THE FUNCTIONAL RESPONSE OF PLANTS TO TRAMPLING PRESSURE

VOLUME I OF II VOLUMES

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ABSTRACT

The responses of Calluna vulgaris, Phleum bertolonii and Plantago lanceolata to trampling pressure, applied at controlled intensities and frequencies, were investigated.

Substantial water stresses develop within shoots of C. vulgaris following damage. The magnitude of such stresses is related to the plant's ability to avoid desiccation and the consequences of such stresses on plant survival are related to its capacity to tolerate desiccation. Both physiological parameters are influenced by plant microclimate, which also has repercussions on the rapidity with which water stresses develop following trampling and the degree of desiccation injury suffered by the plant. Net primary productivity is also reduced by trampling pressures, both through direct mechanical effects and the effects of water stress on plant assimilation and metabolic activity. The susceptibility of the plant to water stress is variable, being related to environmental factors and the stage of development of the plant. Consequently, tolerance by C. vulgaris to trampling may be regarded as variable.

The responses of P. bertolonii and P. lanceolata to simulated pressures have been considered in terms of the plant's capacity to recover following damage. Trampling induced soil compaction may have a detrimental effect on the ability of the plant to grow. Simulated trampling results in damage and defoliation of the shoot. Evidence indicates that following damage, an elevation in growth rate may occur which may be related to an increased photosynthetic capacity. These effects have been discussed in relation to hypothetical consequences of reduced competition for root produced metabolites and the role of assimilates in aiding recovery. It is postulated that increased durability of the plant to trampling pressure is dependent upon increased

growth rates.

Since both tolerance and recovery following trampling are variable and dependent upon plant function, it is suggested that the 'carrying capacity' of vegetation is variable and related to the physiological activity of individuals composing the community.

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Frequently Used Symbols

Symbol	Description	Unit
BRN	Visible damage estimate of trampled plot, at time of measurement	
BRN'	Visible damage increase of trampled plot, related to time of treatment	
CRWC	Critical Relative Water Content, defining desiccation tolerance	
RWC	Relative Water Content, of field shoots	
SCI	RWC at stomatal closure ('Intersection' method)	
SCL	RWC at stomatal closure ('Lopushinsky' method)	
B_1, B_2	Biomass at time 1 and time 2 respectively	(g; kg)
E	Transpiration flux density	
F_n	Net photosynthesis	(μg $g^{-1}s^{-1}$)
F'_n	Estimated rate of net photosynthesis	(μg $g^{-1}s^{-1}$)
P	Water potential pressure chamber value	(MPa)
P_n	Net primary production	(g m^{-2})
P_r	Net primary productivity	(g $m^{-2}y^{-1}$)
R	Total resistance to water movement	
R_l	Resistance to water movement in leaf	
R_r	Resistance to water movement in root	
R_{sl}	Resistance to water movement in soil	
R_x	Resistance to water movement in xylem	
$\bar{R}$	Mean relative growth rate	(g $g^{-1}wk^{-1}$)
$\bar{R}_t$	Mean relative growth rate, total plant	(g $g^{-1}wk^{-1}$)
$\bar{R}_l$	Mean relative growth rate, live	(g $g^{-1}wk^{-1}$)
R_{max}	Maximum relative growth rate	(g $g^{-1}wk^{-1}$)
T	Transpiration	
W_1, W_2	Weight at time 1 and time 2 respectively	(g; kg)

Symbol	Description	Unit
$i\ pt^{-1}\ (wk^{-1})$	Impacts per pot (per week)	
$p\ pl^{-1}\ (wk^{-1})$	Walking passages per plot (per week)	
r_a	Boundary layer (air) resistance to diffusion	
r_c	Cuticular resistance to diffusion	
r_m	Mesophyll resistance to diffusion	
r_s	Stomatal resistance to diffusion	
t_1, t_2	Time 1 and time 2 respectively	
y	Year	
h	Hour	
min	Minute	
s	Second	
ψ	Water potential	
ψ_g	Hydrostatic potential	
ψ_1	Leaf water potential	
ψ_1^{tc}	Leaf water potential, determined by thermocouple psychrometer	
ψ_s	Solute potential	
ψ_s^{xy}	Combined solute & matric potentials	
ψ_{s1}	Soil water potential	
ψ_{tp}	Turgor pressure potential	
ψ_x	Xylem pressure potential ($\equiv$ xylem water potential)	(MPa)
ψ_x^P	Xylem water potential, determined with pressure bomb ($\equiv$ xylem pressure potential)	
π	Osmotic potential	
τ	Matric potential	

(Suffixes 1, 3 and 6 indicate the period (in months) between the time of treatment and the time of parameter measurement. Suffix 0 indicates control value)

(Units refer to those employed in the current study)

PART I - INTRODUCTION

(a) General introduction

(i) Historical perspective

Early workers in the field of recreational ecology were concerned primarily with the vegetational response to recreational pressures, for example, Jeffreys (1917) observed that tall stemmed plants were at a disadvantage with respect to survival on footpaths. Bates (1935) quantified the response of plants to trampling and suggested that treading selectively eliminated those species not structurally adapted to withstand trampling injury. Survival strategies were based either upon plant morphology, e.g. rosette plants, or phenology, for example, disturbed areas were found to be populated in summer by annuals or seedling perennials, e.g. Plantago major, capable of resisting trampling because of life form and habit and thus able to exist through the summer and autumn. In contrast, more susceptible perennials occupied less disturbed areas and either seeded in summer before winter damage eradicated them or existed vegetatively in times of greatest disturbance. Bates also attempted to determine, experimentally, the relative tolerances to trampling of a number of species and it may be justifiably concluded that he pioneered the use of analytical and experimental approaches in combination to determine the response of vegetation to trampling (Liddle, 1975a).

Following the investigations of Bates, little work was done until the mid 1960's when the problem of reconciling ecological value and recreational use became more acute. Increasing mobility and desire for informal recreation resulted in increased pressure on semi-natural areas, often of high conservation value (Liddle, 1975a) and also on areas of less conservation value but equally important from resource user orientation (Burton, 1974). Overuse, leading to resource degradation, confronts planning and management schemes with the need for information concerning the capacity of use which sites will withstand and the vegetation and environmental change resulting from such use. The underlying need is to describe, both qualitatively and quantitatively, the ecological processes involved in the response of vegetation to recreational pressures.

Liddle (1975a) divided the approaches hitherto used into analytical and experimental and it is relevant to briefly review these approaches, (more extensive reviews are given by Speight, 1973; Tivy, 1973 and Liddle, 1975a).

(ii) The Analytical approach

This approach, basically descriptive, involves ecological surveys of paths and other areas of recreational use, e.g. Bates (1935), Davies (1938), Westhoff (1967), Goldsmith *et al*. (1970), Marr & Willard (1970), Willard & Marr (1970), Bayfield (1971), Chappell *et al*. (1971), Streeter (1971), Burden & Randerson (1972), Burton (1974), Dale & Weaver (1974), Liddle & Greig-Smith (1975b).

The assumption inherent in such studies is that differences between pressurized and control areas are attributable to recreational effects and that prior to the start of trampling the area had a homogenous vegetation cover (Chappell *et al*., 1971). Trampling effects may be indirect however, (Burton, 1974), affecting edaphic factors (Canaway, 1975a), altering the microclimate and changing the light regime in the canopy. The intensity of pressure a site has experienced may be ascertained by direct observation e.g. Hammond (1967), La Page (1967), Schofield (1967), Bayfield (1971, 1973), Leney (1974), or indirectly by measuring some parameter which can be related to intensity of use, for example, soil compaction parameters such as bulk density (Chappell *et al*., 1971), or penetration resistance (Liddle & Greig-Smith, 1975a). From such estimates of use it is possible to relate observed biotic differences to varying intensities of use. Various methods have been employed to ascertain vegetational differences, for example, transects across paths, e.g. Goldsmith _et al_. (1970), Bayfield (1971), Chappell _et al_. (1971), Streeter (1971), Dale & Weaver (1974), Liddle & Greig-Smith (1975b), by which cover, frequency or biomass of the plant may be related to trampling intensity. Certain investigations may be more amenable to the study of a limited number of plots, for example, where no precisely defined pathways exist, e.g. Marr & Willard (1970), Willard & Marr (1970, 1971) or where sites of larger areal extent are involved e.g. Leney (1974). Alternatively, permanent plots may be used to study vegetational change over a number of seasons e.g. La Page (1967), Greller _et al_. (1974).

The effects of a given intensity of recreational pressure on a site depends upon the biotic and physical environment, the management regime and the conditions at the time of trampling. Consequently, results obtained tend to be applicable to the site from which they were obtained rather than of general applicability. This may be illustrated by reference to the work of Schofield (1967) who observed that 7,500 people visiting a salt marsh over a season effectively destroyed the vegetation cover whilst only 3,500 to 4,500 on a grey dune system caused soil and sand exposure. In contrast, 11 skiers per hectare per day effectively reduced vegetation cover in the mountain environment of the Cairngorms (Bayfield, 1971) whilst in a deciduous woodland in Hampshire, 50 people passing over a particular area on one day had a deleterious effect, which persisted for a number of months, on _Mercurialis perennis_ (Barker, 1967). One problem immediately apparent in comparing the results of different workers is the lack of a unified terminology for the expression of data and the methods used by Bayfield (1971) have much to commend them. It is evident from the figures quoted above that cause i.e. trampling intensity, and effect i.e. vegetational change, should not be treated in isolation and that different ecosystems respond differently to recreational use. However, principles underlying vegetational reaction to trampling may be formulated.

Although not relevant to list the relative tolerances of all species whose reaction to trampling is known (cf. Speight, 1973), it is of interest to note the generalised reaction of plants to trampling. Certain species, e.g. Asperula cynanchica, Calluna vulgaris, Leontodon hispidus, Mercurialis perennis, Poterium sanguisorba, Thymus drucei, Viola hirta have been reported as being very sensitive to trampling pressures whilst others, e.g. Agropyron pungens, Agrostis stolonifera, Agrostis tenuis, Armeria maritima, Carex flacca, Cerastium semidecandrum, Centaurea scabiosa, Pastinaca sativa, Plantago coronopus, Plantago major, Senecio jacobea, Taraxacum officinale, have been reported, by various authors, as ranging from less sensitive than the aforementioned to tolerant to trampling pressure. Certain species are notably tolerant of trampling, e.g. Bellis perennis, Cynosurus cristatus, Dactylis glomerata, Festuca rubra, Poa annua, Poa Pratensis, Lolium perenne, Plantago lanceolata, Trifolium repens, and a certain level of trampling may even be a prerequisite for their continued survival at a particular location (Westhoff, 1967; Liddle & Greig-Smith, 1975b) by removal of competition from other species less tolerant of trampling. Liddle (1975a) noted that most species which show resistance to trampling survive by adopting vegetative strategies (cf. Bates, 1935) although some, e.g. Poa annua, adapt to flowering when trampling is at a comparatively low level, thus ensuring continued survival of the species even if not of the individual plant.

A distinction may be made between a plant's ability to withstand wear, i.e. 'wear tolerance', and its capacity to recover from wear, i.e. 'recuperative potential', which, in combination, determine the 'durability' of the vegetation (Canaway 1975a). The experimental approach may be employed to determine the relative importance of these components of durability.

(iii) The Experimental approach

This approach has been pursued by a number of workers, e.g. Edmond (1958-1966), Wagar (1964, 1965), La Page (1967), Scott-Williams (1967), Cieslinski & Wagar (1970), Bayfield (1971), Burden & Randerson (1972), Leney (1974), Liddle & Greig-Smith (1975b). The main object of such studies is to quantify and evaluate processes involved in change in the ecosystem resulting from trampling and to identify methods by which such changes may be assessed in an attempt to generate regression equations by which ecosystem changes may be predicted, e.g. Wagar (1964), Cieslinski & Wagar (1970), Liddle (1975a), so enabling the durability of potential recreation sites to be evaluated. Ultimately, the objective is to accurately predict the 'carrying capacity' of a recreational site. In certain respects the approach has the same failings as the descriptive approach, namely, the relationships derived for a particular area are specific for the conditions, both biotic and physical, under which the experiment was performed. However, the methods may be useful, just necessitating a change in the equation constants.

One of the earliest experimental approaches was by Bates (1935) who, by trampling plots of vegetation of known species composition, identified those species resistant to trampling and the adaptations which gave them a selective advantage, such as folded rather than rolled leaves in the bud. The next relevant studies made considered the effect of sheep treading on various pasture species (Edmond, 1958, 1962, 1963, 1964, 1966). From his studies a number of conclusions can be enumerated:

(1) modification of soil structure resulting from treading led to plants being affected indirectly as well as directly (Edmond, 1958);

(2) recovery and reappearance of damaged and buried tillers was remarkably rapid (Edmond, 1958);

(3) lower herbage yield on heavily trodden plots was due to lower tiller density and the inability of immature tillers to grow as much as mature tillers, with the additional complication of possible nitrogen depletion due to the lack of legumes (Edmond, 1958);

(4) soil compaction and puddling resulted in impeded gaseous diffusion, leading to possible oxygen deficiency (Edmond, 1958);

(5) no critical treading threshold was observed, all increases in treading producing increased damage (Edmond, 1958);

(6) changes in botanical composition were more persistent than reduction in yield (Edmond, 1958);

(7) the first treading produced most of the measured effect (Edmond, 1958);

(8) different soil moisture conditions led to different treading effects, (Edmond, 1962);

(9) on dry soil most damage occurred through the direct effects to the shoot, i.e. bruising, crushing, breaking (Edmond, 1962);

(10) wet soil reduced some direct total yield effects but instead, disruption, displacement and burial of the aerial part of the plant occurred, combined with root damage (Edmond, 1962);

(11) in contrast to the initial set of experiments where effects were observable after the first treading treatment as a result of increased soil bulk density, the already high bulk density in the subsequent set of experiments resulted in no significant effects

being observed until the third treatment application (Edmond, 1962);

(12) soil moisture modified the effects of treading on Lolium perenne x, L. multiflorum (ryegrass) and Trifolium spp., the greatest effects occurring where soil was wet (Edmond, 1962);

(13) physical damage to the soil resulted in increased soil bulk density and decreased water holding capacity (Edmond, 1966);

(14) treading produced a significant reduction in herbage yield during most seasons although the effect was slight in summer. Rapid growth in the spring turned a large difference in yield into a smaller proportion of the total growth (Edmond, 1966);

(15) treading effects were greater on fertilized soil (Edmond, 1966);

(16) physical strength of ryegrass, which was found to be more tolerant of treading than the clovers, may be related to sclerenchyma tissue (Edmond, 1966);

(17) damage to elevated growing points of species such as Dactylis glomerata may influence tillering ability and thus yield (Edmond, 1966);

(18) young tillers were the most easily damaged, possibly due to their greater exposure (Edmond, 1966);

(19) although disruption of plants in wet soil may aid dispersal, this was offset by reduced vigour (Edmond, 1966);

(20) recovery follows a sigmoid pattern, faster growing plants overcoming damage most quickly (Edmond, 1966);

(21) rhizomes, for example of Poa pratensis, stolons, for example of Poa trivialis and creeping rather than upright stems, for example of Trifolium repens, may confer some ability to withstand treading (Edmond, 1966);

(22) treading alters plant competition so that treading resistant species such as perennial ryegrass, become increasingly dominant (Edmond, 1966).

Although these observations are derived from the effects of sheep treading, the general principles act as guide lines for studies into the effects of human trampling on vegetation. Human trampling at controlled levels has been employed by a number of workers, e.g. Bayfield (1971), Kellomaki (1973), Liddle (1973), Leney (1974) whilst artificial methods, such as tamps and rollers, have also been extensively used, e.g. Wagar (1964), Cieslinski & Wagar (1970), Bayfield (1971), Kellomaki (1973), Leney (1974), Shearman _et al_. (1974). A brief résumé of their methods and findings will be made to enable the present study to be placed in context.

Wagar (1964) attempted to describe relationships between vegetation, site factors and visitor use by using a tamp. From his results a number of independent variables were isolated which could be used to account for the reduction in plant weight due to the treatment. Although realising the limitations of the method, Wagar suggested that some possible relationships may be determined and some tentative conclusions proposed, namely:

(1) no threshold was apparent at which additional 'tamping' resulted in accelerated site damage (cf. Edmond, 1958);

(2) survival of vegetation decreased as simulated use increased;

(3) in the area studied, large changes in use caused only small changes in damage on areas where use was already heavy. In contrast, in areas of wilderness light use could result in marked changes in plant composition and appearance (cf. Willard & Marr, 1970);

(4) grasses were damaged less than herbs;

(5) shaded plants suffered less than plants in sunny situations, which appeared to be related to moisture retention, although competition for soil moisture and nutrients makes the problem more complex.

Wagar (1965) took this last point further by observing that water, together with other cultural treatments such as fertilizing and mulching, could increase the ability of vegetation to withstand trampling damage. Recovery of crushed vegetation was found to be improved on watered plots, although, if plants were trampled whilst in a turgid state and the ground was softened by an increased water content, greater damage could result. This suggests that the time of the application of such treatments relative to when the plants are trampled may be of importance.

In an experimental site in the Rocky Mountains, U.S.A., Scott-Williams (1967) observed that following the removal of recreational pressures, erect plants such as Polygonum bistortoides and Geum rossii resumed growth in the same season in contrast to cushion type species e.g. Silene acaulis which resumed growth at the margins only. He concluded that, whilst nearly all species exhibited an immediate response when the pressure was removed, some species reacted more quickly than others, to the extent that restoration of the ecosystem, to a state equivalent to that existing before the twenty-five year period of use, would take in excess of five hundred years, perhaps due to the slow growth rates of alpine-arctic plants. In contrast to this approach in which ecosystem reaction to the removal of pressure was investigated, La Page (1967) considered the response of vegetation to trampling for a period of three years following the inauguration of a camp site. Use was quantified in terms of 'camper days' which was subsequently realised to be inadequate. However, his conclusions may be summarised as follows:

(1) following the onset of use there was a heavy loss in cover, e.g. a 10% decrease for 150 'use days' throughout the summer, a 60% decrease for 300 'use days';

(2) species composition was modified, small leaved plants being favoured and small plants being favoured to larger ones. The tolerance of grasses over herbs was superior (cf. Bates, 1935; Wagar,

1964);

(3) those species which were more resistant to soil compaction and drought became increasingly abundant;

(4) although cover was directly related to total use, no relationship existed with second year use alone. During the second year of use trampling resistant species became more dominant so that by the end of the third year of study, the relationship between 'camper days' and cover was non-existent;

(5) continued use resulted in an erratic response of cover although the general trend was for cover to increase with increased use as resistant species became dominant.

Such studies help to predict the durability of species to trampling but it is necessary to simultaneously consider the effect of environmental variables on the response. Cieslinski & Wagar (1970) performed a series of experiments using a roller delivering a force of 41.4×10^{3} Nm^{-2}, and assumed that relationship between roller damage and human trampling was unimportant. Vegetation response to simulated trampling varied according to the intensity of 'trampling' but not the frequency (cf. Edmond, 1958). From an analysis of the variables involved, slope, aspect and elevation were found to make the most significant contribution towards explaining variation in cover in relation to 'trampling', whilst tree trunk basal area and clay and stone composition of the soil consistently appeared in the regression equations. It was concluded that increased vulnerability of trampled vegetation to heat and desiccation resulted in enhanced survival on slopes of cool aspect (cf. Wagar 1964, 1965; La Page, 1967), but increased altitude, shorter growing seasons and reduced vegetational development resulted in a reduction in survival. The study indicated that regression techniques can be of value in predicting vegetation response to trampling (cf. Wagar, 1964; La Page, 1967; Liddle, 1975a), to enable sites to be compared for relative tolerance and, in addition, re-emphasises the importance of environmental factors in considering such responses (Edmond, 1958-1966; Wagar, 1964).

Bayfield (1971) adopted a dual approach to studying cause and effect of trampling in a mountain environment on Cairngorm, N.E. Scotland. In addition to describing vegetational response to actual visitor use, he performed a number of experiments at controlled intensities of trampling in addition to using a tamp for use on greenhouse grown material. Intensities of trampling ranged from 40 to 240 passages over 1.2m x 4.6m plots on a Calluna-Trichophorum heath community. Subsequent analyses twelve and twenty-three months after trampling revealed that frequency of application had little effect so that treatments spread over a number of months were essentially additive (cf. Cielinski & Wagar, 1970) and consequently comparable to treatments applied at one instant in time. Damage to Trichophorum cespitosum appeared directly proportional to trampling pressure (cf. Edmond, 1958; Wagar, 1964), but recovery was relatively rapid being most dramatic in the most heavily trampled plots. This rapidity of recovery was ascribed to the plants 'deciduous habit' and 'basal growth apices' (cf. Edmond, 1966), thus, although damage was severe, few plants were killed so that at the final analysis cover of live T. cespitosum had risen from the pretreatment value of 33% to 35%.

Calluna vulgaris, however, suffered more severely. Again an almost linear relationship between trampling intensity and damage was observed, (the latter being quantified as dead, broken, bruised and leaf stripped shoots). Broken stems were the most serious result of trampling and wind removal of these from the plots resulted in apparent recovery as damage values decreased from 18% to 12% after twelve months. The cover of live *C. vulgaris* decreased from a pretreatment value of 35% to 18% suggesting recovery to be very low.

The use of a tamp was made to simulate trampling at defined levels on *Phleum bertolonii* (S50 Timothy). The higher number of impacts reduced tiller number and dry weight of both aerial and root parts but the lower values of 'tamping' appeared to have a stimulatory effect since tiller number and dry weight were greater than the control valves.

It is evident from Bayfield's work that in assessing vegetational response to trampling, the capacity for recovery may be as important as assessment of immediate post treatment damage. Recovery from, in addition to response to, trampling may affect the competitive advantage or disadvantage of a species in a particular community (Bayfield, 1971). Bayfield noted that although simulated trampling employing a tamp may be of limited application, it facilitates the development of a modelling methodology and enables relative tolerance indices to be developed. Simulated trampling, whether by tamp or human foot, is, however, only an approximation to the damage accruing from actual recreational activities, owing to the complexities of pressures transmitted to the ground by feet (Harper *et al.*, 1961). Bayfield (1971) found that the trends in tolerance of *Calluna vulgaris* and *Trichophorum cespitosum* to trampling were similar for both approaches however. One of the problems he encountered was the definition of criteria for damage since the parameters adopted applied to only small parts of the study area involved. The relationship between damage and traffic was dependent on vegetation type and was further confused by topographic variation, for example, "...downhill walkers appeared potentially more damaging than uphill..." (Bayfield, 1973), possibly due to the greater impetus of footsteps coming downhill (Harper *et al.*, 1961). Despite the problems of relating actual pressures to simulated pressures Bayfield (1971) considered that, owing to the repeatability of the pressures, such techniques are useful from a quantitative viewpoint.

Cultural treatment of plots (cf. Wagar, 1965), by the application of seed and fertilizer to a trampled Calluna - Trichophorum - Molinia community was found to enhance recovery (Bayfield, pers. comm.) although the use of such techniques requires a consideration of the economics involved in relation to the amenity value restored.

Although the dropping of a weight to deliver a known force has been a commonly employed method of simulating the action of a foot e.g. Wagar (1964); Bayfield (1971), Kellomaki (1973), Leney (1974), an approach adopted by Allcock (1973) was to take 'natural' turves and bring them into contact with the foot by raising the turf to deliver a force of $245.2 \times 10^3 Nm^{-2}$, taken as being equivalent to the pressure exerted by a man, weighing 90.6kg. whilst walking. Loss in productivity was curvilinearly related to the pressure applied (cf. Edmond, 1958; Wagar, 1964, 1965; Cieslinski & Wagar, 1970; Bayfield, 1971) but the severity of the response may have been due to the substantially higher impact loading used in the study. This suggests that when simulated techniques are employed care must be exercised in interpreting the results in the light of other workers findings due to the lack of a unified approach to simulating the pressures involved.

Certain studies have simulated vehicular impact on vegetation, e.g. Greller et al. (1974), Liddle & Greig-Smith (1975b). The low potential for recovery of tundra ecosystems had been established by Scott-Williams (1967) and Willard & Marr (1970). Willard & Marr (1971) concluded that a Kobresia turf community would require "...several hundred and possibly even a thousand years for ecological processes to produce a persistent 'climax' ecosystem in some of the areas modified by visitor activities." Greller et al. (1974) attempted to correlate damage and recovery of an alpine tundra ecosystem with a known number of passages (1020) of a snowmobile over it. Damage resulted from direct soil erosion, breakage and crushing of stems and leaves (cf. Marr & Willard, 1970; Bayfield, 1971), ultimately resulting in the denudation of the surface vegetation cover. Differential species susceptibility was not apparent where geomorphology enabled survival, for example, where rocks offered protection to the plant. Where such protection was not apparent species such as *Bistorta bistortoides* with subsurface apical meristems or *Geum rossii* where meristems were on the surface but protected by leaf bases, were best able to survive. In contrast, semi-woody chamaephytes were most susceptible (cf. Bayfield, 1971) whilst cushion plants, which have been observed to be susceptible to trampling by various authors e.g. Scott-Williams (1967), Willard & Marr (1970), were found to be susceptible but in relation to the protection afforded by other plants such as *Kobresia myosuroides*.

Liddle & Greig-Smith (1975b) investigated the effect of car passages on *Festuca rubra* and found that, although fresh weight was reduced by 17%, tiller number increased by 42% (cf. Bayfield, 1971). Soil compaction, however, resulted in an increase of 16% in fresh weight although reduced the increase in tiller number to 37%. The stimulatory effect of low trampling levels on productivity was also noted and a suggestion made that this may have resulted from improved access to water, but it is necessary to consider the effects of soil compaction on gaseous diffusion and thus growth (Edmond, 1958; Liddle, 1975a). The stimulatory effect of 'real' trampling has been observed in the field situation where an increase in biomass of the grass-herb-dwarf shrub layer was observed at low pressures, (one and four passages a week for eight weeks), (Kellomaki, 1973). However, with increased levels of trampling, productivity relationships are modified by changing species composition. Grime (1973) observed that characteristics which tend to maximise dry matter production under productive and competitive conditions, such as tall stature and rapid potential growth rate, are "...of low selection value in circumstances of extreme environmental stress". Thus, under conditons of low environmental stress, productivity is high and species of high competitive index are most vigorous and consequently, due to competitive exclusion, species density is low. However, as stress increases, species of lower competitive ability are able to survive as the competitive species decline in vigour until a point is reached where stress is such that density is limited by the lack of species capable of withstanding the stress imposed. Slow growing species are, however, infrequent in habitats subject to a high intensity of damage or disturbance, e.g. paths, possibly because they are not adapted to the rapid replacement of foliage by regrowth (Grime & Hunt, 1975). Liddle (1975a) noted that in mixed communities following the increase in dicotyledonous species in relation to monocotyledonous species at moderate levels of pressure, further increases in pressure resulted in an increase in trampling resistant species, many of which were monocotyledonous. One of the aims of management of a recreational area is to exert some control over the relationship existing between stress, productivity, species diversity and species density.

Liddle has also investigated the effects of trampling on vegetation microclimate (Liddle & Moore, 1974). Vegetation removal and soil compaction resulted in an increase in path temperature of 9C° during the day in contrast to a decrease of 6C° in air temperature compared to that over the vegetation. The resultant temperature extremes suggested that species capable of tolerating large temperature ranges may be better adapted to survive in such environments, e.g. Poa annua, (Liddle & Greig-Smith, 1975b). However, soil compaction, by increasing soil moisture availability in a sand dune system, may enhance survival at times of drought (Liddle & Greig-Smith, 1975b).

In common with other workers mentioned above, Liddle (1975a) has suggested a methodology for assessing the durability of different habitats to recreational pressures. By administering controlled trampling intensities on a section of the habitat under consideration, a relationship may be derived between the percentage cover and the $\log_e$ number of passages, typically of the polynomial form:

$$y = a + bx + cx^2 \qquad (1)$$

where : y = percentage cover

$x = \log_e$ number of passages

The equation constants may subsequently be used to estimate the number of passages required to reduce cover to 50%. Liddle (1973) derived a number of predictive regression equations for various permutations between season, degree of recovery and type of stress. A similar relationship has been derived relating the number of passages to primary productivity (Liddle 1975b). Burden & Randerson (1972) used similar regression techniques to obtain a relationship between trampling pressure, soil compaction, grazing and standing crop to predict the dry weight of a particular component in a shingle ridge community at Morton Marsh, Norfolk. The model developed from such techniques was used to determine the 'trampling capacity', (i.e. the level of trampling at which standing crop becomes zero), for a particular sward component. Although realising that the overall trampling capacity of the sward was composed of the capacities of several species, and that the real situation was likely to be more complex than the model suggested, they observed that such a method "...allows a tentative prediction to be made of the levels of trampling pressure at which deterioration of the sward is likely to begin". (Burden & Randerson, 1972).

It is evident that similar methods may be applied to describe the susceptibility of a habitat to trampling in terms of species number, density, cover, biomass, productivity, etc., as has been attempted by a number of workers as mentioned above. However, Liddle (1973) appears to be one of the few workers who considered the recovery aspect, the importance of which was stressed by Bayfield (1971). A refinement of model making processes to consider damage and recovery would enable greater flexibility in predicting the intensity of pressure inducing a degree of change with a specified period allowed for recovery, or the degree of pressure resulting in a change which could be stabilised and maintained by a given intensity of use. Theoretically, it should be possible to describe the carrying capacity of an area in terms of the desired ecological composition required and consequently such methodology is potentially a powerful management tool. However, the major disadvantage of the method is the inability to validly extrapolate beyond the range investigated in the field, which implies that a model must be developed for each site under consideration. Providing the model is developed prior to extensive site use, the technique allows management schemes to be developed prior to irreversible damage occurring.

More recently, studies on the response of vegetation to trampling pressure have been undertaken in which cellular aspects have been considered. A wear simulator (Shearman et al., 1974), simulated the effects of tyres and feet and,delivering a force of 7.061 x $10^3 Nm^{-2}$ and 1.422 x $10^3 Nm^{-2}$ respectively, was used to assess the wear tolerance of seven turf grass species (Shearman & Beard, 1975a). Injury was assessed in terms of:

(1) percentage total cell wall fraction;
(2) percentage verdure (g (fresh weight) dm^{-2});
(3) chlorophyll content (mg dm^{-2});
(4) visual rating (arbitrary scale).

Wear tolerance was based on the number of revolutions of the simulator required to reach a predetermined end point for the above criteria. They found that, due to the high correspondence between the above criteria, any one of the measures could be used to evaluate wear tolerance differentials, facilitating the ranking of the species under consideration. Subsequent work on the relationship between cell wall constituents and wear tolerance (Shearman & Beard, 1975b) revealed that total cell wall content, lignocellulose, cellulose and hemicellulose, (expressed as mg dm^{-2}) were significantly positively correlated with wear tolerance of the species under investigation although total cell wall content accounted for a significant proportion of the variation observed between species and was suggested as the best criterion to employ. Beard (1973) suggested that wear tolerance was influenced by the degree of tissue hydration (cf. Wagar, 1964, 1965), the quantity and location of sclerenchyma fibres (cf. Edmond, 1966), lignin content, shoot density and coarseness of the leaves and stems. In response to this, Shearman & Beard (1975c) quantified the following:

(1) verdure, shoot density and leaf width;

(2) load bearing capacity and tensile strength of leaves;

(3) percentage moisture content and relative water content of leaves;

(4) sclerenchyma content and the percentage of lignified cells, (as a percentage of the cross sectional area).

No significant correlations were observed between wear tolerance and parameters in the first three categories listed above although leaf width and tensile strength made a significant contribution to the combined contributions made by all factors to wear tolerance. However, the factors in category (4) were closely associated with species differences in wear tolerance, the greater the lignification the greater the wear tolerance. However, there is evidently a threshold above which lignification leads to increased susceptibility to trampling damage (Bayfield, 1971; Dale & Weaver, 1974; Greller _et al_., 1974).

In terms of the experimental approach to the response of vegetation to trampling, a methodology is evolving by which the response to trampling can be determined and predicted together with the assessment of recovery potential at one end of the spectrum, whilst at the other end an attempt is being made to define the physiological, morphological and anatomical factors which determine the durability of plants to recreational pressures. The methods employed in the two approaches discussed above make comparisons both between and within the approaches difficult. The complexity of recreational activity makes isolation of cause and effect problematical. The analytical approach used by many, e.g. Goldsmith et al. (1970), Marr & Willard (1970), Willard & Marr (1970), Chappell et al. (1971) has resulted in an ordination of plants with respect to their tolerance to trampling in relation to the pressures involved and their physical environment. The experimental approach e.g. Allcock (1973), Shearman & Beard (1975a,b,c) has attempted to define plant response to defined levels of mechanically applied forces, whilst many workers have sought to combine the approaches e.g. Bayfield (1971), Leney (1974), Liddle & Greig-Smith (1975b) to enable species response to be defined, quantified and predicted.

Certain environmental variables have been isolated as being of significance in describing the reaction of vegetation to recreational pressures such as edaphic factors e.g. Bates (1935), Edmond (1958-1966), Cieslinski & Wagar (1970), Chappell et al. (1971), Burden & Randerson (1972), Liddle & Greig-Smith (1975b), shade (Wagar, 1964), aspect, slope angle and altitude (Cieslinski & Wagar, 1970), soil moisture availability (Settergren & Cole, 1970; Liddle & Greig-Smith, 1975b) and microclimate (Liddle & Moore, 1974).

Studies on plants indicate that no critical threshold of pressure is apparent (Edmond, 1958; Wagar, 1964; Bayfield, 1971) and although

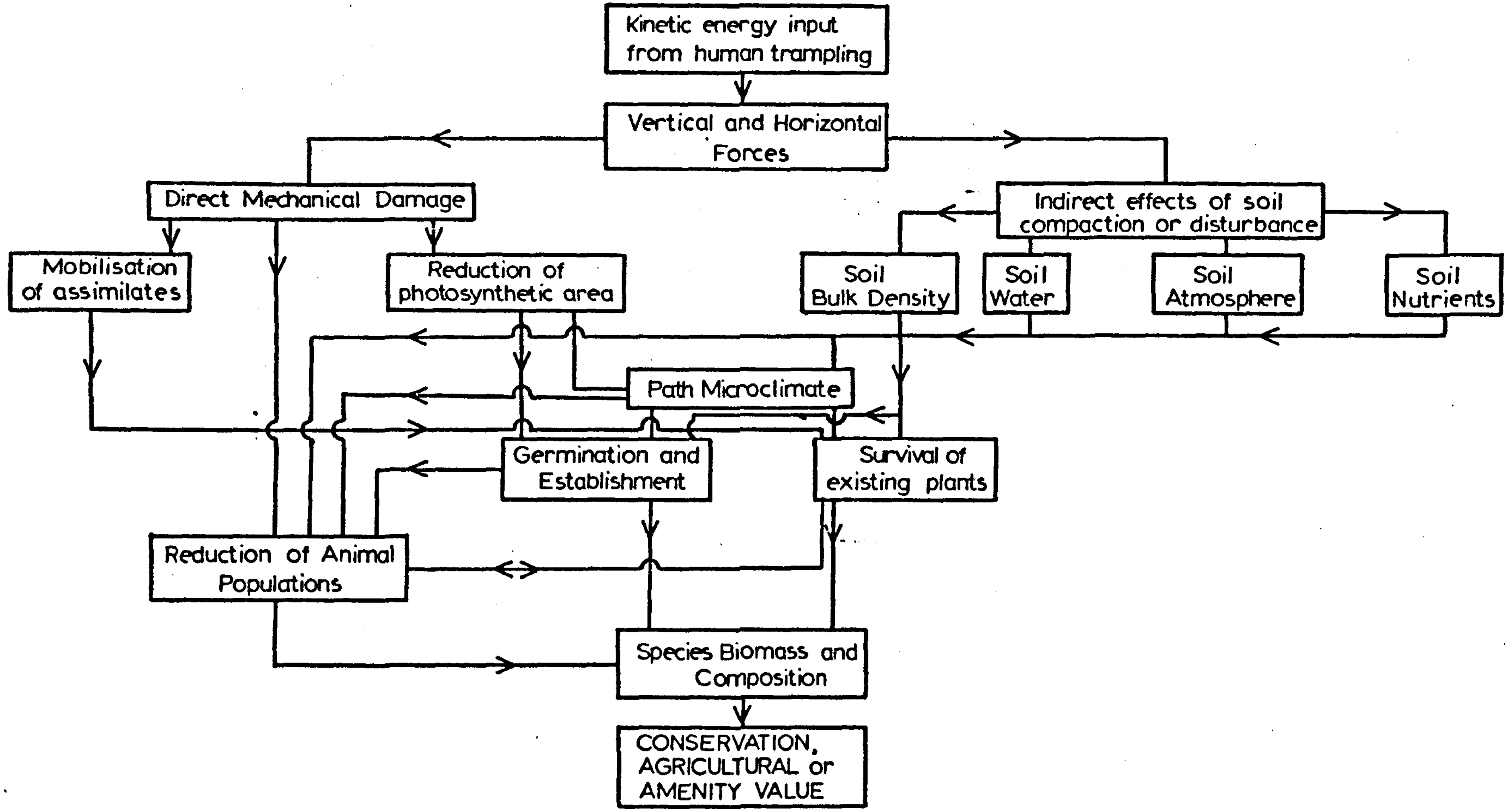


Figure 1 Interrelationships between some of the ecological effects of trampling (from Liddle, 1975a).

yield reduction is a universally observed phenomenon, low pressures may stimulate productivity (eg. Bayfield, 1971; Kellomaki, 1973; Liddle & Greig-Smith, 1975b). The initial treading generally appears to be the most damaging (Edmond, 1958; Wagar, 1964; La Page, 1967) presumably because it is a new variable in what might have been a comparatively stable situtation.

Certain morphological and anatomical characteristics typify plants resistant to trampling (Speight, 1973):

(1) plants procumbent or trailing rather than erect;
(2) flexible rather than woody or rigid stems;
(3) leaves arranged in a basal rosette;
(4) leaves flexible and which fold rather than fracture under pressure;
(5) intercalary, in addition to apical, meristems;
(6) seasonal regrowth dependent on cryptophytic buds rather than aerial parts;
(7) reproduction via suckers, stolons, corms in addition to seeds;
(8) rapid growth rate.

In terms of recovery following removal of pressures e.g. Scott-Williams (1967), Willard & Marr (1970), it would appear that the period required to enable recovery is considerably greater than the period required to produce the damage. In the management of natural areas it would appear important to be able to quantify and manipulate damage-recovery ratios but it would also seem valuable to investigate individual plant responses. The relationships between various factors affecting ecosystem response to trampling have been summarised in a 'logical model' by Liddle (1975a)(Figure 1). In addition to considering certain of the plant factors presented in this model it is hoped that the present study will enable the model to be developed to include seasonal difference in the susceptibility of plants to trampling as a function of seasonal variation in certain physiological variables within the plant, together with the effects microclimatic variables have on influencing the degree of injury sustained by a trampled plant.

(iv) The concept of 'Carrying Capacity'

The ultimate objective in a study of the present kind is to provide a methodology by which the 'ecological carrying capacity' of an area may be defined. The term 'carrying capacity', however, is essentially a management orientated concept. Tivy (1973) observed that recreational carrying capacity is a multidimensional concept dependent upon the character and rate of deterioration of each resource element in an area and the varying attitudes and requirements of the recreationists. Speight (1973) listed four factors affecting the intensity of use of a recreational area:

(1) its physical extent;
(2) its ecological characteristics;
(3) human psychology and behaviour;
(4) economics.

Wagar (1964) observed that 'recreational carrying capacity' has three components:

(1) the impact of the environment on the people;
(2) the impact of the people on the environment;
(3) management methods involved in modifying these reciprocal impacts.

A number of definitions of carrying capacity exist such as "the number of user unit use periods that a recreation site can provide... without permanent biological or physical deterioration of the site's ability to support recreation and without appreciable impairment of the recreational experience." (Tivy, 1973), in which 'user unit use periods' reflect the spatial requirements of a particular activity and the duration of use. A similar definition has been given by the Countryside Commission (1970) in which the carrying capacity is "...the level of recreation use an area can sustain without an unacceptable degree of deterioration of the character and quality of the resource or of the recreation experience", however, a problem arises in defining 'unacceptable'. The Commission also define 'ecological carrying capacity' as "the maximum level of recreation use, in terms of numbers and activities, that can be accommodated before a decline in ecological value, assessed from the ecological viewpoint". Speight (1973) criticised such a definition since it does not include an appreciation of the duration of use, the seasonal distribution of use or the management objectives. He proposed a redefinition as follows, "The maximum intensity of use by specified forms of recreational activity, measured in terms of numbers of people per season per year, a given area will support without undergoing an unacceptable degree of ecological change away from the ecosystem conditions considered desirable." It will be evident that although site deterioration can be predicted, the definition of a carrying capacity depends on defining acceptable thresholds of physical and aesthetic deterioration (Tivy, 1973), consequently, "final definitions of recreational carrying capacity must be of an administrative nature" (Wagar, 1964). Despite the objectiveness of the ecological factor, the ecological carrying capacity is not an absolute value, for example, the preservation of a trample sensitive species at a particular locality would require a minimum of recreational pressure and would indicate a site of low carrying capacity whilst the evolution of a habitat from one of considerable species diversity to a sward composed of a few tolerant species might reflect a site of high recreational carrying capacity (Burden & Randerson, 1972).

Speight (1973) argued that intensity of use is not directly controlled by the ecosystem but needs to be management directed. Although true to a certain extent, a feedback situation at higher intensities of use is likely to operate where ecosystem degradation results in destruction of the experience recreational users are seeking, resulting in a stabilisation or decline in intensity of use. This produces another concept, that of the 'sustained yield capacity' which is the optimum number of user units per unit area a site can accommodate at one instant in time (Tivy, 1973).

Speight (1973) observed that quantification of ecological carrying capacity is complicated by the differential vulnerabilities of different parts of the ecosystem in relation to different recreational pursuits. Consequently, it is only feasible to define a single capacity for an area if one of two assumptions is made:

(1) the lowest capacity may be defined by the intensity of use the least resilient component in the system can withstand;

(2) the highest capacity may be defined by the intensity of use the most resilient component can withstand.

One aspect of the present study is the consideration of whether 'resilience' changes during the course of the year, indicating a dynamic situation with regard to the definition of ecological carrying capacity.

(b) Site selection

Field experimentation required the presence of a homogeneous, even-aged stand of Calluna vulgaris suitable for productivity estimates and capable of protection against the presence of people inadvertently interfering with experimental trampling intensities. Secondly, a site of sufficient area and within easily accessible distance to enable monthly trampling trials to be performed and plant material collected for laboratory based investigations was required.

Cannock Chase, in South Staffordshire is a 25-square mile area of forest and heathland providing an extensive area of C. vulgaris growing on shallow, stony podsols. Burton (1974) has reviewed the vegetation communities of the area and observed that the co-dominants on the dry acid heath are C. vulgaris and Pteridium aquilinum, the former being dominant on the plateau top which rises to heights in excess of 200m. Vaccinium spp. are the only other frequently occurring species. Areas of degenerate heather were found to possess a greater species diversity, Deschampsia flexuosa, Molinia caerulea, and Nardus stricta occurring locally as co-dominants with C. vulgaris.

Despite the existence of large areas of heather, the site requirements were difficult to satisfy since much of the heather is in the mature to degenerate phase. A 7-year-old stand was located which was suitable for monthly investigations but due to the heterogeneity of cover productivity assessments were not possible. Since the area is one of extensive informal outdoor recreation for both local and subregional populations (Burton, 1974), the main experimental site was fenced off to protect both the site and associated instrumentation.

The site, (G.R. SJ983183), was located on a slight north-westerly facing slope approximately 200m. above mean sea level. Up to 1951 the area had been used for Army tank trials resulting in a marked compaction of the soil (Burton, 1974). Consequently, root penetration was restricted to the upper 15-20cm. of soil overlying the Bunter Pebble Beds.

A second site of heather suitable for productivity studies was located on an easterly facing slope at an altitude of 300m. on Kerloch, Kincardineshire (G.R. NO710887). This area, managed as a grouse moor, consisted of four areas of even-aged heather, pioneer (c. 3 years old), building (c. 7 years old), mature (c. 15 years old) and degenerate (in excess of 23 years old), forming a virtual monoculture of *C. vulgaris*.

(Material was aged by ring counts, which may underestimate age by two years (Watt, 1955). This was facilitated by staining sections for 3 minutes in a 1% solution of phloroglucinol, followed by immersion in concentrated hydrochloric acid for 1 minute. After the appearance of a red coloration, the section was washed in distilled water and mounted in a 50% solution of glycerin (Purvis, Collier & Walls, 1966)).

The relative inaccessibility of the site meant that human interference was likely to be minimal but the activities of mountain hares, (*Lepus timidus*) necessitated the encasement of the environmental measurement instrumentation cables in hard walled nylon tubing to prevent damage.

(c) Materials

Calluna vulgaris is a species cited as being sensitive to trampling by a number of authors, e.g. Goldsmith *et al*. (1970), Bayfield (1971), Burden & Randerson (1972), Speight (1973), Burton (1974). Since it is a species which forms extensive 'monocultures' on heathland it was chosen as a suitable species to indicate the response of woody plants to trampling damage.

Calluna vulgaris (L.) Hull (a full account of the species is given by Gimingham (1960)).

This species composes a monotypic genus, having a typical growth form of a 'semi-sedentary' dwarf shrub, although it is variable with respect to size, habit and morphology. In Britain it is usually less than 0.8m in height (Gimingham, 1960). Two distinct types of shoot may be distinguished, short shoots with leaves 1-2mm. in length and active for up to three seasons, and long shoots with leaves 3-4mm. long, widely spaced and active for only one season (Gimingham, 1960). The leaves are usually glabrous although a pubescent fórm exists, (var. hirsuta. S.F. Gray)(Clapham, Tutin & Warburg, 1962). The leaf margins are reflexed so that the abaxial surface is confined to a narrow median groove. It is common throughout the British Isles, dominant over large areas of well drained acid soils, and becomes dominant in the field layer of open woods, e.g. Pinus, Betula and certain Quercus woods, having an altitudinal distribution from sea level to c. 1036m. (Gimingham, 1960; Clapham, Tutin & Warburg, 1962).

Gimingham (1960) observed that optimal development, as evidenced by long shoots, length and regularity of short shoots, long flowering zones with abundant flower production and obvious storeyed growth, occurs where there is a distinct layer of moist humic material overlying well drained sand or gravel or where the whole root region consists of drained peat (e.g. Kerloch Moor, Kincardineshire). In contrast, on poor, stony ground where the 'mor' layer may be non-existent, height may be reduced to less than 0.38m., whilst stems may be twisted and young branches close and short with reduced flowering zones (e.g. Cannock Chase, Staffordshire).

Reproduction is either by seed or regeneration from subsurface 'stools' or rhizomes and its life history may be divided into pioneer, building, mature and degenerate phases (Watt, 1955). It commences growth in April or May, flowers in mid to late August through to September. Most seed is disseminated in October and November, germination occurring in autumn so that seedlings overwinter in various stages of development (Gimingham, 1960).

Early work on the water relations of *C. vulgaris*, cited by Gimingham (1960), indicated that in relation to the area of its transpiratory surface, the water content of the plant is low whilst the cross sectional area of the xylem is comparatively large. Gimingham noted that "*Calluna* is susceptible to summer drought, especially on shallow soils with low organic content".

In contrast to the sensitivity to trampling exhibited by *C. vulgaris*, it was considered desirable to investigate the possible mechanisms exhibited by resistant species. Two herb species suitable for growth in the greenhouse and which could be obtained commercially were chosen, *Plantago lanceolata* and *Phleum bertolonii* (S50 Timothy).

Plantago spp. have been cited as being resistant to trampling by a number of authors, e.g. Bates (1935), La Page (1967), Chappell *et al*. (1971), Streeter (1971), Burden & Randerson (1972), Allcock (1973).

Plantago lanceolata L.

A glabrous to pubescent perennial with leaves 2-30cm. long, lanceolate in shape, the blade narrowing into the petiole. It is to be found on neutral to basic soils, generally distributed throughout Britain (Clapham, Tutin & Warburg, 1962).

Phleum spp. have been used as a component of reseed mixtures in certain recreational areas, e.g. on Cairngorm (Watson, Bayfield & Moyes, 1970), and on Cannock Chase (Burton, 1974). Perring (1967) observed that Phleum nodosum is a trample resistant species to be found in heath grassland. Bayfield (1971) used S50 Timothy (Phleum pratense) in an experimental investigation into trampling effects on herbs, owing to it being "...a convenient experimental plant and is an important component of reseed mixtures..." used on the Cairngorm recreational area. The S50 strain may be more correctly regarded as a strain of Phleum bertolonii (Hubbard, 1968). This species has been considered as a variety of Phleum pratense but Hubbard (1968) noted that whereas P. pratense has a diploid chromosome number of 42, P. bertolonii has a diploid chromosome number of 14.

Phleum bertolonii DC. (Aberystwyth S50 Timothy strain)

Typically a loose to compactly tufted perennial, 10-50cm. high. The species comprises a large number of strains, differing in growth form from prostrate and creeping to stiffly erect, growing on a wide range of soils from heavy clays to sandy or calcareous soils. The S50 strain is prostrate and persistent (Hubbard, 1968).

Table 1. Thermistor probe positions at field trial sites

Site	Probe height with reference to ground level	Condition
Kerloch alt. 300m. (G.R. NO710887)	+ 140 cm	shielded - air temperature reference at standard observing ht.
	+ 20 cm	shielded - canopy height, 25 - 30cms
	+ 15 cm	shielded
	0 cm	unshielded- ground-air interface
	- 5 cm	unshielded- root level
	- 15 cm	unshielded- root level
	stem	unshielded- taped to *C. vulgaris* stem
	0 cm(ttd)	unshielded- placed at ground-air interface in plot receiving 100 passages per plot
Cannock Chase alt. 170m. (G.R. SJ983183)	+ 60 cm	shielded - air temperature reference
	+ 20 cm	shielded - canopy height
	0 cm	unshielded- ground-air interface
	- 5 cm	unshielded- root level
	- 15 cm	unshielded- root level
	stem	unshielded- taped to undamaged *C. vulgaris* stem
	stem(ttd)	unshielded- taped to treated *C. vulgaris* stem

PART II - ENVIRONMENTAL MEASUREMENTS

Plant distribution and reactions to stimuli may be closely linked to climatic factors in the immediate environment. In investigating a plant's reaction to its habitat or an externally applied stress it is useful to assess certain relevant factors in the physical environment. Temperature and precipitation have been considered in the present investigation.

(a) Methodology

(i) Temperature measurement

Temperature measurements were made at the experimental sites using thermistors enclosed in 5cm. long aluminium shields, in conjunction with a nine channel, automatic recorder (Grant Instruments (Developments) Ltd., Toft, Cambridge). The small size of thermistors makes them suitable for point measurements (Long, 1968; Holwill & Silvester, 1973; Painter, 1976). The temperature of each probe is recorded once an hour, the record being made on a waxed paper chart, giving a theoretical running time for the instrument of two months, without requiring attention. The positions of the thermistor probes in the field are given in Table 1.

One of the major errors involved in stratified temperature measurement concerns exposure of the sensing element. Although in a position to exchange heat with the air it is also exposed to radiation. This introduces an error which is directly proportional to the sensor's radiation balance i.e. heat gain from radiation input, and inversely proportional to the heat exchange with the surrounding air, i.e. the heat transfer coefficient which increases as the square root of the wind speed (Geiger, 1965). Radiation gain may be reduced by

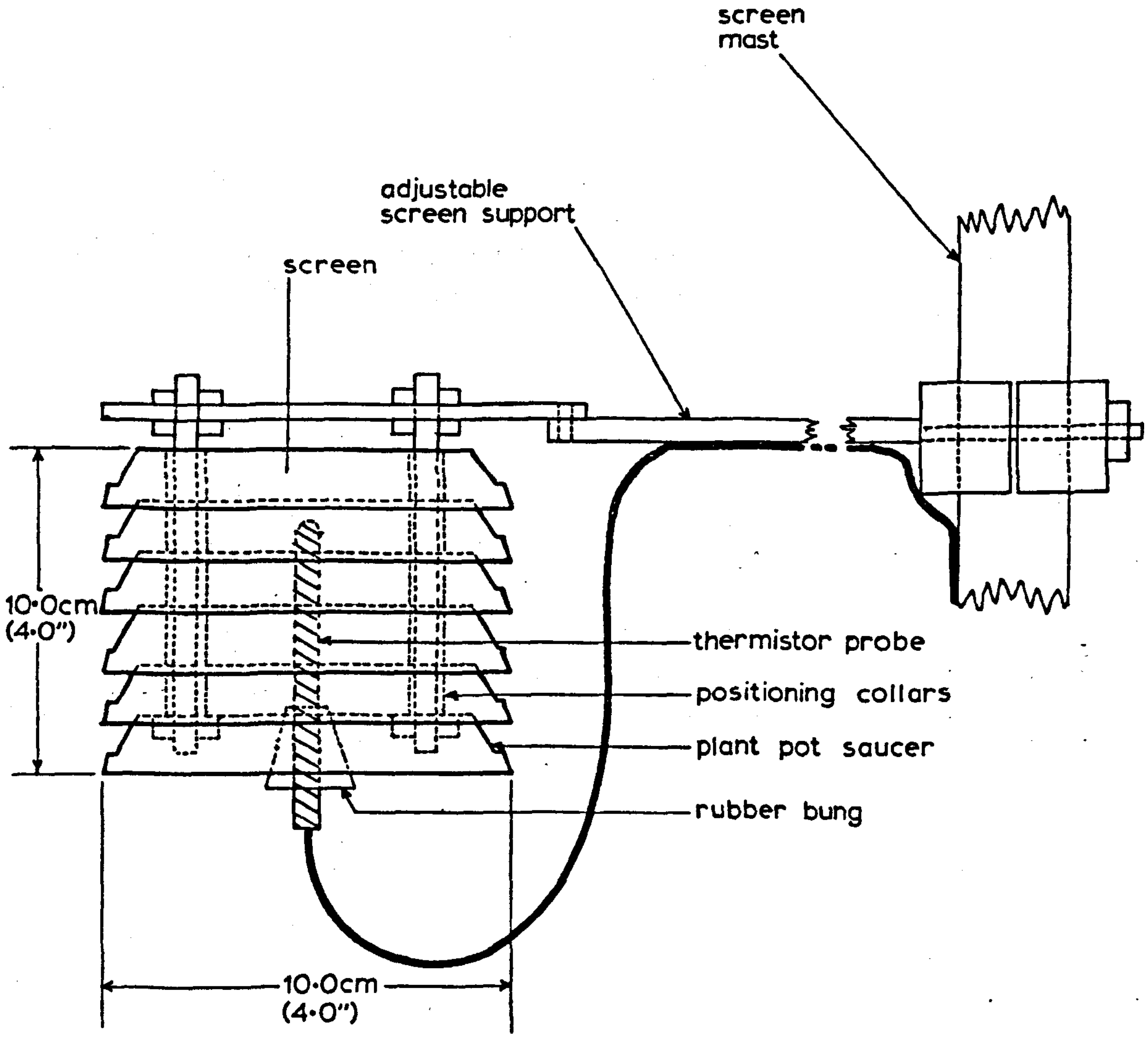


Figure 2 Diagram of thermistor probe shield.

sheltering the sensor but this may interfere with the heat exchange between sensor and air by reducing air flow over the sensor. In addition, the shield may absorb and re-radiate heat, so warming the air flowing over the sensor and therefore introducing a further source of error. Thus, although shielding may reduce the radiation error, it will not be totally eliminated. Standardisation of the shield enables comparability of results even if absolute precision is not achieved.

Platt & Griffiths (1964) enumerated four criteria which a temperature shield or screen should satisfy:

(1) the shield should ensure that no direct radiation can fall on the sensor;

(2) the shield should cut off reflected radiation, e.g. from the ground;

(3) air flow across the sensor should be unimpeded;

(4) to prevent the shield from becoming a radiating body and thus affecting the sensor, it should be doubled walled.

Painting the screen and sensor white will help to alleviate the problem of radiation gain by increasing surface reflectivity, although Platt & Griffiths noted that radiative heating and cooling produce only a slight error in the normal Stevenson screen.

In an attempt to fulfil the above criteria, miniature screens were constructed suitable for use within the plant canopy. The basic design (Figure 2) consists of plastic plant pot bases or pot saucers assembled to give a louvred effect thereby preventing direct radiation impinging upon the sensor whilst offering minimum resistance to air flow around the sensor, which passes up the middle of the shield. The shields were painted with white gloss paint to minimise radiative heat gain and mounted on adjustable alloy arms to facilitate positioning within the plant canopy (Plate 1). The largest screen, (approximately 15cm. in diameter), was employed for the reference air temperature,

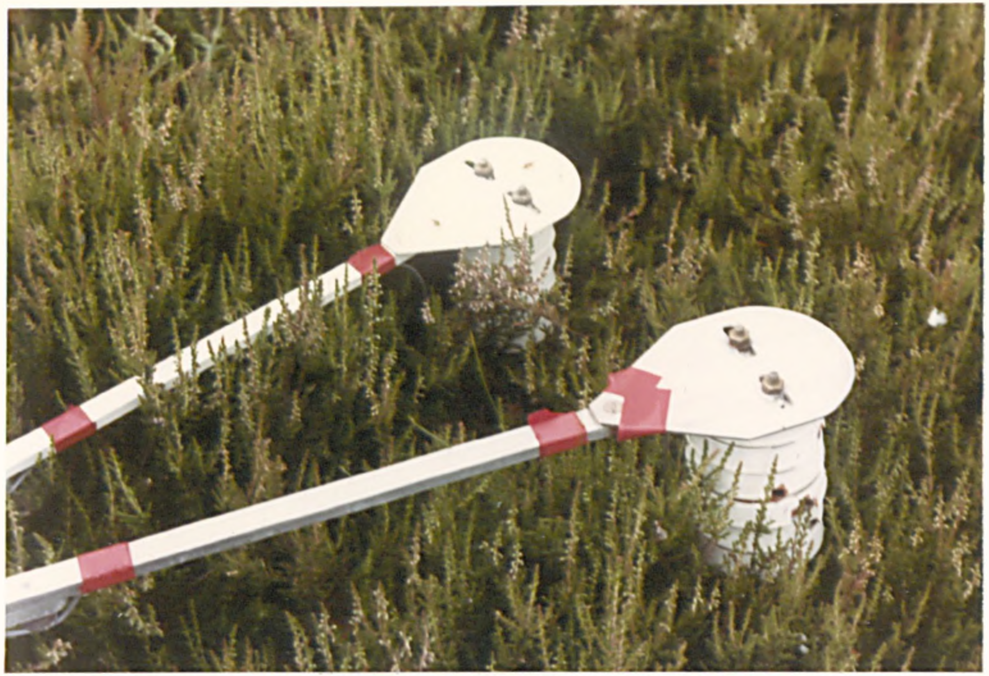


Plate 1. Miniature screens for shielding thermistors employed for air temperature sensing within the vegetation. (Kerloch, July 1975).

140cm. above ground. The smaller screens, (approximately 10cm., 7cm. and 5cm. in diameter) were employed within the plant canopy to minimize disturbance. Direct radiation below the foliage was probably small in comparison to that above the canopy and consequently unimportant as a major source of error.

Although long term sensor instability has been overcome with the continued development of thermistors, checks with a mercury in glass thermometer revealed a slight drift of ± $1C^{o}$ over the period of one year. The high impedence of thermistors, however, enables the use of long extension cables which is of value in a field study, and the problems associated with maintaining an accurate reference temperature for the reference junction of thermocouples are not encountered. These factors, together with their ease of use, especially in remote applications, make thermistors suitable for ecological purposes, where slight errors in precision may be relatively unimportant.

The direct radiation error is absent when measuring soil temperature and the standard metal sheath around the glass bead has been considered sufficient protection in most applications. In the present study the sensors were positioned by digging a small hole and inserting the thermistor horizontally into the side of the hole which was refilled. Soil disturbance may introduce a source of error, since soil temperature depends on the heat conductivity of the soil and presumably, if pore space, water holding capacity and other structural characteristics of the soil have been altered by digging and refilling a hole, soil temperature may be affected. Some degree of disturbance is a necessary consequence of positioning the sensor and is difficult to alleviate. It is also possible that soil heterogeneity, in terms of structure and composition and therefore thermal properties, is such that only an extensive network of sensors may present a relatively accurate indication of the thermal regime of the root medium. The levels of sensing used in this study were considered to give an indication rather than an accurate presentation of the thermal microclimate of the rooting zone.

In contrast to the above, surface temperature measurement is attended with special difficulty. Shielding will obviously affect the radiation balance and thus the thermal microclimate of the ground - air interface whilst the sensor itself will shield the soil surface being measured. The degree of this error will depend upon the size of the sensor, which if small will not have an appreciable effect on soil temperature. Geiger (1965) noted that surface readings may be obtained by drawing flat sensors over the surface but for remote applications this is impractical.

Plant temperature is perhaps the most difficult to assess accurately. With respect to long wave radiation a leaf acts virtually as a black body, absorbing both direct and diffuse radiation and emitting it in proportion to its own temperature in accordance with Boltzmann's Law. Geiger (1965) noted that leaves adjust their temperature within a "few seconds to changes in their surroundings", depending on radiation balance, heat exchange with the surrounding air and latent heat loss involved in evapo-transpiration. Platt & Griffiths (1964) suggested that thermistors or thermocouples mounted in hypodermic needles, which can be inserted into the plant, may be used to sense plant temperature. However, injury at the site of insertion may cause erroneous temperatures to be generated which, combined with the differential thermal characteristics of tissue and sensor, may result in imprecise records being obtained, although comparative values will be indicated. The method is more applicable to instantaneous measurements since the temperature relationships of dead, dying or damaged material may be at variance with those of living material, thus for long term applications the method is unsuitable. The aim of attaching a sensor to a shoot in the current study was to obtain some indication of the temperature range a shoot might experience rather than an accurate indication of shoot temperature.

(ii) Measurement of precipitation

Since water is fundamental to life processes, its measurement is often of great value in ecological studies. Precipitation may fall as rain, hail, sleet, snow and dew, the predominant form in temperate regions being rain.

In accordance with British Meteorological Office regulations, gauges should be installed with the rim height 30.5cm. above ground level, in a position such that it is not unduly influenced by local winds if the results are to be applicable to a large area, and at least twice the height of the nearest obstruction away from that obstruction. Since the gauge rim defines the area over which rain is caught it should end in a knife edge, preferably be of metal to avoid distortion and have a deep rim to avoid splash out. Gauges are generally installed so that the orifice is horizontal but it has been shown, in theory and practice, that it is incorrect to have a horizontal orifice if the ground has an appreciable slope (Leyton *et al*., 1968). However, the difficulties involved in assessing the angle at which a falling rain drop is making with the vertical has resulted in the horizontal installation being favoured.

For remote applications a recording gauge is of most use since it can be left unattended for long periods and may also provide information not only on the amount of rain which has fallen but also on intensity and duration of fall. One such type of gauge is the 'tipping bucket' gauge in which the rain is funnelled into a bucket which is divided into halves and pivoted about its centre. The bucket tips and empties its contents when a predetermined volume of water has entered whilst the other half begins to fill. Each tip is recorded, in the present instance by an electromechanical counter and a chart event recorder (Rustrak Galton Ltd., Brighton). In the latter case the event is recorded on a waxed paper chart, although problems were encountered with the chart drive mechanism. The chart speed of 2.54cm.hr^{-1} gives a theoretical resolution time of 180 seconds between events which, with a bucket size equivalent to 0.25mm. of rain, enables a maximum rate of 7.62mm. rain hr^{-1} to be recorded. In practice, with the aid of a binocular microscope, the resolution is better than this, allowing the resolution of rainfall intensities greater than 7.62mm. rain hr^{-1} such as might be encountered in a storm. At a chart speed of 2.54cm.hr^{-1}, the chart is of sufficient length to last 31 days. The absence of a regulated D.C. motor, however, results in a variable chart speed as the battery discharges so that an unquantified slowing down of the chart speed results in inaccuracies in defining the periods during which rain is falling.

Owing to the finite time taken for the bucket to tip there is a period during which rain does not run into the bucket. This may be circumvented by collecting the rain in a separate container and allowing it to syphon into the buckets at a slower rate (Painter, 1976), whilst Platt & Griffiths (1964) present a formula by which the error, which is dependent on the rate of fall and time taken for tipping, may be estimated.

Ideally, recording raingauges should empty into a collecting vessel containing a few drops of oil to prevent evaporative loss, which may be substantial over long periods. This provides a check on the record provided by the counter and acts as a failsafe should gauge failure occur.

Exposure of raingauges often leads to a serious source of error. Since the gauge represents an obstacle to air movement, turbulence around the orifice of the gauge may result in raindrops being transported across the orifice of the gauge rather than into it (Leyton et al., 1968). In exposed areas such as moorland, where this may be a considerable problem, the underestimate made by a standard gauge may be assessed by comparing the catch with a gauge whose rim is at ground level and surrounded by a non-splash surface, to prevent raindrops splashing into the gauge leading to an overestimate (Painter, 1976). Such a gauge probably gives the most accurate estimate of rainfall. When considering the rainfall of an area the sample measured is comparatively small, e.g. typically 96.8cm^2 to 161.3cm^2 over an area of many thousands of hectares. It may be considered that accuracy would be improved by having larger collecting areas. Leyton et al. (1966) found that collecting areas of a square metre gave close agreement with standard gauges over the long term though the consistency between collecting areas was less than that for gauges. Platt & Griffiths (1964) noted that "...annual totals of rainfall at any one site are usually.....normally distributed....." but that as the time interval over which the rainfall is considered decreases so the complexity and inaccuracies of the distributions increase and errors become greater.

In storm situations the localised nature of the rainfall may introduce a considerable error into point measurements which need to be identified in interpreting precipitation records. The errors involved in sampling rainfall make comparisons between sites difficult in short term investigations, whilst the extrapolation of data from one site to another may be of dubious value, especially when precise relationships such as relating plant water content to water input into the environment are involved. Most errors lead to an underestimate of rainfall. In moorland sites such as those involved in the present study, wind will introduce a major source of error into rainfall measurements and in the hill environment of Kerloch the localised nature of storms may also result in considerable error.

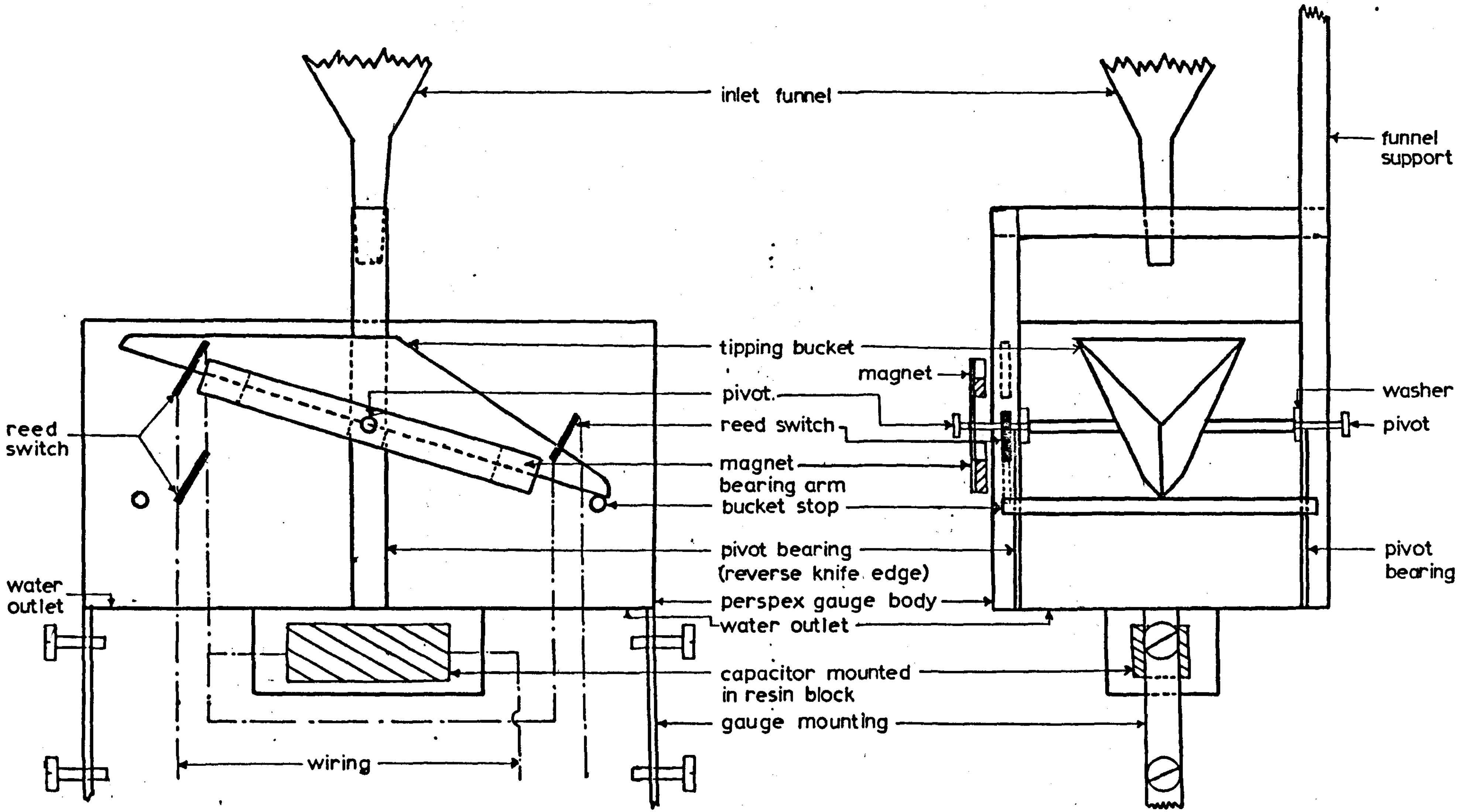
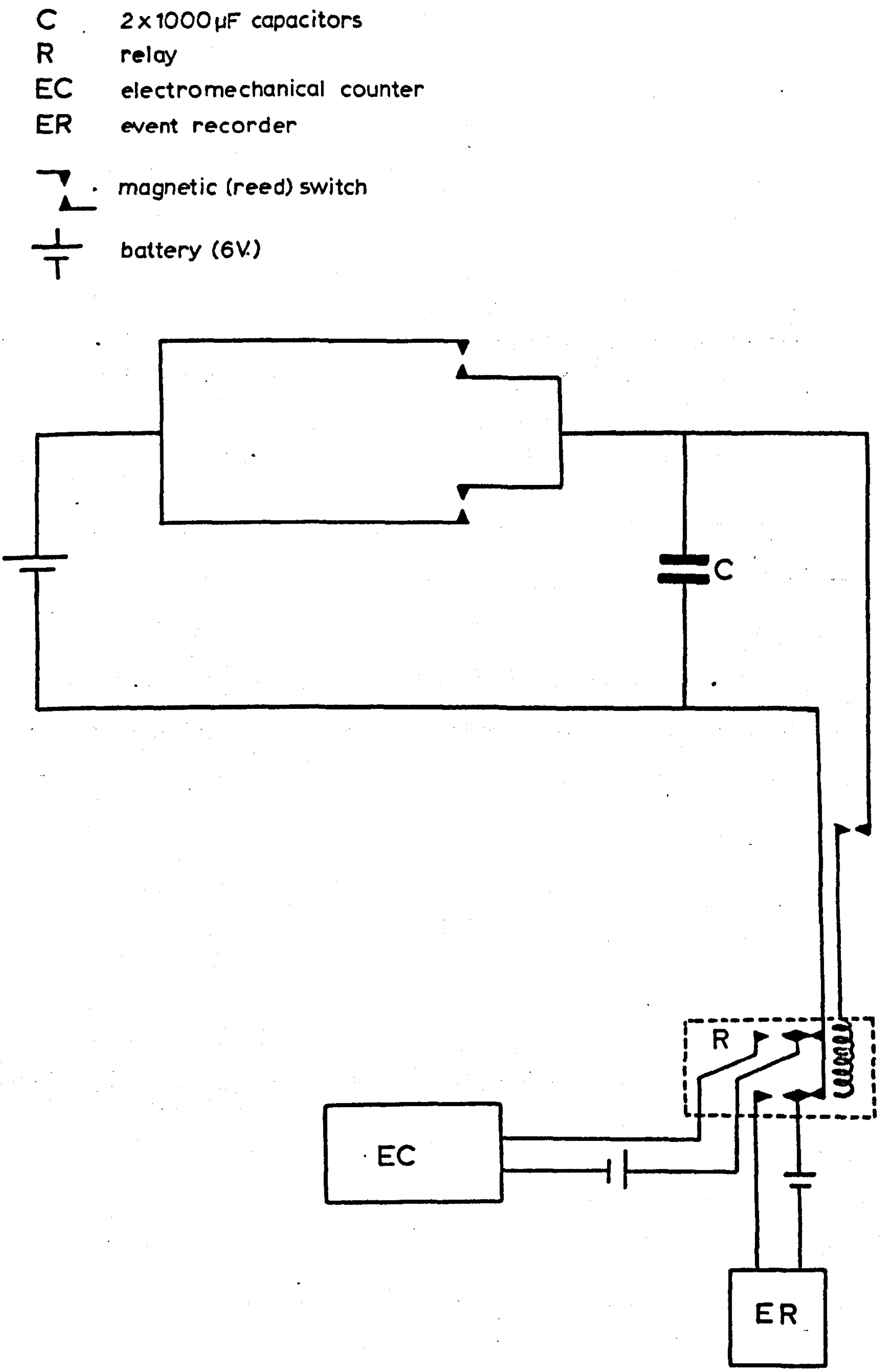


Figure 3 Diagram of the basic construction of the tipping bucket raingauge.

Figure 4 Circuitry used with recording raingauge.



Apparatus

The raingauges used in the present study were constructed from 15.25cm. diameter plastic piping, into which was mounted a 15.25cm. diameter plastic funnel. The funnel was sufficiently smooth to minimize adherence of raindrops to the surface. Damage to the funnel rims by Lepus timidus at the Kerloch site was a problem which could have been overcome by fitting the funnels with metal rims. The large funnel leads to a smaller funnel which drains into a brass bucket. The draining properties would have been improved by using curved buckets (Collinge, 1962), perspex being a suitable alternative to brass (White & Rhodes, 1970). The bucket was connected to a lever bearing magnets which on passing an arrangement of reed switches (Figure 3) actuated a circuit to generate a single electrical pulse (Figure 4). This pulse triggered a relay (Radiospares type 40, 12V, 185Ω) which actuated a five figure, non-resettable, electromechanical counter (6V) and an event recorder (Rustrak-Galton Ltd., Brighton). The system was powered by a 6V. Nickel-Cadmium rechargeable battery, containing sufficient power to record 30cm. of rain. The electromechanical counter was powered by an independent 6V supply since experience indicated that a slight reduction in battery voltage resulted in failure of the counter to operate. Two 1000μF capacitors ensured that when the battery was nearly discharged, the relay would still be actuated.

Although bucket capacity is normally varied to give a unit of rainfall measure, e.g. for a 20.3cm. diameter funnel, 0.25mm. of rainfall is equivalent to 8.2ml. of water (White & Rhodes, 1970), it was more convenient to calibrate the bucket to tip at a constant volume of water and calculate the equivalent depth of rainfall. The bucket was calibrated by placing lead weights on the dividing partitions between the two halves of the bucket. Water retention by the surfaces of the

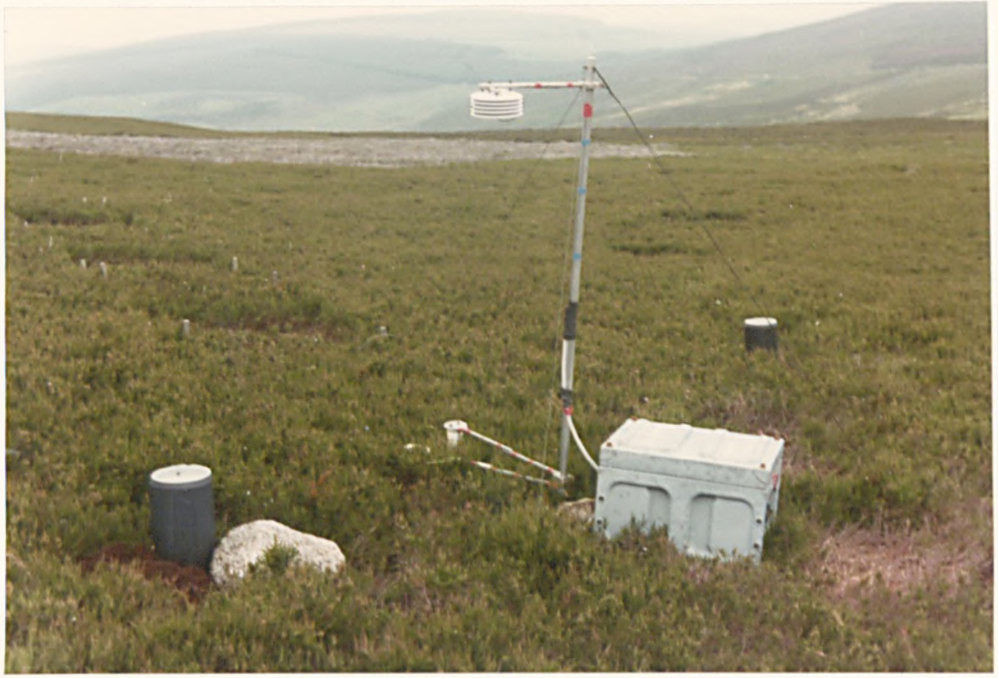


Plate 2. Automatic weather station used for recording on-site measurements of rainfall and temperature. (Recording instruments are housed in the weatherproof grey metal box adjacent to the mast used for positioning of temperature sensors). (Kerloch, July 1975).

bucket was minimized by lacquering those surfaces in contact with water, although the first tip in any series of tips was consistently an overestimate. A mean of twenty replicate tips was used to give a calibration factor. The standard error of the mean for all gauges was consistently less than 1% of the mean. Each tip represented approximately 0.25mm. of rain, although precise calibration factors were used to convert field data into values for rainfall.

Certain problems were encountered in the initial use of the equipment. The failure of the chart drive mechanism of the event recorder was not easily overcome. Despite being powered by a 6V motor cycle battery, the chart drive often failed to operate for periods greater than a few days. This was partly overcome by fitting a counter which continued to operate on failure of the event recorder. Secondly, corrosion and wear on the pivot bearings altered the calibration factors. The initial 'bearings' consisted of a turned down screw resting on a plastic mounting, but this led to large errors in the tipping of the two halves of the bucket. A partial remedy was obtained by letting the screws pivot on a thin brass strip, acting as a reverse knife edge. The small error obtained when the calibration was checked <u>in situ</u> at the termination of the study indicated a degree of success attained by such a modification. A more appropriate method would involve a collecting mechanism in the base of the gauge to check the counter for possible intermittent operation and as a safeguard against failure.

The recording apparatus was housed in a weather-proof, alloy box with an air tight screw-on lid which effectively excluded the penetration of water (Plate 2).

(b) Site microclimate

The malfunction of the on-site weather stations made it desirable to establish relationships between the site and the nearest weather station recording the appropriate parameters. These were located 6.4km. north of the Kerloch site at an altitude of 77m. at Banchory (G.R. NO 700958), and approximately 9km. west of the Cannock site at an altitude of 100m. at Penkridge (G.R. SJ 919115). Two options were available, either to use only the data from the official meteorological observing stations at Banchory and Penkridge or to derive a relationship between these stations and the experimental site.

(i) The thermal microclimate

The intermittent functioning of the temperature recorders, a problem also experienced by Polwart (1970), has necessitated the interpolation of missing data for extensive periods during the course of the investigation. Polynomial regression analysis (Appendix A,2(b)) has been performed relating the available data for each probe of each site to the appropriate daily average temperature for the meteorological station. The respective regression equations (Appendix B, Table 1 & 2) have been used to derive the various temperature parameters for the sites from the station data. A significance level of 0.1% was attached to the acceptance of a polynomial equation, the lowest order of polynomial fulfilling this requirement being used. The proportion of variation accounted for by the Banchory-Kerloch regression varied from 76.1% for the -15cm. probe to 96.3% for the +15cm. probe whilst the variation accounted for by the Penkridge-Cannock regressions varied from 86.1% to 89.6% for the -15cm. probe and 60cm. probe respectively. (In the case of the surface probe at Cannock the highest degree of polynomial calculated only had a significance level of 1%.)

