sum of squares	Mean square	F
regression	2	2068.74011	1034.37006	9.143
residual	15	1696.96387	113.13092	

Variables in the equation:

	B weight	F	Significance
desiccation tolerance	116.83729	8.789	0.01
percent moisture content	0.37279	7.435	0.05
constant	-45.32407	4.009	0.06

Step 3

Variable entered: sublethal water content

Multiple R = 0.752

R^2 = 0.567

Analysis of variance:

Source	d.f.	Sum of squares	Mean square	F
regression	3	2129.98764	709.99588	6.768
residual	14	1635.71634	116.83688	

Variables entered into equation:

	B weight	F	significance
desiccation tolerance	88.307456	2.470	n.s.
percent moisture content	0.3483379	5.935	0.05
sublethal water content	45.076087	0.524	n.s.
constant	-24.037269	0.415	n.s.

Table A4 Multiple regression analysis for browning (treated minus control values) of *C. vulgaris* subjected to 100 walking passages per plot (Cannock Chase, 1974-1976) (cont'd 2)

Summary table:

Variable	F to enter	sign. (p)	Multiple R	R^2	R^2 change	r	Overall F	Overall sign. (p)
desiccation tolerance	7.739	0.05	0.57096	0.326	0.326	0.571	7.739	0.05
percent moisture content	7.435	0.05	0.74119	0.549	0.223	0.534	9.143	0.01
sublethal water content	0.524	n.s.	0.75208	0.567	0.016	-0.569	6.077	0.01

(c) Multiple regression analysis:

The computational procedures employed in the regression of the dependent variable y on more than one independent variable, $x_1 \ldots x_n$ is described by Snedecor (1946). By performing such an analysis, y may be described in terms of a number of x's, thereby enabling a more accurate estimate of y to be obtained or described.

In stepwise multiple regression, as used in the present study, the contribution made by a particular x to the regression is rated in order of decreasing importance.

The programme employed was that, for use with the U.M.R.C.C. ICL 7600/1900 computer, obtained from the Statistical Package for the Social Sciences (Nie et al., 1975). An example of the salient features of the print-out are presented in Table A4.

A choice is made as to the variables to be entered into the analysis. In the present example all three variables are significantly correlated with the dependent variable.

At step 1, the CRWC for desiccation tolerance is entered and accounts for a significant proportion of the variance (32.6%), the F ratio to enter the variable being significant at the 5% level. The multiple correlation coefficient (multiple R) indicates the degree of correlation between y and $\hat{y}$ (the estimated value of y), indicating the 'success of estimating' the value of y from the independent variable (Snedecor, 1946).

At step 2 percent moisture content is entered. The total sum of squares is 3765.7039 and the inclusion of two independent variables into the analysis accounts for 2068.74011 of the total, i.e. 54.9% of the total (= R^2). The overall F ratio for the regression is 9.143, which, with 2 and 15 degrees of freedom, is significant at the 1% level. Variable x_2 (i.e. percent moisture content) explains an additional 22.3% of the variation above that explained by variable x_1 (= R^2 change), i.e.

841.1238/3765.7039 compared to 1227.61636/3765.7039 for x_1. This additional explained variation is significant at the 5% level ($F = 7.435$). The improvement is reflected in the increase in the multiple R value from 0.571 for step 1 to 0.741 for step 2, indicating a closer correlation between y and $\hat{y}$. This value may be examined for significance by reference to the appropriate tables (Snedecor, 1946) which take into account the number of variables in the correlation. The multiple R value at 15 degrees of freedom with 2 variables (step 2) is 0.606 at the 1% level of significance, thus, the correlation may be regarded as significant.

At step 3 the variable, sublethal water content, accounts for a further 1.6% of the variation (61.2475/3765.7039). This is not a significant improvement in the regression ($F = 0.524$), thus, although the overall regression remains significant (F 6.077, $p = 0.01$), there is no statistical justification for including variable x_3 in the regression.

It may be concluded that desiccation tolerance and percent moisture content make significant contributions towards explaining the variation in browning, yielding an equation of the form:

$y = a + bx_1 + cx_2$ (where a, b and c are the B weights of the equation (step 2));

$$\hat{y} = -45.324 + 116.837x_1 + 0.373x_2$$

(3) Comparison of two means for groups of equal size but with unpaired data (Student's t test) (Snedecor, 1946):

Example: Comparison of mean ψ_x of *C. vulgaris* shoots sampled from plots treated at 100 p pl^{-1} one month prior to sampling, and control shoots sampled from adjacent plots.

