BIOCHEMICAL AND PHYSIOLOGICAL CHANGES OCCURRING DURING WILTING AND THE EARLY STAGES OF ENSILAGE

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For Doug, and my parents

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SYMBOLS AND ABBREVIATIONS

Roman Alphabet

A	available energy; R_n - G - S - P
ATP	adenosine triphosphate
B.O.D.	biological oxygen demand
c	concentration of a gas in air (subscripts CO_2, carbon dioxide; H_2O, water vapour)
c_p	specific heat of air at constant pressure
CP	crude protein
D M	dry matter content (%, wet basis)
DNA	deoxyribonucleic acid
e	vapour pressure (mb), at $z = z_R$
e_w	saturation vapour pressure
E	swath evaporation rate (kg m^{-2} s^{-1}, where area refers to swath)
E_o	potential evapotranspiration
EE	ear emergence
F W	fresh weight
G	energy flux into ground
H	sensible heat flux (+ve upwards), at $z = z_R$
H K	Hay Knowes field
I.R.G.A.	infra-red gas analyser
L or l	lower (abaxial) surface of leaf
L.	*Lactobacillus* spp.
L A	lactic acid bacteria
L F	Lower Fulford field
Mrad	megarad; dose unit of gamma-irradiation
o pH	optimum pH

ppm	parts per million
p	net rate of energy absorption by photosynthesis and respiration
P_N	net photosynthetic rate
r	resistance to transfer (subscripts CO_2, H_2O)
$r_{a \text{ or } b}$	boundary layer resistance (swath and leaf; subscripts CO_2, H_2O; L or l, lower and U or u, upper surface of leaf)
r_c	cuticular resistance (leaf; subscripts H_2O, l, u as above)
r_D	bulk aerodynamic resistance of the swath surface to momentum transfer (swath external resistance)
r_i	internal resistance (swath and leaf)
r_I	climatological resistance (Stewart and Thom 1973)
r_s	stomatal resistance (leaf; subscripts CO_2, H_2O)
r_S	surface resistance of the swath (Monteith 1965)
r_{ST}	bulk physiological resistance of the swath to water vapour transfer
R	total resistance (leaf)
R_D	dark respiration rate
R_n	net radiation ($W\ m^{-2}$)
RNA	ribonucleic acid; RNase ribonuclease enzyme
R H	relative humidity (%)
R.Q.	respiratory quotient
R W C	relative water content (%)
S	total energy flux into storage between z_R and ground level
Str.	*Streptococcus* spp.
t	air temperature (^oC); time period (h)
T	leaf transpiration rate (subscripts l, u)
T C A	trichloroacetic acid
TNBS	2,4,6 - trinitrobenzenesulphonic acid
U or u	upper (adaxial) surface of leaf
vpd	vapour pressure deficit (mb); $(e_w(t) - e)$

W S C	water-soluble carbohydrates
W S D	water saturation deficit (%)
z	height above ground; z_R reference level

Greek Alphabet

$\alpha(t)$	temperature-dependent conversion factor for photosynthetic rate calculations
Δ	rate of change of saturation vapour pressure with temperature; $d\, e_w/dt$ (mb $^{o}C^{-1}$)
Δc	concentration gradient (subscripts CO_2, H_2O)
γ	psychrometric constant (c.0.66 mb $^{o}C^{-1}$)
λ	latent heat of vapourisation of water
λE	latent heat flux (+ve upwards), at $z = z_R$
$(\lambda E)_{eq.}$	equlibrium evaporation rate of the swath
ρ	density of air

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ABSTRACT

An intensive study was made of the early stages of silage-making, i.e. the field wilting period and the first few days of fermentation in the silo. Wilting was studied under both field and controlled environmental conditions. Fermentation was studied using axenic and inoculated laboratory silos.

In the Field Studies (Section 1), evaporation rates of grass swaths were determined under a range of environmental conditions which were monitored continuously by microclimate techniques. Evaporation occurred only between 09.00 and 18.00 h, and was highest during the morning then decreased progressively during the afternoon. This evaporation pattern was analysed in terms of the ambient vapour pressure deficit and the swath resistance to evaporation. The decrease in swath evaporation during the day, and throughout the length of the wilting period, resulted from the marked increase in swath resistance.

In the Controlled Environment Studies (Section 2), simulated swath evaporation rates, and single leaf transpiration and net photosynthetic rates, were determined after harvest. Stomatal closure occurred c. 15 min after cutting; thereafter transpiration was entirely cuticular and net photosynthesis was zero. Leaf segment studies using resaturated plant material showed that net photosynthetic potential declined to zero within the first 24 h of wilting and dark respiratory potential increased more than two-fold over the same period. These changes were related to the leaf D M content. The biochemical changes in grass leaves during wilting comprised: 1) an overall decrease in total

soluble carbohydrates, glucose and fructose, although oscillations in sugar levels occurred, and 2) an overall decrease in total soluble protein with a concomitant increase in total free amino groups. Reasons for these changes are discussed.

In the Ensilage Studies (Section 3), the silos containing grass inoculated with lactic acid bacteria showed: 1) a rapid pH fall and 2) an increase in total soluble sugars, glucose and fructose during the first 3 - 6 h of fermentation followed by a decrease, and 3) an overall decrease in total soluble protein and an increase in total free amino groups. However, the irradiated axenic silages showed no change in pH or sugar levels, but a similar pattern of changes in nitrogenous components. The roles of plant and bacterial enzymes, and the mechanism of these changes, are discussed.

GENERAL INTRODUCTION

The ensilage of green crops is essentially a fermentation process in which a specific group of micro-organisms, the lactic acid bacteria, ferment plant cell substrates to a mixture of acid products under anaerobic conditions. The production of fermentation acids (mainly lactic and acetic) renders the plant material unsuitable for the growth of decomposing organisms and thereby preserves it. Some aspects of the ensilage process have been reviewed by McDonald *et al.* (1966), Whittenbury *et al.* (1967), Ohyama (1971), Woolford (1972), Ruxton (1972) and McDonald and Whittenbury (1973), who considered the microbiology and biochemistry, which are interrelated and of fundamental importance. Extensive silage research programmes are underway in many countries, for example W. Germany (Zimmer's group), Belgium (De Vuyst's group), Japan (Ohyama's and Uchida's groups) and the U.K. (McDonald's and Wilkin's groups).

The mechanism of the ensilage fermentation process is well documented regarding the sequence of biochemical and microbial changes which occur inside the silo. The published reviews agree that when herbage is ensiled, the lactic acid bacteria, present in low numbers on the fresh plant material, begin to multiply and establish themselves as the air trapped inside the silo is progressively exhausted through plant and microbial oxidative processes. The aerobic bacteria, originally dominant on the fresh herbage, are suppressed and may die as conditions become anaerobic. The numbers and species of lactic acid bacteria present determine the nature and directions of the biochemical

processes which predominate. Liberation of plant sap supplies the bacteria with their most important fermentation substrates, the soluble sugars glucose and fructose, which are converted into a mixture of lactic and acetic acids by well-defined biochemical pathways. The characteristic pH fall associated with ensilage results in the preservation of the plant material.

However, the fermentation equilibrium can only be maintained in the direction of lactic acid production under certain restricted conditions:-

Firstly, for successful ensilage, anaerobic conditions must be achieved rapidly and maintained strictly. The presence of air during ensilage, either initially through delayed sealing or later through leakage, can cause considerable losses by the oxidative activities of plant and microbial enzymes. Hence, the silage product is of little nutritional value. (McDonald and Whittenbury 1967; Honig 1969; H.M.S.O. 1970; Ohyama *et al.* 1970; Ohyama and Masaki 1971; Weise 1971; Ruxton and McDonald 1974).

Secondly, successful preservation requires a suitable microbial flora. The roles of the various species of lactic acid bacteria in the ensilage process have been reviewed by Whittenbury (1968) and Woolford (1972). In general, a succession of species occurs, those dominant initially giving way to those species better adapted to progressively lower pH conditions. There are two main groups of lactic acid bacteria, the homofermenters producing relatively more lactic acid than the heterofermenters, so that dominance of the silage microflora by the former group is preferable. However lactic acid species may

not be able to develop or persist successfully in the presence of other competitive micro-organisms. Competition from other anaerobic species such as clostridia can be a serious problem, especially in wet herbage. These micro-organisms can tolerate and exploit wet acid conditions causing a secondary fermentation, destruction of the lactic acid, and of amino acids producing amines and ammonia which may be toxic, if present in large amounts. Hence, the pH rises and an unstable silage of little nutritional value is produced (Gibson 1965; McDonald *et al.*1966; Whittenbury 1968; Woolford 1972). Competition from aerobic micro-organisms, for the limiting plant substrates available, can also be a problem, as previously mentioned. Microbial aspects of ensilage are currently under investigation in many countries, for example France (Gouet *et al.*1972), W. Germany (Weise 1967), Japan (Akuta *et al.*1970; Ohyama *et al.*1971; Nishiyama *et al.*1972), U.S.S.R. (Pereverzeva and Lapotyshkin 1971) and in the U.K. (Woolford 1974; Mann 1975).

Thirdly, successful preservation is dependent upon the plant component which has a central place in the ensilage process: plant substrates provide the micro-organisms with their nutritional requirements, both in the silo, and subsequently in the rumen. However, the plant aspects of ensilage, plant biochemical composition and plant enzyme activity, are often overlooked; hence there is little information about this subject in the silage literature. (McDonald 1972; Woolford 1972). Changes in the plant component may occur not only during ensilage itself, but also during the field stages of silage-making from the moment the herbage is cut: the plant aspects of ensilage should be considered throughout the early stages in the field and in the silo, and form the subject of this research programme.

PLANT ASPECTS OF ENSILAGE

The biochemical composition of the plant material depends upon a wide range of plant and environmental factors during growth. Some control over the ensilage process can be achieved by careful choice of plant material of optimum biochemical composition: it is possible to select a plant species, variety and growth stage with suitable levels of fermentable sugars, nitrogen components, and cellulose fibre and hence D-value (McDonald *et al.* 1966; McDonald 1972; H.M.S.O. 1970). This aspect of ensilage is considered in detail below under the heading, 'Biochemical Nature of the Plant Material'. Knowledge of the biochemistry of herbage species is surprisingly vague in many areas in spite of its obvious economic significance (Butler and Bailey 1973).

Relatively few studies have acknowledged and investigated the role of plant enzymes during ensilage. Recently, Woolford (1972) reviewed some relevant literature but considered only U.K. studies directly concerned with silage. In fact conservation studies in many countries, and aspects of plant physiology and biochemistry relating to the field stages in particular, should also be considered. Details are given below under the headings, 'Biochemical Changes during Wilting' and 'Biochemical Changes during the Early Stages of Fermentation'.

The probable significance of plant enzymes in the ensilage process was recognised and stressed by Mabbitt in 1951. Subsequently, there has been much speculation, on the basis of very little experimental evidence, about their role. There have been several attempts to separate and define the roles of the plant and bacterial enzymes in the ensilage process. Usually the bacterial component is removed from the system by

a sterilisation procedure. Alternatively, the plant component can be removed and replaced by an inert substrate (Woolford 1972, 1974).

Basically, there are two methods of obtaining sterile plant material: 1. Cultivation from seed under sterile conditions, and 2. Sterilisation of naturally grown grass. It is important that both the plant biochemical composition and the plant enzyme activity are unaffected, a difficult and exacting requirement. The first method was attempted by Mabbitt (1951), Nilsson (1959), Stirling (1953, 1968) and Playne *et al.* (1967) who managed to produce small quantities of aseptically-grown timothy grass, which was however of atypical biochemical composition. This aseptic plant material was ensiled with and without inocula of lactic acid bacteria, and conclusions were drawn about the role of plant enzymes from the results. More recently, techniques for the cultivation of sterile plant material have improved with the development of elaborate 'isolation systems' originally intended for rearing germ-free animals (Heneghan 1973). The plants are grown in large sterile chambers under controlled conditions, and very recently large amounts of aseptic plant material have been produced in conjunction with the American Space Programme. This plant material is rigorously tested and must satisfy many criteria in order to be considered sterile (Walkinshaw *et al.* 1973; Hale *et al.* 1973). The plant biochemical composition should resemble that of naturally grown plant material, and would be ideal for studying plant enzyme mediated changes involved in silage fermentation, but this has not yet been attempted.

The second method of obtaining sterile plant material, i.e. by sterilisation of naturally grown herbage, is also difficult, as many

conventional sterilisation procedures, such as heat treatment, are too drastic and non-selective in their action: plant and microbial enzymes are destroyed simultaneously and plant biochemical composition is affected (Gouet *et al.* 1970). Bactericidal agents such as chloroform, and antibiotics such as bacitracin and penicillin have been used (Gouet and Fatianoff 1964) but are unreliable and the plant material produced cannot be used subsequently for inoculation experiments (Gouet *et al.* 1970). In principle, it may be possible to find an antibiotic which would selectively inhibit the microbial enzymes alone.

A satisfactory compromise is provided by the use of γ-irradiation, a simple and rapid sterilisation technique developed in France by Gouet *et al.* (1970, 1972). Exposure of plant material to a high dose (1.2 - 2.5 Mrad) of γ-irradiation from a ^{60}CO source effectively inactivates the bacteria but leaves the plant enzymes intact (Haber and Walne 1968; Gouet *et al.* 1970, 1972), and could provide a solution to this problem (Huhtanen and Pensack 1963).

The physiological system which appears to be most sensitive to irradiation is that responsible for cell division. It is thought that the initial lesion is in the DNA itself but this is not yet clearly established. By contrast, enzymes are generally resistant to radiation *in vivo*. A great many studies have been made of irradiation of the more common metabolic enzymes; for example, the effect on respiratory enzymes is small. Inhibition of enzyme replacement is probably involved rather than direct inactivation of enzyme systems. Since micro-organisms can continue most of their enzyme-mediated metabolic functions for a limited time after irradiation, they are termed *inactivated* rather than *dead* (Bridges 1964).

This γ-irradiation sterilisation treatment provides a potentially useful means of studying plant enzyme activity during the early stages of fermentation in the silo. Another approach is needed for studying the plant aspects involved in the field stages of silage-making.

WILTING

The success of the ensilage process is highly dependent upon the moisture content of the plant material. A very important means of regulation can be readily achieved by reducing the plant moisture content prior to ensiling. Under relatively dry conditions of 70% moisture or less, very little clostridial activity occurs and a lactic acid fermentation is induced (McDonald *et al.* 1966). A field-drying treatment termed *wilting* is frequently used as a pre-treatment for ensilage. (The *wilting* term used in an agricultural context for the natural drying process of cut plant material, is related to, but not synonymous with the *wilting* term of plant physiology, which refers to the response of growing plants to moisture stress). The process of water loss from the cells of cut plant material is accompanied by physiological and biochemical changes, so that plant enzyme activity and composition are affected. Despite the frequent use of a wilting pre-treatment for ensilage, detailed information on the nature of these changes is lacking, and the fundamental drying process is very little understood (Warboys 1967; Thaine 1967; Kormos and Chestnutt 1967; Pizarro and James 1972).

Although the use of a wilting treatment during silage-making was once a matter of controversy (Kormos and Chestnutt 1967; H.M.S.O. 1970), the beneficial effects of wilting to DM levels of about 30% are

now well established for temperate herbage species (Murdoch 1960; McDonald and Whittenbury 1967; Catchpoole 1972; McDonald 1972; McDonald *et al.* 1968; Lanigan 1966). For example, apart from the selective effect on the microflora, the extent of fermentation is restricted following wilting, resulting in a decrease in organic acid production (Ohyama *et al.* 1970, Daniel *et al.* 1970), a higher final pH and higher levels of residual sugars than in fresh silage (Anderson and Jackson 1970, Catchpoole 1972). There is little amino acid catabolism: increasing DM content is accompanied by a significant decrease in ammonia and carbon dioxide formation (Daniel *et al.*1970; Gouet *et al.* 1970). Butyric acid formation is completely inhibited in wilted silage above 30% DM (Gouet *et al.* 1965). Furthermore, effluent losses are reduced to a very low level or zero following wilting: this is a most important advantage of wilting since the B.O.D. of silage effluent is extremely high and poses serious pollution problems (Sutter 1955; H.M.S.O. 1970; Catchpoole 1972; Pedersen *et al.* 1973). Hence, total losses from the silo are reduced and a product of high nutritional quality is obtained on ensiling wilted plant material (Nordfeldt 1955; Jones 1966; McDonald *et al.* 1968; Anderson and Jackson 1970; Uchida *et al.* 1970, 1971; Catchpoole 1972). There are further nutritional advantages of wilting since the silage product is eaten more readily by ruminants and their total DM intake increases (H.M.S.O. 1970; Catchpoole 1972). In fact, the high lactic acid content of fresh silage can itself reduce intake (McLeod *et al.* 1970). There are also practical advantages of wilting since lighter herbage is easier to handle and an increased tonnage can be stored (H.M.S.O. 1970).

However, there are a number of disadvantages associated with the wilting process such as the inherent weather risk and the time lost.

The field losses probably represent the most serious potential disadvantage of wilting. In principle these may be of three kinds (Pizarro and James 1972):-

1. *Physical losses* of plant material during harvesting and drying.
2. *Leaching losses* of plant nutrients during rain.
3. *Respiration losses* through plant enzyme activity in the cut herbage.