The proportion of variation which can be accounted for is generally

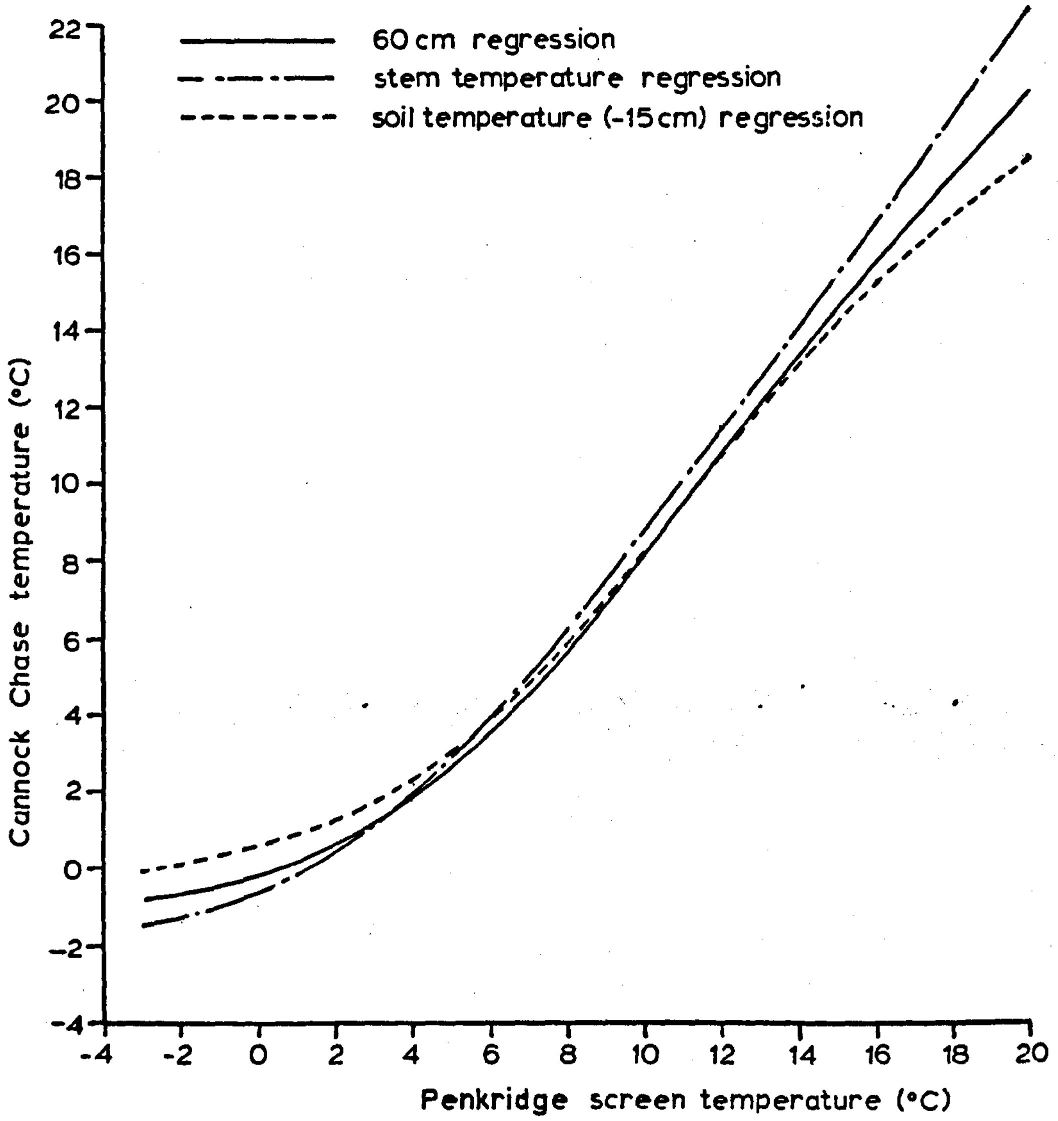


Figure 5 Examples of polynomial regressions relating site temperature measurements at Cannock Chase to screen temperatures at Penkridge meteorological station.

greater at the Kerloch site than at the Cannock site, i.e. the regressions are a better fit at the former site although the average variation accounted for by all probes at the two sites is the same, i.e. 87.05% and 87.9% respectively. Five of the eight probes at Kerloch have relationships with the Banchory data accounting for greater than 90% of the variation. The discrepancy between the Banchory-Kerloch and Penkridge-Cannock regressions may result from the greater distance between site and station in the latter case. It is also noteworthy that the lowest amount of variation accounted for at both sites is with the -15cm. probe, possibly reflecting the suppressive effect the soil has on diurnal fluctuations in temperature compared to the fluctuations experienced in the air leading to a less good fit between site data and station air temperatures. Polwart (1970) employed a direct conversion factor of a 1C° temperature drop for every 167m. increase in altitude rather than the use of a regression relationship which, although significant at the 0.1% level, was based primarily on values in the middle of the temperature range. The lapse rates (i.e. decrease in temperature with altitude) at the two sites correspond to 1C° drop in air temperature for every 79m. increase in altitude at Kerloch and 1C° per 83m. increase in altitude at Cannock. This compares with an average dry adiabatic lapse rate of 1C° per 100m., i.e. the rate of temperature drop in a rising, expanding volume of air (Barry & Chorley, 1968). However, this refers to a dynamic situation in which the air is moving through the environment. The actual decrease in temperature with increasing altitude on any occasion depends upon the local air temperature conditions (Barry & Chorley, 1968). This is reflected in the curvilinear nature of the regressions where the lapse rate varies according to the prevailing temperature, for example, at an air temperature (140cm. probe) of 15°C at Banchory the environmental lapse

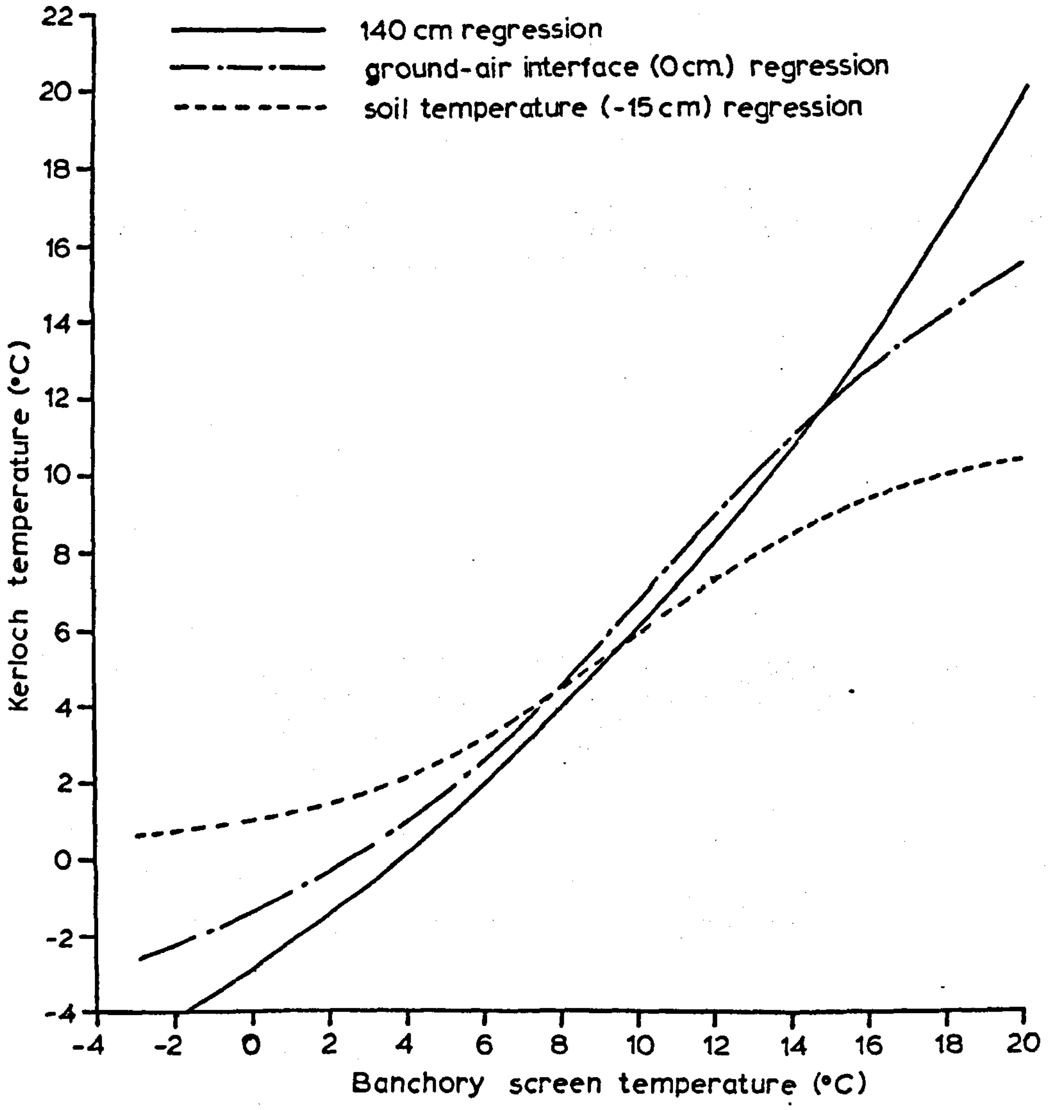


Figure 6 Examples of polynomial regressions relating site temperature measurements at Kerloch to screen temperatures at Banchory meteorological station.

rate of 1C^o per 80.2m. increase in altitude gives a temperature of 12.2^{o}C at Kerloch, in contrast, at a temperature of 5^{o}C the lapse rate is decreased to 1C^o per 55.9m., giving a site temperature of 0.8^{o}C.

Examples of these curvilinear relationships are plotted in Figures 5 and 6 for three probes. The curvilinearity is a phenomenon noted by Polwart (pers. comm.) who compared mountain and lowland temperatures in west Scotland. At Cannock the relationship is linear over the range of temperatures normally encountered but in all cases, at lower temperatures, a decrease in temperature at Penkridge is not accompanied by a linearly proportional decrease in Cannock. This may be an artifact of the statistical method employed, in which the regression is influenced by the comparatively low number of observations at the extremes of the temperature range resulting in an atypical relationship. Alternatively, it may represent a real situation in which cooling of ground, air and stem becomes more difficult at lower temperatures, thus causing departure from a linear relationship. This curvilinearity is marked for the soil probe (-15cm.) especially in the Banchory-Kerloch relationships, indicating that the soil heats up and cools down less readily than the air so causing the curvilinearity in the relationship at the extremes of the temperature range encountered. This may be the result of the low heat conductivity of peaty soil (Barclay-Estrup, 1971) on Kerloch. A fuller analysis of the complex relationships existing between soil and air temperatures is given by Geiger (1965).

Seasonal variation in temperature

The mean monthly temperatures at three probe levels are presented in Figure 7 and 8. At both sites there is a tendency for soil temperature means to lie above those of the air temperature in the winter period, particular on Kerloch. The differences in temperature

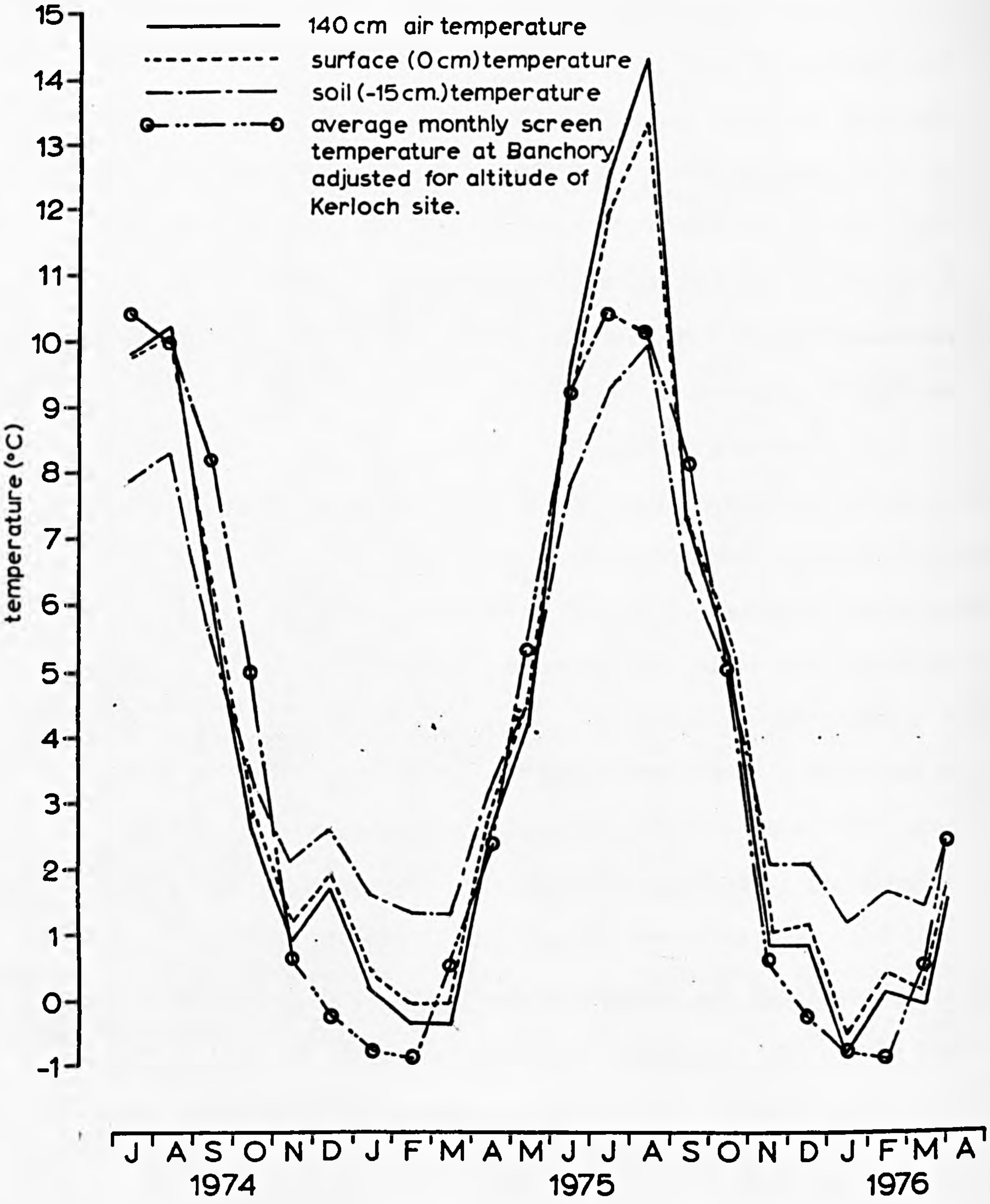


Figure 7 Seasonal course of mean monthly temperature at three levels, Kerloch.

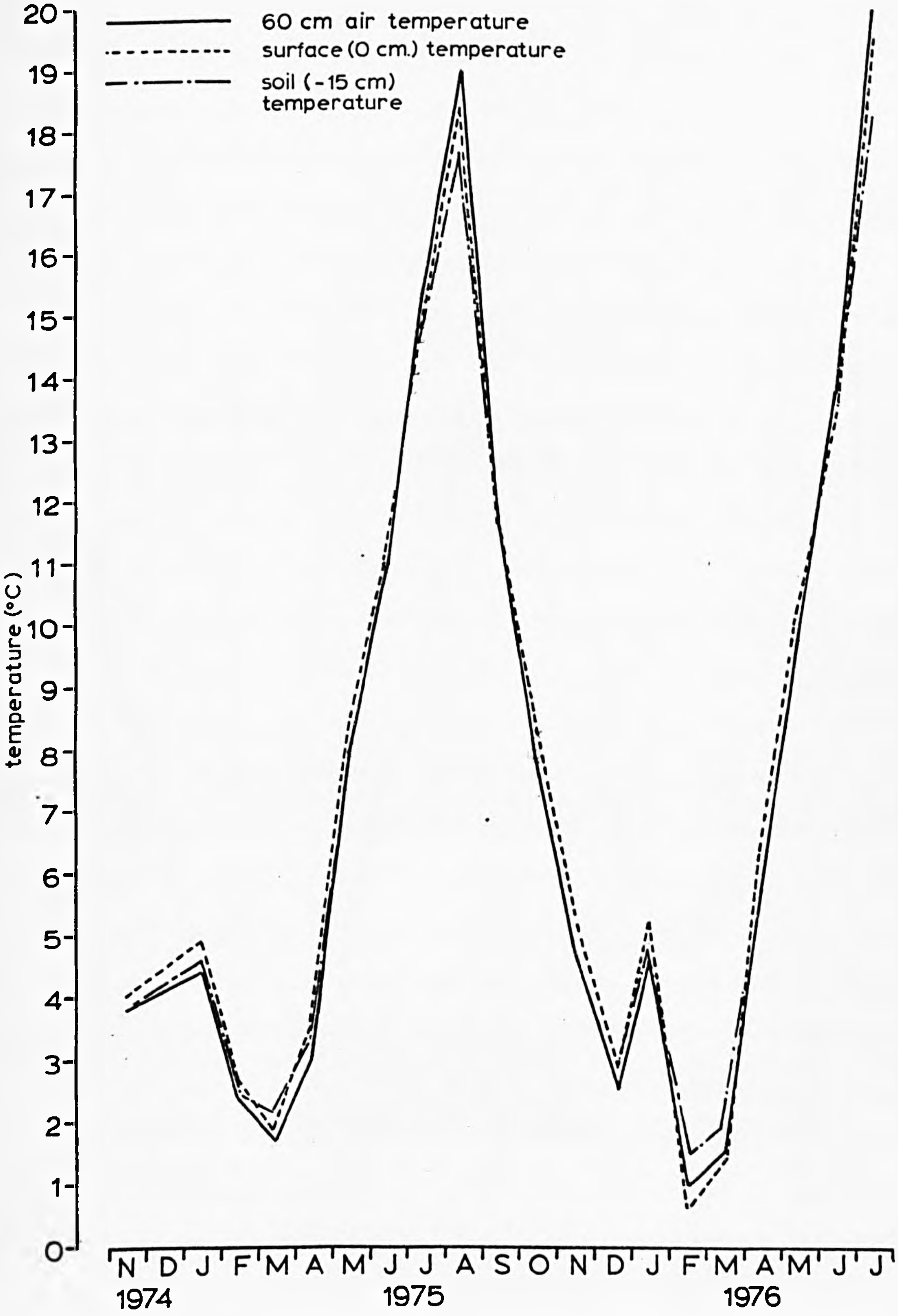


Figure 8 Seasonal course of mean monthly temperature at three levels, Cannock Chase.

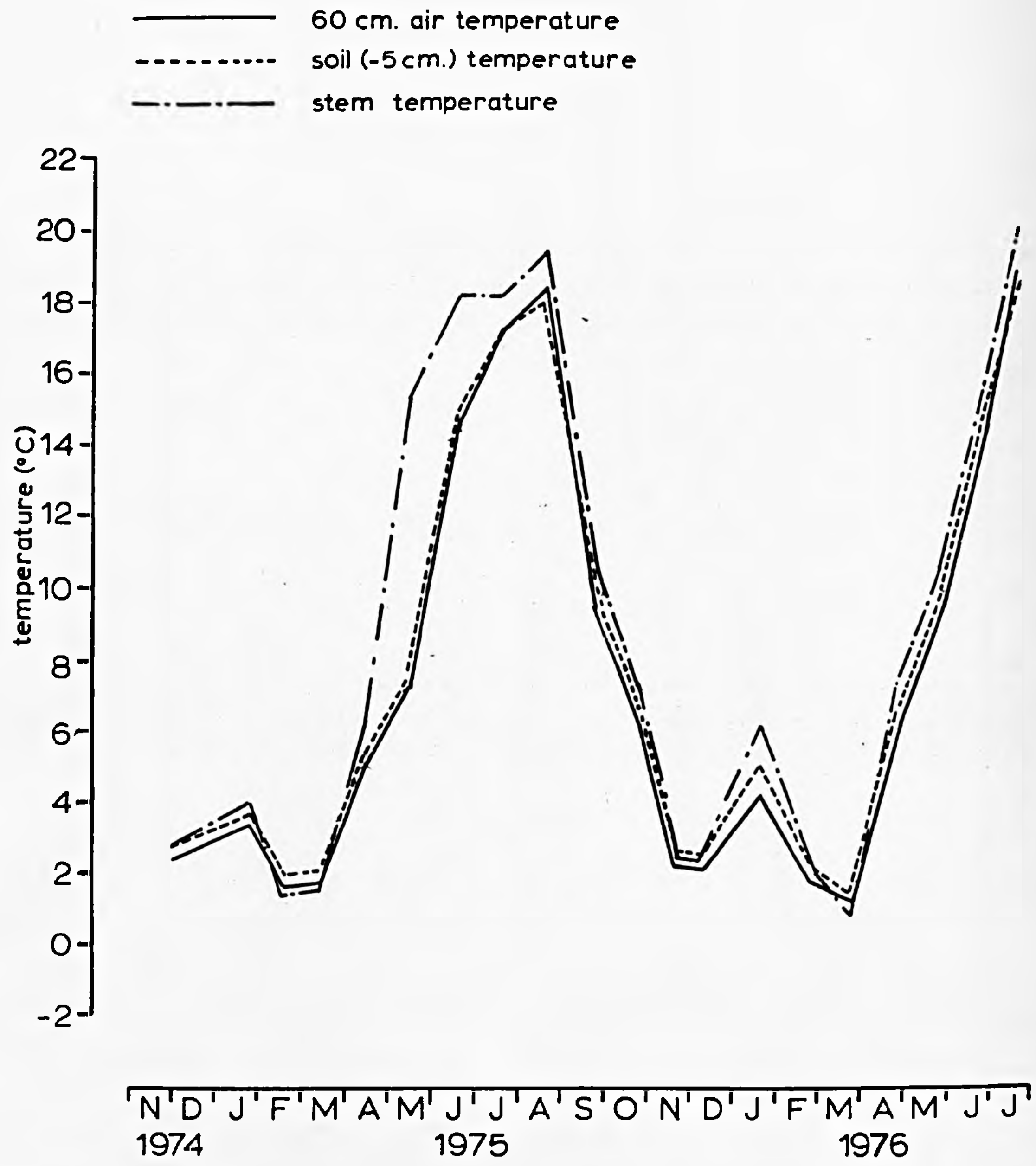


Figure 9 Seasonal variation in mean temperature fourteen days before treatment, Cannock Chase.

at different levels is more marked on Kerloch than on Cannock Chase. It is possible that the denser vegetation cover on Kerloch reduces the depth of penetration into the soil of thermal variations (Richardson, 1958) resulting in higher winter soil temperatures and lower summer soil temperatures in comparison to air temperatures. In contrast, the sparser vegetation cover on Cannock Chase may be resppnsible for the closer relationships between soil and air temperatures suggesting a more rapid equilibration between the two media.

The maximum air temperatures on Kerloch occur after the mid-summer solar zenith, i.e. in August 1974 and 1975 whilst the minimum occurs following the mid-winter solstice, i.e. February 1975, January 1976. Similarly, the maximum and minimum air temperatures on Cannock Chase occur in July 1976 and March 1976, February 1977. This reflects a lag in seasonal variation in temperature similar to that found in diurnal variation in temperature (see below). The growing season, as defined by a temperature of 7.2°C (Grace & Woolhouse, 1970) commences in June and ends in mid September 1975 on Kerloch compared to May to October 1975 on Cannock Chase and recommencing in May 1976. However, this may not accurately reflect the temperature experienced by the shoot. The mean temperature for the fourteen days prior to treatment at Cannock Chase (Figure 9) (chosen on the basis that certain physiological variables are correlated with this value and not the monthly mean), indicate that temperatures allowing shoot growth may be attained before the time suggested by air temperature i.e. April 1975, 1976. Mean shoot temperatures also rise to higher values than air temperatures which has implications with respect to growth rates (cf. Arnold & Monteith, 1974). This may be due to the lack of shielding on the stem temperature probe although shoot temperatures are known to exceed air temperatures by considerable amounts (e.g. Salisbury & Spomer, 1964). It should be re-

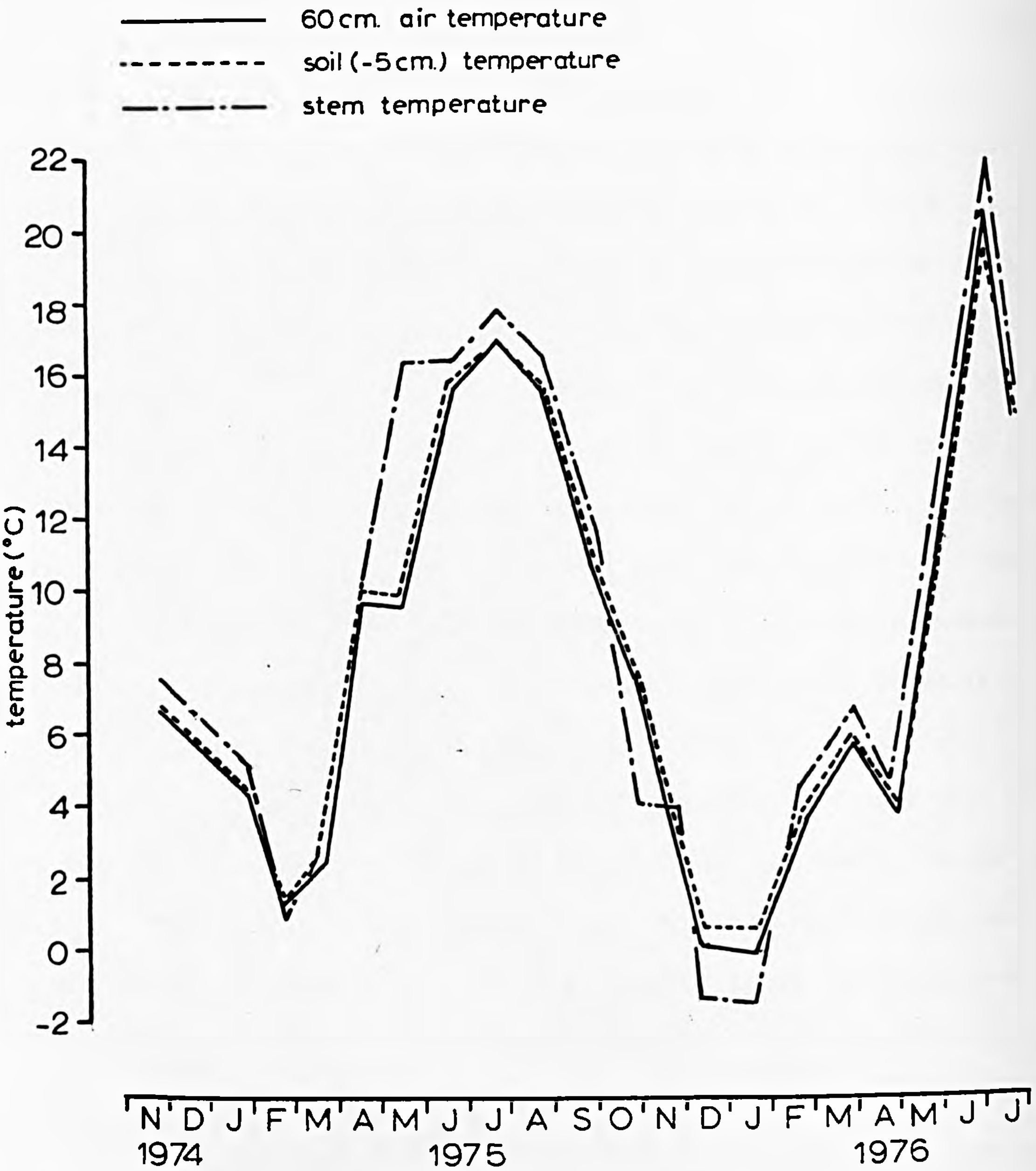


Figure 10 Seasonal variation in mean temperature seven days after treatment, Cannock Chase.

emphasised that whilst mean values may indicate the thermal microclimate of a site the values which may be important may be more specific. For example, day time temperatures may influence photosynthesis whilst thermal extremes, not reflected in the mean value, may affect plant response to certain environmental stresses.

The monthly average for a period of years at Banchory and corrected for the altitude of the Kerloch site is also presented (Figure 7). The monthly means for the period 1974-1976 approximate to these values although discrepencies occur. These are especially noticeable in June, July and August 1975 where the site means are up to 4C° higher than the calculated average.

It is also noteworthy to consider the significance of 4°C, the temperature at which water is most dense (Slatyer, 1967). The implications are more fully discussed below but soil temperature means (-15cm.) at Cannock lie above 4°C between April and October 1975 and after April 1976 and never drop below 0°C, although this does not imply that there are not periods when the ground is frozen and, as a consequence, water is unavailable to plant. Similarly, on Kerloch soil temperatures (-15cm.) fall below 4°C in October 1974, 1975 and rise above 4°C in April 1975 but do not fall below 0°C.

A further temperature extraction with reference to the Cannock Chase site is presented in Figure 10 in which the mean temperature over the seven day period following treatment has been calculated. The seasonal variation closely parallels the fourteen day mean (Figure 9) although some minor discrepancies occur such as sub-zero stem temperatures in December 1975 and January 1976 and a stem mean in excess of 20°C in June 1976. The thermal microclimate experienced by the plant in the immediate post treatment period may affect the plant's reaction to trampling.

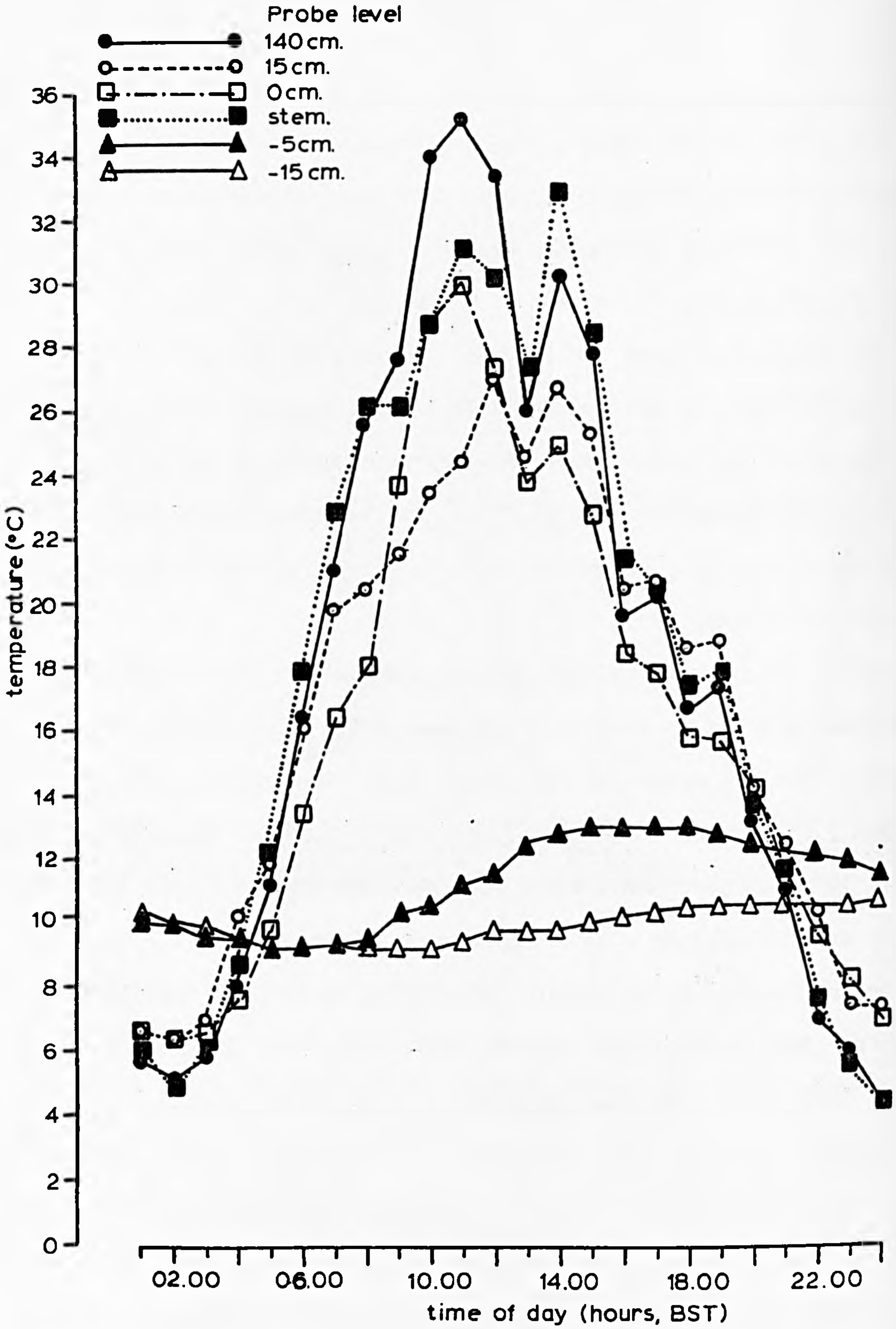


Figure 11 Diurnal variation in temperature, Kerloch (25.06.75.).

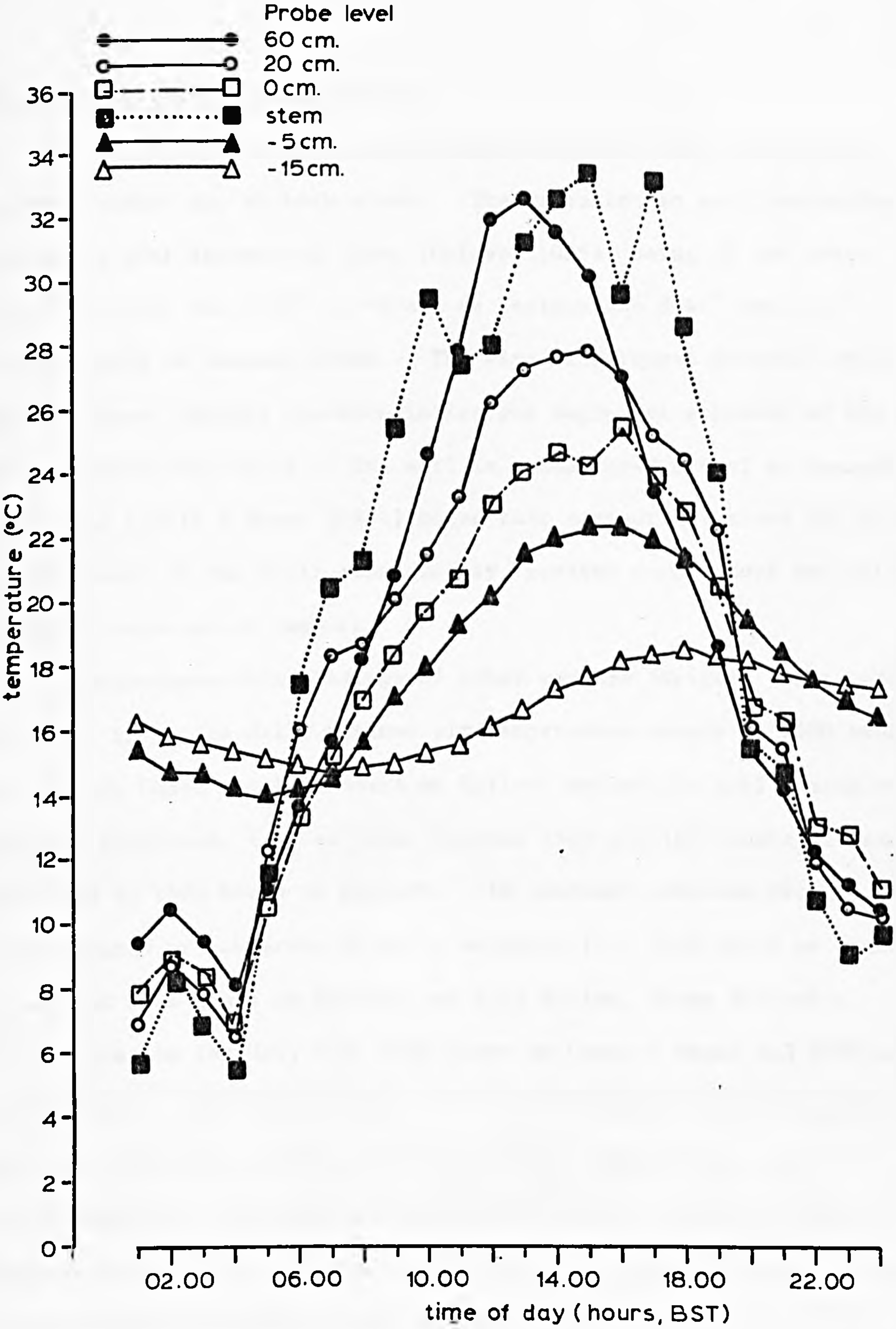


Figure 12 Diurnal variation in temperature, Cannock Chase. (25.06.75.).

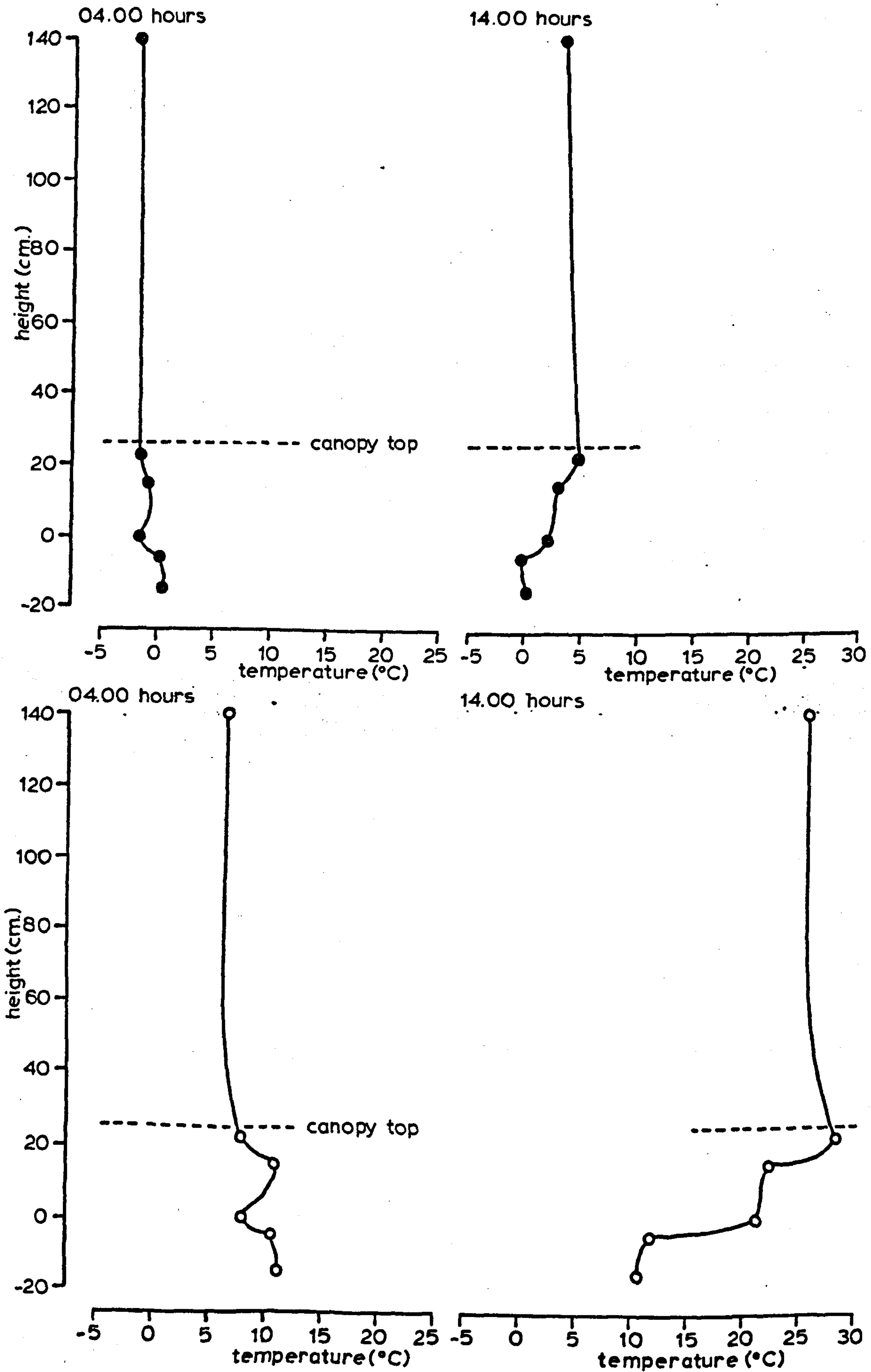
Diurnal variation in temperature

The diurnal patterns of temperature (Figure 11 and 12) are for a typical summer day at both sites. The variation in soil temperature decreases with increasing depth (Geiger, 1965), being of the order of 3.6C° at -5cm. and 1.6C° at -15cm. on Kerloch and 8.4C° and 3.8C° respectively on Cannock Chase. The site differences probably arise from the different thermal characteristics and degree of exposure of the soil at the respective sites. The soil is a compacted podsol at Cannock Chase and Liddle & Moore (1974) noted that compaction raises the thermal conductivity of the soil; consequently, greater temperature variation might be expected at Cannock.

In accordance with findings of other workers (Geiger, 1965; Liddle & Moore, 1974) the daily maximum air temperature occurs at 1300 hours on Cannock Chase and 1100 hours on Kerloch whilst the soil maxima occur in late afternoon, i.e. at -5cm. between 1500 and 1600 hours at Cannock and 1500 to 1800 hours on Kerloch. In contrast, minimum air temperatures are observed in early morning, i.e. 0400 hours on Cannock Chase and 0200 hours on Kerloch and soil minima, where definable occur later in the day, i.e. 0600 hours on Cannock Chase and 0700 hours on Kerloch. This reflects the time lag in heating and cooling between air and soil which, during the winter months especially, may have consequences for the water balance of the plant, for whilst the air temperature may be sufficiently high to permit transpiration, low soil temperatures may inhibit water uptake.

Surface temperatures (0cm.) generally lie between those of the soil and air, in contrast to the findings of Barclay-Estrup (1971), on Cannock Chase the lowest surface temperature (at 0400 hours) lies below the air temperature possibly due to the lack of vegetation to impede night reradiation and therefore promote surface cooling. On Kerloch, surface

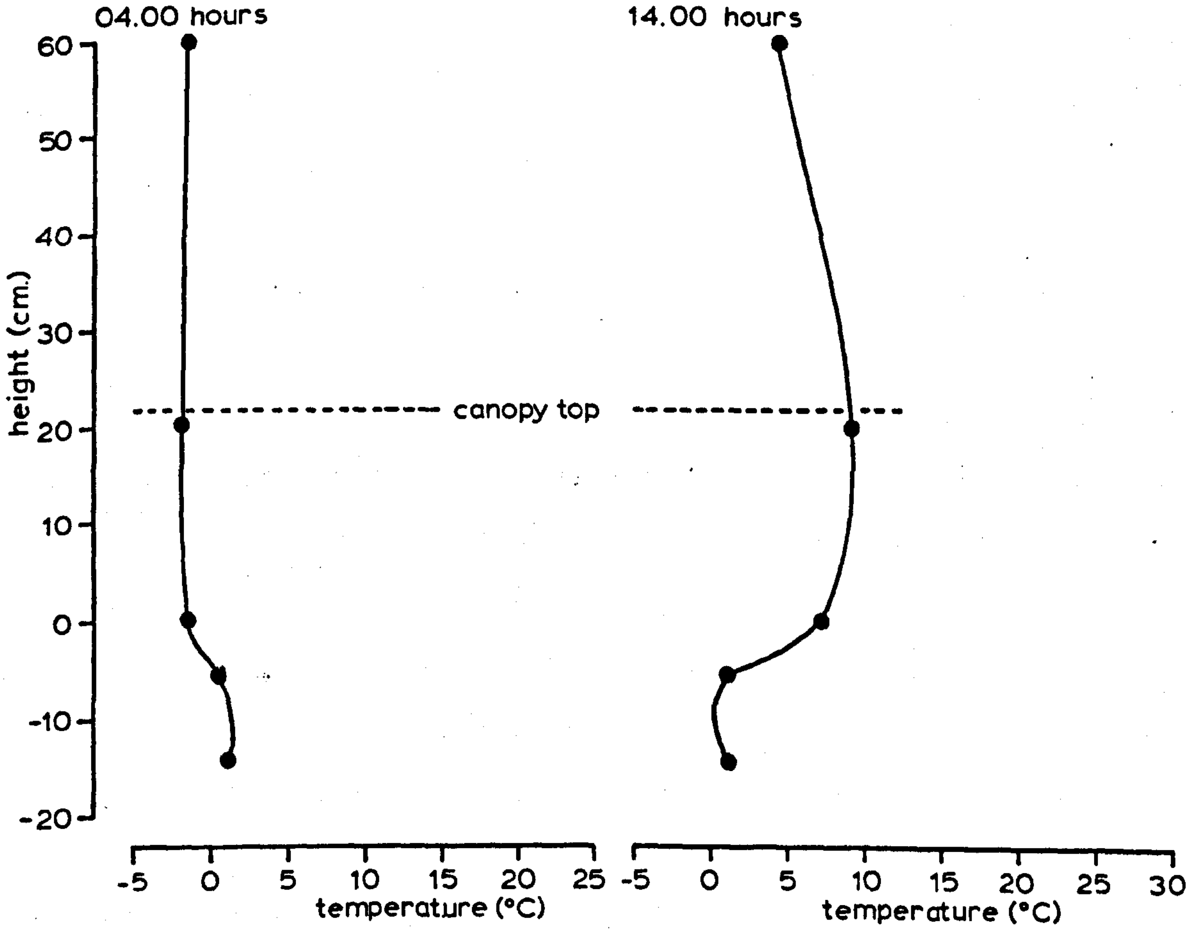
(a) Winter temperature profiles (22.02.75.):

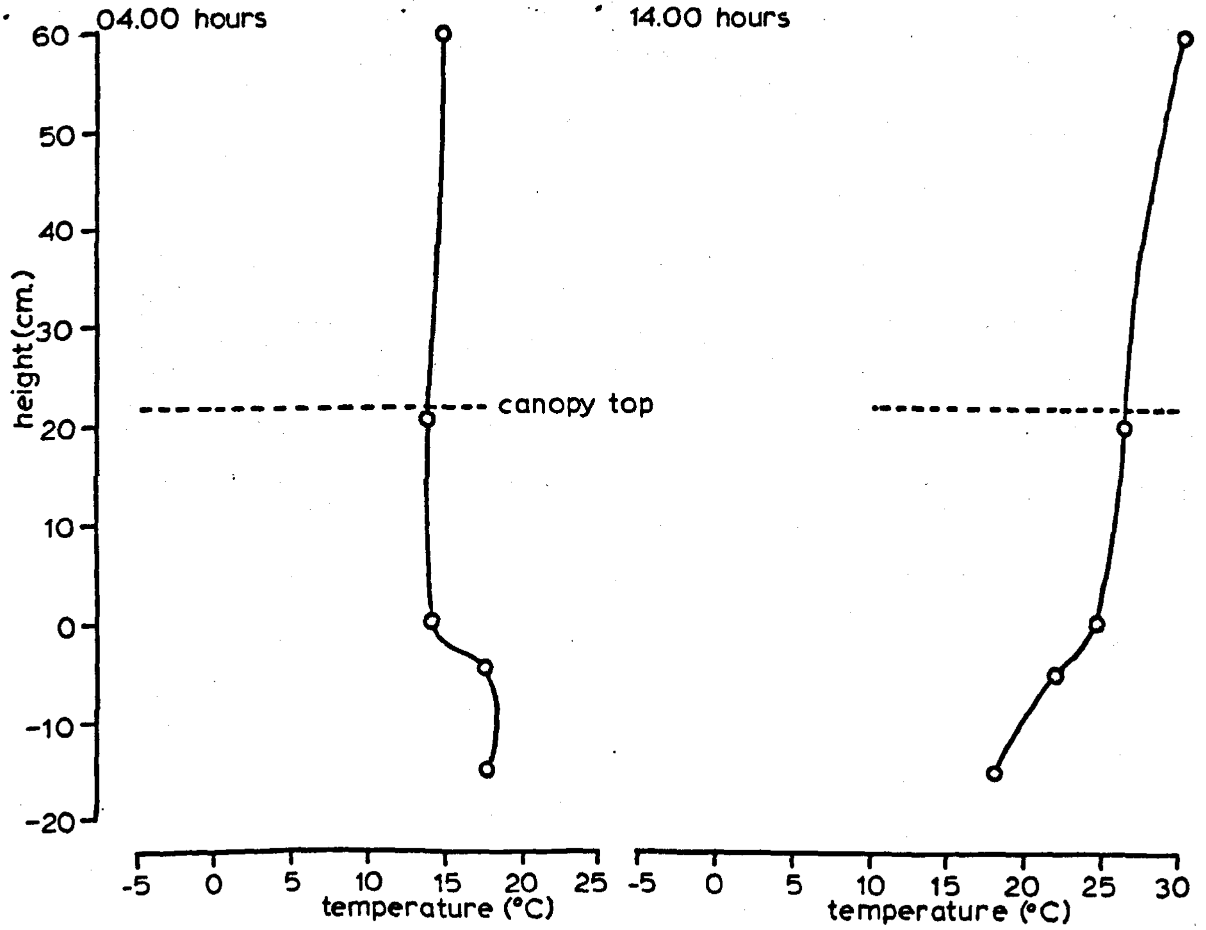


(b) Summer temperature profiles (01.08.75.):

Figure 13 Temperature profiles in a Calluna vulgaris stand, Kerloch.

(a) Winter temperature profiles (22.02.75.):





(b) Summer temperature profiles (01.08.75.)

Figure 14 Temperature profiles in a Calluna vulgaris stand, Cannock Chase.

temperatures do not fall as low as air temperature, possibly because of a denser vegetation cover reducing radiant heat loss at night, and possible reradiation from the vegetation in a downward as well as upward direction.

Whilst it may not be valid to compare the unshielded stem probe with the shielded or subsurface probes, it is of interest to observe that on Cannock the temperature range recorded by this probe is greater than that of any other (i.e. 33.4C°) whilst the stem maximum is the highest recorded on Cannock and the stem minimum the lowest temperature recorded at both sites. This may be consequential in terms of metabolic processes mentioned above especially if the condition is of wider seasonal applicability as it would appear to be, e.g. on the 13.02.75 on Cannock Chase the stem maximum was 9.2°C compared to a soil maximum of 1.6°C.

Temperature stratification

The thermal characteristics of the microclimate may be elucidated by considering profile stratification, (Figure 13 and 14).

The temperature amplitude down the profile is relatively small, an observation corroborated by the findings of Polwart (1970), although the amplitude varies with the time of day and time of year. In summer the temperature range on Kerloch at 0400 hours is 4.0C° compared to 17.6C° at 1400 hours. This compares with a range of 2.0C° and 2.8C° respectively in winter. This accords with similar observations made by Barclay-Estrup (1971) for a 'building phase' community of Calluna vulgaris. Also in agreement with his study is the observation that maximum daytime temperatures occur just within the top of the canopy (20cm.), this being especially marked in the summer profile. This may reflect the absorption of radiation by the upper layers of vegetation combined with long wave re-radiation from below, heating the air which is comparatively still due to the boundary layer resistance to wind movement caused by the canopy. By the same virtue the lower layers of vegetation (15cm.) may be comparatively cooler in summer due to radiation interception and reflection by the upper layers of the canopy. The small variation in soil temperature reflects the insulating properties of the vegetation cover. During the day the temperature decreases with decreasing height down the profile but at night the vegetation cover may restrict heat loss helping to maintain a higher temperature in the soil. In addition, re-radiation from the soil into the air below the canopy top may contribute toward maintaining a higher temperature at 15cm. than at higher levels within the profile (cf. Liddle & Moore, 1974). Surface temperatures (0cm.) may also be lower than air temperatures at 15cm. due to the re-radiation of heat which is then trapped in the air layer close to the ground. The maximum amplitude of temperature is observed at a point just within the canopy whilst the minimum is found within the soil which, due to its covering of dense vegetation and low heat conductivity, is protected from extreme temperature variations (Keen & Russel, 1921; Gloyne, 1950; Geiger, 1965; Barclay-Estrup, 1971).

The Cannock Chase profiles are similar to those described above in which the soil temperatures are higher than the air temperatures at night and lower during the day. The temperature range at 0400 hours is 3.8C^{o} and at 1400 hours 13.0C^{o} for a summer day compared to 3.0C^{o} and 8.2C^{o} respectively for a winter day. The summer temperature range is less than that at Kerloch which may be due to the comparative openness of the plant cover and the thin stony soil which heats and cools in greater sympathy with the air. The greater winter temperature ranges may similarly result from a sparse vegetation cover in which the temperatures above ground level are similar since the plant cover has less effect on air turbulence at Cannock thereby facilitating mixing of the air, whilst direct insolation may raise the surface temperature. Similarly, at night the main distinction is between air and sub-surface temperatures in which the air cools down to a greater extent than the soil.

From this generalised discussion on site thermal microclimate, certain points may be considered directly relevant to the current investigation, namely:

(1) the relationship between the thermal microclimate and plant water balance;

(2) the effect of temperature on the plant following mechanical damage;

(3) the effect of temperature on the development of physiological resistances to environmental stresses;

(4) the effect of temperature and its extremes on plant metabolic processes;

(5) the effect of temperature on growth and the duration of the period in which growth is possible.

The validity of the 'mean' temperature

Arnold & Monteith (1974) justify the use of 'mean' temperatures with the following conditions:

(1) that the rate of a temperature dependent process e.g. growth, is linearly related to temperature between the predefined temperature limits, T_1 and T_2;

(2) deviations in temperature do not exceed the limits defined by T_1 and T_2.

(3) in comparison to the averaging period used to determine the mean temperature, the process response time is negligible;

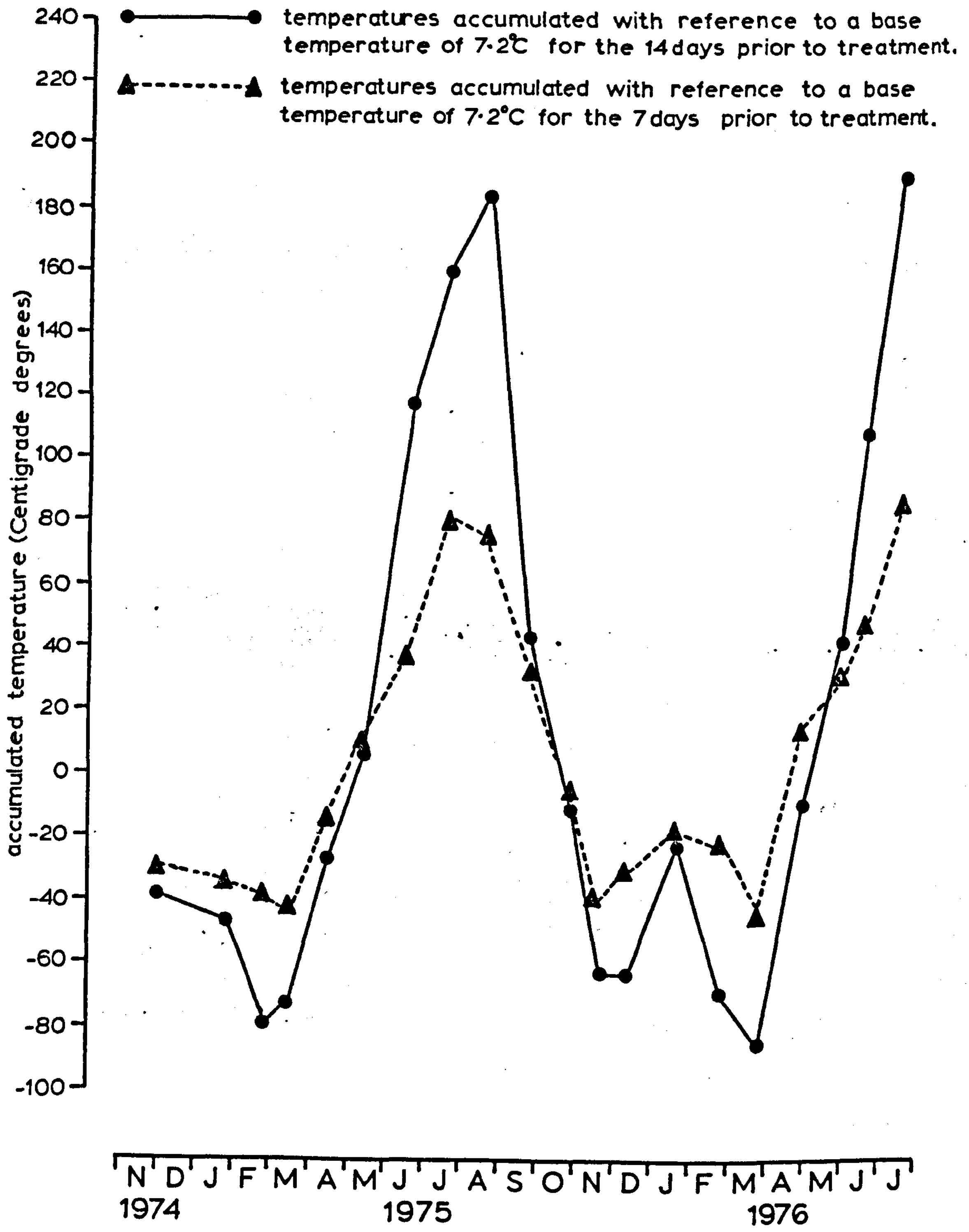


Figure 15 Seasonal variation in accumulated stem temperature, Cannock Chase.

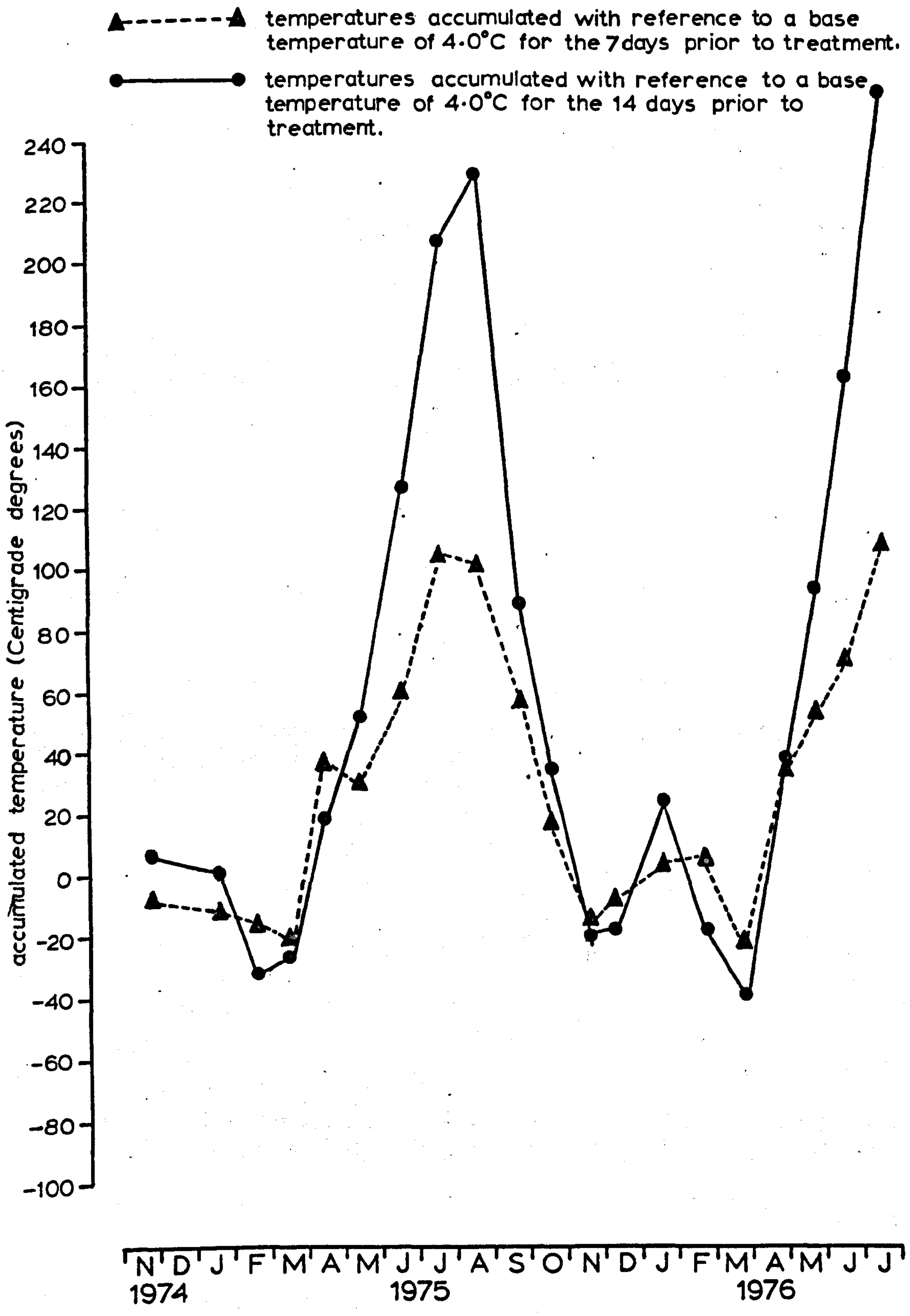


Figure 16 Seasonal variation in accumulated soil temperature, Cannock Chase. (-5 cm. level).

(4) that the process rate is a function of current temperature and is not affected by temperature prehistory.

It is argued that, if these conditions are fulfilled, the relationship between mean temperature and mean rate of a process will not be affected by temperature fluctuations so that "...the mean rate of a temperature-dependent process will be precisely related to the average (i.e. arithmetic mean) temperature..." (Arnold & Monteith, 1974).

The validity of the concept may be questioned. For example, evidence exists that the photosynthetic rate in Calluna vulgaris is affected by temperature prehistory (Grace & Woolhouse, 1970), whilst it may be an oversimplification to relate a physiological process to a single parameter in a multifactorial environment. However, "...details of microclimate in a plant community will often be much less important ecologically than the mechanisms which determine how plants respond.... to a simple discriminant such as average temperature" (Arnold & Monteith, 1974), and in the absence of information regarding the response of processes to field temperatures at a detailed level it is considered justified to employ mean values in an initial investigation of plant macroresponses to environmental stresses.

Accumulated temperatures (Figure 15 and 16)

A parameter often used in studying plant development in relation to temperature is the integral of a mean temperature for a given period, e.g. hourly, daily, above an arbitrary base line. Arnold & Monteith (1974) noted that "....it is not valid in principle unless the physiological process limiting growth is a linear function of temperature". Such an integral has been employed in certain parts of the present study as a possible variable accounting for seasonal variation in parameters such as desiccation tolerance. The values extracted

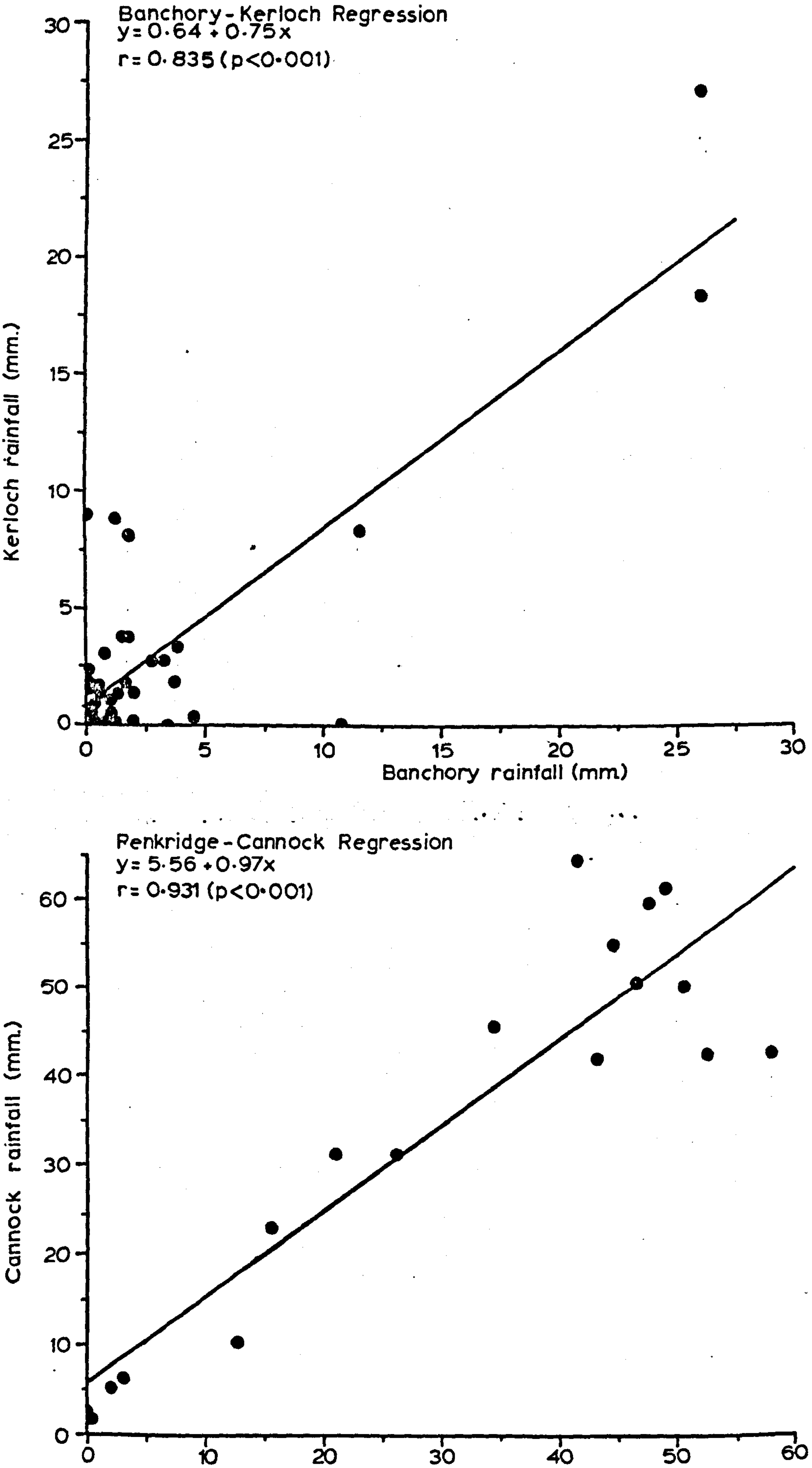


Figure 17 Regression of experimental site rainfall on rainfall data from the nearest meteorological station.

are accumulated temperatures using the daily mean, with reference to a base temperature of 7.2°C and 4°C, the significance of which have been noted above, for the seven and fourteen days prior to treatment. The seasonal pattern of variation is similar for both parameters, and approximates to the seasonal variation in mean monthly temperature, i.e. maximum values in summer and minimum values in winter, the latter often being negative, that is, temperatures lower than the specific reference temperature.

(ii) Rainfall

Linear regression analysis revealed highly significant ($p < 0.001$) correlations between on-site rainfall data, when available, and the rainfall data for the corresponding periods at the meteorological stations (Figure 17). For the Banchory-Kerloch regression, data were obtained for 49 days during which rain fell and was recorded at the site, one day representing the period 0900 hours to 0900 hours the following day, i.e. the standard observer time for the meteorological station at Banchory. The relationship indicates that with increasing rainfall the departure from a 1:1 relationship increases, suggesting that the site receives less rainfall than the station at higher rainfalls whilst at low amounts of rainfall the site appears to have a higher rainfall than the station. This may result from the concentration of points at the lower rainfall values compared to the scarcity of points at the higher values leading to a bias in the analysis. Gauge error may also result in erroneous rainfall data records for the site since with intense rainfall the proportion of rainfall not recorded increases whilst at lower values, calibration error may cause the observed discrepency. However, the effect may be real. With increased altitude precipitation might be expected to increase (Trewartha, 1954), thus a difference in altitude of 223m. from 77m. at Banchory to 300m. on Kerloch, may result in sufficient cooling of the air to induce condensation and thus rain, although in view of the altitudes involved the effect might be expected to be slight. It is more difficult to account for the decrease at higher altitudes at higher amounts of rainfall. Since comparisons are on a daily basis it is probably more appropriate to consider intensity rather than frequency. It might be postulated that under storm conditions associated with high intensities of rainfall, high winds may result in greater errors on the exposed site than at the meteorological station. It is also possible that if and when the site is above the cloud base it may not experience the same intensity of rainfall as the region below the clouds. The Dee valley, in which the Banchory station is situated, may also influence the passage of rainbearing clouds so that the adjacent hill tops lie outside the path of the rain. These effects have been observed on certain occasions whilst visiting the site although such hypotheses need considerable observational verification. The period for which the comparison has been made between site and station is comparatively short in relation to the duration of the experiment and the relationship may be atypical. A further problem is encountered when solid precipitation falls e.g. hail, snow. At higher altitudes it might be expected to be more prevalent than at lower elevations, especially at certain times of the year, and its assessment was not possible with the raingauges employed at the site. Consequently, this represented an unmeasured source of error, especially if precipitation falls as snow at 300m. but has melted to form rain at 77m. However, since this is likely to occur during periods of the year outside the 'growing season', the consequences in terms of water availability to the plant, and growth in relation to water stress, are likely to be small. Since there are a number of problems associated with the use of on-site records at Kerloch precipitation data for Banchory have been presented as indicative of seasonal variation occurring at the site rather than absolute quantities involved.

The relationship between rainfall received on the Cannock Chase site and the rainfall recorded at the Penkridge meteorological station is less problematical, the site receiving consistently higher amounts of rainfall than Penkridge, possibly as a consequence of its greater altitude, although the height difference of 70m. might not be expected to have such a significant effect. However, frequency in addition to intensity may be a complicating factor since the relationship is based upon a period rather than a daily unit. Although intensities of rain falling from a single cloud system may vary, the topographic control over rainfall may be less influential on Cannock than on Kerloch so that higher intensities of rainfall falling from a larger cloud system may encompass both site and station resulting in a narrower discrepency between the two localities. At the lower altitudes involved at Cannock, precipitation falling in the solid state may be of less significance and less likely to affect the relationship. Due to malfunction of the event recorder it is not possible to compare the frequency of rainfall at the Cannock site with the frequency of rainfall at the Penkridge station. However, the Penkridge data have been adjusted by the use of the regression, to represent the precipitation received at the site and are used in subsequent analyses.

At each site two gauges were employed to provide a check and to sustain the record should a gauge fail to operate. The errors resulting from localised turbulence noted above might be expected to result in different readings for the two gauges at a single site. The correlation coefficients of 0.992 ($p < 0.001$) and 0.996 ($p < 0.001$) between the two gauges at the Kerloch and Cannock sites respectively, based on nineteen

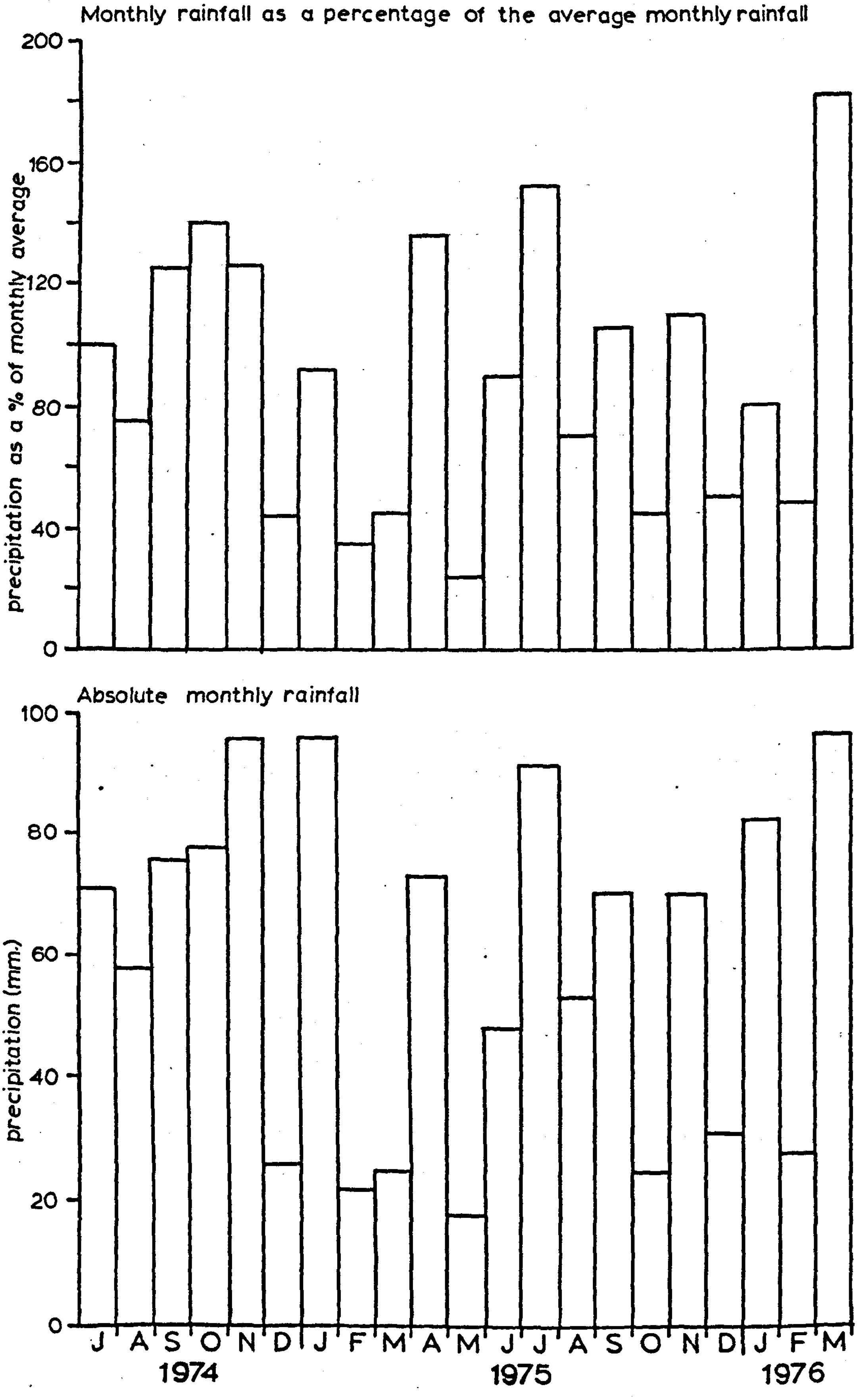


Figure 18 Seasonal course of precipitation at Banchory.

and seven pairs of data items, indicate a good agreement between the two gauges at each site. Following a malfunction in one gauge, the second could therefore be used to provide a reliable estimate of the rainfall, whilst the agreement between the replicates implies a substantial degree of accuracy in recording the rainfall even if precision was not achieved.

Seasonal variation in rainfall

The precipitation data for Banchory are presented in Figure 18 on a monthly basis for the duration of the experimental period. For the growing seasons of 1974 and 1975 the total rainfall corresponds to 100.9% and 106.7% respectively of the average for the period (i.e. the average rainfall received for a particular period over a number of years), indicating that, in gross terms, there was not an appreciable departure from normality. However, rainfall distribution within the period of growth indicates an abnormal situation in 1975. During May, precipitation was less than one third of the average, which may have had an effect on processes associated with the spring flush of growth. June and August received below average rainfall but some compensation may have occurred due to the above average rainfall for July. The monthly total, however, may obscure a situation of greater significance to plant growth. During July the frequency of thunderstorms was above average which may have resulted in 65% of the month's rainfall occurring on five days whilst the remainder of the month was comparatively dry. Much of the year received below average rainfall with concomitant implications for plant growth. Thus it is possible that the time at which rain falls in addition to the amount may be of significance when considering productivity relationships, since a high rainfall in the midst of a dry period may alleviate the problem of drought but to a lesser extent than the same volume of rain spread over a longer period when less is likely to be lost through runoff

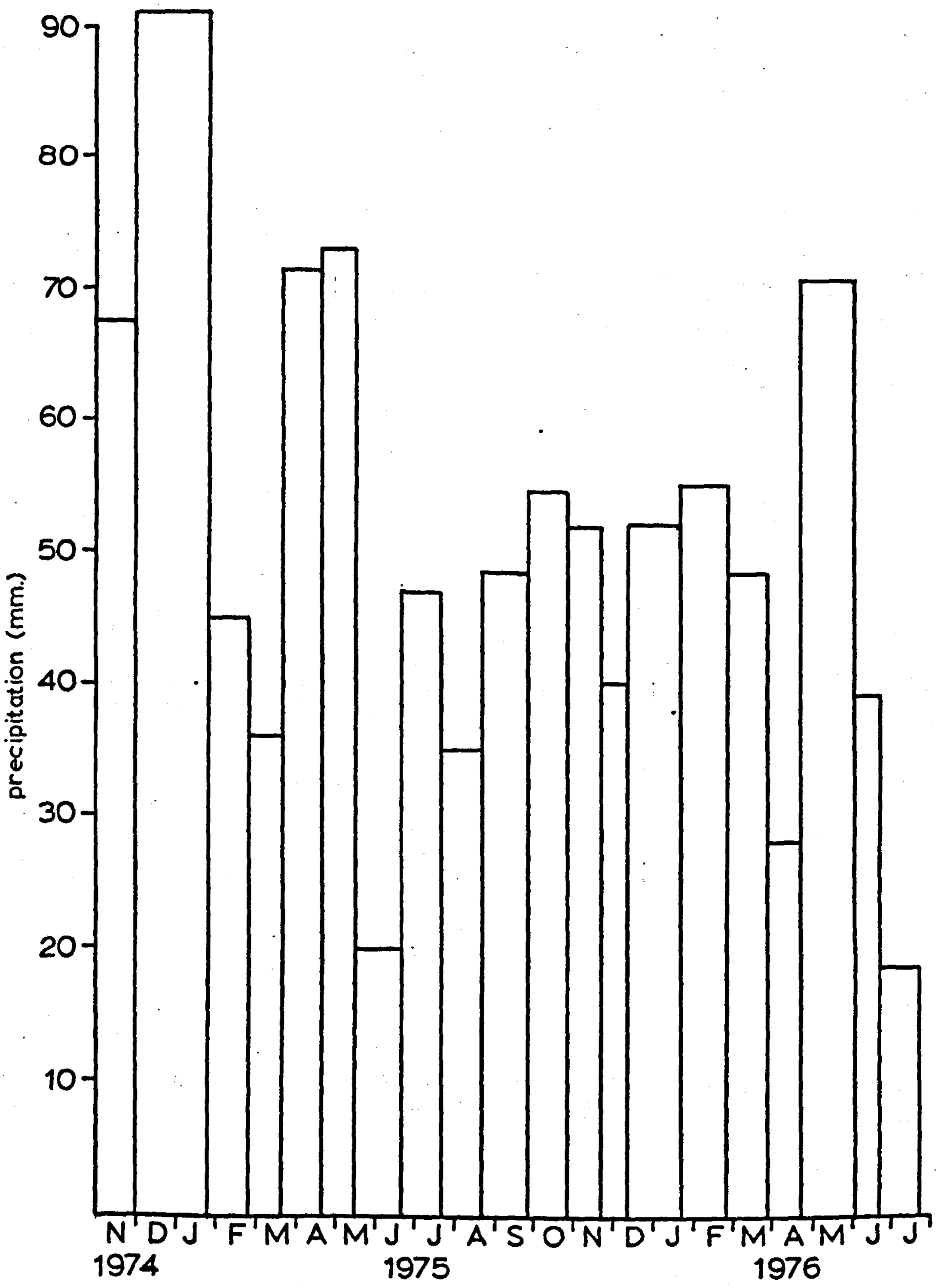


Figure 19 Precipitation received between treatment and sampling dates at Cannock Chase.

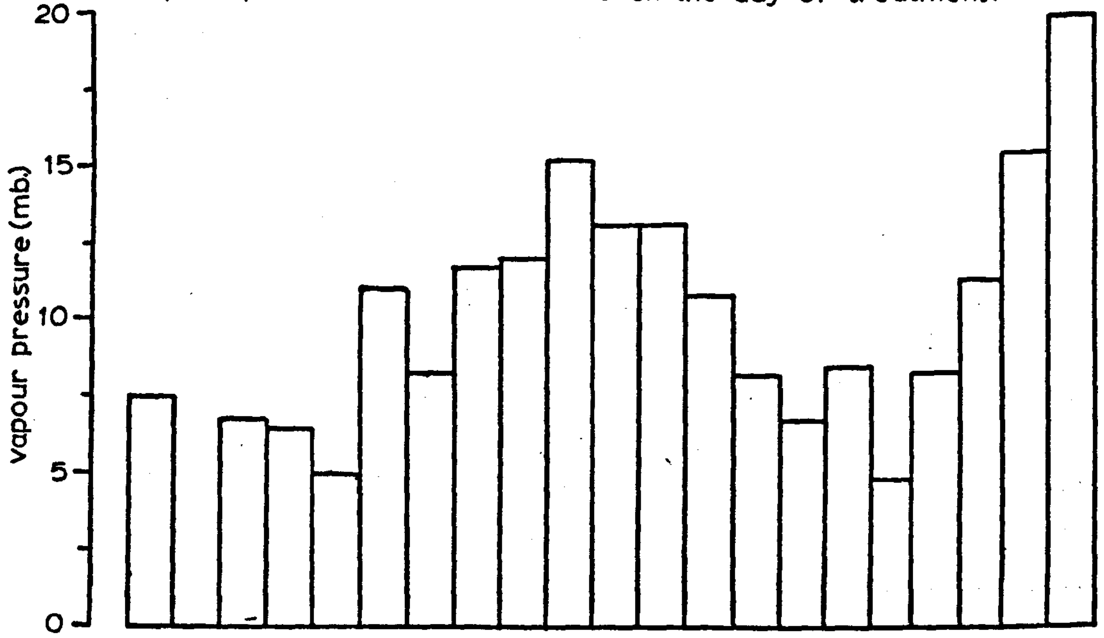
and evaporation.

The precipitation received between the time of treatment and the date of sampling on the Cannock Chase site is presented in Figure 19. As with the Banchory data no clear seasonal pattern emerges and the variation is further confused by unequal periods of representation (indicated by the width of each histogram). Data permitting the rainfall to be expressed in terms of proportion of the long term average are unavailable but it is worthy of note that the summer of 1975 was comparatively dry whilst that of 1976 experienced the most severe drought recorded since measurements commenced at the meteorological station. This has obvious implications with regard to plant growth and survival irrespective of the additional stress imposed by trampling.