Data (in MPa):

control (x_1)		treated (x_2)	
-0.2	-0.15	-1.10	-1.20
-0.3	-0.30	-1.10	-1.10
-0.25	-0.30	-0.20	-0.70
-0.20	-0.30	-0.85	-1.00
-0.20	-0.30	-0.70	-0.80
-0.20	-0.20	-0.60	-0.60
-0.15	-0.30	-0.70	-0.80
-0.25	-0.15	-1.20	-0.50
-0.15	-0.15	-0.80	-0.80
-0.25	-0.35	-0.70	-0.80

Treatment	n	Degrees of freedom	Σx	$\Sigma(x^2)$	$\bar{x}$
control	20	19	4.65	1.1625	-0.23
treated	20	19	17.25	15.8025	-0.86
total	40	38	21.90	16.965	

(i) Correction factor: $\frac{(\Sigma(x))^2}{n}$ (where n is number data items per group)

x_1 = 1.0811

x_2 = 14.878

(ii) Sum of squares: Σx^2_n - correction factor

$$Sx_1^2 = 0.0814$$

$$Sx_2 = 0.9245$$

(iii) Pooled variance: $$(s^2) = \frac{(Sx_1^2 + Sx_2^2)}{2(n-1)}$$

$$= \frac{0.0814 + 0.9245}{38}$$

$$= 0.0265$$

(iv) Variance for difference between means: $$(s\underline{x}^2) = \frac{2s^2}{n}$$

$$= \frac{0.0265 \times 2}{20}$$

$$= 0.00265$$

(v) $$t = \frac{\text{difference between means } (\underline{x})}{\sqrt{s\underline{x}^2}}$$

$$= \frac{0.63}{0.0515}$$

$$= \underline{12.233}$$

For 38 degrees of freedom t (from tables) at $p = 0.05 = 2.025$

$p = 0.01 = 2.713$

$p = 0.001 = 3.570$

The calculated value for t exceeds those from the table, it may therefore be concluded that the means are derived from two populations, i.e. there is a significant difference between them.

Table A5 Summary of Computer Programmes employed

Programme	Author	Reference
One way analysis of variance with equal replication	A. Polwart	Snedecor & Cochran (1967)
One way analysis of variance with unequal replication	A. Polwart	op, cit.
One way analysis of variance, Latin Square	A. Polwart	op. cit.
Analysis of variance, 2 factors with replication	-	University College of N. Wales, Bangor (1974)
Analysis of variance, 2 factors without replication	A. F. Grundy	Rao (1952)
Linear correlation & regression analysis	P. E. Smith	Snedecor (1946)
Polynomial regression analysis	U.M.R.C.C. Applied Statistics Volume 2	Cooley & Lohnes (1971)
Multiple regression analysis	U.M.R.C.C. SPSS	Nie *et al*. (1975)
Temperature data analysis	P. E. Smith	

APPENDIX B. Polynomial Regression Equations

APPENDIX B Table 1 Banchory-Kerloch temperature regression relationships

Probe height	Number of Data Pairs	R^2	Regression equation	Significance (p)
140cm.	311	0.904	$\hat{y} = -2.858669 + 0.656923x + 0.023212x^2$	< 0.001
22cm.	342	0.907	$\hat{y} = -2.93382 + 0.72811x + 0.020345x^2$	< 0.001
15cm.	313	0.963	$\hat{y} = -1.984408 + 0.527295x + 0.026342x^2$	< 0.001
0cm.	342	0.897	$\hat{y} = -1.497861 + 0.061029x + 0.104066x^2 - 0.00324x^3$	< 0.001
-5cm.	342	0.914	$\hat{y} = 0.511931 - 0.346137x + 0.129663x^2 - 0.004267x^3$	< 0.001
-15cm.	342	0.761	$\hat{y} = 1.106740 - 0.301778x + 0.113720x^2 - 0.003825x^3$	< 0.001
stem	303	0.910	$\hat{y} = -3.093795 + 0.684437x + 0.023197x^2$	< 0.001
0cm (treated)	153	0.800	$\hat{y} = -3.232829 + 1.065981x$	< 0.001

(x : measured temperature (°C), Banchory; $\hat{y}$: calculated temperature (°C), experimental site, Kerloch)

APPENDIX B Table 2 Penkridge - Cannock Chase temperature regression relationships

Probe height	Number of Data Pairs	R^2	Regression equation	Significance (p)
60cm.	417	0.897	$\hat{y} = -0.38918 + 0.207842x + 0.088187x^2 - 0.002346x^3$	< 0.001
20cm.	433	0.880	$\hat{y} = -2.020638 + 0.886244x + 0.013022x^2$	< 0.001
0cm.	431	0.871	$\hat{y} = -0.818182 + 0.453173x + 0.059953x^2 - 0.001476x^3$	< 0.001
-5cm.	433	0.873	$\hat{y} = 0.393504 + 0.017402x + 0.110927x^2 - 0.003185x^3$	< 0.001
-15cm.	433	0.861	$\hat{y} = 0.746857 - 0.051982x + 0.113785x^2 - 0.003343x^3$	< 0.001
stem	387	0.886	$\hat{y} = -0.954221 + 0.404553x + 0.07523x^2 - 0.001865x^3$	< 0.001
stem (treated)	391	0.885	$\hat{y} = -1.090711 + 0.330659x + 0.086835x^2 - 0.002243x^3$	< 0.001