In the literature, there are very few attempts to separate these three main causes of field loss. Usually the changes in the plant material are characterised only in overall terms of DM changes. For example, Kormos and Chestnutt (1967) cite various studies in which net losses, no change, or net gains in DM were found depending on the nature of the crop, the prevailing weather conditions and the length of the wilting period. Some DM changes during wilting are summarised in Table 1. Hence, there is no unanimity of opinion about the DM changes associated with field wilting. Some workers consider the field losses to be of little importance (e.g. Fleischmann 1912; Greenhill 1936; Nash 1959) whilst others have recorded losses of considerable magnitude (e.g. Mihin and Tupikova 1940; Murdoch 1953; Dijkstra and Brandsma 1955; Murdoch *et al.*1959; Watson and Nash 1960). Evidence and comments on the effects of plant and environmental factors are rather generalised and vague, probably due to the difficulty in measuring losses accurately, and to complications provided by so many interacting factors which may influence losses under different conditions (Kormos and Chestnutt 1967).

The respiratory losses are likely to be especially important since the respiratory substrates are primarily readily available carbohydrates, which represent valuable nutrients for the silage and rumen microfloras. Few studies of respiratory losses as distinct from total

TABLE 1 DM changes reported

Country	Wilting Period	Wilting Conditions	Final DM %
Germany	1d	good	-
England	54h	good	51
U.S.S.R.	3 - 4d	-	-
Switzerland	1d	good	-
Netherlands	1d	good	-
Germany	-	-	-
Netherlands	3d	-	39 - 47
N. Ireland	1 - 2d	good	58 - 73
N. Ireland	1 - 2d	bad	-
Switzerland	-	good	30 - 35
Netherlands	-	average - bad	31 - 55
Scotland	1d	v. good - v. bad	19 - 30
N. Ireland	18d	bad	80
U.S.S.R.	-	-	-
Denmark	-	-	30 - 37
Denmark	-	-	38 - 51
N. Ireland	1 - 2d	-	-
N. Ireland	3d	bad	30
N. Ireland	1d	average	-
N. Ireland	3 - 6h	average - good	27 - 42
Denmark	5 - 28h	good	33 - 51
Denmark	31 - 74h	bad	32 - 38

during wilting in the field

DM loss %	DM gain %	Reference	
0	2 - 5	Fleischmann	1912
0	0	Greenhill	1936
12 - 17	0	Mihin and Tupikova	1940
0 - 3	0	Crasemann and Heinzl	1949
2.5 - 3.0	0	Bosch and Deijs	1950
+	+	Flieg	1952
12.3 - 13.3	0	Dijkstra	1952
2.5 - 4.0	0	Murdoch	1953
6.5	0	Murdoch	1953
0 - 2	0	Sutter	1955
2.3 - 12.4	0.6	Dijkstra and Brandsma	1955
1	1 - 3	Nash	1959
11.4 - 25.2	0	Murdoch *et al.*	1959
-	+	Kocevyh	1962
1 - 3	0	Stat. Forsogsvirk.	
2 - 6	0	Plkutt.	1963
0,6	+	Brown	1963
8	0	Kormos and Chestnutt	1966
1.1	0	Kormos and Chestnutt	1967
0	0 - 1.1		
0.6 - 3.3	0	Moller and Skovborg	1971
1.5 - 6.0	0		

DM loss have been made (Harberts 1954; Musgrave and Dawson 1946; Greenhill 1959; Wood 1972; Pizarro and James 1972). Pizarro and James (1972) concluded that DM losses by respiration are small and amount to only about 1% in cut swards of *Lolium perenne* under simulated hay-making conditions, but these losses may be quite substantial in terms of sugars. The nature of the biochemical changes to be expected during wilting is discussed in detail below (pp. 21-35).
A further disadvantage of wilting is that an overdry crop can cause overheating in the silo with the formation of an indigestible product of little nutritional value (Watson *et al.* 1961; McDonald *et al.* 1966).

It is generally felt that where wilting is possible it is desirable for high moisture crops since the advantages outweigh the disadvantages. Watson *et al.*(1961) claim that wilting is possible in any weather, and some water loss from cut herbage is inevitable under most conditions. Wilting is the usual pre-treatment for silage-making in many countries including the U.K., France and the U.S.A. (Watson *et al.* 1961; McDonald 1972; Dulphy 1973).

BIOCHEMICAL NATURE OF THE PLANT MATERIAL

Recently, Butler and Bailey (1973) reviewed the chemistry and biochemistry of herbage extensively. Those aspects of the biochemical composition of herbage, with important implications in the early stages of ensilage, will only be considered here. The ryegrasses, *Lolium perenne* L. and *Lolium multiflorum* Lam. species used widely in silage research and practice in the U.K., are taken as examples. All figures are quoted as per cent. of the herbage DM.

1. Plant Carbohydrates

Plant carbohydrates may be broadly divided into the non-structural carbohydrates found mainly in the cytoplasm of plant cells, and the structural carbohydrates which comprise the plant cell walls. The former group, sometimes termed 'available carbohydrates', is of great importance in the ensilage process since it provides an energy source for the silage bacteria and for the plant cells themselves during wilting; the latter group provides a major energy source for ruminant animals and may have a minor role in the ensilage process (McDonald *et al.* 1966).

Non-structural carbohydrates found in forage grasses are the sugars glucose, fructose and sucrose and the polysaccharides fructan and starch. The sugars are mostly metabolic intermediates. They occur in rather low concentrations and do not vary greatly except for sucrose. Glucose and fructose generally comprise virtually all of the free reducing sugars, and occur in field-grown herbage in about a 1:1 ratio at concentrations between 1 and 3%. Sucrose occurs in larger amounts at 2 to 8%, and with glucose and fructose comprises most of the total free sugars (McIlroy 1967).

The major non-structural polysaccharide in temperate grasses is fructan , a storage carbohydrate whose accumulation and degradation accounts for most of the fluctuations in total non-structural carbohydrates in field-grown herbage. Fructans are $\beta 2 \rightarrow 6$ linked D-fructofuranose polymers which occur in a homologous series in grass tissue. Ryegrass is a short-chain fructan accumulator with the highest concentrations occurring in the stems. (Smith and Grotelueschen 1966).

However, temperate grasses can also contain small amounts of starch (Smith 1971; Wilson and Bailey 1971). The occurrence of starch in ryegrass leaves was reported by Wood (1945), and in Italian ryegrass by Rocher (1973, 1974), and Prioul (1974): electronmicrographs show starch grains associated with the chloroplasts.

Many workers concerned with ensilage have measured the water-soluble carbohydrate (WSC) concentration of herbage (mainly fructan, sucrose, glucose and fructose), which is most useful for assessing its ensiling potential. In temperate grasses WSC concentration is very variable, depending on the species and plant organ, and is subject to both diurnal and seasonal variations, and to the influence of environmental factors such as light, temperature and fertiliser application. In general, of the herbage grasses usually grown in the U.K., the ryegrasses have the highest WSC contents occasionally exceeding 30% (Waite and Boyd 1953; McDonald *et al.* 1966).

Since fructan accumulates mainly in the stems, the leaf:stem ratio greatly influences the total WSC content. This is reflected in characteristic seasonal WSC variations, the concentration increasing with maturity as the proportions of stem increases. *L. perenne* growing in Scotland was found to have a low concentration of free hexoses throughout the year, but a sucrose maximum about May - June in the leaves and stems and a fructan maximum in June in the stems (Waite and Boyd 1953; Mackenzie and Wylam 1957). Stage of growth studies usually show that a WSC peak occurs just before or at ear emergence and that fructan is the main non-structural carbohydrate accumulated (Norman and Richardson 1937; Waite 1957; Dent and Aldrich 1967; Terry and Tilley 1964). Temperate grasses have a characteristic

diurnal rhythm: WSC concentration increases during the morning until about mid-afternoon, then decreases until daylight the next day. The diurnal pattern is mostly a result of changes in sucrose concentrations; diurnal fructan changes are not well established (Smith 1973). The ryegrasses exhibit typical diurnal rhythms in sucrose and WSC concentrations (Kingsbury 1965).

The environmental factors, light, temperature and fertiliser application, have a large influence on WSC content, primarily through an effect on fructan levels. It is generally agreed that high WSC concentrations in grass herbage are favoured by high light intensity, low temperature and low applications of fertiliser nitrogen during growth (Smith 1973).

Plant structural components consist primarily of polysaccharides with small amounts of lignin and protein. They differ from the reserve carbohydrates above in that they are not normally remobilised once formed. However, under the unusual circumstances of plant 'starvation' during wilting, and of fermentation during ensilage, changes in the structural carbohydrate component cannot be ruled out. The traditional view is that plant cell wall polysaccharides take little part in the ensilage process, although it has been suggested that they may make a minor contribution (McDonald *et al.* 1966). However their main importance undoubtedly lies in the field of nutrition, in their role in rumen metabolism (Bailey 1973). Since there is some controversy over the significance of the structural carbohydrates in the ensilage process, their nature is now considered briefly, as a basis for further discussion.

The structural polysaccharides may be divided into two main classes, the fibre polysaccharides consisting of cellulose microfibrils embedded in the matrix polysaccharides, consisting of the pectic substances and hemicelluloses.

Herbage cellulose has not been isolated and studied in detail but is assumed to be chemically similar to the cellulose in all other plants: a linear polymer of $\beta 1 \rightarrow 4$ linked D-glucose units of high molecular weight with degrees of polymerisation up to ten thousand (Bailey 1973).

Pectic substances are a group of amorphous polysaccharides present in high levels in the middle lamellae of plant cell walls. Grass pectins have not been investigated structurally but are probably similar to the pectins of other plants, consisting of polymers of uronic acid units, arabans, galactans and various other sugars in small amounts.

Hemicelluloses are closely associated with cellulose in plant cell walls and may be divided into two broad classes; pentosans and hexosans. The pentosans are mainly xylans in herbaceous plants, $\beta 1 \rightarrow 4$ linked chains to which side-chains of arabinose, galactose, glucose and gluconic acid may be attached. The hexosans are primarily mannans containing glucose units and sometimes galactose side-units. β-glucans have also been isolated from plant tissues. These are chains of $\beta 1 \rightarrow 3$ and $\beta 1 \rightarrow 4$ linked glucose units quite distinct from cellulose amd similar to cereal gum. Recently, such a β-glucan has been isolated from oat leaf hemicellulose (Frazer and Wilkie 1971; Bailey 1973).

The literature on herbage composition contains few studies where both the hemicellulose and cellulose fractions have been measured in a meaningful way (Bailey 1973). The data available on structural carbohydrate composition in herbage grasses show that variations occur in the amounts of fractions both absolutely and relative to one another. The main differences are found between species, varieties and plant parts, and are influenced by seasonal changes, climatic factors and fertiliser application.

2. Plant Nitrogenous Components

Proteins. Herbage proteins have not been studied extensively but are thought to be characteristic of plant proteins in general. Leaf proteins are almost entirely in the form of metabolic protein concerned with cell growth and biochemical function, and are found primarily in the cytoplasm. When leaf cells are disrupted, a large proportion of the cell protein is liberated into solution, the 'soluble leaf protein', while the membranous material is broken into fragments containing the 'insoluble leaf protein'. The soluble fraction is a heterogeneous mixture of different proteins in which a single enzyme, ribulose diphosphate carboxylase, constitutes up to half of the total soluble leaf protein. The insoluble protein fraction is much less well characterised but frequently accounts for up to half of the total leaf protein (Lyttleton 1973). Some information is available about the location of proteins in leaf cells, about 50 - 60% occurring in the chloroplasts, less than 5% in the mitochondria, about 1 - 2% in the nucleus, and the remainder in the ribosomes and cytoplasmic matrix, with a small fraction in the cell wall. Hence chloroplast protein is the major component, and the bulk of the insoluble chloroplast protein is

associated with lipid material and pigments such as chlorophyll. Both mitochondria and chloroplasts appear to contain autonomous protein synthesising systems (Boutler 1970; Lyttleton 1973).

The amount of literature dealing with the protein content of herbage is immense, and very variable. Only some major features and general trends in herbage protein studies are considered here.

Herbage proteins are often characterised by measuring the crude protein (CP) content of herbage material, but such a measure will not distinguish between nitrogen present as true protein, or inorganic nitrogen, free amino acids, low molecular weight peptides, nucleic acids, etc. The true protein content will in general be at least 10% lower than the CP and the difference may be greater, up to 25% or more. Unfortunately, in the scientific literature, by far the most information about the protein content of herbage is recorded merely as the CP value (Lyttleton 1973).

Wide variations occur in the protein content of temperate herbage grasses, but as a very general rule, the range 10 - 20% CP may be considered typical. Grasses such as *Lolium* spp. which require a moist, temperate climate can produce high yields of CP. A decline in CP is observed as grasses mature (Mowat *et al.* 1965).

Data on the amino acid composition of herbage proteins are not very extensive, but indicate that in general it remains fairly stable even when protein levels rise or fall dramatically. Analysis of extracted ryegrass protein has shown it to be rich in aspartic and glutamic acids, leucine, alanine, valine and arginine, with intermediate levels

of most other protein amino acids, but poor in cystine, tryptophan and methionine (Gerloff *et al.* 1965). Italian ryegrass protein is of very similar composition, but also relatively poor in tyrosine and proline (Goswami and Willcox 1969). It seems that the amino acid content of leaf protein varies only slightly from plant to plant. It is possible that individual proteins may vary in composition but the aggregated cell protein is remarkably constant (Lyttleton 1972).

Free Amino Compounds. The free amino acids and their amides are the major components of the 'soluble nitrogen' of plants while amines, small peptides, amino acids in bound non-protein form, nucleotides, chlorophyll and ureides are minor components (Hegarty and Peterson 1973).

The free amino acids occurring in herbage include the protein amino acids and their derivatives, and a wide variety of non-protein amino and imino acids. A common feature of plant maturation is the overall decline in total free amino acids as well as total nitrogen, probably through an increase in plant structural material. Generally, there is a decrease in individual amino acids with age, especially in asparagine levels. It seems that there are differences in amino acid values between species and varieties.

The nutritional conditions under which plants are grown can have profound effects on their nitrogen metabolism. Few studies of the effects of environmental factors, such as light, temperature and moisture stress, on the soluble nitrogen component of herbage plants have been carried out (Hegarty and Peterson 1973).

Comprehensive physiological studies on the metabolism and biosynthesis of the protein amino acids have been made (Yemm and Folkes 1958; Pate 1968; Folkes 1970). The non-protein amino acids are of little importance since they are present in low concentrations and do not appear to play an essential role in nitrogen metabolism. Few investigations have been made of the bound amino acids in herbage even though some of them may have important physiological properties, (Hegarty and Peterson 1973).

Plant amines are almost as widespread and diverse as the amino acids but rarely occur in comparable concentrations, and as a result, less is known about them. Herbage amines have not been extensively investigated. Simple aliphatic mono-amines have been detected in trace amounts in many higher plants. The diamines putrescine and cadaverine have been identified in herbage plants, and the presence of the polyamines spermine and spermidine has recently been recognised (Smith 1970). The small amounts of amines in herbage probably make little direct contribution to the overall nitrogen nutrition of livestock, but silage may contain large amounts of amines, for example histamine and tyramine in cocksfoot silage (Macpherson 1962).

BIOCHEMICAL CHANGES DURING WILTING

The drying process involves changes in the cut herbage in terms of both its microbial flora and its biochemical composition (Gouet *et al.* 1965; McDonald *et al.* 1966; Whittenbury *et al.* 1967; Pereverzeva and Lapotyshkin 1971; Mann 1974). Most studies of herbage drying have considered the long-term effects of field-curing for hay, in terms of the gross field losses incurred rather than the detailed changes

in crop quality (Watson and Nash 1960; Hart and Burton 1967). However some hay-making studies have recorded losses in biochemical components such as CP, starch equivalent, carotene and vitamin E in the course of drying (Carter 1960; Akopjan 1962; Hart and Burton 1967).

Some silage wilting studies have reported changes in carbohydrate and nitogenous components in more detail. Although some workers have found an increase in fermentable sugars in grass and legumes during wilting (Watson and Nash 1960), it is now generally held that a net respiratory loss of sugars occurs (McDonald and Whittenbury 1967). Measurements of the respiratory quotient for wilting grass and legumes have given values close to unity (Greenhill 1959; Pizarro and James 1972), indicating that the main respiratory substrate is a hexose sugar. Pizarro and James (1972) suggest that sugar losses during extended wilting may be quite substantial. Wylam (1953), Melvin (1963) and Festenstein (1966) have demonstrated sucrose catabolism to carbon dioxide in wilting grass. Wylam (1953) reported hydrolysis of almost half of ryegrass fructan during 24 hours' wilting to 45% DM. Other workers have reported that fructans and total soluble fructose residues decrease almost continuously throughout the drying period, and that sucrose levels decrease initially and then increase (Greenhill 1959; Melvin and Simpson 1963; Lanigan 1966).