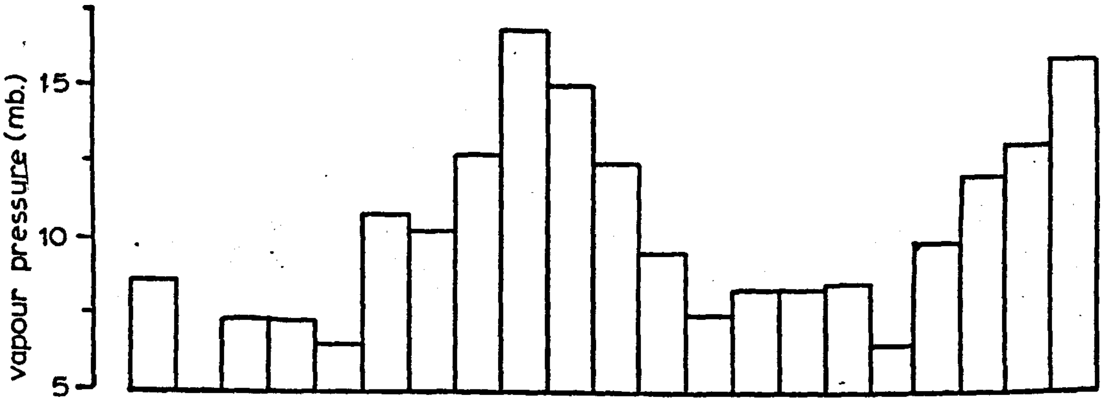
(iii) Vapour pressure (Figure 20)

The vapour pressure is the partial pressure exerted by water vapour in the atmosphere. As the temperature increases so the capacity for air to hold water increases. If water is available an increase in evaporation rate occurs which results in an increase in the vapour pressure. The saturation vapour pressure is the point at which further additions of vapour into the air result in condensation (e.g. Barry & Chorley, 1968; Bannister, 1976). Since the phenomenon is temperature dependent the seasonal course of vapour pressure emulates the seasonal course of temperature. The values (in m.b.) are those for the Penkridge meteorological station at 0900 hours on the day of treatment, the mean value for the seven days prior to treatment and the mean value for the seven days after treatment. (These values have been used in subsequent regression analyses relating plant parameters to environmental variables). A seasonal pattern in vapour pressure is clearly evident, the lowest values occurring in winter i.e. February, March, December 1975, January,

(a) Vapour pressure at 09·00 hours on the day of treatment.



(b) Mean daily vapour pressure for the 7 days prior to treatment.



(c) Mean daily vapour pressure for the 7 days after treatment.

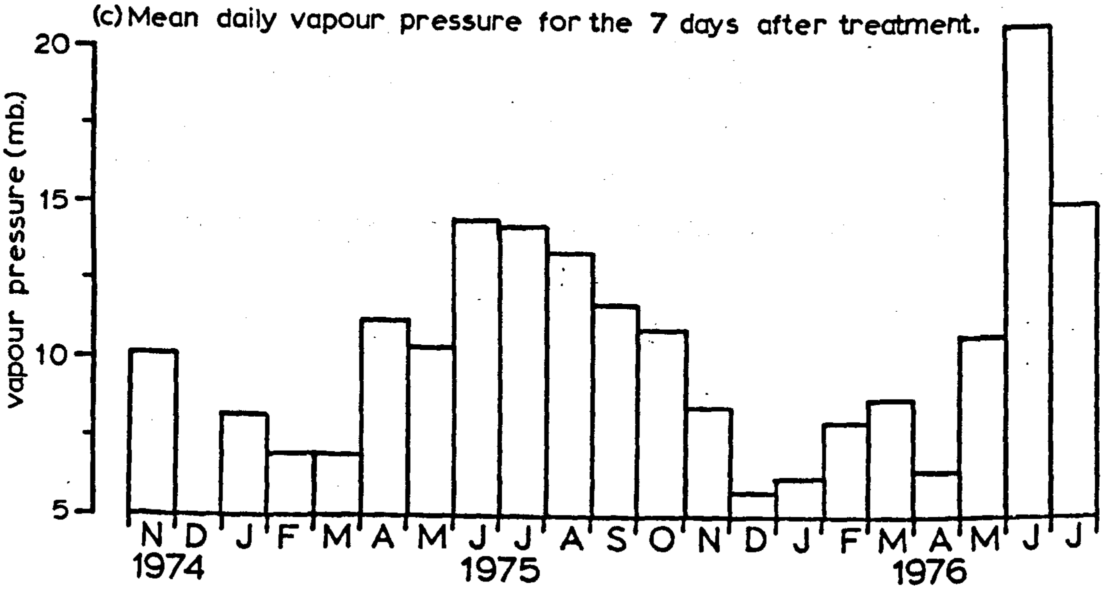


Figure 20 Seasonal variation in Vapour Pressure, Penkridge.

(values for December 1974 omitted)

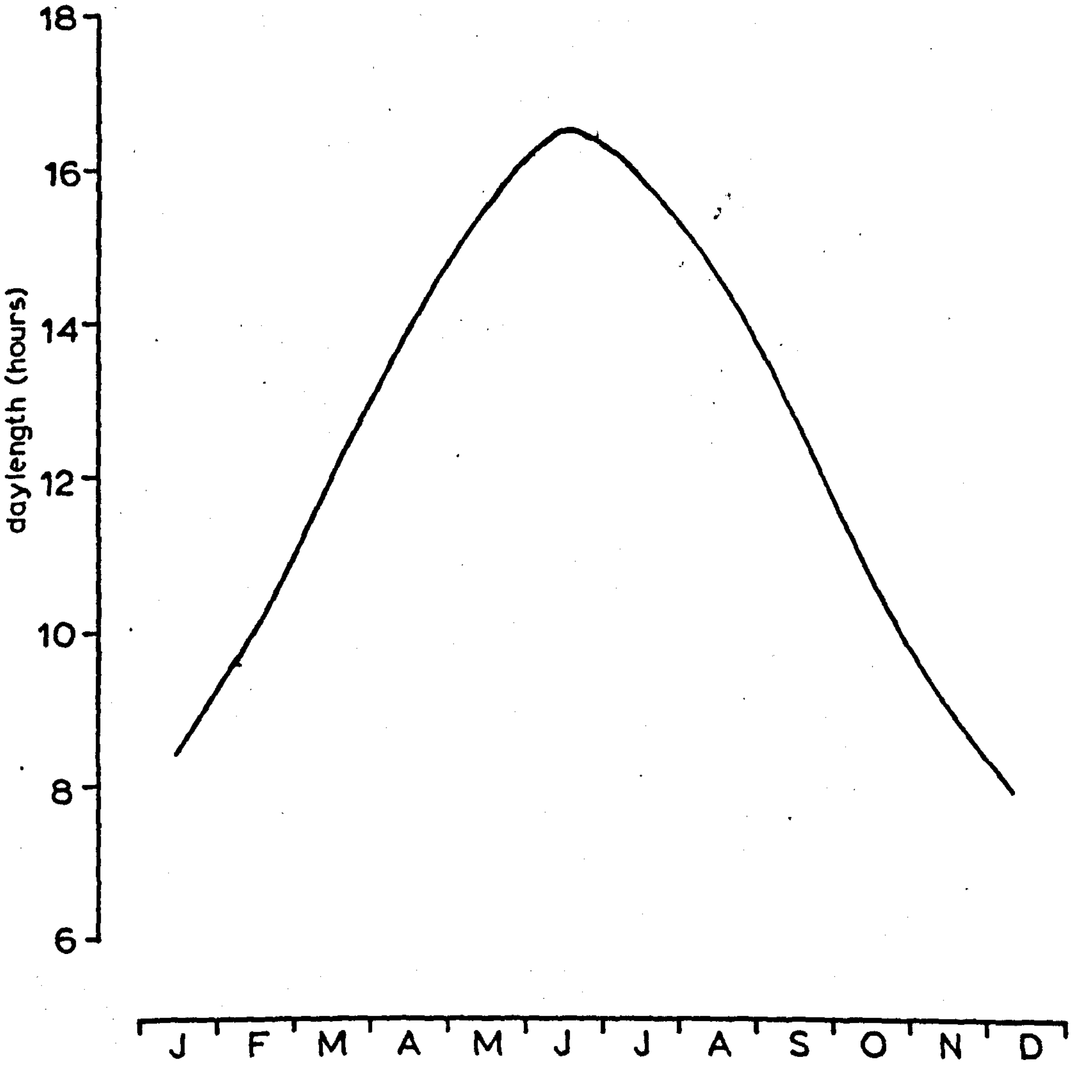


Figure 21 Seasonal variation in daylength,(sunrise-sunset, Greenwich).

April 1976 rising to maximum values in summer, i.e. June, July, August 1975, June, July 1976. Since the value is dependent upon evaporational loss of water into the air the implication is that on days with a high vapour pressure the evaporative demands made by the atmosphere are high. This implies that water loss from a water source, such as a leaf, will be high, consequently, such a parameter may be of significance when considering the water relations of a plant since it reflects the evaporative demands made by the atmosphere on the plant's water content. Consequently, it is possible that water loss will be greater on days of high vapour pressure, thus possibly resulting in higher water deficits if supply is unable to balance demand.

(iv) Daylength (Figure 21)

Seasonal variation in daylength had been based upon the period between sunrise and sunset at Greenwich, extracted from tables. Although it is not precise for the latitude of Cannock Chase, the error likely to arise in the present study is unlikely to be of great significance compared to experimental and sampling errors.

Length of photoperiod has been considered to be of possible significance to the desiccation tolerance of plants by certain workers e.g. Polwart (1970), Bannister (1971) and has been used as an independent variable in accounting for seasonal variation in desiccation tolerance and avoidance in the present study.

PART III - FIELD STUDIES - PLANT WATER RELATIONSHIPS

A. DESICCATION TOLERANCE AND AVOIDANCE

Introduction

Drought resistance may be defined as the "capacity to survive periods of drought with little or no injury" (Meyer & Anderson, 1952), drought being defined as a period in which soil moisture stress is so high that little if any water is available to the plant. Drought resistance is composed of two fractions (Levitt, 1958, 1963, 1972; Jarvis & Jarvis, 1963b), tolerance and avoidance, which together make up the "constitutional resistance" (Stocker, 1961). Parker (1956) noted that in addition to 'Dürreresistenz' (Höfler et al., 1941) or drought resistance, a quantity known as the 'Austrocknungsresistenz' (desiccation resistance) (Höfler et al., 1941) is also involved in the plant's ability to withstand drought. This latter quantity is the resistance of tissue to low water content without adaptation for the control of water loss. Desiccation resistance may be defined as the stress required to produce 50% cell death (the LD_{50} point). Unlike survival time estimates of drought tolerance, where 'killing time' depends on the rate of loss of water, the LD_{50} point is determined under steady state conditions and is devoid of the time element, e.g. Iljin (1927) defined the 'drought killing point' as the relative humidity required to cause 50% cell death. In contrast, Levitt et al. (1960) defined the drought resistance as the time required to produce 50% cell death under standard conditions of 15% relative humidity and 30°C. However, they stated that resistance to water loss, tolerance of decreased water content and enhanced water uptake need to be evaluated in assessing drought resistance. This can only be performed on whole plants, so in their studies whole plants were allowed to desiccate and at pre-determined times shoots were cut and allowed to resaturate for twenty four hours, after which injury was estimated. Shoots, however, are easier to work with and have been commonly used in desiccation resistance studies, e.g. Jarvis & Jarvis (1963b), Pharis (1966), Bannister (1970, 1971).

When a shoot is removed from the plant, the drought avoidance component of drought resistance consists of assessing:

(1) avoidance of stomatal water loss;

(2) avoidance of cuticular water loss.

It is evident that in assessing a plant's relationship to drought, both drought avoidance (A_d), which helps maintain a sufficient water content to enable growth, and drought tolerance (T_d), which allows survival at a water content too low to allow growth, are involved. These two factors may be related to describe the overall drought resistance of the plant (R_d) by the following: (Levitt, 1972)

$$R_d = T_d \times A_{d50} = -\psi_{p50} \times \frac{\psi_{e50}}{\psi_{p50}} = -\psi_{e50} \qquad (2)$$

where: A_{d50} represents the critical drought avoidance of the plant;

T_d represents the drought tolerance of the plant;

ψ_{p50} represents the plant water potential at the 50% killing point;

ψ_{e50} represents the environmental water stress at the 50% killing point.

From this it is apparent that the higher the R_d is the lower the $-\psi_{e50}$ must be (i.e. the more severe the drought) before injury results.

A schema has been proposed by Levitt et al. (1960) for investigating plant response to drought which may be briefly reiterated as follows:

(1) actual drought resistance should be measured;

(2) drought avoidance and tolerance should be assessed independently to

enable the relative contribution of each to the overall resistance to be evaluated;

(3) if avoidance is the primary drought resistance mechanism, transpiration, absorption and translocation of water should be measured to discover which is of paramount importance;

(4) if tolerance is the primary drought resistance mechanism, osmotic potential (π) and bound water content should be measured;

(5) the mechanism by which the plant produces changes in the above in response to drought should be elucidated.

In addition to the direct response to drought a full investigation of 'total drought resistance' must also consider such factors as the prevention of protein denaturation or aggregation (cf. Levitt, 1972).

For the purposes of clarity and relevance to the current investigation it may be pertinent to briefly expand the concepts of tolerance and avoidance as propounded by Levitt (1972). The tolerance situation may be divided into dehydration tolerance and drought tolerance.

(i) Drought tolerance is the drought stress existing at the limit of dehydration tolerance, consequently, since it is an energy concept, it must be quantified in term of water potential or a derivative thereof, e.g. relative humidity resulting in 50% cell death (Iljin, (1927).

(ii) Dehydration tolerance is the strain the plant is under at a particular water content. By determining the critical relative water content (CRWC) (Section IIIA, 1(c)) the maximum strain a plant can withstand and remain viable is assessed. When used in conjunction with the water saturation deficit, information regarding the stress resistance of the plant may be obtained. For example, if the CRWC is low and the plant relative water content approximates to it the resistance of the plant is due primarily to tolerance, conversely, if the relative water content existing in the plant is high in comparison to the CRWC the plant is resisting drought by avoidance. Levitt (1972) noted that tolerance to drought may be determined from measurements of water deficit by deriving a relationship between water deficit and water potential e.g. Jarvis & Jarvis (1963b), Bannister (1971). From such relationships it is possible to determine the critical level of water deficit and water potential causing damage, which has implications for water uptake as well as reflecting the degree of hardening which has occurred (Jarvis & Jarvis, 1963b).

The avoidance situations may likewise be divided into strain and stress situations.

(i) Dehydration avoidance may be quantified by measuring the relative water content (RWC) (Weatherley, 1950) which indicates the degree to which the plant has avoided dehydration. The corollary of RWC, i.e. water saturation deficit (Stocker, 1929), indicates the strain the plant is under as a consequence of drought stress. However, since neither of these quantities "...measures the plant's ability to maintain a thermodynamic disequilibrium with its environment" (Levitt, 1972) a measure of dehydration avoidance is not obtained.

(ii) Drought avoidance. The ability to conserve water was initially considered to be the fundamental cause of drought resistance and plant yield was used as an indicator of such resistance. Maximov (1929) quantified this as the ratio of water lost to dry matter produced ('water requirement'). Neales, Patterson & Hartney (1968) derived a similar function, the transpiration ratio (T):

$$T = \frac{\text{(net efflux of water/time)}}{\text{(net influx of CO}_2\text{/time)}}$$

It was thus feasible to classify plants as 'water savers', having high transpiration efficiencies, in contrast to 'water spenders'. Such measurements also indicate the avoidance of indirect metabolic strain resulting from drought stress.

Levitt (1963) suggested that drought avoidance may be evaluated by comparing environmental and plant water potentials. Drought avoidance may therefore be defined as "the water removing potential of the environment required to lower the water potential of the unstressed plant by one unit" (Levitt, 1972) and may be quantified as the ratio of "the specific water removing potential" (Levitt, 1972) of the environment to the steady state decrease in water potential it produces in the plant, (Levitt, 1972):

$$A_d = \frac{\psi_o - \psi_e}{\psi_o - \psi_p} \approx \frac{\psi_e}{\psi_p} \qquad (3)$$

where A_d represents drought avoidance;

ψ_o represents the water potential of the plant at saturation;

ψ_p represents the water potential of the plant at steady state when exposed to ψ_e;

ψ_e represents the water potential of the environment.

The plant's ability to maintain a disequilibrium with its environment i.e. values greater than unity, indicates its capability to avoid drought. It is apparent that to determine A_d it is necessary to expose the plant to a constant and specific drought of sufficient duration to enable ψ_p to become constant. The difficulty in determining the critical avoidance point at which injury is prevented, has led to the adoption of the 50% cell death point, thus A_d may be redefined as (Levitt, 1972):

$$A_{d50} = \frac{\psi_{e50}}{\psi_{p50}} \qquad (4)$$

where: A_{d50} is the critical drought avoidance of the plant;

ψ_{e50} is the environmental water potential inducing 50% cell death;

ψ_{p50} is the plant water potential at the 50% killing point.

A full review of plant response to water stress is given by Levitt (1972) but from the above summary it is apparent that there are at least four responses a plant may make to drought, avoidance of drought and dehydration and tolerance of drought and dehydration. In the present study the ability to maintain a given degree of hydration, as reflected by the prevention of water loss by stomatal control, and the ability to tolerate low RWC's have been investigated in relation to damage to the plant by trampling, which disrupts the plant's internal water relationships (see section IIIB, 2).

1. Desiccation Tolerance (dehydration tolerance)

(a) Introduction

The relationship between desiccation tolerance and plant development has been reviewed by Levitt (1972) who noted that the drought tolerance of seedlings has been observed to decrease with development whilst in *Triticum aestivum* the period of rapid internode growth has been found to be accompanied by an increase in susceptibility to drought injury. Bannister (1970) observed that age of *Erica tetralix* shoots was a major determinant of desiccation resistance although no such relationship was found in *Calluna vulgaris*. The possibility exists that shoot age may affect seasonal variation in desiccation tolerance. Certain factors have been proposed by various workers to account for the response of plants to drought, three of which are considered below:

(1) Increase in cell wall material

Gaff & Carr (1961) have suggested that "hardening off" of a plant when exposed to dry conditions may be related to the buffering capacity of cell wall water. As the plant dries, apoplastic water is lost first so that the thicker the wall the greater the buffering effect before the symplastic water content is drawn upon.

(2) Reduction in cell size and alteration in cell structure

Reduction in cell volume is associated with increased drought tolerance (Iljin, 1957) although care must be exercised to ensure that water stress has not reduced cell size (Hsiao, 1973) whilst the stress has resulted in another process increasing drought tolerance.

Levitt (1972), in a re-evaluation of Iljin's data, noted that the negative correlation between 'osmotic value' and cell size may produce a relationship between osmotic value and drought tolerance such that relationships between cell volume and drought tolerance are spurious. Iljin (1957) stated that the "...osmotic value is not an indispensable criterion of resistance to drought among all species of plants. It is only one of the means of defence against drought that is inherent in each species to a different degree", the other factors being incorporated into his "Mechanical Stress Theory" of tolerance in which small cell and vacuole size and accumulation of carbohydrates confer upon the cell an ability to resist mechanical disruption. Levitt (1972) considered that such a theory, whilst accounting for many observed desiccation phenomena, leaves many unexplained.

(3) The protective effect of sugars

Levitt (1972) stated that an increase in sugar concentration when a plant is exposed to water stress is a widely observed phenomenon. Decreased growth resulting from moisture stress (Hsiao, 1973) combined with the lesser effect of stress on photosynthesis, may result in accumulation of carbohydrates (Eaton & Ergle, 1948; Hodges & Lorio, 1969). Variations in the drought resistance of Oryza sativa (rice) have been found to be correlated with sugar concentration (Murty &

Srinivasulu, 1968) although starvation effects accompanying moisture stress may reduce carbohydrate reserves. No causative association between sugar concentration and drought tolerance has been demonstrated. Maximov (1929) postulated that the accumulation of substances may protect the protoplasm from coagulation and desiccation whilst high solute concentrations may prevent visible wilting despite increasing water deficits. The protective effect of sugars on membranes has more recently been invoked as a mechanism preventing desiccation damage. Membrane destruction has been considered as a major source of injury in cells subject to desiccation (Levitt, 1972). The cryoprotective effect of sugars has been demonstrated by Heber & Santarius (1964) whilst more recently, the protective effect of sugars on photophosphorylation and electron transport in tissues subject to desiccation has been demonstrated, leading Santarius (1973) to conclude, on the basis of in-vitro experiments, that "...accumulation of sugars in frost hardy plant cells in winter contributes to frost and desiccation resistance". Santarius suggested that since conditions similar to freezing effects occur during desiccation, the mechanism by which the cryoprotective effect occurs may be extrapolated to encompass desiccation resistance. He postulated that the dilution effect of non-toxic solutes, e.g. sugars, on toxic solutes may inhibit membrane inactivation. Secondly, the affinity of hydroxyl groups of the sugar molecule for water may influence the water binding capacity thus preventing protein denaturation. Levitt (1972), in reviewing earlier work by the same authors, criticised the methodology employed, since the desiccation and rehydration rates employed were likely to induce osmotic rupture and would not be experienced in the field situation. Levitt also found it difficult to understand why desiccation inactivation does not occur in soluble enzymes but conceded that non-penetrating solutes such as sucrose, might osmotically prevent damage to membranes. Thus sugar concentration remains as a possible factor influencing desiccation tolerance.

The response of plants to drought has been extensively reviewed e.g. Parker (1956, 1969), Henckel (1964), Levitt (1972). Parker (1969) noted that increased amylase activity in a droughted plant may, providing the enzymes have not been denatured, cause a rapid conversion of starch to sugar thus protecting proteins from desiccation damage and increasing the tolerance of the plant to drought. Rabe (1905) noted that wilting may produce a significant increase in sugar concentration within two hours. In contrast Slatyer (1960), working with Acacia aneura, considered that if carbohydrate breakdown was to occur, long periods would be required since he failed to observe a change in π in desiccation periods up to seventy two hours. Eaton & Ergle (1948) considered that although accumulation of sugar may aid desiccation tolerance it did not prevent desiccation occurring in Gossypium sp.. Although sugars increase the bound water content of cells, Parker (1963) noted that bound water is not well correlated with drought resistance. The response also varies with speed of desiccation; if slow, no increase in sugars may occur (Levitt, 1972) and death from metabolic disturbance may ensue (Henckel, 1964). Stocker (1960) proposed a two phase process in response to desiccation, a 'reaction' phase in which protoplasmic disorganisation occurs followed by a 'restitution' phase in which reorganisation results in an increased protoplasmic viscosity leading to 'hardening'. Henckel (1964) considered that adaptation to drought involves increased hydrophily of the protoplasm, changes in the colloidal state of the protoplasm and changes in π, among other factors, which enhance plant survival in response to desiccation.

Levitt (1972) concluded that "...uncertainty as to the mechanism of drought injury makes it doubly difficult to postulate a mechanism of tolerance and an explanation of how this is achieved during the hardening process". In the confusion of mechanisms by which desiccation tolerance is achieved, the effect environmental parameters are going to have on a biochemical mechanism by which tolerance is achieved must remain obscure.

(b) Methodology

The use of visual damage and critical water content values was employed by Oppenheimer in the 1930's. By saturating droughted leaves for three days following drought, the 'water saturation deficit' of Citrus Bigaradia was calculated and values of 37.6% to 60.2% were obtained which indicated "the very high degree of drought resistance" of the bush (Oppenheimer & Mendel, 1934). Oppenheimer (1932) defined the "submortal water saturation deficit" as the water saturation deficit causing 5-10% necrosis of the leaf. In Citrus Bigaradia this appeared as a well defined dead brown area but in leaves of the Jaffa orange (Citrus sp.) the damaged areas tended to discolour in a gradual transition from damaged to healthy tissue. However, these leaves were found to have lost much of their water absorbing capacity which led Oppenheimer & Mendel (1934) to postulate that "the degree to which the water absorbing capacity is lost may give useful information on the amount of injury suffered at wilting". On finding no strict correlation between water absorbing capacity and the degree of necrosis arising from the "sub-mortal water saturation deficits", they concluded that it was not possible to use water absorbing capacity as a means of determining submortal water saturation deficits. This may be due to the fact that temperature and light intensity during the desiccation process affect leaf browning (Parker, 1953b).

Höfler et al. (1941) derived an equation for determining the water saturation deficit at the drought killing point. The critical saturation deficit (CSD) may be represented by:

$$\mathrm{CSD} = \frac{(\mathrm{SW} - \mathrm{CW})}{(\mathrm{SW} - \mathrm{DW})} \qquad (5)$$

where: SW is the saturated weight of the leaf;

CW is the leaf weight at the lethal water content level;

DW is the dry weight of the leaf.

A problem arising from using such a relationship is when a highly vascularised leaf is compared to one containing fewer vessels and thus more living cells, volume per volume. Although both leaves may lose equivalent amounts of water, cells in the former leaf may remain viable because of water movement from xylem to cell (Ursprung & Blum, 1945). This may be overcome by defining the 'plant water requirement' thus:

$$\text{plant water requirement} = \frac{(\mathrm{SW} - \mathrm{FW})}{(\mathrm{SW} - \mathrm{CW})} \qquad (6)$$

where: FW is leaf fresh weight.

Parker (1956) however, stated that there is no infallible method for comparing the desiccation tolerance of two or more species in terms of lethal levels as represented by percentage values.

Arvidsson (1951), in a study of the vegetation of Öland, used the water absorbing capacity together with visible damage, toassess injury resulting from critical water saturation deficits. Although the water absorbing capacity was reduced prior to the appearance of visible damage a "satisfactory agreement existed between the two measures of drought damage". For example, visible damage of 5-10% of the leaf area in Convalleria majalis and Maianthemum bifolium corresponded to 85-95% resaturation (Arvidsson, 1951). The 'limiting value of deficits' generally resulted in a water absorbing capacity of 40-80% of the maximal water content.

In a study of Meditteranean shrubs, Oppenheimer (1963) found that the water resaturation deficit, i.e. degree of deficit remaining after rehydration, was linearly correlated with the difference between the water saturation deficit reached by the tissue and the point of permanent turgor loss (PPTL). (The PPTL is defined as the point above which droughted leaves fully regain their saturated weight). Below the PPTL, increasing cell damage resulted in an increased water resaturation deficit.

Rychnovská-Soudková (1963) evaluated the drought resistance of *Stipa spp.*, *Bromus spp.* and *Melica spp.* in a study of causal phytogeography in Central Europe. Saturated plants were allowed to desiccate for up to seven hours at 28.3 ± 1°C and 42 ± 7% relative humidity. After desiccation the leaves were cut into segments and allowed to resaturate. It was considered that "water loss due to drying the test leaves under constant conditions and the ability of the plant to compensate the resulting water deficit in the leaves and to re-establish turgescence is an important criterion for the evaluation of the water relations in the plants under study". Thus, water deficits of 45-50% in *Stipa spp.* were fully reversible in contrast to equivalent deficits in *Bromus spp.* and *Melica spp.*. The degree to which water deficits could be compensated for, together with the point at which changes became irreversible, was indicated by a characteristic inflexion in the compensation curve. Using this method Rychnovská-Soudková (1963) and Rychnovská & Květ (1965) defined a 'continental' type response in which certain species e.g. *Stipa spp.* could fully resaturate even from severe water deficits and an 'oceanic' type, as exhibited by *Bromus spp.* and *Melica spp.* which showed irreversible changes.

In addition to the curves observed by Rychnovská-Soudková (1963), a second type of compensation curve was found in some species (Rychnovská, 1965) in which an increase in water deficit enhanced the water holding capacity of the leaf so that the resaturated water content was higher than the initial saturated water content, a situation described as "oversaturation". It was, however, still possible to define a critical water deficit. Rychnovská & Květ (1963) had observed such 'oversaturation curves' in a number of species e.g. *Chrysopogon gryllus*, *Dactylis glomerata* and suggested it was a species characteristic. Rychnovská (1965) found both types of curve among all groups of plants, i.e. xerophytes, mesophytes and hygrophytes, and although the oversaturation type curve occurred in the most mesophytic species, she concluded that it was not a species characteristic. In terms of species ecology however, the 'normal' resaturation curve would be advantageous to plants subjected to prolonged drought whilst the 'oversaturation' curve would favour mesophytic conditions where drought periods are short and where full use of occasional rainfall can be made (Rychnovská & Květ, 1965).

Bannister (1970) employed similar methods in determining the desiccation tolerance of four heath species, including *Calluna vulgaris*. Damage was assessed by resaturation and the measurement of the percentage fresh weight of the damaged portion of the shoot. Tolerance was expressed as the water deficit required to produce 20% damage in the sample. He found little value in using probit analysis over handrawn curves to determine the critical water deficit, which varied from 75% to over 100% percentage resaturation in *Vaccinium myrtillus* stems and leaves corresponding to the 20% damage point. If buds in this species were used instead, a level of resaturation of 86-92% was found which included the 20% damage point for all species, although "each monthly relationship does not necessarily conform to the annual pattern for the species...". Bannister (1971) also observed that visible damage was best induced in a humid environment. Those shoots resaturated under moist conditions became blackened in contrast to those resaturated in a dry atmosphere, which remained green.

Although shoots have often been employed in determining plant response to drought their use has been questioned. Pharis (1966), who used the lethal foliage moisture content (FMC) to determine desiccation tolerance of conifers, noted that it is often assumed that until the shoot tissue is dead, the whole plant is alive. Brix (1960) had realised the requirement for determining the lethal point in terms of the whole plant since he found needle tissue capable of taking up water despite the death of the intact plant. He therefore suggested that the lethal FMC was "merely an indicator of an internal water stress which affects some portion of the roots more significantly than it affects stems". Pharis (1966) found that two possibilities existed in relation to whole plant viability:

(1) if plants were considerably above or below the lethal point, only a few days may be required to reveal if death would result;

(2) if plants were close to the lethal point, the plant may oscillate between life and death and several weeks may be required to determine whole plant viability.

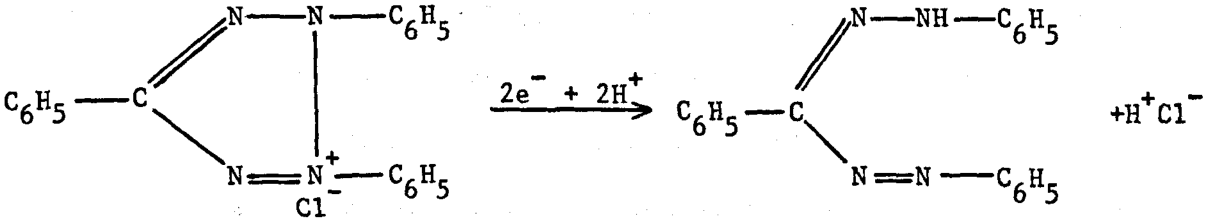
Thus, even if leaf turgidity is regained, it does not ensure survival and conversely, the inability to resaturate does not necessarily imply death. Pharis also found foliage age may be consequential in determining lethal FMC; for example, young shoots may die whilst the plant is still viable. In analysing the relationship between decrease in FMC and visual damage, a direct correlation was found up to approximately 20% damage but in a zone 10% above the lethal point, severe damage occurred which bore no consistent relationship with decreasing FMC (Pharis, 1966). Despite these problems it was concluded that needle moisture content is a "fair index for determining whole plant viability" (Pharis & Ferrel, 1966).

It would appear that despite the disadvantages of using the water content-resaturation relationship in determining desiccation tolerance, it is possible to demonstrate a significant correlation between the two and to determine a sub-lethal water deficit, which is reproducible and specific for the species concerned (Weinberger _et al._, 1972).

The determination of cell viability

The methods described above involve the indirect determination of viability on a gross scale. By employing suitable chemical reagents it is possible to determine cell viability and perhaps relate it to apparent shoot viability as defined above. Parker(1953a, b) reviewed the methods which are available, one of which is enzymic and involves the use of tetrazolium salts. Kuhn & Jerchel (1941) demonstrated that viable organisms, e.g. yeast cells, garden cress, can convert tetrazolium salts to red, insoluble formazan compounds. The validity of this reaction was supported by Mattson, Jenson & Dutcher (1947) who suggested that dehydrogenases were involved but Gunz (1949) cast doubt on the method by reducing tetrazolium salts using non-viable cells and cell free extracts. Parker (1953a) found that conifer needles dried to 40% of their moisture content showed a concomitant decline in intensity of tetrazolium test and found that in the presence of respiratory inhibitors, cells failed to reduce tetrazolium salts. He conceded, however, that 2, 3, 5-triphenyl tetrazolium chloride (T.T.C.) can be reduced by naturally occurring compounds carrying sulfhydril groups, and by light if it is of sufficient intensity (Parker, 1955) but that "...although reduction of triphenyl tetrazolium chloride may not always be a good indication of viability in the sense that the entire respiratory mechanism is still functional, the lack of reduction in cells normally reducing it is a fairly certain sign that death has occurred".

The reaction may be represented as follows (Parker, 1953b):



triphenyl tetrazolium chloride (T.T.C.) formazan

T.T.C. has been found to be reduced by succinic and isocitric dehydrogenases and certain oxidases (Parker, 1956).

Steponkus & Lanphear (1967) refined the technique for use in determining freezing injury in plants. Previous applications of the method relied on subjective estimates of the redness of the solution containing formazan. Steponkus & Lanphear quantified this spectrophotometrically. They found that samples which had a 50% reduction value or greater indicated long term viability in contrast to values of 50% or less where, despite tissue viability at the time of sampling, continued viability was not ensured. A similar technique for determining cell viability has been employed in the present study.

(c) Methods

Shoots of Calluna vulgaris were brought into the laboratory from the field and stood in distilled water in sealed polythene tubes and left to saturate[1] in the dark at 25 ± 1°C for 24 hours. Following the saturation period the shoots were blotted dry, weighed and placed under standard desiccating conditions of 25 ± 1°C, 40 ± 10% relative humidity and 13klx. Groups of five shoots were removed after 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14 and 16 hours, weighed, replaced in the saturating chambers and

---

[1] 'Saturate' refers to the process by which shoots are stood in water and permitted to take up water until water deficits have been eliminated.

placed in the dark at 25 ± 1°C. After a further 24 hours the shoots were removed, blotted dry and assessed for visual damage by estimating the proportion of damaged leaves (browned or blacked) on each shoot (leaves with 50% browning being counted as damaged) before and after the desiccating period, obtaining the difference and calculating the mean visual damage for each shoot. The shoots were oven dried at 90°C for 48 hours and re-weighed. (All weighings were made on a 500mg. torsion balance).

The induced water content produced during the desiccating period, and the percentage resaturation were determined by the following equations:

$$\text{Induced RWC} = \frac{(FW - DW)}{(SW - DW)} \qquad (7)$$

$$\%\ \text{Resaturation} = \frac{(RS - DW)}{(SW - DW)} \qquad (8)$$

where: FW is shoot fresh weight (i.e. weight after desiccating period);

DW is shoot dry weight;

SW is shoot saturated weight (i.e. weight after standing in water for 24 hours in the dark);

RS is shoot resaturated weight (i.e. weight after standing in water for 24 hours in the dark after desiccating period).

The induced RWC and % Resaturation have been related by polynomial regression analysis (Appendix A2(b)). The resultant regression equations (Appendix B, table 3) were used to obtain the 'critical relative water content' (CRWC), i.e. the induced water content allowing 85% resaturation (see section IIIA, 1d(i)).

This procedure was performed monthly (November 1974 to July 1976), and in the latter part of the sampling period (December 1975 to July 1976) similar regression relationships were determined for shoots damaged three months before sampling by trampling at 100 passages per plot (1m x 0.5m)

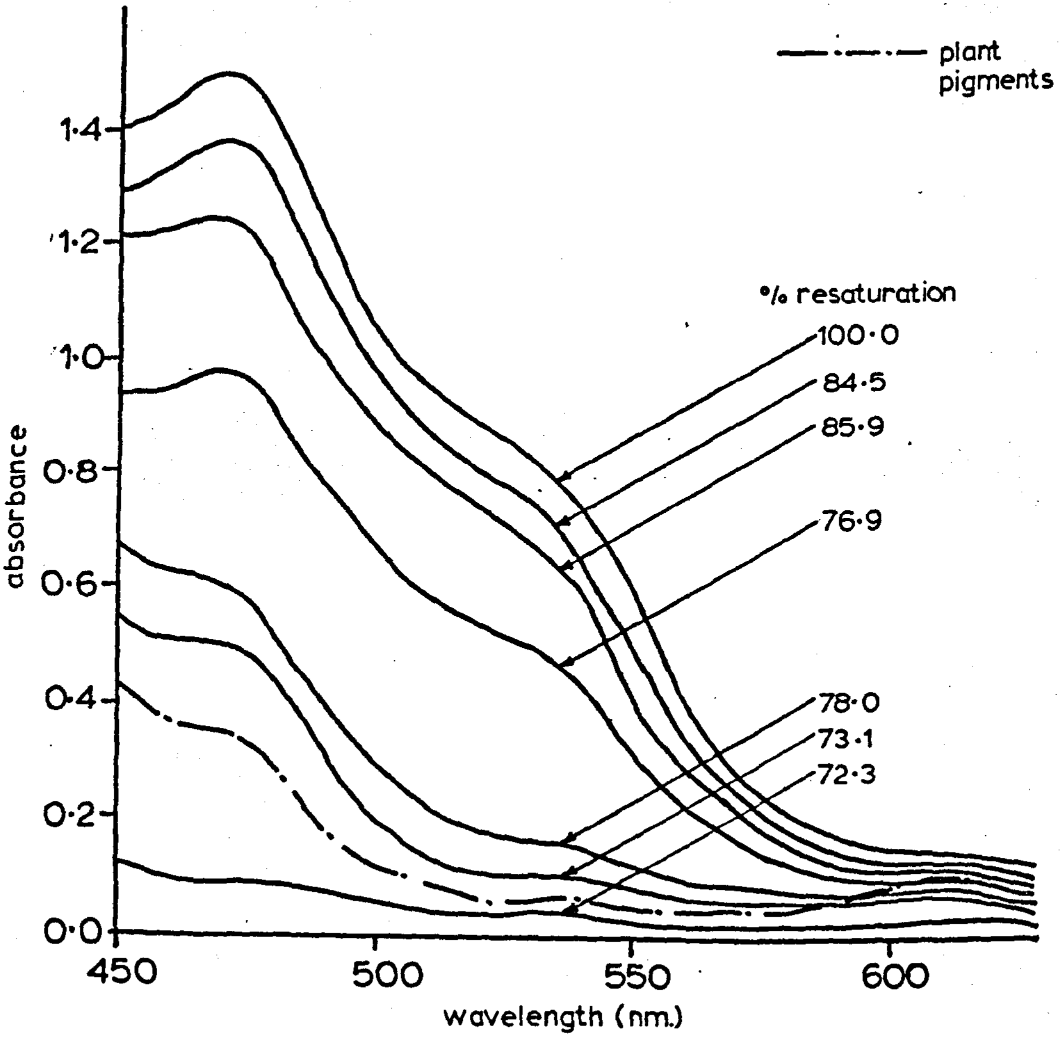


Figure 22 Spectrograph of replicate formazan extracts from desiccated tissue of C. vulgaris.

(Appendix B, table 4).

The relationship between visual damage estimates and % Resaturation was found by regression analysis (Appendix A, 2(a)). These relationships are presented in Appendix B table 5. A modified T.T.C. test was performed to relate % Resaturation and visual damage to cell viability.

Following the normal desiccation and resaturation procedure for twelve desiccation periods, (0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12 and 14 hours with three replicate shoots per treatment), leaf material was cut up into small sections of less than 1mm. in length. The material was weighed to give an estimated dry weight of 25mg. (This necessitated the prior determination of the dry weight to fresh weight ratio on a sample of shoots by which to convert resaturated weights of the experimental shoots to give 25mg. dry weight). After cutting up the material it was placed in 15.25cm. x 1.27cm. test tubes to which was added 3ml. of triphenyl tetrazolium chloride solution (T.T.C.). This solution was a 0.6% (wv^{-1}) T.T.C. in a 0.05M Na_2HPO_4 - KH_2PO_4 buffer (pH 4.0) plus 0.05% (vv^{-1}) Tween 80 (wetting agent). The tissue was infiltrated under vacuum for 1h. Care was exercised at this stage to prevent the solution rising out of the tubes and carrying the material with it by placing perforated 'Parafilm' caps over the ends of the test tubes.

Following infiltration, the material was incubated at 30°C for 15h. after which the T.T.C. solution was decanted from the tubes and the plant material washed several times with distilled water to remove residual T.T.C. solution. The formazan was extracted using 7ml. of 95% (vv^{-1}) ethanol heated in a boiling water bath until the tissue turned pale (approximately 0.5h.). The extract was cooled, made up to 10ml. and the absorbance of the formazan measured at 535nm. on a spectrophotometer (Pye Unicam SP800) using an ethanol reference blank

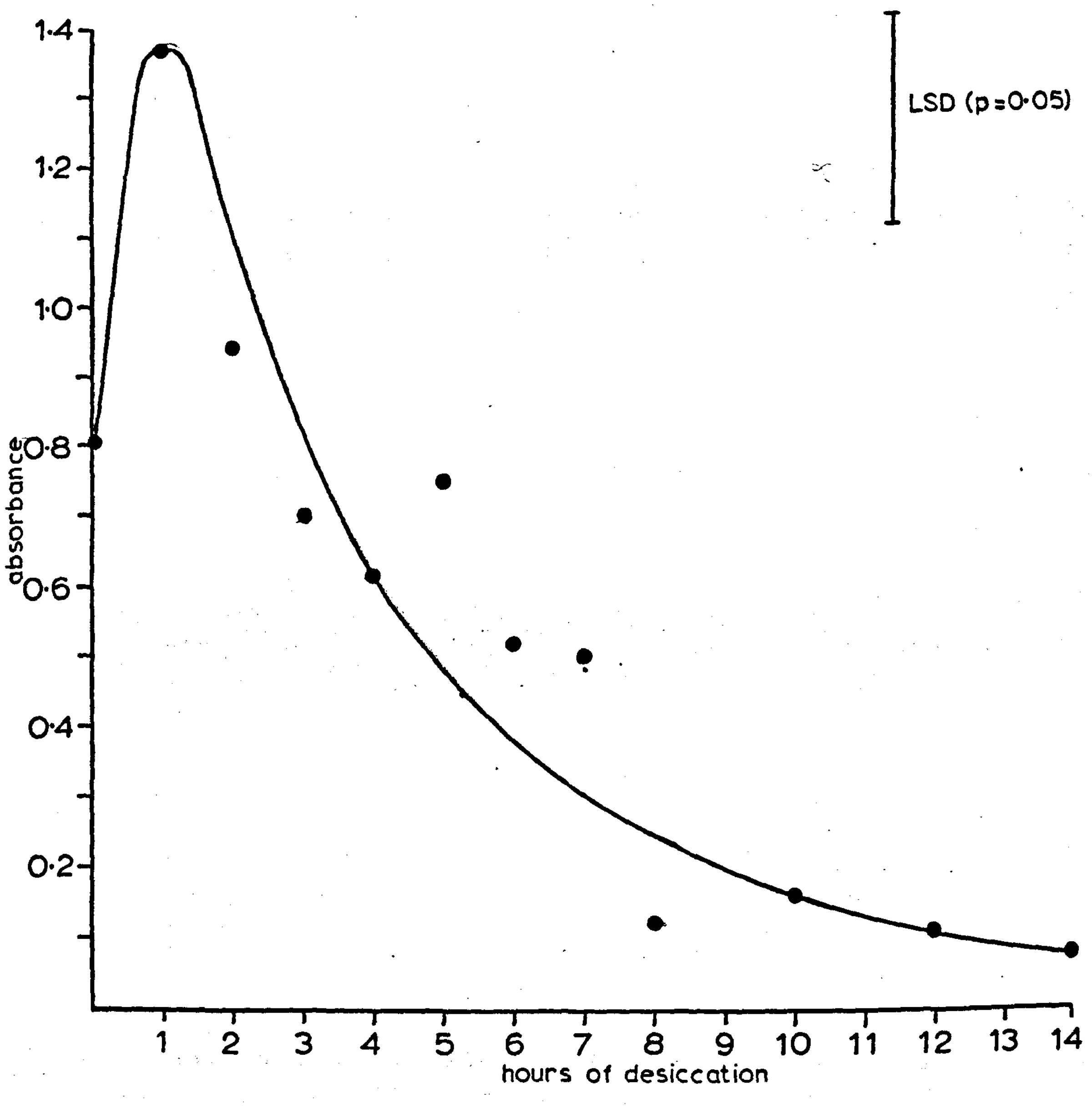
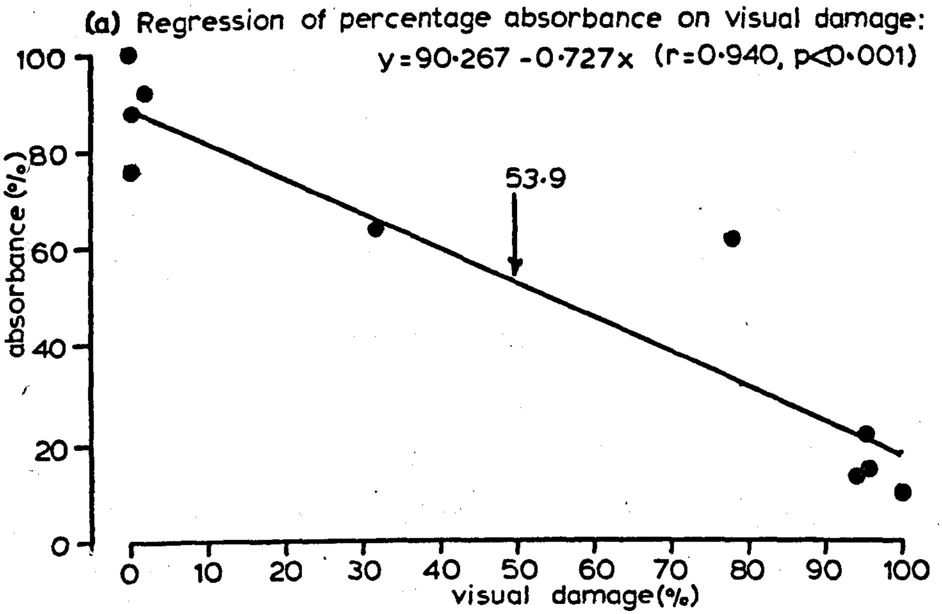
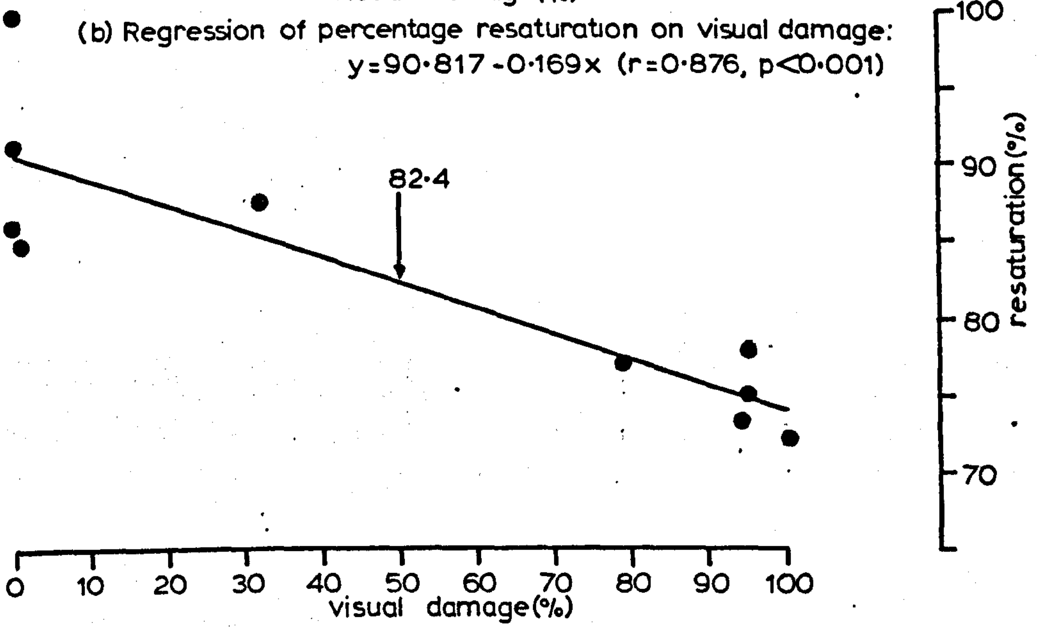


Figure 23 The effect of desiccation period on cellular ability to reduce TTC, C.vulgaris.


(a) Regression of percentage absorbance on visual damage: $y = 90{\cdot}267 - 0{\cdot}727x$ $(r = 0{\cdot}940,\ p < 0{\cdot}001)$


(b) Regression of percentage resaturation on visual damage: $y = 90{\cdot}817 - 0{\cdot}169x$ $(r = 0{\cdot}876,\ p < 0{\cdot}001)$

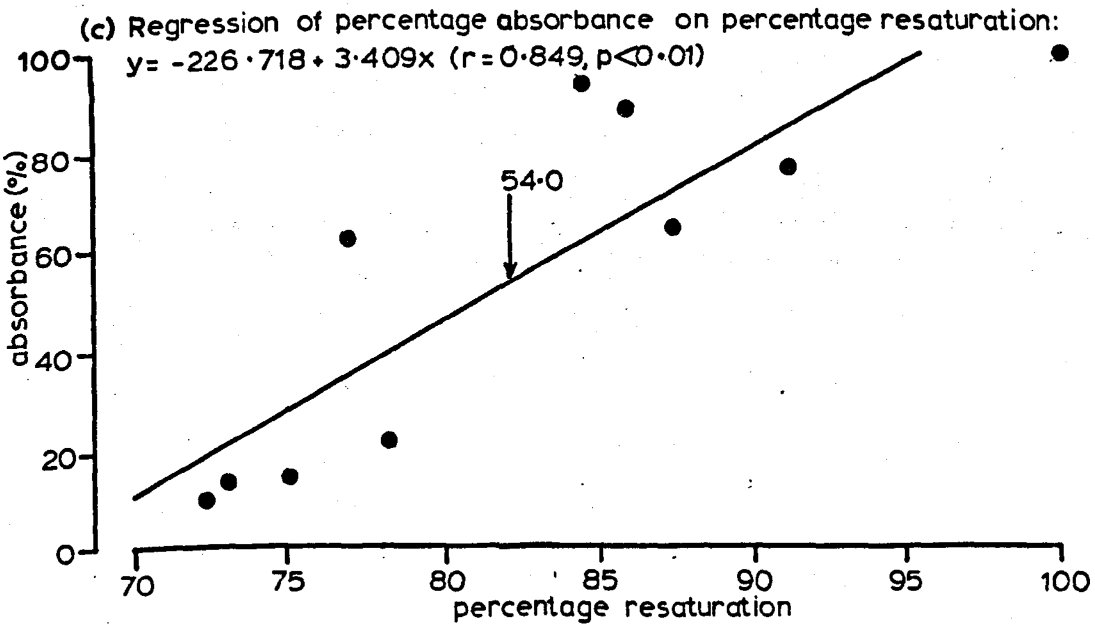

(c) Regression of percentage absorbance on percentage resaturation: $y = -226{\cdot}718 + 3{\cdot}409x$ $(r = 0{\cdot}849,\ p < 0{\cdot}01)$

Figure 24 Regressions relating visual damage, percentage resaturation and absorbance of formazan extracts of C. vulgaris desiccated for varying periods.

(Figure 22). Although the peak absorbance of reduced T.T.C. is 485nm., interference from plant pigments occurs at this wavelength, consequently 535nm was used since interference is minimal (Steponkus & Lanphear, 1967).

The absorbance of the treated shoots was expressed as a percentage of the control value and the values obtained related to the percentage visual damage and estimated percentage resaturation.

(d) Results

(i) Biochemical test for cell viability

A modified sigmoid curve results from plotting absorbance at 535nm. against desiccation period (Figure 23)(which should reflect induced water content). There is a significant ($p < 0.001$) decrease in enzyme activity, as indicated by T.T.C. reduction, especially over the three to eight hour desiccation period, after which the curve levels off, indicating metabolic inactivation. The absorbance indicated for the long desiccation periods may result from residual enzyme activity, or the physical reduction of T.T.C. to formazan which does not require the presence of living organisms. A short period of desiccation appears to stimulate formazan production, a phenomenon also observed by Polwart (1970) in relation to frost injury. This may result from mechanical damage rupturing some cells and so removing the permeability barrier between the T.T.C. solution outside the cell and the site of reduction within the cell. In view of this unexplained stimulation it was considered justified in excluding those treatments where T.T.C. reduction exceeded that of the control from subsequent analyses and to base all calculations on the control values.

From the resulting significant correlations between the variables, visual damage, percentage resaturation and percentage absorbance, regression equations were used to predict the percentage absorbance and percentage resaturation at a visual damage value of 50% (Figure 24). If

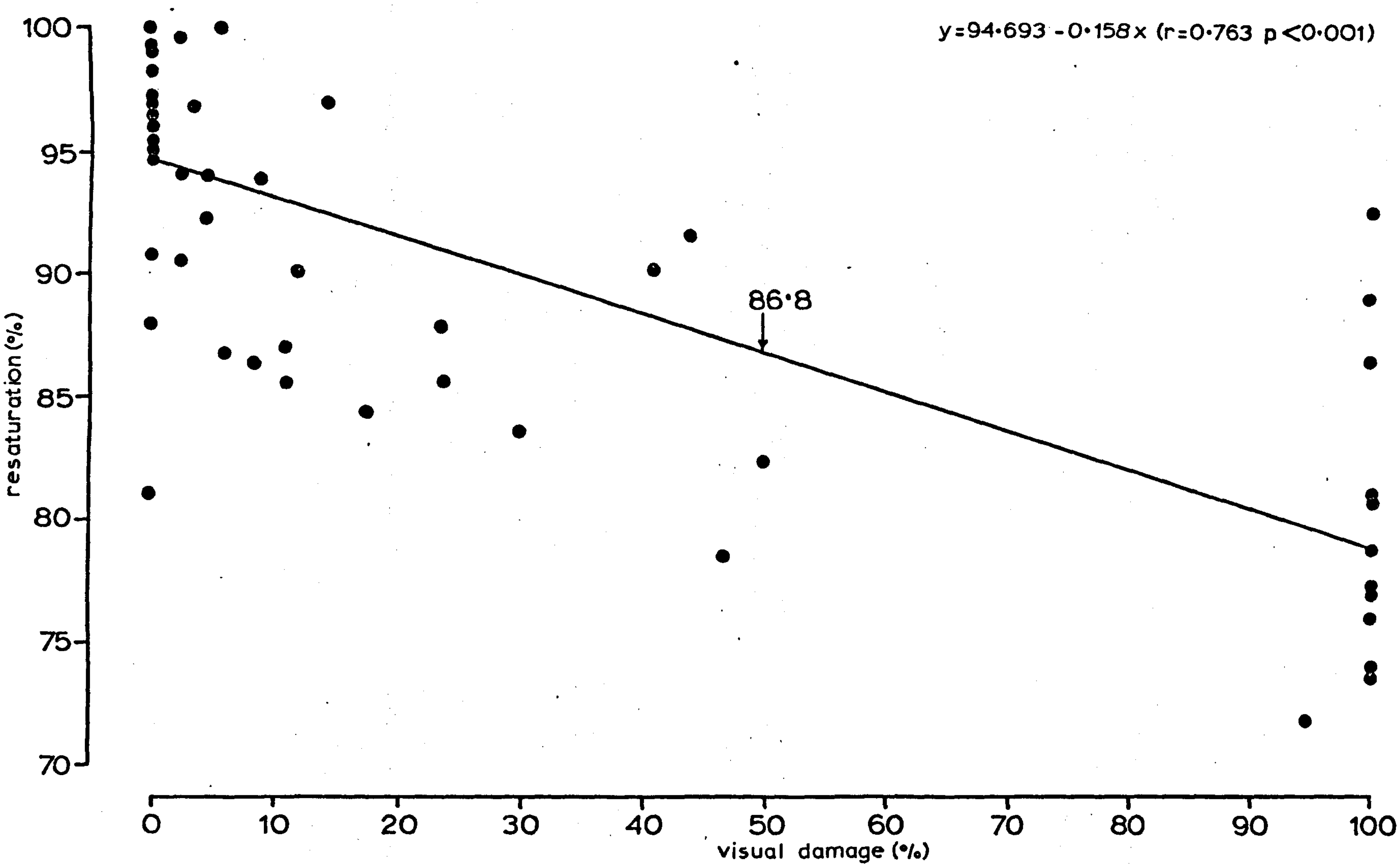


Figure 25 Regression of Resaturation on Visual damage in C. vulgaris. (Cannock Chase 18.04.75.)

it is assumed that 50% absorbance indicates the point at which 50% of the cells have been killed, it is apparent that 50% visual damage (which corresponds to 53.9% absorbance) may indicate the point at which c.50% of the cells have been killed (Figure 24a). The visual damage-resaturation regression (Figure 24b) suggests that 50% visual damage corresponds to a resaturation value of 82.4%, similarly, the relationship between % resaturation and % absorbance (Figure 24c) indicates that this level of resaturation approximates to the LD_{50} point as determined using T.T.C. test. It may be concluded that visual damage in the case of Calluna vulgaris is a reliable indicator of the LD_{50} point and consequently of survival in relation to desiccation tolerance. It is also evident that resaturation levels, as validated by both visual damage and T.T.C. reducing ability, are related to the LD_{50} point.

To test the constancy of the visual damage - % resaturation relationship, a number of correlations were carried out on the monthly data, an example of which is presented in Figure 25 where the 50% visual damage point corresponds to a resaturation value of 86.8%. The monthly values for resaturation when visual damage equals 50% (Appendix B, table 5) reveal that the range extends from 78.5% to 90.8% with a mean value for eighteen such correlations of 84.9 ± 0.9%.

It may be concluded that, from the relationships established between cell viability, visual damage and resaturation, it is justifiable to use the 85% resaturation value of induced RWC to represent the CRWC below which damage to desiccated shoots is in excess of 50%, and their continued survival is in doubt. It should be realised, however, that such a value indicates short term viability. It would require long term viability determinations of desiccated shoots to ascertain the relationship between the LD_{50} point, as defined here, and continued survival following a period of desiccation. It is possible that estimates

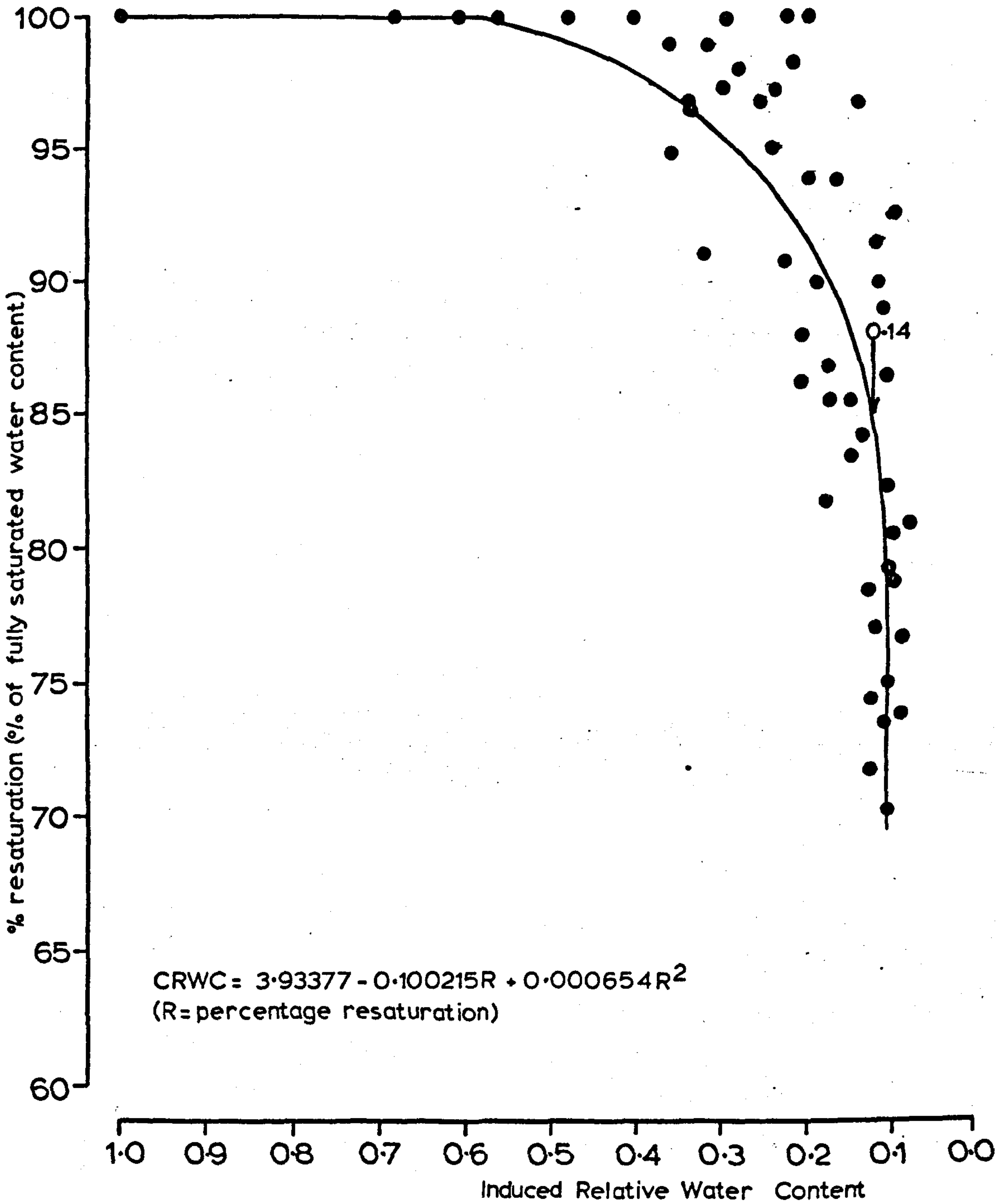


Figure 26 Resaturation curve for determing the Critical Relative Water Content below which visual damage to shoots of C. vulgaris exceeds 50%. (Cannock Chase 18.04.75.)

based on short periods of desiccation do not realistically indicate plant response to dehydration and consequently, such indices as used in the present study may not portray the precise reaction of Calluna vulgaris to prolonged periods of dehydration.

(ii) Determination of Critical Relative Water Content

A typical resaturation curve is presented in Figure 26. The curve has been fitted using the appropriate regression equation which is significant at the 0.1% level and accounts for 52.7% of the variation in the data. At the point at which the curve intersects the 100% resaturation value, it is extended horizontally to the 100% resaturation/1.0 RWC value. Since the important part of the relationships is that part which is curvilinear, the appropriate regression equations have been employed to determine the CRWC for each month. Although the "oversaturation" type curve was characteristic of Calluna vulgaris, in which resaturation values in excess of 100% were obtained, for the purposes of the regression analyses such values were taken as 100%.

The mechanism by which 'oversaturation' occurs is obscure but is most probably due to biochemical changes in the tissue (Rychnovská & Květ, 1965). It is possible that cellular osmotic relationships may be affected by desiccation, a conversion of starch to sugars occurring (Rabe, 1905; Parker, 1969) resulting in a lowering of the π so that on contact with water, the ability to absorb water is enhanced and is manifested in resaturation values in excess of 100%. At greater stresses, other factors such as cell death may occur, thus limiting water uptake thereby providing an index of shoot viability.

(iii) Seasonal variation in Desiccation Tolerance (Cannock Chase)

No clearly defined seasonal pattern emerges in the desiccation tolerance of C. vulgaris (Figure 27) although a tendency exists in which

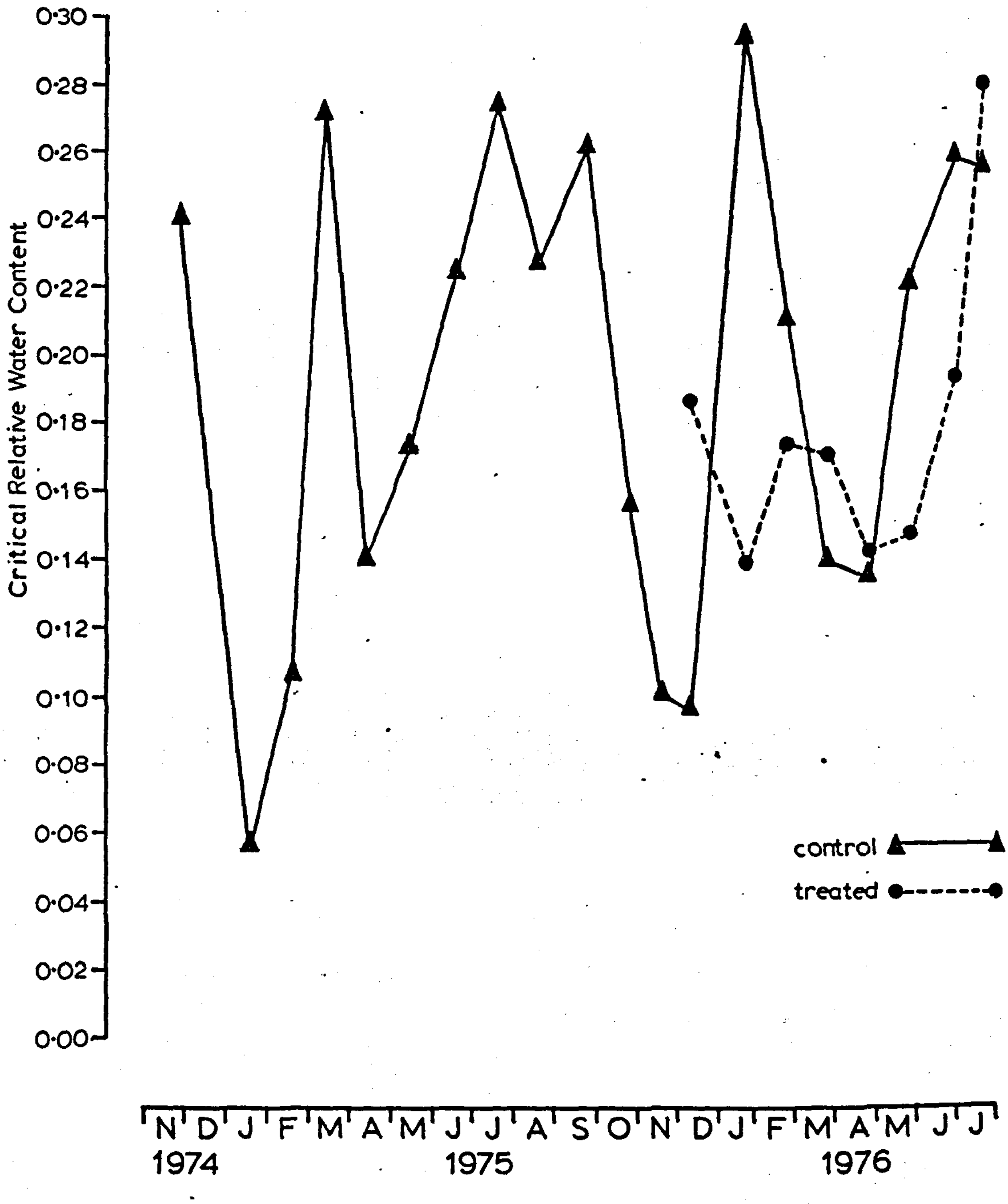


Figure 27 Seasonal variation in Desiccation Tolerance, (as defined by the Critical Relative Water Content), of <u>C. vulgaris</u>. (Cannock Chase).

the lowest CRWC values, indicative of the greatest tolerance, occur in the winter months, e.g. January, November and December, 1975, whilst the highest CRWC's are found in the summer months July, September 1975, June, July, 1976. Exceptions to this trend are observed in March 1975 and January 1976 where CRWC's of 0.2715 and 0.2934 occur which are equivalent to or greater than the summer values. Since each point is derived from a single polynomial regression the variance in the data has not been analysed and no statistical significance can be attached to seasonal differences in CRWC. The amplitude of variation is similar to that existing for the RWC at stomatal closure for which statistically significant differences over time do exist, it is therefore probable that the seasonal variation in CRWC is real and not an artifact of the method employed (cf. Polwart, 1970) (Section IIIA, 2(d)).

The unknown rate at which a physiological variable responds to a change in an environmental parameter necessitates a subjective partitioning of the data into appropriate units, e.g. the mean temperature for the 7 day, 14 day and 28 day period before sampling. Of the environmental variables considered to have a possible influence on seasonal variation in CRWC, the various temperature parameters and vapour pressure are the primary variables significantly correlated with CRWC (Table 2). The temperature prehistory over the fourteen days prior to sampling accounts for the highest proportion of variance in the data although the maximum r (-15cm. temperature averaged over 14 days before sampling), whilst accounting for a significant proportion of the variance, only explains 33.7% of the total variation in the data. Air temperature, in being significantly correlated with soil temperature ($r = 0.999$, $p < 0.001$) might also be expected to be significantly correlated with CRWC and although it accounts for a smaller proportion of the variation (33.4%), the difference is unlikely to be significant. The vapour pressure on the day before sampling is significantly correlated with CRWC although

Table 2. Correlation coefficients (r) between desiccation tolerance in *C. vulgaris* and Environmental and Physiological variables (Cannock Chase)

Parameter	r	Significance
Environmental		(p)
Mean Temperature:		
60cm: 7 days before sampling (mean)	0.5377	< 0.05
14 days before sampling (mean)	0.5783	< 0.05
28 days before sampling (mean)	0.5334	< 0.05
- 5cm: 7 days before sampling (mean)	0.5413	< 0.05
14 days before sampling (mean)	0.5735	< 0.05
28 days before sampling (mean)	0.5395	< 0.05
-15cm: 7 days before sampling (mean)	0.5374	< 0.05
14 days before sampling (mean)	0.5809	< 0.05
28 days before sampling (mean)	0.5396	< 0.05
stem: 7 days before sampling (mean)	0.5286	< 0.05
14 days before sampling (mean)	0.5343	< 0.05
28 days before sampling (mean)	0.5054	< 0.05
Accumulated temperature:		
7 days before sampling (base 7.2°C)	0.5365	< 0.05
14 days before sampling (base 7.2°C)	0.5774	< 0.05
7 days before sampling (base 4.0°C)	0.5410	< 0.05
14 days before sampling (base 4.0°C)	0.5869	< 0.05

/...

Table 2. Correlation coefficients (r) between desiccation tolerance in *C.vulgaris* and Environmental and Physiological variables (Cannock Chase) (cont'd)

Parameter	r	Significance (p)
Environmental		
Vapour Pressure:		
0900h on day of sampling	0.3369	n.s.
0900h on day before sampling	0.5863	< 0.05
7 days before sampling (mean)	0.5396	< 0.05
Between sampling dates (mean)	0.4912	< 0.05
Rainfall (total):		
7 days before sampling	0.0509	n.s.
14 days before sampling	-0.1467	n.s.
28 days before sampling	-0.2113	n.s.
Daylength:		
7 days before sampling (mean)	0.3904	n.s.
Physiological		
Sublethal water content (SCI - CRWC)	-0.7032	< 0.01
Sublethal water content (SCL - CRWC)	-0.5805	< 0.05
Dry weight : Fresh weight ratio	-0.0646	n.s.
Maximum net photosynthesis	0.8185	< 0.01

(All correlations based on 18 observations except for 'Maximum net photosynthesis based on 9 observations) (n.s. - not significant) this is not the case for the vapour pressure existing at the time of sampling. Neither daylength nor rainfall are significantly correlated with CRWC.

Of the derived temperature parameters, accumulated temperatures in relation to a temperature base of 7.2°C, (the temperature at which bud break occurs (Grace & Woolhouse, 1970)), and 4°C, (the temperature at which water is at its maximum density), for the seven and fourteen day period before sampling are significantly correlated with CRWC, although the temperature prehistory over the longer period accounts for a greater proportion of the variance in the data.

Of the physiological variables which have been considered, the maximum rate of photosynthesis attained under optimal conditions is significantly correlated with CRWC but dry to fresh weight ratio, which may affect the buffering capacity of the plant, is not. However, in relation to the buffering zone generated between the point of stomatal closure, (SCI/SCL; Section IIIA, 2(d)), and the RWC at which water deficits become lethal, i.e. the sublethal water content, significant negative correlations are observed, the larger accounting for 49.5% of the variation in the data. Thus, as the desiccation tolerance of the plant increases (i.e. lower values of CRWC), there is a significant tendency for the buffering zone to increase in magnitude.

When the relative contributions made by each variable in explaining the variation in CRWC over the period 1974-1976 are considered (Table 3) using multiple regression analysis, (Appendix A, 2(c)), no improvement is obtained by considering more than one variable. On the basis of the measurements available, the mean values for the 7 days prior to sampling have been entered into the analysis and temperature at a depth of -5cm. has been employed. In the analysis where soil temperatures have been included together with accumulated air temperatures (base 7.2°C), (Table 3a), the mean soil temperature over the 7 days prior

Table 3 Multiple Regression Analyses relating Desiccation tolerance (CRWC) to Environmental variables, *C. vulgaris* (Cannock Chase)

Step	Environmental variables Variable	Significance to enter (p)	Multiple R	Multiple R^2	R^2 change	Simple R	Overall Significance (p)
(a)							
X_1	Soil temperature (−5cm) (mean related to 4°C)	< 0.05	0.5817	0.3384	0.3384	0.5817	< 0.05
X_2	Soil temperature (−5cm) (mean 7 days before sampling)	n.s.	0.5929	0.3516	0.0132	0.5413	< 0.05
X_3	Daylength	n.s.	0.5982	0.3578	0.0062	0.3904	< 0.05
X_4	Vapour Pressure (7 days before sampling)	n.s.	0.6027	0.3632	0.0054	0.5396	n.s.
	Regression equation:	$\hat{Y} = 0.1713 + 0.0063 X_1$					< 0.05
(b)							
X_1	Vapour Pressure (7 days before sampling)	< 0.05	0.5396	0.2911	0.2911	0.5396	< 0.05
X_2	Rainfall (7 days before sampling)	n.s.	0.5404	0.2920	0.0009	0.0509	n.s.
X_3	Daylength	n.s.	0.5421	0.2938	0.0018	0.3904	n.s.
	Regression equation:	$\hat{Y} = 0.0786 + 0.0113 X_1$					< 0.05

to sampling, with reference to a base temperature of 4°C (i.e. mean soil temperature minus 4°C) makes the most significant contribution towards explaining the variation in CRWC, accounting for 33.84% of the total variation in the CRWC. Of the five variables considered only four are entered into the analysis, the F ratio of the final variable (accumulated temperature) being insufficient for further computation. The maximum variation accounted for is 36.32%, but the variables X_2 to X_4 account for a nonsignificant 2.48% of the variation.

In contrast, when temperature is removed as a variable from the analysis (Table 3b), it is only vapour pressure which makes a significant contribution, explaining 29.11% of the total variance, rainfall and daylength contributing a nonsignificant 0.27%. The exclusion of vapour pressure when temperature is entered as an independent variable may be due to the significant correlation existing between them ($r = 0.989$, $p < 0.001$), so that they account for similar proportions of the variation in the data and consequently do not complement each other. Neither rainfall nor daylength make a significant contribution to the regression. It may be concluded that there is no statistical justification for employing more than one of the measured independent variables in the regression, and that, in addition to random variation in the data, other factors not considered in the present investigation may be influencing seasonal variation in desiccation tolerance.

(iv) The effect of trampling on Desiccation Tolerance

Variations in the desiccation tolerance of shoots damaged three months prior to sampling by trampling, (100 walking passages per 0.5m^2 plot), appear to be independent of those in control shoots both for the date of sampling ($r = 0.211$, n.s.) (Figure 27) and for the CRWC of the

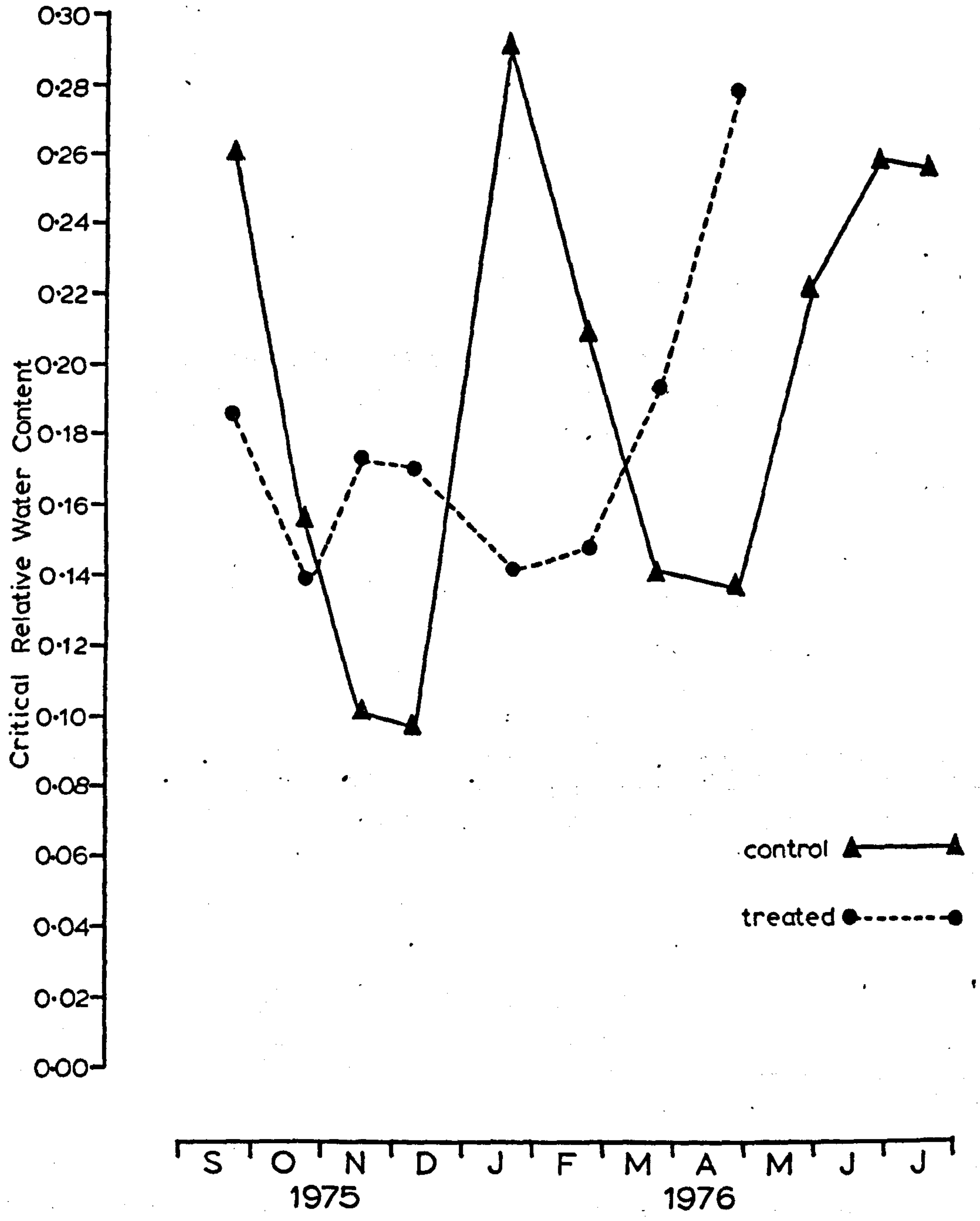


Figure 28 The effect of trampling on the seasonal change in desiccation tolerance of C. vulgaris. (Cannock Chase).

(Data for treated shoots are related to the date of treatment, shoots for desiccation tolerance determinations were sampled three months after treatment, i.e. December to July) damaged shoots related to the time of treatment (r = -0.294, n.s.) (Figure 28). A similar seasonal trend is evident in which desiccation tolerance rises in the winter and decreases with the onset of the growing season to reach a summer minimum. Since there is no replication it is not possible to determine whether statistically significant differences exist between control and damaged shoots. No consistent pattern emerges in relation to the CRWC of control shoots. Trampling on C. vulgaris would appear to have an effect on desiccation tolerance although it is not a unidirectional effect.

(e) Discussion

The general trend in seasonal variation in desiccation tolerance agrees with the findings of Bannister (1970) who observed a decline in tolerance in the early part of the growing season followed by a rise to a winter maximum for C. vulgaris. The absolute values for CRWC are considerably lower in the present study, e.g. a minimum of 0.055 compared to c. 0.2 in Bannister's study. The discrepancy may arise from differences in methodology. Although Bannister determined percentage resaturation after 24 hours, damage was assessed after one week in contrast to 24 hours in the present study. Although the 85% resaturation value and 50% damage are significantly correlated, Bannister employed 20% damage as an expression of desiccation tolerance as a consequence of which the water deficit required to induce the damage would be less than that required to produce the damage level used in the present study, resulting in differences between the studies in the absolute CRWC obtained.