(x : measured temperature (°C), Penkridge; $\hat{y}$: calculated temperature (°C), experimental site, Cannock Chase)

APPENDIX B Table 3 Resaturation-CRWC Regression relationships for *C. vulgaris* (control) (Cannock Chase)

Sampling Date	Nos. of pairs of data	R^2	Regression equation	Significance (p)
20.11.74.	50	0.149	$\hat{y}=0.230229-0.00251x+0.00031x^2$	< 0.05
11.12.74.	68	0.397	$\hat{y}=1.131564-0.034472x+0.000277x^2$	< 0.01
23.01.75.	65	0.350	$\hat{y}=8.449614-0.200739x+0.0012x^2$	< 0.01
21.02.75.	60	0.365	$\hat{y}=3.167723-0.080461x+0.000523x^2$	< 0.01
18.03.75.	70	0.778	$\hat{y}=2.099832-0.062905x+0.000487x^2$	< 0.001
18.04.75.	60	0.527	$\hat{y}=3.93377-0.100215x+0.000654x^2$	< 0.001
15.05.75.	60	0.632	$\hat{y}=3.033995-0.082697x+0.000577x^2$	< 0.001
19.06.75.	60	0.582	$\hat{y}=2.404291-0.067117x+0.000488x^2$	< 0.01
21.07.75.	60	0.773	$\hat{y}=3.381384-0.090617x+0.000636x^2$	< 0.001
20.08.75.	59	0.397	$\hat{y}=-1.03304+0.014835x$	< 0.001
24.09.75.	60	0.258	$\hat{y}=-0.799118+0.012484x$	< 0.001
24.10.75.	70	0.348	$\hat{y}=-1.287261+0.016997x$	< 0.001
19.11.75.	70	0.440	$\hat{y}=3.822384-0.097231x+0.000629x^2$	< 0.01
10.12.75.	70	0.759	$\hat{y}=-10.456545+0.441109x-0.006087x^2+0.000028x^3$	< 0.01
21.01.76.	70	0.661	$\hat{y}=0.000592+0.020615x-0.000542x^2+0.000004x^3$	< 0.01
25.02.76.	70	0.340	$\hat{y}=-0.807072+0.011976x$	< 0.001
24.03.76.	70	0.284	$\hat{y}=1.820015-0.053586x+0.000398x^2$	< 0.001
21.04.76.	70	0.726	$\hat{y}=5.520186-0.139489x+0.000896x^2$	< 0.001
27.05.76.	70	0.730	$\hat{y}=2.233159-0.063179x+0.000465x^2$	< 0.001
22.06.76.	65	0.884	$\hat{y}=1.238378-0.036933x+0.000299x^2$	< 0.001
19.07.76.	65	0.585	$\hat{y}=4.424846-0.115754x+0.000785x^2$	< 0.01

(x : percentage resaturation of desiccated shoots; $\hat{y}$: CRWC)

APPENDIX B Table 4 Resaturation-CRWC Regression relationships for *C. vulgaris* (treated) (Cannock Chase)

Sampling Date	Nos. of pairs of data	R^2	Regression equation	Significance (p)
10.12.75	36	0.163	$\hat{y}=-0.352643+0.006337x$	< 0.05
21.01.76	36	0.379	$\hat{y}=2.483754-0.064562x+0.000435x^2$	< 0.01
25.02.76	36	0.472	$\hat{y}=1.676429-0.048281x+0.00036x^2$	< 0.01
24.03.76	36	0.388	$\hat{y}=1.666755-0.047345x+0.00035x^2$	< 0.05
21.04.76	35	0.454	$\hat{y}=1.391935-0.040289x+0.000301x^2$	< 0.001
27.05.76	35	0.630	$\hat{y}=2.778454-0.077109x+0.000543x^2$	< 0.001
22.06.76	39	0.837	$\hat{y}=1.519779-0.043912x+0.000333x^2$	< 0.001
19.07.76	39	0.239	$\hat{y}=-0.407536+0.00708x$	< 0.01

(x : percentage resaturation of desiccated shoots; $\hat{y}$: CRWC)

APPENDIX B Table 5 Visual Damage - Resaturation regressions, *Calluna vulgaris* (Cannock Chase)