Under drought conditions, the CP content of growing herbage can fall to a very low level (Lyttleton 1973) and there can be large effects on free amino acid levels (Hegarty and Peterson 1973). Although several workers have reported no, or negligible, change in the

total nitrogen and CP contents of wilting herbage (Greenhill 1936; Eriksson 1953; Dijkstra and Brandsma 1955), a decrease in true protein and an increase in simpler nitrogen compounds is usual during wilting (Macpherson 1952; Singh 1962; Brady 1960, 1965). Many workers have attributed such changes in nitrogen components to a rapid post-harvest protein hydrolysis, giving rise to an increase in peptide, free amino acids and amides, (Kemble and Macpherson 1954; Kemble 1956; Brady 1960, 1961; Ohyama 1970; Hegarty and Peterson 1973). The large increase in free amino acid levels during wilting may include a marked proline accumulation, as found by Barnett and Naylor (1966) in Bermuda grass, and Kemble and Macpherson (1954) in ryegrass. Large increases in amides can also occur.

The effects of water stress on both nitrogen and carbohydrate components of herbage are reviewed in more detail below (pp.26-29).

Greater insight into the wilting process can be gained only if the fundamental plant responses are considered: when the leafy shoots of herbage plants are severed from the roots at harvest, disturbance of normal physiological and metabolic processes occurs. The plant responds to cutting in several ways (Kramer and Brix 1965):

1. *Physical changes,* such as loss of turgidity and premature closure of stomata.
2. *Physiological changes,* in transpiration, photosynthesis and respiration, for example.
3. *Biochemical changes,* in the plant carbohydrate and nitrogen metabolism, for example.

These changes probably depend on the plant response to injury on detachment; to water stress during the early stages of wilting, and the

onset of plant senescence during extended wilting. The plant physiology literature contains information on these topics which is relevant. Crafts (1968) has reviewed the effects of water deficits on physiological processes and plant composition; Brady (1973) has provided information on the water stress aspects, and has reviewed the process of plant senescence. Many of the responses to water stress are parallel to those of senescence, and in many tissues water stress induces premature senescence. These aspects of plant physiology are usually studied in whole living plants, but detached plant parts are also used and are more relevant to the wilting situation.

Plant Responses to Water Stress

Many plant functions are impeded when the water content of the plant is reduced. Physiological responses to water stress will be considered first, since they determine the biochemical changes which occur.

Photosynthesis. Since the end of the last century, many workers have found a reduction in net assimilation rate with wilting (used in the physiological sense, referring to attached leaves), (Heath 1969). Photosynthesis and transpiration rates often decline to comparable extents in water-stressed plants, suggesting that stomatal behaviour is controlling both functions (Brix 1962; Boyer 1970). However, reduced photosynthesis still persists in stressed leaves irrespective of stomatal behaviour (Scarth and Shaw 1951; Wardlaw 1967; Slavik 1965). There is some evidence to suggest that chloroplasts from water-stressed plants are deficient in Hill activity and photophosphorylation. There are strong indications that photosynthesis is inhibited in water-stressed plants by factors additional to CO_2 supply (Brady 1973).

Respiration. The effect of water stress on respiration rate has been studied at length in many species, but it is by no means resolved. Different workers have found that water stress causes an increase, a decrease, or has no effect on the dark respiration rate of plants (Heath 1969; Pizarro and James 1972; Brady 1973).

Brady (1973) states that respiration has generally been found to fall with the water content of plants. Greenhill (1959) found a decreased respiration rate in *Lolium* leaves with reduced moisture content, and more recently Meidner (1967) provided direct evidence of a decrease in dark respiration rate with increasing water deficit in maize leaves. However, Smith (1915), Iljin (1923) and Heath (1951) found that respiration rate increased with reduced leaf moisture content, but ultimately respiration rate fell when the moisture content became sufficiently low. Heath (1951) found a four-fold increase in dark respiration rate in *Pelargonium* leaves, and Iljin (1923) found a progressive rise in the dark respiration rate of *Ranunculus* leaves, with reduced water content. Of other species investigated, six including wheat showed a rise, and seven a fall. Short-term observations may reveal a period of increased respiratory activity when stress is applied suddenly (as on excision of the leaf), probably resulting from cell damage (Brady 1973).

Protoplasmic Effects. When plant cell water content falls, the chemical potential of water falls as the turgor, osmotic and matrix potentials vary (Slatyer 1967). The concentrations of solutes and the properties of protoplasm also change (Brady 1973). Henckel (1964) emphasises the physical and chemical characteristics of protoplasmic resistance to water stress. These include high protoplasmic viscosity and elasticity

coupled with a low respiration rate. The degree of hydration of cell proteins is also important, high resistance being associated with high levels of protein-bound water. Kessler *et al.* (1964) report a causal ralationship between the stability of proteins and their ability to bind water: the degree of hydrolysis depends on the rate and level of water loss. Ribosomal proteins and RNA are more stable than related nuclear and soluble cytoplasmic constituents. High osmotic pressures induce conformational changes in proteins through modification of the water-protein system essential for maintaining the secondary and tertiary protein structures. These changes paralleled changes in enzyme activities.

Biochemical Effects. Water stress may have both qualitative and quantitative effects on plant constituents. Physiological changes in respiration and photosynthesis are reflected in biochemical changes in plant constituents and enzyme activities. A shift towards the hydrolysis of macromolecules and a decline in synthetic reactions commonly occur, resulting in a reduced content of macromolecules in water-stressed tissues (Brady 1973). For example, there is evidence of inhibition of starch, nucleic acid and protein synthesis, increased hydrolytic activity of proteases, ribonucleases etc. and therefore a reduction in protein, starch and nucleic acid levels under stress conditions (Crafts 1968; Brady 1973).

Carbohydrates. Probably the most direct effect of water stress on plant constituents is on carbohydrates through the inhibition of photosynthesis. The decreased synthesis of starch (Brady 1973) and cellulose (Ordin 1960) is associated with a diversion of carbon to sucrose synthesis. Sucrose, and in grasses fructans, may accumulate

in stressed tissues as utilisation is suppressed (Brady 1973). Woodhams and Kozlowski (1954) found rapid hydrolysis of starch to sugars in tomato and bean plants, but no increase in amylase or phosphorylase activity has been reported (Brady 1973). The high percentage of starch in their control plants compared with the stressed plants was not compensated by sugars in the latter, which they attributed to high respiration in the stressed plants. Zholkevich and Koretskaya (1959) found changes in carbohydrates, phosphated sugars and organic acids in stressed pumpkin roots suggesting that sugar conversion was restricted by blocking glycolysis and the Krebs cycle. They suggested that interruption of phosphorylation is the primary response to water stress, leading to a decrease in ATP, phosphated sugar esters, RNA and DNA and thus to suppression of protein synthesis.

Nitrogen components. Large changes in nitrogen metabolism affecting both anabolic and catabolic reactions, occur under conditions of moisture stress. Changes in the metabolism of nucleic acids and proteins and amino acids are intimately linked.

Gates and Bonner (1959) found a decrease in both RNA and DNA in young tomato leaves subjected to water stress, and attributed this to increased breakdown. Kessler (1959) reported that water stress is inversely related to nucleic acid level in sunflower seedlings, and later (1961) found a net reduction in RNA and an increase in ribonuclease activity in stressed tomato plants. Since RNA synthesis was unaffected, Kessler assumed that the change in RNA level resulted from hydrolysis.

The effect of water stress on protein levels is much less clear: although it is generally agreed that protein synthesis is particularly

susceptible to stress, the effect on proteolysis and total protein content is still a matter of controversy. Petrie and Wood (1938) found that formation of proteins from amino acids decreased with increasing moisture stress, and more recently Ben-Zioni *et al.* (1967) demonstrated the inhibition of protein synthesis in water-stressed tissues. In a detailed report, Kessler (1967) states that the net rate of protein formation from amino acids decreases regularly with developing water stress in tomato plants. Mothes (1956) reports a relationship between water content and proteolysis. Proteases activated by reduced SH groups have increased activity in stressed plants with closed stomata because of lowered oxygen tension. In other cases no increase in protease acitivity has been found although the protein content declines (Brady 1973).

Chen *et al.* (1964) found that the total nitrogen level in the stems and leaves of rough lemon and sweet lime plants increased with increasing water deficit. The protein level increased at the beginning of dehydration, decreased then increased slightly after permanent (irreversible) wilting. Protein level and water content of the tissue were positively correlated; protein level and rate of water loss were negatively correlated. Shah and Loomis (1965) found that RNA and protein levels were affected in stressed sugar beet plants before wilting was observed. Gaff and Levitt (1965) found that the soluble protein fraction in cabbage leaves had decreased by about half at the death point. However, a decline in protein content is not invariably seen in water-stressed tissues although this may be the general response in the older leaves of a plant (Brady 1973).

Water stress is known to induce changes in free amino acid levels, particularly a great increase in free proline and in amides

(Kemble and Macpherson 1954; Barnett and Naylor 1966; Morris *et al.* 1969; Brady 1973). Barnett and Naylor (1964) suggested that, in Bermuda grass, proteolysis caused by water stress releases amino acids that upon deamination yield free ammonia which is synthesised into amides. $^{14}CO_2$ studies showed that amino acids were continually synthesised during drying but protein synthesis was inhibited and protein level decreased.

Todd and Yoo (1964) studied the effects of detachment and desiccation in wheat leaves, particularly in relation to enzyme activities. Both these treatments decreased saccharase activity rapidly. Phosphatase activity decreased, most rapidly in dried leaves. Peptidase activity decreased slightly with drying. Peroxidase activity increased in detached turgid leaves and decreased in dried leaves. The particulate enzyme dehydrogenase showed a sharp decline when leaves had lost 60% of their water. Cell viability decreased with drying time. Protein content decreased rapidly in both treatments: their content was 50% when leaves had lost 87% of their original water. Todd and Basler (1965) found that breakdown of protoplasmic constituents, including nucleic acids and organelles, was similar in intact and detached wheat leaves subjected to stress. They concluded that stress injury and death involve the breakdown of synthetic machinery. Tissues that resist dehydration probably have less hydrolytic enzyme activity on proteins and nucleic acids and possibly other structural materials.

Shaw and Loomis (1965) suggested that moisture stress and age have similar effects on RNA metabolism in sugar beet leaves.

Plant Senescence

Many studies of leaf senescence which have used detached leaves are relevant to the swath wilting situation. Detached leaves undergo a precipitous metabolic decline (Varner 1965). In the early literature, this was considered as a 'starvation' phenomenon in which extensive degradation of macromolecules and organelles took place (Wood 1945; Lugg 1949; Brady 1973). This view has been modified since with the recognition that changing rates of synthesis and turnover are also important (Brady 1973). Detached wheat leaves have been studied intensively with respect to the structural and chemical changes accompanying senescence (Shaw and Manocha 1965; Shaw *et al.* 1965).

Photosynthesis. The photosynthetic apparatus, the dominant metabolic system in the leaf, shows the earliest physiological and biochemical changes during senescence (Woolhouse 1967). The yellowing associated with chlorophyll loss and breakdown of chloroplast proteins is an obvious symptom of senescence which is closely related to the decline in normal photosynthetic function of the leaf (Yemm 1961). Many leaves show extensive yellowing within a few days of detachment. Ultrastructure studies have shown that changes in chloroplast membranes occur at the stage when net losses of chlorophyll and protein can be recorded, and at later stages of senescence plastid membranes are few and disperse (Barton 1966).

Degradation of other macromolecules is also extensive: RNA, protein, lipid and various carbohydrates, the latter being involved in respiratory metabolism (Brady 1973).

Respiration. After reviewing a large volume of literature, James (1953) and Yemm (1965) described the distinctive pattern of changes in respiratory metabolism which accompany senescence in detached leaves. The early literature contains many studies of the 'respiration drift' (measured as CO_2 production or oxygen uptake) of detached leaves re-supplied with water in the dark. F. F. Blackman systematically analysed the respiration drift curves of cherry laurel and nasturtium and identified six phases (James 1953). The drift curves of barley, wheat, tropical grasses and succulents show the same pattern. Later studies involving several cereals and grasses attempted to resolve the metabolism underlying these changes and paid particular attention to variations in carbohydrate, protein and other leaf constituents (James 1953). The overall pattern and the nature of the successive respiratory phases in 'starving' detached leaves is as follows:-

Phase 1: Mature leaves detached after a period of active photosynthesis show a high sometimes rising respiration rate at first. These leaves normally contain plentiful supplies of readily available carbohydrate, primarily sucrose and starch, which form the principle respiratory substrate for the first 12 - 24 hours, as indicated by R.Q. values close to unity. Detached leaves of wheat, beans, barley, etc., kept in continuous darkness rapidly lose carbohydrates. However some drift curves show no Phase 1, e.g. leaves low in carbohydrates initially, and the grasses examined by Wood *et al.* (1943, 1944) showed an excess of CO_2 production over carbohydrate loss even on the first day.

Phase 2: Almost all leaves show a rapid decline in respiration with progressive exhaustion of available carbohydrates. At the same time

the decline in R.Q. from 1.0 to 0.8 indicates the use of alternative respiratory substrates.

Phase 3: A period of slow steady CO_2 evolution may represent a basal respiration level associated with low available carbohydrate.

Phase 4 and 5: Together these constitute the characteristic secondary peak in CO_2 production, the 'senescent hump', possibly caused by partial breakdown of cell organisation allowing enzymes to reach substrates not normally accessible. At this stage rapid yellowing generally occurs in adult leaves. There is strong evidence, particularly in barley, that carbohydrates now form only a minor part of the respiratory substrate, although less readily available sources of carbohydrate may be drawn into catabolism, such as hemicelluloses in runner beans and maize. No loss of pectin and insoluble hemicelluloses was detected after prolonged periods of isolation but considerable breakdown of alkali-soluble hemicelluloses (largely pentosans, uronic anhydrides and hexosans) occurred accounting for 14.7% of the dry matter in maize leaves (James 1953). Cellular disorganisation may allow hydrolytic enzymes access to the hemicelluloses previously inaccessible in the cell walls. However the fall in R.Q. to about 0.8; the accumulation of free amino acids, peptides and amides; the rise in free ammonia and the corresponding fall in amino nitrogen during the later stages, all strongly indicate that protein hydrolysis and catabolism provide a large part of the respiratory substrate. James (1953) and Yemm (1965) concluded that complete protein degradation to ammonia and CO_2 occurs. Phase 5 ends with the collapse of cell organisation.

Phase 6: Autolysis of cell contents leads to tissue degradation. The rising CO_2 output is largely due to the respiration of saprophytic moulds

and bacteria which gain access to the cell contents.

The respiration drift in detached senescing leaves depends entirely on aerobic respiration. Chlorophyll and protein breakdown may be greatly retarded by an atmosphere of nitrogen (Wood 1945; James 1953). Ultrastructure studies have shown that active mitochondria persist in senescent cells (Brady 1973). The gradual decline in biosynthetic activity often associated with high respiration rates in ageing plant tissue may result from the breakdown of cellular organisation and the failure of regulating mechanisms dependent upon oxidative processes (Yemm 1965).

Biochemical Effects. The metabolism of proteins and their products in detached leaves has been studied extensively (James 1953). From consideration of a large volume of work on the protein metabolism of 'starving' leaves Wood (1945) concluded that the general course of events is always the same. Protein hydrolysis commences promptly and continues smoothly until the chloroplasts begin to disintegrate; proteins of the particulate and soluble fractions are broken down at much the same rate; the amino acid and amide contents successively reach maximum values and then decline; the ammonia content rises slowly from a low to a high value. The rapid protein loss and concomitant, increase in α-amino nitrogen after excision (Lugg 1949; James 1953; Anderson and Rowan 1968; Brady 1973) is especially marked in the grasses and other monocotyledonous plants in which it is detectable within the first few hours (Steward and Durzan 1965). Protein loss occurs before visible yellowing and lowering of R.Q. However, proteins are not completely removed from 'starving' leaves but decline to a steady value,

possibly because a highly resistant insoluble protein component persists (James 1953).

The declining levels of RNA and protein in senescent leaves may reflect changes in the activities of RNases and proteases (Brady 1973). There is a great deal of evidence that RNase activity increases before or as net protein hydrolysis is initiated. Protein synthesis is also affected in detached leaves. Steward and Durzan (1965) stated that protein synthesis ceased due to exhaustion of sulphur-rich amino acids and breakdown of chloroplast structure resulting in cessation of amino acid production and the accumulation of amides. Using $^{14}CO_2$, Racusen and Aronoff (1954) found a decreasing ability to convert amino acids into protein in excised soybean leaves. Hardwick *et al.* (1968) stated that the failure to synthesise photosynthetic proteins is more important than increased proteolysis in explaining the net loss of protein from detached leaves. However, Brady (1973) is diametrically opposed to this view in stating that protein loss is not related to decreased synthesis as cells remain capable of protein synthesis at advanced stages of senescence.

These facts indicate an alteration in the balance of anabolic and catabolic processes in the detached leaf (Carr and Pate 1967), both at the physiological level in the balance of photosynthesis and respiration, and thus also at the biochemical level expressed in the leaf composition and enzyme activities. Usually the capacity of the leaf for synthesis declines while hydrolytic and degradative processes become dominant. These functional changes are reflected in the leaf ultrastructure, such as the rapid degradation of the endoplasmic reticulum and ribosomes in detached wheat leaves (Shaw and Manocha 1965). In

general, structural and storage entities and components associated with photosynthesis are catabolised while those metabolic entities concerned with mobilisation and respiration are preserved (Brady 1973).