Contrary to the findings of Bannister (1970), the significant correlations observed with certain environmental parameters suggest that environmental factors may affect desiccation tolerance, although Bannister noted a strong association with monthly accumulated temperature. In interpreting such relationships however, care must be exercised. As noted above, environmental factors are interrelated and a significant relationship with one that has been measured may obscure a more meaningful relationship with one that has not due to mutual correlation, thus leading to erroneous interpretation of cause and effect. Such relationships may also be a by-product of a more meaningful correlation between an environmental factor and another physiological variable. Bannister, for example, found a "strong correlation between heat resistance, desiccation resistance, and/or average damage in both Vaccinium and Calluna which suggests that the two resistances are closely related", similarly desiccation hardening may be paralleled by an increase in frost resistance (Henckel, 1964) and whilst the lack of a significant correlation between frost and drought resistance in Vaccinium spp. led Polwart (1970) to consider that the two resistances respond differentially to changes in the environment he concluded that "...in general, the frost and drought hardiness of V. vitis-idaea ... show a positive correlation...".

The variation in desiccation tolerance may reflect the seasonal progression of growth and development in the plant (Bannister, 1970; Levitt, 1972). The shoots employed in the present study, although of current and previous season's growth, are composed of leaves with a relatively wide age range. Consequently, the determination of the desiccation tolerance of a shoot may reflect the mean response of leaves which, in reality, are responding differentially, and changes in age structure may result in changes in desiccation tolerance. This may account for the absence of significant correlations with physiological variables since the tissues may be in varying physiological states at any one time.

Seasonal changes in desiccation tolerance may be related to structural changes within the cell. An increase in dry to fresh weight ratio with age (cf. Bannister, 1964b; Kozlowski & Clausen, 1965; Polwart, 1970) may reflect an increase in cell wall material increasing the buffering capacity of the cell against water loss (Gaff & Carr, 1961). This may be the explanation for the significant correlation between the sublethal water content and CRWC, in which the former represents the water available in the cell walls. This may increase with age as the cell wall thickens but the postulate is not supported by the nonsignificant correlation between dry to freshweight ratio and sublethal water content, nor by the nonsignificant correlation between dry to fresh weight ratio and CRWC. This assumes that dry to fresh weight ratio represents changes in cell wall volume, which may be invalid. It is also of note that seasonal variation in this ratio may invalidate the determination of CRWC since if water content remains constant but dry weight fraction increases, a lowering of RWC results so that seasonal changes in dry weight per unit volume may lead to errors in determining RWC (see below). There is no evidence to substantiate the postulate that cell wall buffering capacity is causally related to desiccation tolerance.

Cell size may also be related to desiccation tolerance (Iljin, 1957). In the present study, the reduction in desiccation tolerance in late spring-early summer may be related to vacuolation and elongation associated with growth whilst nonvacuolate cells in overwintering buds and the decrease in π as a result of the accumulation of reducing sugars over winter (Grace & Woolhouse, 1970) may result in an increase in desiccation tolerance.

In view of the correlations existing between CRWC and environmental variables it is of interest to consider the possible effects such variables have upon the physiology of the plant to produce a change in desiccation tolerance. The positive correlation between vapour pressure and CRWC is contrary to what might be expected since the likelihood of larger water deficits resulting from increased evaporative demand might be expected to be related to increased desiccation tolerance. However, the relationship may be a consequence of the relationship between vapour pressure and time of year so that vapour pressures are maximal at the time of the year when growth rates are likely to be high. Thus, the correlation between vapour pressure and CRWC may be spurious since younger and growing tissues are generally less resistant to desiccation than older or dormant tissues (Bannister, 1970). The association may also be an artifact of the relationship existing between vapour pressure and stomatal closure, (see Section IIIA, 2(e)), so that a low summer tolerance to desiccation is accompanied by an increased resistance to the formation of deficits by effective stomatal control (cf. Bannister, 1970).

The effect of lower temperatures in the soil is to increase the viscosity of water and decrease membrane permeability thus reducing water availability to plants (Kramer, 1942; Abd El Rahman _et al_., 1959; Ehrler, 1963; Kuiper, 1964; Kleinendorst & Brouewer, 1972; Anderson & McNaughton, 1973). Although plants may adapt to water uptake at lower temperatures (Kuiper, 1964) decreasing temperature may still lower uptake (see Section IIIB, 2(ii)). This, together with reduced root growth and suberization of ageing root tissue causing decreased permeability (Slatyer, 1967), may result in a reduction in plant water content and the development of water deficits. The correlation between soil temperature and CRWC may represent an adaptive response to reduced water absorption as a result of the above mentioned factors. That this is not the sole mechanism accounting for seasonal variation in CRWC is indicated by the observation that on the basis of the regression between soil temperature and CRWC, ($\hat{y} = 0.1435 + 0.00642x$), a soil temperature in the order of -14°C would be required to account for the lowest CRWC recorded, whereas unavailability of soil moisture is likely to occur at a temperature much higher than this.

It is of interest to note that high CRWC's (Figure 27) are associated with positive accumulated stem temperatures above base 7.2°C (Figure 15) which occur from May to September and define the growing season whilst lower CRWC's are associated with negative accumulated temperatures. Whilst this may reflect the normal progression of seasons, the high January 1976 CRWC is associated with a peak in accumulated temperatures, which become positive for accumulated soil temperatures (Figure 16), whilst the decrease in the March 1976 value of CRWC corresponds to a trough in the accumulated temperatures. Although the relationship is not consistent, e.g. a low CRWC in April 1975 is associated with a relatively higher accumulated temperature than the high CRWC for March 1975, the suggestion that desiccation tolerance is solely a function of the general progression of seasons may be oversimplistic and there is the possibility that accumulated temperature and the development of desiccation tolerance are causally related. The temperature response may fall into two categories in which higher values of CRWC are related to temperatures in excess of 7.2°C whilst low CRWC's are associated with temperatures less than 4°C. The higher values of CRWC would appear to be significantly correlated with temperatures in excess of 7.2°C ($r = 0.7895$, $p < 0.01$ for correlation between number of degrees in excess of 7.2°C for the mean temperature 7 days prior to sampling and corresponding CRWC). No such correlation exists between CRWC and temperatures less than 4°C ($r = 0.3615$, n.s.). This suggests that at temperatures allowing growth, desiccation tolerance decreases, agreeing with the observations made by Bannister (1970).

In relation to growth effects on desiccation tolerance it is relevant to consider seasonal variation in photosynthesis and associated sugar content. Grace & Woolhouse (1970) noted that "...seasonal increases in photosynthesis were accompanied by corresponding decreases in the sugar content of the shoot..." which may explain the significant correlation between CRWC and the rate of photosynthesis. The relationship may not be causal but an artifact of the increased demand for assimilates by growing tissue which reduces sugar concentrations in the shoot and stimulates photosynthesis. Unfortunately sugar concentrations have not been measured in the present study. Photosynthesis in C. vulgaris does not cease below 7.2°C but a reduction in growth rate may result in decreased utilization and increased storage leaving a large reserve of sugars overwinter which show a marked drop at budbreak (Grace & Woolhouse, 1970, 1973b). Although the evidence is indirect, the seasonal variation in sugar content may be associated with the seasonal variation in CRWC. When utilization is high and reserves low i.e. at temperatures in excess of 7.2°C, CRWC's are also high, whilst in the winter when temperatures are low and reserves are high, CRWC's are low. This invokes the hypothesis regarding the protective effect sugars may have against desiccation damage (Parker 1963, 1969; Santarius, 1973).

Age of material and daylength have been found to be associated with variations in CRWC (Bannister, 1970; Polwart, 1970). Although age and daylength did not have a significant effect in C. vulgaris they accounted for a significant proportion of the variation in desiccation tolerance of Erica cinerea and E. tetralix although the relationship was not simple but related to the equinoxes and solstices of the year (Bannister, 1970). In the present study daylength accounted for a small and insignificant proportion of the variance, which agrees with the findings of Bannister. The absence of such a relationship is, in some respects, unexpected since daylength reflects the progression of seasons and plant development.

The development of desiccation tolerance as discussed above may be regarded as an anticipatory effect in which tolerance develops in response to factors which may result in drought rather than in response to moisture stress itself (cf. Parker, 1963). Bannister (1970) considered that the maximum desiccation resistance in winter reflects the plant's more continental and northerly distribution where winter drought may develop.

When the plant is subjected to a sudden moisture stress, as when trampled (Section IIIB, 2(i)), mechanical injury may develop as a result of rapid desiccation. Controversy exists as to whether hardening in response to moisture stress results in increased protoplasmic viscosity making death by rupturing on dehydration more difficult or whether increased fluidity of the protoplasm decreases the likelihood of rupture (Parker, 1956). Henckel (1964) considered that increased protoplasmic elasticity reduces the likelihood of mechanical damage. It would appear that the physiological state of the protoplasm at the time of trampling may affect the hardening response. This suggests that when desiccation tolerance is low the development of irreversible damage may prevent hardening and vice versa when tolerance is high. However, the results suggest that when tolerance is low the damaged plants have a capacity to harden further, whilst when damaged when tolerance is high the plants appear to deharden or develop a lower tolerance of desiccation. The situation is somewhat confused by the three month period of adjustment between treatment and measurement though the trend remains and is accentuated when the CRWC of damaged shoots is related to the CRWC at the time of treatment. This apparent dehardening may result from metabolic disturbances. Elevation of respiration rates as a result of desiccation (Brix, 1962) or mechanical injury (see Section V) may deplete sugar reserves which are not replaced because the photosynthetic capacity is low at the time of the year when tolerances are high (Section IVA(d)). In the summer, induction of deficits may retard growth more than photosynthesis because of a relatively greater sensitivity to moisture stress (Hsiao, 1973) thus, because of the higher photosynthetic capacity compared to the winter period assimilates may build up resulting in a higher desiccation tolerance in trampled shoots in May and June, 1976. Although this is not consistent i.e. July 1976, it is of interest to note the higher tolerance of damaged shoots in January 1976 coincides with a high photosynthetic capacity of control shoots (Section IVA(d)). However, such proposals require extensive experimental verification.

(f) Conclusions

Seasonal variation in desiccation tolerance occurs although a mechanistic explanation for such variation remains obscure. Such variation has implications for plant survival when the plant is subjected to rapid dehydration, for example as a result of trampling, and may also affect seasonal susceptibility to trampling effects. There is tentative evidence that damaged shoots may be capable of hardening to the imposed desiccation condition thus enhancing chances of survival but the response is not consistent.

2. Desiccation Avoidance (dehydration avoidance)

Stomatal Closure Point (RWC at Stomatal Closure)

(a) Introduction

One assessment of the plant's ability to avoid desiccation is the determination of the RWC at which stomatal closure occurs thereby reducing further water loss. Interpretation of seasonal variation in the stomatal closure point depends, to a certain extent, upon an understanding of how stomata function which has been reviewed by Meidner & Mansfield (1968) and Raschke (1975).

Threshold values of ψ^1 or RWC may exist above which leaf resistance and thus stomatal opening remain constant. Hsiao (1973) quoted values ranging from a ψ of -0.7 to -1.8MPa, suggesting stomata may be insensitive to mild stress, whilst Meidner (1975) noted that stomatal reaction to sudden changes in ψ may be comparatively rapid (20-60s), due to an upset in the continuity of water throughout the plant. Once the threshold ψ has been reached, leaf resistance may increase sharply, indicating stomatal closure (Hsiao, 1973). The relationship between stomatal resistance (r_s) and leaf water potential (ψ_l) has been found to vary, Jordan & Richie (1971), for example, finding that growing environment affected stomatal response to stress. Brown, Jordan & Thomas (1976) suggested four mechanisms by which such response changes may be effected:

(1) osmotic adjustment;
(2) changes in guard cell wall elasticity;
(3) changes in guard cell wall thickness;
(4) changes in the level of hormonal activity.

Hygen (1953a) observed that age affects stomatal reaction to water deficits, and suggested that during the drying of a shoot, the free water evaporated during the initial stages of drying and that upon exhaustion of these fractions, stomatal closure ensued, further water loss occurring at the expense of more firmly bound water and indicated by a reduction in transpiration rate.

Classically, stomatal opening and closing is related to turgor pressure differentials existing between guard and subsidiary cells (Meidner & Mansfield, 1968), possibly mediated through changes in solute accumulation within guard cells (Raschke, 1975). Differential rates of

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[1] ψ represents plant water potential (see Section IIIB, 1(a)).

water supply and loss to and from cells of the stomatal apparatus, altering the turgor pressure relationships of the cells, may be affected by alterations in internal factors (Edwards et al., 1976). These authors noted that "...a small reduction in turgor of all cells is thought to be equivalent to a two-fold or greater increase in the difference in turgor between subsidiary and guard cells..." due to the mechanical advantage which subsidiary cells have over guard cells. By reducing tissue turgor pressure potentials stomatal opening may therefore be induced and since epidermal turgor may be more effective in determining pore width than guard cell turgor (Edwards, et al., 1976) a decrease in ψ_1 may produce a decrease in r_s which results in stomata remaining open at lower ψ_1 or RWC's.

Brown et al. (1976) presented evidence that decreased ψ_1 is accompanied by decreased π. RWC at stomatal closure may be lowered by a decrease in π affecting the comparative turgor pressures of guard and subsidiary cells but cell wall elasticity and thickness may also affect turgor pressure relationships in such a way that a relatively weaker wall may result in greater opening at a given pressure (Edwards et al., 1976). This implies that with increased cell wall flexibility, lower pressure differentials are required to induce a change in shape of the guard cells as a consequence of which the guard cells may be reactive over a smaller range of ψ_1 or RWC, in which a more rapid response effects closure at a higher RWC. Alternatively, diffusion from guard to subsidiary cells may take longer in cells with thickened walls resulting in slower stomatal reactivity, greater water loss and a lower RWC at stomatal closure.

Stalfelt (1955) noted that the water deficit required to induce stomatal closure is greater the higher the light intensity, reflecting the equilibrum between hydroactive closure and photoactive opening.

The situation is not simple for stomatal opening has been shown to be independent of substomatal carbon dioxide concentration under certain conditions (Meidner & Mansfield, 1968); thus it may be oversimplistic to regard the phenomenon solely in terms of increased photosynthetic rate in the guard cells. In addition to the effects of light, humidity gradients may act as a control system for preventing water loss by regulating r_s (Lange et al., 1971; Schulze et al., 1972; Camacho-B, Hall & Kaufmann, 1974; Hall & Kaufmann, 1975; Johnson & Caldwell, 1976). Watts et al. (1976) found that when light was not limiting, the vapour pressure deficit was the main variable affecting r_s in Picea sitchensis. The mechanism by which humidity gradients act is based upon peristomatal transpiration in which cuticular water loss from the guard cells may be greater than water loss from other epidermal cells. Guard cell vacuoles may therefore compete with water loss from guard cell walls to maintain their turgidity (Meidner, 1975). As humidity gradients from the inside to the outside of the leaf increase, water loss from the guard cells increases more than water loss from the other epidermal cells, resulting in a decrease in turgor pressure relative to the subsidiary cells, and stomatal closure. The existence of an epidermal water supply route independent of that of the mesophyll cell walls (Meidner, 1975) in which the evaporative power of the atmosphere controls stomatal aperture, could result in a regulatory mechanism preventing the development of water deficits in the mesophyll which might be metabolically disruptive. However, this implies that water content differentials existing between guard and subsidiary cells affect stomatal closure independently of the RWC of the tissue in general which would invalidate the concept of a threshold RWC at which stomatal closure occurs. Despite the possible importance of localised water contents in controlling stomatal aperture, ultimately the stomata will close as a result of water stress (Lopushinsky, 1969). Bulk leaf water stress may therefore be regarded as a mechanism by which hydroactive stomatal closure inhibits further desiccation following the development of substantial water deficits (Camacho-B et al., 1974; Johnson & Caldwell, 1976). The two mechanisms, i.e. hydropassive stomatal closure and humidity gradient control of stomatal closure, are neither mutually exclusive nor dependent since both have been isolated in different species from different habitats (Johnson & Caldwell, 1976).

Variation in stomatal closure point may be considered in terms of the photosynthetic capacity and desiccation tolerance of the plant. Bannister (1971) enumerated three characteristics which may influence the performance of three heath species, including C. vulgaris, under shaded conditions:

(1) the ability to retain open stomata over a wide range of water deficits as a prerequisite for efficient gaseous exchange and assimilation;

(2) the ability to resist large deficits forming which would cause stomatal closure or physical damage;

(3) the ability to recover from field water deficits without damage.

Similarly, Hall & Kaufmann (1975) suggested that the response of stomata to humidity gradients is co-ordinated with the prevention of water stress in subepidermal tissues set against the needs of evaporative cooling and gaseous exchange, the relative importance being determined by the plant-environment complex. The mechanisms by which stomata respond to the imposition of water stress include elevation of internal CO_2 concentrations and an increased sensitivity of the stomatal apparatus to CO_2 (Meidner & Mansfield, 1968) and increased levels of abscisic acid in stressed leaves resulting in loss of potassium ions, a decrease in guard cell turgidity and consequent stomatal closure (Raschke, 1976). However, plants may adapt to long term deficits, permitting gaseous exchange at lower water contents. Brown et al. (1976) preconditioned plants of Gossypium hirsutum to water stress by exposing them to conditions inducing ψ_1 of -2.8 to -3.2MPa compared to -1.2MPa in the controls. This resulted in a 0.7MPa downward adjustment in the π of the guard cells resulting in the abaxial stomata remaining open at a ψ_1 1.4MPa lower than that of nonstressed plants. Thus, stomatal closure, as affected by π, may be regarded as a function of water stress prehistory.

Meidner & Mansfield (1968) noted that in some circumstances, continued water shortage could result in the stomata remaining closed. Stomatal opening may also be inhibited for some period following the recovery in plant water status (Meidner & Mansfield, 1968; Hsiao, 1973). Studies on Vicia faba and Nicotiana tobaccum indicated that although most stomatal activity was regained within one day of rewatering following stress, total recovery took up to five days (Fischer et al., 1970). Glover (1959) found that prolonged drought resulted in permanent after-effects on Zea mays stomata, the stomata opening to only half their prestressed aperture despite regaining full turgidity. In contrast, stomata of Glycine max were unaffected by a fourteen day drought. Such mechanisms whereby immediate stomatal opening is inhibited may be advantageous in preventing rapid consumption of renewed supplies over a short period but the inhibition of CO_2 uptake may result in a negative assimilation balance. Irrespective of the mechanisms by which changes in stomatal closure point are produced it is apparent that seasonal variation in this parameter may be conducive to the survival of an individual in a particular habitat.

(b) Methodology

Hygen (1951) suggested that the most rapid and accurate gravimetric method for measuring transpirational water loss was weighing excised shoots or plants whose water absorption was inhibited during the experiment. Although certain errors are involved in the use of such a method for determining transpirational rates, e.g. hydropassive closure (Stalfelt, 1955) and increased water loss resulting from decreased tension of water columns (Scholander et al., 1964), the attendant increase in water stress eventually results in hydroactive stomatal closure (Stalfelt, 1955). Thus, by following the transpiration decline of cut shoots an indication of the stress which the plant can tolerate before restricting water loss may be obtained.

The transpiration decline of cut shoots may be classified into three phases (Hygen, 1951):

(1) 'stomatal' phase in which water loss occurs through the stomata and cuticle;

(2) 'closing' phase when the water deficit is sufficient to induce stomatal closure;

(3) 'cuticular' phase representing water loss when stomatal transpiration is zero.

Assuming constant stomatal aperture, the decrease in loss rate is related to a decreasing water content which introduces a problem into the determination of the cuticular transpiration rate. With increasing water stress the vapour pressure at the surface of the organ will be reduced which, together with adsorption, hydration binding and osmotic effects, results in the decline not being directly proportional to the decrease in water content. Hygen (1951) considered that, for this reason, stomatal effects on transpiration rate should be determined at the highest water content feasible. Hygen suggested that the cuticular transpiration rate may be determined by inducing a water deficit of sufficient magnitude to induce stomatal closure, but realised that the reduction in diffusion gradient from the inside to the outside of the leaf will lower the cuticular loss rate in comparison to the cuticular component when the stomata are closed at high turgidities and the diffusion gradient is steeper. Pisek & Berger (1938) considered that the point of complete stomatal closure may be defined more precisely using loss rate or 'decline' curves than by microscopical methods. The induction of a water deficit may therefore be employed to determine stomatal closure although precise estimates of transpiration rates may not be obtained.

The measurement of the 'transpiration decline curve' permits the quantification of transpiration rates, the duration of the 'closing' phase and the effectiveness of stomata in controlling transpiration. The duration of the 'closing' phase may have some ecological significance for the plant's water balance at times of drought. Its derivation requires the use of controlled, standardized conditions since external factors will determine the rate at which water is lost and thus determine the rate at which the stomata close and stomatal transpiration ceases. Stalfelt (1932) found that the 'closing' phase is initiated when a threshold value of RWC has been reached. If only a short time period is required to attain this threshold value, heterogeneity in material and conditions under which transpiration decline is followed may obscure differences between plants in closing phase duration. Hygen (1951) however, determined the rapidity of closure from the curvature of the 'closing' phase which reflected the efficiency of stomatal control on the rate of transpiration, permitting xeromorphic, mesomorphic and hygromorphic populations of Vaccinium myrtillus to be distinguished.

Williams & Amer (1957), in a re-evaluation of Hygen's hypothesis, recognised a 'constant rate period' during which, in the absence of changes in stomatal aperture, water loss was constant despite a fall in water content. After a critical value for water content had been reached the transpiration rate fell (i.e. 'falling rate period') but not in a predictable relationship with water content. Gregory et al. (1950) found such a pattern in Pelargonium sp., a water content of 90% being required to induce the rate change. Williams & Amer (1957) considered that Hygen's hypothesis represented a "...situation in which transpiration changes brought about by stomatal movements will greatly outweigh those due to changes in water content", but the demonstration of a 'constant rate phase' in the early stages of wilting in Pelargonium zonale despite a falling water content tended to invalidate the hypothesis until the 'falling rate phase' was entered. Providing the transpiration rate-water content curve is linear Williams & Amer considered that Hygen's methods are applicable for the duration of the 'falling rate phase' whilst in plants of low cuticular resistance the 'constant rate phase' may be of such a short duration as to be insignificant. Williams & Amer's main contribution is that the complex computations used by Hygen to determine the transpiration rate which is independent of water content may be legitimately simplified without affecting the ecological meaning of the result.

Hygen (1951, 1953a,b) could also be criticised in his use of fresh weight, which as a unit for comparing plant water contents is open to criticism (Section IIIB 2(a)). Subsequent studies have used saturated water content as a basis for measurements. Jarvis & Jarvis (1963a), working with Lupinus albus, used Hygen's rapid weighing method to derive the transpirational decline curve and after plotting, semi-logarithmically, RWC against time, defined the three phase curve and by extrapolating the linear stomatal and cuticular phases obtained the intersection point from which the RWC at stomatal closure could be obtained. They noted that although the definition of the stomatal closure point may be enhanced by such techniques "the intersection depends for its precise placing on the rates of stomatal and cuticular water loss, especially the latter".

A further re-appraisal of Hygen's method by Bannister (1964a) was critical of the necessarily laborious treatment of the data, in addition to which the correction to standard evaporating conditions limited the application of the results to field plant responses. Bannister (1964a) suggested that the decline in RWC could be used to determine stomatal closure and make its determination easier. The rate at which the stomatal response occurs may depend upon the intensity of evaporation but although the time taken to produce stomatal closure may vary, Bannister (1964a) argued that stomatal closure would occur when the shoot had lost a given amount of water irrespective of the rate at which it was lost (cf. Gregory et al., 1950; Williams & Amer, 1957). Consequently, providing shoots are fully saturated at the commencement of the experiment the relative water content at which stomatal closure occurs is independent of the desiccating conditions and may therefore be a useful parameter for assessing stomatal behaviour of plants in the field situation (Bannister, 1964a).

Bannister (1964a) considered that the semi-logarithmic plots of Jarvis & Jarvis (1963a, b) did not facilitate the determination of stomatal closure point but rather imposed a limitation by requiring all determinations to be performed under identical conditions to enable extrapolation of the linear portions of the curve. Bannister (1964a) suggested that a plot of water loss rate against time gave the best estimate of the stomatal closure point since little extrapolation was necessary, total or partial stomatal closure coinciding with the inflexion in the water loss curve. The time at which closure occurred could then be related back to the initial curve of RWC decline over time.

A break of slope in the percentage transpiration decline curve for conifers was interpreted as representing stomatal closure by Lopushinsky (1969). The closure point was determined by extrapolation of the linear cuticular phase and taking the point at which the curve departs from linearity as indicative of stomatal closure. By relating this point in time to the RWC decline curve it was possible to determine the RWC at stomatal closure. Although total closure did not occur, Lopushinsky concluded that the stomata no longer had effective control over water loss after stomatal closure.

Although some authors, e.g. Heath (1959), considered that localised water content differentials in the epidermal cells were responsible for controlling stomatal aperture, thereby invalidating a technique dependent upon bulk tissue RWC, Lopushinsky (1969) concluded that hydroactive stomatal closure resulting from desiccation would occur at a threshold value of RWC which might be expected to vary between species.

Mathematical precision may be given to Bannister's method (1964a) by calculating regression lines for the stomatal and cuticular phases, but a comparison between this method and the ocular fit of curves indicated no significant improvement was achieved by using regression methods (Bannister, 1971).

An objection which might be raised against the determination of stomatal closure by transpiration decline is the inherent assumption that the decline results from increased stomatal resistance although it might also be affected by an increased mesophyll resistance (r_m). Although such 'incipient drying' of the mesophyll cell walls is not totally precluded, a mechanism is not known which results in the vapour pressure in the sub-stomatal cavities being substantially below saturation values, although water potentials at cell wall surfaces may be far from zero (Meidner & Mansfield, 1968). Deficits of considerable magnitude

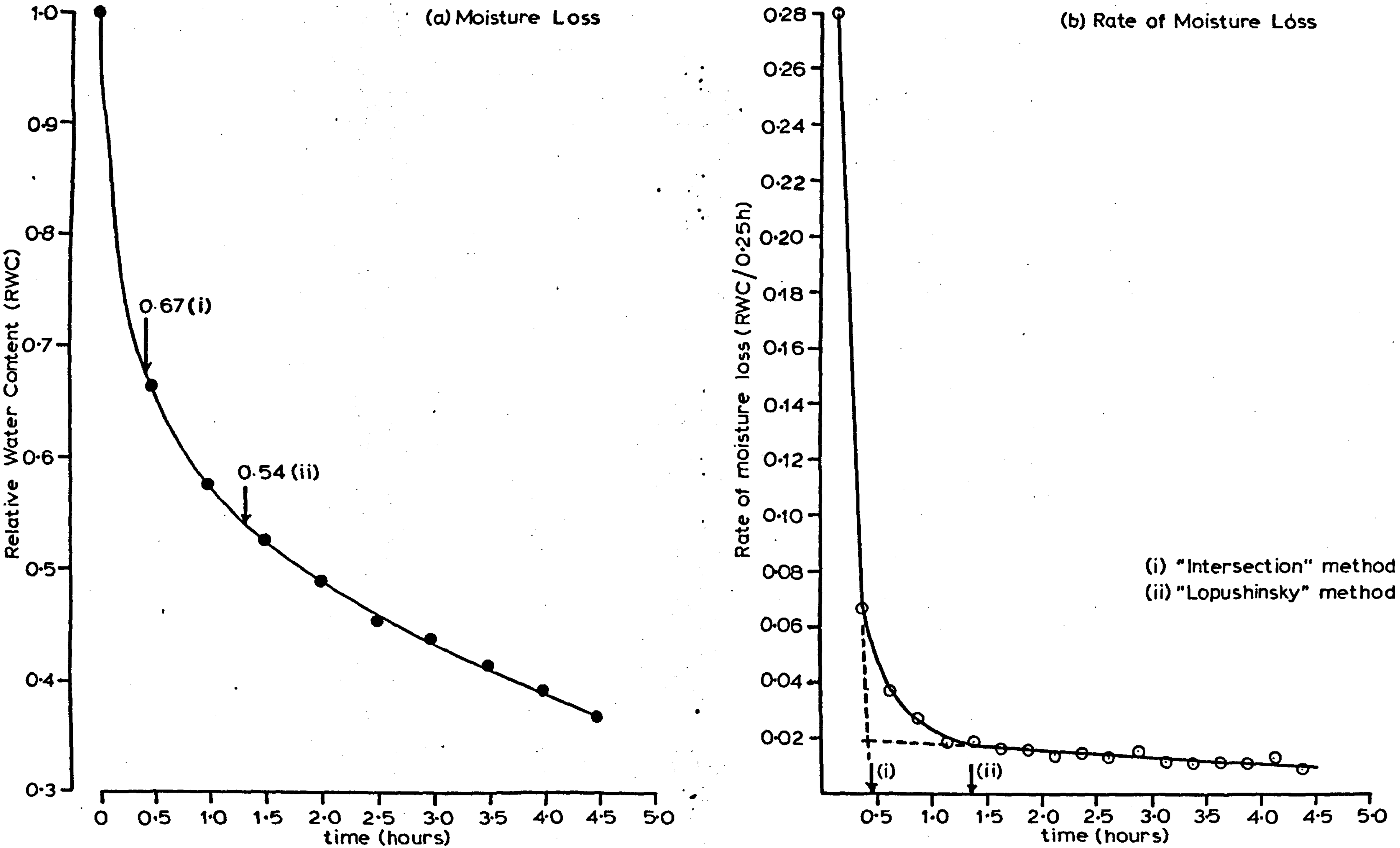

Figure 29 Determination of the Relative Water Content at Stomatal Closure in Calluna vulgaris. (24.09.75.)

are required before r_m outweighs the effects of r_s although the subject is controversial (e.g. Troughton, 1969; Troughton & Slayter, 1969; Redshaw & Meidner, 1972; Section IVA (a)). Bannister (1964a) realising that the transpiration decline curves could arise from a different mechanism, simultaneously followed stomatal aperture, with the aid of a porometer, and water loss on Betula pendula, Vaccinium myrtillus and Rhododendron sp. and found the two estimates of stomatal closure to be in agreement.

It would appear from the work of a number of authors, e.g. Hygen (1951, 1953a, b); Jarvis & Jarvis (1963a,b), Bannister (1964a, 1971), Lopushinsky (1969), Polwart (1970) that the method of 'transpiration decline' is capable of detecting species differences in stomatal reaction to water deficit and it is used in the present study to determine seasonal differences in the ability of C. vulgaris to avoid the formation of sublethal water deficits.

(c) Methods

Shoots of C. vulgaris, taken into the laboratory from the field, were stood in distilled water in stoppered polythene tubes and left to become fully saturated in the dark at 25 ± 1°C for 24 hours. In the final 2 hours of the saturation period the shoots were illuminated to induce stomatal opening (cf. Hygen, 1951). Ten saturated shoots were removed from their chambers, blotted dry and placed under standard conditions of 25 ± 1°C, 40 ± 10% relative humidity and a light intensity of 13klx in a growth chamber. Weight loss was recorded at half hourly intervals for 4.5 hours after which shoots were oven dried at 85°C for 48 hours. The RWC at each weighing was calculated (Equation 7) and an RWC-time curve plotted for each shoot (Figure 29a). From this curve the rate of loss per 15 minute period was determined and plotted (Figure 29b).

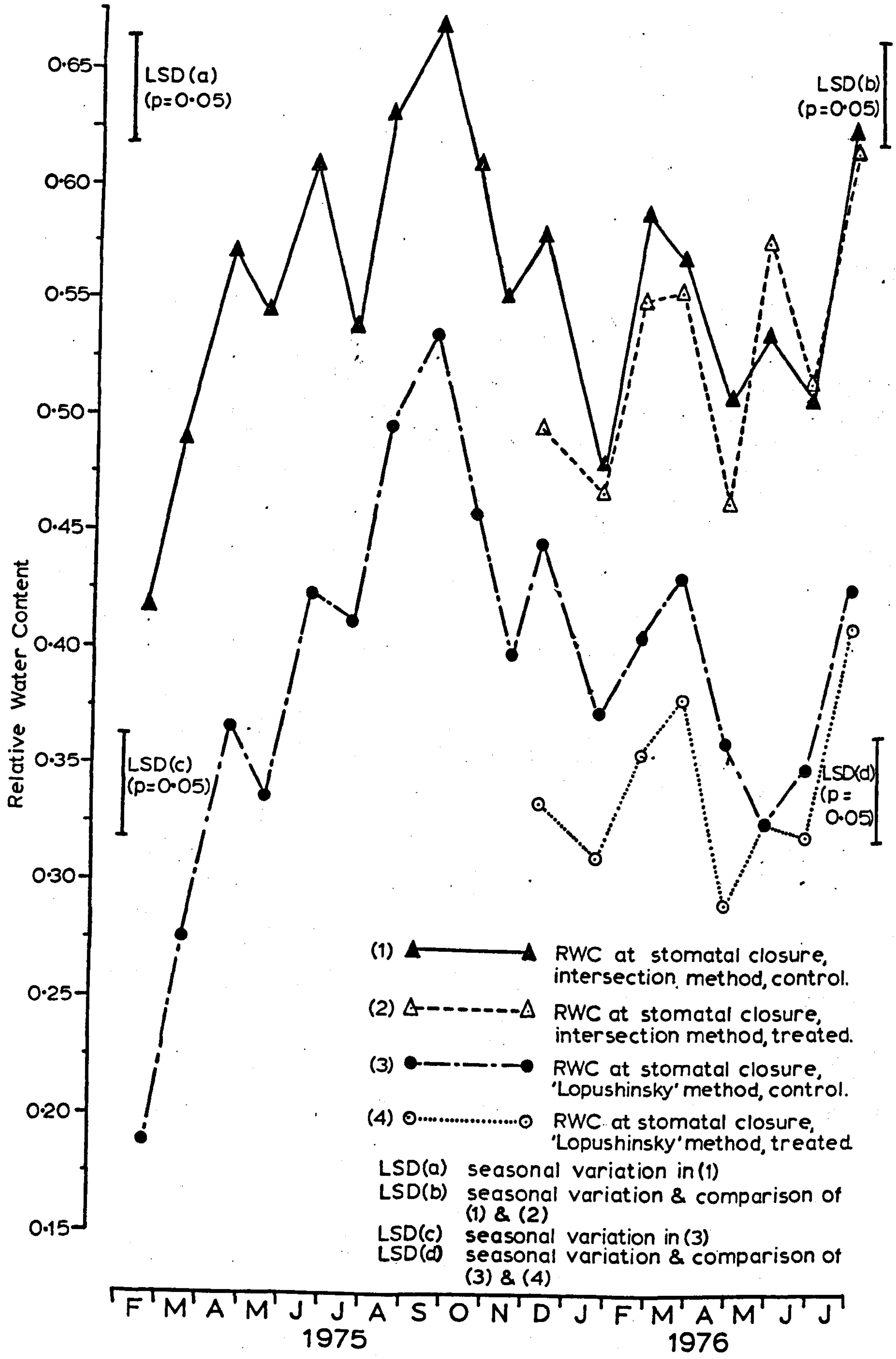

Figure 30 Seasonal variation in the Relative Water Content at Stomatal Closure of C. vulgaris. (Cannock Chase).

Extrapolation of the linear phases of stomatal and cuticular transpiration to the point of intersection gave the time at which stomatal closure occurred (i.e. Figure 29b, point (i), 0.45h). This point related back to Figure 29a gave the RWC at stomatal closure (i.e. 0.67). In view of Bannister's observations (1971), it was considered that the use of regression lines to define the linear phases was not justified especially as a difference of some minutes in the intersection point has a minor effect on the RWC at stomatal closure.

In addition to the 'intersection method', a second point was defined at the end of the closing phase and beginning of the cuticular phase, i.e. the point at which the cuticular phase departs from linearity (cf. Lopushinsky, 1969), (figure 29b, point (ii), 1.35h). The time at which this change occurs may similarly be related to Figure 29a to give a second RWC at stomatal closure (i.e. 0.54) which, because of the time difference, lies somewhat below that of the first. These two points ((i) and (ii)) will be referred to as SCI and SCL respectively. Variations in stomatal closure point were followed from February 1975 to July 1976 on control material.

For the period December 1975 to July 1976 trampled material was also sampled and variation in stomatal closure point followed. The material sampled had been treated three months prior to sampling.

Each stomatal closure point represents the mean of ten replicates, differences having been analysed by analysis of variance (Appendix A(1)).

(d) Results

(i) Seasonal variation in stomatal closure point (Cannock Chase)

Significant seasonal differences ($p < 0.001$) exist in both SCI and SCL values (Figure 30). The lowest values of SCI are observed in the winter months of February 1975 and January 1976 whilst the highest

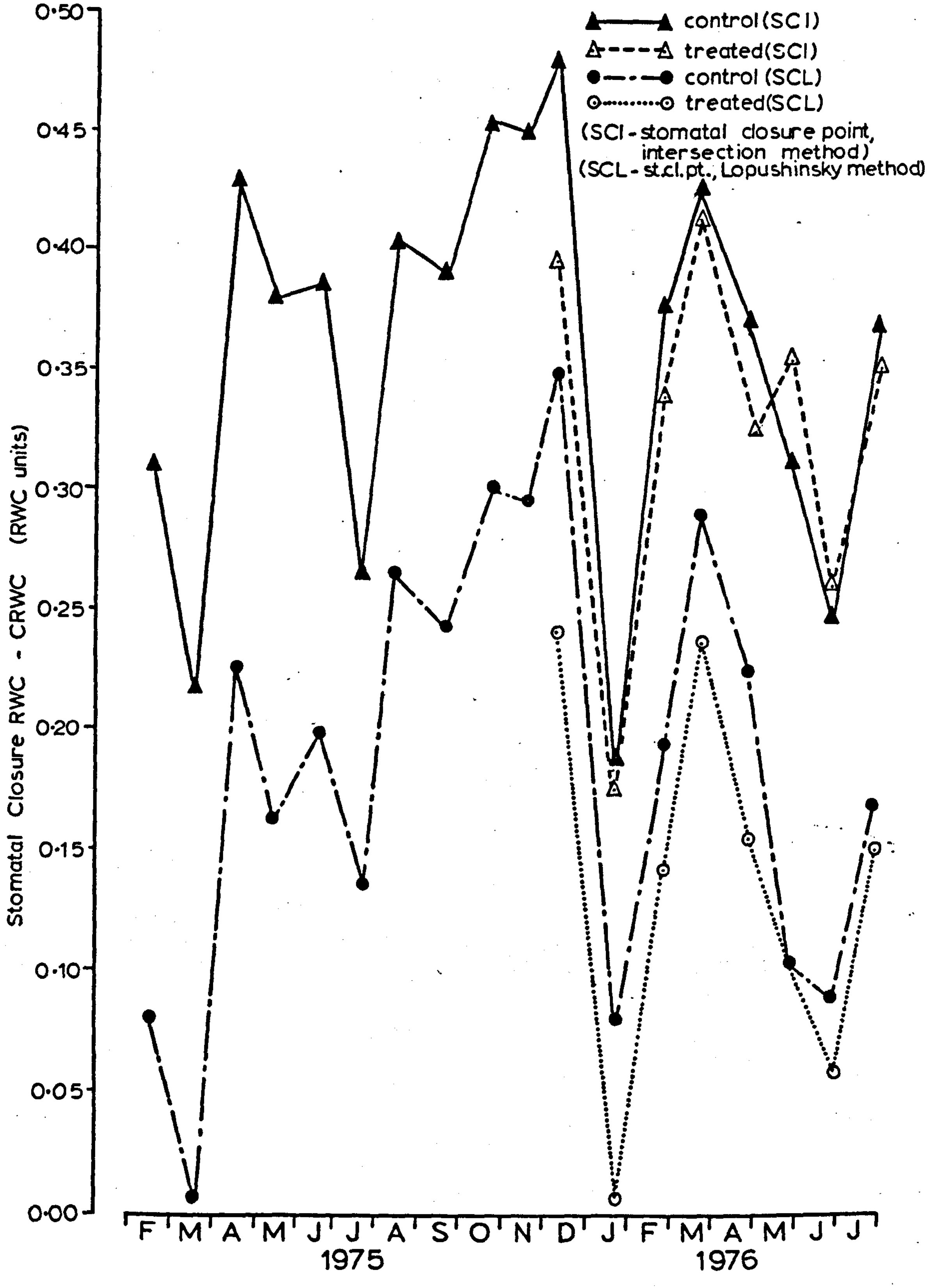

Figure 31 Seasonal variation in the difference between the Relative Water Content at Stomatal Closure and the Critical Relative Water Content (CRWC) defining desiccation tolerance, <u>C. vulgaris</u>. (Cannock Chase).

values recorded are found in September 1975 and July 1976. A similar trend exists for SCL although the lowest value in 1976 occurs in May. The two parameters are significantly correlated ($r = 0.880$, $p < 0.001$), the latter lying an average of 0.1695 ± 0.0086 RWC units below that of SCI.

For the period December 1975 to July 1976 two way analysis of variance has been performed to take into account the effects of trampling on stomatal closure point. In addition to significant seasonal differences in SCI and SCL for both control and treated shoots, ($p < 0.001$), a significant treatment effect is also observed. This is just significant for SCI, ($p < 0.05$), the treatment causing a significant reduction in SCI in December 1975 and April 1976 (the least significant difference between two points (LSD) is represented by LSD(b)). The treatment effect is more apparent for values of SCL in which a highly significant ($p < 0.001$) reduction in SCL occurs in the treated shoots. The significant reduction is maintained until April 1976 after which, although the SCL of the treated shoots is equal to or less than that of the control, the difference is not significant (LSD(d)). Although the seasonal variation in stomatal closure of control and treated shoots is similar, a significant ($p < 0.05$) time x treatment interaction in both instances indicates that the effect trampling has upon stomatal closure point is not constant but varies with the time of the year or possibly the environmental conditions existing at that time.

The RWC at stomatal closure never falls below the CRWC for desiccation tolerance as indicated by the difference between the stomatal closure point and the CRWC, henceforth known as the 'sublethal water content' (Figure 31). This implies that the plant is always capable of avoiding desiccation although the sublethal water content with reference to SCL is small in March 1975 (0.0061) and for damaged plants in January 1976 (0.0184). This suggests that at certain times,

Table 4 Multiple Regression Analysis relating seasonal variation in Stomatal Closure Point 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)
X_1	Vapour Pressure (0900h on day of sampling)	< 0.05	0.5696	0.3245	0.3245	0.5696	< 0.05
X_2	Percentage Moisture Content	n.s.	0.6511	0.4239	0.0994	-0.2208	< 0.05
X_3	Daylength	n.s.	0.6791	0.4611	0.0372	0.1557	< 0.05
X_4	CRWC	n.s.	0.6951	0.4832	0.0221	0.1109	n.s.
X_5	RWC	n.s.	0.7055	0.4977	0.0145	0.2521	n.s.
X_6	Stem Temperature (mean for 7 days prior to sampling)	n.s.	0.7093	0.5031	0.0054	0.4533	n.s.
X_7	Dry weight/Fresh weight	n.s.	0.7124	0.5075	0.0044	0.1568	n.s.
X_8	Soil temperature (-5cm) (mean related to 4°C)	n.s.	0.7131	0.5085	0.0010	0.4639	n.s.
	Regression equation:	$\hat{Y} = 0.4646 + 0.0087\ X_1$					< 0.05

the ability to tolerate desiccation may be important when the ability to avoid desiccation is limited, although the values quoted correspond to periods when desiccation tolerance is abnormally low for the time of year (Figure 27). Each point is based on the mean value for the other two parameters, consequently analysis of variance has not been performed. Although the amplitude of seasonal variation is marked no clearly evident seasonal trend emerges in sublethal water content variation, the value fluctuating around a mean of 0.3586 ± 0.0198 and 0.1892 ± 0.022 for SCI and SCL sublethal water contents respectively. The significant correlation between sublethal water content and CRWC ($r = -0.7032$, $p < 0.01$) indicates that as the desiccation tolerance decreases i.e. higher values of CRWC, the sublethal water content decreases so that the buffering effect produced by stomatal closure against desiccation decreases as desiccation tolerance decreases. This is a reflection of the independent seasonal variation in CRWC and stomatal closure point as indicated by a low and non-significant correlation coefficient.

Seasonal variation in stomatal closure point is correlated with few other measured variables. No significant correlations exist with temperature, rainfall, CRWC or percentage moisture content (Section IIIB, 4) or their derivatives, but a significant correlation is found between vapour pressure at 09.00 hours on the day of sampling and SCI ($r = 0.5696$, $p < 0.05$) and SCL ($r = 0.6632$, $p < 0.01$).

Eight independent variables have been considered in accounting for seasonal variation in SCI in a stepwise multiple regression (Table 4). Together they account for 50.85% of the total variation in SCI but only three of these variables result in a significant regression equation accounting for 46.11% of the total variance. However, only vapour pressure at 0900 hours on the day of sampling makes a significant independent contribution to the equation, accounting for 32.45% of the

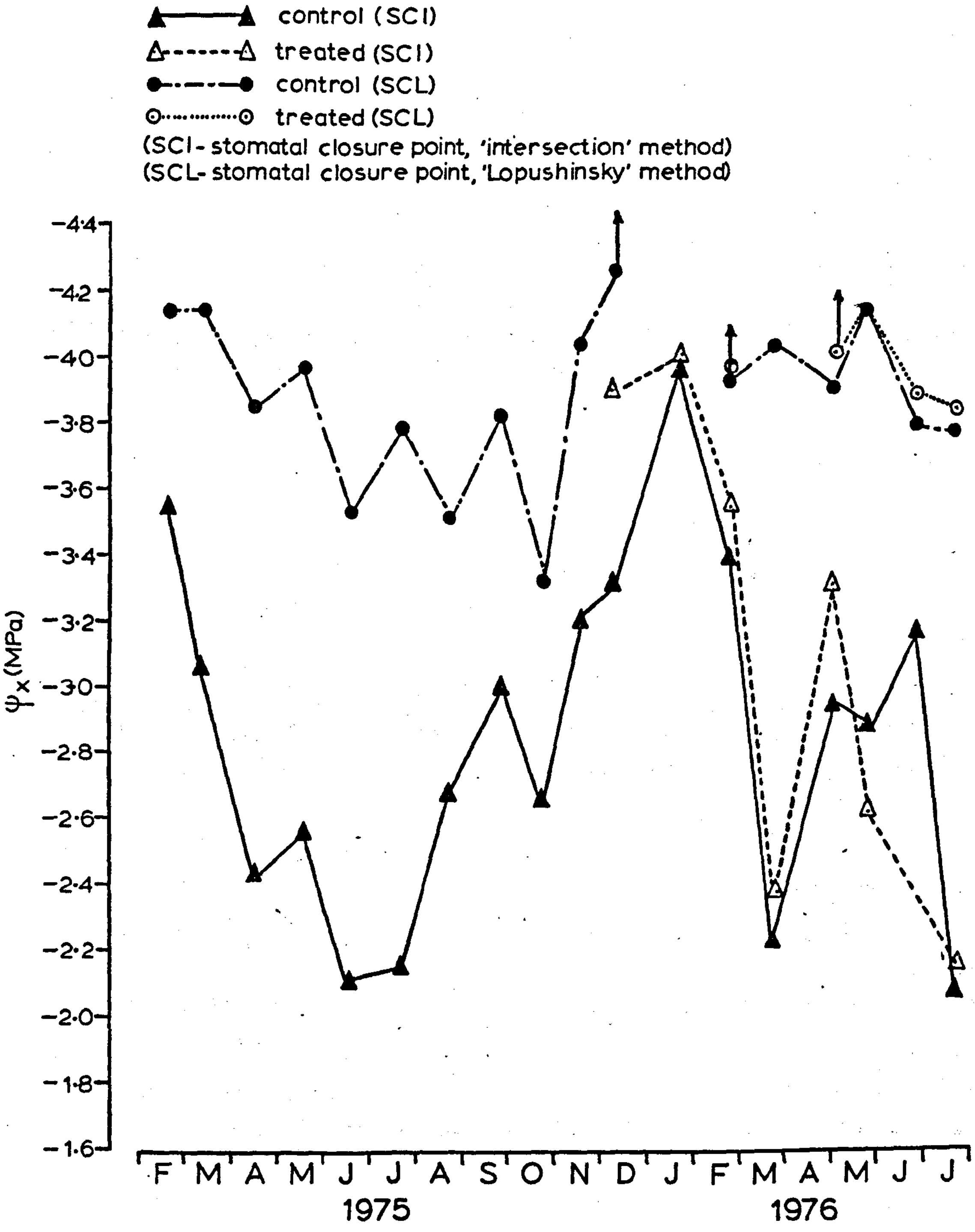

Figure 32 Seasonal variation in ψ_x at Stomatal Closure, C. vulgaris (Cannock Chase).

total variance. Thus, although by considering the variables X_1 to X_3 a better regression is obtained, only one factor may be regarded as making a significant contribution.

(ii) The ψ_x at stomatal closure

The ψ_x at stomatal closure, as determined from the appropriate polynomial regressions relating ψ_x to RWC (Appendix B, Table 6), shows an erratic seasonal pattern (Figure 32). The amplitude of variation is greater for SCI and the significant correlation between ψ_x and SCI ($r = -0.5327$, $p < 0.05$), indicates that when the stomatal closure point is high, e.g. in summer, the ψ_x is high, which is to be expected if the stomata are contributing toward controlling the water stress developing in the plant at any particular time. There would appear to be no stabilisation in the stress required to induce stomatal closure as might be suggested by the seasonal shift in the relationship between ψ_x and RWC (Section IIIB 3). Rather the stress, in terms of ψ_x, required to induce stomatal closure undergoes marked seasonal variation.

The amplitude of variation in ψ_x at SCL is comparatively less, possibly as a result of the seasonal change in the RWC - ψ_x relationship. It is worthy of note that the January 1976 value exceeds the capacity of the instrument used to measure ψ_x.

(iii) The time taken to stomatal closure

The stomatal reaction time (time to stomatal closure) exhibits a significant ($p < 0.001$) seasonal variation for control shoots and damaged shoots (Figure 33). The seasonal trend is slight but is significantly correlated with SCI and SCL respectively ($r = -0.5065$, $p < 0.05$ and $r = -0.7582$, $p < 0.05$ respectively), which indicates that the lower the stomatal closure point the longer the time taken to

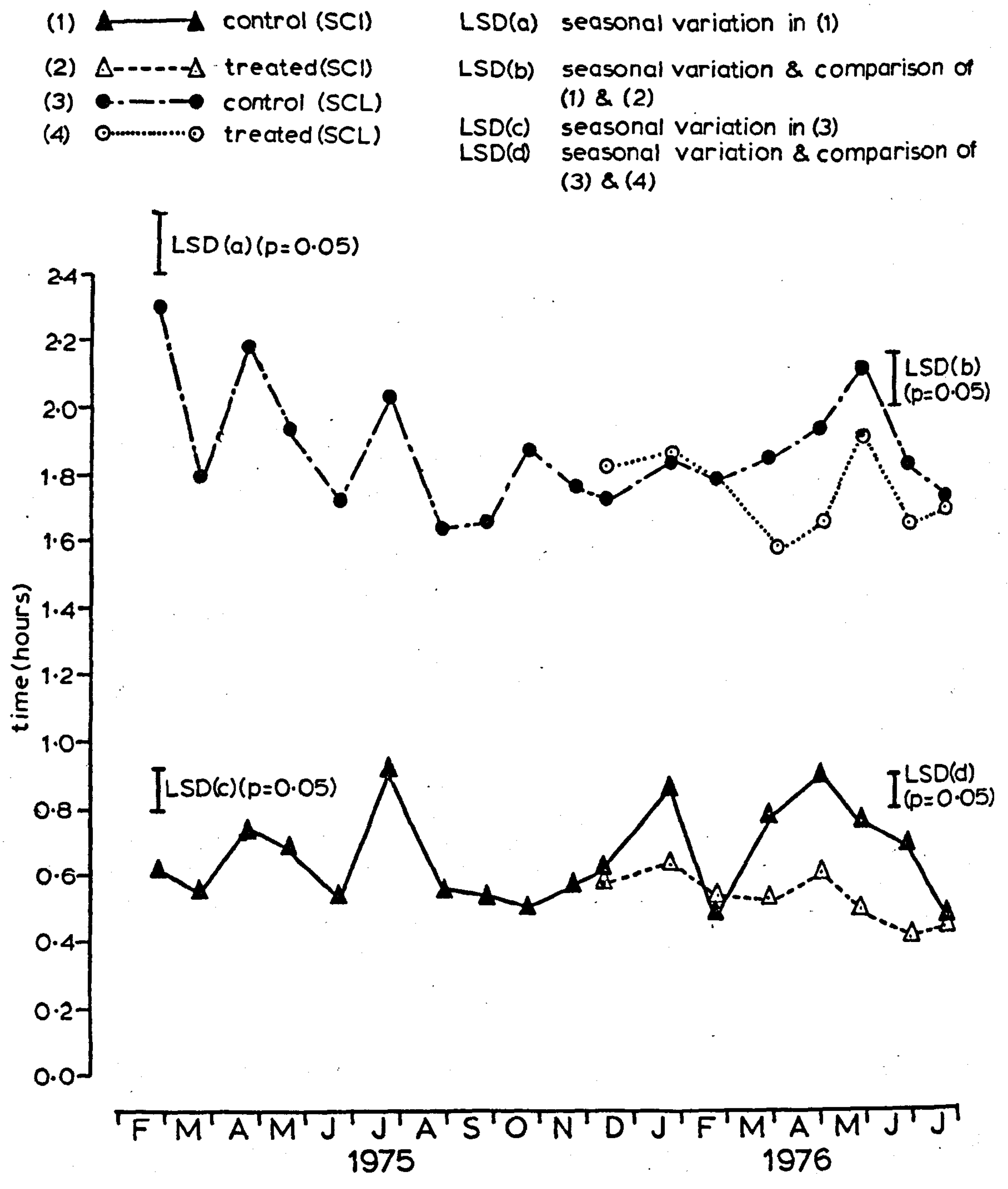

Figure 33 Seasonal variation in the time taken to stomatal closure, C. vulgaris. (Cannock Chase).

stomatal closure. Seasonal differences in time to stomatal closure may also result from different evaporating conditions existing during the measurement of transpiration decline despite conditions being nominally identical. Thus seasonal comparisons may not be valid. However, the time taken to stomatal closure in damaged shoots is significantly lower than that for control shoots, ($p < 0.001$ and $p < 0.01$ for SCI and SCL respectively) although the stomatal closure points are lower in damaged shoots indicating a more rapid stomatal reaction. The phenomenon is not consistent, e.g. in February 1976 damaged shoots take longer than undamaged shoots to attain an RWC sufficient to induce stomatal closure (SCI) but since the determinations on control and treated shoots were performed simultaneously, differences may be real. The significant time x treatment interactions ($p < 0.001$ and $p < 0.01$ for SCI and SCL) indicate that seasonal effects influence the effect trampling has upon stomatal reactivity with the consequence that they may not be considered independently. Thus, in December 1975 and February and July 1976 trampling does not have a significant effect on stomatal reaction time for SCI, significant differences being even fewer for SCL.

(iv) Cuticular Transpiration Rate

The cuticular transpiration rate (Figure 34) reflects water loss after stomatal closure. The rates plotted are the mean loss rate per 15 min. period over the 120 min. period following stomatal closure (SCL). Trampling results in a significant increase in cuticular transpiration ($p < 0.001$). There are also significant seasonal differences ($p < 0.001$) which are more marked in control shoots, rising from a winter minimum in January 1976 to a peak in April 1976 after which a significant decline occurs. Again, a highly significant time x treatment interaction ($p < 0.001$) implies that time of year influences the effect trampling

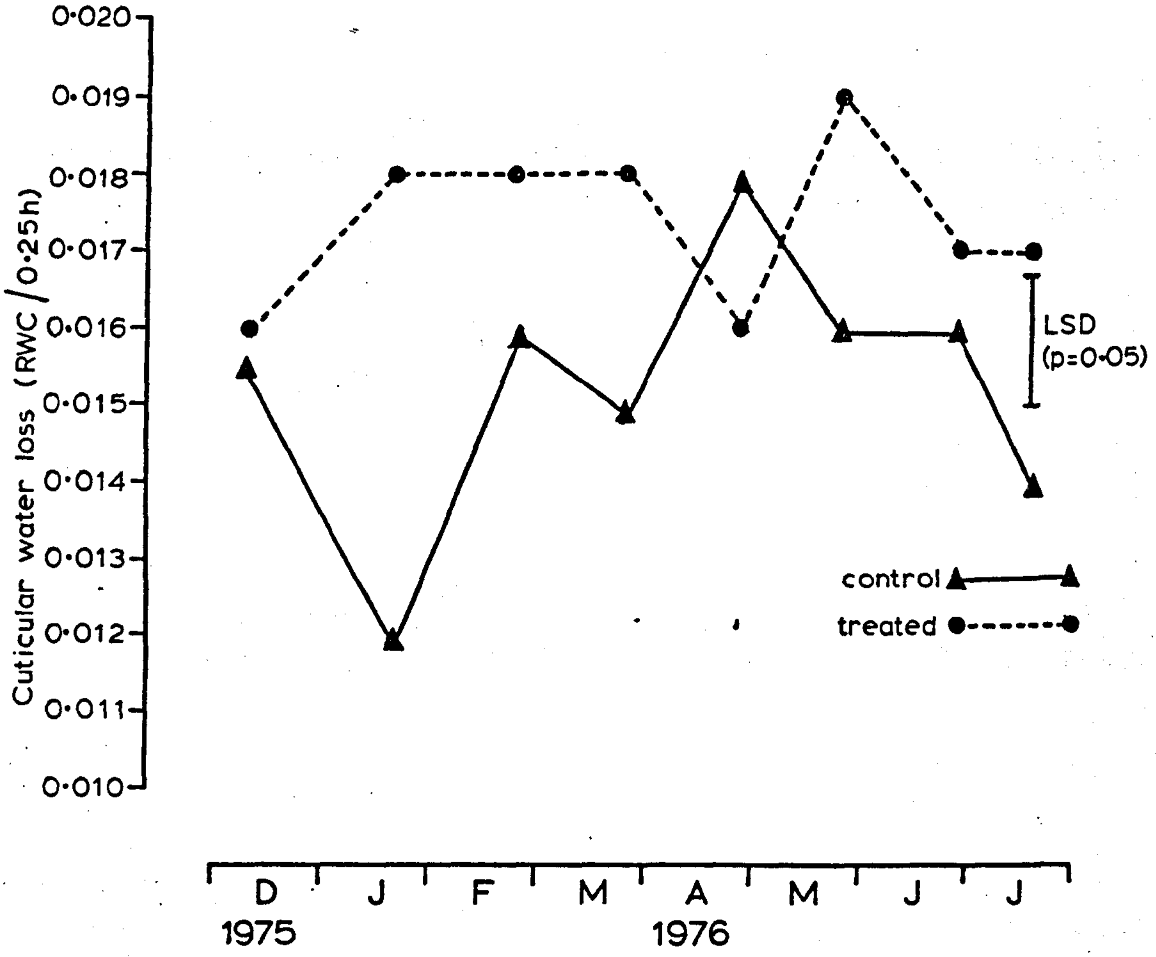

Figure 34 Seasonal variation in cuticular water loss, C. vulgaris, (Cannock Chase).

has upon cuticular water loss.

(e) Discussion

Low stomatal closure points in winter may be associated with a possible increase in soluble sugar content of cells as observed by Grace & Woolhouse (1970), thereby affecting the π differentials and consequently the turgor pressure potential, so bringing about a decrease in the ψ_1 required to induce stomatal closure (Brown et al., 1976; Edwards et al., 1976), but since π's were not measured in the present investigation the possible role π may have on seasonal changes in stomatal closure point cannot be evaluated.

Cell wall elasticity and thickness changes may explain the higher stomatal closure points of shoots sampled at the beginning of the growing season (cf. Edwards et al., 1976). Younger tissues may have thinner and more flexible cell walls permitting more rapid stomatal reaction to water loss so facilitating the maintenance of a high RWC at stomatal closure. Conversely, older tissue with thicker, less flexible cell walls may retard stomatal closure leading to lower RWC's before closure is complete, as found in samples collected in winter. It might be expected that dry to fresh weight ratio would give an indication of cell wall thickness, an increase reflecting an increase in dry weight per unit volume, a proportion of which would lie in the cell wall. The absence of a significant negative correlation between stomatal closure point and dry weight/fresh weight ratio does not substantiate the postulate.

Hormonal control of stomatal response may also be invoked as a mechanism causing seasonal variation in stomatal closure. Stomatal opening may be promoted by an increase in cytokinin concentration (Pallas & Box, 1970), whilst a reduction in concentration may have the reverse effect. Seasonal changes in cytokinin availability may therefore have repercussions on stomatal closure. Such changes may be direct, e.g. a decreased demand for cytokinins in winter by the photosynthetic apparatus (Section IVA, a(ii)) leading to elevated cytokinin concentrations so that larger deficits may be required to induce stomatal closure as found in winter. However, this postulate must be balanced against the possible decrease in cytokinin synthesis by the roots due to low soil temperatures.

The correlation between vapour pressure on the day of sampling and the stomatal closure point suggests that the humidity gradient response of stomata (Lange _et al_., 1971) may operate in _C. vulgaris_. However, since determinations were performed under standard conditions of temperature and humidity and the threshold RWC causing stomatal closure is independent of evaporating conditions employed in its determination (Bannister, 1964a), an adaptive mechanism must be postulated whereby the response of peristomatal transpiration to humidity gradients may be regulated to a rapidly changing environment. The correlation suggests that the steeper the humidity gradient the higher the stomatal closure point thereby preventing excessive water loss. Possible hormonally induced changes in membrane permeability to potassium affecting guard cell turgor (Raschke, 1976) may also affect water loss from the guard cells which if restricted may result in a greater water loss from the mesophyll tissue with a consequent reduction in bulk water content of the tissue before hydroactive stomatal closure occurs. Such permeability changes may be related to the humidity gradient hypothesis but a mechanistic explanation whereby a field humidity gradient response is maintained under laboratory situations remains obscure since it may involve biochemical and structural aspects of the cell.

Irrespective of the mechanisms responsible for producing seasonal changes in the stomatal closure point, the high values in summer may be a response to preventing young tissue, susceptible to lower damaging water deficits because of a lower desiccation tolerance, from experiencing such deficits. With increased tolerance of water deficits in winter, stomatal closure points are lower, thus facilitating gaseous exchange. In summer when humidity gradients are likely to be steep because of the greater evaporative demands of the atmosphere, sensitivity to water loss may be high resulting in high closure points which corresponds to the period in which desiccation tolerance is low. Although periods of summer moisture stress may result in a retardation of net photosynthesis due to stomatal closure (cf. Bannister, 1971), in the present study, despite drought conditions in the summer of 1976, field RWC's of control shoots were always greater than the RWC at stomatal closure (Figure 45) although these measurements were taken at midday and the RWC's may have subsequently become lower. This suggests that photosynthesis would not have been totally impeded by stomatal closure even under conditions of soil moisture stress and high stomatal closure points although the positive RWC's with respect to the stomatal closure point may have resulted from the water deficit, sufficient to cause stomatal closure, being partially satisfied by a restriction in water loss by stomatal closure and continued water uptake, especially as stomata do not immediately reopen following recovery in plant water status (Meidner & Mansfield, 1968; Hsiao, 1973).

Bannister (1971) noted that in "...winter, water conservation would appear to be important as large deficits may be found in the field". Whilst a trend was evident for lower field RWC's to exist in the winter period (Figure 44), the RWC's at stomatal closure were also lower. Although stomatal closure may help conserve water, increased tolerance to desiccation is accompanied by a downward shift in stomatal closure point, resulting in potentially larger water deficits occurring but which would not be damaging. The sublethal water content may be regarded as enabling a buffer to exist between the point at which gaseous exchange is inhibited and that at which damage to the tissues as a result of desiccation develops. The mechanism would appear to be adapted to protect sensitive tissues against the development of damaging water deficits whilst allowing hardier tissues to benefit from gaseous exchange over a wider range of deficits when assimilation capacity may be reduced by other factors, (cf. Bannister, 1971). The temperature optimum for photosynthesis in C. vulgaris does not alter appreciably with a change in temperature regime (Grace & Woolhouse, 1970), consequently in the colder parts of the year temperature may be limiting photosynthesis. By maintaining stomatal opening for as long as possible during the day despite the development of large water deficits, the assimilation capacity is increased by maximising gaseous exchange. This may result in lower RWC's but by lowering the stomatal closure point, these will have a negligible effect on gaseous exchange rates. In principle this is similar to Bannister's comment (1964a) that "...lower closure relative turgidities found in plants from drier areas...suggests that these plants are adapted to the drier conditions not by any greater transpirational control, but rather by an ability to forgo a degree of stomatal protection for the sake of continued assimilation".

Although stomatal closure point and RWC of shoots in the field appear to be independent, in trampled shoots the lower RWC's are accompanied by a significant decrease in RWC at stomatal closure. The immediate response to stress may result in rapid stomatal closure but the results suggest that adaptation to long term deficits may occur, permitting gaseous exchange at lower water contents thus facilitating CO_2 uptake but without risk of desiccation damage since the stomatal closure points remain above the CRWC for desiccation tolerance. The mechanisms by which this may be achieved remain obscure (Brown et al.,

1976) and the possibility that stomata remain closed for varying periods after the relief of the stress (Glover, 1959; Meidner & Mansfield, 1968; Fischer _et al_., 1970; Hsiao, 1973) may invalidate the determination of stomatal closure point on damaged material. The errors involved in the method combined with the small number of samples exhibiting a significantly lower stomatal closure point compared to the control shoots makes the postulate that damaged shoots adapt to lower RWC's to facilitate CO_2 uptake extremely tentative.

The comments made regarding seasonal changes in stomatal reaction time make the trends of dubious significance. If the differences are real the tendency for quicker closure in younger tissue may reflect greater elasticity of younger cells, facilitating a more rapid restriction of water loss and greater control over the water deficit developing. There is no experimental evidence in support of this and the argument may be somewhat circular in that it is not possible to determine whether rapidity of reaction determines the deficit developing at stomatal closure or whether the RWC at stomatal closure is a predefined quantity and the rapidity with which water loss occurs to lower shoot RWC to this value determines the time taken to stomatal closure (cf. Bannister, 1964a). Similarly, the significant differences between control and treated shoots may be an artifact of the method employed. In those instances where this effect is observed, 75% of the cases have a significantly lower percentage moisture content (Section IIIB, 4) than control shoots which may affect the result. The significance of such an effect, if real, remains obscure in the light of the foregoing discussion unless water loss rate from the treated shoot is greater than in the control, in which instance rapidity of reaction may be of significance in preventing the development of water deficits close to the lethal point.

The increased cuticular transpiration rates at the onset of the growing season in control shoots may reflect thinner cell walls and less cuticularisation of the epidermis of young tissue. The decrease in cuticular transpiration rate from May to July 1976 corresponds to the development of drought conditions. Levitt (1972) stated that such conditions may result in decreased cuticular transpiration rates by a decrease in the permeabiltiy of the cuticle and by the deposition of epicuticular lipids restricting water loss. Contrasting with this is the significantly increased rate of cuticular transpiration in damaged plants. Hall & Jones (1971) noted increased transpiration rates in Trifolium repens, the leaves of which had lost much of their epicuticular wax by being in abrasive contact with the ground. Grace (1974) suggested that increased transpiration with increased wind speed in Festuca arundinacea may have resulted from increased cuticular damage. He observed that abrasion and surface wear resulting from the increasing number of leaf collisions could result in epidermal cells being punctured, reducing their turgidity with respect to guard cells, leading to an increase in stomatal aperture, with the consequence of increased transpiration rate. Grace suggested that this may explain why deficits required to induce stomatal closure in wind treated plants were greater than for controls. Abrasion and damage to epidermal cells when plants are trampled may explain why cuticular transpiration rates are higher and RWC at stomatal closure lower in damaged plants.

The definition of two points SCI and SCL has been considered to be justified on the basis that there is no porometric confirmation as to when the stomata have closed and since the inflexion in the loss rate curve is often not acute the SCL represents the point at which the stomatal component of transpiration may be regarded as having ceased whilst the SCI may represent the mid point of stomatal closure rather than complete closure.

(f) Conclusion

Significant variation occurs in the desiccation avoidance ability of *C. vulgaris* as indicated by the stomatal closure point although the mechanisms by which this is achieved remain obscure. Such variation has implications for CO_2 exchange capacity both in undamaged and damaged shoots. There may be some compensation in damaged shoots in terms of a lowering of the stomatal closure point to facilitate continued gas exchange but this may render the plant more susceptible to desiccation damage as it reduces the buffer zone as defined by the sublethal water content. This situation is aggravated by the elevated cuticular loss rates over which the plant has little control which, depending upon the environmental conditions and the degree of damage, may reduce the plants desiccation avoidance ability to an ineffectual level. This, combined with the reduced assimilatory capacity of the plant, may endanger the continued survival of the trampled plant.

3. Total Desiccation Avoidance

The level of water deficit induced over a given time period

(a) Introduction

The total desiccation avoidance capacity of a shoot may be indicated by the water deficit which has developed after a given period of desiccation (cf. Bannister, 1971). This parameter obviously incorporates a stomatal component, i.e. the rapidity with which the stomata close and restrict water loss but it may also reflect variation in the cuticular component of water loss and internal factors controlling water retention.

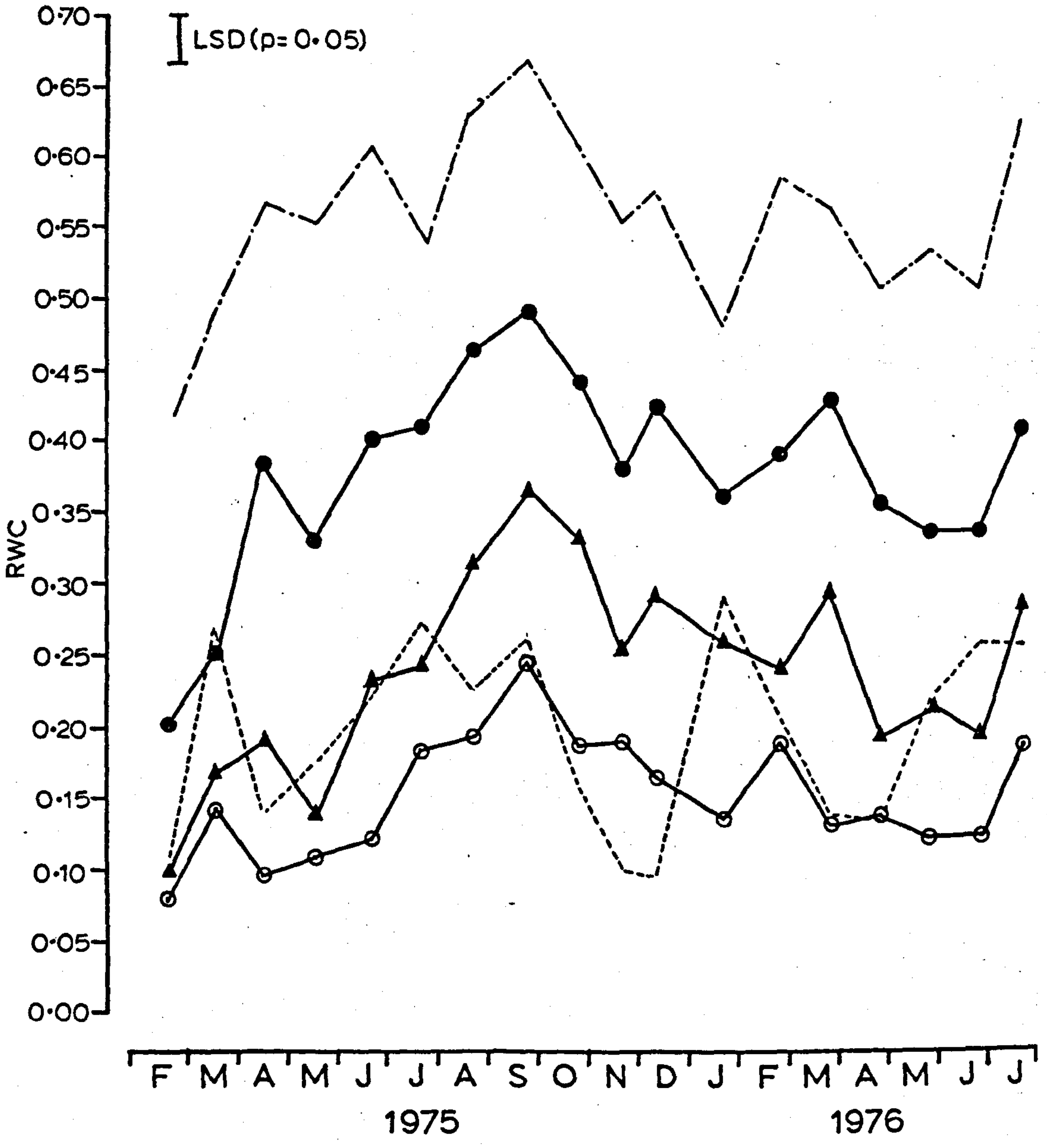

Figure 35 Seasonal variation in RWC of <u>C. vulgaris</u> shoots after specific desiccating periods, indicating total 'desiccation avoidance' capacity. (Cannock Chase).

●——● RWC after a desiccation period of 2·0 hours
▲——▲ RWC after a desiccation period of 4·5 hours
○——○ RWC after a desiccation period of 8·0 hours
—·—·— RWC at stomatal closure (SCL)
--------- CRWC (desiccation tolerance)

(b) Method

Shoots of C. vulgaris, sampled from Cannock Chase, were brought to full saturation at 25 ± 1°C in the dark by standing their bases in distilled water in the saturation chambers.for 24 hours. In the final two hours of the saturation period the shoots were illuminated to induce stomatal opening. At the end of the saturation period the shoots were removed, blotted dry, weighed and placed under standard desiccating conditions (25 ± 1°C, 40 ± 10% relative humidity, 13klx light intensity). Ten shoots were removed at time intervals of 2.0h., 4.5h. and 8.0h, reweighed and ovendried at 85°C for 48 hours, following which the dry weight was determined. (All weighings were made on a 500mg. torsion balance). The RWC was calculated (Equation 7) for each shoot and the results subject to analysis of variance.

(c) Results

There is a significant ($p < 0.001$) seasonal variation in the RWC developed after specific desiccation periods (Figure 35), the greatest ability to avoid the formation of large water deficits being shown in September 1975 whilst the lowest occurs in the winter month of February 1975. (The LSD presented on Figure 35 applies to the three solid graph lines representing the RWC after specific desiccating periods, the stomatal closure point and CRWC having been included for comparison). The seasonal trend is similar for all three time treatments (i.e. 2.0h., 4.5h. and 8.0h.) although whilst the 4.5h. treatment is significantly correlated with the 2.0h. treatment ($r = 0.909$, $p < 0.001$), this is not the case for the 2.0h. and 8.0h. treatments ($r = 0.417$, not significant). There is also a significant correlation between the RWC after 2.0h. and the stomatal closure point ($r = 0.868$, $p < 0.001$) but not between the RWC after 2.0h. and CRWC ($r = 0.104$, not significant).

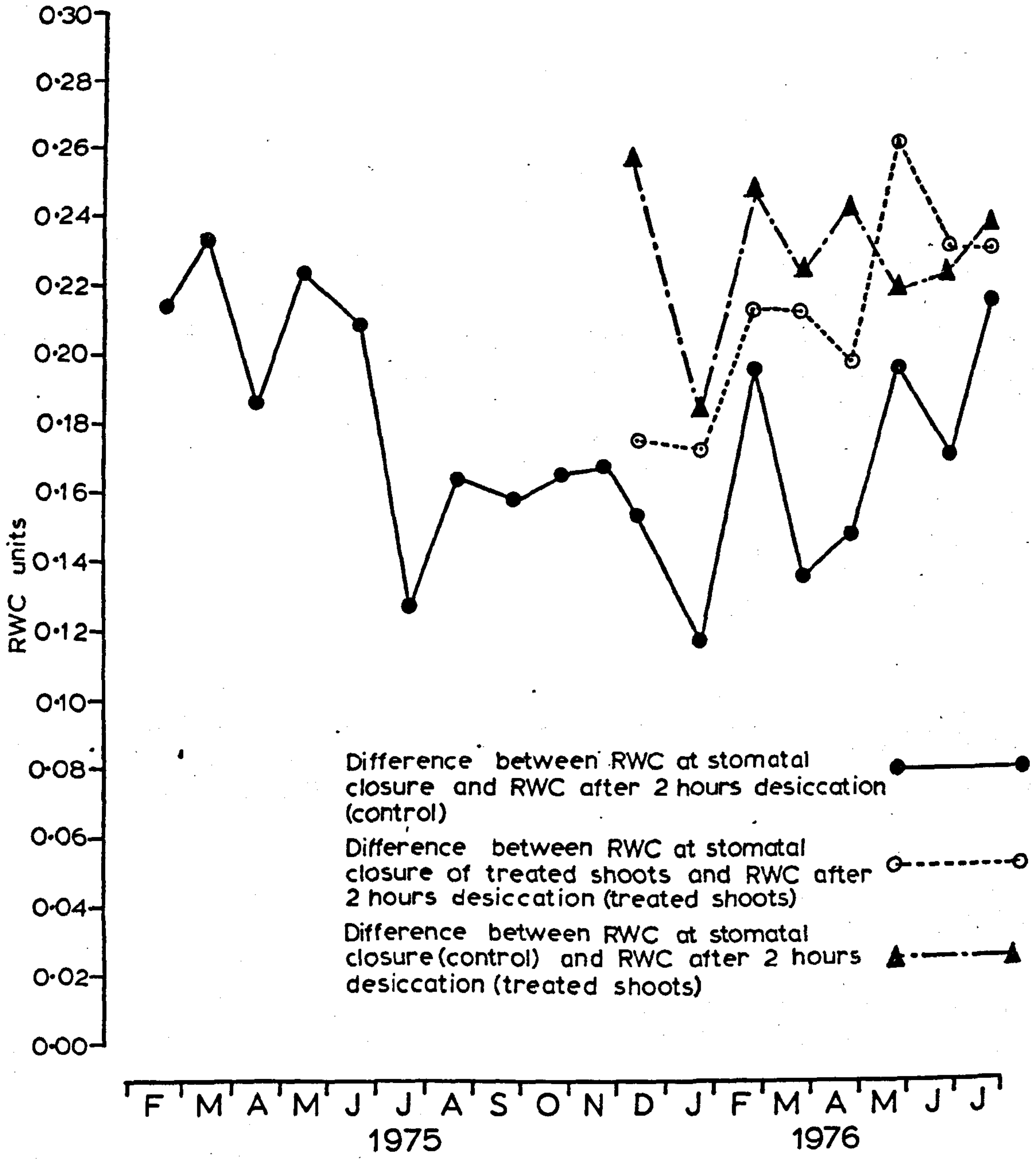

Figure 36 Seasonal variation in the difference between the RWC at stomatal closure (SCI) and the RWC after a desiccation period of 2 hours, C. vulgaris, (Cannock Chase).

The RWC after 4.5h. is consistently significantly ($p < 0.001$) lower than the RWC after 2.0h. and only in February, March and May 1975 are there no significant differences between the RWC after 4.5h. and 8.0h. Although monthly differences in the ability to avoid desiccation exist, the amplitude of variation decreases with increasing length of desiccation period, possibly because, with a decreasing RWC, there is less water to be lost.

The difference between the stomatal closure point and the RWC after 2.0h. (Figure 36) does not show any evident seasonal trend or correlation with an environmental factor. (The differences are based on the mean response of the two parameters and have not been subjected to analysis of variance procedures). The deviation about the mean response (0.1777) is comparatively small (0.034) and suggests that the residual variation of 24.7% not accounted for by the correlation between stomatal closure point and RWC after 2h. may not be due to variations in cuticular losses. The differences referrred to in Figure 36 indicate the ability of the plant to control water loss by stomatal control, i.e. the lower the RWC at stomatal closure the smaller the difference, thus the values between July 1975 and January 1976 reflect lower stomatal closure points than for the February to June 1975 and May to July 1976 periods. This is suggested by the significant negative correlation between the difference between stomatal closure point and the RWC after 2h. ($r = -0.560$, $p < 0.05$). Similar trends are observed with damaged material ($r = -0.7765$, $p < 0.05$ for the correlation between time to stomatal closure and the difference in RWC at stomatal closure and after 2h.), however, the differences between the two RWC parameters are somewhat greater for damaged material than for control material, despite shorter times to stomatal closure (Section IIIA, 2(d)), suggesting that although the stomata may be

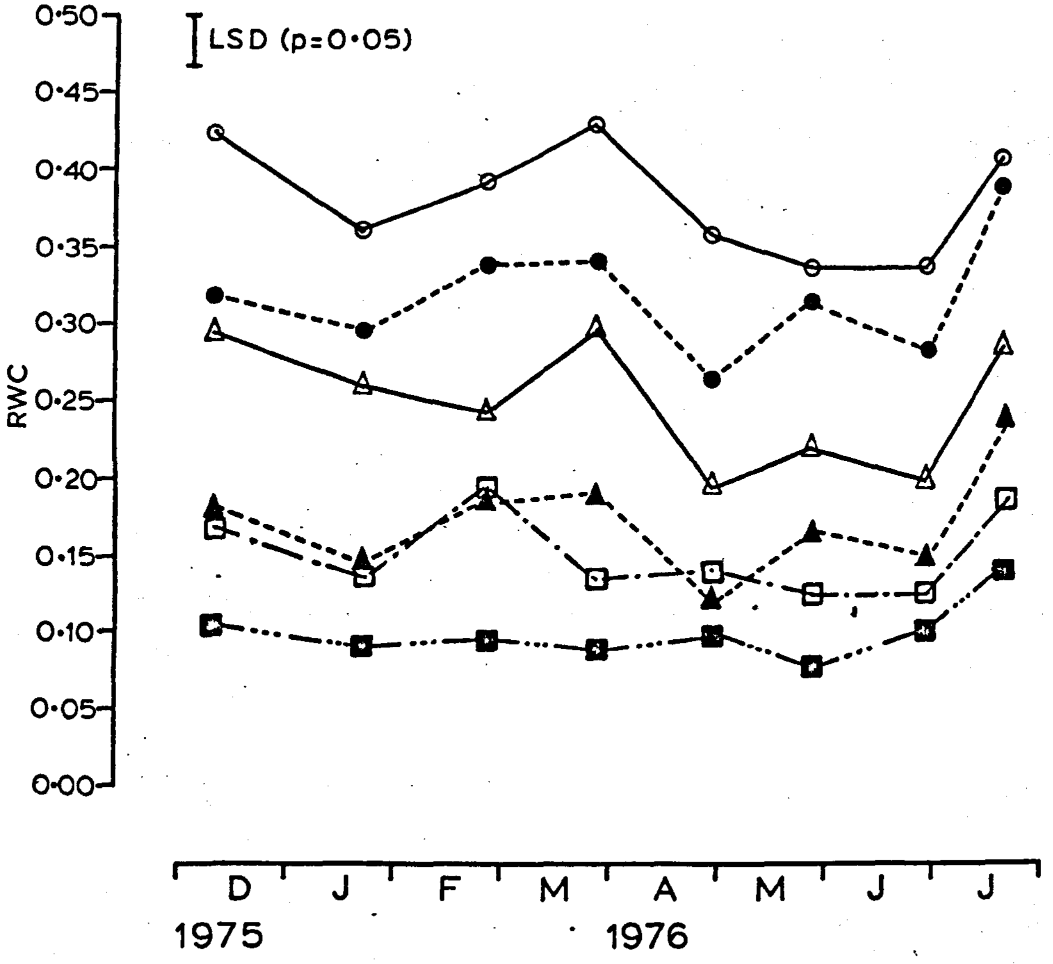

Figure 37 The effect of trampling on the total desiccation avoidance capacity of C. vulgaris as indicated by the RWC developing over specific desiccating periods, (Cannock Chase).