Sampling Date	Nos. of pairs of data	r	Regression equation	Significance (p)	Resaturation at visual damage = 50%
10.12.74	19	-0.881	$\hat{y}=91.934 - 0.228x$	< 0.001	80.5
18.03.75	55	-0.763	$\hat{y}=94.693 - 0.158x$	< 0.001	86.8
18.04.75	60	-0.762	$\hat{y}=87.879 - 0.788x$	< 0.001	78.5
15.05.75	60	-0.691	$\hat{y}=92.641 - 0.142x$	< 0.001	85.5
19.06.75	60	-0.855	$\hat{y}=93.249 - 0.226x$	< 0.001	81.9
21.07.75	60	-0.760	$\hat{y}=91.296 - 0.177x$	< 0.001	82.4
20.08.75	60	-0.663	$\hat{y}=94.693 - 0.155x$	< 0.001	86.9
24.09.75	60	-0.505	$\hat{y}=94.454 - 0.134x$	< 0.001	87.7
24.10.75	70	-0.657	$\hat{y}=96.535 - 0.113x$	< 0.001	90.9
19.11.75	70	-0.792	$\hat{y}=98.093 - 0.148x$	< 0.001	90.7
10.12.75	70	-0.637	$\hat{y}=93.278 - 0.159x$	< 0.001	85.3
21.01.76	70	-0.694	$\hat{y}=88.787 - 0.175x$	< 0.001	80.2
25.02.76	70	-0.577	$\hat{y}=92.144 - 0.113x$	< 0.001	86.5
24.03.76	70	-0.399	$\hat{y}=92.055 - 0.077x$	< 0.001	88.2
21.04.76	70	-0.816	$\hat{y}=94.651 - 0.156x$	< 0.001	86.9
27.05.76	70	-0.771	$\hat{y}=89.429 - 0.156x$	< 0.001	81.6
22.06.76	64	-0.861	$\hat{y}=92.669 - 0.248x$	< 0.001	80.3
19.07.76	65	-0.402	$\hat{y}=87.351 - 0.079x$	< 0.001	83.4

(x: visual damage estimate; $\hat{y}$: percentage resaturation of desiccated shoot)

APPENDIX B Table 6 RWC-ψ_x regressions, *C. vulgaris* (Cannock Chase)

Sampling Date	Nos. of pairs of data	R^2	Regression equation	Significance (p)
03.12.74	50	0.903	$\hat{y}=27.438607-122.484019x+164.469976x^2-69.705268x^3$	< 0.001
15.05.75	67	0.967	$\hat{y}=-2.761174-13.361143x+35.682428x^2-19.719267x^3$	< 0.001
21.07.75	35	0.918	$\hat{y}=-12.042119+25.907089x-14.000313x^2$	< 0.001
20.08.75	31	0.965	$\hat{y}=16.961689-101.715913x+158.494759x^2-73.878707x^3$	< 0.01
24.09.75	45	0.876	$\hat{y}=-4.460494-2.002631x+6.481787x^2$	< 0.05
24.10.75	26	0.911	$\hat{y}=12.321605-79.508006x+126.097971x^2-59.044252x^3$	< 0.05
19.11.75	52	0.936	$\hat{y}=3.190789-43.997655x+81.016614x^2-40.345162x^3$	< 0.001
10.12.75	61	0.622	$\hat{y}=-7.414865+7.073569x$	< 0.001
21.01.76	38	0.867	$\hat{y}=7.741863-60.761107x+96.808662x^2-43.899351x^3$	< 0.05
25.02.76	46	0.601	$\hat{y}=-2.964402-5.941059x+8.858864x^2$	< 0.001
24.03.76	31	0.840	$\hat{y}=-13.058562+27.008945x-13.954219x^3$	< 0.001
21.04.76	68	0.717	$\hat{y}=-0.283206-28.096776x+62.489091x^2-34.332409x^3$	< 0.05
27.05.76	74	0.741	$\hat{y}=-6.103792+6.025041x$	< 0.001
22.06.76	56	0.939	$\hat{y}=-4.435057+0.505289x+3.891139x^2$	< 0.01
19.07.76	96	0.875	$\hat{y}=3.362859-46.19775x+88.792818x^2-46.137133x^3$	< 0.001

(x : shoot RWC; $\hat{y}$: ψ_x)

APPENDIX B Table 7 RWC - F_n regressions, *C. vulgaris* (Cannock Chase)

Sampling Date	Nos. of pairs of data	R^2	Regression equation	Significance (p)
19.11.75	56	0.339	$\hat{y}=-1.603188+5.018464x-2.853795x^2$	< 0.001
10.12.75	65	0.617	$\hat{y}=0.676162-4.99495x+11.572376x^2-6.927543x^3$	< 0.001
21.01.76	85	0.648	$\hat{y}=-0.127405-2.96653x+12.665297x^2-9.03o403x^3$	< 0.001
25.02.76	61	0.548	$\hat{y}=-1.391841+5.97972x-4.43098x^2$	< 0.001
24.03.76	66	0.591	$\hat{y}=-2.0970646+8.2779808x-6.3125375x^2$	< 0.001
24.04.76	84	0.558	$\hat{y}=-1.1960472+5.1315078x-3.7003482x^2$	< 0.001
27.05.76	85	0.629	$\hat{y}=-2.4197886+8.9266689x-5.681374x^2$	< 0.001
22.06.76	75	0.704	$\hat{y}=1.2841446-10.586929x+24.698457x^2-15.005386x^3$	< 0.001
19.07.76	91	0.643	$\hat{y}=-3.793988+11.848909x-7.594065x^2$	< 0.001

(x : shoot RWC; $\hat{y}$: F_n ($\mu g\ g^{-1}\ s^{-1}$))