BIOCHEMICAL CHANGES DURING THE EARLY STAGES OF FERMENTATION

There is very little information in the silage literature about the biochemical and associated microbial changes which occur during the early stages of fermentation. Yet this critical early period involves the changeover from aerobic to anaerobic conditions, the establishment of suitable microbial populations and the onset of fermentation of the plant cell substrates. In studies of the biochemical and microbial changes occurring in small laboratory silos, the first analyses have been performed after two days (Ohyama and Masaki 1971; Gomez 1972; Gouet *et al.* 1972; Wilson and Wilkins 1973), after three days (Gouet *et al.* 1964, 1970), after four days (De Vuyst *et al.* 1971; Wilson and Wilkins 1972, 1973) or even longer. However, limited information about earlier stages of ensilage is provided by studies in which the first analyses were performed after 24 - 30 hours (McDonald *et al.* 1968; Henderson *et al.* 1972; Ruxton and McDonald 1974).

Most of the evidence about early biochemical changes concerns the nitrogenous components. De Vuyst *et al.* (1971) found that protein degradation was greatest during the first four weeks of ensilage. The marked reduction in lysine, arginine and histidine indicated the transamination or catabolism of amino acids during this phase. Ohyama and Masaki (1971) found a rapid decrease in protein during the first 4 to 7 days of ensilage but little change thereafter. Since an increase in volatile basic nitrogen did not occur until much later, they concluded

that the early protein loss resulted from hydrolysis rather than degradation. In further studies, Ohyama *et al.* (1973) found the extent of protein loss in the early stages to be temperature dependent. Gomez (1972) also reported early protein hydrolysis causing a reduction in protein nitrogen from 86% to 60% of total nitrogen during the first few days of ensilage.

Lanigan (1966) stated that the soluble carbohydrates of herbage which provide the primary substrates for respiration and fermentation are almost completely fermented within the first two days of ensilage. Greenhill (1964 a,b) claimed that oxygen is consumed within the first hour so that sugar losses through aerobic respiration should be negligible.

Playne and McDonald (1966) found that the buffering capacity, dependent primarily on organic acids, doubles within the first three days of ensilage through the formation of lactates and acetates. The rapid pH changes which characterise ensilage are also observed within the first few days.

Details of the timecourse of biochemical changes occurring during the very early stages of ensilage are lacking. There is no information about the respective roles of the plant and microbial enzymes. McDonald and Whittenbury (1967) stated that the activity of plant enzymes continued while aerobic conditions persisted and the pH remained unchanged. Gouet *et al.* (1970, 1972) separated and characterised the roles of these two groups of enzymes but only after at least two days of ensilage had elapsed.

RESEARCH PROGRAMME

An important area for research is indicated: the biochemical changes occurring during the field wilting period and the first few days in the silo.

The physiology and biochemistry of wilting has been neglected; hence a research programme was devised to investigate in detail the field wilting process, supplemented by studies of wilting under controlled conditions.

Section 1: Field Studies

Field studies were planned to investigate the wilting process from the moment of harvest, throughout the 24- and 48- hour wilting periods generally used in silage-making, under a range of environmental conditions.

Environmental aspects of wilting were studied using microclimate techniques to monitor the environmental parameters temperature, relative humidity, windspeed , light penetration and solar radiation in the swath of cut herbage and in the ambient air.

Plant aspects of wilting were studied, namely the swath evaporation rate and DM content (mean and stratified) throughout the wilting period.

Section 2: Controlled Environment Studies

Series of experiments were carried out under controlled conditions (growth cabinet, assimilation chamber) to investigate intensively the physiology and biochemistry of wilting at the levels of simulated swaths,

individual leaves and leaf biochemical processes throughout 48-hour wilting periods. Plant physiology techniques were used to measure leaf transpiration, photosynthesis and respiration; biochemical techniques were used to measure changes in soluble carbohydrates, nitrogen components and chlorophyll.

Since little is known about the early stages in the silo, a series of experiments was designed to investigate biochemical changes during the first week of ensilage.

Section 3: Ensilage Studies

Laboratory silos were used to study the timecourse of changes in various plant constituents, including soluble carbohydrates, nitrogen components and pigments, in relation to pH and DM changes from zero time until 48 hours to seven days of ensilage. The effect of several treatments such as wilting and inoculation of lactic acid bacteria was studied. The roles of plant and microbial enzymes during this early period were separated using a γ-irradiation sterilisation technique.

SECTION 1

FIELD STUDIES

SECTION 1: INTRODUCTION

The process of water-loss during the field-drying of grass is dependent upon plant factors (internal variables), natural environmental factors, and man-made factors (external variables). The plant factors include the nature of the leaf surface and stomatal behaviour which influence the plant resistances to water loss. The man-made factors include the mechanical treatment of the crop during harvesting and conditioning. The natural environmental factors include the energy balance and the vapour pressure gradient from the cut crop into the ambient air. The precise roles and relative importance of these factors during drying is largely unknown, especially in the early stages of drying which are of interest in silage-making.

The environmental factors are thought to be particularly important in controlling the rate of moisture loss from cut crops (Warboys 1967; Hart and Burton 1967; Spatz *et al.* 1970; Elderen *et al.* 1972). The influence of meteorological conditions on cereals during the harvest period was described by Voigt (1955), Hesselbach (1968), Baumgartner (1969), Kampen (1969) and Bruck *et al.* (1969). The last two authors mentioned radiation as a significant influence. Many studies of hay drying have been reported but few have considered the effect of weather and crop factors on drying rate (Hart and Burton 1967). In the U.S., Hart and Burton (1967) reported relationships between the moisture content of cut coastal Bermuda grass and weather factors such as solar radiation, vapour pressure deficit, windspeed and rainfall. Regression analysis was used to evaluate the effect of several weather factors on

the moisture content of the curing grass at the end of the day or night. Significant effects of the initial moisture content, the accumulated vapour pressure deficit of the air, total solar radiation and total DM yield were established. Work in Germany on the relationship between drying rate and several meteorological factors was summarised by Spatz *et al.* (1970). In a pasture dominated by ryegrass and white clover, strips of herbage were cut for hay and sampled at 3-hourly intervals during the day. The air vapour pressure deficit and solar radiation were well correlated with the drying rate, whereas sunshine hours were less well correlated. An equation was given relating decrease in herbage moisture content to these three factors. An influence of windspeed could not be established. The drying rate also varied significantly with plant age and initial moisture content.

British work was summarised by Smith (1968) and Brown and Charlick (1972). Warboys (1967) discussed the effect of climatic factors on the field drying process and measured the drying rates of ryegrass under poor, average and good drying conditions for normal and lacerated herbage. He suggested that a critical moisture content exists, below which the drying rate is dependent on plant factors rather than weather factors. Burroughs *et al.* (1968) estimated the resistance to water loss of two grass species cut for hay during two successive days of good evaporating conditions. Resistance increased throughout day 1 as the moisture content fell rapidly. On day 2 however, after evaporation of the surface moisture, resistance increased very rapidly with only slight fall in the crop moisture content. The importance of maintaining a low crop resistance throughout the drying cycle by frequent working of the swath was stressed.

The potential evapotranspiration (E_o) of a growing crop can be calculated from meteorological data using the classic Penman equation (Penman 1948, 1956), later modified by McIlroy (McIlroy and Angus 1964). As this includes evaporation from the soil and transpiration from the growing plant, further modification is necessary before applying the equation to a cut crop. Roy and Wilkins (1968) measured the drying rate of ryegrass swaths and related this to various weather factors. The full Penman equation was used to estimate the drying potential of the environment; errors of between 4 and 15% may result. Regression equations were calculated to give the crop moisture content at various values of accumulated E_o. However with E_o constant, the drying rate decreased progressively indicating that the crop resistance to evaporation increased during drying. The use of radiation data for estimating the drying potential of the air was suggested.

Brown and Charlick (1972) investigated the effect of different crop mechanical treatments on the drying rate, and used the accumulated vapour pressure deficit to define the drying potential of the air. A good correlation between crop moisture content and ambient vapour pressure deficit was found.

LIST OF SYMBOLS

A	available energy; $R_n - G - S - P$
c_p	specific heat of air at constant pressure
e	vapour pressure (mb), at $z = z_R$
e_w	saturation vapour pressure
E	swath evaporation rate
E_o	potential evapotranspiration
G	energy flux into ground
H	sensible heat flux (+ve upwards), at $z = z_R$
P	net rate of energy absorption by photosynthesis and respiration
r	resistance to transfer
r_b	boundary layer resistance of the swath
r_D	bulk aerodynamic resistance of the swath to momentum transfer
r_I	climatological resistance
r_s	stomatal resistance of a leaf
r_S	surface resistance of the swath
r_{ST}	bulk physiological resistance of the swath to water vapour transfer
R_n	net radiation
t	air temperature (^oC)
vpd	vapour pressure deficit (mb); $(e_w(t) - e)$
z	height above ground; z_R reference height
Δ	rate of change of saturation vapour pressure with temperature; $d\, e_w / dt$ (mb $^oC^{-1}$)
γ	psychrometric constant (c. 0.66 mb $^oC^{-1}$)
λ	latent heat of vaporisation of water
λE	latent heat flux (+ve upwards), at $z = z_R$
ρ	density of air

THEORY

The complex of environmental factors influencing the cut crop may be collectively considered as the 'swath microclimate' which incorporates the swath energy balance, water balance, etc. and is related to the prevailing ambient weather conditions. Some aspects of microclimate theory, as expounded by Monteith (1973), Thom (1971, 1972, 1973) and Burrage (1971) for single leaves and growing crops, will now be extended and applied to the swath situation.

The swath energy balance is of the first importance for the drying process, since it determines the amount of energy available for the evaporation of water. When the swath is exposed to radiation, the energy which it absorbs can be used in three ways: for heating, for the evaporation of water and for photochemical reactions. Heating of the swath implies a transfer of heat by conduction or convection (sensible heat, H). Evaporation involves a transfer of water vapour molecules using the energy supplied by latent heat (λE, latent heat flux). The energy available for heating and evaporation is a function of the net radiation on the swath (R_n, incoming - outgoing radiation), and is given by

$$A = R_n - G - S - P = H + \lambda E \quad \text{---------- 1.}$$

(Stewart and Thom 1973). For the swath situation, A is expected to be very close to R_n in value since the other terms in the equation, G, S and P, are very small or negligible (Burrage 1971; Thom 1973).

Therefore $A \simeq R_n \simeq H + \lambda E$

Each of these terms may be either positive or negative: the swath may gain or lose heat to the surrounding air, or may gain or lose water vapour; incoming radiation may be greater or less than outgoing radiation (Burrage 1971).

The swath evaporation rate may be considered in terms of the water vapour transfer or latent heat flux (λE) from an unsaturated surface, which differs from the classic case of evaporation from an open water surface in that considerable additional resistances to transfer may be expected. The swath evaporation rate depends on the water vapour concentration gradient from the swath surface into the air and on the resistances. Surrounding the swath is the thin skin of air known as the 'boundary layer' in contact with the surface, in which water vapour transfer is limited by molecular diffusion. This boundary layer presents a resistance to transfer (r_b), the magnitude of which depends upon plant factors (size, shape, orientation, surface topography) and environmental factors (windspeed, temperature). Additional internal plant resistances to transfer may be expected, such as the stomatal resistance (r_s) assuming that the stomata begin to close after harvest.

Following Monteith (1965), Stewart and Thom (1973) and Thom (1973), and using the notation of the latter, the Penman combination equation for latent heat flux from an unsaturated surface can be written in the form

$$\lambda E = \frac{\Delta A + \rho c_p \dfrac{(e_w(t) - e)}{r_D}}{\Delta + \gamma\left[1 + \dfrac{r_S}{r_D}\right]} \qquad \text{---------- 2.}$$

where A is given by equation 1. r_D is defined as the bulk aerodynamic resistance of the surface to momentum transfer (the external resistance of the swath, including the boundary layer resistance to water vapour flux). r_S is the Monteith surface resistance (the internal resistance of the swath, representing a purely physiological resistance to the water vapour flux alone).

Let the numerator of equation 2. be represented by $x + y$.

If $r_S >> r_D$ and $y >> x$ (which implies that $r_I >> r_D$; see equation 4), then equation 2. simplifies to

$$\lambda E = \frac{\rho c_p (e_w(t) - e)}{\gamma r_S}$$

that is $\lambda E = \frac{\rho c_p}{\gamma} \cdot \frac{\text{v.p.d.}}{r_S}$

so that $r_S = \frac{\rho c_p}{\gamma} \cdot \frac{\text{v.p.d.}}{\lambda E}$ ---------- 3.

r_S is a function of time after harvest and will be a good approximation of r_{ST}, the *bulk physiological resistance of the swath to water vapour transfer.* Changes in r_S (or r_{ST}) will adequately describe changes in the bulk stomatal resistance of the vegetation. Equation 3. derived above is a particular limiting relationship which should apply in conditions which are relatively dry and windy but not necessarily sunny, but cannot of course apply in general.

r_I is a climatological parameter, a convenient measure of the overhead conditions, referred to as the 'climatological resistance' (Thom 1973), namely,

$$r_I = \frac{\rho c_p}{\gamma} \cdot \frac{\text{v.p.d.}}{R_n} \quad \text{---------- 4.}$$

which enables equation 3. to be written in the convenient form

$$\frac{\lambda E}{R_n} = \frac{\Delta r_D + \gamma r_I}{\Delta r_D + \gamma r_D + \gamma r_S} \quad \text{---------- 5.}$$

If r_I is negligible, in conditions with air near saturation (very humid), light winds or calm, and hazy sunshine (non-zero solar radiation), then

r_s will usually be negligible also, especially prior to cutting or after when wet with rain or dew, so that equation 5. reduces to

$$\lambda E = \frac{\Delta}{\Delta + \gamma} \cdot R_n \quad \text{------ 6.}$$

This is called the equilibrium evaporation rate, (λE) eq. which may give the maximum likely value of λE (Thom 1973). Near this equilibrium evaporation rate, λE is independent of r_D. When λE is less than this equilibrium value, it will decrease with increasing windspeed (Thom 1973).

Therefore, in summary, the swath evaporation rate or latent heat flux depends upon the net radiation, the vapour pressure deficit of the air, and various resistances to water vapour flux of which the bulk physiological resistance of the swath is thought to be the most important usually.

The aim of the Field Programme undertaken in Section 1 was to investigate the relationship of swath drying rates to the swath micro-climate and the ambient weather conditions. The variables selected for measurement, throughout the 24- and 48- hour wilting periods generally used in silage-making, were the following:-

Swath variables:	Evaporation rate
	DM content or moisture content
	Swath description
Environmental variables:	Temperature
	Relative humidity
	Light penetration
	Solar radiation
	Windspeed

SECTION 1: MATERIALS AND METHODS

FIELD EQUIPMENT

Measuring apparatus

One 30 m tape; two 150 cm tapes; a measuring stick graduated in cm intervals up to 30 cm.

Weighing frame and spring balance

A weighing frame, with 50 x 50 cm base made of removable supporting rods, was used in conjunction with a 4 kg spring balance (Perry) graduated in 10 g divisions. This balance was accurate to 5 g, one revolution of the pointer per kg giving increased sensitivity. Plate 1.1

Sample storage equipment

Polystyrene ice buckets; polythene bags.