○——○ RWC after a desiccating period of 2·0hours (control)

●- - - -● RWC after a desiccating period of 2·0hours (treated)

△——△ RWC after a desiccating period of 4·5hours (control)

▲- - - -▲ RWC after a desiccating period of 4·5hours (treated)

□–·–·–□ RWC after a desiccating period of 8·0hours (control)

■···–···■ RWC after a desiccating period of 8·0hours (treated) regulating water loss, the total desiccation avoidance capacity is somewhat less for damaged than for control material.

This latter suggestion is substantiated by the effect of trampling on the desiccation avoidance (Figure 37) in which, in addition to a significant ($p < 0.001$) seasonal variation in total desiccation avoidance, treated shoots have a significantly ($p < 0.001$) lower RWC than control shoots, except in May and July 1976, for the RWC after 2h. It is of note that the RWC after 4.5h. for damaged shoots is, for the most part, not significantly different from the RWC after 8 h. for control shoots.

(d) Discussion

The results suggest that stomatal control over water loss significantly influences the degree of water deficit developing over a specified time interval. The differences between the stomatal closure point curve and that of RWC after specific desiccating periods, due to continued water loss following stomatal closure, probably results from cuticular transpiration losses. Seasonal changes in cuticular transpiration rates may account for the seasonal pattern of variation in total desiccation avoidance capacity. Larcher (1960) postulated differences in cuticular transpiration rate as being responsible for drought resistance differences between Quercus ilex and Q. pubescens, the latter having a higher cuticular transpiration rate and lower drought resistance. Levitt (1972) noted that cuticular transpiration is not constant for a specific leaf, drying reducing the transpirational losses until the cuticle becomes virtually impervious to water. He also noted that 'pseudohardening' may occur in which lipids are deposited on the leaves to inhibit cuticular losses, whilst intense respiration on droughting has also been invoked as a mechanism for maintaining plant water content (Genkel et al., 1967). The second and third propositions are feasible in the present investigation but would have to occur in the few hours during which the plant is exposed to desiccation. The other possibilities imply premature adaptation to reducing cuticular water loss. The absence of a seasonal trend in the difference between RWC at stomatal closure and the RWC after 2h. suggests variation in cuticular losses may not be responsible. It is also necessary to approach these values as indicators of desiccation avoidance with caution since the variations in RWC after specific periods may result from variations in the desiccating conditions despite their being nominally identical for each monthly determination.

The comparisons between control and treated material suggest that despite desiccation avoidance being affected by stomatal closure in both control and treated shoots, the ability to restrict water loss and thus avoid dehydration is greater in the former than in the latter. The implication of this conclusion in terms of plant survival is apparent when RWC's over varying desiccating periods are considered. Desiccation of treated material for 4.5h. results in an RWC lying mostly below the CRWC for desiccation tolerance. In shoots with an effective desiccation avoidance mechanism it is likely that RWC's will not reach CRWC and impair survival. This is partly substantiated by the observation that when avoidance ability is low, tolerance is high. In contrast, if water supplies to the plant are restricted, the decreased ability to avoid desiccation by damaged plants may result in RWC's that approach or lie below the CRWC, increasing the likelihood of desiccation injury. This will be most critical when, in control shoots, avoidance ability is high since tolerance may be low at such times and damage from comparatively small deficits may result. This suggests that the seasonal change in the balance between desiccation tolerance and desiccation avoidance may be paralleled by a seasonal change in the susceptibility of C. vulgaris to induced water deficits.

4. General discussion - The comparative importance of tolerance and avoidance of water deficits

Parker (1956) stated that there "...seems to be all gradations in different plants in their water holding capacity and in their lethal level of water content..." and that these two factors frequently determine the drought resistance of a plant. This conclusion may be modified, in the light of the present investigation, to suggest that the balance between water conservation i.e. desiccation avoidance, and desiccation tolerance may undergo seasonal changes within a species.

Heth & Kramer (1975) suggested four possibilities as to why some plants can survive drought better than others, namely:

(1) a more efficient water absorbing system;

(2) better control over water loss;

(3) better tolerance of dehydration;

(4) a combination of the above.

Oppenheimer (1968) found that when Pinus radiata was exposed to drought, rapid stomatal closure occurred but because of its low tolerance, death occurred sooner than in P. pinea which had a lower cuticular loss rate. Some species, however, were found to possess good avoidance and tolerance mechanisms. Thus Quercus ilex possessed a higher drought resistance than Q. pubescens since stomatal closure occurred at an RWC of 0.83 compared to 0.8 and the sublethal RWC of 0.29 in the former was greater than the 0.5 value for the latter species.

Avoidance would appear to confer certain advantages which tolerance does not, e.g. the maintenance of sufficient turgor pressure to allow growth (cf. Hsiao, 1973) and the avoidance of stresses which may result in irreversible injury. It is a pertinent observation that maximum avoidance occurs at a stage in the Calluna plant life cycle when the plant is growing. A corollary of this argument is that the maximum water stress at which a plant is able to continue growing is a function of turgor pressure potential and cell wall plasticity. Jarvis & Jarvis (1963b) found that stomatal closure occurred at a higher ψ in Populus tremula (-0.5MPa) and Betula verrucosa (-1.0MPa) than in Picea abies (-1.5MPa) and Pinus sylvestris (-3.7MPa) which resulted in higher turgor pressures in the former and a greater potential for cell elongation, although assimilatory capacity would obviously be reduced as a result of stomatal closure, which may have repercussions on growth.

Avoidance would appear to be of greatest benefit in the growing season whilst tolerance would be advantageous when assimilation is restricted by environmental and possibly physiological limitations. Tolerance would also be of benefit when absorption capacity may be reduced e.g. in winter, whilst when conditions are conducive to water loss e.g. under high evaporative conditions on a summer day, avoidance would be advantageous. The trends in avoidance and tolerance suggest that such a mechanism may be present in C. vulgaris, although the lack of any significant correlation between the two parameters implies that they may be operating independently. This might be expected if they are controlled by different factors and affect different aspects of the plant's water relations. They may be regarded as complementary. Jarvis & Jarvis (1963b), for example, found an inverse correlation between avoidance and tolerance in Pinus sylvestris, Picea abies, Populus tremula and Betula verrucosa. Levitt (1972) suggested that certain aspects of tolerance and avoidance may be mutually exclusive, e.g. high RWC's indicate avoidance whilst low π's indicate high tolerance. Levitt considered that plants develop primarily one or the other, avoidance being more important in higher plants. Efficient avoidance of drought implies that tolerance may have no selective value, especially if the development of tolerance depends on the induction of sublethal deficits, since drought avoiders will not be able to harden (Levitt, 1972). The oversaturation type of response, as shown by C. vulgaris, reflects an ability to make maximum use of available water between drought periods, but only if avoidance of water loss is efficient will the plant's survival be enhanced, since a species unable to increase its tolerance to desiccation is susceptible to drought injury from water loss over which the plant has no control, e.g. cuticular losses (Rychnovská & Květ, 1965). Levitt (1972) concluded that drought tolerance is important only if accompanied by drought avoidance.

To a certain extent, the dynamic situation described above is at variance with the suggestions of Bannister (1971). He considered that whilst the ability to retain open stomata, the ability to resist the formation of large deficits and the ability to recover from deficits without damage may all be important in summer, the first may be less important in winter when environmental factors are limiting photosynthesis, whereas "conservation" would appear to be important where large winter deficits may be formed. However, since "Most winters afford conditions that could lead to the formation of large water deficits..." (Bannister, 1971), it would appear that tolerance of deficits is important since plants would not appear to be effectively avoiding drought if large deficits are found (Section IIIB, 2(ii)).

The desiccation resistance of a plant may be viewed in terms of two components, tolerance and avoidance, the relative importance of which may change seasonally and as a result may enhance survival. The complexity of the response suggests that it is "...a great mistake to try to find a single cause of drought resistance in any one woody plant. There may be contributing factors anywhere from the roots to the leaves, from the finest part of the cytoplasm to the most complete tissues, from the embryo to the mature plant". (Parker, 1956).

B. THE DEVELOPMENT OF WATER STRESS IN *C. VULGARIS*

Introduction

Water movement in a plant occurs along gradients of decreasing 'free energy', thus the difference between the free energy of water in the plant compared to that of pure free water i.e. the water potential (ψ), quantifies the water stress involved. This quantity is applicable to many physical systems and may have the same physiological significance for a range of tissues, in contrast to the water deficit (i.e. the water content existing compared to that of a fully saturated plant) which is not directly comparable to other stresses and may have different physiological significances in different plants (Kramer & Brix, 1965). Macklon & Weatherley (1965), however, argued that ψ gradients may not always be the most appropriate parameter to consider. Slatyer (1960) for example, found that a ψ of -2.0MPa represented a relative water content (RWC) of approximately 90% in *Acacia aneura* compared to approximately 50% in *Lycopersicon esculentum*. In this instance RWC was a more sensitive index of stress than ψ. Barrs (1968) suggested that both parameters are valid measurements of water stress and both have been quantified in the present study.

1. Xylem water potential (ψ_x) as an indicator of plant water stress

(a) Introduction

Taylor & Slatyer (1962) provided a terminology by which plant water status could be expressed on the basis of thermodynamic principles in which ψ may be represented as:

$$\psi = \frac{(\mu_w - \mu^o{}_w)}{\bar{V}_w} \qquad (9)$$

where : μ^o_w is the water potential of pure water (≡ ϕMPa);

$(\mu_w - \mu^o_w)$ is the capacity of water to do work with respect to pure water;

$\bar{V}_w$ is the partial molar volume of water.

In the xylem vessels this may be expanded to take into account a series of other potentials:

(i) the gravitational potential ($-\rho gh$) which depends on the density of water (ρ), the acceleration due to gravity (g) and plant height (h);

(ii) the frictional potential ($\sum_{s1}^{p} f_i r_i$) which results from partial fluxes and resistances, in the xylem element, to water moving from the soil to the point of measurement in the plant;

(iii) the osmotic potential (π);

(iv) the matric potential (τ);

(v) the soil water potential (ψ_{s1}).

Xylem water potential (ψ_x) may be rewritten as:

$$\psi_x = -\rho gh - (\sum_{s1}^{p} f_i r_i) - \pi - \tau - \psi_{s1} \qquad (10)$$

The pressure chamber method of determining ψ specifically measures the gravitational component (negligible in plants of limited height), and the frictional component, thus ψ_x may be related to the pressure chamber value by (Boyer, 1969):

$$\psi_x = P - \psi_s^{\text{xylem}} \qquad (11)$$

where: P is the combined gravitational and frictional potentials and is given by the apparatus;

ψ_s^{xylem} is the combined solute and matric potentials.

A number of reviews have been made concerning the passage of water through plants and the resistances it encounters, e.g. Cowan (1965), Slatyer (1967), Cowan & Milthorpe (1968), Richter (1973). The flux of water through the soil-plant-atmosphere continuum is proportional to the ψ gradient and inversely proportional to the flow resistances along the pathway (Elfving et al., 1972). To move water from one point in the system to another requires a difference in potential between the two points, which will be largest when the resistance is highest and the flow maximal. Although an imbalance between influx and efflux of water is required to induce an alteration in a cell's water economy, when transpirational losses equal supply a driving force or potential is required to move the water through the flow path resistances (Jarvis, 1976). If the resistances remain constant the potential will vary with the rate of transpiration. Water deficits may be regarded as a result of the equilibration in ψ between cells adjacent to the flow path and the water in the path, the magnitude of deficits depending on the "... moisture characteristics of the cells, the resistance to movement of water out of the cells and the water potential in the flow pathway" (Jarvis, 1976).

Frictional resistances to the movement of water occur on the internal surfaces of vessels and tracheids, in cell walls and membranes, in the symplasm and in cell pits (Jarvis, 1976). The water potential required to move water through a plant may be broken down into, (Richter, 1973):

$$\psi_1 = \psi_{s1} + \psi_g - T(R_s + R_x + R_1) \qquad (12)$$

where: ψ_1 is leaf ψ;

ψ_{s1} is soil ψ;

ψ_g is hydrostatic ψ;

T is transpiration;

R_{s1} R_x and R_1 are resistances to water transport in the soil, the xylem and the leaf.

In a reassessment of this 'Ohm's Law' analogy, Richter evaluated the total resistance to water movement (R) in the following terms:

$$R = R_r + R_x + R_l = \frac{(\psi_l - \psi_{sl})}{E} \qquad (13)$$

where: R_r, R_x and R_l represent the resistances in root, stem and leaf; E is the transpiration flux density, i.e. volume per unit time per unit area.

He then considered the implications of varying the components of R whilst maintaining E constant, the effect depending upon which component of R was altered. Richter quotes, for example, the effect of obstructing half the stem xylem vessels, thus effectively doubling R_x. To compensate for this, ψ_l will decrease if E is to remain constant. The proportion by which ψ_l decreases will be related to the contribution R_x makes to R, for example, if R_x makes up the greater proportion of R an alteration in R_x will have a proportionately greater effect on ψ_l than an equivalent change in R_r. Removal of half the leaves however, will have the reverse effect. If R_l is the major component of R and the volume of water passing through the plant remains constant, the potential water loss will double since the transpiration flux (i.e. the volume of water lost from all leaves per unit time) is doubled, but since the volume per unit area per unit time (i.e. transpirational flux density) is double, the losses are divisible by twice the transpirational flux density, R consequently remains unaltered as does ψ_l. If, however, $R_r + R_x$ is greater than R_l, the volume of water passing through $R_r + R_x$ is not affected by the decrease in leaf area but since the flux density is twice its previous value R will be half of its original value with the consequence that no decrease in ψ is required to maintain the flow of water (Richter, 1973).

Restated, it may be said that an observed decrease in ψ_1 may result from one of at least two causative factors:

(1) an increase in resistance somewhere along the pathway whilst the transpirational flux density remains unaltered;

(2) an increase in transpirational flux density whilst the resistance remains constant.

The stem or xylem resistance may be divided into two components, the resistance resulting from water movement through the vessel lumen and end walls and the resistance to water movement in the vessel walls. Resistance to flow is comparatively much larger in the walls than through the lumen, but since "in angiosperms most of the water undoubtedly moves through the vessels in the sapwood provided the water columns in these vessels are not cavitated" (Sheriff, 1974), the major component of R_x is provided by the lumen resistance since water movement in the cell walls is comparatively severely restricted unless water flow is prevented in the cell lumen. Cowan & Milthorpe (1968) give the conductivity of the xylem (k_x) as:

$$f = \frac{-k_x A}{1} \Delta\phi \qquad (14)$$

where: f is flow rate;

A is the cross sectional area of the xylem;

1 is the length of xylem;

$\Delta\phi$ is the hydraulic potential over length 1, (defined as the work required to change the state of free water to that of the pore water).

An alteration in A (cf. Richter, 1973), e.g. by damage to the functional xylem, may result in a decrease in ψ to overcome the increase in R_x.[1] An alteration in the functional value of A may result from cavitation of the continuous water columns in the xylem or mechanical damage.

The stability of xylem sap under tension has been the subject of much controversy, e.g. Stocking (1945) considered sap had a low stability under tension, Scholander et al., (1965) suggested that tensions up to 10.13MPa may not rupture xylem sap continuity whilst Oertli (1971) was of the opinion that tensions between 10MPa and 100MPa were required to cause xylem sap cavitation and concluded that "... fracture of xylem water columns, under usual conditions, is improbable". Milburn & McLaughlin (1974) however, observed cavitation "in fresh turgid leaves....at negative pressures of 5-15 bars..." (-0.5 to -1.5MPa) which would suggest sap stability is not as great as once thought.

Preston (1961) listed the possible consequences of a water column under tension:

(1) "the columns and vessels may withstand the tension";
(2) "the water may break and the vessel cease to function in conduction";
(3) "gas bubbles may be drawn in from intercellular spaces or from neighbouring gas filled cells again breaking continuity";
(4) "the vessel terminations in leaf lamina may collapse".

Mechanical damage may also lead to an effective increase in R_x by decreasing A. This may result from, (Milburn & Johnson, 1966):

(1) crushing cell walls and septa of xylem vessels and tracheids;
(2) destruction of connections between vessels and tracheids;
(3) breakage of whole cells.

It is apparent that ψ_x may therefore be affected by a mechanical stress imposed upon the plant, in addition to the environmental effects

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[1] Where R_x and k_x are inversely correlated.

mediated through changes in transpiration rate.

(b) Methodology

ψ_x may be measured using a pressure chamber apparatus. Water under tension in the xylem as a result of "transpirational pull", recedes from the severed end of a shoot to a distance proportional to the tension in the xylem vessels at the instant of cutting. Although Dixon (1914) exerted pressures on leaves, the problems inherent in his method forced him to abandon it and it was not until Scholander et al. (1964) demonstrated 'hydrostatic pressure' in plants using pressure exerted on the leaves that the method was rediscovered. Scholander et al. (1965) proposed that the tension existing in the xylem elements could be ascertained by exerting a pressure on the leaves sufficient to force the xylem sap meniscus back to the cut end of the shoot.

Scholander et al. (1965) argued that for ψ_x to equal ψ_1 the pressure must be directly transmitted from outside the leaf to the liquid within. Since there is a direct continuity from the air in the substomatal cavity to the xylem via the apoplastic water pathway of the parenchyma, the leaf is ideally suited to their technique. However, this assumes ψ_x equals ψ_1 which is not necessarily valid since the resistance between the two is often unquantified, although Tyree et al. (1975) have attempted to remedy this.

Gardner & Rawlins (1965) were dubious of the assumption that the pressure chamber even measures xylem sap tensions but their argument involves cell membranes acting as a barrier between the inside and outside of the plant and therefore affecting the relationship between ψ_x and P (the pressure applied). However, if water movement is apoplastic, cell membranes are not necessarily involved but a new source of error, that of long range adsorption forces existing in cell walls, may become apparent. Gardner & Rawlins argued that Scholander's technique could "prove the existence of adsorption forces just as readily as the existence of negative pressures" (Gardner & Rawlins, 1965).

On the assumption that xylem solute potential is negligible or constant (Boyer, 1967), it has sometimes been supposed that P is directly equivalent to ψ_x and is a measure of various plant parameters, e.g. plant moisture stress (Waring & Cleary, 1967). Certain workers have realised that it does not represent the total water potential, e.g. Klepper & Ceccato (1968), Begg & Turner (1970). Various other descriptive terms have been used, e.g. "xylem pressure potential" (Kaufmann, 1968a), "sap stress" (De Roo, 1969), "negative hydrostatic pressure" (Waggoner & Turner, 1971). Since "xylem pressure potential" may be regarded as thermodynamically correct (Ritchie & Hinckley, 1975), this is the term used in the present study (represented by ψ_x) but does not include the π exerted by the xylem sap.

The Relationship between ψ_x and psychrometric measurements of ψ_l

The psychrometric estimation of ψ_l (determined by vapour equilibration using a thermocouple psychrometer), is used as a reference value against which other methods may be compared. Although a consistent relationship between ψ_x^P (ψ_x determined by the pressure chamber) and ψ_l^{tc} (leaf water potential determined by thermocouple psychrometry) is often observed it is not necessarily 1:1. Boyer (1967) found that ψ_x was usually more negative than ψ_l and suggested that this was the result of pith filling with sap and the compression of vascular tissues by pressure necessitating higher pressures than might be expected to force the sap back to the severed end of the shoot. This suggests that the assumption that the spatial arrangement of water in the shoot remains the same whether the shoot is under pressure or not, may be invalid.

The relationship between ψ_x^P and ψ_1 may not remain constant and the point at which ψ_x^P equals ψ_1^{tc} varies with species, e.g. - 2.0MPa in Taxus sp. (Boyer, 1967), -0.3MPa in Pinus taeda (Kaufmann, 1968a). The discrepancy between species may result from differential filling of voids and non-conducting elements, which were dry before measurement.

The use of excised shoots in the method has also been questioned. On severance, an increase in transpiration may result due to the release of tension in the transpiration stream, which ultimately results in a more negative shoot ψ. It is implicit in the method that the relationship between ψ_x^P and ψ_1^{tc} is the same before and after excision. Duniway (1971) questioned this, and West & Gaff (1971), using Pyrus malus, showed the assumption to be invalid. Evaporative loss from the cut end of the shoot was found to have a greater effect on ψ_x^P than ψ_1^{tc} with the consequence that pressure is required to fill the dried vessels with sap in excised tissue in comparison to naturally desiccating tissue (West & Gaff, 1971). Waring & Cleary (1967) found that in Pseudotsuga menziessi ψ_x^P and ψ_1, as determined using vapour pressure equilibration, corresponded to within 0.1MPa, whilst Blum et al., (1973) and Frank & Harris (1973) concluded that a linear relationship existed between ψ_x^P and ψ_1^{tc} but that the two were not directly equivalent. Calibration curves, specific for the species and age of plant, may be produced but values of ψ_x^P in the uncorrected state may be useful as relative indicators of plant water stress (Ritchie & Hinckley, 1975).

Errors attending the method

1 Sampling errors

A certain amount of variation is inherent in the plant material. Waring & Cleary (1967) observed a small variation in ψ_x in a stand of

Pseudotsuga menziesii under conditions of low atmospheric and soil moisture stress but if atmospheric stress increased variation was functionally related to stand microclimate. Rooting depth, as reflected by tree size, became important when soil moisture stress was critical, thus to minimize variations, samples should be obtained from plants of similar size and environment.

The relationship between frictional resistances and ψ_x^P implies that with increased height of the tree ψ_x^P should decrease. Scholander et al. (1965) found such a 'hydrostatic pressure gradient' to exist in Pseudotsuga menziesii and Sequoia gigantea. Errors resulting from such gradients would be of less significance in dwarf shrubs e.g. Calluna vulgaris.

Leaf age may also affect ψ_x^P through stomatal resistance and reactivity and it may be concluded that "...theory predicts and data support the existence of appreciable spatial variability of P (ψ_x^P) within the crowns of herbaceous plants as well as large trees" (Ritchie & Hinckley, 1975).

2 Technique errors

(i) Stem trimming subsequent to cutting:

When the stem is cut sap withdraws from the cut end to a distance proportional to the tension existing at the instant of cutting (Scholander et al., 1965). If the shoot is trimmed to improve the viewing area this relationship will be destroyed and the pressure required to return the meniscus the shorter distance will be less, so giving an erroneous value of ψ_x. Scholander et al. noted that this source of error is of less consequence in plants with short tracheids, e.g. conifers.

(ii) Time interval between excision and measurement:

Artifically raised ψ_x may result from water loss during the interval between excision and placing the shoot in the pressure chamber. For example, Scholander et al. (1965), working with Pseudotsuga menziesii, found that the tension increased by up to 0.04MPa min^{-1} of exposure. In contrast, Waring & Cleary (1967) found, for the same species, that shoots could be left for up to five minutes without an appreciable decrease in ψ_x. Storage in humid chambers may reduce the error, e.g. Ritchie & Hinckley (1971) found a negligible error of 0.004MPa min^{-1} after short term storage in humid chambers whilst Hellkvist et al. (1974) observed that Picea sitchensis shoots stored in polythene bags for 6 and 26 hours produced errors of only 0.05MPa and 0.1MPa respectively. Ritchie & Hinckley (1975) noted that deciduous plants may exhibit a rapid decrease in ψ_x following excision and suggested that the difference between deciduous and conifer plants may result from a greater resistance to water loss in the latter. The water stress which the plant is under at the time of sampling may also influence the decrease in ψ_x on excision. It would seem good practice to minimize the time between excision and measurement and to standardize the time interval involved.

(iii) Exclusion errors: (Millar & Hansen, 1975)

Measurements of ψ_x have usually been made with 0.8-2.0cm. of sample protruding from the chamber, e.g. Scholander et al. (1965), Boyer (1967), Waring & Cleary (1967), Kaufmann (1968a, b) since this is the length required to pass through the chamber top. This part of the shoot may be defined as 'portion I' whilst that within the chamber and subjected to the increase in pressure as 'portion II' (Millar & Hansen, 1975). Exclusion errors refer to the former and may be appreciable, e.g. Boyer (1967) and Waring & Cleary (1967) reported an additional 0.02-0.03MPa cm^{-1} of portion I to obtain the balancing pressure (i.e. pressure at which the meniscus reaches the cut end of the shoot). They attributed the errors to the amount of stem voids which water can occupy under no pressure. Waring & Cleary (1967) suggested that, whilst the amount of stem within the bomb is not critical, the length protruding should be constant and minimized to not more than 2cm.

In addition to the length of 'excluded' tissue, Millar & Hansen (1975) found species differences in the exclusion error and suggested that such errors may be due to cells absorbing water following excision and not being subjected to the applied pressure. In addition, water forced from portion II may be absorbed by the cells of portion I and consequently to return the sap meniscus to the severed end requires a greater pressure on portion II than that equivalent to the tension prior to excision.

Departures from a 1:1 ratio between ψ_x^P and ψ_1^{tc} may result from exclusion errors since these are not involved in psychrometer determinations of ψ.

Although Waring & Cleary (1967) argued that the amount of stem inside the chamber is not critical, Boyer (1967) and Kaufmann (1968a, b) suggested that this may not be so. Boyer (1967) found that for Rhododendron roseum ψ_x^P versus ψ_1^{tc} in 3-5cm. and 10-12cm. stems differed by 0.22MPa and 0.36MPa respectively whilst Kaufmann (1968b) found differences up to 0.88MPa depending on the length of portion II in Citrus spp.

(iv) The effect of rate of pressure increase on the pressure equilibration point:

The balancing pressure represents a point at which no excess water is expressed from the symplast to the apoplast, all parts of the symplast and apoplast then assuming equivalent ψ (Tyree & Hammel, 1972; Tyree *et al.* 1973), theoretically giving the ψ in the leaf cells at the time of severance. Boyer (1967) found that the time required to achieve this balancing pressure did not affect its magnitude. From this he concluded that there was rapid equilibration between mesophyll and xylem ψ. In contrast, Waring & Cleary (1967) noted that equilibrium may not be achieved if the pressure is raised too rapidly, whilst if not raised with sufficient rapidity erroneous readings may result. They suggested standardizing the rate of increase to 0.069MPa s^{-1}.

Various rates of pressure increase have been used, e.g. 0.02-0.025 MPa s^{-1} (Kaufmann, 1968a, b), 0.069MPa s^{-1} (West & Gaff, 1971), whilst Blum _et al._ (1973) in experiments on _Sorghum bicolor_ used two rates of increase, 0.033MPa s^{-1} and 0.038MPa s^{-1}, and found better estimates of ψ_1 were obtained using the higher rate of pressure increase. Hellkvist _et al._ (1974) found that rate of pressure increase had no affect on the endpoint determination in _Picea sitchensis_ and suggested using a high rate of increase to within 0.6-0.7MPa of the estimated balance pressure in material with low ψ_x.

(v) _Errors resulting from temperature changes during pressure application and release_:

Puritch & Turner (1973) found that a temperature range of 100C° may occur in a single pressure charge-discharge cycle. The amount of heating is determined by the rate of pressure increase which, even at low rates, e.g. 0.007MPa s^{-1}, could exceed 8C° whilst the amount of cooling is directly related to the level of pressure in the chamber prior to release. Rapid pressure release may result in subzero temperatures but heating and cooling effects are ameliorated by the presence of foliage within the chamber. Puritch & Turner found that instant pressure release from pressures in excess of 2.4MPa resulted in death of the terminal part of needles from _Abies grandis_, whilst a temperature drop of 64.5C° occurred from the release of gas at a pressure of 4.1MPa.

Puritch & Turner suggested that a slow rate of pressure increase will minimize the temperature rise whilst efficient heat exchange through the chamber wall will help reduce the increase in temperature. Similarly, slow pressure release or release in steps to allow equilibration between room and chamber temperature will avert too great a temperature drop. Although temperature drop is of little consequence if the shoot is to be discarded, Puritch & Turner observed that temperature changes may affect the osmotic and matric potentials, and turgor pressure. Such changes may contribute to the discrepancy between pressure chamber values and psychrometer measurements of ψ_1.

Temperature increases within the chamber may also result in increased evapotranspiration from the shoot so artifically raising ψ_x. Slow rates of pressure increase will maintain elevated temperatures for longer so magnifying this error, consequently, some workers e.g. Boyer (1967), Duniway (1971), moistened the chamber walls during measurements to reduce the diffusion gradient between leaf and chamber air.

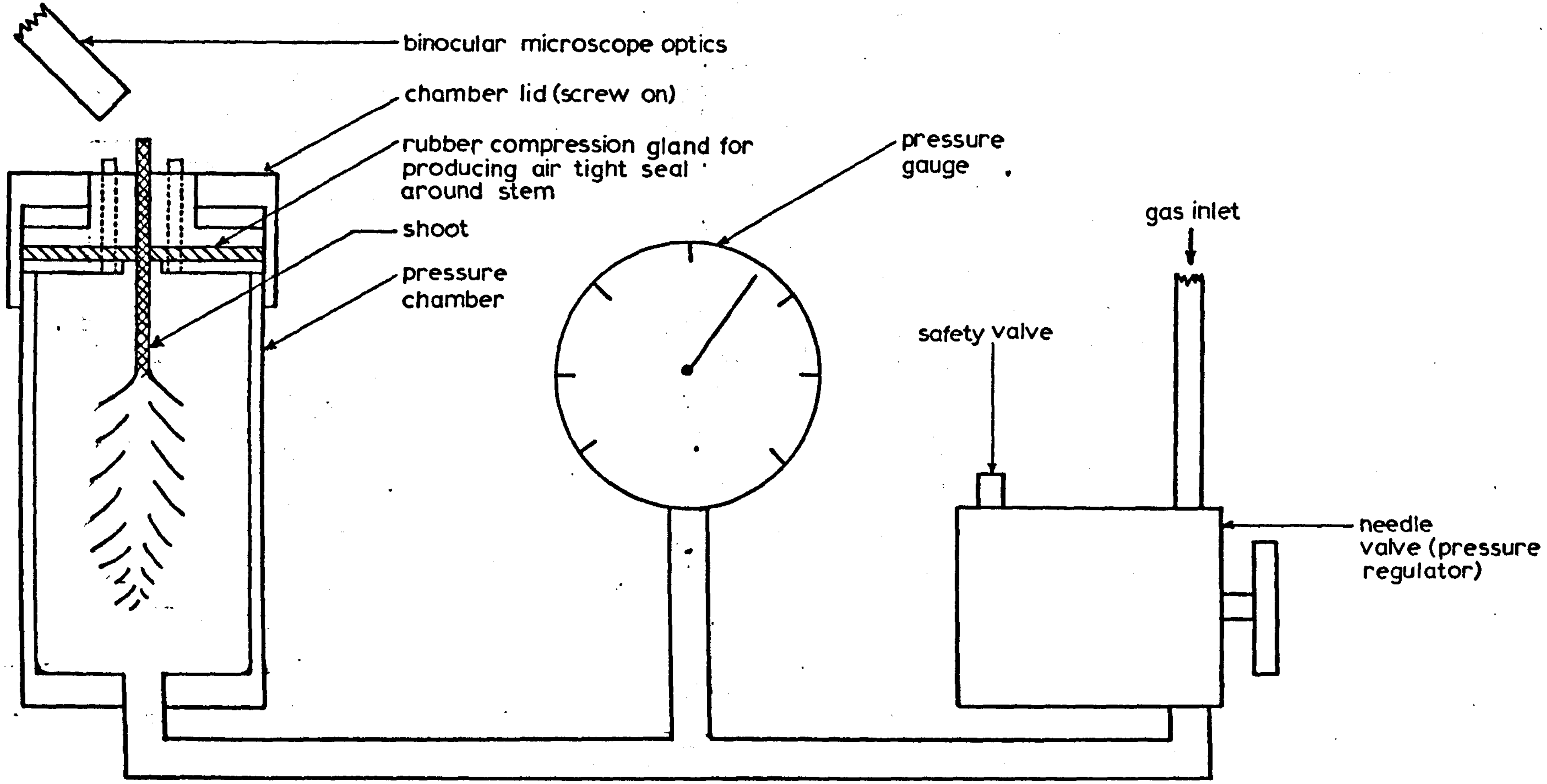
(vi) Recognition of the 'end point':

The appearance of the meniscus at the cut end of the shoot is a recognisable phenomenon, visible with a lens, although increase in conductivity has been used to indicate this point (Richter & Rottenburg, 1971). Certain problems have been encountered such as resin exudation in conifers (Kaufmann, 1968a) and bubbling of air passing out of the xylem before fluid exudation occurred (Ritchie & Hinckley, 1975).

(vii) Xylem sap osmotic and matric potentials (ψ_s^{xy}):

Boyer (1967) noted that workers often regard ψ_s^{xy} to be sufficiently small to be ignored but he found that it may exert a stress of 0.2-0.3MPa in *Rhododendron roseum* and *Taxus baccata*. Hellkvist *et al*. (1974) found this component to be greater than 0.02MPa in *Picea sitchensis* whilst Gee *et al*. (1974) concluded that since it is of such small magnitude and the xylem and cell sap π often approach equilibrium, "...the bomb reading can be used as a direct measure of ψ_1". Meiri *et al*. (1975) also assumed ψ_s^{xy} to be negligible but Ritchie & Hinckley

Figure 38 Pressure bomb apparatus used for the determination of xylem water potential.



(1975) considered, in view of Boyer's observations, that if the pressure chamber is used to estimate ψ_1 then ψ_s^{xy} should also be quantified.

(ix) The effect of internal resistances on ψ_x:

Differences in ψ between xylem and leaves may result from flow resistances between these points, although rapid equilibration between stem and leaf ψ suggest these may be small (Boyer, 1967). Tyree *et al.* (1973), however, found several hours were required for equilibration between ψ_1 and ψ_x. They suggested that rapid apoplastic ψ changes may have been the cause of Boyer's results. Tyree *et al.* (1975) found that in *Tsuga canadensis*, two thirds of the entire shoot resistance resulted from the xylem venation up to the leaves. Meiri *et al.* (1975) considered that measurements of ψ on complex organs represented the highest value for the organ rather than the average due to the resistances to water flow between stem and leaf. Leaf resistances have also been found to increase with age (e.g. Slatyer & Bierhuizen, 1964; Kaufmann, 1968b).

It is apparent that ψ measurements made with the pressure chamber are subject to limitations and errors but they provide an indication of the water stress a plant is under and may be determined with relative ease and rapidity.

(c) Methods

The pressure bomb apparatus (Figure 38) was employed with compressed nitrogen, to determine ψ_x of *C. vulgaris* shoots in the field. To avoid removing leaves and upsetting the ψ_x within the shoot, material was sampled such that the internode distance was sufficient to allow the stem to pass through the chamber top without mutilation. This required a length of 1.5cm., allowing 0.1cm. to protrude for observation. This was standardised to minimize exclusion errors. A rate of pressure increase of approximately 0.07MPa s^{-1} was employed but at low ψ's the pressure increase was 0.1 - 0.2MPa s^{-1} to within 0.5 - 1.0MPa of the estimated ψ_x, at which point the slow rate was employed.

Two approaches were adopted for measuring the appearance of the meniscus at the cut end of the shoot, direct observation using a hand lens (x 10 magnification) and the use of cobalt chloride papers. The latter method involved soaking strips of filter paper in a saturated solution of cobalt chloride which were then oven dried at 85°C. The blue strip turned pink on contact with moisture. By placing a strip over the cut end of the shoot it was possible to detect the point at which the sap meniscus reached the end of the shoot by a sudden change in colour. The method has an advantage over the optical method when one operator is involved in raising the pressure and determining the end point since the paper strip gives an instant and easily recognised colour change from a distance permitting simultaneous observation of the pressure gauge. However, several disadvantages attended the method. It was assumed that the colour change occurred with sufficient rapidity to denote the appearance of the meniscus at the cut end of the shoot, but although this may not be justified, the time lag involved should be constant for all determinations and make treatment comparisons valid. On days of high atmospheric humidity the paper strips had a tendency to lose their blue colour before the balancing pressure had been reached, especially if this pressure was high. Also, the expulsion of moisture saturated air from the xylem vessels prior to the appearance of the meniscus resulted in the colour change occurring slowly. Under such circumstances it was a matter of operator experience to determine when the true end point had been reached.

A further problem encountered in the use of the pressure chamber in the field concerned sample variation. In attempting to determine the effects of trampling treatments on ψ_x it was necessary to contend with treatment variation as well as spatial and temporal variation in ψ_x. Attempts were made to overcome the problem of temporal variation by sampling each treatment sequentially to avoid diurnal variation in ψ_x but since sampling was performed on days when environmental conditions were likely to maximise diurnal variation, possible treatment effects may have been obscured. Due to the length of time required for each determination a problem of adequate replication also arose. These problems led to the abandonment of field determinations of ψ_x in October 1975 and the reliance on the calculated relationship between RWC and ψ_x (Section IIIB, 3) since the determination of field RWC permitted greater replication.

Material was sampled from Cannock Chase block 1, (treated on 20.08.74, at 0, 50, 100 and 200 passages per $0.5m^2$ plot (p pl^{-1})) on 19.10.74, 22.10.74, 05.11.74. and 10.12.74. This was repeated for Cannock Chase block 2 (treated on 24.04.75. at 0, 50, 100 and 200 p pl^{-1}) on 21.05.75. (These blocks were laid out as Latin Square blocks, two shoots being sampled from each replicate, the mean value of which was used to give the value for each of the four replicates for each of the four treatments). In addition to the measurements made on material sampled from the main block experiments, material was sampled from plots having received 100p pl^{-1} one month before sampling and compared with control shoots sampled from the vegetation adjacent to the treated plot. There were four replicate plots for the treatment and each plot was sequentially sampled, the number of samples obtained depending on the time available for measurements. These comparisons were made between February and October 1975.

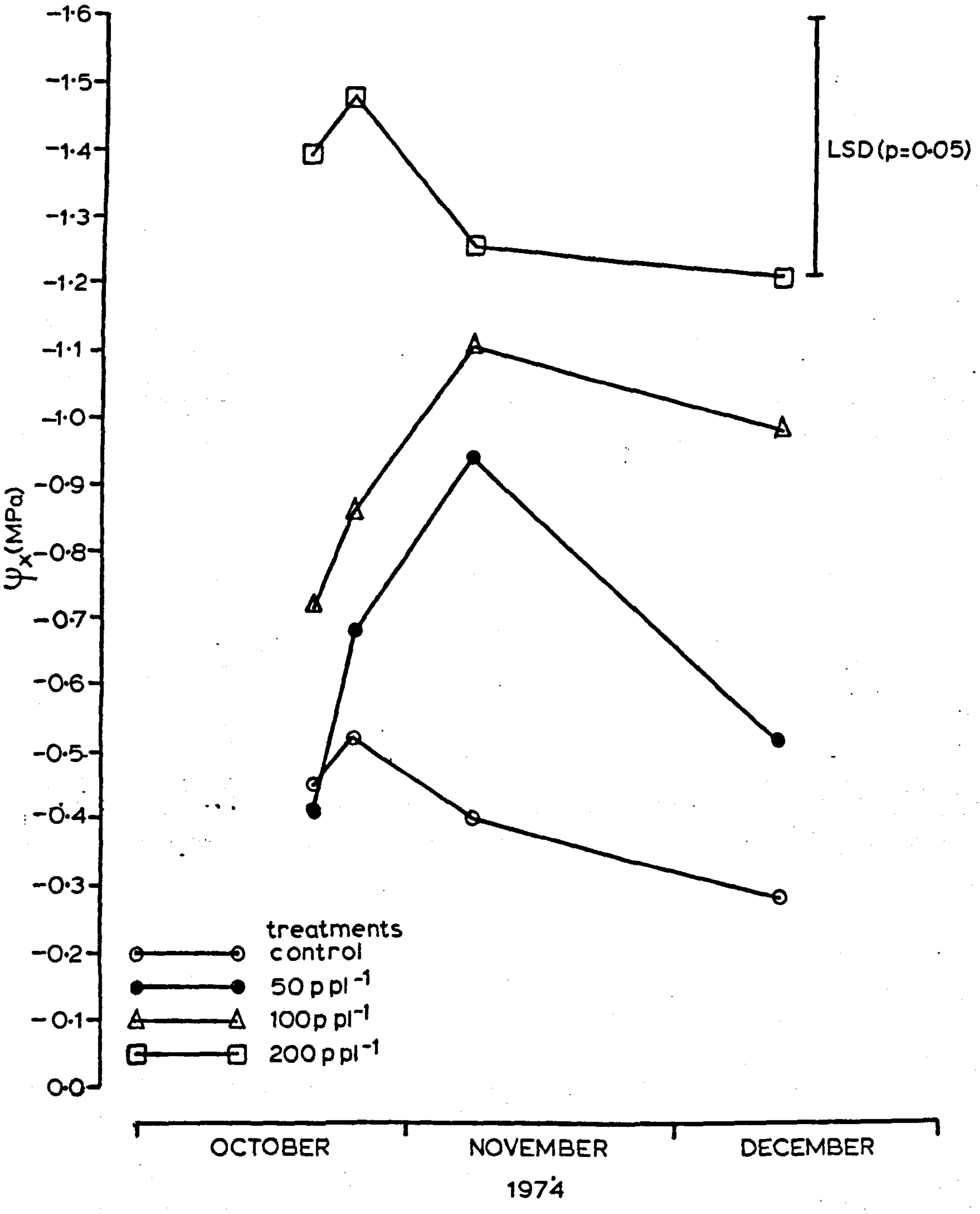


Figure 39 The effect of trampling intensity on ψ_x of <u>C. vulgaris</u> over time. (Cannock Chase).
(Treatments applied 27.08.74.)

(d) Results

(i) The effect of trampling intensity on ψ_x

The results of block 1 (Figure 39) indicate a significant ($p < 0.001$) decrease in ψ_x (i.e. more negative value indicative of higher stress) with increase in trampling pressure. For the October 1974 measurements, the only significant treatment effect is that between 200 p pl^{-1} and all other treatments. The November 1974 measurements reveal that all trampling treatments result in a significant decrease in ψ_x compared to the control whilst in December 1974 the control and lowest trampling treatment (50p pl^{-1}) result in a ψ_x significantly higher than the ψ_x associated with the two higher trampling intensities.

These measurements were made between 60 and 112 days after treatment and although a certain amount of variation is present in ψ_x over this time period there is no overall time effect on ψ_x, neither does the effect of the treatment on ψ_x vary significantly over time. The relationship between trampling intensity and ψ_x is significantly linear ($r = -0.812$, $p < 0.001$; $r = -0.706$, $p < 0.01$; $r = -0.742$, $p < 0.001$; $r = -0.888$, $p < 0.001$ for the respective sampling dates, October to December, 1974). In contrast to these negative correlations existing, a positive correlation ($r = +0.780$, $p < 0.001$) exists for the results of block 2 in which all treatments are associated with a significantly higher ψ_x than the control ($p < 0.001$) (Figure 40).

(ii) Seasonal differences in the effect of a constant trampling intensity on ψ_x

The ψ_x existing in *C. vulgaris* shoots, one month after having been treated, indicate that, with the exception of measurements made on 18.04.75., treated shoots possess a lower ψ_x than control shoots (Table 5).

In an attempt to establish if the onset of growth had an effect on ψ_x, shoots exhibiting new growth were sampled on 21.05.75. and compared to those which had not broken bud. The results indicate that shoots

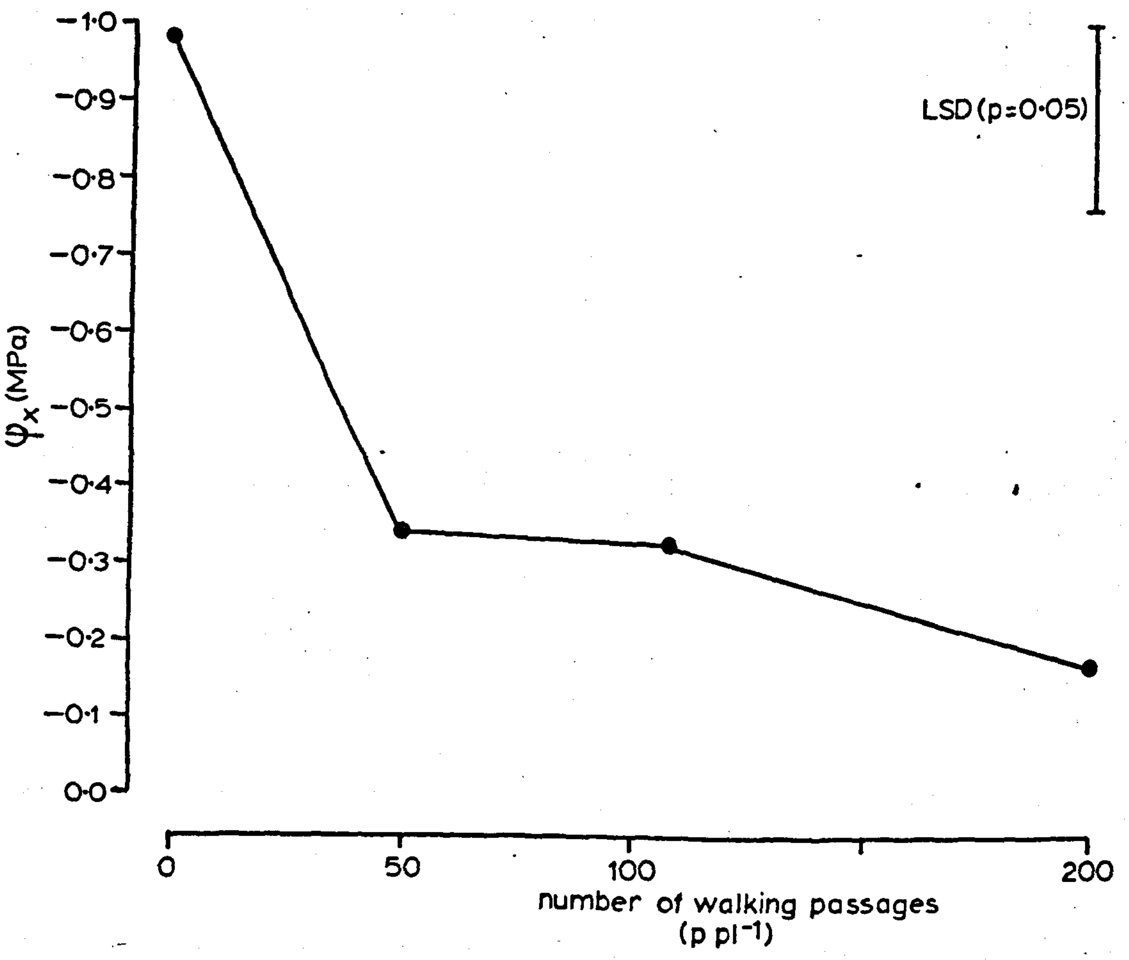


Figure 40 The effect of trampling intensity on ψ_x of C. vulgaris (Cannock Chase).
(Treated 24.04.75, measured 21.05.75)

Table 5 The ψ_x of control and trampled shoots of *C. vulgaris* treated at a trampling intensity of 100p pl^{-1}, one month prior to measurement

Treatment Date	Sampling Date	Mean control ψ_x(MPa)	Mean treated ψ_x(MPa)	N.	T.	Significance
23.01.75	21.02.75	-0.83	-1.35	16	4.026	$p < 0.01$
21.02.75	18.03.75	-1.03	-1.57	18	1.963	n.s.
18.03.75	18.04.75	-0.86	-0.23	40	12.246	$p < 0.001$
21.07.75	20.08.75	-0.74	-0.90	20	2.030	n.s.
24.09.75	24.10.75	-0.43	-0.67	22	3.429	$p < 0.01$

(N = number of samples; n.s. = not significant).

bearing new growth possessed a significantly higher ψ_x (-0.27MPa) than shoots not bearing new growth (-1.25MPa) ($p < 0.001$).

(e) Discussion

The absolute magnitude of the ψ_x induced is of little significance in the current discussion since, although it will have an effect on the plant's metabolism, this has not been quantified in the present study.

The comparatively lower ψ_x of treated shoots suggests that mechanical damage resulting from trampling has an adverse effect on the water balance of the plant.

The resistances to the passage of water from soil to air through the plant (Cowan, 1965; Richter, 1973) suggest certain possibilities accounting for the effects of trampling on ψ_x:

(1) trampling affects the soil in a manner so as to increase the resistance to water uptake (i.e. an increase in R_{sl});

(2) trampling has a directly damaging effect on the root vascular

system (i.e. an increase in R_r);

(3) trampling damages the stem vascular system (i.e. an increase in R_x);

(4) trampling damages the petiolar and leaf vascular systems (i.e. a variable effect on R_1);

(5) an alteration in canopy micro-environment leads to an increase in transpirational flux density, resulting in a decrease in ψ_x.

These proposals must remain hypothetical in the absence of experimental verification but certain tentative postulates may be suggested.

The nature of the substrate in which the plants were growing at Cannock Chase, combined with the comparatively light trampling pressures involved, suggest that soil moisture relationships (R_{sl}) and plant roots (R_r) were unlikely to be affected. Similarly, although there is some experimental justification that canopy micro-environment changes associated with trampling may alter the equilibrium between water loss and gain (Section IIIB, 2(i)), it is suggested that shoot density at the Cannock Chase site was not sufficiently high for water loss to be increased by an improvement in the evaporating conditions resulting from disruption of the plant canopy by trampling.

The results suggest that the xylem sap is under considerable tension in treated shoots, rendering the column liable to cavitation (cf. Preston, 1961; Milburn & McLaughlin, 1974). Such tensions may arise from mechanical damage to the xylem elements (Milburn & Johnson, 1966) resulting in an increase in R_x and a concomitant reduction in ψ_x to overcome the increased resistance and maintain the flow of water through the plant (Richter, 1973). Cavitation resulting from mechanical vibration is feasible when a plant is subject to trampling and may also increase R_x by reducing the cross sectional area of the functional xylem. Although Milburn & Johnson (1966) considered that sudden cavitation does not occur at some point in the development of a water stress due to the differentiation of water pathways through the stem, the occurrence of a sudden, severe water stress may induce a rapid rate of cavitation (Milburn, 1973). Milburn also observed that in Ricinus sp. stomatal opening rather than closure resulted from such stress, thus decreasing the ability of the plant to reduce the severity of the stress, thereby enhancing cavitation and increasing R_x.

The normal resistances to water loss are the resistances offered by the leaf boundary layer, stomatal and cuticular diffusive resistance, substomatal and cell wall resistances (Cowan & Milthorpe, 1968). In addition to the possible decreased effectiveness of the stomata in controlling water loss, the cuticular diffusion resistances, normally in the order of 50s cm^{-1} and thirty to fifty times greater than the minimal stomatal resistances (Cowan & Milthorpe, 1968) may be reduced by bruising, tearing and abrasion of the cuticle (Section IIIA, 2) so water loss over which the plant has little control may occur until the internal stress within the plant is equivalent to the atmospheric stress providing the gradient down which water vapour leaves the plant.

Although no significant trends over time are found on block 1, there is a consistent decrease of -0.86 to -0.96MPa between the ψ_x of the control and that of shoots trampled at 200ppl^{-1}, thus the decrease in ψ_x of the control shoots on 22.10.74. is paralleled by a decrease in ψ_x of shoots from the most heavily pressurized plots, similarly, the increase in ψ_x in November and December 1974 is paralleled by an increase in the ψ_x from the treated plots (200p pl^{-1}). Although variations in the control ψ_x are not precisely paralleled by the ψ_x of shoots from the intermediate treatments, the trend suggests that a dynamic equilibrium has been established between supply and loss of water which varies, in accordance with environmental conditions, around a value that is dependent upon the increase in R_x induced by trampling damage.

The linear relationships between ψ_x and treatment suggest that the possible increase in R_x resulting from crushing and breaking of vessels and bending of the stem resulting in cavitation and displacement of vessel and walls (Preston, 1961; Milburn & Johnson, 1966) may increase proportionally with increased trampling intensity. Ultimately a saturation of the response might be expected such that with increased trampling pressure no further decrease in ψ_x occurs, although this is not evident in the results.

The data for block 2 conflict with these postulates. The low ψ_x's of control shoots measured on 21.05.75 on block 2 suggest conditions favourable for the development of low ψ_x. Alternatively, it may reflect increased physiological activity in the plant at the beginning of the growing season in which increased net photosynthesis (Section IVA, d(ii)) is necessarily accompanied by increased water loss. The control values appear abnormally low compared to the ψ_x for control shoots at other times of the year and may reflect the reduced cross sectional area of the xylem available for supplying water to a shoot of increasing metabolic activity compared to the previous season's situation, due to the blockage, either physically or by cavitation, of some of the xylem vessels over the winter, thereby increasing R_x and resulting in a decrease in ψ_x to meet the water requirements and losses by the re-activated leaves. This phenomenon was observed in April and May 1975 but shoots which had actually broken bud had a higher ψ_x than control shoots not having broken bud. This may reflect the extreme sensitivity of cell elongation to water stress (Hsiao, 1973; Hsiao _et al._, 1976) such that shoots which have broken bud have increased the functional xylem content possibly by cambial activity, thus lowering R_x and reducing the water potential gradient necessary to transport water from the roots to the leaves. It is of note that the comparatively high ψ_x of the control plant in May 1975 corresponds to a comparatively high desiccation tolerance which may reflect physiological inactivity (Section IIIA, 1). A decrease in π may result from increased soluble sugar concentrations in the vacuolar cell sap which may be manifested in a decreased plant ψ (cf. Slatyer, 1967) whilst increased soluble sugar concentrations may be associated with desiccation tolerance (Santarius, 1973). It is evident that the phsyiological activity of the plant, as reflected in the development of new growth may have an appreciable affect on ψ_x.

The situation is further complicated by the higher ψ_x of treated shoots compared to the control in contrast to the situation existing at the end of the 1974 growing season. The time interval between treatment and measurement may account for this (i.e. 27 days for block 2 compared to 60 days for block 1). Alternatively, the depletion of soluble sugars in damaged plants may result in an increase in π and a possible increase in ψ. Grace & Woolhouse (1970) observed a decrease in soluble sugars during the growing season. The use of such carbohydrate reserves may be a contributory factor to the high ψ_x of shoots bearing new growth. In addition, a decrease in carbohydrate reserves may occur in damaged shoots, possibly as a result of respiratory depletion and the inhibition of photosynthesis as a result of severe water deficits causing stomatal closure. This may lead to an increase in π and ψ_x but such a proposition must remain hypothetical in the absence of measurements of π on control and treated tissues. The proposal requires, for its validity, a sufficient supply of water to the leaves to satisfy the decrease in π resulting from increased solute concentration which is a consequence of dehydration. This may be provided by root pressure and capillarity rather than 'transpiration pull'.

Tissue death may also alter the relationship between cross sectional area of the xylem, water demand and ψ_x (Equation 14). If the area of functional xylem elements remains unaltered but demand is reduced because of tissue death, the ψ gradient required to generate a rate of flow sufficient to satisfy the demands of the remaining living tissue will be less and may be manifested in an increase in ψ_x. It is also possible that the ψ_x response time to changes in water demand and loss from the leaf may be affected by trampling. Sheriff & Sinclair (1973) observed that when xylem sap columns are disrupted, large time lags in ψ gradients occur. Control shoots may therefore respond more rapidly to environmental changes than treated shoots. However, these arguments apply equally to shoots sampled at the end of 1974 when the opposite effect was observed. A mechanistic explanation of the differential response remains obscure.

2. Relative Water Content (RWC) as an indicator of plant water stress

(a) Methodology

The water content of tissue can be given by the difference between fresh weight and dry weight of the sample but comparisons between and within a species are facilitated by a common basis of expression.

Some workers e.g. Tranquillini (1963) have expressed water content as a percentage of the dry weight, but diurnal and long term fluctuations in this latter quantity may invalidate its use as a basis of expression (cf. Weatherley, 1950). Fresh weight is also an unsatisfactory basis of expression since not only is it more variable than dry weight but the extent of changes in actual water content will be minimized, the lower the water content compared to full saturation.

The most valid basis for the expression of water content has been the fully saturated weight (Stocker, 1929). The method involves weighing the shoot immediately upon severance from the plant (fresh weight (FW)), saturating it by standing it in water to obtain the turgid or saturated weight (SW) and finally obtaining the oven dry weight (DW). The 'Wasser-defizit' (water deficit) (Stocker, 1929) may be given by:

$$WD = \frac{SW - FW}{SW - DW} \times 100 \qquad (15)$$

This quantity is referred to as the water saturation deficit (w.s.d.) by some, e.g. Larcher (1965), Slavik (1965), but the term 'water deficit' has also been applied to quantities derived using different formulae (Barrs, 1968).

Weatherley (1950) reappraised the determination of plant water content. Using leaf discs cut from the leaves of <u>Gossypium hirsutum</u>, the discs were floated on distilled water, after the initial weighing, for twenty four hours in darkness or diffuse light, after which they were blotted dry, weighed to give SW, and finally oven dried. Three phases of water uptake were observed, an initially rapid uptake in response to the water stress, a slower phase in which full turgidity was attained and a final slow, steady uptake ascribed to growth. The relative turgidity (RT) (Weatherley, 1950) was obtained by:

$$RT = \frac{FW - DW}{SW - DW} \times 100 \qquad (16)$$

This quantity is related to the water deficit by:

$$WD = 100 - RT \qquad (17)$$

As with the quantity 'water deficit', the quantity 'relative turgidity' has been extensively used but has similarly been corrupted. It has also been criticized on the grounds that it implies a relationship with turgor pressure which may not exist (Slatyer & Barrs, 1965).

Weatherley (1965) has accepted relative water content (RWC) as a preferable term for the quantity given by Equation 15 but although used by some workers, e.g. Ehlig & Gardner (1964), Jarvis & Slatyer (1966), care must be exercised since Jarvis & Jarvis (1965) defined RWC as the field water content divided by the dry weight of the tissue as a percentage of the control water content.

Errors in determining the RWC of plant tissue

(i) The determination of the saturated weight

The phases of water uptake by water stressed tissue noted by Weatherley (1950) have been observed by others, e.g. Barrs & Weatherley (1962), Catsky (1965a). Barrs & Weatherley (1962) suggested that the two distinct phases of rapid and slow uptake be known as Phase I and Phase II. Barrs (1968) noted that discs and whole leaves exhibit both phases whilst Slatyer & Barrs (1965) observed that water uptake continued for the duration of the floating period. The use of metabolic inhibitors (Barrs & Weatherley, 1962) substantiated the postulate of Weatherley (1950) that Phase II uptake was due to growth, and it was suggested that the best time to obtain the saturated weight was at the point where Phase I was satisfied and Phase II had made no contribution to plant weight, on the assumption that Phases I and II were consecutive. However, Yemm & Willis (1954) and Catsky (1959) suggested that Phase II was operative from the moment the discs were placed on water, but were unable to provide experimental justification of the postulate. Yemm & Willis (1954) used a mathematical method of eliminating the Phase II contribution whilst Catsky (1959) suggested a floating period of three hours after which Phase I would be completed and Phase II would not be making a significant contribution. Hellkvist (1973) found such saturation times for woody shoots to be insufficient for them to achieve full saturation and extended the period to twenty-four hours. Molz, Truelove & Peterson (1975) have suggested that a dynamic equilibrium exists between Phase I and Phase II uptake. The gradient along which water diffuses is controlled by both phases but initially the contribution of the latter is small. As Phase I is satisfied so the relative contribution of Phase II increases; thus, in relative terms, the contribution of Phase II increases in magnitude to become maximal at the completion of Phase I uptake.

The floating disc technique assumes that water does not enter intercellular spaces and Weatherley (1950) concluded, from experimental evidence, that such 'injection' errors were negligible in contrast to the findings of Ashby & Wolf (1947). Barrs & Weatherley (1962) using discs of two sizes, cut from Ricinus sp. and Sambucus sp., concluded that injection errors could be minimized by reducing the circumference/area ratio, since greater infiltration was observed the smaller the disc. Spomer (1972) corroborated this by finding that different water content values for the same leaf could be obtained with discs of different sizes. Injection errors are not a consistently observed phenomenon; Rutter & Sands (1958) found they were negligible in Pinus sylvestris, whilst Carr & Gaff (1962) found a small but consistent error which could be allowed for. Hewlett & Kramer (1963), however, considered that injection and infiltration led to excessive water uptake by leaf discs. Catsky (1960) regarded the error to be sufficiently serious to merit devising an alternative method, in which polyethylene foam rings were employed, to enable uptake of water by the disc through the cut edges.

The effect of temperature on water uptake has been reviewed by Milburn & Weatherley (1971). Certain workers, e.g. Millar (1966) found that saturation levels varied with temperature. Barrs & Weatherley (1962) investigated the possibility of eliminating Phase II uptake by the use of low temperature ($3^{o}C$) but differential species responses invalidated the method. Milburn & Weatherley (1971) found that an induced water deficit in Ricinus sp. was satisfied by water uptake at 1°C. They found that response to prolonged water deficits could be separated into two fractions by temperature, a recovery of turgidity and a residual uptake in response to delayed cell expansion resulting from inhibited growth during the imposed stress (cf. Hsiao, 1973), the latter being temperature sensitive. In contrast to Weatherley's (1963) suggestion that the low and high temperature fractions of the saturation curve are due to satisfaction of deficits in the apoplastic and symplastic systems respectively, the low temperature phase may be regarded as satisfying the true water deficit whilst the second phase satisfies the residual growth effect resulting from prolonged stress. Although Barrs & Weatherley (1962) suggested that the temperature effects on growth alone were insufficient to account for the temperature effect on turgid water content of leaf discs, Milburn & Weatherley (1971) argued that uptake resulting from arrested growth could be of considerable magnitude, with possible effects on the determination of RWC.

Millar (1966) suggested that a flotation temperature identical to that at which the plants were grown could overcome the problem but this would only be applicable to plants grown under defined conditions. Low temperature flotation might be more applicable since this reduces the growth phase fraction, especially when leaves of varying ages are being compared. However, Milburn & Weatherley (1971) suggested that more extensive studies are required before the technique of determining RWC is modified.

The blotting dry of material after saturation may also introduce considerable error (Ashby & Wolf, 1947). Leaves which are hirsute or which have large venation may aggravate the problem and care must be exercised to prevent 'overdrying' (Slatyer & Barrs, 1965). These workers adopted a standard procedure of sandwiching the leaf discs between filter papers and applying a standard weight (500g.) for one minute.

Osmotic potential (π) of the cell contents may also result in error. Weatherley (1950) stated that although the ψ of two samples may be equivalent if the π's are different they will absorb different quantities of water to attain full turgidity. If a decrease in π led to a permanent decrease in ψ, more water would be absorbed, with a concomitant effect on the RWC.

Cell wall extensibility may also affect water uptake. Weatherley (1950) found that similar RWC's were obtained from plants of different ages under a standard environment but he did not exclude the possible effects of cell wall extensibility leading to an error in the determination of SW.

(ii) The determination of fresh weight

The major source of error is due to surface water present on the tissue at the time of collecting. Comparison of dew laden leaves with the same leaves blotted dry resulted in an increase of 1.4-3.1% in RWC (Weatherley, 1950) but Slatyer & Barrs (1965) suggested that absorbed dew represents an actual contribution to the water status of the plant.

If surface moisture is not removed before weighing, considerable error may result and although Weatherley (1950) considered this to be a slow and laborious process, Slatyer & Barrs (1965) found that drying with absorbent tissue was rapid, non-damaging and gave values which did not depart significantly from material dried in the standard method used for saturated weight determinations.

(iii) The determination of dry weight

If flotation periods are in excess of a few hours respiratory loss of dry weight may ensue. Weatherley (1950) used duplicate samples to overcome this. Mathematical methods were used to relate the dry weight of the samples dried immediately to those dried after saturation.

It is also feasible to assume a relatively constant dry:fresh weight ratio thus:

$$DW_a = \frac{DW_b \times FW_a}{FW_b} \qquad (18)$$

$$RWC = \frac{FW_a - DW_a}{SW_a - DW_a} \times 100 \qquad (19)$$

where samples with the suffix (a) are used for determining the saturated weight and samples with the suffix (b) are oven dried immediately after sampling.

Barrs & Weatherley (1962) postulated that an imbalance between photosynthetic gain and respiratory loss resulted in a dry weight loss. By exposing floating Ricinus sp. discs to varying light intensities, the light compensation point was found at which neither loss nor gain in weight occurred. By floating discs for twenty four hours at this light intensity, the error resulting from dry weight change was reduced. By reducing the floating period to four hours, the error became inconsequential. Catsky (1965a) found that dry weight losses over a flotation period of six hours were not significant. However, dry weight losses in whole leaves, where saturation times are longer, may be more serious and more difficult to allow for.

(iv) Miscellaneous errors

Milthorpe & Spencer (1957) suggested that upon wilting, an irreversible contraction in the cell wall may occur leading to an error in the determination of the RWC. Barrs & Weatherley (1962) were sceptical of this notion and considered that changes in cell walls represented a form of 'recovery'. Experiments on Ricinus sp. indicated cell wall contraction to be an important source of error but Barrs & Weatherley suggested sampling only mature leaves owing to the possible effects of 'plastic flow' of cell walls in young tissue.

Gaff & Carr (1961) postulated that up to 40% of the water content at full saturation was located in the cell walls. This is able to buffer the protoplast from short term dehydration effects since much of it can be lost without compensation from the symplast. It was therefore argued that RWC is not valid for estimating protoplast water deficit. Studies by Slatyer (1955) and Catsky (1965a) indicated little buffering effect of the symplast by the apoplastic water content. Slatyer & Barrs (1965) considered it difficult to conceive of changes in cell wall water content without compensating changes in the symplastic water content since drainage of the cell walls would generate a considerable water potential gradient from symplast to apoplast. The relationship between RWC and ψ also suggests symplastic water is in equilibrium with apoplastic water.

(v) Leaf discs versus whole leaves

Hewlett & Kramer (1963) stated that leaf discs take up comparatively more water than whole leaves, giving rise to erroneously low RWC's. However, RWC's in the region 98-100% have been obtained using discs (Weatherley, 1951; Slatyer, 1955) whilst Catsky (1965a) found agreement in the RWC obtained using discs and leaves.

Use of shoots in determining RWC

With some species it is impractical to determine the RWC of leaves due to their small size e.g. Calluna vulgaris, consequently, shoots may be used. Bannister (1964b) used shoots of C. vulgaris consisting of the current season's growth except early in the growing season when shoots also bore previous season's growth. Bannister noted that it was not possible to achieve the same homogeneity with shoots as with leaf discs, consequently the finer points of Weatherley's technique were not applicable.

There is a certain historical precedent in the use of shoots. Thoday (1921) used whole shoots of Passerina sp. to compare the ratio of water content to fresh/dry weight ratio. Runyon (1936) used shoots of the creosote bush to determine water deficits and Killian (1947) saturated shoots collected in the field by placing them in water for forty eight hours. Weatherley (1950) criticized the use of whole shoots on the grounds that the associated errors would obscure variations in RWC of smaller magnitude than found in the desert plants used by the above workers. However, Bannister (1964b) was able to demonstrate diurnal variation in RWC of three heath species using the shoot method.

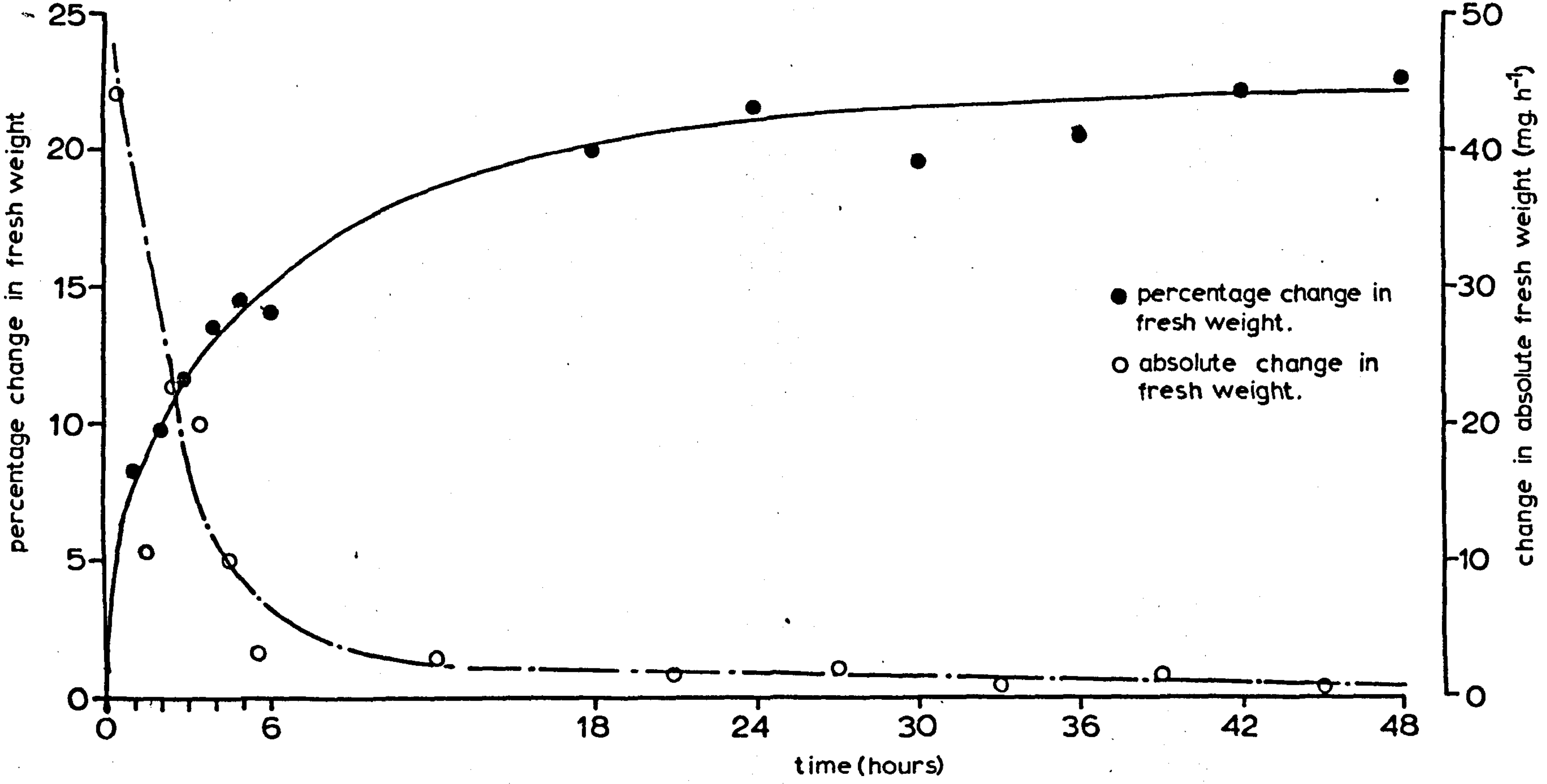
(b) Methods

Shoot RWC is used in the present study to determine desiccation tolerance, stomatal closure point (Section IIIA) and field water stress of Calluna vulgaris.

Shoots sampled in the field consisted of current and current plus previous season's growth. The shoot was immediately weighed in the field on a 500mg. torsion balance (White Electric Inst. Co. Ltd.) which was used for all subsequent weighings. After weighing, the shoot was placed in a stoppered polythene tube (8cm. x 4cm.) to prevent shoot desiccation on transport to the laboratory. (Bannister (1964b) noted that samples stored for 24 hours without water gave reasonable estimates of field RWC).

Upon return to the laboratory distilled water was placed in the tube to a depth of approximately 1cm., the tubes re-stoppered and placed in the dark at 25°C. After 24 hours the shoots were removed, blotted dry between absorbant tissues using hand pressure to remove surplus water.

Figure 41 Change in fresh weight over time of water stressed, severed shoots of <u>Calluna vulgaris</u>, standing in distilled water.



After weighing the shoots were oven dried at 85°C for 48 hours and reweighed. The RWC was calculated according to Equation 16.

Standardised saturating conditions were employed since deviations from such conditions may result in erroneous values of RWC (Bannister, 1964b). Field sampling time was standardized to between 1100 hours and 1200 hours to avoid diurnal variation masking seasonal variation in RWC.

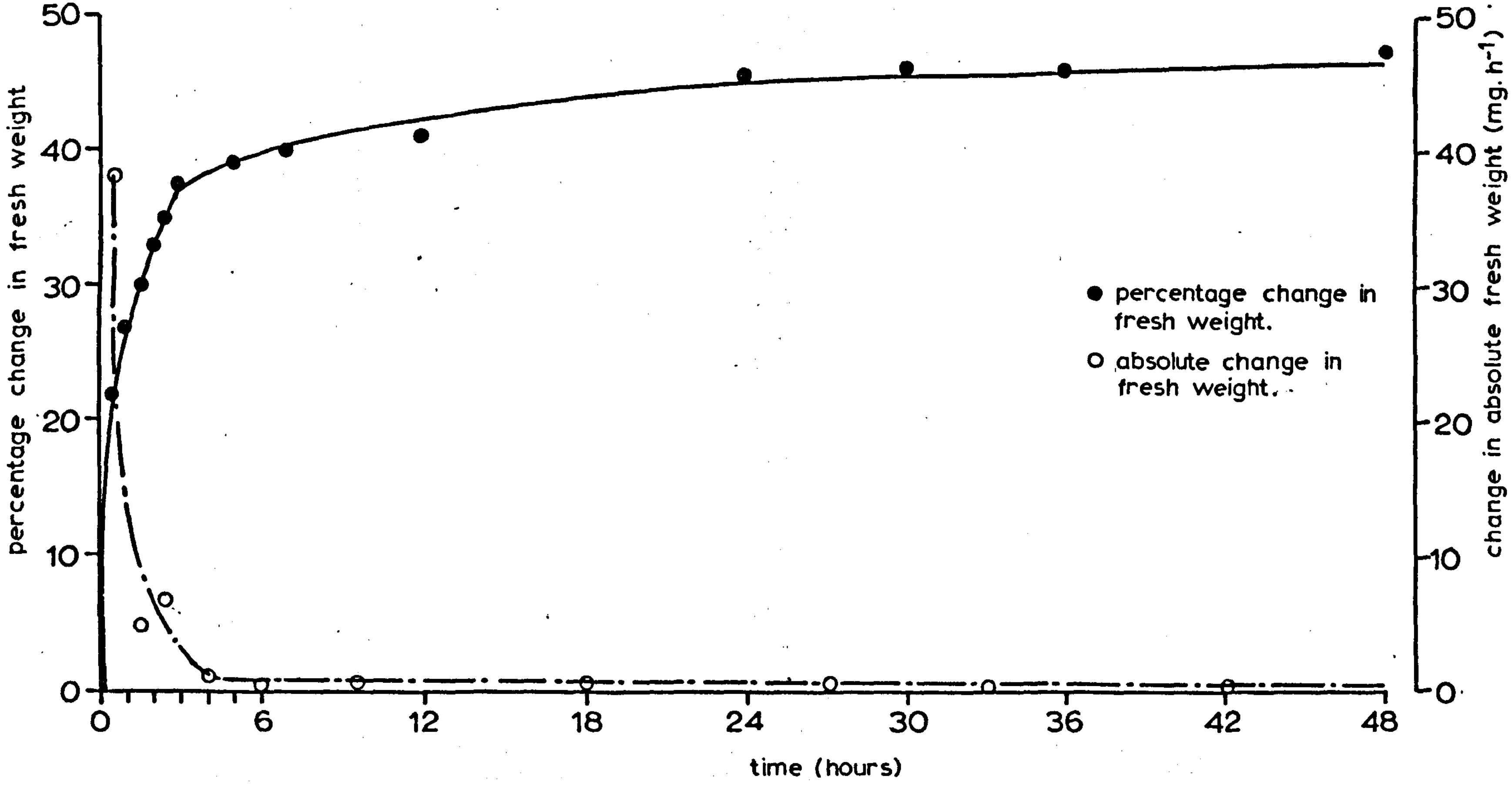
Determination of time required for material to achieve full saturation

To determine the period required for shoots to become fully saturated a water deficit was induced in ten shoots which were then placed in stoppered bottles, containing distilled water, in the dark at 25°C. Each shoot was removed, blotted dry and weighed every hour for the initial 6 hours and thereafter 18h., 24h., 30h., 36h., 42h. and 48h. after the shoots had been first stood in water.

Changes in fresh weight (Figure 41) indicate that an initially high rate of water uptake i.e. Phase I, gives way to a slower rate after approximately 5 hours, and this gives way to a constant rate of uptake after 10 hours, i.e. Phase II. Although it is evident that Phase I uptake is satisfied after 5-10 hours, it was decided, for convenience, to standardize the time to saturation at 24 hours.

A similar experiment was performed to determine the saturation time for leaf discs of *Plantago lanceolata*. A cork borer was used to punch 0.8cm. discs from the leaves. There were three replicates consisting of 10 discs each, which, after the initial weighing, were floated on distilled water in petri dishes in the dark at 20°C. Change in weight was followed from 0.5-48 hours after the start of the experiment. At each weighing the discs were removed, placed between two pads, made up of five filter papers, with a 500g. weight placed on top for one minute,

Figure 42 Change in fresh weight over time of 8 mm. diameter discs cut from water stressed leaves of *Plantago lanceolata*, floating on distilled water at 20°C in the dark.



to dry them and weighed. A clear inflexion in the rate of water uptake (Figure 42) occurred after 3-4 hours. In subsequent experiments 4 hours was allowed to full saturation as this would probably not result in serious dry weight losses for which correction would have to be made.

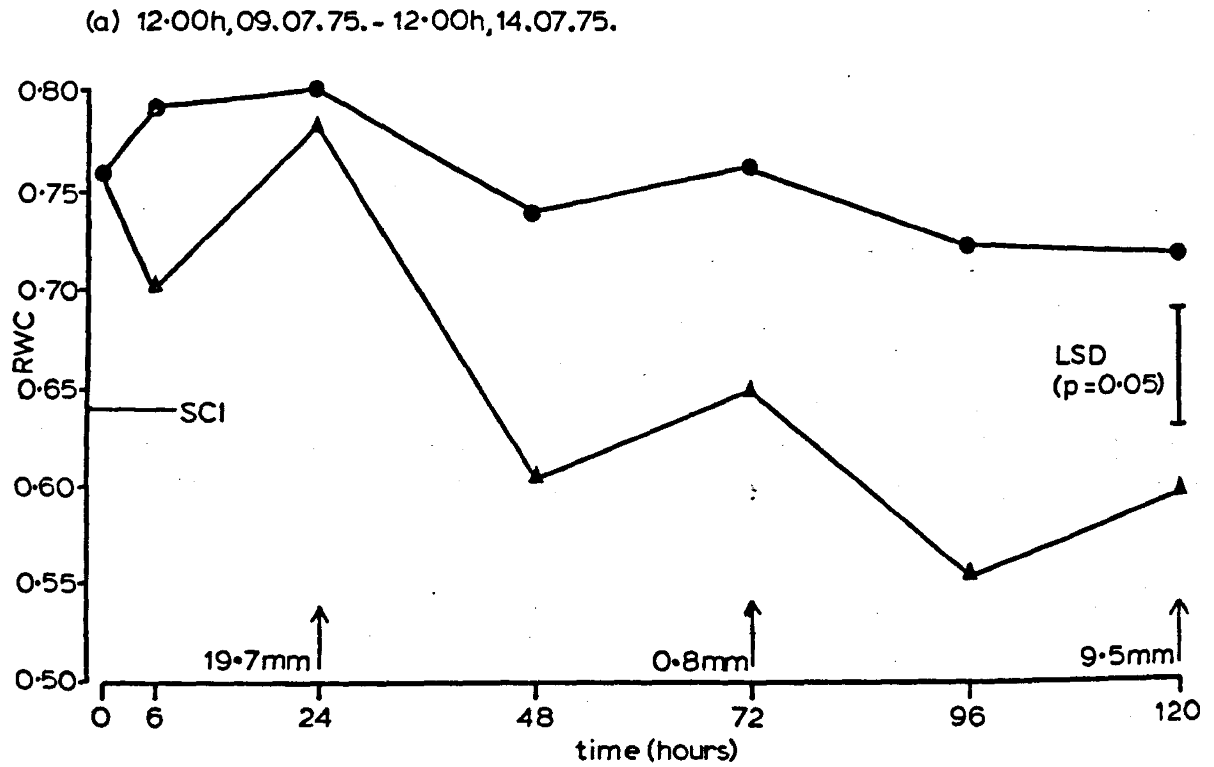
(i) The development of water deficits in *C. vulgaris* following trampling:

(a) Methods

Plots (2m. x 0.5m.) of even aged *C. vulgaris* at Cannock Chase were subjected to a trampling pressure of 100 walking passages, applied at one moment in time. Four replicate plots were treated on a particular treatment date. Five shoots per plot (i.e. twenty shoots per treatment) were sampled up to ten days following the application of the treatment. Twenty control shoots were sampled from the vegetation adjacent tp the trampled plots. The RWC of sampled shoots was determined according to the method described in Section IIIB, 2(b).