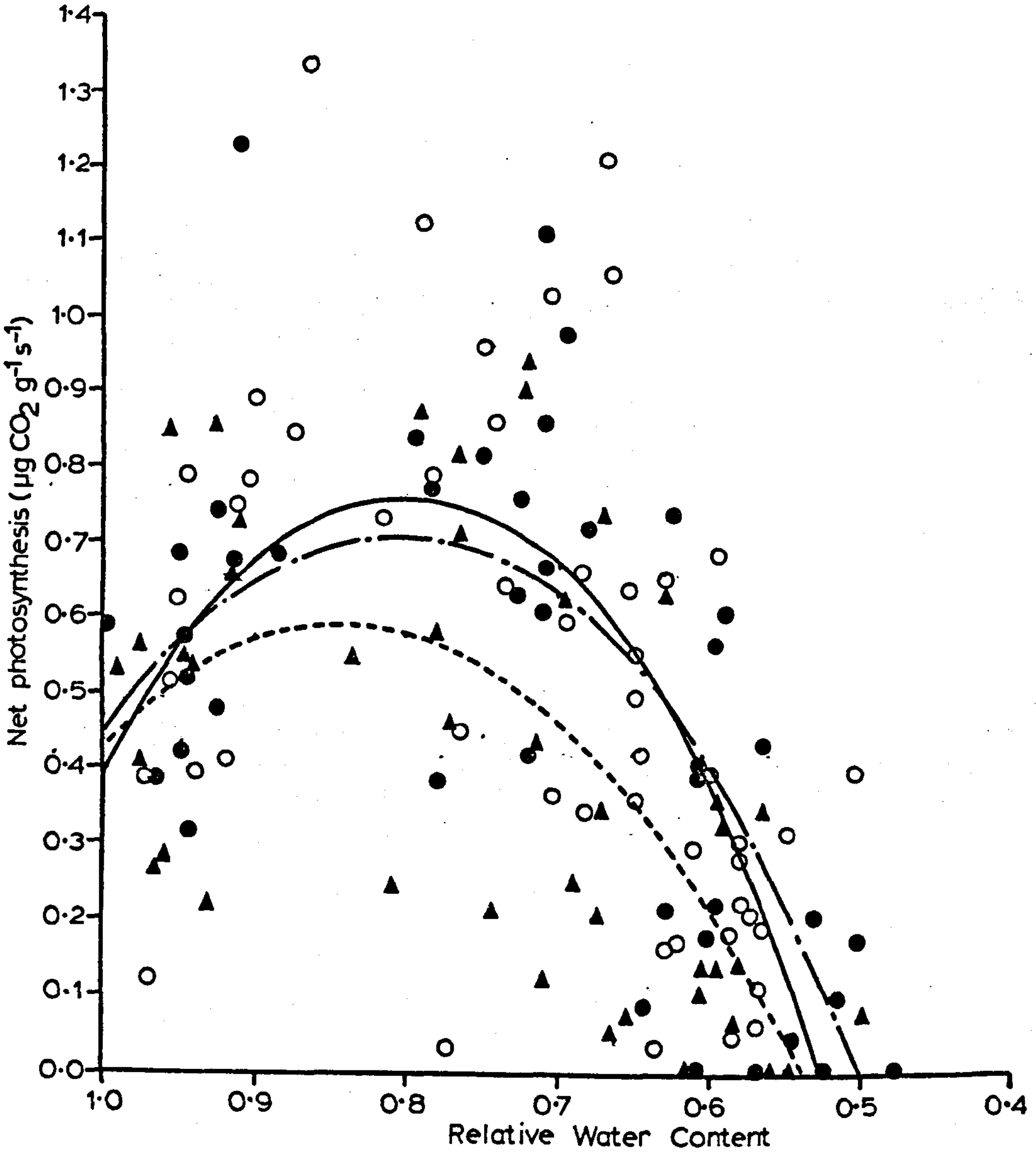

Figure C1 The effect of shoot storage on the relationship between RWC and net photosynthesis, C. vulgaris, (Cannock Chase).

○ ——— 24h storage period, $y = -5{\cdot}488379 + 15{\cdot}553547x - 9{\cdot}660425x^2$

$R^2 = 0{\cdot}345$ ($p < 0{\cdot}001$)

● –·–·– 48h storage period, $y = -4{\cdot}207908 + 12{\cdot}135360x - 7{\cdot}453474x^2$

$R^2 = 0{\cdot}478$ ($p < 0{\cdot}001$)

▲ ------- 72h storage period, $y = -3{\cdot}574652 + 9{\cdot}764278x - 5{\cdot}720125x^2$

$R^2 = 0{\cdot}374$ ($p < 0{\cdot}001$)

APPENDIX C SOURCES OF ERROR ASSOCIATED WITH THE DETERMINATION OF CO_2 UPTAKE BY C. VULGARIS

(i) Effect of storage of shoots on CO_2 uptake

(a) Method

30 shoots were sampled from the field and subject to the following experimental regimes:

(1) 10 replicates were placed in the saturation chambers and stood for 24 hours in the dark at $25 \pm 1^{\circ}C$ to allow them to become fully saturated, (24 hours storage period);

(2) 10 shoots were stored for 24 hours at $3^{\circ}C$, followed by the saturation procedure as in (a), (48 hours storage period);

(3) 10 shoots were stored for 48 hours at $3^{\circ}C$ in the dark, followed by saturation after storage as in (a), (72 hour storage period).