Light/Temperature Meter

This was a self-contained, manual, battery-operated instrument with a 7.5 cm scale and knife-edge pointer. The meter scale was calibrated in one degree intervals from 10° to 30° C for temperature measurements, and in five percent intervals from 0 to 100% of incident light intensity for light measurements. This instrument was equipped with two interchangeable probes: a sensitive thermistor with response time of c. 2 s; and a miniature light cell (Cd S photoconductive cell with 0.6 cm^2 sensitive area) responding to end-on incidence of illumination within the visible wavelengths, with response time of <1 s. The light cell was mounted in a circular holder on the end of a 20 cm metal arm for ease of manipulation. Plate 1.2

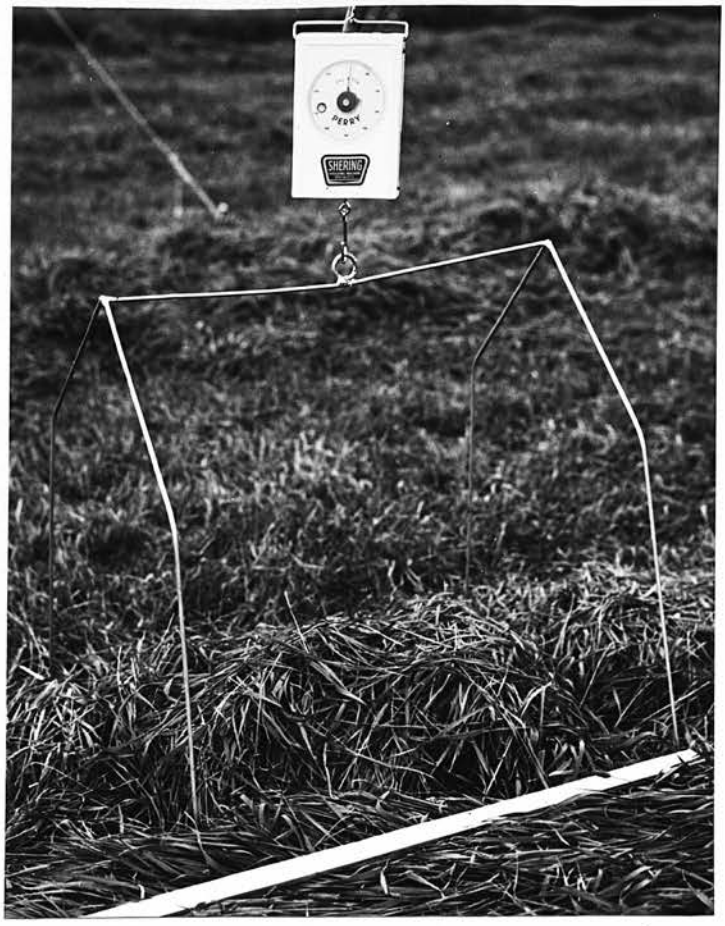


PLATE 1.1 Weighing frame and spring balance for swath evaporation measurements

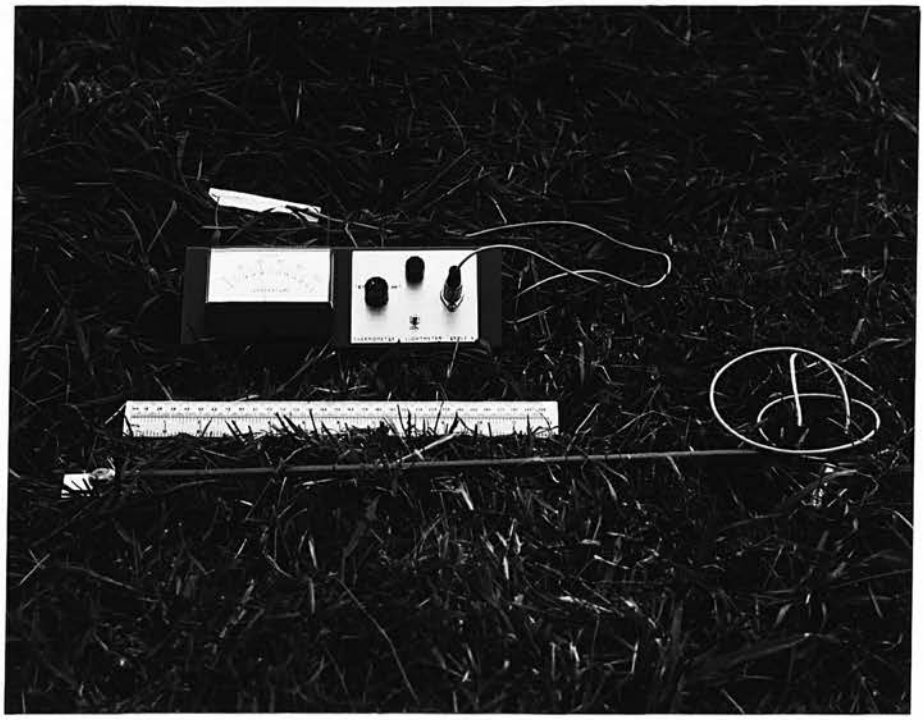


PLATE 1.2 Light/Temperature meter

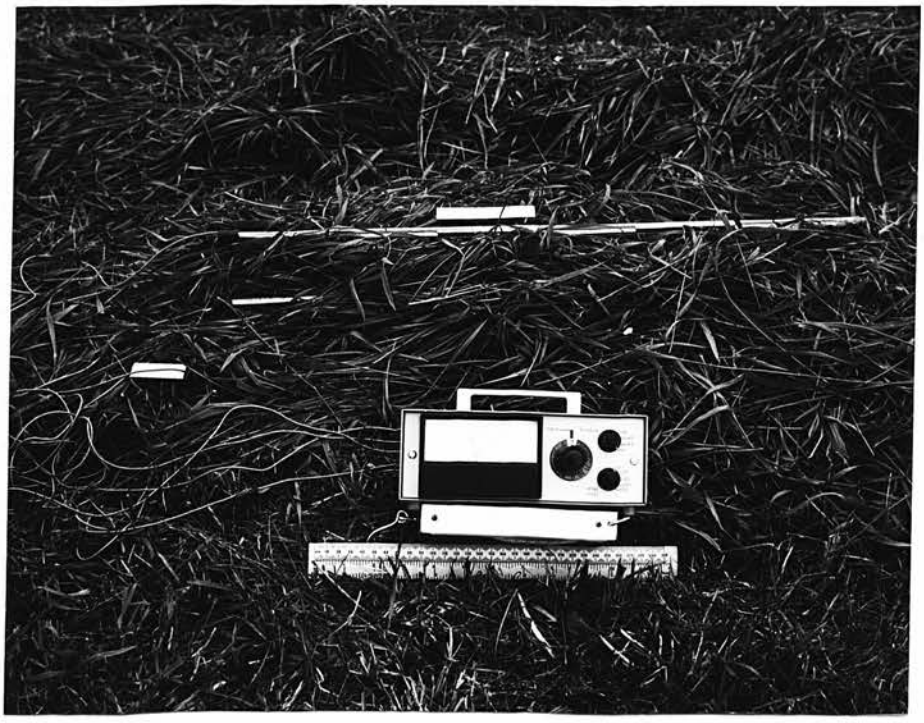


PLATE 1.3 Tube solarimeters and mV meter

Tube solarimeters and mV meter

Four tube solarimeters were constructed by the author as described by Szeicz *et al.* (1964), but with thermopile dimensions 60 x 0.6 cm and mounted inside pyrex tubes of 0.8 cm internal diameter. These tube solarimeters provided an integrated measure of radiant flux density in energy units, rather than in photometric units of illumination as in the case of the light cell. They were used in conjunction with a 12-channel mV meter with a 6 cm scale and knife-edge pointer. The meter had five differential mV scales to cover the ranges 0 to 3, 10, 30, 100 and 300 mV, and was a self-contained battery-operated instrument. Plate 1.3

Kipp and Zonen solarimeter

This commercial solarimeter, complete with base and screen, was used with the mV meter described above.

Microclimate Recording Station

This instrument was constructed by Grant Instruments (Developments) Ltd. according to the specifications of the author, and was designed for outdoor use with a climate mast and accessories. It provided automatic hourly, or continuous, recordings of the following environmental variables: temperature, relative humidity, windspeed and relative light intensity. The Microclimate Recording Station was a self-contained, battery-operated system which consisted basically of two slave recorders and a control box:-

Control Unit. The central control unit was powered by two rechargeable Ni/Cd batteries, and had outputs for the two slave recorders. It contained a clock, with a precision battery-wound clock movement, and its controls which provided a cycle interval of 1 h; and an advance timing

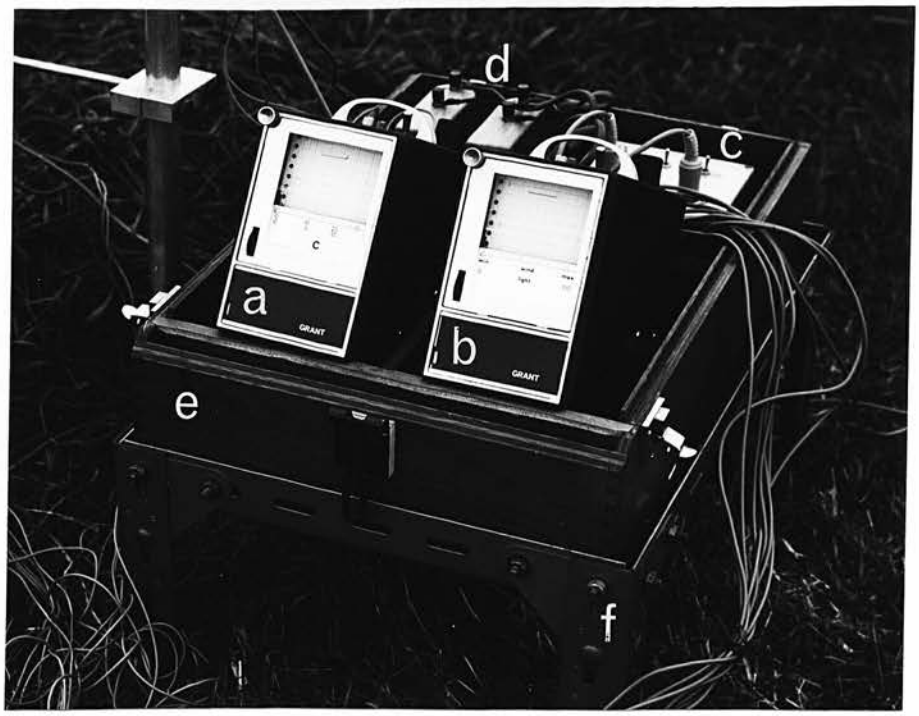


PLATE 1.4 Microclimate Recording Station

a = Recorder A for temperature and humidity measurements

b = Recorder B for wind and light measurements

c = Control unit

d = Ni/Cd batteries

e = Weatherproof box

f = Metal stand support

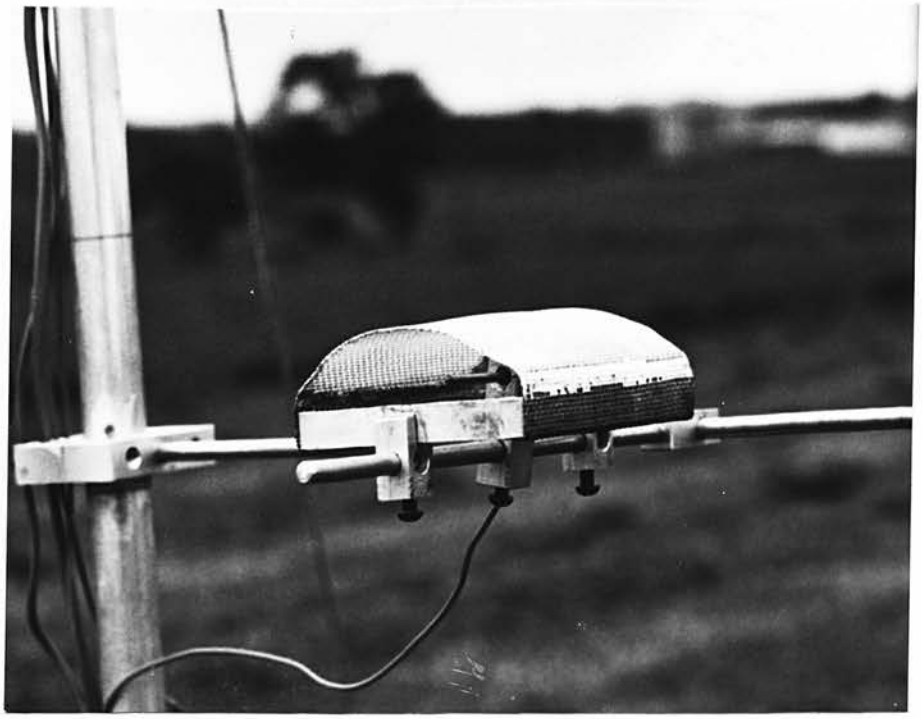


PLATE 1.5 Ambient thermistor with sun-shield

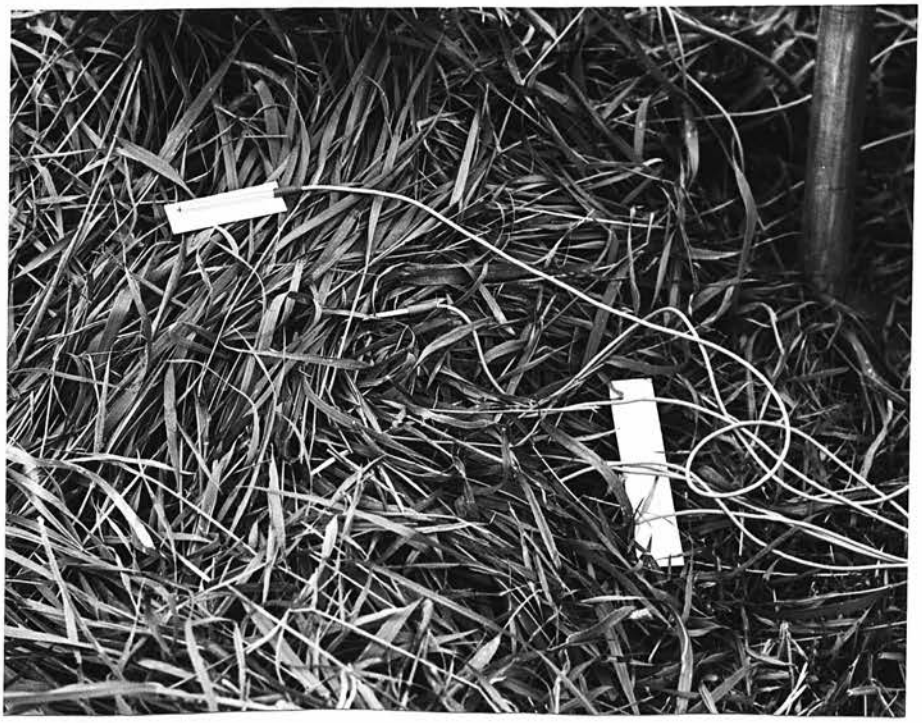


PLATE 1.6 Swath thermistors

unit to switch on the wet bulb ventilating fans (see below) 4 min before the cycle began.

Chart Recorder A for temperature measurements. This multipoint chart recorder registered temperatures directly, and also temperature differences between pairs of probes, used as wet and dry bulb pairs for humidity measurements. There were 10 input channels for dry bulb temperatures, and 2 difference channels for wet bulb depressions; and 2 temperature ranges, 0 - 30°C for the former and 0 - 15°C for the latter.

Chart Recorder B for wind and light measurements. The second multipoint chart recorder was designed for use with sensors generating mV signals, namely, 2 run-of-wind anemometers and 4 light-difference sensor pairs. Hence, there were 2 input channels for wind and 8 input channels for light measurements.

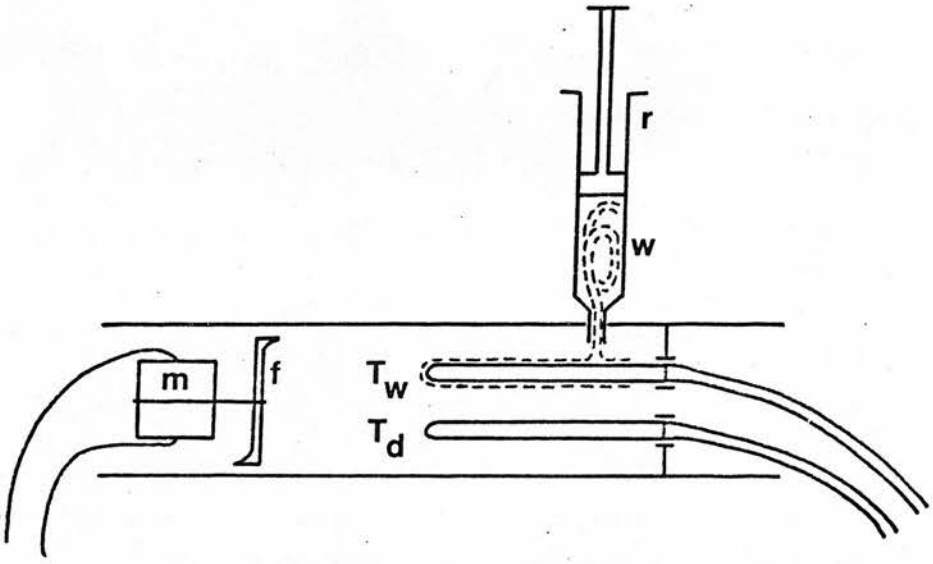
Both recorders A and B used a chopper-bar method of recording on pressure-sensitive paper, with the advantages of a dry marking process. The probes were monitored consecutively, the recording of a complete cycle taking <1 min.

A weather-proof box housed the control unit, batteries and the two slave recorders. Plate 1.4

Sensors. The sensors used with the station were the following:-

Thermistor probes. 12 thermistors (Grant type AM, 50 x 3.2 mm) with 2 m leads were used for air temperature measurements. Plate 1.5, ambient air thermistor; Plate 1.6, swath thermistors.