A short term trampling experiment was performed at the Kerloch site to determine the rapidity with which water deficits develop in *C. vulgaris* following trampling. Four trampled plots (2m. x 0.5m.), separated by four control plots (2m. x 0.5m.), were treated, following an initial determination of shoot RWC at 1200 hours (09.07.75), at a pressure of 100 walking passages. A second sample, consisting of five shoots from each of the four replicate plots for each treatment (i.e. trampled and control), was obtained at 1800 hours on the same day. Subsequent samples were obtained, at 24 hourly intervals, at 1200 hours, for a total of 120 hours after the initial sampling.

The above experiment was repeated on 04.04.76. Following an initial sampling at 1200 hours, the plots were treated in the same manner and shoots sampled hourly for the succeeding 5 hours, and then at 24 hourly intervals at 1200 hours each day for 72 hours. The effect of increased



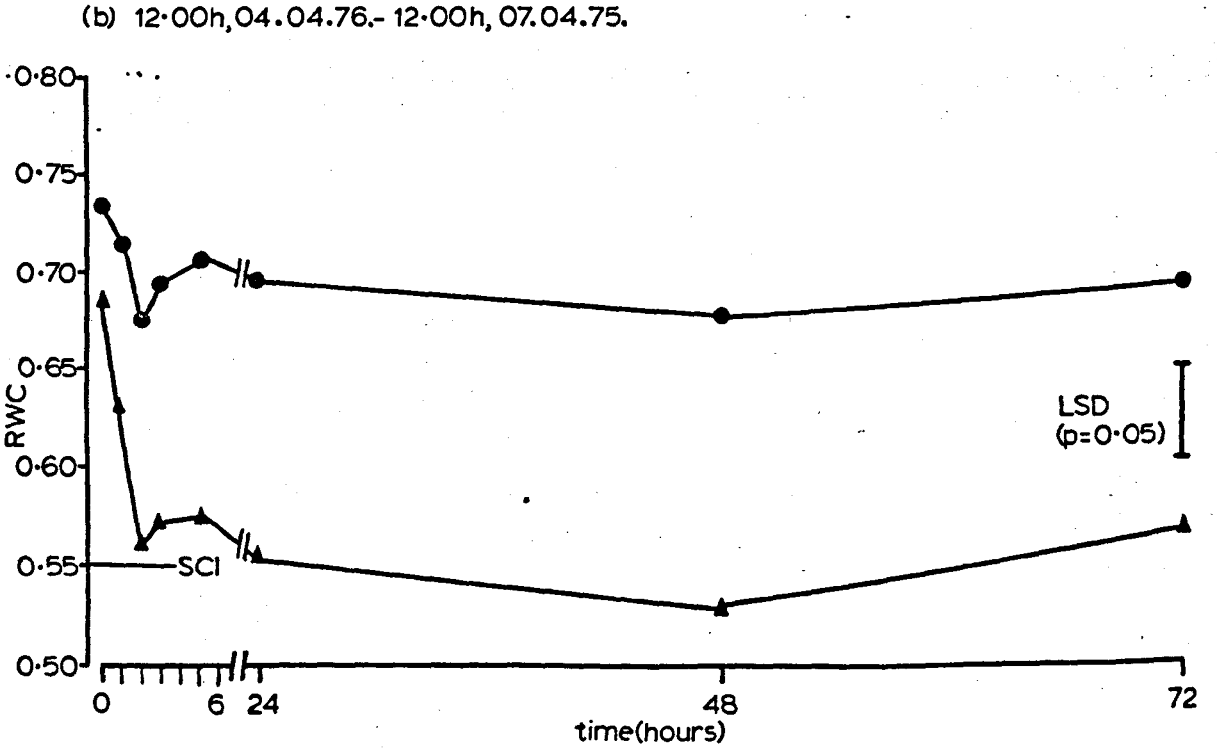


Figure 43 Development of Water Deficits in C. vulgaris shoots after trampling (100 p pl^{-1}) at 0 hours, (Kerloch).

(upward arrows, ↑, indicate rainfall for the previous 24 hours)
(SCI - stomatal closure point, intersection method)

evaporation potential on shoot RWC as a consequence of the destruction of the canopy was ascertained by removing the vegetation in a one metre wide strip surrounding four plots (2m. x 0.5m.). Shoots were sampled from the edges of the plots, i.e. the region adjacent to the cleared vegetation, where the evaporative potential might be expected to be greater due to the reduced canopy resistance to air movement compared to the control plots.

A simple estimate of potential evaporation on trampled and control plots at the Kerloch site was made. 8cm. long pipe cleaners were saturated, blotted dry to remove excess moisture, weighed and placed on wire stands randomly positioned in the vegetation at a uniform height corresponding to the mid point of the leaf height in the canopy of the control plots. At the end of 60 minutes the pipe cleaners were reweighed and the moisture loss calculated. During the course of the 60 minute period humidity and wind velocity at canopy height were determined.

(b) Results

The results of the Cannock Chase study (Table 6) indicate that trampled shoots have a consistently significantly lower RWC than control shoots.

The varying number of days that the shoots were sampled after trampling reflect access to the site but the results indicate that significantly higher water deficits are found in trampled plants within a short period of damage being inflicted.

The results of the Kerloch plots, treated 09.07.75, (Figure 43a) indicate that a significant ($p < 0.001$) reduction in RWC of trampled shoots occurs within 6 hours of treatment. No significant differences in the RWC of control shoots occurs over the 120 hour measuring period, with a mean response of 0.7623 ± 0.0107. In contrast, the RWC of

Table 6 The development of water deficits in shoots of *C. vulgaris* following trampling at a pressure of 100p pl^{-1} (Cannock Chase)

Date of treatment	RWC (control)	RWC (trampled)	No. of days after treatment	T	Sign.
23.04.75	0.7267	0.6015	4	6.128	<0.001
21.05.75	0.6659	0.5177	5	9.721	<0.001
24.07.75	0.7441	0.5605	3	7.236	<0.001
31.07.75	0.6742	0.4913	10	8.034	<0.001
27.08.75	0.6763	0.5434	7	6.079	<0.001

(N = 38)

trampled shoots decreases from a maximum of 0.7712 to a minimum of 0.5558. The greater variation in the RWC of trampled shoots is reflected in the greater standard error associated with the lower value for the mean response, 0.6718 ± 0.0328. There is a tendency for the difference between the RWC of control and treated shoots to increase over time from a difference of 0.0908 after 6 hours to 0.1190 at 120 hours, but this trend is obscured by the occurrence of rainfall over the experimental period. The control shoots appear to be unaffected by precipitation, although a slight tendency for an increase in RWC is evident. The treated shoots exhibit a marked response in relation to the incidence of rainfall although the increase is only significant in response to the 19.7mm. of rainfall received up to 24 hours after the initiation of the experiment. The differential response of control and treated shoots over time, as reflected in a significant time x treatment interaction ($p < 0.001$) may be a reflection of the rainfall response noted above.

The findings presented above are corroborated by the experiment performed on 04.04.76. (Figure 43b). A substantially lower RWC of treated shoots develops within one hour of the treatment being applied, which becomes significant ($p < 0.001$) within two hours. Over the following 70 hours the difference between the RWC of control and treated shoots remains at a relatively constant level (0.1346 ± 0.0041); this is reflected in the small variation in RWC of control and damaged shoots between 3 and 72 hours after trampling, i.e. 0.6926 ± 0.0051 and 0.5579 ± 0.0082 respectively.

A significant time effect is observed in the first two hours of the experimental period, after which no significant variation in RWC over time occurs. The RWC of control shoots decreases to a minimum at 1400 hours, thereafter exhibiting a recovery from 0.6762 to 0.7050 at 1700 hours. In contrast, the recovery exhibited by treated shoots is slight.

The effect of increasing evaporative potential, by increased exposure, results in a lowering of shoot RWC compared to control shoots taken from an undisturbed canopy (Table 7).

Table 7 The effect of increasing evaporation potential, by destruction of the canopy, on RWC of *C. vulgaris* (Kerloch)

Day	RWC (control)	RWC (trampled)	RWC of shoots from edge of de-vegetated strips
1	0.6741	0.5273	0.6292
2	0.6908	0.5642	0.6529
3	0.7343	0.6337	0.6976
mean response	0.6997	0.5751	0.6599

(L.S.D. = 0.0491)

Although significant ($p < 0.001$) treatment effects exist, these are

Table 8. Percentage moisture loss of pipe cleaners over 60 minutes indicative of potential evaporation rate (Kerloch)

(a) Potential evaporation data for the period 11.07.75 to 13.07.75

Date	Control plot	Trampled plot	Significance of difference	Temperature	Humidity	Windspeed
11.07.75	26.5	29.7	<0.01	19.6°C	74.7%	(c) 0.53m s^{-1} (t) 1.05m s^{-1}
12.07.75 (am)	6.4	9.8	<0.001	14.6°C	88.3%	(c) 0.10m s^{-1} (t) 0.12m s^{-1}
12.07.75 (am)	8.4	14.2	<0.001	15.6°C	85.7%	(c) 0.06m s^{-1} (t) 0.24m s^{-1}
13.07.75	5.5	9.2	<0.001	15.1°C	91.0%	(c) 0.27m s^{-1} (t) 0.72m s^{-1}

(b) Correlation coefficients between environmental variables and potential evaporation (11.07.75 to 13.07.75, Kerloch)

Variable		r	Significance
c	humidity	- 0.982	< 0.05
t	humidity	- 0.991	< 0.01
c	temperature	0.991	< 0.01
t	temperature	0.990	< 0.01
c	windspeed	0.854	n.s.
t	windspeed	0.718	n.s.
t + c	windspeed	0.729	< 0.05

c - percentage moisture loss, control plots (n = 4)

t - percentage moisture loss, trampled plots (n = 4)

(c) Potential evaporation data for the period 05.04.76 to 07.04.76

Date	Control plot	Trampled plot	Significance of difference	Temperature	Humidity	Windspeed
05.04.76	34.1	37.3	n.s.	12.8°C	60%	2.19m s^{-1}
06.04.76	48.8	59.3	<0.001	6.7°C	60%	1.52m s^{-1}
07.04.76	43.8	52.9	<0.001	7.8°C	50%	1.88m s^{-1}

primarily between control and trampled shoots. Shoots from the edges of de-vegetated strips, although having a lower RWC than control shoots, only differ significantly from shoots from trampled plots.

The potential evaporation rate (analysed using two way analysis of variance with unequal replicates[1]) was significantly greater in plots which had been subjected to the trampling treatment on both experimental occasions (Table 8a, c). The potential evaporation rate also differed significantly ($p < 0.001$) on different days, similarly on both occasions on control and treated plots. The associated environmental measurements (temperature, humidity and windspeed) may help to explain the significant variation in evaporation rate over time. Humidity and temperature were measured in control plots only whilst windspeed was measured, at the same height, in control (c) and treated plots (t) for the period 11.07.75. to 13.07.75. The windspeed data for July 1975 are consistently greater in treated than in control plots which may be associated with the greater moisture loss rates. Linear correlation coefficients calculated between these environmental parameters and the percentage moisture loss (Table 8b) indicate that air temperature and humidity account for a significant proportion of variation in the data. The correlation with humidity is not unexpected since as relative humidity decreases, the water demanding capacity and thus the evaporative demand of the atmosphere increases. The correlation with windspeed is less perfect but significant when the data are bulked. Since evaporative water loss may be related to a complex of environmental factors, which may be interrelated, the correlations are not unexpected. Variations, over time, in evaporation may be related to variation in the environmental factors measured but the differential response of treated and control plots may be attributable to windspeed differences. As this was the only factor measured in both

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[1] Computer programme for ICL 4130, author: F. Grundy.

control and treated plots it is not possible to decide upon its relative importance since temperature and humidity differences are likely to be associated with changes in canopy structure arising from trampling effects.

The relationship between potential evaporation and environmental parameters is less apparent in the data for April 1976 than for July 1975, for example, the highest windspeed and humidity recorded are associated with the lowest moisture loss rate, i.e. on 05.04.76., whilst the highest loss rate, on 06.04.76. is associated with the lowest windspeed and equally high humidity.

(c) Discussion

In addition to illustrating the rapidity with which water deficits develop in trampled plants with respect to control values, the results of the April 1976 experiment indicate part of the diurnal variation in RWC of *C. vulgaris*. The afternoon recovery is in accordance with the findings of Bannister (1964b) who concluded that "...stomatal responses of the plants have an important role in determining the daily course of transpiration". The RWC by mid-afternoon reached a value of 0.6762 which may result in an increase in r_s. The reduction in transpiration rate associated with stomatal closure may arrest a further decline in RWC, which occurs if water loss exceeds water supply. Continued supply of water to the plant when loss had been restricted may lead to an alleviation of the water stress, as manifested by an increase in RWC. That the regulatory mechanism controlling RWC in the plant may be comparatively sensitive is suggested by the constancy of RWC at the same time on successive days over a short period of time. An equilibrium between loss and supply would appear to be established which results in the plant being under a slight water deficit even when soil moisture is not limiting, as evidenced by the lack of response by the shoot to rainfall. Such a deficit may be expected in order to generate the necessary water potential gradient to raise water from roots to leaves; it may also be advantageous in inducing optimal stomatal opening for carbon dioxide uptake (Section IVA, d(i)).

The marked response of damaged shoots to rainfall may be due to a number of possible reasons:

(1) precipitation alleviating a soil moisture stress, enabling the plant to reduce the water deficit;

(2) reduction in transpiration, thus preventing severe deficits forming, may result from an increase in atmospheric humidity associated with precipitation;

(3) a restricted water supply within the plant may be alleviated by water absorption by the foliar portions of the plant;

(4) experimental error.

The first two possibilities appear unlikely since they might be expected to have repercussive effects on control plants, which are not apparent. Destruction of the canopy does increase potential evaporation but does not significantly increase the water deficit in the plant. Damage to the plant, as it affects water loss and supply, would appear to make the most significant contribution to the lowered RWC's. A situation such as foliar absorption of water, may circumvent the problems associated with damage to the vascular system, by providing water directly to dehydrated tissue. The exposure time with which the leaves are in contact with free water may affect the degree of recovery, for example, the prolonged duration of rainfall between six and twenty four hours in the July 1975 experiment, is likely to permit greater foliar absorption than in the latter instances. However, such a postulate must be considered in the light of the efficacy of foliar absorption. Greene & Bukovac (1974) considered that stomatal penetration of aqueous solutions with surface tensions near that of water, is not an important pathway of entry, although Bannister (1964b) considered that water can be directly absorbed through the aerial portions of the plant. The abrasive effect of trampling on the foliage may lower the cuticular resistance to water transfer (cf. Hall & Jones, 1971; Grace, 1974), which, together with tearing and rupturing damage, may aid penetration of water when present on the surface of leaves in a manner similar to the enhancement of water loss as reflected in elevated cuticular transpiration rates (Section IIIA, 2(d)). If the effect is real, the application of water to damaged plants may alleviate water stresses incurred as a result of trampling, a conclusion similar to that of Wagar (1965).

The effectiveness of stomatal regulation of RWC is indicated by the proximity of the RWC of damaged shoots to the stomatal closure point. Effective stomatal control over water loss may occur before complete stomatal closure (Meidner & Mansfield, 1968). The RWC of control shoots might therefore be expected to lie above or at an RWC equivalent to an RWC at which stomatal control commences to be effective, i.e. at the onset of the 'closing phase' which in July 1975 occurred at an RWC of 0.692 and in April 1976 at an RWC of 0.620. The control RWC for July 1975 lies considerably above the value at which the closing reaction commences as does the control RWC for April 1976. The onset of stomatal closure may therefore be limiting the deficit allowed to develop in the plant. It would appear that stomatal closure may be defining a minimum base line in damaged plants. The difference between the RWC of control and treated plants may be due to enhanced cuticular losses in damaged plants, which at stomatal closure, are balanced by supply so preventing a further reduction in RWC. However, this assumes bulk tissue RWC is regulating stomatal closure which may not be the case. The hypothesis proposed by a number of workers e.g. Lange et al. (1971), Schulze et al. (1972), Hall & Kaufmann (1975), Johnson & Caldwell (1976) regarding the humidity control of stomatal aperture may invalidate bulk tissue RWC as a controlling mechanism for stomatal aperture (cf. Lopushinsky, 1969). Meidner (1975) however, considered that overall ψ_1 might be expected to affect guard cell turgor "when it approaches in magnitude the difference between the osmotic potential of the guard cell sap and the matric potential of the epidermal and guard cell walls". Such potentials are likely to develop at low RWC's and may represent values at which hydroactive closure ensues. Thus, the conclusion with respect to stomatal control of RWC in damaged shoots may be valid, hydroactive closure resulting from a disequilibrium between water supply and loss. The validity of the RWC at the initiation of closing phase as a regulator of control RWC remains dubious and does not explain the constancy of RWC over time of the July 1975 data despite varying environmental conditions. Under standard conditions, the time required to develop a specific water deficit in shoots of a specific age and physiological state might be expected to be constant, but under varying evaporative conditions it might be expected to vary. Thus, a constant time interval between stomatal opening and time of measurement may result in varying RWC's when environmental conditions vary. The techniques employed were possibly not sufficiently sensitive to determine such variation. Alternatively, a more subtle control over plant RWC may exist which has not been considered in the present study.

Although occurring in a short period of time, the rapidity with which the lowered RWC's develop will depend upon the degree of damage to the vascular system, damage to the epidermal tissue and the extent to which the equilibrium between supply and loss is shifted in favour of loss, which will depend on environmental conditions. Although the daily variations in RWC tend to be associated with the environmental measurements, those made are inadequate to allow a rigorous analysis, but increased wind velocities would appear to enhance evapo-transpiration. Destruction of the canopy, whether by trampling or clipping, reduces canopy resistance to water loss which may result in lowered RWC's if absorption of water lags behind loss rate. Bannister (1964b) noted that transpiration does not follow evaporating conditions exactly, and the variations in these conditions in the present study may have been insufficient to have a significant effect on plant RWC. Similarly, the differences between evaporating conditions on clipped and control plots do not significantly affect RWC and it would appear unlikely that destruction of the canopy by trampling alone is the sole cause of lowered RWC's in trampled plants.

(ii) Seasonal variation in RWC of *C. vulgaris* and the effect of trampling on RWC:

(a) Methods

Plots (2m. x 0.5m.) of *C. vulgaris* at the Cannock Chase site were trampled at a pressure of 100 walking passages per plot. A group of four plots were demarcated and treated each month from November 1974 to June 1976, (omitting December 1974). Shoots were sampled, for the determination of RWC, between 1100 hours and 1200 hours on each sampling date, from plots treated 1 month (RWC 1), 3 months (RWC 3) and 6 months (RWC 6) before sampling. Five shoots were sampled from each plot giving 20 replicates per treatment (where treatment represents time between trampling the plot and sampling the shoots). Twenty control shoots (RWC 0) were randomly sampled from the undamaged vegetation adjacent to the treated plots.

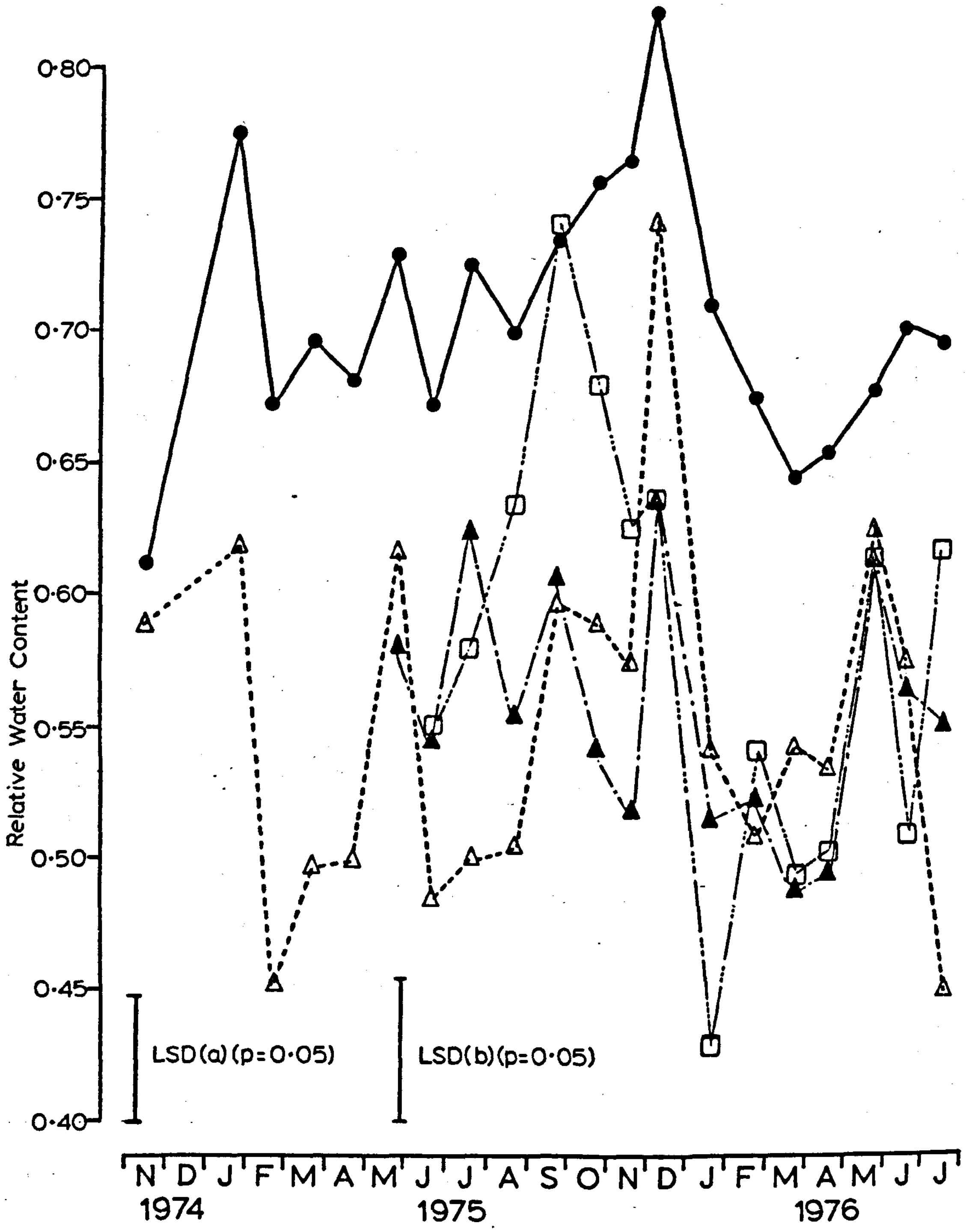


Figure 44 Seasonal variation and the effect of trampling on the Relative Water Content of C. vulgaris, (Cannock Chase).

●———●	control shoots (RWC 0)
△-------△	shoots treated 1 month before sampling (RWC 1)
▲-·—·—·▲	shoots treated 3 months before sampling (RWC3)
□—···—□	shoots treated 6 months before sampling (RWC6)
LSD(a)	seasonal variation in and differences between RWC of control shoots and shoots treated 1 month before sampling
LSD(b)	seasonal variation in and differences between RWC of all treatments, June 1975 - July 1976.

(b) Results

Subsequent analysis of the data is complicated by the varying time periods for which data from different treatments are available. One way analysis of variance performed on the seasonal variation in RWC 0, RWC 1, RWC 3 and RWC 6 indicate highly significant ($p < 0.001$) monthly differences in each case. Two way analysis of variance reveals that, in addition to highly significant time effects, significant ($p < 0.001$) treatment effects and time x treatment interaction exist. The mean response over the period June 1975 to July 1976, of 0.7106, 0.5512, 0.5561 and 0.5873 for RWC 0, RWC 1, RWC 3 and RWC 6 respectively, indicates that trampled plots have a significantly ($p < 0.001$) lower RWC than the RWC of control shoots, although only RWC 1 and RWC 6 are significantly different within the trampling treatments. On a monthly basis (Figure 44) significant differences, in which damaged plants have consistently lower RWC's than control plants, (except for September 1975 when RWC 6 is greater than RWC 0), occur on eleven of the fourteen sampling dates for RWC 0 and RWC 1, and RWC 0 and RWC 3 and seven of the fourteen sampling dates for RWC 0 and RWC 6. Significant differences between the trampling treatment data are few, i.e. July 1975 (RWC 1 and RWC 3); September 1975 and July 1976 (RWC 1 and RWC 6) and October 1975 (RWC 3 and RWC 6).

Seasonal variation in RWC appears erratic. The lowest values for RWC 0 are observed in the winter months, i.e. November 1974, February 1975, March 1976. However, the pattern is not consistent since the values for January and December 1975 are comparatively high (0.7782 and 0.8221 respectively) whilst that for June 1975 is low (0.6721). Following the low values at the beginning of the year, RWC 0 tends to increase over the course of the year to a maximum in December, 1975.

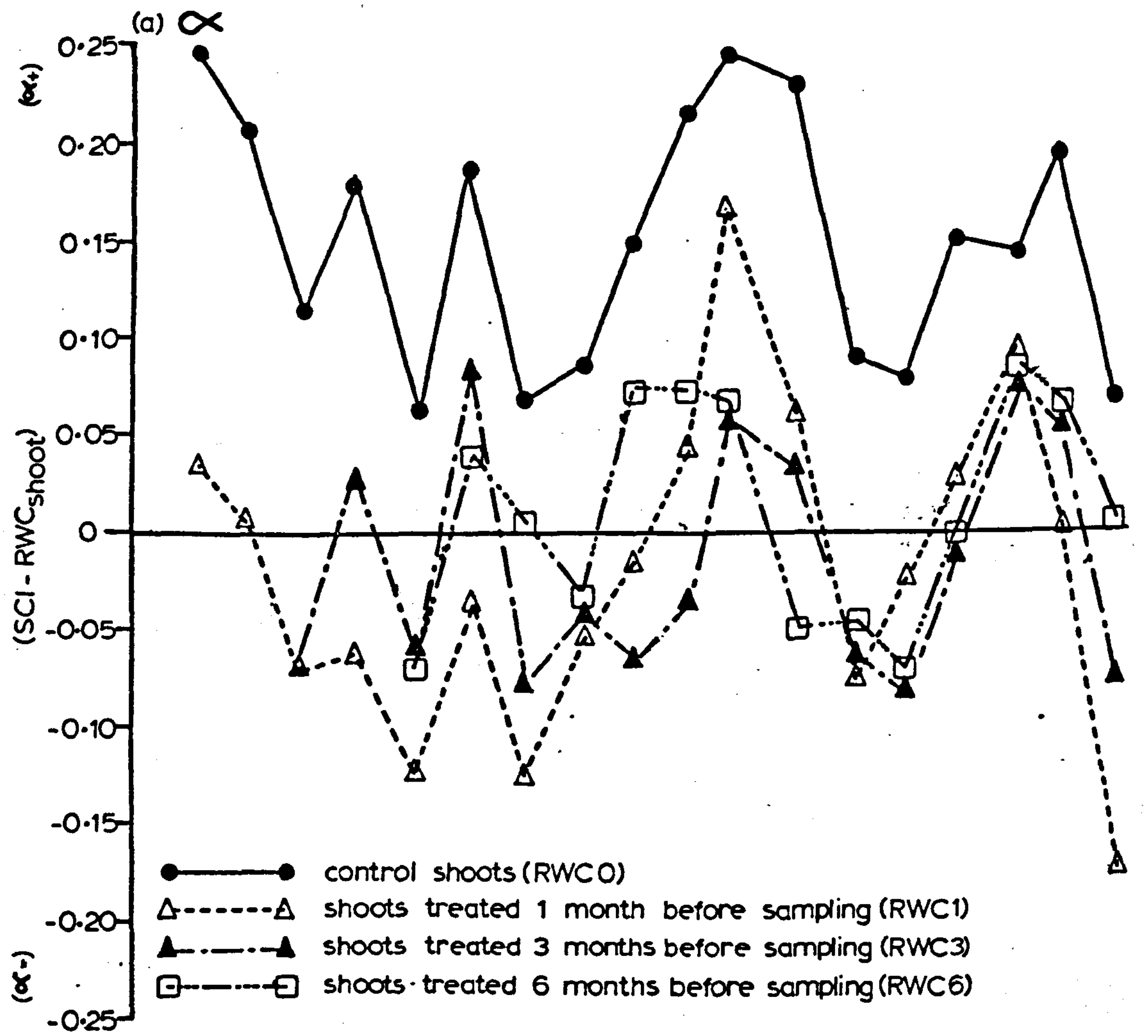
Seasonal variation in the RWC of treated shoots is equally erratic, with no obvious seasonal trend. Although the RWC's of treated shoots exhibit a seasonal variation similar to that of RWC 0 (as indicated by significant correlations (Table 9)), the proportion of variance accounted for by such a correlation, and thus possibly due to the same cause, is small, being at maximum 33.9%.

RWC 0 is not significantly correlated with any of the measured temperature parameters, rainfall or vapour pressure, nor with stomatal closure point ($r = 0.321$, n.s.), desiccation tolerance ($r = -0.169$, n.s.), or percentage moisture content ($r = -0.443$, n.s.). However, RWC 0 and daylength are negatively correlated ($r = -0.4828$, $p < 0.05$) indicating that there is a tendency for lengthening days to be associated with lower shoot RWC.

Table 9 Correlation coefficients (r) between RWC 0, RWC 1, RWC 3, RWC 6 of *C. vulgaris* (Cannock Chase)

Variable	r	Significance
RWC 0, RWC 1	0.5824	< 0.01
RWC 0, RWC 3	0.5315	< 0.05
RWC 0, RWC 6	0.5426	< 0.05
RWC 1, RWC 3	0.5080	< 0.05
RWC 1, RWC 6	0.3490	n.s.
RWC 3, RWC 6	0.5801	< 0.05

No significant correlations occur between RWC 1 and environmental variables nor the physiological variables stomatal closure point ($r = 0.321$, n.s.), desiccation tolerance ($r = -0.290$, n.s.) or percentage moisture content ($r = -0.245$, n.s.). Since stomatal closure might be expected to exert some control over the water deficit developing within the plant, but since the RWC at stomatal closure appeared unrelated to



(b) Seasonal variation in Desiccation Tolerance

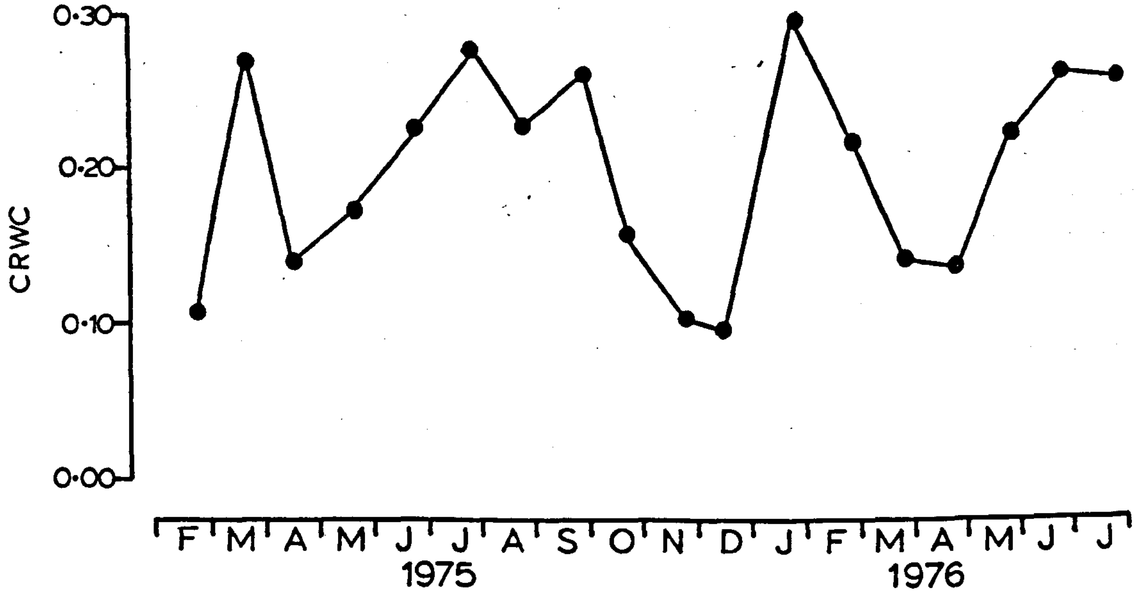
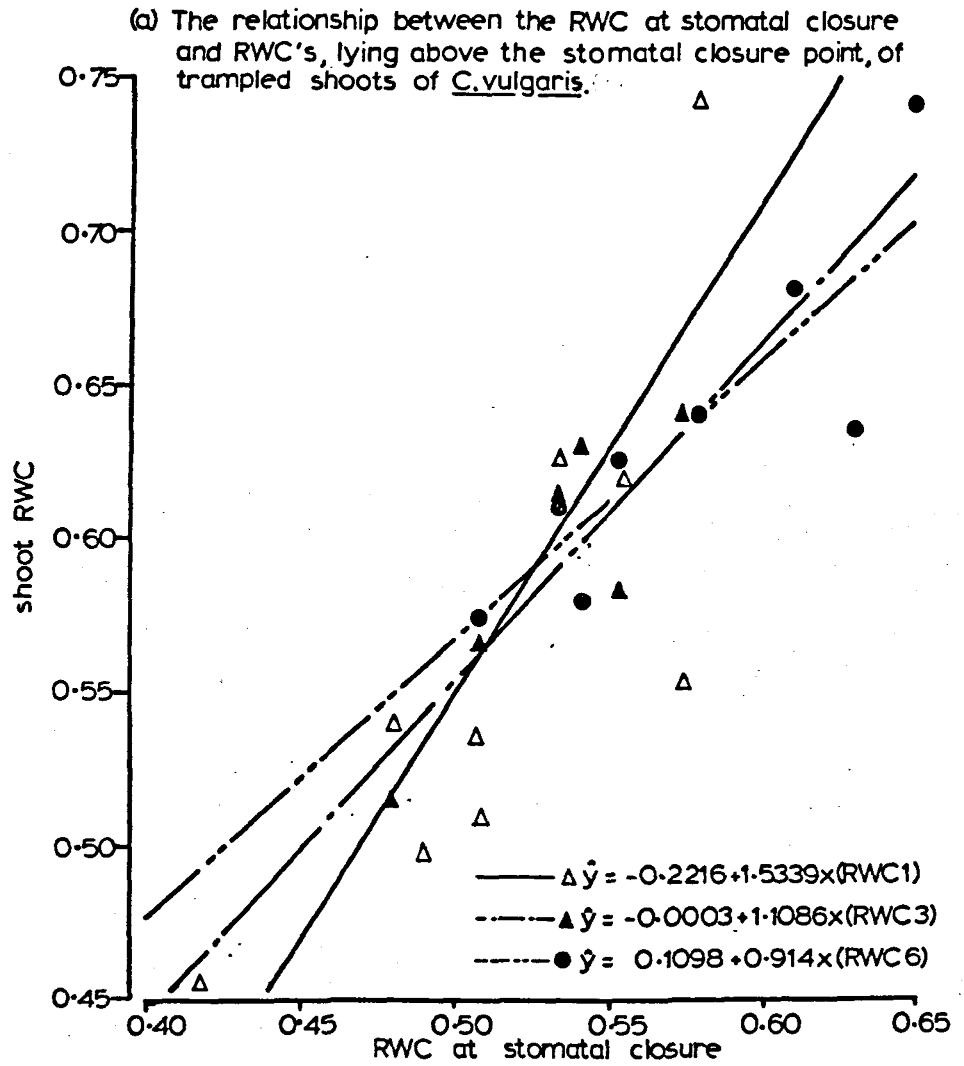


Figure 45 Seasonal variation in the difference between the RWC at stomatal closure and shoot RWC of C. vulgaris in the field (α).

RWC 1, a more subtle explanation is suggested. The difference between RWC in the field and the RWC at stomatal closure might be expected to give some indication of the effectiveness of stomatal closure in controlling water loss. Consequently, the mean RWC for each treatment at each sampling date has been subtracted from the RWC at stomatal closure (SCI) for that date. The difference (α) is positive when the shoot RWC lies above SCI (α+) and negative when shoot RWC lies below SCI (α-). The results (Figure 45a) indicate that RWC 0 is consistently positive, although at certain times the difference is not great, e.g. June, August 1975; March, July 1976. In contrast, the results for treated plots show an erratic seasonal variation in which nine out of eighteen values for RWC 1 are negative, ten out of sixteen are negative for RWC 3 and six out of fourteen are negative for RWC 6. (This does not allow for any possible adjustment in SCI of treated plants (Section IIIA, 2(d)). The seasonal variation is somewhat erratic but for RWC 1 in 1975, the negative values occurred between April and October. The pattern in 1976 is less apparent, the values between April and June being positive, although the value for July was the largest negative value recorded. The general seasonal pattern of variation is similar for other treatments including RWC 0.

Correlation analysis was performed relating shoot RWC to stomatal closure point and vapour pressure, where RWC + indicates shoot RWC greater than the RWC at stomatal closure and RWC - indicates shoot RWC less than the RWC at stomatal closure (Table 10).

Significant correlations exist between SCI and RWC + for the three treatments under consideration, and between SCI and RWC - for RWC 3 and RWC 6. RWC - was also significantly correlated with vapour pressure (VP) for RWC 6, indicating that with increasing vapour pressure the shoot RWC increases, but remains below the RWC at stomatal closure.


(a) The relationship between the RWC at stomatal closure and RWC's, lying above the stomatal closure point, of trampled shoots of C. vulgaris.

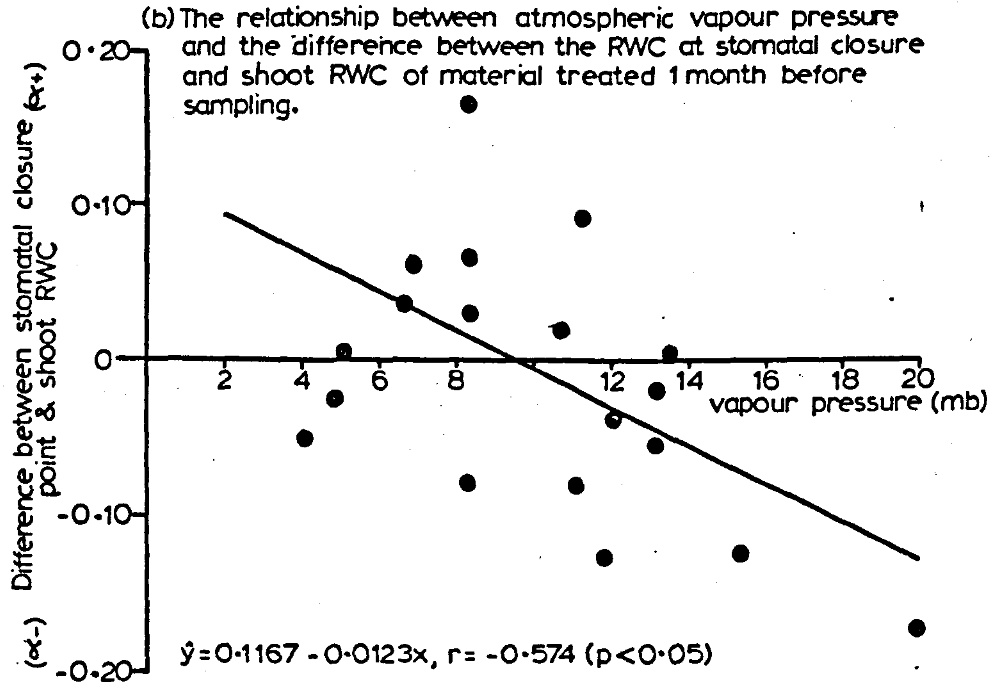

(b) The relationship between atmospheric vapour pressure and the difference between the RWC at stomatal closure and shoot RWC of material treated 1 month before sampling.

Figure 46 The relationship between Stomatal Closure Point and the RWC of trampled shoots of C. vulgaris, and the influence of the evaporative demand of the atmosphere on the proximity of treated shoot RWC to the stomatal closure point, (Cannock Chase).

Table 10 Correlation coefficients (r) between RWC, SCI and Vapour Pressure (VP) *C. vulgaris* (Cannock Chase)

Variable	RWC 1	RWC 3	RWC 6
SCI, RWC	0.321	0.138	0.679*
SCI, RWC+	0.857**	0.838*	0.854**
SCI, RWC-	0.215	0.793*	0.883*
VP, RWC	0.239	0.249	0.566*
VP, RWC+	-0.013	0.545	-0.037*
VP, RWC-	-0.327	0.593	0.856*
VP, $\alpha+$	-0.136	0.544	-0.337
VP, $\alpha-$	-0.675*	-0.185	0.203
VP, $(\alpha+) + (\alpha-)$	0.576*	-0.151	0.301

(* $p < 0.05$; ** $p < 0.01$)

The positive correlations referred to above indicate that with increasing RWC at stomatal closure so the RWC of the damaged shoot increases (Figure 46a). For RWC 1 the relationship is not 1:1 so that with decreasing RWC at stomatal closure the difference between field RWC and stomatal closure point becomes less. This trend is not so evident in RWC 3 and RWC 6.

For RWC 1 a negative correlation exists between vapour pressure and $\alpha-$ but not between vapour pressure and $\alpha+$, although the overall correlation with α is significant. This negative relationship (Figure 46b) indicates that with an increase in vapour pressure the difference between RWC 1 and SCI becomes less until, at a value of 9-10mb., it becomes zero, thereafter becoming increasingly negative with increasing vapour pressure. Thus, at low vapour pressures field RWC might be expected to lie above the RWC at stomatal closure whilst at high vapour pressures the reverse situation is expected.

This significant environmental effect might be expected to result in the significant time x treatment interaction observed between RWC 0, RWC 1, RWC 3 and RWC 6. Although RWC 1 is not significantly correlated with daylength ($r = -0.357$, n.s.), seasonal changes in environmental variables reflected in daylength, i.e. vapour pressure and daylength ($r = 0.688$, $p \lesssim 0.01$), stem temperature and daylength ($r = 0.738$, $p < 0.001$), together with specific environmental conditions on the day of sampling, might be expected to affect the treatment response on different sampling dates.

Temperature differentials between air and soil might also have an effect on water uptake and loss. Temperature maxima available for sampling dates (Table 11) reveal that in winter, air temperature maxima may be two to three times greater than soil temperature maxima, e.g. January, February 1975. The differences in summer, although greater in magnitude, are relatively less prounounced e.g. June 1975, 1976. It is also of note that stem maxima may be a number of degrees above those of the air.

Table 11 On-site temperature maxima, available for specific sampling dates (Cannock Chase)

Date	Temperature 60cm. (^{o}C)	Temperature stem (^{o}C)	Temperature -5cm. (^{o}C)
29.11.74.	3.4	3.6	2.2
23.01.75.	3.6	3.8	1.0
21.02.75.	5.6	8.0	3.8
18.03.75.	5.2	12.8	4.2
19.06.75.	21.8	22.0	16.2
24.10.75.	13.0	-	9.6
10.12.75.	4.4	6.4	3.2
24.03.76.	4.0	6.0	2.0
22.06.76.	26.4	33.0	20.6

Table 12 Multiple Regression Analysis relating seasonal variation in the RWC of control shoots of *C. vulgaris* (RWC 0) to Environmental and Physiological variables (Cannock Chase)

Step	Variable	Significance to enter (p)	Multiple R	Multiple R^2	R^2 change	Simple R	Overall Significance (p)
(a)	Environmental variables						
X_1	Daylength	< 0.05	0.4828	0.2331	0.2331	-0.4828	< 0.05
X_2	Vapour Pressure (mean over 7 days before sampling)	< 0.05	0.6619	0.4382	0.2051	-0.0039	< 0.05
X_3	Accumulated temperature (7.2°C) over 7 days before sampling)	n.s.	0.6909	0.4773	0.0391	-0.0840	< 0.05
X_4	Rainfall (total over 7 days before sampling)	n.s.	0.7063	0.4989	0.0216	-0.3666	< 0.05
X_5	Stem temperature (mean for 7 days before sampling)	n.s.	0.7184	0.5161	0.0172	-0.0802	n.s.
	Regression equation:	$\hat{Y} = 0.7900 - 0.0136\,X_1 + 0.0087\,X_2$					< 0.05

Step	Variable	Significance to enter (p)	Multiple R	Multiple R^2	R^2 change	Simple R	Overall Significance (p)
(b)	<u>Physiological variables</u>						
X_1	SCI	n.s.	0.2521	0.0636	0.0636	0.2521	n.s.
X_2	Browning (control plots)	n.s.	0.3327	0.1107	0.0471	-0.2491	n.s.
X_3	CRWC	n.s.	0.3835	0.1471	0.0364	-0.1789	n.s.

Multiple Regression Analyses

The variables entered into the analyses (Table 12), in an attempt to explain seasonal variation in RWC 0 and RWC 1, have been separated into environmental and physiological variables. Although combining the variables into one analysis improves the regression, the dependence of physiological on environmental parameters may confuse the analysis.

(1) RWC 0 on environmental variables (Table 12a)

Of the five variables entered into the analysis, accounting for a maximum of 51.6% of the variation in the data, only daylength and vapour pressure, which together account for 43.8% of the variation, make a significant contribution towards explaining seasonal variation in RWC 0. Although daylength and accumulated temperature do not exactly parallel each other, since daylength is longest in June whilst temperatures are maximal in August, they are significantly correlated ($r = 0.744$, $p < 0.001$) and may therefore be expected to emulate each other in explaining seasonal variation in RWC 0. Although vapour pressure is similarly correlated with daylength ($r = 0.688$, $p < 0.01$), the fit is less perfect. Vapour pressures, whilst exhibiting a seasonal trend, are more susceptible to fluctuations as a result of local micro-climatological conditions and thus, part of the variation unaccounted for by daylength as an indicator of seasonality, may be accounted for by local changes in the evaporating conditions to which the water relations of the plant may be sensitive. The two variables make an approximately equal contribution to the regression.

(2) RWC 0 on physiological variables (Table 12b)

Of the three variables entered into the analysis, none make a significant contribution towards explaining the variation in the data.

Table 13 Multiple Regression Analysis relating seasonal variation in the RWC of shoots of *C. vulgaris* trampled one month before sampling, (RWC 1) to Environmental and Physiological variables (Cannock Chase)

Step	Variable	Significance to enter (p)	Multiple R	Multiple R^2	R^2 change	Simple R	Overall Significance (p)
(a)	Environmental variables						
X_1	Daylength	n.s.	0.3571	0.1275	0.1275	-0.3571	n.s.
X_2	Rainfall (total over 7 days before sampling)	n.s.	0.3579	0.1282	0.0007	-0.1179	n.s.
(b)	Physiological variables						
X_1	Browning (on treated plot)	< 0.05	0.5498	0.3023	0.3023	-0.5498	< 0.05
X_2	SCI	n.s.	0.6550	0.4291	0.1268	0.1952	< 0.05
X_3	CRWC	n.s.	0.6734	0.4535	0.0244	-0.3401	< 0.05
	Regression equation:	$\hat{Y} = 0.6579 - 0.0023 X_1$					< 0.05

No significant regression is obtained.

(3) RWC 1 on environmental variables (Table 13a)

Of the five variables entered, the F ratios permit only daylength and rainfall to appear in the final regression at the 5% level of significance. Neither make a significant contribution towards explaining the variation in RWC 1. No significant regression is obtained.

(4) RWC 1 on physiological variables (Table 13b)

In contrast to RWC 0, it is the physiological variables which explain variation in RWC 1, accounting for a maximum of 45.4%. However, it is only browning, which is an indicator of the stress the plant is under and the damage the plant has suffered, which makes a significant contribution towards explaining the variation in the data, accounting for 30.23% of the total variance. Stomatal closure point and desiccation tolerance make a small and non-significant contribution (i.e. 15.1%) so that only browning may be statistically validly employed in the resultant regression equation.

Integration of environmental and physiological variables into the analysis increases the total explained variation to 58.99% and 48.19% for RWC 0 and RWC 1 respectively, but does not affect the significance of those variables explaining variation in RWC 0 and RWC 1.

(c) Discussion

The low winter RWC's observed accord with the findings of Bannister (1964b), who found the lowest values of RWC between December and May rising to a summer maximum followed by a decline to low values in winter. The trends are less evident in the present study, and appear to be displaced in as much as they rise from a late winter minimum to an early winter maximum over the course of the year. Bannister considered that departures from the general trend were the result of wet conditions, e.g. rain, snow, fog, dew, melting frost. Similar reasons may be advanced to account for the erratic pattern observed in the present study. Surface moisture on the shoots at the time of sampling may have resulted in erroneous measurements of fresh weight, although sampling was performed on dry days at a time when surface moisture, e.g. dew, rime, should have disappeared. Low evaporating conditions may have resulted in reduced transpirational losses so that deficits made up over night did not develop to the same extent by the standard time of sampling on sampling days on which apparently erroneously high RWC's were recorded. Bannister (1964b) observed, however, that removal of surface moisture still resulted in high RWC's so that errors from surface moisture seem unlikely. Rather, a possibility exists that foliar absorption of dew and frost melt may occur.

Unlike the findings of the present study, Bannister (1964b) observed that RWC of C. vulgaris fell below the stomatal closure point on a number of occasions. In the present study, although shoot RWC appeared independent of stomatal closure, the greatest differences between shoot RWC and stomatal closure point tended to occur in winter, implying that deficits developing on winter days, may, to some extent, be related to stomatal closure. Since stomatal control is a metabolic process it is likely to be influenced by temperature. Schulze et al. (1974) observed that stomatal opening is enhanced by an increase in temperature. The relationship existing between substomatal CO_2 concentration and stomatal opening (Meidner & Mansfield, 1968) implies that at low temperatures, rates of photosynthesis may be so low as to retard stomatal opening by reducing the rate at which the substomatal

CO_2 concentration is lowered. This may inhibit water loss and may result in high RWC's. Loftfield (1921) observed that the time taken to full stomatal opening was lengthened by low temperatures and Stalfelt (1962) also observed stomatal opening was slow at low temperatures. The low stem temperatures in January and December 1975 may be associated with reduced stomatal opening and thus high RWC's. Although there is a lack of any correlation between RWC and temperature, this might be expected in view of the specificity of the response. However, the RWC at stomatal closure decreases during the winter months, consequently, a greater imbalance between water loss and supply would have to occur before the RWC was lowered sufficiently to result in stomatal closure, and conditions in winter may not be conducive to reducing water content to such a value, resulting in the larger values of $\alpha +$ in winter for RWC 0. Thus, two options may exist accounting for high RWC's in winter, retarded stomatal opening and conditions not conducive to rapid water loss.

The lack of any relationship between environmental variables and RWC agrees with Bannister's (1964b) observations, although day length and vapour pressure emerge as variables accounting for variation in RWC 0. Nevertheless, it is possible to propose a tentative explanation accounting for the increase in RWC over the course of the year. Metabolic consumption and transpirational loss of water may be enhanced in summer when active growth and enhanced evaporating conditions exist, together with the possibility of certain soil moisture tensions limiting uptake. The days are also longer and stomatal opening may occur earlier in the day resulting in lower RWC's developing by midday. In contrast, in winter the evaporative potential (as indicated by vapour pressure) decreases and the days shorten so water losses may be less, resulting in higher RWC's and longer overnight periods in which deficits, induced during the day time, are made up. However, this does not account for the low RWC's observed in the winter months. Bannister (1964b) noted that soil and atmospheric moisture were not restricted in winter when low RWC's were observed. He considered that soil moisture tension was unlikely to reduce water uptake over winter whilst physical changes in water at low temperature were unlikely to completely explain any reduction in uptake. Bannister considered that the inability to satisfy water deficits may result from resistances to water uptake in the plant. The association between low soil temperature and reduced water uptake has been widely observed. Kramer (1940) considered that low soil temperature was one of the most important environmental factors affecting water absorption, the response being due to a "..decrease in root growth, and in water permeability of the cell membrane and an increase in the viscosity of water and of protoplasm". (Kramer, 1942). Many other workers share similar views, e.g. Abd El Rahman *et al*. (1959), Ehrler (1963). Kuiper (1964) observed, in studies on *Phaseolus vulgaris*, a 'critical temperature' which varied with conditions during growth, above which changes in viscosity of water limited water uptake whilst below which temperature sensitive membrane permeability affected water uptake. Anderson & McNaughton (1973) however, found that even at temperatures as low as 3°C root permeability to water of a number of species was sufficient so as to prevent water deficits arising at which transpiration rate became sensitive to RWC, although they observed that root chilling lowered leaf RWC. In contrast, Bannister (1964b) found that, by root chilling, transpiration could be reduced although RWC remained constant. Bannister also noted that the formation of an impervious secondary endodermis in the roots of *C. vulgaris* has been demonstrated and that suberization of the root, after growth has ceased at the beginning of the dormant season, may result in high resistances to water uptake.

A difference in water loss by transpiration and water uptake may result in a lowering of RWC, although equilibration between water reservoirs and pathways in the plant may reduce the rapidity with which such deficits develop (Kleinendorst & Brouwer, 1972), and thus may buffer the plant against the rapid formation of large water deficits. However, the imbalance between uptake and loss referred to above may account for the low RWC's observed in winter when soil temperatures are low and the plant, because of dormancy, has a high resistance to water uptake, while stem temperatures may generate conditions conducive to transpirational water loss, resulting in a decrease in plant RWC. The inability of C. vulgaris to make up water deficits in winter, resulting in desiccation damage, has been suggested as the cause of 'frosting' in winter (Bannister, 1964b).

Even if soil moisture is not limiting, observations suggest that water uptake occurs only when a transpiration stress develops (Bannister, 1964b; Jarvis, 1976). This implies that water deficits may be satisifed only if stresses within the plant are conducive to the satisfaction of such deficits. During long winter nights the imbalance between water supply and loss may not be rectified, due to reduced transpirational demands on the water supplies of the plant, with the consequence that water deficits are not satisfied.

The response of the plant's water balance to the plant/environment complex may make a consideration of the relationship between RWC and environmental variables on a seasonal basis meaningless, since at certain times the plant may be responding to one factor and at other times to another. This makes the establishment of relationships between the few environmental factors measured and RWC futile, the more so since the seasonal pattern is based on one observation a month and may not represent actual relationships existing between RWC and seasonal changes in the environment. It is of interest to note however, that vapour pressure emerges as a factor contributing towards seasonal variation in RWC, and that the correlation with daylength may reflect a general seasonal trend in environmental conditions and the physiological state of the plant. However, the observation that it is negative suggests that during the period of the year when high RWC's are required for growth, RWC's are lower than in the dormant season. This may be a factor which could contribute towards the phenomenon of osmotic adjustment by which the plant may be able to adapt its potential for elongation to water limiting conditions (cf. Hsiao _et al._, 1976).

The absence of any significant correlations between RWC 1 and environmental variables suggests that the RWC of damaged plants may be under the control of more subtle regulatory mechanisms, and stomatal closure has been invoked as a possible mechanism in controlling plant RWC. Stalfelt (1955) stated that a causal relationship existed between leaf water deficit and stomatal movement. Raschke (1976) was sceptical of the postulate that passive water loss lowers the ψ to an extent resulting in stomatal closure and Meidner (1975), on the basis of observations on water pathways in leaves, considered that stomata may function independently of bulk tissue water content. However, that dehydration ultimately results in stomatal closure is a widely held view, e.g. Lopushinsky (1969), Johnson & Caldwell (1976). Lopushinsky (1969) reported threshold RWC values resulting in closure of 0.81 to 0.85 for _Pinus ponderosa_ and _P. contorta_ whilst a value of 0.82 has been reported for _P. sylvestris_ (Jarvis & Jarvis, 1963b).

Bannister (1964a) noted that "...stomatal closure and a consequent reduction of transpiration begin very shortly after a shoot is allowed to develop a water deficit", consequently, high closure values might be expected to result in high field RWC's. The significant correlations between stomatal closure point and RWC 3, and stomatal closure point and RWC 6 suggest that stomatal closure may be controlling RWC, but this only occurs when RWC's have been partitioned into those above and those below the stomatal closure point. However, in both instances the higher stomatal closure points are associated with higher field RWC's of shoots. This also applies to RWC 1 values lying above the stomatal closure point but for values lower than the stomatal closure point, stomatal control does not appear to have any effect on RWC, implying that, under these circumstances, stomatal control was ineffectual in enabling desiccation avoidance. The extent to which RWC 1 lies below the stomatal closure point is related to the evaporating potential of the environment, the greater this is, the larger the difference between shoot RWC and the stomatal closure point whilst under low evaporating conditions stomatal control regulates water loss effectively. The large negative differences occur when potential desiccation avoidance is high and when desiccation tolerance is low, i.e. in the summer months, which, combined with the low RWC's for the same period, renders the tissue susceptible to desiccation damage. The decreased desiccation avoiding ability may result from the higher cuticular loss rates of new tissues and the mechanical damage to tissues which increases cuticular transpiration losses (Section IIIA, 2(d)). A steepening of the humidity gradient between the inside of the leaf and the atmosphere will enhance water loss even when stomata are closed. Although, with increasing water loss, this gradient will be reduced and desiccation may lead to decreasing permeability of the cuticle (Levitt, 1972), this may account for the relationship between vapour pressure and RWC 1. The RWC 3 and RWC 6 values suggest partial recovery since the α- values are smaller; possibly long term exposure to water deficits results in some adaptation to the stress. The present analysis has not taken into account any readjustment of stomatal closure point which may result from desiccation. However, repair of damage or a decrease in cellular π steepening the ψ gradient between root and shoot may enhance the water balance of the leaves and lower the water deficit existing in the tissue. RWC 6 shows an increasing tendency to be positive with respect to the stomatal closure point compared to RWC 1 and RWC 3, suggesting either recovery or hardening over prolonged exposure to high water deficits. Although hardening implies increased desiccation tolerance, decreased cuticular permeability may result in 'pseudo-hardening' (Levitt, 1972) by increasing water loss avoidance capacity. This is implied by the regression of positive RWC's on stomatal closure point, in which the ability of the shoot to maintain a positive RWC with respect to the stomatal closure point increases with the length of time the shoot has been damaged, as indicated by the decreasing slope of the regression for RWC 1, RWC 3 and RWC 6 (Figure 46a). This may reflect an adaptation to desiccation which renders the plant less susceptible to desiccation damage since its RWC is more likely to be above the stomatal closure point. This has certain repercussions on the assimilatory capacity of the plant. At low stomatal closure points the 6 month damaged shoots will be at an advantage relative to the 1 month damaged shoots since their RWC's will be higher and thus not result in the likelihood of stomatal closure and reduced CO_2 uptake. However, at higher stomatal closure points the reverse situation may apply but, depending upon the RWC threshold at which stomatal closure occurs, the effect on CO_2 uptake may be negligible since CO_2 uptake and water loss respond differently to increases in r_s (Section IVA, a(i)). Although at higher stomatal closure points the RWC of the plant may be higher (which may, itself, have metabolic advantages), the lower RWC 6 values may not result in an increase in r_s which impairs CO_2 uptake.

The correlations between RWC 3, RWC 6 and stomatal closure point suggest that, over time, stomatal control over water loss in damaged shoots becomes more effective, possibly as a result of damage repair and pseudo-hardening but that control is not so effective as in undamaged plants. Although this discussion has centred upon SCI, the same principles might be expected to apply if SCL is used to indicate stomatal closure, although, since all field RWC lie above SCL, the discussion regarding α+ and α- becomes irrelevant.

These observations must be viewed with the possibility that water supply is restricted. This will result in a decrease in RWC until the stress is sufficient to inhibit transpiration. The stress at that point may be sufficient to result in stomatal closure but, given the appropriate environmental conditions, passive water loss over which the plant has little control will result in a further lowering of plant RWC. The recovery over time however, results in a reduction in the water deficit of the damaged plant. Although RWC 1, RWC 3 and RWC 6 are generally not significantly different, the mean response increases from RWC 1 to RWC 6, such that fewer significant differences exist between RWC 0 and RWC 6 than between RWC 0 and RWC 1, although substantial and significant water deficits remain 6 months after trampling.

The seasonal variation in RWC of treated shoots, although modified by trampling, follows the same pattern as that for control shoots. Explanations for seasonal variations are somewhat complex. The reaction of the plant under stress is obviously different from that of the unstressed plant, and is perhaps affected, to a greater extent, by physiological variables. The physiological susceptibility of the plant to desiccation, as influenced by desiccation tolerance and avoidance, may be reflected in the damage, as evidenced by 'browning', experienced by the plant. Bannister (1964b) noted that browning is associated with low RWC's and this is substantiated by the present investigation (Section IIIC, 4(b)). Consequently, it is not unexpected that seasonal variation in RWC of damaged plants may be described in terms of seasonal variation in browning, which is a summation of the physiological response of the plant to desiccation. It is also of note that a negative correlation exists between these two parameters (Section IIIC, 4(b)). This implies that water deficits observed in the tissue are real and not an artifact of dead tissues competing with living tissues under a high water deficit for water, and thus preventing the alleviation of such living tissue deficits (cf. Bannister, 1964b).

(iii) The effect of trampling intensity on RWC of *C. vulgaris*

(a) Methods

Plots (2m. x 0.5m.), laid out in a Latin Square arrangement, received a range of trampling pressures. The plots at the Cannock Chase site were trampled at 0, 50, 100 and 200 walking passages per plot, with four replicates per treatment. The plots at the Kerloch site received a range of pressures of 0, 100, 200 and 300 walking passages per plot, with four replicates per treatment.

On the respective sampling dates shoots were sampled from each plot for the determination of RWC. In subsequent analyses the mean value for each group of five shoots per plot is employed.

(b) Results

No significant differences in RWC between treatments are found for the Cannock Chase block 1 (Figure 47a), and although a trend for decreasing RWC with increasing trampling pressure up to 100p pl^{-1} exists, the relationship is obscured by an increase in RWC of shoots receiving the highest trampling pressure employed (200p pl^{-1}).

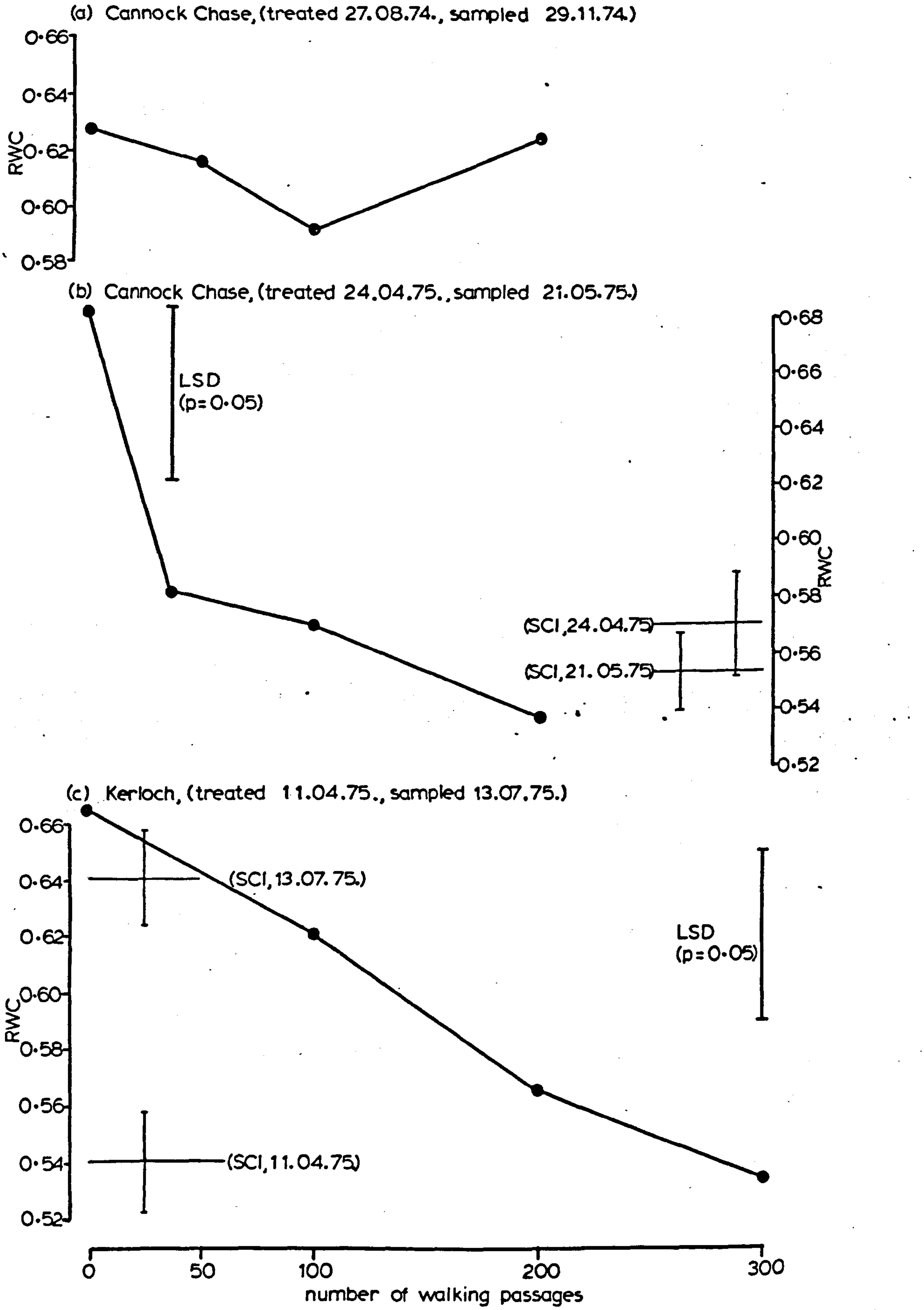


Figure 47 The effect of trampling intensity on shoot Relative Water Content of C. vulgaris.
(SCI - RWC at stomatal closure; bar lines indicate one standard error each side of the mean.)

Shoot RWC's on block 2 at the Cannock Chase site (Figure 47b) exhibit a significant ($p < 0.01$) decrease with increasing pressure, all trampling treatments resulting in a significantly lower RWC compared to the control. The relationship exhibits curvilinear tendencies in which a pressure of 50p pl^{-1} results in the largest decrease in RWC, thereafter, although a slight downward trend exists, the RWC at 200p pl^{-1} is not significantly lower than that at 50p pl^{-1}.

The RWC of shoots over the range of intensities applied at the Kerloch site (Figure 47c) shows a significant linear decrease with increasing pressure ($r = -0.674$, $p < 0.01$). Significant ($p < 0.01$) differences exist between treatments in which the next but one increase in pressure results in a significant lowering in RWC (i.e. the RWC of shoots from plots receiving 200p pl^{-1} is significantly lower than the control, similarly shoot RWC from the 300p pl^{-1} treatment is significantly less than shoot RWC from the 100p pl^{-1} treatment).

Also presented on Figure 47 are the stomatal closure points for the time of treatment and time of sampling which are significantly ($p < 0.001$) different at the Kerloch site but not at the Cannock Chase site.

A pressure of 50p pl^{-1} at the Cannock Chase site, block 2, results in an RWC of the shoot which does not differ significantly from the stomatal closure point either at the time of treatment or sampling. In contrast, the RWC's for the Kerloch plot lie considerably below the stomatal closure point at the time of sampling. The lowest RWC recorded, at a pressure of 300p pl^{-1}, does not differ significantly from the stomatal closure point at the time of treatment.

(c) Discussion

The increase in trampling pressure is associated with a lowering in the RWC of *C. vulgaris* shoots, corroborating the measurements of ψ_x

(Section IIIB, 1(d)). The reasons advanced for the relationship between trampling intensity and ψ_x are probably applicable to the relationship between trampling intensity and RWC, although certain paradoxes arise.

Although significant decreases in ψ_x were observed on block 1 at the Cannock Chase site up to two months following treatment, no significant effect on shoot RWC is apparent after a similar intervening time period. A second paradox arising is the significant decrease in RWC on block 2 at the Cannock Chase site whilst there is a significant increase in ψ_x for shoots sampled on the same day i.e. the trends, in terms of stress, are in opposite directions (cf. Section IIIB, 1(d)). It is pertinent to note that the threshold effect observed for the two parameters occurs at the same trampling intensity (i.e. $50p\ pl^{-1}$), but a mechanistic explanation, other than sampling errors, remains obscure.

Although the relationship between ψ_x and RWC for May 1975 is curvilinear (Section IIIB, 3(c)), the theoretical ψ_x calculated from the observed RWC shows a substantial decrease with increasing trampling pressure (i.e. from -1.52MPa to -2.69MPa), with a curvilinear tendency, (the ψ_x at a trampling pressure of $50p\ pl^{-1}$ being -2.34MPa). It would appear that the observed RWC lies outside that portion of ψ_x/RWC relationship in which a decrease in RWC is accompanied by only a small decrease in ψ_x. The discrepency may arise from a differential response between the apoplastic water pathway and the symplastic water content. The diffusion resistance to water movement between the apoplast and symplast may result in a differential time response between the increase in ψ_x and the alleviation of symplastic water deficits. Although large time lags in the transmission of ψ_x through the leaf to the xylem vessesl may occur (Sheriff & Sinclair, 1973), if stomatal closure were to occur and restrict water loss, a recovery in terms of ψ_x may be more rapid than an increase in symplastic water content due to the aforementioned resistance. The situation is complicated by the fact that different aspects of plant water stress are being considered, i.e. ψ_x and RWC, and the relationship existing between ψ_x and ψ_1 is unknown. Because the major water movement pathway through the plant may be regarded as apoplastic, while RWC measurements involve deficits existing in the symplast, a direct correspondence between ψ_x and ψ_1 may not exist. In addition to the differential responses which may exist, a sampling time error may occur. Whereas RWC's were measured at midday, ψ_x was measured in the afternoon by which time RWC and ψ_x may have begun to increase (cf. Bannister, 1964b). If stomatal control over water loss were to become effective subsequent to RWC determinations, a discrepancy might be expected. However, the results imply that over a given time period damaged plants would have to respond either before or more rapidly than control shoots. This might occur, given that at the time of sampling for RWC, the water deficits may have been sufficient to induce hydroactive closure in damaged shoots but not in control shoots. This would result in premature stomatal closure compared to the control shoots and a comparatively higher rate of reduction in ψ_x, while the control shoots may not develop deficits sufficient to induce stomatal closure until later in the day or possibly not at all. However, this assumes the shoot is able to recover from water deficits and react as an undamaged plant, which may not be valid on the basis of the short term investigations reported above (Section IIIB, 2(i)). Neither does such an explanation take into account the possibility of reduced stomatal closure points occurring in damaged shoots. The discrepancy may arise from the differing physiological states between damaged and undamaged plants in terms of their metabolic activity and growth status, which has not been evaluated, and the effect tissue dehydration and death have upon RWC determinations.

and the development of ψ_x.

The conclusions made regarding stomatal control over water loss (Section IIIB, 2(i)) may be invoked in explaining the level of water deficit developing in shoots from the Cannock Chase block 2 experiment, the threshold RWC corresponding to the stomatal closure point. The slight downward trend observed with further trampling pressure, may be related to the degree of mechanical damage to the xylem and leaf tissue, the latter resulting in water loss over which the plant has little control. The response at the Kerloch site is slightly different, no threshold effect being apparent. The data do not support the postulate regarding stomatal control over water loss, since all RWC's of damaged shoots lie below the stomatal closure point for the sampling date. An interesting situation arises when the stomatal closure point at the time of treatment is considered. The inference is that the RWC of shoots sampled from plots receiving a trampling pressure of 300p pl^{-1} may be determined by stomatal closure, the damage at lower trampling pressures being insufficient to reduce the RWC to a level where it is controlled by stomatal closure. By implication this requires that no seasonal adjustment in the stomatal closure point has occurred, in the manner that it occurs in control shoots, or that the stomatal closure point has decreased by adaptation to the water stress. The metabolic disturbance resulting from moisture stress may inhibit seasonal adjustments in stomatal closure point. On the basis of the suggestion that RWC of treated shoots is controlled by the stomatal closure point at the time of treatment, a relationship between the seasonal course of water deficits and the stomatal closure point at the time of treatment for the Cannock Chase data for the period 1975-1976 might be expected. The absence of any significant correlations between RWC of trampled shoots and stomatal closure point at the time of treatment does not substantiate the hypothesis but the role of environmental conditions as they affect shoot RWC complicate the relationships discussed above. The hypothesis is therefore not totally invalidated.

A discrepency also exists between the two sites in terms of the threshold at which further decrease in RWC is reduced. It is not possible to define a threshold for the Kerloch site since the trampling pressures applied were not great enough to yield such a threshold, if such a saturation of the response occurs. Although the damage to the plant may be related to the number of impacts it receives, it will also be related to the morphological, anatomical and physiological state of the plant at the time of treatment, for example a plant with a rigid stem is liable to suffer greater damage than one with a pliable stem, while plants with procumbent stems may suffer less than ones with upright stems. A thick litter layer, as at the Kerloch site, may cushion the effects of trampling whilst a dense community structure may help buffer the plant against damage. Such factors may account for site differences in response since at the Cannock Chase site the community is open and the substrate harder than at the Kerloch site. Conditions conducive to high rates of evapotranspiration, e.g. the open community at the Cannock Chase site, may result in RWC's being lowered to the stomatal closing point despite less severe damage than for a plant more heavily trampled but under a lower evaporative demand. At low levels of tissue damage supply may counterbalance water loss to a greater extent resulting in less severe water deficits from forming. Under such conditions, a higher level of damage resulting in a higher loss rate, may be required to lower the RWC to the stomatal closure point. Canopy structure may influence such a response. Barclay-Estrup (1971) observed that in the building phase of the <u>Calluna</u> cycle, the development of a dense canopy results in smaller temperature ranges, lower vapour pressure deficits and slower air speeds than in the degenerate phase. The Kerloch site corresponds to the building phase but the Cannock site, although of similar age, is of a structure comparable to the degenerate phase which may enhance evaporative losses from the plant, so that given comparable levels of damage, and despite destruction of the canopy structure on Kerloch, loss rates may be greater at the Cannock Chase site because of environmental effects.

(iv) General Conclusions

Measurements of plant ψ_x and RWC of C. vulgaris indicate that, following trampling, severe water stresses develop within the plant, Internal and external mechanical damage to the plant might be expected to alter plant resistances to water supply and loss, consequently contributing to the development of water stresses within the plant. The development of water deficits occurs comparatively rapidly following trampling and may be defined as an 'acute' response, although the rapidity with which such deficits develop will depend on the environmental conditions at the time of treatment, and possibly the anatomical, morphological and physiological characteristics of the plant. The increased water deficits associated with trampled shoots may arise from a reduction in water supply to the leaves as a result of stem damage and/or decreased stomatal control over water loss, possibly due to increased cuticular transpiration. Although there is some evidence suggesting that plants may adapt to reduce the severity of the stress, the effects are still apparent six months after treatment. This long term effect may be referred to as a 'chronic' response by the plant to trampling. There is a tendency for increased levels of trampling pressure to result in lower RWC's, although some evidence suggests that the minimum value is controlled, to some extent, by the stomatal closure point. Superimposed upon this relationship is the effect environmental factors, physiological factors and canopy structure may have upon water loss from the plant. Data from the two sites suggests that a high canopy density may be beneficial in retarding water loss. Seasonal change in environmental and physiological variables implies that the effect of a constant intensity of trampling applied at different times of the year may vary, as suggested by the time x treatment interactions observed between treatments. In terms of plant water relations, this suggests that the susceptibility of C. vulgaris to trampling may vary seasonally.

3 The Relationship between RWC and ψ

(a) Introduction

The use of RWC as an indirect determinant of ψ_1 was suggested by Weatherley & Slatyer (1957), and has been used extensively in a number of species, e.g. for Pinus sylvestris, Picea abies, Betula verrucosa, Populus tremula (Jarvis & Jarvis, 1963b); Cornus florida (Knipling, 1967); Gossypium hirsutum (Jordan & Ritchie, 1971), Calluna vulgaris, Erica cinerea and Vaccinium myrtillus (Bannister, 1971); Atriplex polycarpa and Salsolla vermiculata (Sankary & Barbour, 1972a, b); Pinus sylvestris (Hellkvist, 1973); Brassica oleracea var. acephala (Pospisilova, 1973a, b); Eucalyptus viminalis (Ladiges, 1974); Picea sitchensis (Hellkvist et al., 1974).

Millar et al. (1968) used the term 'Leaf Moisture Characteristic Curves' (L.M.C.C.) to describe the relationship between RWC and ψ in Hordeum vulgare and followed the effects of age, environmental conditions and growth stage on the relationship. Weatherley & Slatyer (1957) observed that "80% relative turgidity in different species might mean quite different degrees of 'water stress expressed as the diffusion pressure **deficit**' * of the leaves". Thus, a ψ of -3.04MPa represented

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* Diffusion pressure deficit = $-\psi$.

an RWC of 0.5 in Lycopersicum esculentum and 0.7 in Ligustrum japonicum, indicative of differential cell water relationships. Jarvis & Jarvis (1963b) commented that any factor affecting the π of the plant will have repercussions on the RWC/ψ relationship. This suggests that it is desirable to determine L.M.C.C.'s which are specific for the experiment, season and species.

Jarvis & Jarvis (1963b) considered that the magnitude of ψ which is able to develop without tissue damage occurring reflects the degree of drought hardening which has occurred, since it indicates the plant's ability to obtain water, the lower the ψ, the greater its capacity to obtain water from the soil. A seasonal effect on the RWC/ψ relationship may be of importance when considering the survival potential of the plant over the course of the year. Knipling (1967) found that in addition to environmental effects on the relationship in Cornus florida, a seasonal change, paralleled by a change in the dry weight/fresh weight ratio and the π, occurred. He suggested that cell wall elasticity decreased with leaf age due to an increase in dry matter with concomitant effects on the RWC/ψ relationship. When cell walls are thin and elastic, a given loss of water will result in a given volume decrease, so maintaining the turgor pressure on the cellular contents. When the cell walls are thick and inelastic there will be little decrease in cell volume and the pressure exerted on the inside of the cell wall will be reduced. Also, in terms of total plant ψ, an increase in cell wall material will affect the matric potential, thereby affecting the relationship between RWC and ψ. The data of Millar et al. (1968) supports the effects of ageing and environment on the relationship. Hellkvist (1973) also noted that ψ may be affected by decreases in cell wall elasticity, increases in dry weight/fresh weight ratios and increasing resistances to water transport associated with secondary thickening.

Jordan and Ritchie (1971) observed that, in Gossypium hirsutum, changes in RWC lagged behind changes in ψ, the latter falling more quickly than the former. The highest ψ was observed by 2400 hours (midnight) whereas maximal RWC's occurred at sunrise. Diurnal changes in RWC and ψ_1 revealed a closed hysteresis loop in which the desorption portion was linear whereas, after initial uptake, the absorption portion was curvilinear, resulting in an appreciable differential between the two curves. The difference was ascribed to differential resistances existing between water flow to evaporation sites within the leaf, affecting desorption, and water flow to leaf mesophyll tissue affecting absorption. Jordan & Ritchie also suggested that the decrease in transpiration in the afternoon enables a greater proportion of the water absorbed by the plant to be available for rehydration of the cells. If this is a widely occurring phenomenon it is important to know, when determining ψ_1 from RWC, whether the tissue is in the desorption or absorption portion of the curve.

The shape of the L.M.C.C. is also of interest. Knipling (1967) found that each 0.1MPa decrease in ψ was associated with a 0.5 - 1.5% decrease in RWC until a 'critical point' was reached at which the decrease in RWC became 3-4% per 0.1MPa. This continued until, as RWC approached zero, ψ tended to infinity. This was explained as the result of an abrupt change in cell wall elasticity. Jarvis & Jarvis (1963b) similarly found a curvilinear relationship in which a critical value was obtained, although a given decrease in RWC was accompanied by a greater decrease in ψ. Bannister (1971) found nearly linear relationships between RWC and ψ in three heath species, but he observed a difference between sun and shade plants. In Calluna vulgaris a small change in ψ over the range 0 to -0.5MPa was accompanied by a larger decrease in RWC of sun than of shade plants but at low ψ the trend was reversed. It was suggested that if apoplastic water buffers the protoplast and is lost with greater ease than symplastic water, plants with thicker cell walls might be expected to exhibit larger reductions in RWC at high ψ than thin walled cells. Sun plants were found to have greater dry to fresh weight ratios, possibly indicative of thicker cell walls, and possibly accounting for differences between sun and shade plants. Such an argument may also apply to seasonal changes in the RWC/ψ relationship.