Following saturation, the CO_2 uptake/RWC relationship was determined as described in Section IVA (c). The results were subjected to polynomial regression analysis to give three relationships between the two variables for the respective time periods.

(b) Results

(The results have not been subjected to analysis of variance, consequently, differences existing between the three sets of shoots are by inference).

The regression relationships are presented in Figure C1 and indicate that with increasing length of storage time there is a tendency for CO_2 uptake to be reduced. The difference in maximal CO_2 uptake is not marked between the 24h. and 48h. storage periods, and the effect of water deficits on CO_2 uptake appears similar on both days. The relationship for shoots stored for 72h. shows a more substantial decrease in CO_2 uptake, and F_n seems generally more sensitive to water stress, indicated by the lower water deficits required to reduce CO_2 uptake. However, whereas the range of maximum CO_2 uptake is appreciable between the three analysis times the range at an RWC of 1.0 is appreciably smaller and the RWC range over which CO_2 uptake becomes zero is also narrow.

(c) Discussion

Storage of *C. vulgaris* shoots at 3°C in the dark prior to the measurement of CO_2 uptake, appears to have an adverse effect on CO_2 uptake, such that with increased storage time the maximal rates of CO_2 uptake are reduced. This may result from enzyme depletion in cut shoots, possibly due to removal of a cytokinin supply from the roots, which is involved in D-ribulose-1, 5-diphosphate carboxylase synthesis (Wareing *et al.*, 1968).

Storage in the dark at low temperatures may retard stomatal opening (cf. Loftfield, 1921; Stalfelt, 1962), and thus limit maximal CO_2 uptake by increasing r_s, assuming r_s effectively limits CO_2 uptake in *C. vulgaris*.

The evidence suggests that storage time between sampling and measurement of CO_2 uptake should be minimized. In the current investigation (Section IVA (c)) shoots were stored for 24 hours followed by saturation for 24 hours (i.e. a 48 hours storage period). The maximal CO_2 uptake rates measured on a monthly basis are likely to be slightly depressed compared to the potential maximum rates attainable under optimum conditions. However, the difference observed is not great and possibly of little significance. The monthly determinations have been made on shoots stored for a standard period of time, thus, although F_n

maximum values may not be precise, comparisons between them are valid.

(ii) Effect of temperature on CO_2 uptake

(a) Introduction

The temperature response of F_n is defined by three points, the low and high temperature compensation points at which F_n is zero, and an optimum temperature at which F_n is maximal (Larcher, 1969). Seasonal changes in these points have been observed by a number of workers, e.g. Neilson *et al.* (1972) found that the temperature optimum for F_n in *Picea sitchensis* decreased to a minimum of 10°C in May and a peak of 20°C in October, associated with which were changes in the high temperature compensation point, such that temperatures of 29-30°C between February and July resulted in a large reduction in shoot F_n which was not so evident between August and January. Pearcy *et al.* (1974) similarly found that increases in temperature above the optimum caused a marked decrease in F_n in *Atriplex hastata*. Studies on *Encelia californica* by Mooney & Harrison (1970) revealed that cold treated plants had a lower F_n capacity at high analysis temperatures than warm treated plants but that a warm pretreatment before analysis increased CO_2 uptake at all temperatures. This was associated with biochemical changes affecting r_m and they commented that "... not only are the absolute photosynthetic values attained for any given plant dependent on its thermal history, but that the entire configuration of the photosynthetic temperature response curve may be altered". The implication of such a response is that any seasonal shift in the temperature optimum for F_n, as observed by Neilson *et al.* (1972), Lange *et al.* (1974), Sawada & Miyachi (1974), may result in erroneous values for maximum rates of F_n when measured at a standard temperature which is other than the optimum temperature for

F_n. In C. vulgaris the temperature optimum of F_n does not appear to vary with thermal prehistory, although under standard conditions F_n is related to the temperature regime from which plants have been taken, and may be modified by a few days exposure to a different regime (Grace & Woolhouse, 1970).

(b) Method

Six shoots of C. vulgaris, (sampled from the Cannock Chase site), were sequentially exposed to an increase in temperature from 12.5°C to 30°C. Relative humidity was maintained at 75 ± 10%, by adjusting the dew point temperature of the humidity control unit (Section IVA (b)). The long period required for temperature stabilization following adjustment (i.e. up to 60 minutes) resulted in each shoot being subjected to a period of experimentation of considerable duration (i.e. in excess of 5 hours). Between successive determinations shoots were stood in humid chambers to maintain a high RWC, but this necessarily exposed them to fluctuating temperatures which may have modified the CO_2 uptake/temperature response.