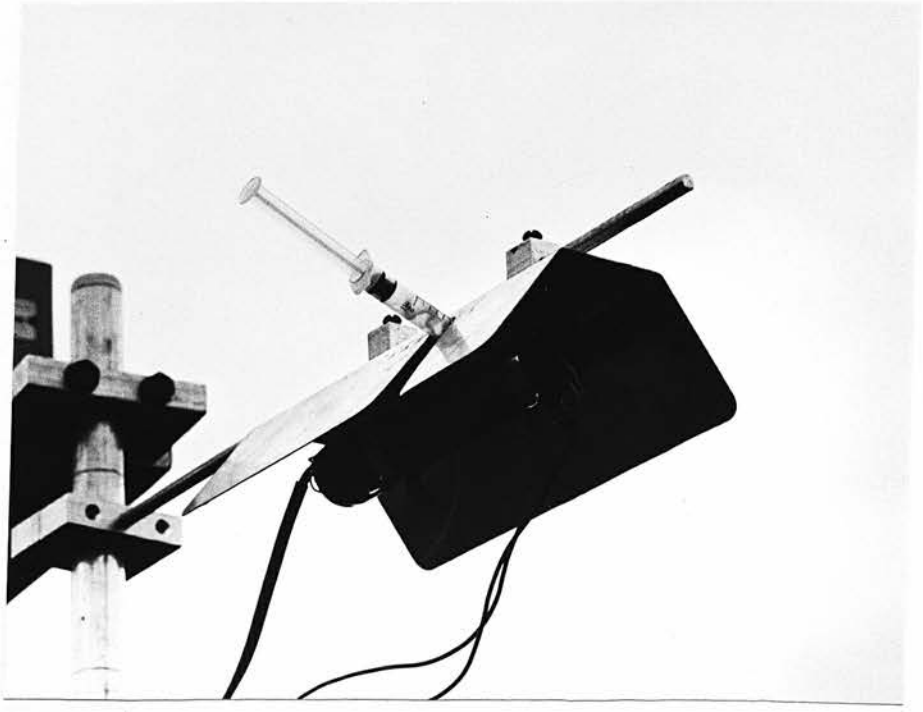
Fig. M.1 Diagram of psychrometer unit



Key

T_w = wet-bulb thermistor
T_d = dry-bulb thermistor
m = motor
r = distilled water reservoir
f = fan
w = wick in reservoir

Plate 1.7 Psychrometer unit



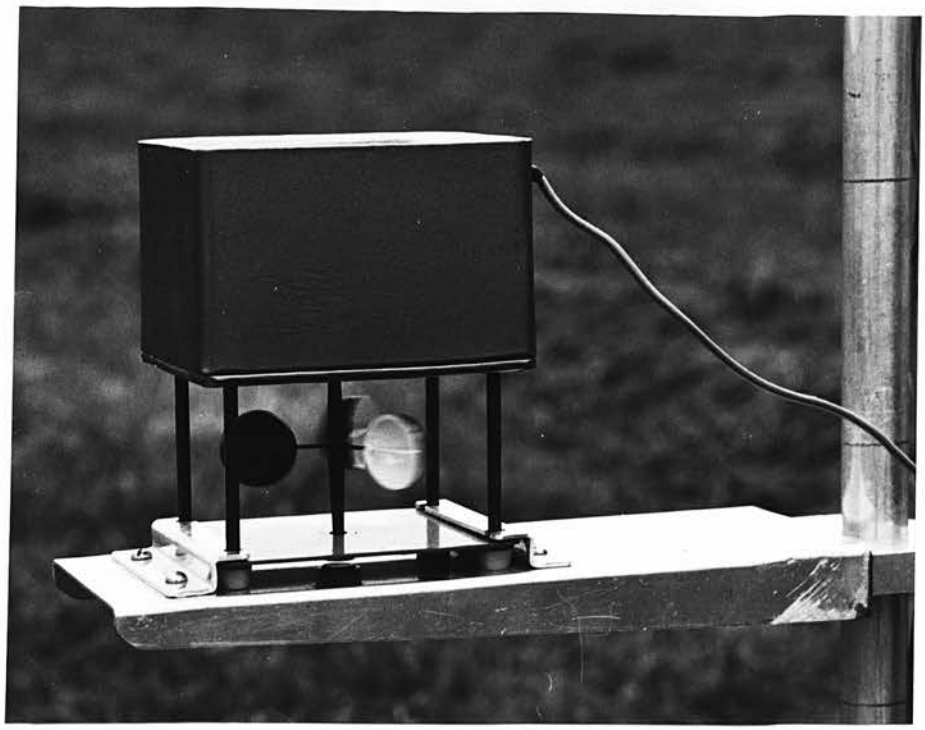


PLATE 1.8 Anemometer

PLATE 1.9 Light cells

Psychrometers. Two psychrometer units, each comprising a dry bulb and a wet bulb difference thermistor mounted in a copper tube with a miniature ventilating fan, were used for relative humidity measurements. A distilled water reservoir was connected to the wet bulb by a wick. The fan motor was switched on 4 min before the recording of each cycle so that the wet bulb attained a steady temperature. Plate 1.7; Fig. M.1

Anemometers. Two miniature integrating cup anemometers, which accumulate a resistance during each hourly period, were used to monitor hourly run-of-wind. Plate 1.8

Light sensors. Eight light cells, mounted in round plastic blocks with point attachments for ease of positioning in the field, were used to measure ambient and swath light levels. Plate 1.9

Climate Mast and Accessories

A climate mast was constructed for use with the Microclimate Recording Station, to carry the ambient sensors. It comprised two 1 m x 3 cm aluminium mast sections which screwed together. The lower section had a pointed extension and collar for anchorage in the soil. The mast was held up by 4 guy-ropes, anchored by anodised aluminium pegs, and was provided with accessories including side-arms to support the sensors. Each side-arm, a 40 cm x 6 mm aluminium rod, was attached to the mast by a screw clamp allowing the height of the arm to be adjusted independently. Plate 1.10

Each ambient thermistor was provided with a white wire gauze sun-shield, which was attached to the side-arm and hence the climate mast. Plate 1.5

Each psychrometer unit was provided with a protective metal weather shield. The whole unit was attached to a side-arm by means of two screws, and hence to the mast. Plate 1.7

Each anemometer was supported on a flat plate and attached to the mast by means of a screw clamp. Plate 1.8

CALIBRATION OF FIELD INSTRUMENTS

Light/Temperature Meter and mV meter

Slight changes in the calibration of both of these instruments may be expected with variation in ambient temperature and battery power. Therefore both meter calibrations were checked routinely on full scale deflection, and adjusted when necessary, before use.

Tube Solarimeters

The mV signals generated by the tube solarimeter thermopiles must be converted into solar radiation energy units of $W\ m^{-2}$. Calibration was effected using the Kipp and Zonen Solarimeter in conjunction with the four tube solarimeters and mV meter. The location chosen for calibration was the shadow-free roof of the University of Edinburgh Darwin Building, at an approximate height of 100 m above ground level. A clear sunny day and a dull day were chosen for the operations, and calibration measurements were made throughout the day, thus covering a wide range of natural solar radiation values.

The orientation and direction of the tube solarimeters was standardised: orientation of the sensitive surface perpendicular to the sun's rays (the orientation with respect to the ground surface

changing therefore with the time of day); direction E - W. The four tube solarimeters were placed side by side, next to the Kipp Solarimeter, and the five sensors were connected to the first five channels of the mV meter. The procedure employed was to switch the channel selector rapidly through channels 1 to 5, simultaneously recording the mV signal generated by each of the four tube solarimeters and the Kipp solarimeter in turn. This operation was repeated about five times and at about 15 minute intervals throughout the day. On each sampling occasion, any change in weather conditions, such as fractional cloud amount and visibility, was recorded.

It was possible to convert the mV signal generated by the Kipp solarimeter into solar radiation energy units since its calibration was known. The mV signal simultaneously recorded for each tube solarimeter was plotted against the solar radiation energy value derived from the Kipp solarimeter. A calibration curve for each tube solarimeter was obtained using the data from the clear and dull days. A typical calibration curve is shown in Fig. M.2 b.

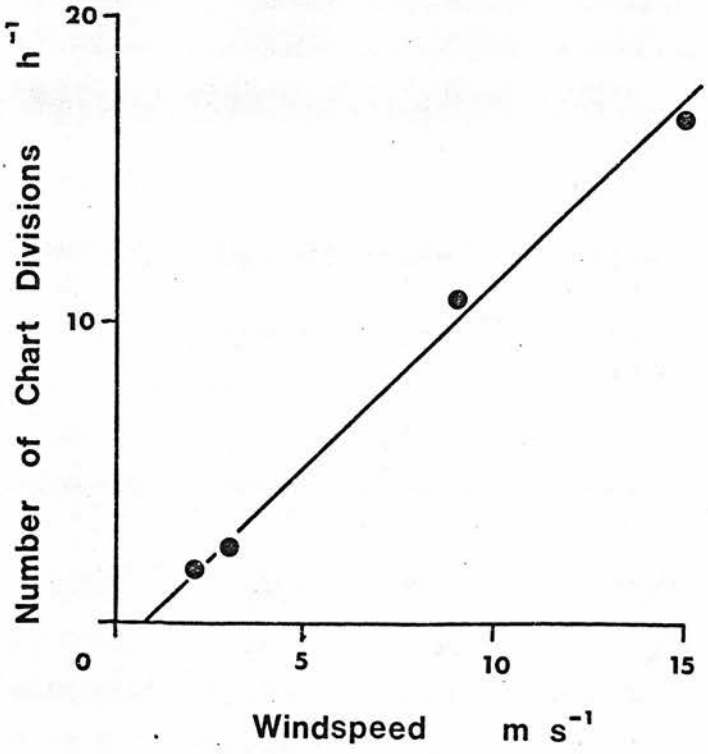
Kipp and Zonen Solarimeter

This instrument, serial no. CM3-683239, was recalibrated at Kew in May 1970, when it was established that radiation of 1 g cal cm^{-2} min^{-1} produces an EMF of 8.0 mV.

Anemometers

The two run-of-wind cup anemometers were calibrated using the wind tunnel in the University of Edinburgh Department of Forestry and Natural Resources. This wind tunnel can be controlled to produce a

a.



b.

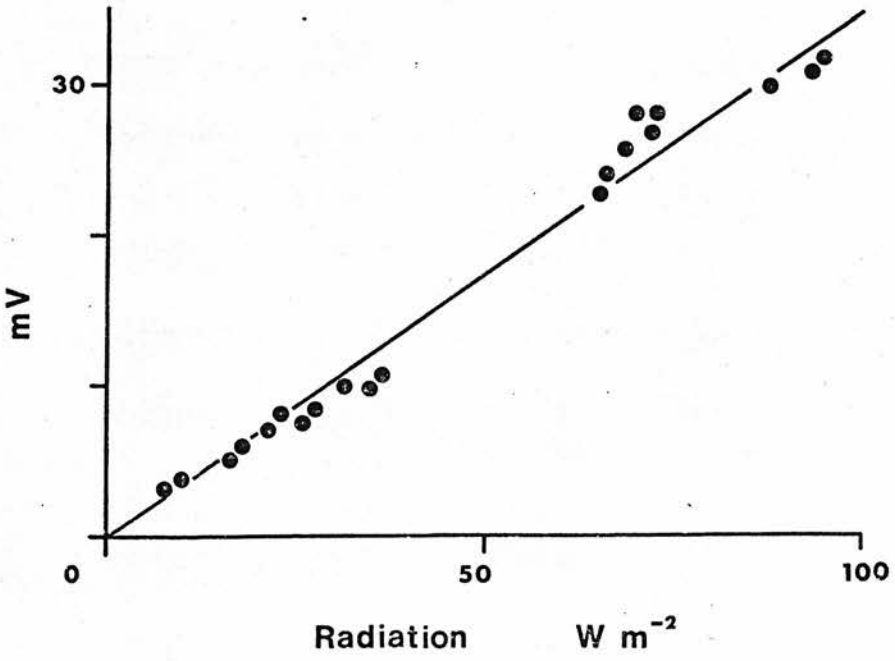


Fig. M.2 a. Windspeed calibration curve
b. Tube solarimeter calibration curve

wide range of windspeeds. The two anemometers were mounted in the wind tunnel experimental chamber and connected to recorder B, the control unit and battery supply. The windspeed in the tunnel was adjusted to 15, 9, 3 and 2 m s^{-1} in turn, and left for at least four hours at the two highest speeds and overnight at the two lowest speeds. During this time the recorder took automatic hourly readings from both anemometers, and hence it was possible to calculate the mean number of chart divisions corresponding to each windspeed. Anemometer calibration curve is shown in Fig. M.2 a. The windspeed is expressed in m s^{-1} allowing the hourly run-of-wind to be derived.

Recorders A and B

Before these instruments were set to record temperatures or mV signals, they were calibrated to allow for the effects of changes in battery voltage and ambient temperature. This was carried out by using two calibration reference points at fixed locations on each chart. The pointer should mark the paper at the positions shown by yellow and blue arrows on the scales. Corrections were made using the yellow and blue screw adjusters. All the temperature and mV ranges were then correctly calibrated. This calibration may be tested at any time, and was checked routinely at least twice a day in the field.

FIELD TECHNIQUES

Swath description

This was carried out using the measuring apparatus.

Swath profiles were constructed in order to characterise the swath shape in transverse section. This was performed by measuring swath height at 10 cm intervals across the complete width of the swath, and the stubble height at each side.

Mean swath measurements of swath width, interswath distance, swath maximum height were derived based upon at least ten measurements for each determination. Knowledge of the first two parameters enables the field cover to be estimated, i.e. the proportion or percentage of the field covered by the harvested crop.

Swath description was performed immediately after harvesting, and after 24 and 48 h of wilting, at random number locations in the field.

Swath evaporation rate

Swath evaporation rate was followed from the time of harvesting throughout the wilting period, using the weighing frame and spring balance. A 50 x 50 cm swath section was cut out from a discrete swath half, at a random number location in the field, immediately after harvesting. This swath portion was transferred carefully to the weighing frame, disturbing the swath structure as little as possible in the process. The precise time of cut was recorded, then the swath portion was weighed giving the initial swath fresh weight at zero time. The swath portion was left in place on the weighing frame which was lowered into the swath space from which it had been taken. The same swath portion was reweighed at hourly intervals between 09.00 and 18.00 h and at 3-hourly intervals thereafter until nightfall, simply by suspending the weighing frame from the spring balance for a few seconds. Thus the decline in fresh weight of an intact swath sample was followed in the field, giving the swath evaporation rate directly.

Swath dry matter content

Stratified swath D M content was determined immediately after harvesting and at 3-hourly intervals, at random swath locations in the field. On each sampling occasion, the swath was cut through transversely from the surface to ground level. The swath face or profile thus exposed was sampled at different heights. Samples of about 50 g of grass were taken from the following layers: 5 - 10, 10 - 15, 15 - 20 and 20 + cm above ground level (the 0 - 5 cm layer consisted of stubble beneath the swath). The samples from each layer were stored separately in sealed polythene bags on ice, in order to minimise water loss and respiratory loss, before processing in the lab. During processing, each sample was chopped, mixed and rapidly subsampled in triplicate for fresh weight and dry weight determination. Dry weights were recorded after drying for 48 h at 80° C in a forced-draught oven, and cooling in a desiccator. The D M content of each layer was derived by expressing the dry weight of each subsample as a percentage of its fresh weight, and then taking the mean of the triplicate samples.

Mean swath D M content was calculated from the knowledge of the stratified swath D M content, by using a weighted mean, to allow for the proportion of swath material occurring in each layer. The D M content of the standing crop was also determined routinely, sampling from ten random locations in the field and treating the bulk sample as before.

Preliminary Investigation of Swath Microclimate

This was carried out using the Light/Temperature Meter. Temperature and light readings were taken manually inside the swath and in the ambient air. Thus, profiles of temperature and light penetration could be constructed from outside the swath through the swath mass down

to ground level. Studies were made of swaths produced by mowing (4-drum rotary mower), and mowing followed by crimping treatment (to condition the crop and accelerate drying).

Investigation of Swath Radiation Environment

This was carried out using the tube solarimeters and mV meter. Measurements were made manually under contrasting conditions of bright sunshine and overcast skies. Solar radiation was measured using the tube solarimeters at different heights inside the swath, and at the swath surface. The direction and orientation of the solarimeters was standardised: direction parallel to the long direction of the swath; orientation perpendicular to the sun's rays as far as possible. The solar radiation measurements were integrated values along 60 cm of swath. Solar radiation gradients from surface to ground were constructed. Inverted solarimeters were used to record reflected radiation at the swath surface, at different heights inside the swath, and in the stubble. Hence, albedo values and quantity of net absorbed radiation of the entire swath and of the different constituent layers could be derived. Hourly measurements of the swath solar radiation profile were made between the hours of 09.00 and 18.00 wherever possible. Values for light extinction may be calculated from these solar radiation data.

Solar Radiation Measurements

Daily totals of incoming solar radiation from sun and sky (0.3 - 2.5 μm) were recorded automatically using a Kipp and Zonen solarimeter with an integrator, located at Bush Estate, about one mile from the site of the field experiments. 24 h totals were recorded as total counts then converted into energy units.

Hourly totals of incoming solar radiation were obtained from a solarimeter with integrator located on the roof of the Darwin Building about ten miles from the site of the field experiments.

Microclimate Monitoring of Swaths

This involved the use of the climate mast, to hold sensors at fixed heights, and the microclimate recording station, described previously.