Hodges & Lorio (1971) also observed that a given decrease in RWC was accompanied by a slight decrease in ψ until a critical point was reached, after which a given decrease in RWC resulted in a larger decrease in ψ. One of the implications of such a relationship is that species, and seasonal differences, in the ability to maintain a certain moisture stress at various levels of RWC may exist. However, as Bannister (1971) noted, slight differences in RWC may be converted into large differences in ψ. Thus, seasonal differences in RWC may be translated into differing degrees of stress due to seasonal changes in the RWC/ψ relationship. Sankary & Barbour (1972a), for example, working with Atriplex polycarpa found, in agreement with Hodges & Lorio (1971), a relationship which varied with age and environment. For drought hardened plants a ψ of -2.2MPa was equivalent to -1.6MPa for normal plants in terms of stress, and shedding of winter foliage occurred at -4.8MPa to -5.5MPa in six month old plants whilst mature plants retained 20-40% of the winter foliage despite a ψ of -5.0MPa.

Pospisilova (1973b) similarly found the RWC/ψ relationship to vary with leaf age, the younger and older leaves exhibiting a near linear relationship in contrast to the curvilinear one of the middle leaves. The latter relationship was similar to that of Knipling (1967) in which, after a critical point, a large decrease in RWC was accompanied by a small decrease in ψ. The youngest leaves were observed to possess lower ψ's at a given RWC than the older leaves in the middle and lower portions of the plant. Pospisilova regarded this as beneficial in terms of enabling the young leaves to retain a water supply during periods of stress since their ability to draw water would be greater. However, this conflicts with Knipling's (1967) suggestions regarding the effects of cell wall elasticity on the relationship between RWC and ψ since young leaves might be expected to have the shallowest slope for the L.M.C.C.

Two types of L.M.C.C. may be identified:

(1) Type 1 curve: an initial decrease in ψ is accompanied by a slight decrease in RWC until a critical point is reached at which the same decrease in ψ represents a greater decrease in RWC;

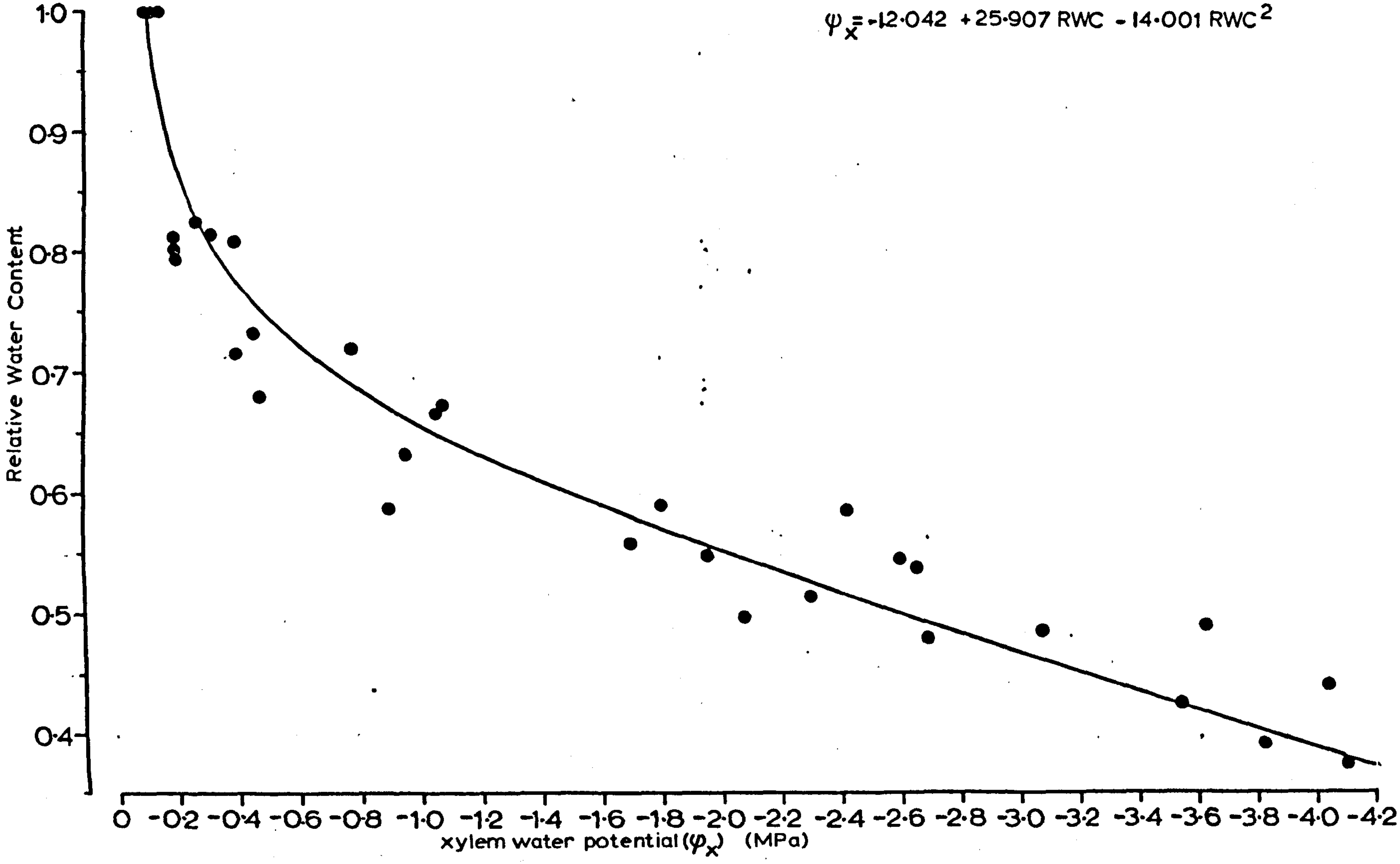
(2) Type 2 curve: an initial given decrease in ψ is accompanied by a large decrease in RWC until a critical point at which a given decrease in ψ results in a slight further decrease in RWC.

(b) Methods

The methods employed in the present study are similar to those employed by Ladiges (1974) in a study using *Eucalyptus viminalis*.

Shoots of *C. vulgaris*, sampled from Cannock Chase, were allowed to become fully saturated according to the method described above (Section IIIB, 2(b)). After determining the saturated weight, shoots were allowed to desiccate under standard conditions (25 ± 1°C, 40% relative humidity, 13klx light intensity). At random time intervals a shoot was removed, weighed to allow the determination of its RWC, and placed in the pressure chamber to determine ψ_x. The sampling interval was

Figure 48 Relationship between Water Potential and Relative Water Content in C. vulgaris. (Cannock Chase 31.07.75.).



determined by the time taken for the previous shoot to come to its balancing pressure. A rate of pressure increase of approximately 0.07MPa s^{-1} was employed but at low ψ, the pressure increase was 0.1 - 0.2MPa s^{-1} to within 0.5 - 1.0MPa of its estimated ψ, at which point the lower rate was employed.

To avoid removing leaves and possibly upsetting the RWC/ψ relationship, shoots were chosen such that the internodal distance was sufficient to allow the stem to pass through the chamber compression gland and seal without mutilation. This required a length of approximately 1.5cm., allowing 0.1cm. to protrude for observation. A binocular microscope (x 10 magnification) was used to determine the appearance of the meniscus at the cut end of the shoot.

No shoot had more than three determinations carried out on it, and care was exercised to regulate pressure release to prevent undue cooling of the shoot (cf. Section IIIB,1 (b)).

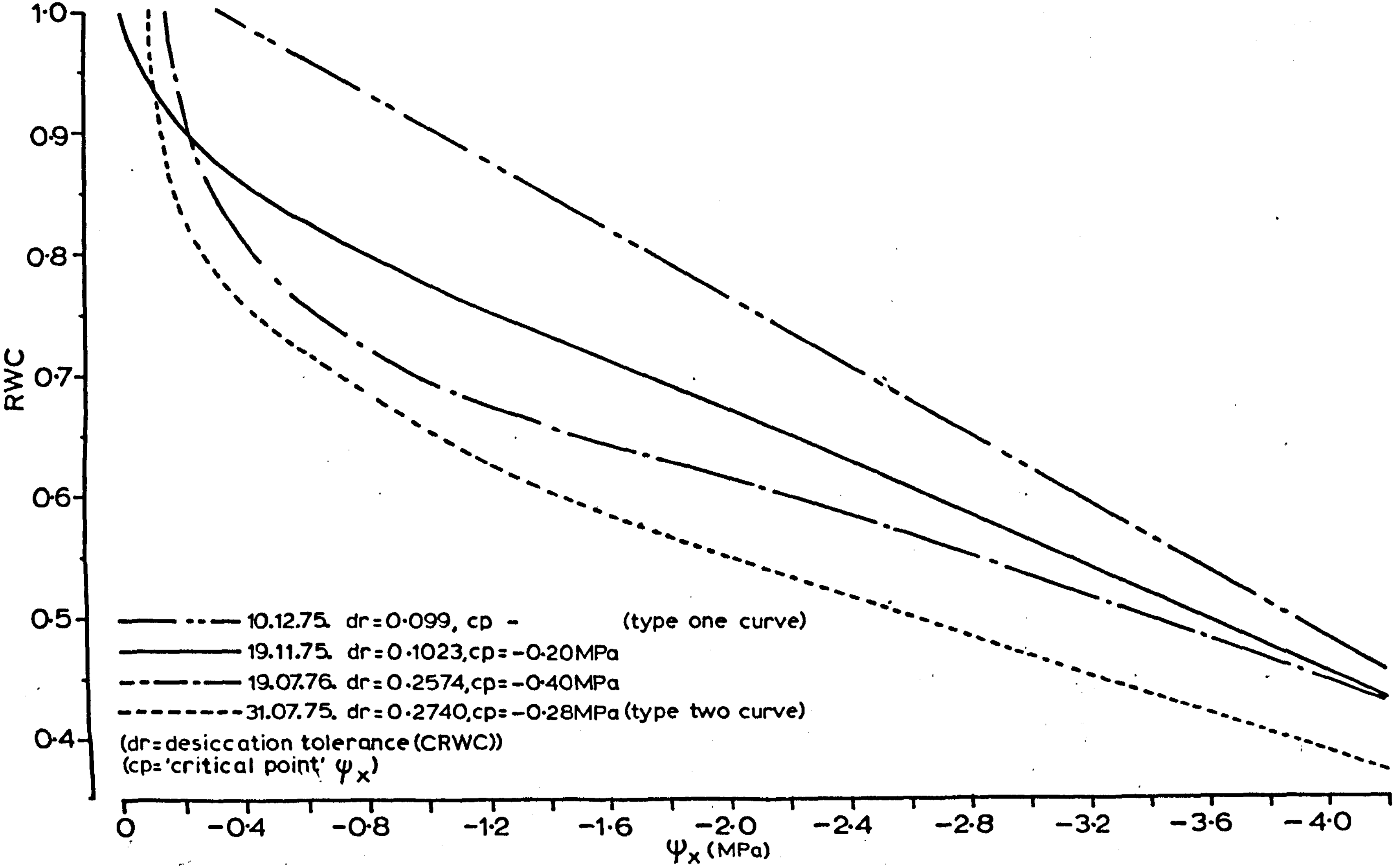
Polynomial regression analysis (Appendix A 2(b)) was performed relating RWC to ψ_x (Appendix B, table 6). These equations have been employed to attach ψ values to measured RWC parameters.

(c) Results

A typical curve relating RWC to ψ_x (Figure 48) indicates that an initially large decrease in RWC is accompanied by a relatively small decrease in ψ. After a 'critical point' of c. -0.4MPa (0.8RWC) a greater decrease in ψ per unit decrease in RWC occurs (type 2 curve). This indicates that with decreasing water content there is a disproportionate decrease in ψ representing a greater stress for the plant.

Not all relationships were found to be curvilinear (Figure 49) (cf. Appendix B, Table 6). In certain instances the regressions are

Figure 49 Seasonal differences in ψ_x - RWC relationships in *C. vulgaris* (Cannock Chase).



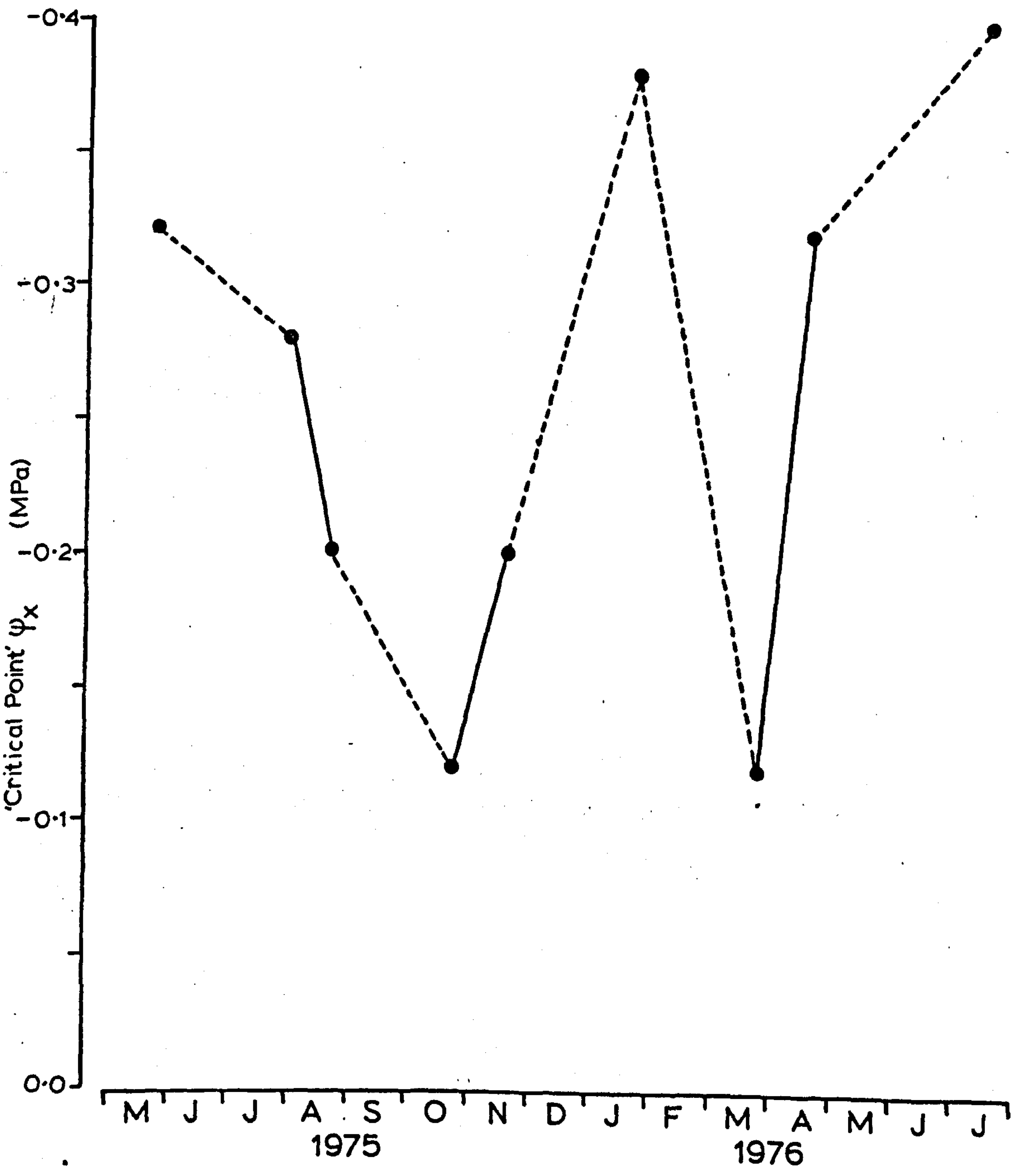


Figure 50 Seasonal variation in the 'Critical Point' ψ_x, C. vulgaris. (Cannock Chase).

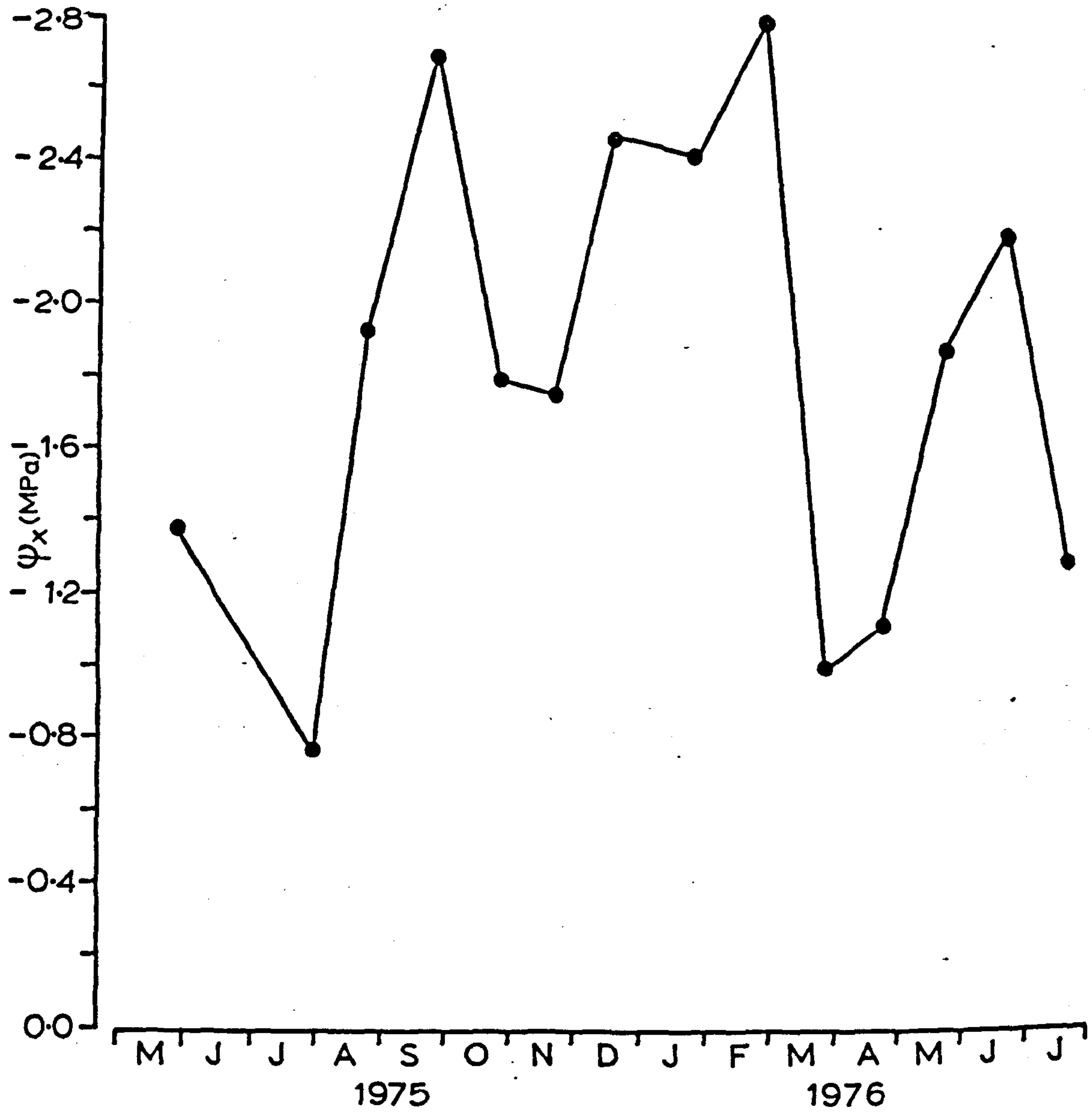


Figure 51 Seasonal variation in ψ_x at a constant RWC (0·7) in <u>C. vulgaris</u> (Cannock Chase).

linear, e.g. December 1975, whilst in others the non-linearity is not marked, e.g. November 1975. There is a tendency for increasing curvilinearity to be associated with decreasing desiccation tolerance (Figure 49).

In instances where it is possible to define a 'critical point' the appropriate ψ_x has been extracted (Figure 50) although no clear trend emerges in the data. A similar erratic pattern emerges when the ψ_x corresponding to an RWC of 0.7 is plotted (Figure 51). The range of values, from -0.76MPa to -2.78MPa, may be an artifact of the method or an error incurred between monthly determinations. Superimposed upon the erratic variation in ψ at a constant RWC is a general trend for a decrease in ψ_x toward the end of the growing season and over the winter period although the data for October, November 1975 are comparatively high, whilst the data for May and June 1976 are comparatively low.

(d) Discussion

The relationship between RWC and ψ_x indicates that the two parameters differ in their sensitivity as indicators of water stress, the former being more sensitive at low levels of stress, the latter at high levels of stress. As a consequence of this type of relationship, small variations in RWC derived parameters i.e. stomatal closure point, desiccation tolerance, may be translated into greater variations in terms of ψ, (cf. Bannister, 1971) which may reflect the stress situation more accurately. It might also be noted that an RWC of 1.0 does not correspond to a ψ of 0.0, which may be due to inaccuracies in the pressure gauge, or internal resistances to water flow in the shoot.

The decrease in ψ_x, at a constant RWC, over the course of the year accords with the findings of Knipling (1967) and may be due to an increase in dry to fresh weight ratio (Section IIIB, 4(c)), and possible associated decreases in cell wall elasticity. Hellkvist et al. (1974) argued that water in the plant is distributed in at least two compartments, the cell walls (apoplast) and the cell vacuoles, each with a specific RWC/ψ relationship since water is retained by different forces, i.e. matric and osmotic potentials, in the respective compartments. Changes in cell wall composition and hygroscopicity, indicated by increases in the 'dry weight fraction' and bound water content of the plant (Knipling, 1967; Hellkvist et al., 1974) may increase the matric volume of water, leading to a "...depression in water potential at a particular relative water content, below that expected for a single phase system of water in solution..." (Hellkvist et al., 1974). This reflects the greater pressure required to remove a given proportion of the water content to yield a specific RWC.

Jarvis & Jarvis (1963b) noted that a decrease in π is accompanied by a decrease in the ψ for a given RWC. Although π was not measured in the present study, the increase in soluble sugar content of C. vulgaris during the winter months (Grace & Woolhouse, 1970) may result in a lowering of π and consequently a decrease in ψ, which may contribute toward the general seasonal trend in ψ_x at a specific RWC. The erratic nature of the graph may result from a shedding of plant organs which might be consequential in determining the ψ_x in relation to RWC. The relationship between the volume of xylem elements and the supply of water to the cell might also be upset as a consequence of the inactivation of vessels, which might also have repercussions on the RWC/ψ_x relationship. These postulates, however, require experimental verification.

The seasonal variation in the RWC/ψ_x relationship is due to a shift in the curves and may be of adaptive value to the plant. Jarvis & Jarvis (1963b) discerned a range of such curves from those in which a large amount of water is lost for a small decrease in ψ (type 2 curves) to those in which a large decrease in ψ is accompanied by a small volume loss (type 1 curves). A similar range is observed in the present study. The possession of a type 1 curve will enhance survival at times of severe moisture stress since a small water loss will establish a relatively large ψ gradient, enabling the plant to obtain water with greater ease. In less stressful conditions, type 2 curves will be of greatest advantage by permitting the plant to lose relatively large amounts of water, which can be replenished relatively easily, without inducing damaging ψ in terms of cell survival. Jarvis & Jarvis (1963b) concluded that the type of curve a plant exhibits "is a compromise depending on its environment, between these two conflicting requirements". The gradation from type 1 to type 2 curves is paralleled by a decrease in desiccation tolerance, which, itself, is related to the growing season. The trend, although not consistent, suggests that at the height of the growing season the plants are able to lose appreciable quantities of water before an appreciable, and possibly damaging ψ develops. When desiccation tolerance is high, environmental conditions are not favourable for growth and water may be a limiting factor, an adjustment may occur enabling the plant to maximise the soil to plant water gradient while minimising water loss. However, set against this is the necessity for high water contents during the growing season to enable extension growth (cf. Hsiao, 1973) and lower stomatal closure points in winter, when water may be limiting, which are conducive to maximising carbon dioxide uptake but may also lead to excessive loss of water.

It is evident that considerable elucidation of the relationships between water content, water potential, assimilation and growth is required. The amount of water a plant cell loses before the stress, in terms of water potential, becomes damaging to the cell would appear to be of some importance to the present study, since if it is not

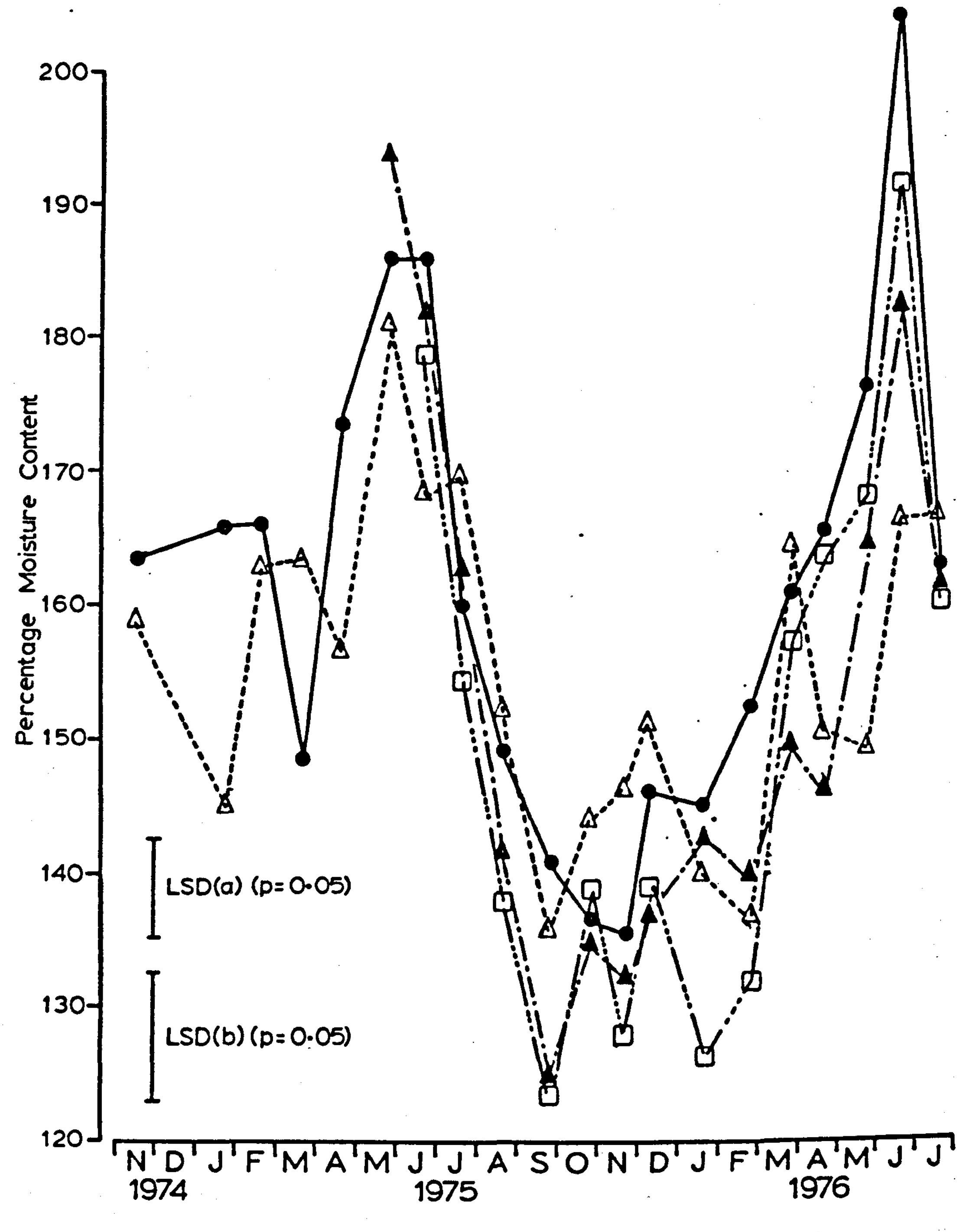


Figure 52 Seasonal variation in the Percentage Moisture Content of C. vulgaris, (Cannock Chase).

●——● control shoots (PMC0)

△- - - -△ shoots treated 1 month before sampling (PMC1)

▲—·—▲ shoots treated 3 months before sampling (PMC3)

□·····□ shoots treated 6 months before sampling (PMC6)

LSD(a) seasonal variation in control shoot Percentage Moisture Content

LSD(b) seasonal variation in and differences between Percentage Moisture Content of all treatments, June 1975 - July 1976.

constant, the susceptibility of C. vulgaris to water stresses arising from trampling may be variable.

4. Seasonal variation in the Saturated Percentage Moisture Content (PMC) of C. vulgaris

(a) Introduction

The ratio of saturated water content to dry weight, referred to as 'percentage moisture content' (PMC), may be used as an indicator of shoot 'succulence' (cf. Bannister, 1964b).

(b) Methods

Shoot weight parameters obtained from the measurement of RWC at the Cannock Chase site (Section IIIB, 2(II)) have been used to determine PMC according to the formula:

$$\text{PMC} = \frac{\text{SW} - \text{DW}}{\text{DW}} \times 100 \qquad (20)$$

Four time treatments may be identified, PMC of controls (PMC 0), PMC of shoots treated 1 month before sampling (PMC 1), PMC of shoots treated 3 months before sampling (PMC 3) and PMC of shoots treated 6 months before sampling (PMC 6). Each treatment, for each sampling date, consisted of four plots from which five shoots per plot were sampled, giving twenty replicates per treatments. The trampled plots were treated at a trampling pressure of 100p pl^{-1}.

(c) Results

Significant ($p < 0.001$) seasonal variation in PMC occurs (Figure 52) in addition to significant ($p < 0.001$) treatment and interaction effects. The seasonal pattern of variation is similar in all treatments, trampled plot PMC's being significantly correlated with PMC 0 ($r = 0.638$, $p < 0.01$; $r = 0.850$, $p < 0.001$; $r = 0.948$, $p < 0.001$ for PMC 1, PMC 3 and PMC 6 respectively).

The control shoots have a low PMC over the winter period, from September 1975 to February 1976, rising to a maximum summer value in June of both 1975 and 1976. This rise commences in March 1975 and February 1976, and following a summer peak, a significant decrease in July of both years is observed, decreasing to a winter minimum. Similar patterns of variation are shown by damaged shoots, although their PMC's generally lie below those of the control. The mean response for damaged shoots shows no significant difference from the control value of 153.1%, PMC 3 and PMC 6 (150.4% and 150.1% respectively) are significantly lower than PMC 1 which shows the highest mean value of 158.7%. The mean response obscures the observation that PMC 1 lies close to or above PMC 0 during the winter period but from April 1976 to June 1976 has a markedly lower value than PMC 0, a trend also shown by the data for 1975 but which is not so apparent.

The independent variables found to be significantly correlated with PMC 0 and PMC 1 (Table 14) indicate that daylength is the only environmental variable significantly correlated with PMC.

Five environmental parameters were selected for step-wise multiple regression analysis in an attempt to account for seasonal variation in PMC 0 (Table 15a). Together they account for 74.9% of the variation in the data but only daylength and accumulated temperature (base 7.2^{o}C, accumulated over the 7 days prior to sampling) make a significant contribution to the regression. Accumulated temperature alone is not significantly correlated with PMC 0 ($r = 0.333$,n.s.) and accounts for only 12.4% of the variation in the data compared to 58.4% accounted for by daylength. Although accumulated temperatures are related to

Table 15 Multiple Regression Analysis relating seasonal variation in the PMC of control (PMC 0) and trampled (PMC 1) shoots of *C. vulgaris* to Environmental variables (Cannock Chase)

Step	Variable	Significance to enter (p)	Multiple R	Multiple R^2	R^2 change	Simple R	Overall Significance (p)
(a)	PMC 0						
X_1	Daylength	< 0.001	0.7642	0.5840	0.5840	0.7642	< 0.001
X_2	Accumulated Temperature (7.2°C) over 7 days before sampling	< 0.05	0.8413	0.7078	0.1238	0.3331	< 0.001
X_3	Stem temperature (mean for 7 days before sampling)	n.s.	0.8564	0.7336	0.0258	0.3473	< 0.001
X_4	Vapour Pressure (mean for 7 days before sampling)	n.s.	0.8645	0.7474	0.0138	0.2809	< 0.001
X_5	Rainfall (total over 7 days before sampling)	n.s.	0.8659	0.7499	0.0025	0.3293	< 0.001
	Regression equation	$\hat{Y} = 67.7119 + 7.4085 X_1 - 0.2270 X_2$					< 0.001

Step	Variable	Significance to enter (p)	Multiple R	Multiple R^2	R^2 change	Simple R	Overall Significance (p)
(b)	<u>PMC 1</u>						
X_1	Daylength	< 0.01	0.6032	0.3638	0.3638	0.6032	< 0.01
X_2	Stem temperature (mean for 7 days before sampling)	n.s.	0.7048	0.4967	0.1329	0.1991	< 0.01
X_3	Vapour Pressure (mean for 7 days before sampling)	n.s.	0.7871	0.6196	0.1229	0.2318	< 0.01
X_4	Rainfall (total over 7 days before sampling)	n.s.	0.8269	0.6839	0.0643	0.1181	< 0.01
X_5	Accumulated temperature (7.2°C) over 7 days before sampling	n.s.	0.8272	0.6843	0.0004	0.2419	< 0.01
	Regression equation	$\hat{Y} = 122.7671 + 2.5677 X_1$					< 0.01

Table 14 Correlation coefficients (r) between PMC 0, PMC 1 and environmental and physiological variables, *C. vulgaris* (Cannock Chase)

Variable	PMC 0		PMC 1	
	r	significance	r	significance
daylength	0.764	<0.001	0.603	<0.01
DW/FW ratio	-0.917	<0.001	-0.580	<0.05
RWC after 8h of desiccation	-0.673	<0.01	-0.491	<0.05
Browning, 1 month after treatment related to time of treatment	0.544	<0.05	0.636	<0.01

season, they are imperfectly correlated with daylength ($r = 0.744$, $p < 0.001$) and thus the inclusion of the two variables in the regression improves the 'fit' obtained.

A similar analysis on PMC 1 (Table 15b) reveals a similar ordering of variables, although accumulated temperature is the variable accounting for the smallest percentage variation in the data (0.04%) and daylength, accounting for 36.4% is the only variable to make a significant contribution to the regression. Although the remaining four variables account for a further 32.0% of the variation, their individual contributions are not significant.

The significant correlation between DW/FW ratio and PMC 0, accounting for a high proportion of variation in the data (84.1%) is to be expected since PMC reflects water content per unit volume while dry to fresh weight ratio reflects the weight of structural material per unit volume. Although the two are related the latter does not take into account the

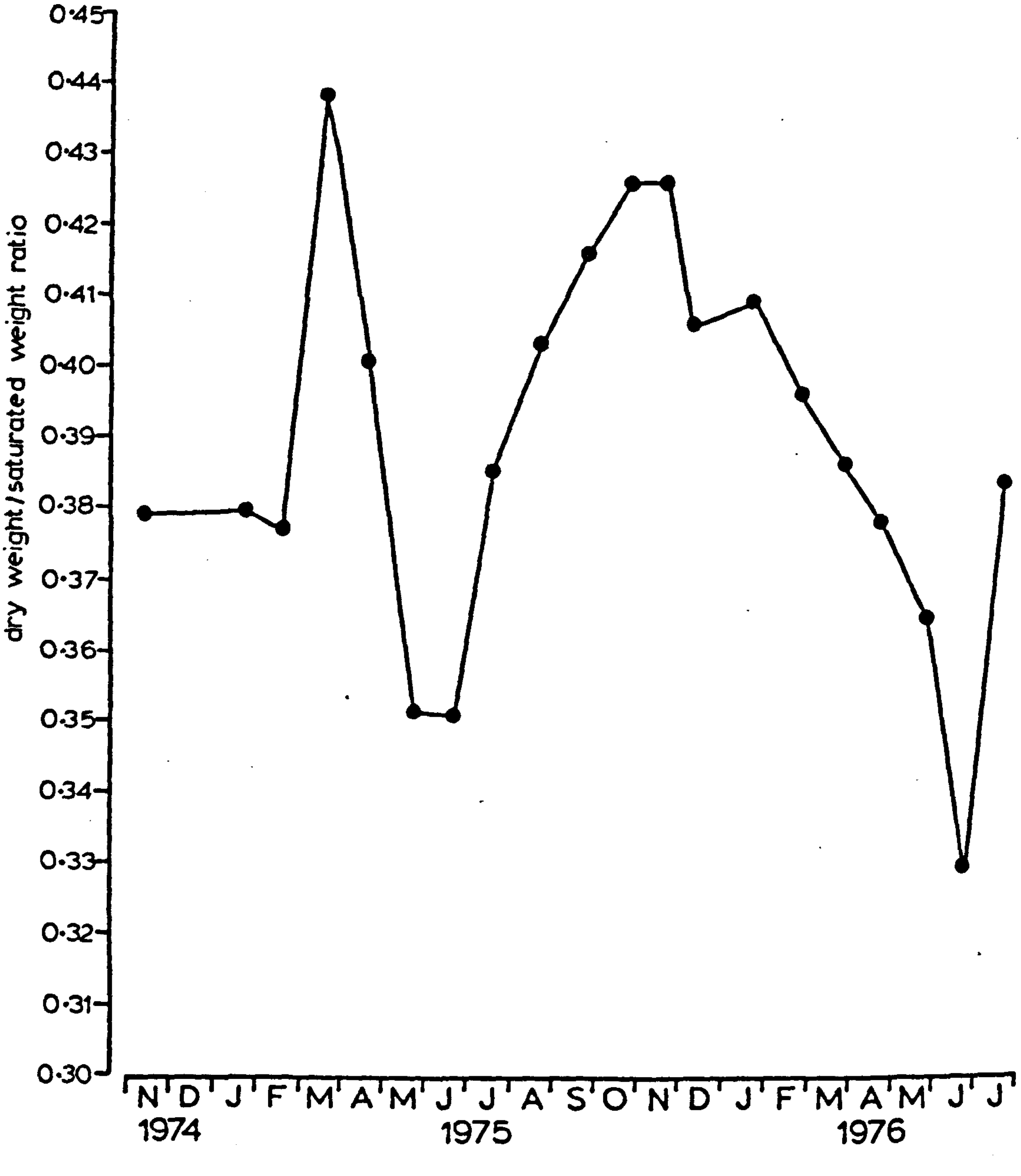


Figure 53 Seasonal variation in the dry weight / saturated weight ratio of <u>C. vulgaris</u> shoots (control), (Cannock Chase).

effect changes in cellular elasticity and water holding capacity might have on PMC, and which might be affected by dry weight changes per unit volume. Since the two parameters are related, the significant correlation between DW/FW ratio and daylength is not unexpected ($r = -0.702$, $p < 0.001$) but whereas with longer days PMC increases, DW/FW ratio exhibits a tendency to decrease (Figure 53). Seasonal variation in this quantity is not smooth and may be affected by the physiological state of the plant at the time of sampling, and the effect this has upon water holding capacity. However, the ratio is high over winter, indicating high dry weight per unit volume, possibly as a result of storage products and the increased structural materials content of older cells, while in the summer months the ratio decreases to a summer minimum in June of both years. Anomalies are present such as the high ratio in March 1975, although this may reflect the comparatively low values over the 1974-1975 winter compared to the 1975-1976 winter. The marked rise observed in July of both years parallels the marked and significant decrease in PMC between June and July of both 1975 and 1976.

(d) Discussion

The observations on PMC are consistent with the findings of Bannister (1964b) who, in addition, found a significant positive correlation between shoot RWC and their succulence. No such relationship was found in the present study, rather the trend is in the opposite direction ($r = -0.444$, n.s.) in which a rise in RWC is accompanied by a decrease in PMC. Bannister (1964b) attributed the rise in RWC and PMC to increased physiological activity coincident with new growth. Kozlowski & Clausen (1965) found that an increase in PMC of the buds of a number of gymnosperm and angiosperm species could be attributed to the rapid translocation of moisture into the bud. Since it is the balance between absolute water content and dry weight which determines

PMC, although dry weight increase in the buds was occurring, it did so at a slower rate than the increase in water content. In contrast, in Quercus ellipsoidalis they observed a decrease in PMC of the leaves which was attributed to a relatively greater increase in dry weight compared to water uptake rather than a significant decrease in absolute water content. They found this to be true in angiosperm leaves in the early part of the growing season whilst Polwart (1970) attributed the fall in PMC of Vaccinium vitis-idaea between August and October to dry weight increasing at a faster rate than increases in absolute moisture content.

The increase, observed in the present study, in PMC at the start of the growing season is consistent with the role water has in providing the necessary force for cell expansion (Hsiao, 1973), for although structural materials e.g. celluloses and hemicelluloses, are required for cell wall construction, together with lipids, proteins and carbohydrates for membranes and cytoplasm which will result in an increase in dry weight, water is a pre-requisite for cell expansion. Consequently, the spring flush of growth in May-June is associated with water uptake and an increase in water content of the tissues associated with vacuolation and elongation. Following this initial burst of growth elongation growth may give way to the build up of assimilates as reserves, and the direction of assimilates into seed production, thus leading to a more rapid increase in dry weight than in water content and a decrease in PMC, observed in July. Assimilation may continue when growth ceases (Grace & Woolhouse, 1970), leading to an accumulation of reserves for overwintering. The possible accumulation of sugars associated with the development of frost and desiccation resistances (Santarius, 1973) and the reduction in cell vacuolation (Iljin, 1957) may result in water content being at a minimum relative to dry weight, as reflected in low

PMC's. Kozlowski & Clausen (1965) found that PMC declined seasonally in gymnosperm needles of the current years growth but one year old needles exhibited an increase in PMC followed by relative stability. Bannister (1964b) and Polwart (1970) also observed differences in succulence between current and previous seasons tissues. Kozlowski & Clausen (1965) ascribed such differences to dry weight changes due to translocation of carbohydrates from old needles into new needles, thus altering dry to fresh weight ratios. In the present investigation, shoots were not partitioned into current and previous seasons growth but were composed of leaves with a wide age range. The relationship between such leaves, together with leaf death and litter fall (cf. Cormack & Gimingham, 1964) may affect the moisture content of shoots and may result in the minimum values recorded in September.

Although damaged shoots do not differ from control shoots in PMC to a great extent, the tendency for lower values, especially in summer, may reflect a reduction in growth potential. Plots treated one month before sampling exhibit a lower production of new shoots at the beginning of the growing season (Section IVB, 2) which may indicate an inability to obtain sufficient water to facilitate cell elongation or a reduction in the rate of assimilation due to water stress. The former is more sensitive to water stress than the latter, Acevedo, Hsiao & Henderson (1971) for example, found that short term water stresses of an hour could reduce shoot elongation, but following the removal of the stress, a rapid period of growth could compensate for any losses. This suggested that continued synthesis of new materials for growth occurred and that only the force to produce cell elongation was lacking. Although long term stresses may have an adverse effect on metabolic processes, continued assimilation in the absence of elongation by vacuolation may increase the dry/fresh weight ratio and lower the PMC, such an effect being most noticeable in the period of maximum elongation.[1] This is substantiated by the observations on damaged shoots, although the situation is confused by the length of period between treatment and sampling. It is necessary to relate the effect of trampling on PMC to the physiological state of the plant at the time of treatment. For example, in June 1976 the smallest difference between control and treated shoots occurs in shoots trampled six months previously, i.e. in January, when growth would be minimal, whilst shoots exhibiting the largest difference were treated at the height of the growing season i.e. July, August. Although the relationship is not consistent, e.g. large differences exist for shoots treated in March while small differences occur in shoots treated in May and June, there is a possibility that the ability to assimilate may affect the PMC of damaged shoots. When conditions are conducive to assimilation, dry weight may increase in the absence of cell elongation, resulting in a relative reduction in water content. When the plant is dormant, however, dry weight may not increase at the expense of water content and the difference in PMC of control and treated shoots becomes less. Obviously, these relationships may be modified by the effect water stresses have on photosynthesis, and the seasonal variations in such relationships. The significant time x treatment interaction observed, indicates that the effect trampling has upon PMC varies over time. This may be related to the magnitude of water stress developing within the plant and the variation in the effect of a particular stress on the physiological activity of the plant. The higher PMC's of recently treated material in winter compared to control values may reflect a

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[1] Tissue death, resulting from trampling induced desiccation, may upset the relationship between dry weight and saturated weight (from which PMC is calculated) by reducing the capacity of the plant to absorb water, resulting in a decrease in absolute water content relative to the dry weight giving rise to a decrease in PMC relative to that of undamaged plants.

reduction in the ability of treated plants to harden by the accumulation of soluble carbohydrates and the exclusion of free water from the cell, resulting in a high degree of succulence.

Any significant difference existing between PMC's of control and treated shoots may upset the determination of any RWC dependent parameters, making comparisons invalid. In addition, the determination of RWC's for damaged and undamaged shoots may be liable to an error associated with differences in PMC. The absence of a significant difference between the PMC of damaged and control shoots suggests that the significance of RWC and its dependent parameters remain unaffected.

The observations regarding the role of daylength and accumulated temperature in accounting for seasonal variation in PMC is consistent with the conclusion made regarding the influence of growth on PMC. As days lengthen and temperatures rise so growth is initiated while the increase in dry weight and the development of dormancy is associated with shorter days and lower temperatures. In contrast, in damaged material, while the seasonal progression as reflected in daylength remains the most significant factor accounting for variation in PMC 1, the removal of accumulated temperature from the regression suggests that the effects of water stress on growth, and thus PMC, may be outweighing the effects of temperature on growth, resulting in a relative reduction in PMC of damaged shoots during the growing season.

The significant correlation between PMC and visual damage, as reflected by browning, on trampled plots is also noteworthy. It suggests that the PMC at the time of treatment may have a significant effect on the damage occurring over the following month. In this context, the PMC of the control shoot is of most importance since it suggests that a high PMC at the time of treatment is related to a high degree of visual damage. This may be related to the state of the plant in terms of growth potential and resistance to desiccation, there being a tendency for the two to be inversely correlated.

The inverse correlation between RWC after an 8 hour desiccation period and PMC indicates that with an increase in PMC, a lower RWC over a standard drying period develops. This latter parameter reflects the water retaining potential of the plant, i.e. desiccation avoidance. While avoidance is related to stomatal closure, factors resulting in water loss following stomatal closure, e.g. cuticular transpiration, bound water related to osmotically active substances within the cell which is a reflection of desiccation tolerance, may be less effective in a less hardy plant, and although PMC is not significantly correlated with stomatal closure nor desiccation tolerance, high PMC's may reflect a greater physiological activity which increases the plants susceptibility to desiccation as reflected in desiccation avoidance, which is indicated by the RWC developed over a standard drying time.

(e) Conclusions

Percentage moisture content of C. vulgaris varies over the course of the year and may be related to the seasonal variation in the physiological activity of the plant, low values being associated with periods of low growth potential, high values being related to likely periods of maximum cell elongation, requiring maximum water contents. Trampling has an effect on shoot PMC which varies with the time of the year, although the difference in shoot PMC of control and treated shoots is relatively small. The tendency for treated shoots to exhibit a lower PMC than control shoots over the summer period may reflect an inability by the shoot to obtain sufficient water to facilitate cell elongation due to trampling induced water stresses, whilst in winter higher PMC's of damaged shoots may result from an inability to accumulate carbohydrate and exclude free water from the cell due to the effects of water stress on the assimilatory mechanism. This variation in response may arise from the differential effect of water stress on assimilation, and cell vacuolation and elongation, the former being less sensitive to water stresses. Damage to the plant arising from trampling, as indicated by PMC, would appear to be related to the physiological state of the plant at the time of trampling, the greatest difference between shoot PMC of control and trampled plants occurring when the plant is likely to be most physiologically active and susceptible to water stresses.

C. DESICCATION INJURY IN *C. VULGARIS* ARISING FROM TRAMPLING INDUCED WATER STRESSES

Introduction

A number of reviews have surveyed the extensive literature concerning the response of plants to drought, e.g. Parker (1956, 1969), Iljin (1957), Stocker (1960), Kramer (1963), Henckel (1964), Gates (1964, 1968), Crafts (1968), Levitt (1972), Hsiao (1973), Slatyer (1973a), Hsiao & Acevedo (1974), Sullivan & Eastin (1974), Hsiao *et al.* (1976). The present review summarises those aspects of drought injury of immediate relevance to the present study.

Loss of water results in (Hsiao *et al.*, 1976):

(1) reduction in the chemical potential of water;

(2) concentration of macromolecules and solutes;

(3) changes in spatial relationships of membranes and organelles through a reduction in cell volume;

(4) reduction in hydrostatic pressure inside cells.

The relative importance of the above factors will depend upon the degree of stress the plant is under. Under mild stress, a reduction in hydrostatic pressure is likely to be the main effect, resulting in a reduction in cell elongation (Hsiao & Acevedo, 1974; Hsiao *et al.*, 1976). In contrast, the 'Reaction - Restitution' hypothesis of Stocker (1960) involved the biochemical aspects of drought injury. The structural integrity of the protoplasm depends upon intra- and inter-molecular bonds. Since the globular macromolecules depend upon water for their stabilisation, dehydration may be expected to lead to protoplasmic shrinkage and structural change. The 'reaction phase' is one in which disruption of the protoplasmic structure, as a result of dehydration, results in metablic disorganization. This is followed by a 'restitution phase' in which the protoplasm is restructured and restablized, ultimately leading to increased metabolic productivity. Stocker associated the notion that enzymic processes are oxidative-hydrolytic in the first phase and reductive-synthetic in the second with his two phase theory.

Iljin, in a series of papers in the 1930's (reviewed by Levitt, 1972) considered that mechanical tensions resulting from water loss, resulting in destruction of bonds and tearing of protoplasm, were the main cause of desiccation injury. His 'Mechanical Stress' theory proposed that on drying, rigidity of the cell walls opposed the shrinkage of the protoplast, resulting in tensions within the cell, which could ultimately result in tearing. If cell walls were flexible, as the cell dried the walls would fold and prevent damage occurring. The tensions generated would depend on the quantity of water lost, for example, large cells decrease in volume 5-10 times on desiccation in contrast to 1.8-2.0 times in small cells, consequently, disturbance will be greater in the former (Iljin, 1957). On rehydration similar damage may occur through too rapid distension of the membranes and protoplasm, since severe desiccation may lead to increased protoplasmic viscosity (Iljin, 1957). Iljin also observed that gradual drying had less severe effects than rapid desiccation. A similar conclusion was reached by Stocker (1960) who observed, in experiments on Lamium maculatum, that when desiccation was slow no reaction phase occurred because restitutional processes increased the hardiness of the plant from the time dehydration commenced, and kept pace with desiccation. Obviously the time scales are relative. Hsiao & Acevedo (1974) stated that water stresses usually develop slowly, changes requiring several hours being gradual relative to molecular events in the cell. However, Stocker (1960) used desiccation periods of 26 hours and 6 days for fast and slow drying in terms of soil drought.

Both concepts may be valid, depending upon the aspect under consideration. For example, responses occuring too rapidly to be mediated by metabolic disruption e.g. elongation, will be affected by desiccation over a few hours (Hsiao & Acevedo, 1974) while metabolic disruption may only result after longer periods of desiccation. Stocker (1960) observed that a sudden decrease in protoplasmic viscosity occurred at a lower soil moisture value if soil drying was rapid than if it was slow. However, although the threshold stress values at which a plant parameter might be affected depend upon the rapidity of desiccation, the relative sensitivities of physiological processes to desiccation are likely to remain the same (Hsiao, 1973; Hsiao et al., 1976).

The mechanism of drought injury is controversial. Levitt, dubious of Stocker's 'Reaction - Restitution' hypothesis, and of the opinion that Iljin's theory required elucidation in terms of molecular concepts of drought injury, proposed the Sulfhydryl or Protein Aggregation hypothesis in which irreversible denaturation could result from the formation of intermolecular bonds between 'sensitive groups' e.g. sulfhydryl groups exposed by cytoplasmic shrinkage and unfolding of protein molecules. An increase in sulfhydryl content of free chloroplasts has been observed upon desiccation (Santarius & Heber, 1967) suggesting unfolding of the molecules leading to exposure of sulfhydryl groups. Santarius (1969) suggested that an increase in the concentration of toxic electrolytes in the cytoplasm as a consequence of desiccation may cause protein injury.

Stocker (1960) considered that a general 'physiological resistance' to water, cold, heat and salts, to which Levitt appears to subscribe, is of dubious validity. However, certain stresses may be related in the effects they have on the plant, e.g. desiccation and frost resistance. The mechanism resulting in increased resistance to one stress may result in increased resistance to other stresses, e.g. frost, drought and heat resistance may be increased as a result of the increase in cryoprotective compounds (Santarius, 1973).

In addition to the direct effects of dehydration on protoplasmic structure and composition, drought resistance may also include the ability to withstand overheating which may modify the colloidal-chemical properties of the protoplasm and consequently metabolism (Henckel, 1964). High plant temperatures may result from reduced transpirational cooling due to stomatal closure at high water deficits. This may cause protein denaturation, destruction of protoplasmic structure and possibly ammonia toxicity owing to protein breakdown. This might ultimately result in a disruption of physiological processes and the effects may be manifested as brown 'scorch' marks on the leaves (Henckel, 1964).

Desiccation may also affect the chemical composition of the plant. Structural cause and functional effect are linked through enzymatic activity (Stocker, 1960). Disruption of the synthetic machinery,rather than protoplasmic coagulation, is considered by some to be the chief cause of drought injury e.g. Todd & Basler (1965). Drought resistant tissues may have a reduced hydrolytic enzyme activity (Crafts, 1968) while the hydrolytic tendency of Stocker's 'reaction' phase may be reflected in a tendency for starch to be hydrolysed to sugar, which may increase the plants' resistance to drought (Section IIIA, 1(a)). However, in water stressed plants proteolytic enzyme activity may increase, resulting in the liberation of free amino acids, which upon deamination yield toxic ammonia (Crafts, 1968). Protein synthesis has been observed to be inhibited by water stress, for example, Barnett & Naylor (1966) found that proline accumulated in stressed plants. They suggested that slow proline turnover may reflect inhibition of proline anabolism and that proline may act as a storage compound for carbon and nitrogen when protein synthesis is inhibited. Levitt (1972) suggested that proline may be able to accumulate without causing injury. Consequently, the increase in amino acid concentration may not result from protein breakdown but rather a retarded protein synthesis leading to a pool of reserves which can be immediately utilized following rewatering e.g. Barnett & Naylor (1966), Morris et al. (1969), Singh et al. (1973a,b,c).

Water stress has an adverse effect upon the protein synthesizing apparatus, the polyribosomes. Hsiao (1973) considered that protein synthesis, together with photosynthesis, were among the earliest metabolic processes to be affected by water stress. Changes in nucleic acid content have also been observed with desiccation (Shah & Loomis, 1965). Slatyer (1973a) noted that RNA synthesis may be impaired and degradation enhanced prior to the water stress becoming visibly apparent. This will have repercussive effects on protein synthesis so that enzymes with a high turnover rate may rapidly decline when protein synthesis is retarded by water stress (Hsiao & Acevedo, 1974). Gates (1964) considered that the relationship between the structure and function of a protein and its water envelope may, in drought tolerant plants, enable processes to be suspended and resumed with minimal damage; under such conditions water acts as a medium by which macromolecules may be co-ordinated and thus plays a more than passive role in unifying the response of the plant.

In addition to considering the effects of desiccation at the molecular level of organisation, it is pertinent to consider the effects at the process level. Stocker (1960) postulated that respiration would increase in reaction to water stress, followed by a decrease in the restitution phase, so that during hardening it falls below the normal rate. Brix (1962) found that the respiration rate of Lycopersicon esculentum decreased with a decrease in ψ but the respiration rate of Pinus taeda rose to peak at low ψ's, following an initial decrease with increasing water stress, after which the respiration rate exhibited a sudden decline with a further decrease in ψ. Although the relationship between water stress and respiration may be contradictory, Hsiao (1973) stated "Recent data demonstrate that dark respiration is generally suppressed, more or less proportionately but not very markedly, by moderate to severe stress".

The response of photosynthesis to water stress (discussed more fully in Section IVA, a(i)) is, in some respects, a little clearer, since it is almost universally accepted that photosynthesis declines with increasing stress, although very mild stresses may enhance CO_2 uptake. Crafts (1968) enumerated three possibilities for the cause of decreased assimilation:

(1) hydroactive stomatal closure;
(2) water stress in the cytoplasm affecting enzyme activity;
(3) dehydration of cuticle, epidermal walls and cell membranes, increasing their resistance to CO_2 diffusion.

The relative importance of these mechanisms is controversial. Hsiao (1973) noted that an almost unanimous opinion exists that stomatal closure impedes CO_2 uptake and is the main cause for reduced assimilation when assimilating tissues are water stressed. Slatyer (1973b) considered this to be the primary reason until severe stresses exist. This is corroborated by the observations of Santarius & Heber (1967) who found that phosphorylation and electron transfer (in vitro) were not affected until severe stresses occurred. Similarly, Troughton (1969) considered that r_m (Option 3 above) was largely independent of RWC in Gossypium hirsutum until an RWC of 0.75 was attained, at which point the value of r_m increased from 2.5 to $12 s.cm^{-1}$, over the RWC range 0.75 to 0.56. However, it was emphasised that before r_m increased, the rate of photosynthesis decreased from 13.9×10^{-2} to $3.0 \times 10^{-2} \mu g CO_2 \, cm^{-2} s^{-1}$, leading to the conclusion that changes in r_m were of only minor significance. Hsiao (1973) considered that although non-stomatal effects of water stress on CO_2 assimilation can be demonstrated, care must be exercised in interpreting such effects.

Duration and severity of desiccation may also affect the response, via the degree to which restitution occurs. In a study on Caltha palustris, drying to 70% of its original water content resulted, after recovery, in an increase in respiration to its pre-stress level but photosynthesis was restored to only 80% of its former value, whilst drying to 59% of its water content resulted in irreversible damage to both processes (Stocker, 1960). For plants subjected to a short, mild stress recovery may occur within a few hours but for plants subject to prolonged or severe stress, recovery on re-watering may take a number of days (Hsiao, 1973). Stocker (1960) considered that, providing the duration or severity of desiccation does not exceed a critical value, the tensions and deformations in the protoplasm may remain latent, but owing to the lability of proteins, increasing duration of desiccation will result in the increasing disrupture of the protein and thus the protoplasmic structure of the plant.

Associated with assimilation is the effect water stress has upon assimilate translocation. Zholkevich, Prusakova & Lizandr (1958) considered that inhibition of translocation by water stress may result in the accumulation of sugars which in turn would reduce the rate of photosynthesis, (although the role of sugars in controlling photosynthesis is controversial (cf. Neales & Incoll, 1968)). Experiments by Wardlaw (1969) on Lolium temulentum indicated that such a reaction was not part of the photosynthetic response to water stress although mild stress might reduce growth which could lead to the accumulation of assimilates at source. Wardlaw noted that although transport of assimilates to the phloem may be retarded by water stress, once in the phloem the materials are readily transported. Thus, observed effects of water stress on phloem transport are likely to be due to the effects of stress on photosynthesis, loading of assimilates into the conducting system and growth rather than a direct effect on the phloem. A corollary of this is that if sugars are important in protecting the plant against desiccation, areas distant from the site of assimilation which will be dependent on adequate sugar translocation for protection, may not have their desiccation tolerance directly impaired by the effects of stress on the phloem. However, Wardlaw noted that the protective effect of sugars on the photosynthetic apparatus (cf. Santarius & Heber, 1967) may imply a reduction in transport of sugars from the site of synthesis, especially if water stress directly impairs the photosynthetic machinery, in order to maintain the integrity of the assimilitory apparatus. Thus, the equilibrium between retention and transfer may differentially affect the response of the various parts of the plant to desiccation.

The balance between photosynthesis, translocation and respiration, as they are affected by water stress, may result in 'starvation' effects (Levitt, 1972). Although drought injury often occurs when appreciable reserves exist within the plant, prolonged stress may result in reserve depletion. Also, due to the often more rapid recovery of respiration rate than of the rate of photosynthesis, a negative assimilation balance may result on recovery (Levitt, 1972).

Ultimately, the effects of water stress will become apparent on growth, although cell growth is probably one of the most sensitive of all processes to water stress (Hsiao, 1973). Stocker (1960), in reviewing the observations of earlier workers, noted a number of morphological and anatomical adaptations to drought. Physiological adaptations facilitating growth may also occur. The role of turgor pressure in elongation growth, and the high sensitivity of such a process to water deficits has been reviewed by Hsiao (1973), Hsiao & Acevedo (1974) and

Hsiao et al. (1976). Changes in soil water potential may induce an almost instantaneous reaction in leaf elongation at a rate too rapid to be metabolically mediated (Hsiao & Acevedo, 1974). Although growth is proportional to the gross extensibility of the cell, which is metabolically dependent, turgor pressure provides the force necessary to extend the cell. The loss of a small amount of water may reduce the turgor pressure potential (ψ_{tp}) and thus have an adverse effect on cell growth (Hsiao & Acevedo, 1974). However, a threshold value of ψ_{tp} may exist above which changes in ψ_{tp} do not affect elongation. This will obviously depend on the yield stress characteristics of the cell, but it may be adjustable so that growth can be maintained despite small changes in ψ_{tp} (Hsiao et al., 1976). A process of 'osmoregulation' (Hsiao, 1973) may also occur by which a lowering of ψ_s may maintain an effective ψ_{tp}. Decreases in ψ_s may be brought about by increased concentration of solutes within the cell by dehydration, increased solute uptake or production of osmotically active substances, although a continued lowering of ψ_s may reduce ψ_{tp} to zero and thus not achieve the desired response (Hsiao et al., 1976). Although not a widely accepted phenomenon, Hsiao et al. considered that it may be one of common occurrence, even among mesophytes where changes in ψ_s may occur in response to water availability and changes in resistances within the plant to water uptake, for example as a result of age.

Growth is not solely dependent on ψ_{tp} since structural materials are also required. Although protein and wall synthesis are sensitive to stress, in the absence of growth their accumulation may be retarded by a feedback mechanism. However, continued synthesis in the absence of elongation, because of differential susceptibilities to stress, may result in the phenomenon of 'stored growth' so that on rewatering, losses in growth as a result of water stress, may be rapidly recuperated

(Hsiao & Acevedo, 1974). Prolonged stress may result in a situation in which the rapid transitory growth phase does not compensate for losses resulting from water stress, so reducing the productivity of the plant. Stocker (1960) recognised the same phenomenon in which growth potential is increased during the restitution and hardening phases. He also considered that the productive ability of plants was greater in hardened than unhardened plants when intensification of drought occurred because unhardened plants were more susceptible to desiccation damage.

Although the expression of growth in terms of cell enlargement is of importance, certain workers have contended that "...the embryonic stage is the chief growth process of the organism and although elongation due to incoming water is a very important phenomenon, it is to a certain extent of secondary importance..." (Maximov, 1941). The effect water stress has upon the embryonic stages may play an important role in plant growth response to water stress. Gates (1964) considered that since both processes are involved in growth it is difficult to divorce the two approaches. From his own studies he concluded that plant response to water stress may be "described as a senescent decline in growth during wilting and the development of a physiologically young condition upon re-watering" (Gates, 1964). He noted that although an integrated pattern characteristic of the whole plant as an organism occurs, the degree of damage resulting from water stress is influenced by the species, the degree of stress and the physiological age of the plant, upon which is superimposed the observation that the young tissues may determine the whole plant response.

One area of importance with respect to embryonic development of tissue is the effect water stress has upon plant apices, which are known to be extremely tolerant of desiccation, possibly as a result of a lack of vacuolation (Iljin, 1957), but are, nevertheless, very sensitive to water stress. Studies on Lupinus albus indicated that primordial

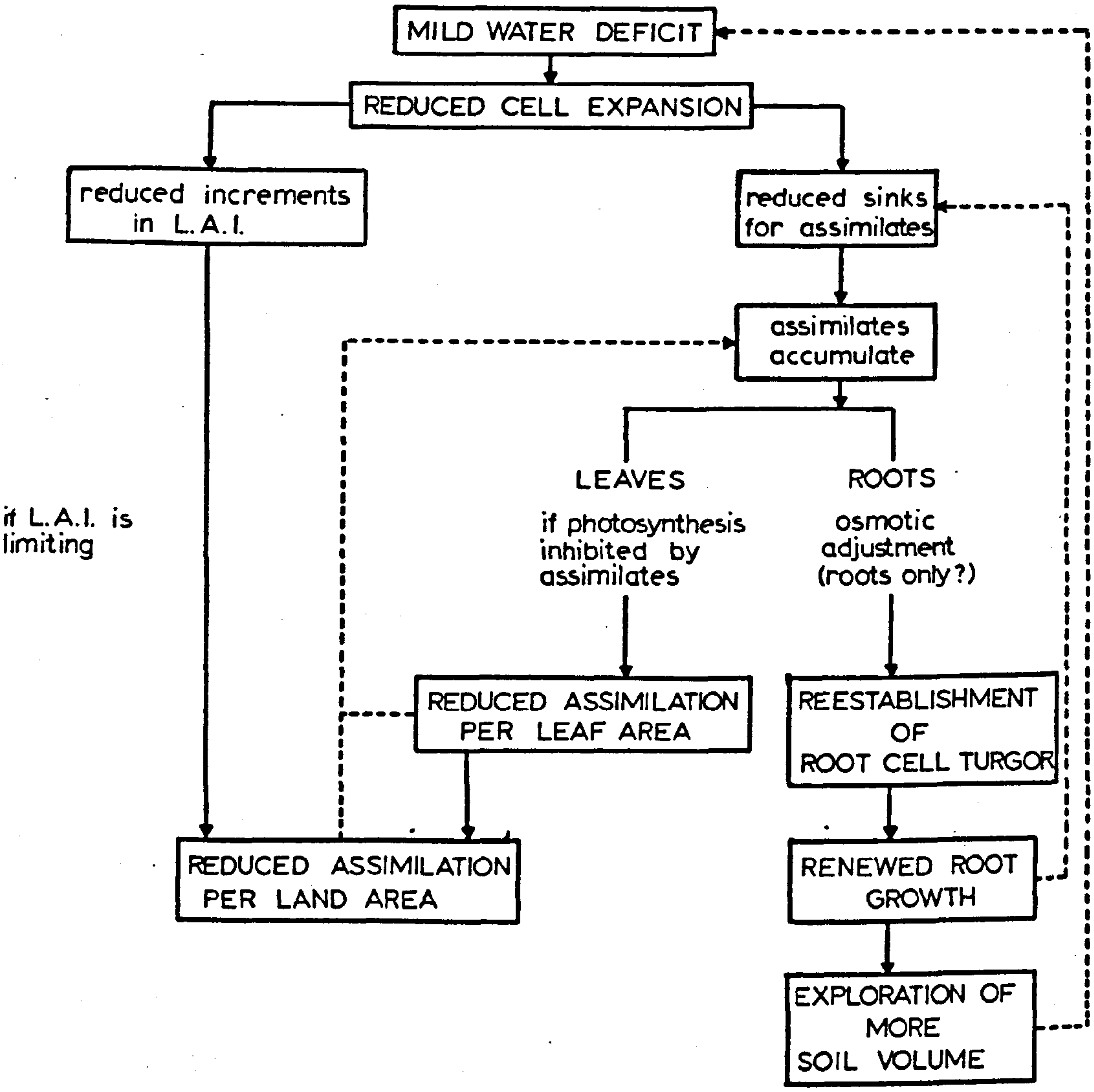

Figure 54 The possible effects of mild water deficits on leaf area development, CO_2 assimilation and root growth, (from Hsiao & Acevedo, 1974).

(The water deficit is assumed to be short and insufficiently severe to affect stomata and photosynthesis directly).

(L.A.I. - Leaf Area Index, -------- negative feedback effects).

initiation is halted by the imposition of a water stress but that relief of stress resulted in a rapid resumption of apical development (Gates, 1968). Prolonged atress may have effects on seed production by reducing the rate at which floral primordia are initiated (Gates, 1968; Slatyer, 1973a), in addition to delaying the overall ontogeny of the plant. Inhibited primordial development will also retard the production of photosynthetic areas so reducing productivity even if the stress is ultimately removed. Thus, the effects of stress on leaf initiation and subsequent development, due to the sensitivity of cell elongation to stress, may ultimately reduce the potential photosynthetic capacity of the plant. Hsiao (1973) emphasised that yield should not be considered solely in terms of the effect water stress has upon CO_2 uptake via stomatal control since the stomata may remain open over a range of ψ's which may nevertheless limit leaf growth and thus assimilation potential.

The effects of a mild water stress on plant assimilation have been schematically presented by Hsiao & Acevedo (1974) (Figure 54). The scheme indicates how a mild stress, solely through its effect on leaf development, may limit productivity of the aerial portions of the plant and affect root development. Any physiological adaptation facilitating root growth may eventually lead to the elimination of such deficits and an increase in normal growth of the aerial portions of the plant. However, superimposed upon this scheme are the biochemical and metabolic disorganisation resulting from severe or prolonged stress and the retardation of the embryonic stages of growth, which may ultimately be responsible for the reduction in yield of a stressed plant.

Hsiao (1973) was of the opinion that many investigations into plant response to desiccation employ stress periods of too long a duration, such periods obscuring the shorter term, primary effects of desiccation on the plant. In the present study, in the absence of data reflecting

Plate 3. Visible evidence, in the form of 'browning' of the vegetation, of desiccation injury on plots of Calluna vulgaris three months after being trampled at 100 walking passages per plot (Cannock Chase, June 1975).

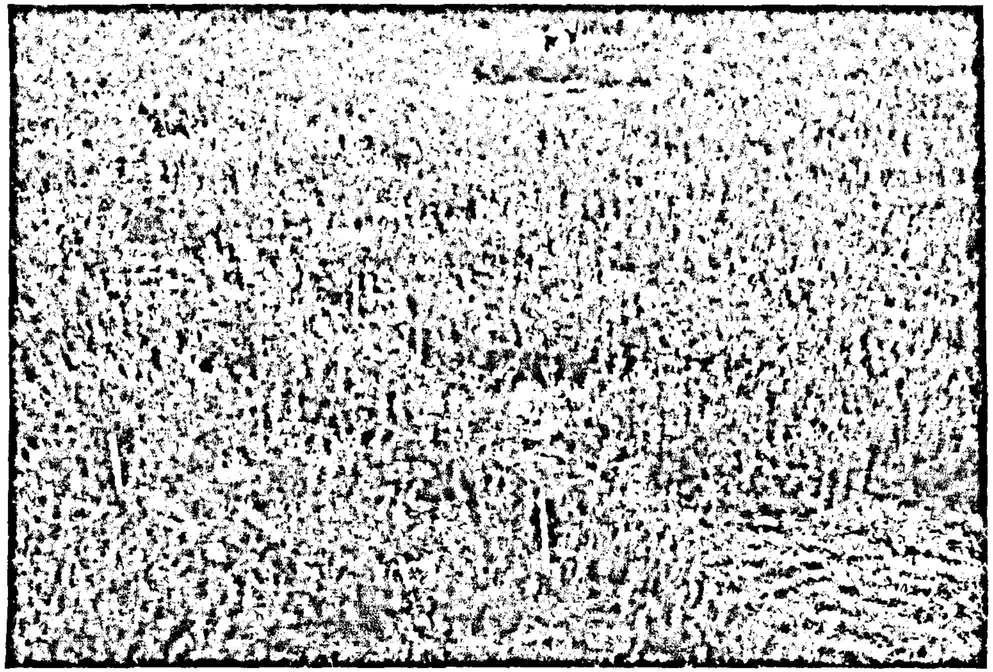

Plate 3. Visible evidence, in the form of 'browning' of the vegetation, of desiccation injury on plots of <u>Calluna vulgaris</u> three months after being trampled at 100 walking passages per plot (Cannock Chase, June 1975).

plant response to stress over a number of months, it is necessary to extrapolate the findings of shorter term studies to the prolonged and comparatively severe water stresses existing in trampled plants of C. vulgaris.

It will be apparent that drought injury falls into two main categories (Levitt, 1972):

(1) Direct Drought Injury which occurs too rapidly to be metabolically mediated (e.g. elongation responses) but which may have indirect effects;

(2) Indirect Drought Injury which may be regarded as synthetic disorientation, depletion of reserves leading to starvation, and disruption of protoplasmic structure (and possibly overheating effects).

Levitt (1972) considered that metabolic disruption alone is generally insufficient to cause injury but may enhance other effects of dehydration. The ability of the plant to adapt to water stress, combined with its tolerance of desiccation, may facilitate survival when water stress develops, for example, as a consequence of trampling pressures.

2. Visible Damage (browning) in trampled shoots of C. vulgaris

The relationship between visual damage and desiccation injury has been discussed above (Section IIIA, 1(c)) where it was concluded that a reliable estimate of desiccation damage could be assessed from a visual determination of injury. Extrapolated from the single shoot to the trampled plant level, this index of injury has been used in the present study to obtain rapid assessments of desiccation damage in the field.

Plate 4. A shoot of *Calluna vulgaris*, obtained from a plot three months after being trampled at 100 walking passages per plot, exhibiting signs of desiccation injury and death. New growth, however, can be observed at the tips of shoots bearing dead leaves at their bases. (Cannock Chase, June 1975).

The brown 'frosting' of C. vulgaris has been associated with low RWC's in winter (Bannister, 1964b) whilst Watson, Miller & Green (1966) ascribed 'winter browning', which may also be observed in summer, as due to desiccation of foliage. They also made the observation that bruised heather, e.g. as a result of being run over by a vehicle, also underwent extensive browning when atmospheric humidity was low and suggested that mechanical damage to shoots increased water loss. Winter browning has, however, been considered as a secondary phenomenon, occurring after shoot death (Bannister, 1964b). He noted that in a dry atmosphere desiccated shoots remained green and that water may be a pre-requisite for the development of brown coloration (Bannister, 1964b, 1971). Light intensity during desiccation has also been observed to affect the development of colour following desiccation (Parker, 1953b), whilst Henckel (1964) ascribed brown marks developing on desiccation to overheating effects. These observations may invalidate the use of visual damage as a reliable indicator of desiccation damage in the field, since environmental conditions, especially the presence of water, may modify the relationship between injury and visible damage. Bannister (1971) employed visual damage in a field study of the response of C. vulgaris and Erica cinerea to drought. He found that the incidence of browning was low in E. cinerea but that browning in shaded plants of C. vulgaris was marked and supported conclusions made regarding the susceptibility of sun and shade plants of both species to drought.

Two observations made in the present study, but not substantiated by experimental investigation, are that browning extends from the basal leaves of the shoot to the tip and that totally brown shoots, which appear dead, may produce new growth in the subsequent growing season. Bannister (1964b) also made the observation that, although 'frosting' affects the whole plant, the youngest parts (i.e. terminal leaves) are least affected. This apparent discrimination, of older leaves over younger, to succumb to desiccation damage may be due to two causes, the higher desiccation tolerance of young tissues (Sun, 1958) or the preferential supply of water to young tissues (Catsky, 1962). Catsky noted that a number of workers have observed that, during desiccation, the older leaves wilt before the younger, leading to the postulate that old leaves act as a water 'reservoir' for young leaves. Studies on Brassica oleracea, B. napus and Nicotiana sangerae confirmed the unequal development of water deficits in leaves of different ages, endorsing the view of a preferential water supply to young leaves during a reduction in water supply to the plant (Catsky, 1962). This phenomenon is most pronounced when deficits develop slowly and older leaves may die off as a result of water stress while growth continues in the younger leaves. Catsky also noted that the development of sublethal water contents may result in irreversible damage to the tissue so that it is unable to attain full turgidity upon re-watering. This property has been employed in studies on seasonal variation in desiccation tolerance (Section IIIA, 1(c)) but may result in erroneous determination of field RWC's in damaged shoots. However, the error tends to underestimate the water deficit existing in the tissue (Catsky, 1962) and Bannister (1964b) also noted that possible water uptake by dead tissue does not invalidate the observation of low winter RWC's in plants. The deficits existing in trampled tissues may therefore be an underestimate of those actually existing. The preference of young leaves for water has also been noted by Pospisilova (1973b). No distinct gradient in ψ from upper to lower leaves was found, rather a given decrease in ψ was associated with a lower decrease in water content of upper (younger) leaves than in lower (older) leaves; the change in ratio between water content and ψ enabled young leaves to retain a preferential water supply during wilting. To ascribe a high desiccation tolerance to younger tissues may therefore be an erroneous interpretation of a situation in which young tissues receive a preferential water supply.

The tolerance of young tissues to desiccation is somewhat controversial. Sun (1958) noted that as seedlings developed, their desiccation resistance decreased. This is not unexpected since substrates which may have been osmotically active and acting as protection against desiccation would be utilized in seedling development and possibly not replaced either by assimilation or from cotyledonous reserves, since the experiments were performed on excised seedlings. In addition, vacuolation, associated with elongation is conducive to a lowering of desiccation resistance (Iljin, 1957). Gates (1964), however, remarked that young tissues seem better able to tolerate water deficits than older tissues. Whatever the mechanism, protection of young tissue is advantageous from the viewpoint of apical development, growth and photosynthetic activity since young tissues are actively growing, and may also have higher photosynthetic rates than older tissues (cf. Grace & Woolhouse, 1970).

The second observation (i.e. apical viability on apparently dead shoots) is also corroborated by others. Bannister (1964b) stated that "Branches of heather that were thoroughly brown and apparently dead.... produced fresh green shoots on the brown portions...' and Watson, Miller & Green (1966) made a similar observation. This accords with the comments of Iljin (1957) and Gates (1964, 1968). Iljin (1957) noted that buds are very resistant to drought, possibly due to the absence of vacuoles in the cytoplasm, which is conducive to the development of extreme drought tolerance. As vacuoles form when the bud develops and grows so drought resistance decreases. Gates (1964) considered that in addition to these factors, structural and compositional changes in the cytoplasm may also affect drought resistance (cf. Stocker, 1960). The meristematic and vacuolate stages in embryo development differ in their desiccation tolerance, the former being most tolerant but tolerance involves only the ability to survive, not to develop (Gates, 1968).

It would appear, from present observations, that buds are capable of withstanding deficits harmful to the rest of the plant and that following the re-establishment of a favourable water balance in the season following damage, buds may be able to develop normally.

3. Seasonal variation in Desiccation Injury, as indicated by the degree of Visible Damage (browning), of *C. vulgaris*

(a) Methods

Plots of *C. vulgaris* (2m. x 0.5m.) were subjected to a walking prèssure of 100 passages per plot. Four replicates for each treatment date were trampled. A separate set of four plots were treated each month from November 1974 to June 1976. Visual damage was assessed on plots treated 1, 3 and 6 months previously. The plots demarcated for a particular month's trampling treatment were assessed for visual damage prior to treatment to give a control value for browning. Visual damage was assessed using a point quadrat frame of ten points lowered onto a 1m. x 0.5m. area five times to give fifty hits per $0.5m^2$. The colour of the leaf with which the point made contact was recorded. If a stem was hit a subjective assessment of the predominant colour of the leaves attached to that stem was made and recorded. The number of brown leaves struck has been expressed as a percentage of the total number of vegetation hits made.

(b) Results

As in previous analyses, the situation is made complex by the varying sampling periods under investigation. One way analysis of variance (performed on angularly transformed data) for each treatment (where

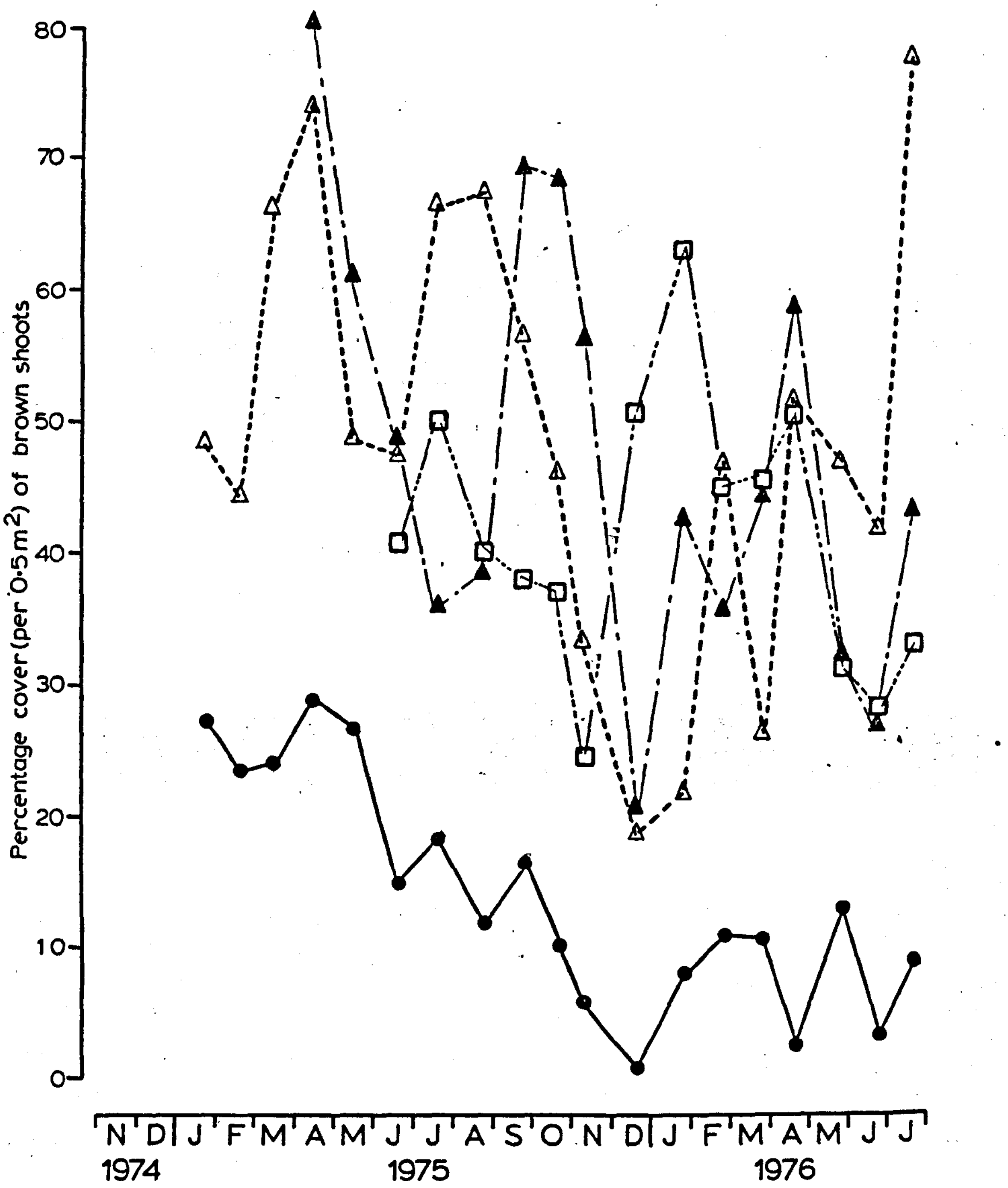

Figure 55 Seasonal variation in visible damage (indicated by browning of the shoot) of C.vulgaris shoots resulting from trampling of the stand at a pressure of 100 walking passages, (Cannock Chase).