The possible effects of enzyme depletion on CO_2 uptake over time in severed shoots may cause an erroneous decrease in CO_2 uptake. For this reason it was decided to progress from a low temperature upwards since, if the reverse procedure were to be adopted, it would not be possible to discriminate between the effect of enzyme depletion and the effect of decreasing temperature on the reduction of CO_2 uptake by the plant.

During the determination of CO_2 uptake on a single shoot, a water supply to the shoot was maintained by nylon capillary tubing. At the end of each determination, the shoots were weighed for RWC determinations.

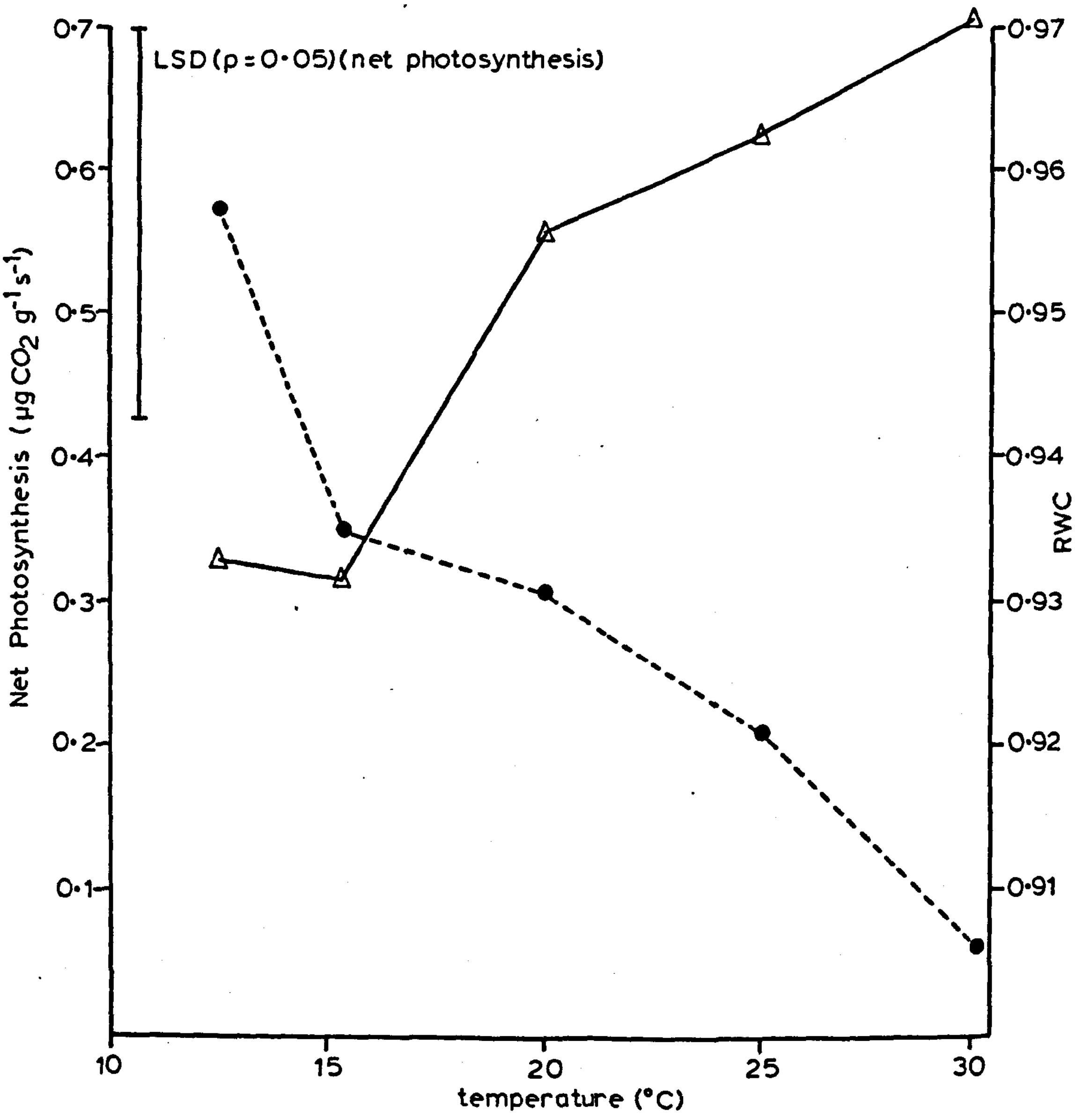

Figure C2 The effect of temperature on net photosynthesis and RWC of <u>C. vulgaris</u> shoots, (Cannock Chase).