Climate mast arrangement: The climate mast was designed to give flexibility of arrangement with respect to the positioning of the sensors. An arrangement was chosen to allow simultaneous monitoring of

(i) ambient weather conditions;

(ii) swath microclimatic conditions (intensively studied);

(iii) soil microclimatic conditions beneath the swath;

and occasional measurements of

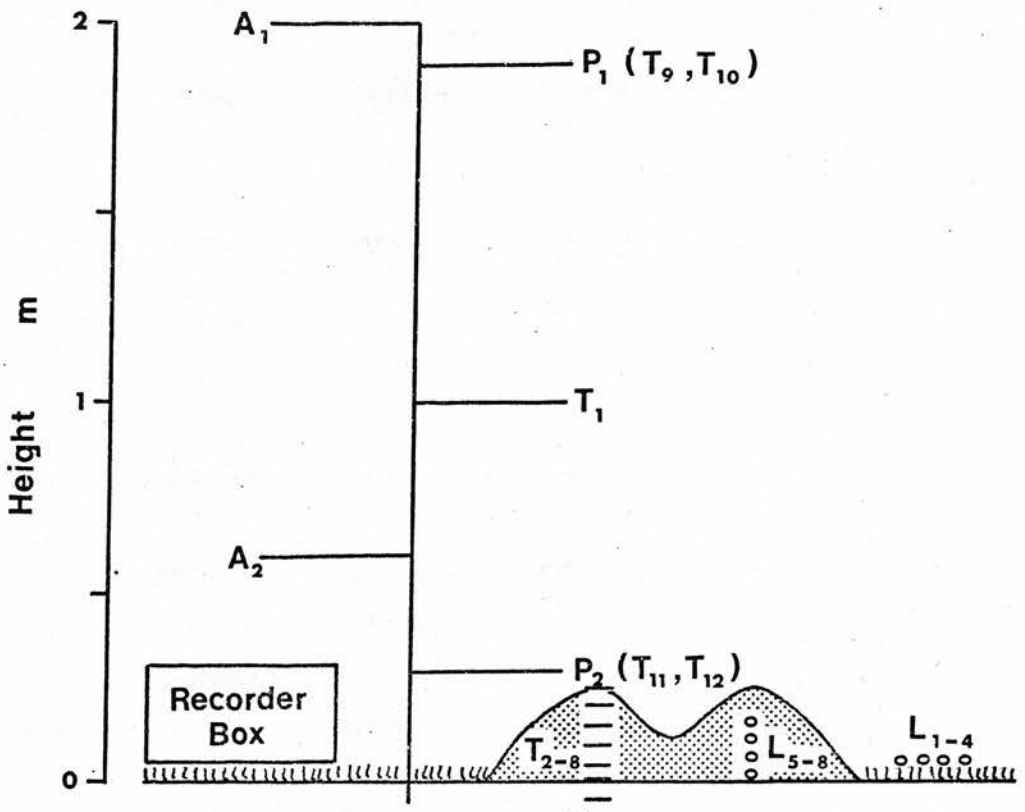
(iv) stubble microclimatic conditions were also made.

The arrangement of sensors generally used was as follows:-

Sensor	Height above the ground (cm)
Anemometers	+200, +60
Thermistors	+190, 100, 30, 20*, 15*, 10*, 5*, 0*, -2, -5 (* = inside the swath)
Psychrometers	+190, 30
Light cells	+ 15, 10, 5, 0 (inside the swath) + 5, 5, 5, 5 (in stubble, beside the swath)

The arrangement of the sensors according to this scheme is shown in Fig. M.3 and Plate 1.10. Thus, provision was made for measurements of the wind profile; ambient temperature, humidity and light intensity;

Fig. M.3 Microclimate mast arrangement



Key

A = anemometer

P = psychrometer

T_1 = ambient thermistor

T_{2-7} = swath thermistors

T_8 = soil thermistor

L_{1-4} = ambient light sensors

L_{5-8} = light difference sensors

= stubble

= swath

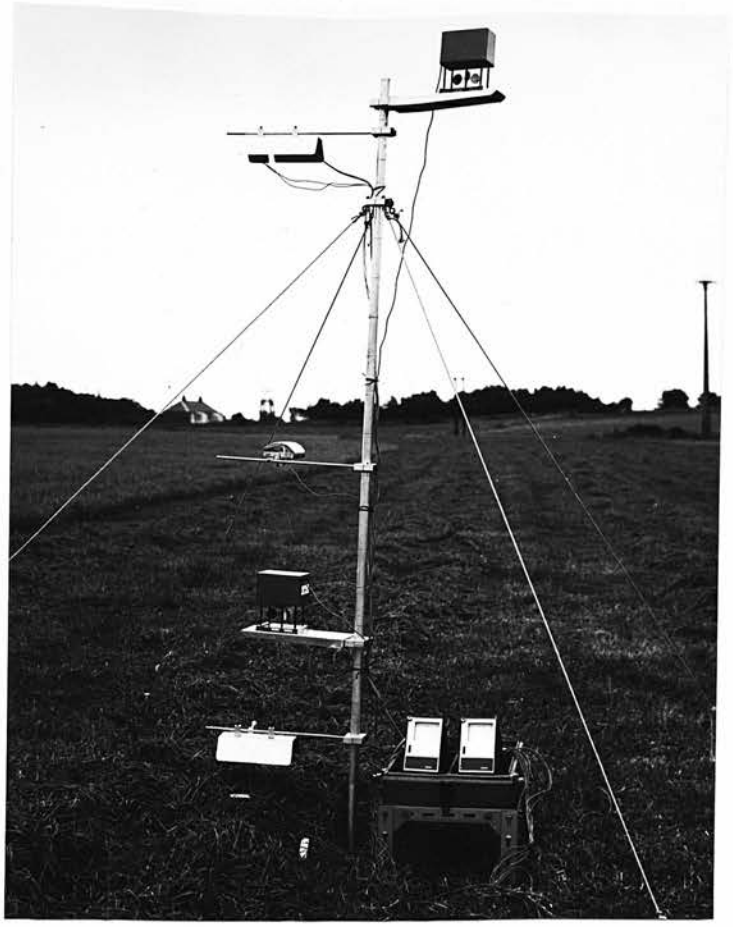


PLATE 1.10 Microclimate Recording Station and Climate Mast

The arrangement of the sensors corresponds to that shown in Fig. M.3, and all sensors are connected to the recorders for automatic hourly monitoring of the environmental variables: temperature, relative humidity, windspeed and light intensity.

swath temperature, humidity and light intensity at several heights, and soil temperature. Therefore the changing gradients of these four environmental variables, from swath to air, could be followed throughout the wilting period. The erection of the mast, positioning of the sensors, and connection to the microclimate recording station took about an hour, so that it was not possible to begin intensive microclimate monitoring until 1 h after harvest.

Microclimate Recording Station. The station was used to measure and record the environmental factors automatically, at hourly intervals, during the wilting period. The mode of connection of the sensors to the input channels of the two recorders A and B was always according to the same pattern, as follows:-

Recorder A

Sensor type

Dry-bulb thermistor	height cm:	+190, 100, 30, 20, 15, 10, 5, 0, -2, -5
	channel no.:	9, 1, 11, 2, 3, 4, 5, 6, 7, 8
Wet-bulb thermistor	height cm:	+190, 30
	channel no.:	10 12

Recorder B

Sensor type

Ambient light sensor	height cm:	5, 5, 5, 5
	channel no.:	1, 2, 3, 4
Difference light sensor	height cm:	15, 10, 5, 0
	channel no.:	5, 6, 7, 8
Anemometer	height cm:	200, 60
	channel no.:	1, 2

Therefore the chart recordings always appeared in the same order, and each sensor could be identified readily from its position on the chart.

Method of operation. The sequence of operations performed when using the microclimate recording station in the field was as follows. Calibration of both recorders A and B was carried out always, as soon as the recording station and mast were assembled. Then one complete cycle was recorded using the 'continuous' control switch in order to check that all connections were good. This was followed by one cycle triggered by the 'start cycle' control switch in order to check that the psychrometers were giving good depression readings. Then the controls were switched to 'automatic' operation and the exact time of the first automatic cycle was noted. After this, the station recorders and control unit with battery supply were enclosed in the wooden box, and the whole unit was covered with a protective weatherproof polythene sheet, and was left to run automatically throughout the wilting period. The calibration was rechecked usually once or twice a day, and the batteries were replaced when necessary. Battery life was between 24 and 30 h, a relatively short period since the two 12 v psychrometer fans were powered from them. The recorder charts needed replacing only infrequently: the chart length was sufficient for about two weeks of recording on automatic operation.

Other Information Recorded

(i) National weather reports for Scotland from *The Scotsman* giving the overall weather picture and forecast and figures from the Turnhouse weather station for the previous day; the monthly weather report issued by the Meteorological Office.

(ii) Local weather report from the weather station records of Bush Estate.

(iii) Weather observations made in the field during the wilting period, such as fractional cloud amount, visibility, etc., at regular intervals.

(iv) Location of experiment including map reference, farm and field names and field position.

(v) Site description including topography, aspect, soil type, drainage, etc.

(vi) Species composition of herbage undergoing wilting, year of growth, first or second crop, stage of growth at harvesting.

(vii) Date, and time of day at which harvest took place.

(viii) Details of harvesting procedure, including the machinery used for harvesting and conditioning the herbage.

(ix) Description of the nature of the swath produced, and any changes observed during the wilting period.

FIELD EXPERIMENTS

Field experiments were carried out on the Edinburgh School of Agriculture's experimental farms, Easter Howgate and Boghall, five miles south-west of Edinburgh in the foothills of the Pentlands, during the summers of 1972 and 1973. The experiments were performed in conjunction with series of silage experiments involving a wilting pretreatment.

Pilot Programme 1972

A preliminary survey was carried out during June 1972, involving swath description, and manual environmental measurements using the Light/Temperature Meter. Other information was recorded. Data were collected on two consecutive days during the early stages of wilting.

Field Programme 1973

The principle field studies were carried out during the summer of 1973 at Boghall Farm. This involved use of all the field equipment

and the corresponding field techniques described above. An intensive study of swath characteristics and microclimate, in relation to the swath evaporation rate, was carried out throughout the 24 h and 48 h wilting periods. Other information was recorded for each wilting period.

June 1973: six 24 h wilting periods were monitored on six consecutive days in two different fields.

July and August 1973: two 48 h wilting periods were monitored.

SECTION 1: RESULTS

PILOT PROGRAMME 1972

Table 1.1 gives details of the location of the field studies, the crop, the harvesting methods, the prevailing weather conditions, the swaths produced and the swath microclimate during the pilot programme.

Preliminary observations indicated that the grass swaths produced by mowing, and mowing plus conditioning, were quite different in character. In general, the mower cut the grass at 3 - 5 cm above ground level and left the plants intact and arranged in two parallel swaths, in which the plants were orientated in the same direction and overlapping by about half their lengths. Conditioned ('crimped') swaths were more irregular and loosely-packed, and the plants suffered some breakage and crushing injury. Maximum swath height was 12 and 20 cm for mown and crimped swaths respectively; swath width was always about 1 m. The swaths were bimodal in transverse section, the two peaks being separated by a trough of c. 10 cm in height. Table 1.1 a.

Microclimatic conditions in fresh and wilted grass swaths were studied by recording swath and ambient temperature, relative humidity and light intensity. Table 1.1 b. Only selected representative results are given.

(i) Temperature

On a sunny morning, the swath surface temperature was 2 - 3° higher than the swath ground level temperature. The form of the temperature gradient was of a relatively low constant temperature (e.g. 16° C)

at ground level rising steadily to within 2 - 4 cm of the surface, then rising rapidly to a peak at the surface (e.g. 19° C) and falling off slightly into the ambient air, e.g. wilted swath no. 6, Table 1.1 b.

(ii) Relative humidity

Swath-air relative humidity gradients were found in crimped and mown swaths immediately after harvesting, with a difference of 15 to 20 percentage units between the swath mass and the ambient air. Conditions were near saturating at ground level inside the swath, falling to about 70% at the swath surface at 10.00 h on a sunny day. However, the relative humidity gradient decreased as drying progressed with a difference of less than 10 percentage units between the swath mass and the air after three hours of wilting, 13.00 h, 18.7.72.

(iii) Light intensity

Light penetration appeared to follow a sigmoid curve, little reduction in intensity occurring within the first few cm of the surface in crimped swaths, but then falling steeply from 80 to 30% of incident light through the next ten cm and diminishing to a plateau at c. 5 to 10% in the ground layer, e.g. wilted swath no. 6.

In general the slopes of the light and temperature gradients were related to swath height and structure, being steeper in smaller dense swaths.

The variable nature of swath shape and microclimatic conditions indicated that a more intensive investigation of these swath characteristics, in relation to drying rates under a range of weather conditions, was justified. It was considered unnecessary to enlarge further on the results of the pilot programme since the results of the main field programme, 1973, covered all these aspects in more detail and will now be given in full.

TABLE 1.1 Pilot Programme 1972: Swath Data

Location: Stackyard field, Easter Howgate Farm.

Crop: Italian ryegrass (EF 486 Dasas) at 25 - 45% ear emergence.

Harvesting machines: 4 - drum rotary mower, M.
Haymaster conditioner, C.

Prevailing weather conditions: warm, sunny and calm on 18 July; early mist clearing by 10.00 h then warm and sunny, heavy dew on grass, on 19 July.

a. Swath description

Date	Wilting period h	Swath no.	Swath width cm	Swath max. ht. cm	Stubble ht. cm	Harvesting method
18 July	0	1	100	12	2	M
		2	100	19	2	M + C
		3	110	17	3	M + C
		4	120	20	2	M + C
19 July	24	5	100	20	4	M + C
		6	100	19	4	M + C
		7	100	11	2	M

TABLE 1.1 Pilot Programme 1972: Swath Data

b. Swath microclimate: typical gradients of swath temperature, light penetration and relative humidity.

	Temperature °C		Light % of incident		Relative humidity %	
Swath no.	3	6	4	6	2	2
Wilting period h	0	24	0	24	0	3
G.M.T. Sensor ht.	11.00	11.00	11.00	11.00	10.00	13.00
Ambient	19.8	20.0	100	100	72	69
Swath surface	19.5	19.4	100	100	77	71
15 cm	19.2	17.6	90	85	-	-
10 cm	18.8	17.2	80	30	-	-
5 cm	18.2	16.7	28	20	-	-
Ground level	16.4	16.4	7	7	90	76

FIELD PROGRAMME 1973

LOCATION OF THE FIELD EXPERIMENTS

The map overleaf, Fig. M.4, shows the location of Hay Knowes and Lower Fulford fields, the sites of the 1973 field experiments. The numbers 1 - 6 indicate the positions of the climate mast during the six 24 h wilting periods in June; the numbers 7 and 8 indicate the positions used during the two 48 h wilting periods in July and August respectively. Mast positions were chosen to represent a range of field conditions (altitude, slope, aspect) and were always at least 100 m from the field boundary in order to avoid local edge and shelter effects.

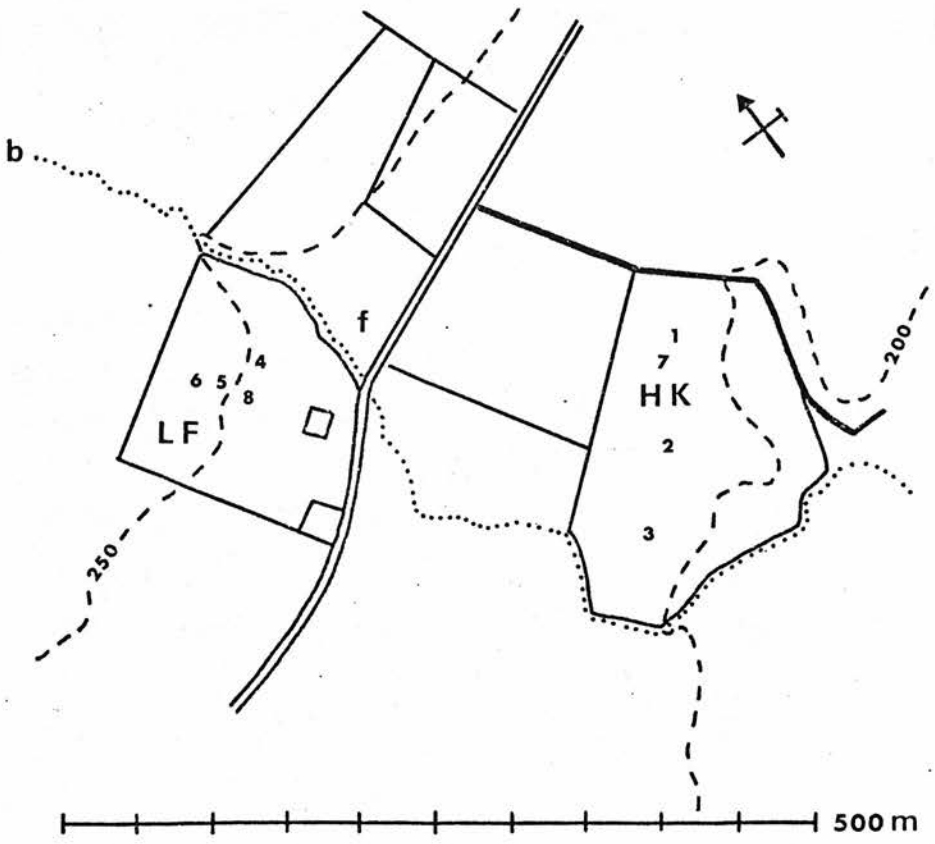
Hay Knowes field is situated at about 200 m in the valley; it is of fairly flat topography on imperfectly drained clay-loam soil. Lower Fulford field is situated between 215 and 275 m on a gentle S.E.-facing slope, on alluvial soil rising to imperfectly drained clay-loam gley above 250 m.