●——● control plot browning (BRNO)

△-----△ browning of plots treated 1 month before measurement (BRN1)

▲·—·—▲ browning of plots treated 3 months before measurement (BRN3)

□·——·--□ browning of plots treated 6 months before measurement (BRN6) treatment refers to the period between treatment and assessment of visual damage, i.e. BRN 0, control plots; BRN 1, plots treated 1 month before measurement; BRN 3, plots treated 3 months before measurement and BRN 6, plots treated 6 months before measurement) indicates significant seasonal variation in all four treatments (all $p < 0.001$). Two way analysis of variance (on data for the period June 1975 to July 1976) reveals highly significant treatment effects in addition to time effects ($p < 0.001$), the mean value of percentage browning for each treatment being 8.6%, 47.7%, 44.4% and 41.2% for BRN 0, BRN 1, BRN 3 and BRN 6 respectively. All trampled plots have significantly higher levels of visual damage than control plots but are not significantly different between themselves. There is a tendency for the value for the mean damage response to decrease as time increases after treatment, from 47.7% in BRN 1 plots to 41.2% in BRN 6 plots.

(i) Seasonal variation in Visual Damage of trampled shoots of *C. vulgaris*, - data plotted with reference to the date of measurement (Figure 55)

A highly significant time x treatment interaction ($p < 0.001$) indicates that the effect of trampling, at a constant intensity applied at one instance in time, as manifested in visible damage to the shoots of *C. vulgaris*, varies over time, resulting in considerable seasonal variation in browning which is not precisely paralleled by variation in natural browning, i.e. of control plots.

Browning of control plots (BRN 0) decreases from a late winter/early spring maximum in January to May 1975 to a minimum in December 1975. Thereafter the value rises, in January-March 1976, but never to a level comparable to that of early 1975. Between April and July 1976 the browning percentage fluctuates between 2% and 15%.

Seasonal variation in browning of trampled plots is erratic and trends are obscure. The highest damage values for BRN 1 occur in spring and summer, i.e. April, July, August 1975; July, 1976 and the lowest in winter, i.e. December 1975, January 1976. The pattern, however, is not consistent, with relatively low and high values departing from the seasonal trend e.g. June 1975, 1976; February 1976. The pattern of browning for BRN 3 and BRN 6 is equally erratic. Peaks for BRN 3 occur in April, September, October 1975, April 1976 and troughs in July, August, December 1975 and June 1976. For BRN 6 the highest values are recorded in July, December 1975, January, April 1976 and the lowest in November 1975 and June 1976. A trend is evident for damage to become less pronounced with increasing length of time since the treatment. 62.5% of the BRN 3 browning values lie above those of BRN 1 but 6 months after treatment the situation is reversed, with 79% of the BRN 6 values lying below BRN 1. This suggests that browning increases after one month, but after reaching a peak declines. The varying response of the treatments is indicated by the lack of significant correlations existing between them (Table 16).

It will be observed that the correlation coefficients between treatments are small, although in the case of BRN 1 and BRN 3 seasonal variation (with reference to the time of measurement) is significantly correlated with variation in BRN 0.

(ii) Seasonal variation in Visible Damage of trampled shoots of *C. vulgaris* - data plotted with reference to the date of treatment (*Figure 56*):

The complex relationship existing between time and treatment reported above (Figure 55) is clarified by plotting the data with reference to the date on which the plots were treated, i.e. the BRN 1 values are plotted for the month prior to measurement whilst the BRN 6

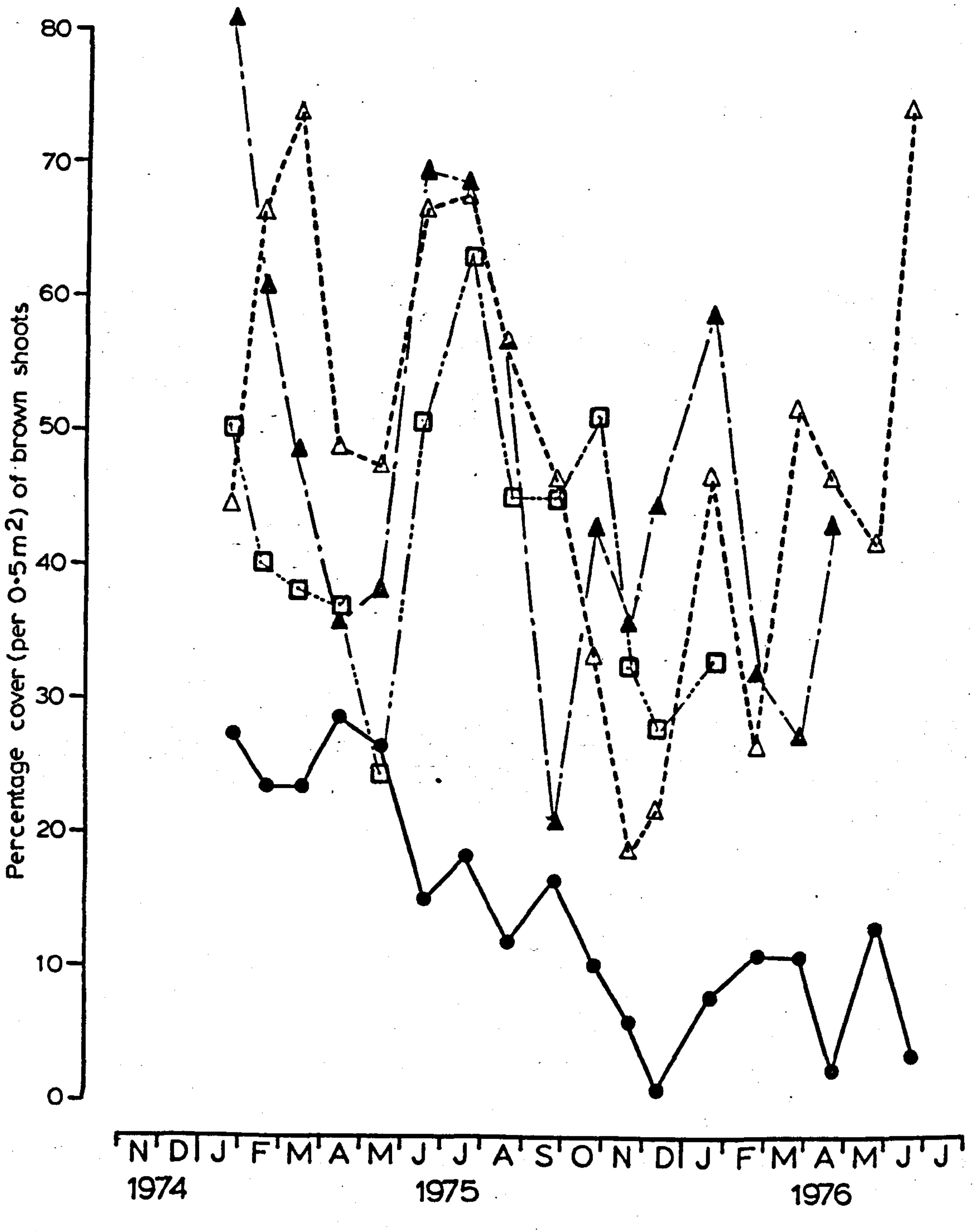

Figure 56 Seasonal variation in visible damage of trampled shoots of C. vulgaris; measurements made 1, 3 and 6 months after trampling of the plots but plotted for the date of treatment application, (Cannock Chase).

●———● control plot browning (BRN0)
△------△ browning of plots treated 1 month before measurement (BRN1)
▲—·—·—▲ browning of plots treated 3 months before measurement (BRN3)
□·····—·····□ browning of plots treated 6 months before measurement (BRN6)

Table 16 <u>Correlation coefficients (r) between visual damage estimates of *C. vulgaris* for different periods after trampling</u> (Cannock Chase)

Variables	With reference to time of measurement	With reference to time of treatment	Increase in browning (i.e. trampled-control) with reference to time of treatment
BRN 0 v BRN 1	0.509*	0.331 (n.s.)	-
BRN 0 v BRN 3	0.554*	0.257 (n.s.)	-
BRN 0 v BRN 6	-0.017(n.s.)	0.144 (n.s.)	-
BRN 1 v BRN 3	0.349(n.s.)	0.449 (n.s.)	0.435 (n.s.)
BRN 1 v BRN 6	-0.291(n.s.)	0.443 (n.s.)	0.529 (n.s.)
BRN 3 v BRN 6	-0.123(n.s.)	0.497 (n.s.)	0.507 (n.s.)

(* - significant at p = 0.05; n.s. - not significant)

values are plotted for the date 6 months prior to measurement; consequently the data for a particular month refers to the same groups of plots, but measured after differing time periods.

The correlations between trampled and control plots account for a smaller percentage of the variation in the data of trampled plots but the trampled plots are more closely correlated, although not significantly so (Table 16). The general trends of the treated plots are of greater similarity (Figure 56), low damage values occurring in late winter (April, May 1975) followed by a rise for plots treated in June, July 1975 then a general drop for plots trampled in the period September - December 1975. There is a trend for the damage values to rise for plots treated from April - June 1976 but a noteworthy departure from the low winter values occurs in January 1976 when all trampled plots exhibit comparatively high values of browning.

As in the previous analysis there are highly significant time, treatment and interaction effects (all $p < 0.001$). The mean response for each treatment (i.e. the mean value for each treatment over the period 1975-1976) differs from the previous analysis, with browning values of 15.4%, 49.1%, 49.9% and 42.1% for BRN 0, BRN 1, BRN 3 and BRN 6 respectively. All trampled plots have significantly higher damage values than the control and there is a tendency for BRN 3 values to be greater than those of BRN 1 and BRN 6, with a substantial decrease in mean response from BRN 3 to BRN 6. However, this reflects percentage damage and obscures the observation that 50% of the values of BRN 3 lie below those of BRN 1 (cf. 37.5% when data plotted with reference to the time of measurement) whilst the number of instances in which BRN 6 values lie below BRN 1 values has fallen from 79% to 56%. The situation is confused in that the precise effect of time on the development of damage, and therefore browning, remains unquantified.

In addition to seasonal differences in the susceptibility of the plant to damage, it is necessary to consider seasonal variation in the length of time taken for damage to become apparent. This may be influenced by environmental conditions, and any recovery that is occurring reducing the apparent values of browning. The lack of significant correlations between browning values of trampled plots and BRN 0 (Table 16), in contrast to the correlations existing when values at the time of measurement are used, suggests that factors other than those causing seasonal variation in undamaged shoots, are affecting browning in trampled shoots. This conflicts with the significant correlations existing in Table 16 which imply that factors causing variation in control shoots also affect browning in damaged plots, although to a limited extent, accounting for a maximum of 30.7% of the variation in the data.

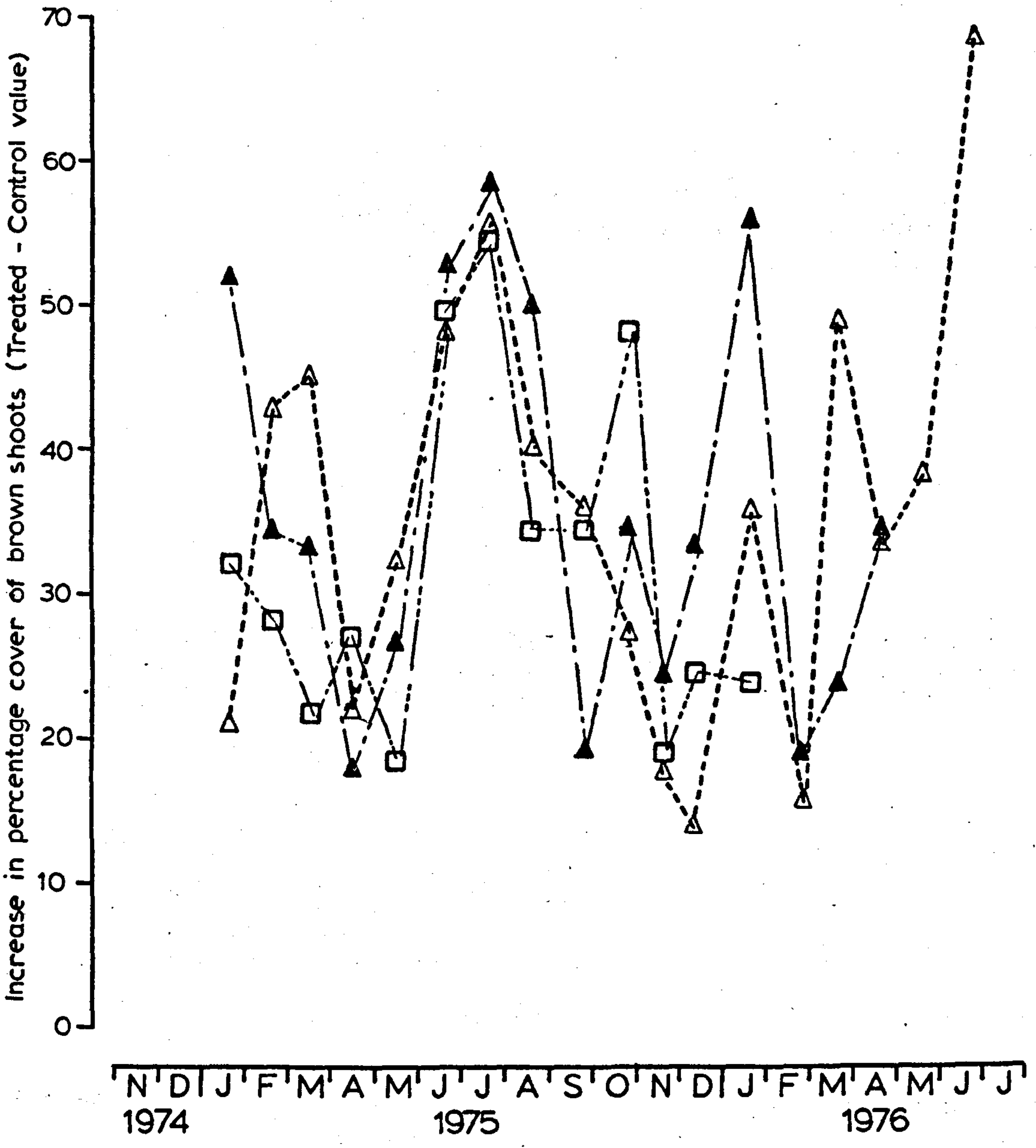

Figure 57 Seasonal variation in the increase in visible damage of shoots of C. vulgaris subjected to a trampling pressure of 100 walking pressure, plotted for the date of treatment application, (Cannock Chase).

△--------△ browning of plots treated 1 month before measurement (BRN1')
▲—·——·—▲ browning of plots treated 3 months before measurement (BRN3')
□···——···□ browning of plots treated 6 months before measurement (BRN6')

(iii) Seasonal variation in the increase in Visible Damage in *C. vulgaris* shoots associated with trampling (treated minus control browning values, plotted for date of treatment) (Figure 57):

The variation in browning of trampled plots of *C. vulgaris* when the control browning value has been removed from the treated plot value[1] (i.e. BRN 1', BRN 3' and BRN 6' respectively) reveals a seasonal pattern similar to that described above. The pattern is again erratic but indicates the actual magnitude of damage. The lowest value of BRN 1' recorded during the 1975-1976 sampling period was 14.0% (10.12.75.) whilst the highest value was 68.9% (22.06.76.). (It is of note that the highest browning value in 1975 occurred on plots treated on 21.07.75.). The lowest BRN 3' value was 17.7% (18.04.75.) and the highest 58.8% (21.07.75.) while the values for BRN 6' are 18.5% (15.05.75.) and 55.3% (21.07.75.) respectively. While this suggests maximum damage occurs in summer and minimum in winter, values of 42.8%, 45.0% and 49.1% were recorded in * February, March 1975 and March 1976 and a low value of 22.2% in April 1975, for BRN 1', but despite the inconsistencies, the general trend remains apparent.

It is also of note that browning values in excess of 50% (cf. desiccation tolerance determination, Section IIIA, 1(c)) occur in 2 out of 19 instances for BRN 1', 5 out of 16 instances for BRN 3' and 1 out of 14 instances for BRN 6'.

(iv) Linear correlation and regression analyses

The analysis of factors accounting for seasonal variation in browning falls into two parts, that responsible for the development of damage in the period between treatment and analysis and related to environmental

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[1] The browning values employed are those for the date of measurement; the difference is plotted for the date of treatment.

Table 17 Simple linear correlation coefficients (r) between Visual Damage estimates (percentage browning) of trampled plots of *C. vulgaris* and Environmental and Physiological variables (Cannock Chase)

Variable	BRN 1 (i)	BRN 1 (ii)	BRN 1'
Temperature			
60cm (a)	0.6303	-	0.5216
60cm (b)	0.5452	0.5184	0.6161
60cm (c)	0.5223	-	0.5734
60cm (d)	0.5239	-	0.6482
-5cm (a)	0.6263	-	0.5259
-5cm (b)	0.5386	0.5040	0.6079
-5cm (c)	0.5169	-	0.5771
-5cm (d)	0.5307	0.5349	0.6411
stem (a)	0.6219	-	0.4857
stem (b)	0.5142	0.4853	0.5582
stem (c)	0.5106	-	0.5467
stem (d)	0.5241	0.5292	0.6149
Accumulated temperatures			
base 7.2°C, 7 days before treatment	0.6299	-	0.5166
base 7.2°C,14 days before treatment	0.5494	0.5021	0.5974
base 4.0°C (soil), 7 days before treatment	0.6322	-	0.5082
base 4.0°C (soil),14 days before treatment	0.5569	0.4945	0.6002
Vapour Pressure (VP)			
VP (a)	0.6088	-	0.4896
VP (c)	0.5556	-	0.5357
VP (d)	0.4985	0.5357	0.6697
Daylength	0.5783	0.6298	0.6535
RWC 0	-	-0.5041	-
RWC 1	-0.5498	-0.6688	-0.5237
PMC 0	-	0.5441	0.5341
PMC 1	-	0.6356	0.5787
dry wgt/fresh wgt ratio	-	-	-0.5341
CRWC	-	0.6298	0.6535
ψ_x SCI	-0.5351	-	-
Sublethal water content	-	-0.6152	-0.5690

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Table 17 Simple linear correlation coefficients (r) between Visual Damage estimates (percentage browning) of trampled plots of *C. vulgaris* and Environmental and Physiological variables (Cannock Chase) (cont'd)

Notes:

BRN 1 (i) - browning, related to the time of assessment

BRN 1 (ii) - browning, related to the time of treatment

BRN 1' - increase in browning associated with trampling, i.e. treated minus control value, related to the time of treatment

Suffixes:

(a) - mean value for the 7 days before treatment or damage assessment

(b) - mean value for the 14 days before treatment or damage assessment

(c) - mean value for the 28 days before treatment or damage assessment

(d) - mean value for the 7 days after treatment or damage assessment and physiological variables existing over this time, and secondly, the physiological characteristics of the plant at the time of treatment and the environmental factors affecting the plant in the immediate post-treatment period.

The simple correlation coefficients (r) relating browning values to environmental and physiological variables are presented in Table 17. To avoid confusion non-significant coefficients for a particular variable are omitted (i.e. represented by a dash) while the coefficient accounting for the highest proportion of variance when a particular parameter and its derivatives are considered is underlined, e.g. of the four 60cm. temperature parameters considered, that for the mean temperature 7 days prior to measurement is the most significant for BRN 1'. The analysis has been confined to a consideration of variables accounting for variation in browning determined one month after treatment (BRN 1 and BRN 1').

(1) Factors related to BRN 1 for the date of measurement

The average variance accounted for by any one of the twenty two variables correlated with browning is 31.5 ± 1.1%. Certain correlations are spurious, e.g. the correlation between browning and mean temperature over the 7 days after analysis, and may reflect general seasonal trends to which environmental parameters may be related.

(1a) Environmental correlations

Mean temperature: Browning is positively correlated with mean temperatures of air, soil and stem, the most significant being with that of air (60cm.) and the most significant time period being for the 7 days prior to the date of measurement. In view of the possibility of overheating effects occurring, the analysis has been refined by considering maximum temperatures. When BRN 1 is correlated with the maximum temperature occurring between the time of treatment and the date of measurement the proportion of variance accounted for is reduced ($r = 0.4743$, $p < 0.05$), but the same correlation performed using BRN $1'$ indicates an improved explanation of the variation in the data ($r = 0.6701$, $p < 0.01$). If stem maxima are used for the 7 days before assessment of browning, the explained variance is increased to 57.1% ($r = 0.7557$, $p < 0.001$). It would appear that the maximum temperature experienced by the plant between trampling and measurement of damage gives a more accurate prediction of plant damage than mean temperature.

Accumulated temperature: Accumulated temperature above base 7.2°C and 4.0°C both account for a significant proportion of the variance in the data (39.7% and 39.9% respectively), an increase in accumulated temperature being associated with increased browning. This may be a reflection of higher average and maximum temperatures occurring as a result of the seasonal progression of temperature, or it may reflect the physiological status of the plant, which may also account for the significant positive correlation with daylength, suggesting that longer days are associated with higher levels of visual damage.

Vapour Pressure: The higher the vapour pressure the greater the degree of damage in the trampled plant, possibly as a result of the greater demand made on the plant's water resources by the atmosphere. (This is supported by the significant negative correlation between RWC and browning, indicating that higher water deficits are associated with higher values of damage (Figure 60)).

(1b) Physiological correlations

ψ_x at stomatal closure point (SCI): This is the only measured physiological variable with which BRN 1 is significantly correlated.

Table 18 Multiple Regression Analysis relating seasonal variation in Visible Damage (BRN 1(i)) of trampled plots of *C. vulgaris* to Environmental and Physiological factors (Cannock Chase)

Step	Variable	Significance to enter (p)	Multiple R	Multiple R^2	R^2 change	Simple R	Overall Significance (p)
(a)	Environmental variables						
X_1	Daylength	< 0.05	0.5783	0.3344	0.3344	0.5783	< 0.05
X_2	Vapour Pressure (mean for 28 days before assessment)	n.s.	0.6481	0.4201	0.0857	0.5557	< 0.05
X_3	Stem Temperature (mean for 28 days before assessment)	n.s.	0.7299	0.5329	0.1128	0.5106	< 0.05
	Regression equation:	$\hat{Y} = 6.2051 + 3.3271 X_1$					< 0.05
(b)	Physiological variables						
X_1	RWC 1	< 0.05	0.5498	0.3023	0.3023	-0.5498	< 0.05
X_2	SCI	n.s.	0.6703	0.4493	0.1470	0.2687	< 0.05
X_3	PMC 1	n.s.	0.6916	0.4783	0.0290	0.2520	< 0.05
X_4	CRWC	n.s.	0.7026	0.4937	0.0154	0.3624	< 0.05
	Regression equation:	$\hat{Y} = 119.5991 - 129.6449 X_1$					< 0.05

Step	Variable	Significance to enter (p)	Multiple R	Multiple R^2	R^2 change	Simple R	Overall Significance (p)
(c)	Combined variables						
X_1	Daylength	< 0.05	0.5783	0.3344	0.3344	0.5783	< 0.05
X_2	Dry wgt/fresh wgt ratio	< 0.001	0.8591	0.7379	0.4035	0.0469	< 0.001
X_3	BRN O	n.s.	0.8853	0.7838	0.0459	0.5095	< 0.001
X_4	Vapour Pressure (mean for 28 days before assessment)	n.s.	0.9168	0.8406	0.0568	0.5557	< 0.001
X_5	Stem temperature (mean for 28 days before assessment)	n.s.	0.9435	0.8901	0.0495	0.5106	< 0.001
X_6	ψ_x SCI	n.s.	0.9544	0.9109	0.0208	-0.5351	< 0.001
X_7	CRWC	n.s.	0.9668	0.9348	0.0239	0.3624	< 0.001
X_8	Accumulated temperature (7.2°C for 14 days before assessment)	n.s.	0.9726	0.9459	0.0111	0.5494	< 0.001
X_9	PMC 1	n.s.	0.9745	0.9496	0.0037	0.2520	< 0.001
X_{10}	Time to stomatal closure	n.s.	0.9753	0.9511	0.0015	-0.1919	< 0.001
X_{11}	SCI	n.s.	0.9753	0.9513	0.0002	0.2687	< 0.001
	Regression equation:	$\hat{Y} = -243.7376 + 6.9252\,X_1 + 521.0331\,X_2$					< 0.001

The lack of a significant correlation between BRN 1 and RWC at stomatal closure ($r = 0.2687$) re-emphasises the difference between RWC and ψ_x as indicators of the stress the plant is under. The correlation between ψ_x at SCI and browning indicates that the lower the ψ_x (i.e. the greater the stress) the less visible damage occurs.

(1c) Multiple Regression Analyses (Table 18)

(1) Environmental variables: Of the three variables entered, all of which are individually significantly correlated with browning, only daylength makes a significant contribution towards explaining seasonal variation in BRN 1, accounting for 33.4% of the total variation. Vapour pressure and stem temperature means for the period between treatment and measurement account for a further but non-significant 19.8% of the variation, raising the total variance explained to 53.2%. Since vapour pressure and stem temperature are correlated with daylength ($r = 0.5326$, $p < 0.05$; $r = 0.6437$, $p < 0.01$) it is not unexpected that they do not make significant contributions towards explaining the variance.

(2) Physiological variables: Of the variables entered, of which only RWC 1 is significantly correlated with browning, the total explained variance is 49.4% but only RWC 1 make a significant contribution. However, although the water deficit developing in the period between treatment and analysis is the variable most closely associated with the degree of visible damage developing after trampling, it may not be totally independent of the dependent variable and consequently has been omitted from subsequent analyses.

It is of interest to note the order of successive variables as they influence variation in BRN 1. The observation that the RWC at stomatal closure (SCI) is the next most important variable has implications for the role desiccation avoidance has in preventing injurious deficits developing. The trend between BRN 1 and SCI however, also indicates that with lower values of SCI, lower levels of damage occur which may reflect the tolerance of the protoplasm to water stress, although the variable CRWC (which is indicative of the plant's tolerance of water deficits) makes the least significant contribution to the regression.

(3) Combined variables (Environmental plus Physiological): Of the eleven variables entered into the analysis, accounting for a total of 95.1% of the variation in the data, only daylength and dry to fresh weight ratio make a significant contribution, explaining 73.8% of the total variation. The remaining nine variables account for 21.3% of the variance. Although this is not significant it is of interest to note that CRWC makes a more significant contribution than SCI (cf. above) while environmental factors such as temperature and vapour pressure are relatively insensitive indicators of the damage likely to occur following trampling at a particular time. Rather, season, as indicated by daylength, and the implications arising from this concept in terms of environmental characteristics, and the general physiological status of the plant, which in a number of respects is integrated in the dry to fresh weight ratio, have the most significant bearing on damage developing in C. vulgaris following trampling.

(2) Factors related to BRN 1/BRN 1' for the date of treatment (Table 17)

In many respects this is a retrospective predictive analysis which attempts to describe the response of C. vulgaris to trampling, as reflected in browning, in terms of physiological and environmental parameters existing before the damage is apparent; that is, the browning likely to occur after one month in contrast to describing the browning which has occurred and is affected by variables in the period between treatment and measurement.

The variables correlated with BRN 1 (related to the date of treatment) are identical to the correlations with BRN 1', although in the latter case significant correlations occur more frequently. Consequently, the discussion will centre on variation in BRN 1'. The average variance accounted for by any one of the individual correlations is 33.0 ± 1.2%.

(2a) Environmental correlations

Mean temperature: The temperature parameters for the 7 days following treatment are the most significantly related to browning, although the temperature regime experienced by the plants prior to trampling also appears to have a significant effect on subsequent damage development. Air temperature (60cm.) again accounts for the highest percentage variation. It is also of note that mean daily temperature on the date of trampling is significantly correlated with browning ($r = 0.5212$, $p < 0.05$) but maximum temperatures are not significantly correlated with browning values. The temperature prehistory of the plant, as reflected in accumulated temperatures over the 7 and 14 days prior to treatment, also has a significant influence on subsequent damage development following treatment, but it is problematical to determine whether such correlations arise from the general seasonal trends in environmental variables, or whether they are of significance in themselves. The observation that stem temperature for the 7 days following treatment accounts for a greater percentage of the variation than the temperature for the 7 days prior to trampling (37.8% and 23.6% respectively) suggests that temperature may have a direct effect on the damage developing rather than an intermediary effect.

Vapour Pressure: Positive correlations exist between browning values and vapour pressure and again it is the value for the 7 day period following treatment that appears most important.

Daylength: The value used in the correlations is the mean value for the 7-day period prior to trampling. It is positively correlated with browning so that with increasing daylength there is a greater probability of increased damage. As noted above, this value represents an integration of seasonal changes in environmental and physiological parameters, but may also influence the period over which water loss, due to photoactive stomatal opening, occurs.

(2b) Physiological correlations

CRWC and Sublethal water content: The CRWC, in representing the desiccation tolerance of the plant, might be expected to be significantly correlated with browning. The lower the desiccation tolerance at the time of trampling (i.e. the higher the value of CRWC) the more severe the desiccation injury as reflected in browning, this variable independently accounting for 42.7% of the variation in BRN 1'. The relationship is expected in view of the significant correlation between desiccation tolerance and visual damage (Section IIIA, 1(c)).

The sublethal water content (i.e. the difference between the RWC at stomatal closure and CRWC) is also of interest. The greater the sublethal water content the lower the desiccation injury. It is of note, however, that neither RWC at stomatal closure nor ψ_x at stomatal closure are significantly correlated with browning.

PMC, Dry weight/Fresh weight ratio and RWC: The greater the succulence of the shoot (as indicated by PMC) the greater the damage to the shoot

Table 19 Multiple Regression Analysis relating seasonal variation in Visible Damage (BRN $1'$) of trampled plots of *C. vulgaris* to Environmental and Physiological factors (Cannock Chase)

Step	Variable	Significance to enter (p)	Multiple R	Multiple R^2	R^2 change	Simple R	Overall Significance (p)
(a)	Environmental variables						
X_1	Vapour Pressure (7 days after treatment)	< 0.01	0.6697	0.4485	0.4485	0.6697	< 0.01
X_2	Daylength	n.s.	0.7114	0.5061	0.0576	0.6535	< 0.01
X_3	Stem temperature (7 days after treatment)	n.s.	0.7344	0.5393	0.0332	0.6149	< 0.05
X_4	Rainfall (7 days after treatment)	n.s.	0.7385	0.5454	0.0061	-0.1762	< 0.05
	Regression equation	$\hat{Y} = 11.6852 + 2.4147 X_1$					< 0.01
(b)	Physiological variables						
X_1	CRWC	< 0.05	0.5709	0.3260	0.3260	0.5709	< 0.05
X_2	PMC 0	< 0.05	0.7412	0.5494	0.2234	0.5341	< 0.01
X_3	RWC 0	n.s.	0.7550	0.5701	0.0207	-0.4314	< 0.01
X_4	SCI	n.s.	0.7617	0.5802	0.0101	-0.1708	< 0.05
	Regression equation	$\hat{Y} = -45.3241 + 116.8373 X_1 + 0.3728 X_2$					< 0.05

Step	Variable	Significance to enter (p)	Multiple R	Multiple R^2	R^2 change	Simple R	Overall Significance (p)
(c)	<u>Combined variables</u>						
X_1	Vapour Pressure (7 days after treatment)	< 0.01	0.6697	0.4485	0.4485	0.6697	< 0.01
X_2	Sublethal water content	< 0.01	0.8439	0.7122	0.2637	-0.5690	< 0.001
X_3	RWC O	n.s.	0.8696	0.7562	0.0440	-0.4313	< 0.001
X_4	CRWC	n.s.	0.8785	0.7717	0.0155	0.5709	< 0.001
X_5	Accumulated temperature (7.2°C) (14 days before treatment)	n.s.	0.8810	0.7762	0.0045	0.5974	< 0.001
X_6	PMC O	n.s.	0.8825	0.7782	0.0020	0.5341	< 0.01
X_7	Daylength	n.s.	0.8838	0.7811	0.0029	0.6535	< 0.05
	Regression equation	$\hat{Y} = 46.0836 + 2.2552\ X_1 - 91.1045\ X_2$					< 0.01

following trampling. This is substantiated by the negative correlation with dry weight to fresh weight ratio. High values of PMC imply greater water contents which might be expected to buffer the plant against desiccation damage, but high PMC's are also associated with young growth susceptible to desiccation damage.

The value for PMC is also somewhat artificial in that, unless the shoot is at an RWC of 1.0, it represents a hypothetical maximum water content unlikely to exist often in the field especially during the day. Rather, the actual water content of the shoot at the time of trampling, (as indicated by RWC 0), is more representative of the water that must be lost before desiccation injury ensues. The RWC is not significantly correlated with BRN $1'$ ($r = 0.4314$, n.s.). The relationship with browning *per se* is, however, significant and indicates that lower RWC's of shoots when they are trampled are associated with the development of more severe desiccation injury. This relationship is further substantiated by considering the difference between CRWC and RWC 0 at the time of treatment and browning. This difference may be taken to represent the total buffering capacity of the plant's water content, encompassing the sublethal water content, but determined by environmental factors to a greater extent than sublethal water content. The difference between RWC 0 and CRWC is significantly correlated with BRN 1 and BRN $1'$ ($r = -0.6606$, $p < 0.01$; $r = -0.6447$, $p < 0.01$ respectively).

(2c) *Multiple Regression Analyses* (Table 19)

(1) *Environmental variables*: The four variables entered into the analysis account for a total of 54.5% of the variance in the data (Rainfall is included in view of its possible relevance to the development of browning following desiccation injury). Of these four variables only 44.9% of the variance is significantly explained, by mean vapour pressure for the

7 day period following treatment.

(2) Physiological variables: The main parameters describing desiccation tolerance and avoidance have been included in the analysis and account for 58.0% of the total variance. Only CRWC and PMC make a significant contribution to the regression, explaining 54.9% of the total variance.

(3) Combined variables: When environmental and physiological variables are combined in the analysis, the total variance accounted for is 78.1%. (The F ratio is too small to include stem temperatures for the 7 days following trampling, in the analysis). Only two variables, vapour pressure and sublethal water content, make a significant contribution towards explaining variation in browning in relation to the factors existing at the time of treatment or in the immediate post-treatment period, accounting for 71.2% of the total variance. The remaining variablesaccount for only 6.9% of the variance but although not significant it is of interest to note the ordering of the variables in which RWC, which is an expression of the total buffering capacity of the plant to water loss, is of greater significance than PMC (cf. above) and in which accumulated temperatures, and daylength make a relatively small contribution to the analysis.

(c) Discussion

(i) Seasonal variation in treatment effects:

The apparent decrease in browning between BRN 1 and BRN 6 may result from a changing ratio of green to brown shoots occurring as a result of litter production or new growth rather than a recovery in damaged tissue.

With respect to litter fall Cormack & Gimingham (1964) noted that litter fall in C. vulgaris occurs in two phases, in October-November when short shoots are shed, and in February when capsules are shed. Forrest (1971) found short shoot fall was maximal from June to October but, despite variations in the precise time at different locations, it is evident that, although evergreen, Calluna exhibits deciduous seasonal patterns. Shoots damaged by trampling may therefore be shed over winter so that the ratio of green to brown shoots increases resulting in relatively more green shoots per unit area. This effect may be enhanced by new growth in the spring. Watson et al. (1966) commented upon a similar situation when they stated that "...brown shoots fall off when pioneer heather starts growing in April, so that no visible damage may remain by early May. On older heather, the dead shoots stay longer but are usually hidden by new growth...". This may be the reason for the decline in percentage browning of control plots over the course of the year, in which new growth conceals brown shoots developing over winter, followed by a fall of dead shoots at the end of the year resulting in an increase in green to dead ratio, evident in the low December browning values. The slight increase in visual damage in January, February and March 1976 may result from browning in response to low winter RWC's (Bannister, 1964b). Although these were not recorded in the field, the sampling dates may not have provided a representative indication of RWC's experienced by C. vulgaris during the winter. The levels of control browning in the period January to April 1976 were less than half of those in 1975. Although this difference might be due to higher water deficits in the 1975 period, the data do not support this. The hot,comparatively dry conditions of the 1975 summer may have resulted in the preferential desiccation of older leaves (cf. Catsky, 1962) resulting in abscission of leaves which might normally have been susceptible to winter desiccation, and which consequently resulted in the low winter values of 1976 compared to 1975. Premature leaf abscission has been observed as a response to drought, e.g. Runyon (1934), Parker (1968). However, these suggestions require empirical validation.

It is possible that part of the reason for the significant time x treatment interaction resides in the phenomenon of leaf abscission of injured tissue so that six months after damage has occurred the most severely desiccated leaves have fallen while recovery in the supply of water to the leaves may result in new growth, so obscuring the effects of prior injury. Obviously, this effect is likely to be time dependent in terms of season of trampling, for example, the low value of BRN 6 in June 1976 relates to plots trampled in December 1975 when the desiccation tolerance was very high (CRWC 0.0991). The physiological state of the plant may have been such that physiological processes were undamaged and although browning after 1 month may have been apparent, and after 3 months considerable, the reduction between the 3 and 6 month measurements may have resulted from leaf fall and unimpaired growth at the onset of the growing season. In contrast, the highest browning values after 6 months occurred on plots trampled in July 1975 when desiccation tolerance was low (CRWC 0.2740) and browning assessed in January 1976. Although this tends to argue against the role of autumn litter fall in changing green to brown shoot ratios, it supports the postulate that new growth following damage may not have been possible, thus apparent recovery did not occur. In this respect the results are not totally consistent, since plots treated in the early part of the year, when desiccation tolerance was high, and assessed for damage following the spring flush of growth, may have high visual damage values, but it is of interest to note that one of the lowest BRN 6 values recorded was for plots trampled in May 1975, and although desiccation tolerance was not high, the following 6 months span the height of the growing season and may have facilitated apparent recovery.

It is also evident that the length of time taken for maximum damage to become apparent varies over the course of the year. For example, for plots trampled in January, June, November and December 1975, and January and February 1976, maximal damage develops after 3 months, for plots treated in February - May, August and September 1975, and March and April 1976 maximal damage develops after 1 month. There is a tendency for more rapid appearance of injury in summer than in winter, although the trend is not consistent. It may reflect the general physiological status of the plant and the environmental conditions prevailing which affect the rate at which water deficits develop in the plant. There is no apparent association between the speed at which injury is manifested and the plant's ability to tolerate desiccation, although the period of the year associated with dormancy in the plant is also associated with longer periods taken for injury to become apparent.

The maximum damage values recorded imply that the LD_{50} point has been exceeded and that, theoretically, the RWC in the shoots had dropped below the CRWC defining the 50% damage point. A comparison of CRWC and field RWC values indicates that RWC's of shoots from plots in which browning values exceed 50% are consistently greater, and often twice the CRWC value. This suggests that long term reaction to desiccation is considerably different from reaction to desiccation over the short term, which is used to determine desiccation tolerance of the plant, which questions the validity of CRWC as indicative of tolerance to long term desiccation.

(ii) Seasonal variation in BRN 1 - damage development between the date of treatment and the date of damage assessment:

The results indicate that trampling, at a constant intensity at different times of the year, has a varying effect on C. vulgaris. The mean temperature response, together with the relationships with accumulated temperature and daylength, may reflect a 'softening' response in which, as temperatures became favourable, the tissues pass from a dormant to a physiologically active state. The greater susceptibility to desiccation of young, rapidly growing tissue with a high degree of vacuolation (Iljin, 1957) may result, in tissues subjected to severe water deficits, in a greater degree of desiccation injury. At the end of the growing season, as the plant enters into the dormant phase, the reduction in vacuolation and the build up of sugars together with other processes involved in the increase in desiccation tolerance, will reduce desiccation injury to the plant induced by trampling. Obviously, the relationship between accumulated temperature and injury will not be perfect since there will be an upper limit to the effect an increase in temperature will have in facilitating growth, above which high temperature damage to processes and structure, e.g. thermal denaturation of proteins, may result. This may be responsible for the relationship between injury and maximum temperatures. Young tissues are generally less tolerant of environmental extremes than older or dormant tissues (Bannister, 1970), thus the development of deficits sufficient to induce stomatal closure and reduce transpirational cooling, may lead to overheating with the consequence that some browning, ascribed to desiccation injury, may be analogous to the 'scorch' marks noted by Henckel (1964) developing from heat injury. The stem temperatures measured indicate that the maximum temperatures experienced by the stem are likely to be considerable. These values are possibly an underestimate of true internal temperature since they do not take into account the absorbance of long wave radiation by dark surfaces or heat generated internally from metabolic processes, which may be considerable if respiration rates increase in response to water deficits and direct mechanical injury arising from trampling. In this context the heat resistance of the plant may play a significant role in seasonal susceptibility to injury resulting from trampling. This parameter has not been investigated in the present study but Bannister's studies (1970) suggested that heat resistance is inversely related to the annual course of temperature, with a minimum from May to July and a maximum from November to December. Consequently, in summer when, as a result of induced water deficits, high internal temperatures are most probable, the ability of the plant to tolerate such temperatures is least, increasing its susceptibility not only to desiccation but also to heating effects arising from desiccation.

The relationship between injury and vapour pressure is probably mediated through the effects vapour pressure has upon RWC. At a time when the desiccation tolerance of the plant is low, the evaporative demands placed upon the plant's water content are greatest, so increasing the possibility of damaging water deficits developing, even if hydroactive sotmatal closure has occurred, since loss through the cuticle and damaged epidermal tissues is likely to be high when evaporative demands are great.

The ψ_x at stomatal closure indicates the stress at which the plant responds to desiccation by avoiding further water loss. As such it is an integral part of the concept of desiccation resistance, although it is not correlated with desiccation tolerance. When the ψ_x at stomatal closure is low (i.e. high stress) appearance of desiccation injury is slight. When the ψ_x at stomatal closure is high, as during the growing season when the stress that can be tolerated is small, the comparative ease with which the ψ_x at stomatal closure can be exceeded will increase the probability of desiccation injury occurring, as suggested by the results. However, the positive tendency for high ψ_x at stomatal closure to be associated with larger sublethal water contents indicates that when ψ_x at stomatal closure is high a larger buffer zone exists, in terms of water content, between the point at which desiccation avoidance becomes operative and the point at which desiccation injury ensues. This neglects, however, the relationship between RWC and the stress it represents in physiological terms. The ability to accomodate high stresses, as indicated by ψ_x, and avoid desiccation injury is of adaptive advantage when CO_2 uptake is limited by external factors. Thus, trampled plants may be able to assimilate at low RWC's with a relatively small risk of desiccation injury if the plant is able to withstand high stresses, indicated by low ψ_x, without risk of injury before stomatal closure occurs. In addition, the low ψ_x at stomatal closure may be associated with the time taken for visible signs of injury to appear. For example, the ψ_x for January 1976 is relatively low (-3.97MPa) and for plants treated at this time, maximal damage becomes apparent after 3 months compared to the reverse situation in March 1976 when ψ_x is relatively high (-2.24MPa) and maximal damage develops within 1 month. This may, in part, reflect the time required for water loss to become of sufficient magnitude for the associated stresses to become lethal. Although dependent upon environmental conditions, the greater the stress required to induce injury, the longer the time required to induce such a stress, especially when environmental conditions may not be conducive to water loss, e.g. in winter.

The multiple regressions indicate that seasonal variation in browning may be described by considering daylength, or the RWC of damaged shoots. Both may be regarded as a reflection of the plant's physiological status, the former indicating the general physiological activity of the plant in terms of seasons when growth and dormancy occur, and possibly the generalised environmental conditions experienced by the plant, while the second is an indication of the damaged plants' water relations with the implications this has in terms of the stress the protoplasm is likely to be under. However, this variable, in being dependent upon the treatment applied, is descriptive rather than a factor which may be considered to predict the response of the plant, such as the desiccation tolerance or avoidance measurements. The importance of the dry to fresh weight ratio, evident from the combined variables analysis, may arise from its implication in a number of previously described parameters, i.e. CRWC, PMC, RWC at stomatal closure, and, in a manner similar to that described for daylength, may be regarded as a value indicative of the general physiological status of the plant. The lack of significance of any of the more specific parameters may reflect a situation in which the gross response of the plant to desiccation is not influenced by variations in a particular and specific parameter over the time period considered. For example, high temperatures may enhance the development of injury but may not, in themselves, influence the absolute magnitude of injury apparent after one month. Thus, it may be postulated that the conditions experienced by the plant and the physiological state of the plant, are integrated to produce a generalised damage response to trampling over the period between treatment and damage assessment.

(iii) Seasonal variation in BRN 1' - damage development influenced by the conditions at the time of treatment:

The relationship between browning, related to the time of treatment, and various independent variables appears more specific than in the above situation. The conclusions made with regard to the general response of the plant to various environmental parameters is equally applicable but the conditions experienced by the plant in the immediate post-treatment period would appear to be of especial importance. For example, daylength may define the period over which the stomata are open, longer days enhancing water loss and reducing the length of the overnight recovery period in which deficits are made up. Similarly, high temperatures in the immediate post-treatment period, together with associated evaporative demands on the plant's water resources, may determine the rate at which water is lost from the plant and the degree to which the plant suffers from overheating effects. The rapidity with which desiccation occurs may affect the injury the plant sustains. Rapid desiccation may prevent the restitution phase occurring (Stocker, 1960) while slow drying may result in less severe injury (Iljin, 1957; Stocker, 1960), enabling the restitution phase to keep pace with the rate of desiccation (Stocker, 1960).

When susceptibility to desiccation injury at the time of treatment is considered, the capacity to tolerate desiccation is of importance, as indicated by the multiple regression analysis, the higher the tolerance the lower the injury. Since desiccation tolerance varies seasonally it is not unexpected that injury following trampling also varies seasonally. Although browning is not correlated with stomatal closure point RWC, the sublethal water content, which is derived from the desiccation tolerance and avoidance capacities, is the physiological factor of primary importance (as indicated by the combined variables regression analysis). Although the seasonal trends of tolerance and avoidance are similar, they are not correlated and the plant's ability to maximise the difference between the two enhances its survival under conditions of desiccation. Thus, the greater the difference between the CRWC and the stomatal closure point RWC, the less likely is desiccation injury since a relatively large volume of water must be lost before injury occurs. The significance of the sublethal water content existing at the time of trampling is substantiated by the RWC relationships. It may therefore be postulated that the amount of water which must be lost at the time of trampling influences the degree of desiccation injury developing in the plant. This value is dependent upon the desiccation tolerance of the plant, the desiccation avoidance of the plant and the conditions determining the water content lying above the RWC at stomatal closure, consequently, it will be influenced by the conditions prevailing immediately prior to trampling. The suggestion that high RWC's may be beneficial to 'withstanding' trampling is contrary to suggestions made regarding RWC and tolerance to trampling in herbs. Wagar (1965) noted that increased damage can result if plants are trampled while turgid, although Shearman & Beard (1975c) found no correlation between RWC and inter-species tolerance to wear. However, Wagar (1964) noted that shaded plants suffered less trampling damage than plants on sunny sites, and suggested that water can enhance survival (Wagar, 1965). The present hypothesis is not contradictory to these suggestions. In herbs, high RWC's will increase the brittleness of the organs and render them liable to breakage. In woody stemmed plants, the nature of the lignified stem makes them susceptible to mechanical damage. Thus, following a restriction in the supply of water the ability to prevent water loss and to tolerate desiccation is of advantage in enhancing survival, and the greater the water content of the shoot the greater the possibility of avoiding injury.

The suggestions made above regarding the relationship between physiological activity and injury are substantiated by the relationship between browning and PMC. High PMC's are associated with young, physiologically active tissues which are more susceptible to desiccation injury because of lowered tolerances since, in a number of respects, active growth and the development of high desiccation tolerance are incompatible.

The combined variables regression analysis indicates that the effect the environment has upon water loss from the plant, which determines the rapidity with which desiccation occurs, and the sublethal water content, which integrates the plant's ability to avoid and tolerate deficits and represents the amount of water which must be lost before injury occurs, are the parameters of most significance in predicting plant response to trampling, in terms of desiccation injury.

4. Variation in Desiccation Injury, as indicated by the degree of Visible Damage (browning) of C. vulgaris related to the intensity of trampling

(a) Methods

Three blocks of C. vulgaris at the Kerloch site were laid out in a Latin Square arrangement. Block 1, composed of 2m. x 1m. plots, was trampled at four intensities, 0, 50, 100 and 200 passages per plot (p pl^{-1}). Block 2 and 3, composed of 2m. x 0.5m. plots, were trampled at four intensities, 0, 100, 200 and 300 passages per plot. Visual damage was measured, by the point quadrat method (Section IIIC, 3(a)) on 1m. x 0.5m. quadrats within the trampled area. Dates of treatment and visual damage assessments are given in Table 20. Desiccation tolerance and RWC at stomatal closure were measured on undamaged shoots sampled when blocks 2 and 3 were treated (Table 20).

(b) Results

The relationships between trampling intensity and visible damage are approximately linear, increased trampling intensity resulting in significantly increased damage ($p < 0.001$ for all blocks) (Figure 58). The blocks are not directly comparable owing to the different trampling intensities employed. Two way analyses of variance reveal a significant

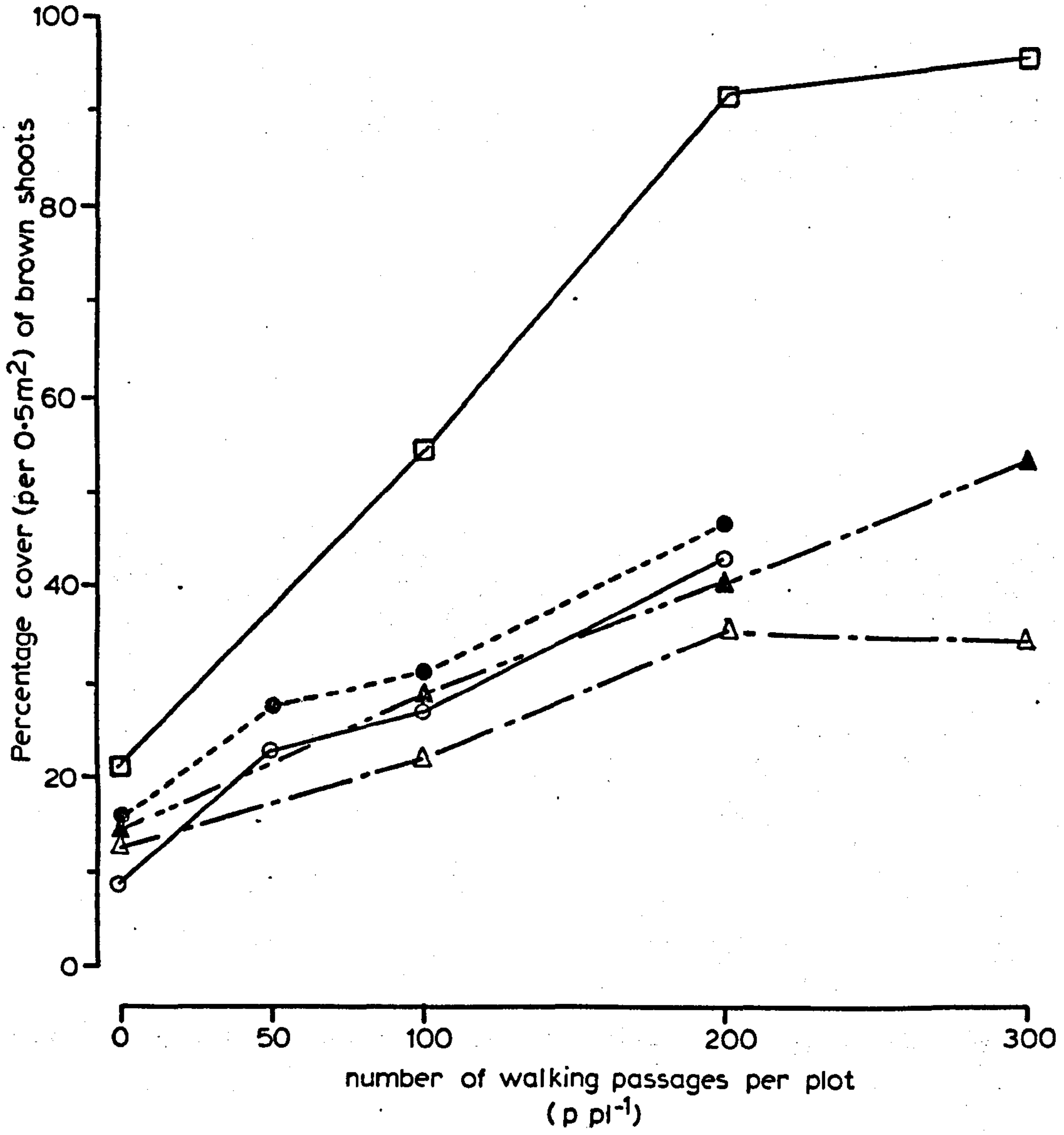


Figure 58 The effect of trampling intensity and date of application on the development of visible damage in shoots of C. vulgaris, (building phase, Kerloch).

○———○ Block1A (treated 04.07.74., measured 01.09.74., time interval 2 months)
●- - - -● Block1B (treated 04.07.74., measured 09.07.75., time interval 12 months)
△— · — · —△ Block2A (treated 11.04.75., measured 09.07.75., time interval 3 months)
▲- · · — · · -▲ Block 2B (treated 11.04.75., measured 05.04.76., time interval 12 months)
□———□ Block 3 (treated 09.07.75., measured 05.04.76., time interval 9 months)

Table 20 Treatment and Visual Assessment dates for plots of *C. vulgaris* treated over a range of trampling intensities (Kerloch)

Block	Treatment Date	Measurement Date	CRWC at time of treatment	RWC at stomatal closure at time of treatment
1A	04.07.74.	01.09.74.	-	-
1B	04.07.74.	09.07.75.	-	-
2A	11.04.75.	09.07.75.	0.2140	0.5466
2B	11.04.75.	05.04.76.	0.2140	0.5466
3	09.07.75.	05.04.76.	0.2833	0.6410

($p < 0.01$) increase in visual damage from block 1A to block 1B, similarly, a significant ($p < 0.01$) increase occurs between block 2A and block 2B. There is a tendency for plots treated in the growing season (i.e. block 1 and 3) to have higher damage values. This is less apparent for block 1 which has a mean value for all treatments after 12 months of 33.9% (block 1B) compared to 30.2% for block 2 after 12 months (block 2B). The mean damage value of block 3 of 69.3% is significantly greater than that of block 2A or 2B ($p < 0.001$). This is emphasised by the maximum damage values observed of 53.3% on block 2B at a trampling pressure of 300p pl^{-1} compared to 94.6% on block 3 at the same trampling pressure. There is some evidence of a saturation in response on blocks 2A and 3 at a trampling pressure of 200p pl^{-1}, above which no significant increase in visual damage occurs. There is a time effect in which the saturation trampling value for both plots is similar but the absolute value of damage is substantially lower on block 2A than block 3. In addition, the effect on block 2 is apparent 3 months after treatment (block 2A) but not 12 months after treatment (block 2B).

The trampling intensities employed on block 1 may not have been sufficiently high to exceed the threshold value. It is of interest to

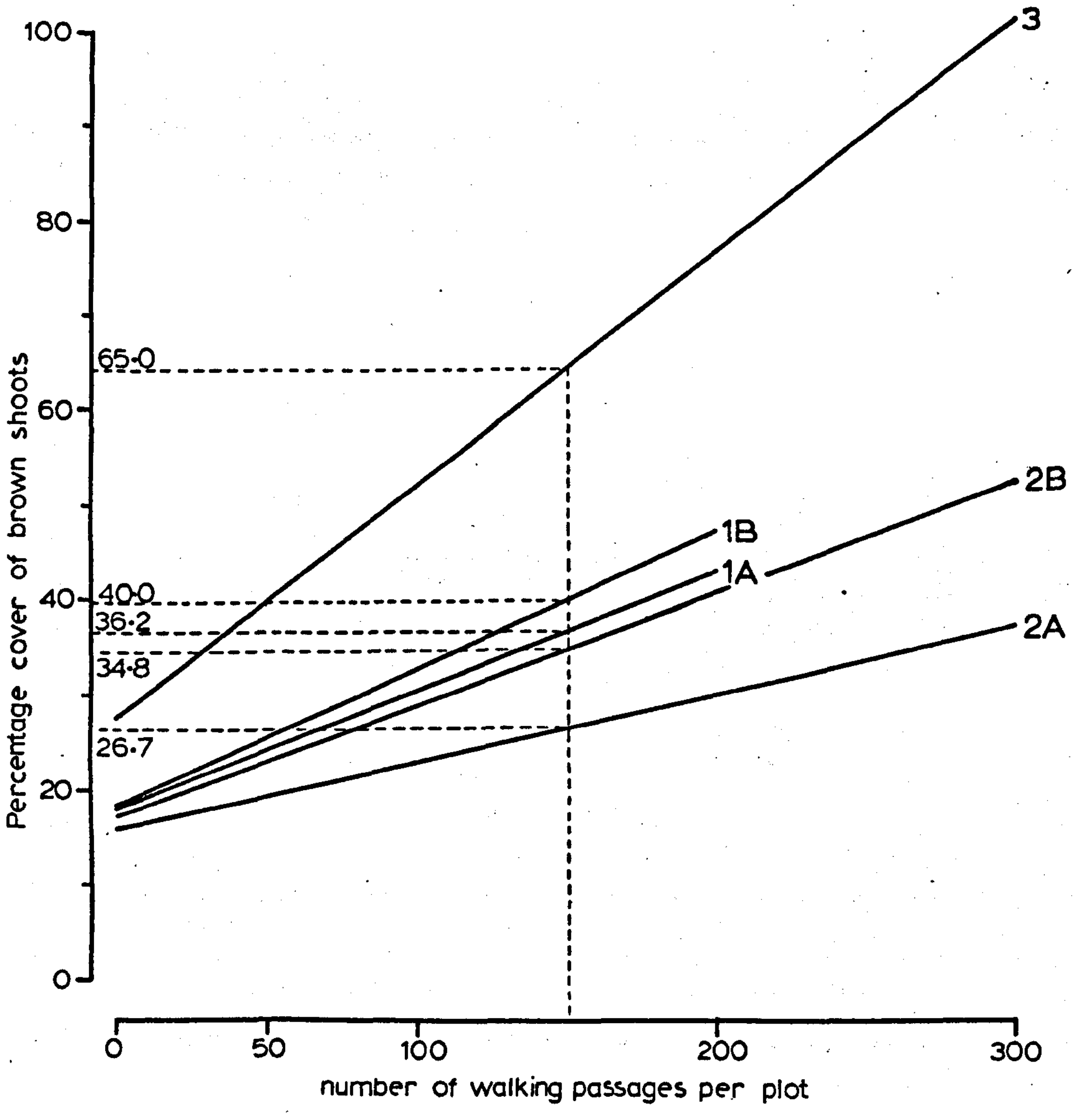


Figure 59 Regressions relating visible damage, resulting from trampling pressure, to trampling intensity, C. vulgaris, (building phase, Kerloch).

Block 1A, $\hat{y} = 19{\cdot}8720 + 0{\cdot}1091x$, $r = 0{\cdot}926$ $(p<0{\cdot}001)$

Block 1B, $\hat{y} = 17{\cdot}8371 + 0{\cdot}1498x$, $r = 0{\cdot}891$ $(p<0{\cdot}001)$

Block 2A, $\hat{y} = 16{\cdot}1284 + 0{\cdot}0703x$, $r = 0{\cdot}727$ $(p<0{\cdot}001)$

Block 2B, $\hat{y} = 16{\cdot}3539 + 0{\cdot}1229x$, $r = 0{\cdot}894$ $(p<0{\cdot}001)$

Block 3, $\hat{y} = 27{\cdot}9667 + 0{\cdot}2469x$, $r = 0{\cdot}927$ $(p<0{\cdot}001)$

note that for blocks 1 and 2 damage increases with time since treatment. In contrast to the narrow range over which browning of control plots varies on the five measurement dates (mean value: 15.1 ± 2.1%), the range of variation for treated plots at the different measurement dates is considerable (e.g. at an intensity of 200p pl^{-1} the mean response is 51.9 ± 10.1%).

Although the threshold effects observed imply a curvilinear relationship between visible damage and trampling intensity, the relationships are significantly linear and linear regressions have been computed to facilitate block comparisons (Figure 59). At a standard trampling intensity of 150p pl^{-1} the estimated damage decreases from 65.0% for block 3, treated in the middle of the growing season to 34.8% for block 2 treated before the start of the growing season but assessed for visible damage after a similar time interval between treatment and assessment. It is of interest to note that the respective desiccation tolerances of material sampled from block 2 and 3 at the time of treatment are 0.2140 and 0.2833 whilst the RWC's at stomatal closure are 0.5466 and 0.6410 respectively, thus the shoots treated in April 1975 had a lower desiccation avoidance capacity but higher desiccation tolerance than plots treated in July 1975. However, the sublethal water contents for the respective treatment dates are not appreciably different (i.e. 0.3326 and 0.3577).

The relationship between visual damage and RWC for block 2A indicates a significant correlation (Figure 60) in which, with increasing damage a decrease in RWC is observed. Compared to the percentage variation accounted for by a similar relationship based on monthly values from Cannock Chase (30.2%) a higher percentage of the variation is explained (51.5%), possibly because the different trampling intensities at Kerloch were applied on a single date, the conditions at the time of sampling were similar for all plots and, following treatment, the plots have

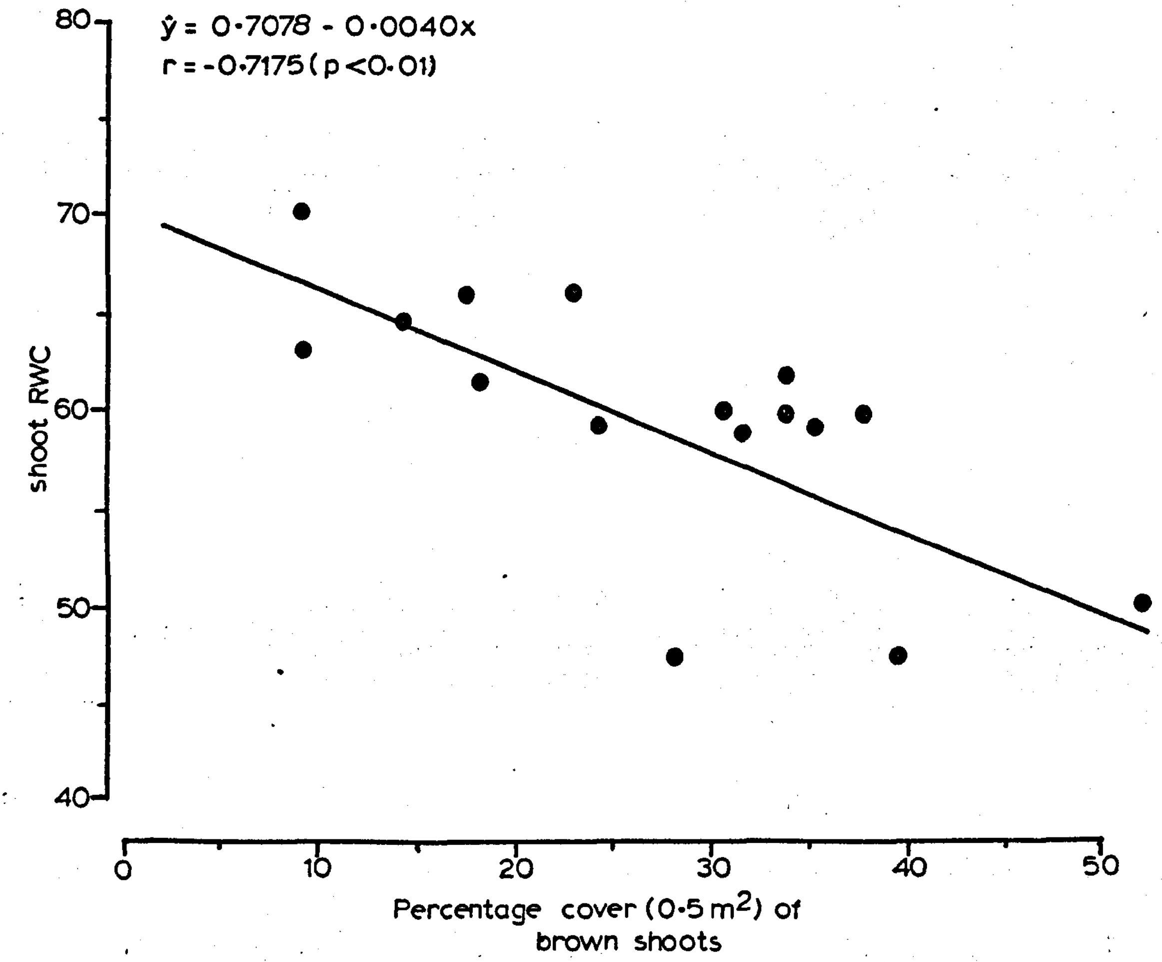


Figure 60 The relationship between plot visual damage and shoot RWC of C. vulgaris, (building phase, Kerloch).

(plots treated 11.04.75, visual damage assessed 09.07.75., shoot RWC measured 13.07.75.)

experienced similar macro-environmental changes.

(c) Discussion

The results of the present study are at variance with the observations of Bayfield (1971) who found an apparent decline in damage over time. This decline was attributed to wind removal of broken stems. In the present study there is a tendency for visible damage to increase over time. The situation for plots treated in April 1975 is consistent with the observations made at the Cannock Chase site, namely, that when the plant is hardy, damage may take longer to become apparent. Damage just before the beginning of the growing season may induce chronic deficits which retard growth so that apparent recovery due to new growth does not occur. There is also a possibility of low winter RWC's (Bannister, 1964b) aggravating a pre-existing stress situation leading to an increase in visual damage. Although this is not evident from the browning data for control plots, water deficits may adversely affect the development of desiccation tolerance in damaged plants making them more susceptible to winter water deficits (cf. Section IIIA, 1(d)). The time of the year at which damage is inflicted obviously modifies the response. The differential response exhibited between plots treated in April and July 1975 may be due to the higher desiccation tolerance of the former, resulting in reduced susceptibility to desiccation injury. The conclusions made regarding the value of the sublethal water content are not supported by the present results since, although a marked difference in response is apparent, the sublethal water contents are similar.

It is possible that environmental conditions may have also modified the response, Below average rainfall in the period following treatment i.e. August 1975, together with high temperatures possibly leading to overheating (Henckel, 1964) may have induced more severe desiccation in the less hardy summer shoots than in plants treated in April 1975.

Although below average rainfall occurred in May, 1975 any stress effects arising from this may have been offset by the above average rainfall value for July 1975. Similarly, the temperatures in April 1975 were unlikely to have risen to a value inducing heat injury. However, the results suggest that the physiological state of the plant is of greatest significance in determining the level of injury suffered by the trampled plant.

The reasons for the improved relationship between RWC and visual damage have been mentioned above. It has also been noted that RWC's of damaged material may be in error (cf. Catsky, 1962). However, the relative values indicate that, for a particular block of C. vulgaris treated and analysed as a unit at a particular time, increasing intensity of trampling leads to increasing water stress and associated with this is an increase in visible damage. Seasonal variation in the relationship between water stress and visible damage implies that a particular stress, possibly arising from a particular level of trampling, will induce a degree of visual damage which is dependent upon the time of year, or, that a particular stress may have a more adverse effect on physiological processes at certain times of the year than at others (cf. Section IIIA, 1(d)). A corollary of this is that the severity of the stress in physiological terms is a seasonal variable and consequently determines the level of pressure required to induce a given degree of damage at any particular time. For example, the intensity of trampling required to induce a given level of damage is less in July 1975 than in April 1975. The relationship is obscure at lower levels of damage where the regressions converge, but, with increasing damage values, the required levels of trampling to induce a given level of damage become increasingly divergent depending upon season.

5. Cumulative trampling effects on C. vulgaris

Bayfield (1971), in a trampling study on a Calluna-Trichophorum heath community, considered that trampling effects were additive over a period of three months. Leney (1974) concluded that trampling of an Ammophiletum was more damaging when the pressure was applied instantaneously than if spread out over time.

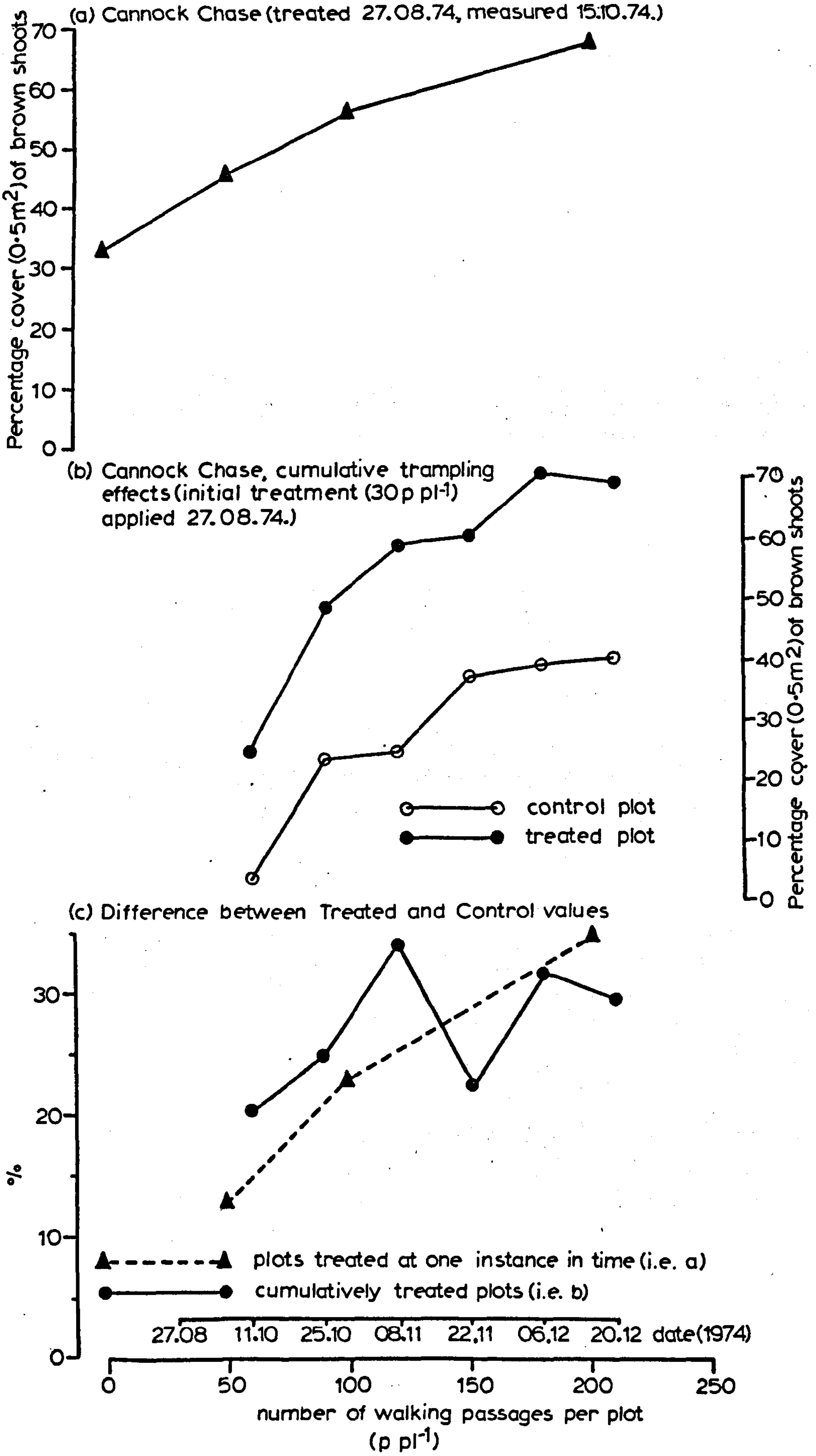
A major criticism of the visual damage variation observed at different intensities of trampling is that, despite care being exercised to trample each plant at every passage, the increase in damage at higher intensities of pressure may reflect an increased probability of the plant being trampled rather than a true response of the plant to increased pressure. This is suggested to some degree by the observation in the previous experiment that, when plants were trampled in July, the smaller plots employed in 1975 resulted in higher levels of damage than the plots used in 1974.

The RWC data (Section IIIB, 2(iii)) suggests that the trampling effect may or may not be an "all or nothing" effect, the controversy arising from variations in physiological and environmental parameters leading to differential plant responses.

The present investigation attempts to examine the cumulative effects of regular trampling on C. vulgaris at Cannock Chase.

(a) Methods

Eight plots (2m. x 1m.) were randomly located in a Calluna stand at Cannock Chase. Four plots were used as controls and four plots were trampled at an intensity of 30p pl^{-1} every 14 days from 27.08.74. to 20.11.74., giving a total accumulated pressure of 210p pl^{-1}. Prior to trampling on each occasion visual damage was estimated by the point quadrat method on a 1m. x 0.5m. quadrat within the trampled and control



(a) Cannock Chase (treated 27.08.74, measured 15.10.74.)

(b) Cannock Chase, cumulative trampling effects (initial treatment (30 p pl-1) applied 27.08.74.)

(c) Difference between Treated and Control values

Figure 61 The comparative effects of trampling pressures applied at one instance in time (a) and accumulated over a period of time (b), C. vulgaris, (Cannock Chase).

plots. At the midpoint of the investigation, between the fourth and fifth treatment dates, visual damage assessments were made on a Latin Square block treated, at 0, 50, 100 and 200p pl^{-1} (each plot 2m. x 1m.), on 27.08.74. and measured on 15.10.74..

(b) Results

The results of the Latin Square block, for plots treated at one instance in time (Figure 61a) indicate a significant ($p < 0.001$) increase in browning with increasing trampling pressure. With increasing cumulative trampling pressure there is a significant ($p < 0.001$) increase in browning (Figure 61b). The browning of trampled plots is consistently, and significantly ($p < 0.001$) greater than the browning of control plots. However, over the trampling period from August to November 1974 the visual damage of control plots also exhibits an increase which is similar to that of treated plots, the increase in browning of treated plots being 24.4% of total vegetation hits to 69.6% compared to 3.8% to 40.2% for control plots. The difference between control and trampled plots (Figure 61c) exhibits an erratic pattern with increased trampling intensity, the mean difference between control and treated plots being 27.2 ± 2.2%. It is of interest to note that the damage values compare favourably with those of the Latin Square experiment at the higher trampling pressures, i.e. at a cumulative pressure of 210p pl^{-1} a damage value of 69.6% was recorded compared to 67.6% at 200p pl^{-1} in the Latin Square block. Similarly, there is a general agreement between the differences between control and treated plots for the two experimental regimes. The discrepencies at lower trampling intensities in absolute browning values (i.e. 45.9% browning at a pressure of 50p pl^{-1} on the Latin Square block compared to 24.4% at 60p pl^{-1} on the cumulative trampling plots) may reflect a time factor since the cumulative trampling total of 60p pl^{-1} was applied on

10.09.74., and between that date and 15.10.74. (when the Latin Square block was assessed for visual damage) control browning increased from 3.8% to 32.7%.

(c) Discussion

The results suggest that the increase in visual damage with increased cumulative trampling intensity is entirely accommodated by an increase in browning of the control plots and that, consequently, maximum damage occurs at a low intensity of trampling above which no further appreciable increase occurs. Assuming the control values to be accurate, the results of the plants trampled over a range of intensities at one time suggest that the significant increase in visual damage with increasing trampling intensity reflects a greater number of plants being damaged per plot rather than greater damage per plant. However, this does not explain why RWC's of single shoots decrease with increasing trampling intensity (Figure 47). With increased intensity the probability that a particular plant will be struck, and struck more frequently, than at lower intensities will increase, consequently, damage to the plant is likely to be greater. However, the significance of the first impact remains unresolved. The levelling off of graphs showing the relationships between trampling intensity and RWC or visual damage suggests that below a threshold trampling pressure, damage to the individual plant increases with increasing number of impacts. The absence of such marked levelling off in the Kerloch data may result from differences in community and plant structure between the Kerloch and Cannock Chase sites.

The differences existing between control and treated plots for instantaneously trampled and cumulatively trampled plots are similar in magnitude and trend. The results imply that a correspondence exists, in terms of damage, between cumulative and instantaneous trampling intensities, and that a cumulative pressure has a similar effect as the same pressure applied at one instance at the Cannock Chase site (cf. Bayfield, 1971). Although at variance with the observations of Leney (1974), the discrepancy may arise because of the low recuperative potential of C. vulgaris resulting from its low growth rate (cf. Gimingham, 1972; Grime & Hunt, 1975) so that unlike certain herbs and grasses, it is unable to repair damage between wear periods. The mechanism of injury may also be different e.g. water deficits impairing assimilation and growth, in contrast to the effects of trampling on herbs (Section V). Thus, up to the saturating level of pressure, the inability to repair damage rapidly, results in a cumulative increase in injury. Above the saturation level damage may be maximal in terms of the degree of cavitation in the xylem vessels, mechanical damage to tissues, and the water deficit arising from an imbalance between water supply and loss. The degree of injury resulting, however, is a protoplasmic variable which is seasonally dependent upon the physiological state of the plant.

The damage has been assessed on intact plants only in the present study, but breakage and detachment of stems will also occur (cf. Bayfield, 1971). Again a saturation effect is likely in which breakage occurs to a maximum level above which no further stems are broken, possibly because they are procumbent or have all been destroyed.

6. General Conclusions

Trampling on C. vulgaris results in increased browning of the shoots due to low RWC's. The desiccation injury at a particular intensity of trampling is not constant but varies throughout the year. The variables of greatest value in predicting the injury the plant is likely to suffer is the relationship between desiccation avoidance, desiccation tolerance and RWC at the time of trampling, which reflects the plant's ability to buffer itself against water loss. These variables depend upon the age and stage of development of the plant, but allow the susceptibility of the plant to be estimated prior to damage occuring. However, their influence is modified by the environment experienced by the plant following damage. The immediate post-treatment period would appear to be especially relevant as it determines the rate of water loss and consequently the possible ability of the plant to repair damage and harden to impending desiccation. Temperature and humidity effects appear especially relevant in this context.

The rate at which desiccation injury becomes apparent is not constant but also varies seasonally, a tendency being shown for more rapid development when the plant is least drought hardy. Similarly, the magnitude of damage is greater when the plant is least drought hardy.

Despite desiccation injury, growth potential is not totally removed. The apparent recovery over time may not be the result of recovery of injured tissues but the development of desiccation tolerant buds when the water stress had been alleviated, combined with abscission of injured and dead leaves which are of little further use to the plant in terms of assimilation capacity.

An increase in visible damage also appears to be related to increasing trampling pressure. The results suggest that saturation values of injury occur, but the absolute damage resulting from such a threshold level of pressure depends upon the time of the year at which the plant is trampled. As a corollary of the conclusion that C. vulgaris varies seasonally in its susceptibility to trampling, the results of C. vulgaris stands treated over a range of trampling pressures suggest that for a predefined level of damage, the trampling intensity required to induce the injury would vary seasonally. This re-emphasises that although environmental conditions may modify the response, the carrying capacity of C. vulgaris in terms of walking pressure may vary seasonally and in response to physiological changes within the plant.

The response of C. vulgaris to cumulative trampling pressure remains obscure. The evidence suggests that a trampling intensity dependent effect exists which applies to the individual plant, although it is conceded that with increasing number of passages across the plot, an increasing number of plants will be damaged and to a greater degree.

It is possible that the threshold intensity of use defining a given level of damage, although varying seasonally, does not vary according to whether the required intensity is applied at one instant in time or spread over a number of weeks. This may arise from the low recuperative potential of C. vulgaris, but it is evident from the foregoing investigations that this grossly oversimplifies a more complex plant reaction to trampling in which environmental effects, seasonal changes in the physiological state of the plant or adaptation to desiccation occurring over the trampling period, which may reduce the likelihood of protoplasmic injury, are integrated to determine the susceptibility of the plant to trampling damage.