△———△ net photosynthesis

●- - - - - - -● shoot RWC

(c) Results

There is a significant ($p < 0.05$) increase in CO_2 uptake with increase in temperature. The main increase occurs between a temperature of 15.5°C and 20°C, above which the rate of increase in CO_2 uptake declines. Between a temperature of 12.5°C and 15.5°C there is no significant change in CO_2 uptake. Despite maintenance of a water supply to the shoot the RWC decreases from 0.9575 to 0.9059 over the temperature range 12.5°C to 30°C. The decrease is not significant (Figure C2).

(d) Discussion

In the monthly determinations of CO_2 uptake by shoots of C. vulgaris, the air temperature at which CO_2 uptake was measured was standardized to 20 ± 1°C. Leaf temperatures are commonly higher than ambient temperatures e.g. Gaastra (1959), Mellor et al. (1964), Barthakur (1975). This effect may be aggravated in assimilation chambers, where low air velocities reduce heat transfer so that the difference between leaf and air temperatures may be greater than those experienced in the field, and may be increased by increasing light intensity. For example, with an air temperature of 12°C, an irradiance of $300 Wm^{-2}$ and an air flow rate of $0.0831 s^{-1}$, turnip leaf temperatures of 21.6°C were found (Gaastra, 1959). In the absence of small thermocouples with which to measure leaf temperature in the present study, the difference between air and leaf temperature remains unquantified. Thus, an initial source of error in CO_2 uptake determinations in the present study is the absence of a defined leaf temperature. Although air temperature was standardized, and leaf temperature was assumed to be the same under constant environmental conditions in the monthly determinations, this assumes a constant energy balance of the leaves from month to month. This may be invalid, for example, as a result of monthly changes in leaf coloration.

It is also necessary to evaluate the effects of temperature prehistory on the measurement of CO_2 uptake at a standard temperature over several months. Although the observations of Grace & Woolhouse (1970) suggest that measurement of CO_2 uptake at a standard temperature will have a constant effect on F_n from month to month, the rapid acclimation which may occur during the 24 hour saturation period may introduce an error. If F_n increases when shoots are transferred from a cold to a warm regime (Grace & Woolhouse, 1970) winter measurements may overestimate the true photosynthetic capacity of shoots under field conditions. Such acclimation may be responsible for the present results since there is no evidence of a decline in CO_2 uptake at 30°C whereas Grace & Woolhouse (1970) reported a temperature optimum of 18°C above which F_n declined. However, the effect may also arise from hydropassive stomatal opening. The decrease in RWC at higher temperatures, possibly as a result of increased transpiration, may result in decreased r_s so that the increase in CO_2 uptake is responding to changes in stomatal resistance rather than directly to temperature, whereas at low temperatures stomatal opening may be inhibited (Loftfield, 1921; Stalfelt, 1962) so reducing CO_2 uptake. However, this depends upon how sensitive CO_2 uptake is to changes in stomatal aperture (cf. Section IVA, a(i)).

The problems of adjusting measured rates of F_n to seasonal changes in temperature makes it desirable to adopt a standard temperature for initial investigations. Seasonal variation in the temperature response of r_s (cf. Neilson _et al._, 1972; Lange _et al._, 1971, 1974; Schulze _et al._, 1973) complicates the relationship between temperature and CO_2 uptake and further justifies the use of a standard temperature when such responses have not been quantified.

(iii) Miscellaneous Errors

Diurnal rythms in CO_2 uptake in C. vulgaris (Grace & Woolhouse, 1970) make it expedient to restrict CO_2 uptake measurements to daylight periods only, although this does not take into account any diurnal rythms in stomatal aperture occurring during daylight hours which may affect CO_2 uptake.

Grace & Woolhouse also found that removal of shoots with roots from plants maintained under standard conditions did not affect F_n while Neilson et al. (1972) found that shoot detachment in Picea sitchensis had little effect on shoot F_n for the succeeding 72 hours. Clark (1954) however, found that following severance of Picea abies branches, F_n remained constant for 20 minutes,and then declined, thus the method of using cut shoots may, itself, lead to error.

Since it was necessary to express CO_2 uptake on a dry weight basis, seasonal changes in dry to fresh weight ratio may lead to error, if the apparent increase in dry weight is unrelated to the assimilatory mechanism. Grace & Woolhouse (1970) considered the possibility that changes in F_n per unit dry weight were associated with changing dry weight of single leaves caused by changes in sugar content, but concluded that seasonal changes in F_n were too large to be fully accounted for in this way. Lange et al. (1974) expressed rates of F_n on a dry weight, surface area and chlorophyll content basis and concluded that "... seasonal changes (in F_n) cannot be explained by variations in the base values of the calculations." The absence of a significant correlation between F_n maximum and dry to fresh weight ratio in the present study suggests that seasonal variation in F_n does not result from variations in the basis of expression.

Flowering also has a significant effect on F_n in C. vulgaris leading to an increase which is related to the quantity of flowers per unit dry weight of shoots (Grace & Woolhouse, 1970). Flower removal was observed, by these workers, to reduce F_n but by autumn the effect of flowering on F_n was no longer apparent. Since the present investigation runs from November 1975 to July 1976 the possible effects of flowering may therefore be considered non-existent.

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