CROP SPECIES

The grass crop grown in Hay Knowes field was a second-year ley consisting of a ryegrass-clover mixture: 60% Italian ryegrass; 35% perennial ryegrass; 4.5% clover and a trace of other species. Lower Fulford field contained a first-year ley of 98% Italian ryegrass and a trace of other species. Both crops were estimated to be at 50% ear emergence on the first day of cutting for silage in each field (10, 13 June, 23 July), but on cutting for hay in Lower Fulford the grass was more mature at c. 100% ear emergence (15 August). The June experiments were carried out on the first cut, and the later experiments on the second cut in each field.

Fig. M.4 Map showing location of field experiments, 1973

(O.S. ref. 647241)



Key

= = main Boghall Road

= farm track

= field boundary

– – – = contour line in m

..... = Boghall burn

HK = Hay Knowes field

LF = Lower Fulford field

1 – 6 = positions of climate mast in June 1973

7 = position of climate mast in July 1973

8 = position of climate mast in August 1973

f = Boghall Farm

TABLE 1.2 Field Programme 1973: Crop and Harvesting Information

Location: Hay Knowes field, Boghall Farm, H K.
Lower Fulford field, Easter Howgate, LF.

Crop: Ryegrass mixture.

H K: mixture of Presto, Barvestra and S.23 Perennial ryegrass, EF 486 Dasas and Manawa H Italian ryegrass, Altaswede red clover and N. Z. white clover.

L F: Tetila Tetraploid and EF 486 Italian ryegrass.

Harvesting machines: 4 - drum rotary mower, M.
Haymaster conditioner, C.

Date	Field	Cut	Yield $kg\ FW\ m^{-2}$ (swath area)	Harvesting Time G.M.T.	Harvesting Method
10 June	H K	1	5.48	10.00	M
11 June	H K	1	4.60	09.30	M
12 June	H K	1	4.36	14.00	M
13 June	L F	1	3.52	16.30	M
14 June	L F	1	2.32	11.30	M
15 June	L F	1	3.68	08.30	M
23 July	H K	2	2.90	13.30	M
15 August	L F	2	2.90	15.40	M + C

TABLE 1.3 Weather Conditions on Wilting Days in June, July and August 1973

Date	Description[4]	Temperature[2] °C max.	min.	Pressure[2] mb 09.00	21.00	R.H.[4] % 12.00	Cloud[1] eighths 10.00	Rain[1] mm	Wind[1] Speed Beaufort	Direction	Total miles day^{-1}	Sun[2] rise	Sun[2] set	Sunshine[2] hours	Solar[3] Radiation W m^{-2} day^{-1}
10 June	Overcast, sunny intervals, fresh breeze, rather cool	16	8	1012	1015	81	7	0	5	SW	150	04.28	21.58	2.1	4540
11 June	Overcast, occasional showers, strong breeze, rather cool	15	7	1016	1011	75	7	tr.	6	SW	190	04.28	21.58	1.1	4931
12 June	Overcast, occasional showers, strong breeze, normal temperatures	18	12	1007	999	-	8	1.4	6	SW	282	04.27	21.59	1.4	5894
13 June	Showers, sunny intervals, high winds, cool	14	6	1007	1023	65	6	0.6	7	SW	197	04.27	21.59	14.1	-
14 June	Sunny spells, becoming cloudy, fresh breeze, normal temperatures	16	4	1029	1028	55	8	0.6	5	SW	109	04.26	22.01	0.1	3588
15 June	Dry and sunny, moderate breeze, warm	23	12	1029	1024	67	7	tr.	4	SW	61	04.26	22.01	5.9	5957
23 July	Cloudy, sunny intervals, cool, light breeze, heavy dew on grass	18	5	1009	1009	65	7	0	1	NE	28	05.02	21.35	6.4	4931
24 July	Dry and sunny, warm, light breeze, slight dew	17	7	-	-	65	4	0	1	NE	18	05.02	21.35	6.6	5226
25 July	Dry and sunny, warm, light breeze	19	9	1013	1018	62	5	0	1	SW	53	05.04	21.33	10.0	6015
15 August	Dry and sunny, very hot, calm, dew on grass	24	9	1023	1020	75	3	0	0	-	18	05.43	20.50	8.7	5053
16 August	Dry and sunny, hot, calm, light or moderate breeze	25	9	1016	1012	65	3	0	0	-	118	05.45	20.48	5.6	3564
17 August	Cloudier, sunny spells, cooler, fresh breeze	16	10	1016	1021	68	1	0	4	WSW	181	05.47	20.45	8.8	5307

Key

1 = Bush weather records

2 = Turnhouse weather records

3 = Forestry and Natural Resources Dept., Edinburgh University, solarimeter records

4 = Observations recorded during field experiments

R.H. = Relative humidity

HARVESTING

Table 1.2 gives details of the date of each field wilting experiment, the harvesting method and harvesting time, and the initial crop yield.

PREVAILING WEATHER CONDITIONS

The monthly weather reports of the Meteorological Office stated that during June high pressure systems affected the British Isles periodically; between dry spells rainfall was sometimes heavy and thundery; winds were occasionally fresh or strong; in E. Scotland mean monthly temperatures and sunshine amounts were above average. During July, depressions alternating with ridges of high pressure affected the British Isles periodically; dry and sunny spells occurred during the last week; winds were primarily light or moderate and most of Scotland was drier than usual. During mid-August, pressure remained high although a cold front crossed the whole country on 16th and 17th; it was often sunny and warm; winds were generally light or moderate; temperatures were well above average; it was drier than usual and particularly sunny in E. Scotland.

Table 1.3 gives details of the local weather conditions prevailing during the field wilting experiments in June, July and August.

SWATH DESCRIPTION

Plates 1.11 and 1.12 show general and close-up views respectively of grass swaths produced by mowing. Table 1.4 contains the swath description data collected on fresh mown and 24-h wilted swaths during June; and fresh mown, 24-h and 48-h wilted swaths during July and August. In

PLATE 1.11 General view of freshly mown grass swaths cut by 4-drum rotary mower

PLATE 1.12 Bimodal structure of freshly mown grass swaths 1 m wide

TABLE 1.4 Swath Description Data for Fresh and Wilted Swaths

Date	Wilting period h	Width of cut cm	Mean Swath Width cm	Inter-Swath Distance cm	Field cover %	Max. Swath Ht. cm	Stubble Ht. cm
10 June	0	140	103	48	68	28.9	8.0
12 June	24	150 150	116 116	30 26	80 82	16.2 11.6	- 3.3
12 June	0	150	105	45	70	25	-
23 July	0	-	-	-	-	13	3.6
25 July	48	145	115	31	63	10.1	4.2
15 August	0	-	54	87	38	27.7	-
16 August	30	-	75	200	27	50	-

Spreading and Subsidence of Wilted Swaths cut by Rotary Mower

Wilting period h	Spreading %	Subsidence %
24	9.5	36 - 52
48	9.5	60

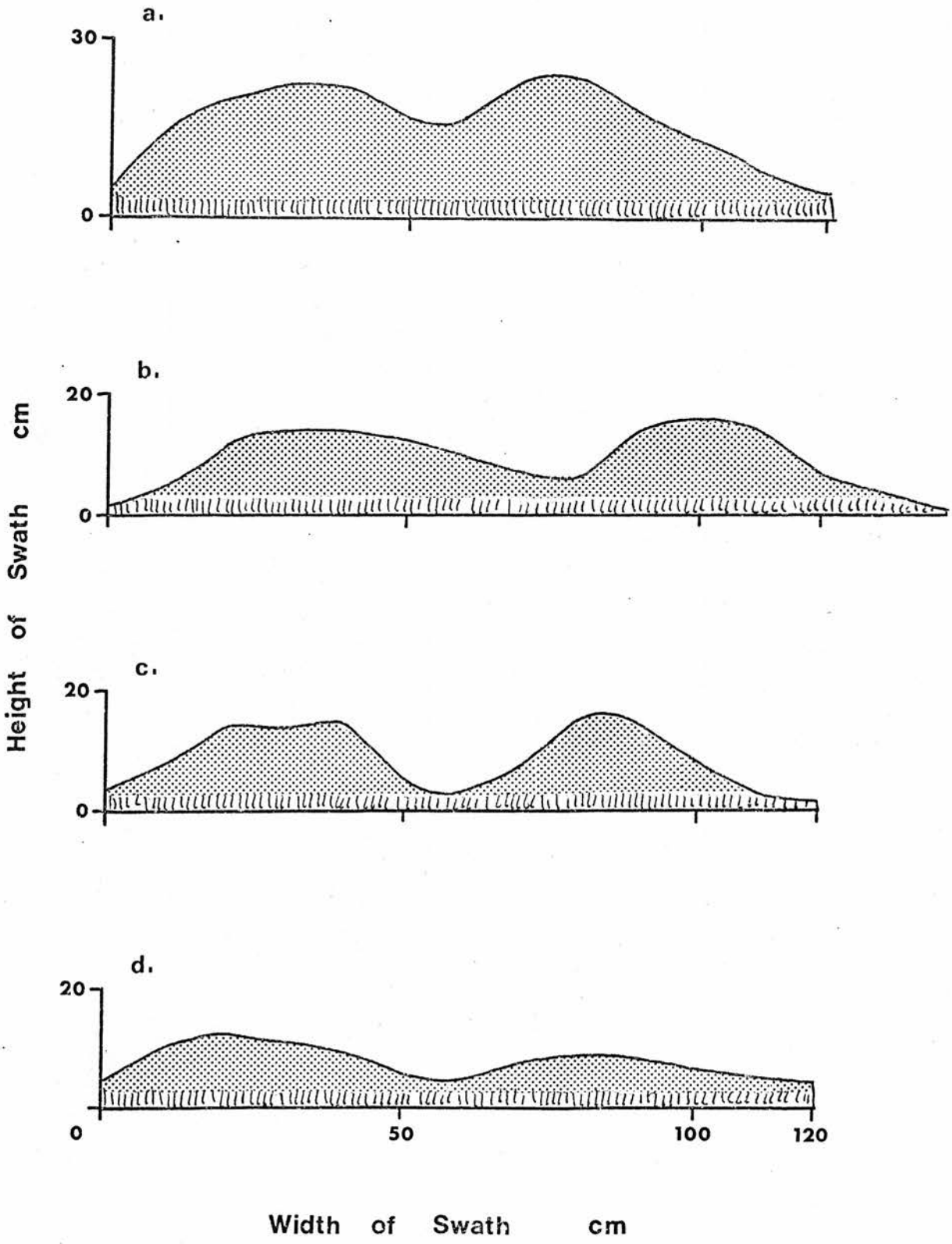


FIG. 1.1 Profiles of typical swaths in transverse section

a. Freshly mown swath, H K, 10 June, cut 1
b. 24 h wilted swath, H K, 11 June, cut 1
c. Freshly mown swath, H K, 23 July, cut 2
d. 48 h wilted swath, H K, 25 July, cut 2

Vertical shading indicates stubble; Stippled shading swath

general, bimodal swaths c. 1 m in width (Plate 1.12) and 20 - 30 cm in height, lying on a 3 - 8 cm layer of stubble, were obtained from freshly mown grass. After 24 h of wilting both spreading and subsidence had occurred, with a 10% increase in width and a 25% reduction in height, particularly after rain when subsidence could exceed 50%. After 48 h of wilting, spreading and subsidence was marked, and the interswath distance was reduced by up to 50% so that the field cover increased greatly.

Fig. 1.1 shows typical bimodal profiles of fresh mown, 24- and 48-h wilted swaths in transverse section. The swath dimensions were dependent upon crop yield: the fresh mown swaths produced in H K field from cut 1 in June, with a yield of 5.48 kg m^{-2} of swath, were significantly greater ($P = 0.05$) in bulk and maximum height than the fresh mown swaths from cut 2 in July, with a yield of 2.90 kg m^{-2} of swath area. The cut 1 swaths were not discretely separated into two halves whereas the cut 2 swaths were. However, both cut 1 and cut 2 swaths showed considerable spreading and subsidence after 24 and 48 h of wilting.

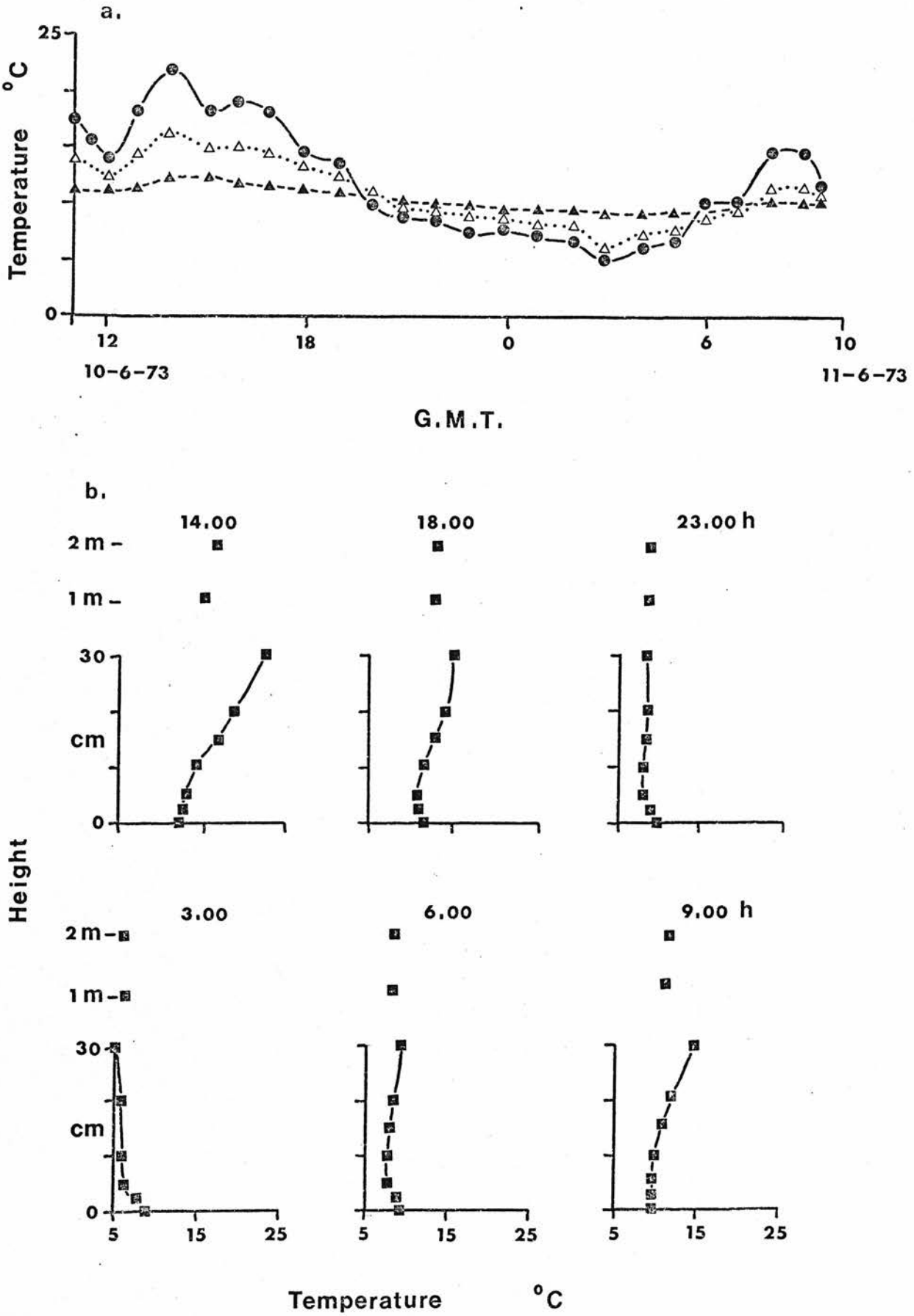
SWATH MICROCLIMATE

General features of the swath microclimate throughout a typical 24-h wilting period of favourable weather conditions (i.e. no rain, positive vpd, fresh breeze, occasional sunny intervals on 10 and 11 June, Table 1.3) are illustrated in Figs. 1.2 - 1.6, and Table 1.5. Figs. 1.2 - 1.5 show the characteristic diurnal pattern and swath gradients of temperature, relative humidity, windspeed and light penetration. Fig. 1.6 and Table 1.5 show aspects of the swath solar radiation environment.

(i) Temperature

Fig. 1.2 shows the diurnal pattern of ambient, swath surface and swath ground level temperature during the first 24 h wilting period, 10 June.

FIG. 1.2 Temperature: a. diurnal pattern and b. gradients



24 h wilting period, H K, 10 June

▲, ground level; △, ambient; ●, swath surface; ■, gradient

The temperature at ground level beneath the swath remained relatively constant throughout; day temperatures were only 1 - 2° C higher than night temperatures. Both ambient and swath surface temperatures showed a maximum at 14.00 h (16° and 22° respectively) and a minimum at 03.00 h (6° and 7° respectively). Variations were greatest at the swath surface with day temperatures up to 6° C higher and night temperatures up to 2° C lower than ambient. At 19.00 h and 06.00 h swath and ambient temperatures were at equilibrium.

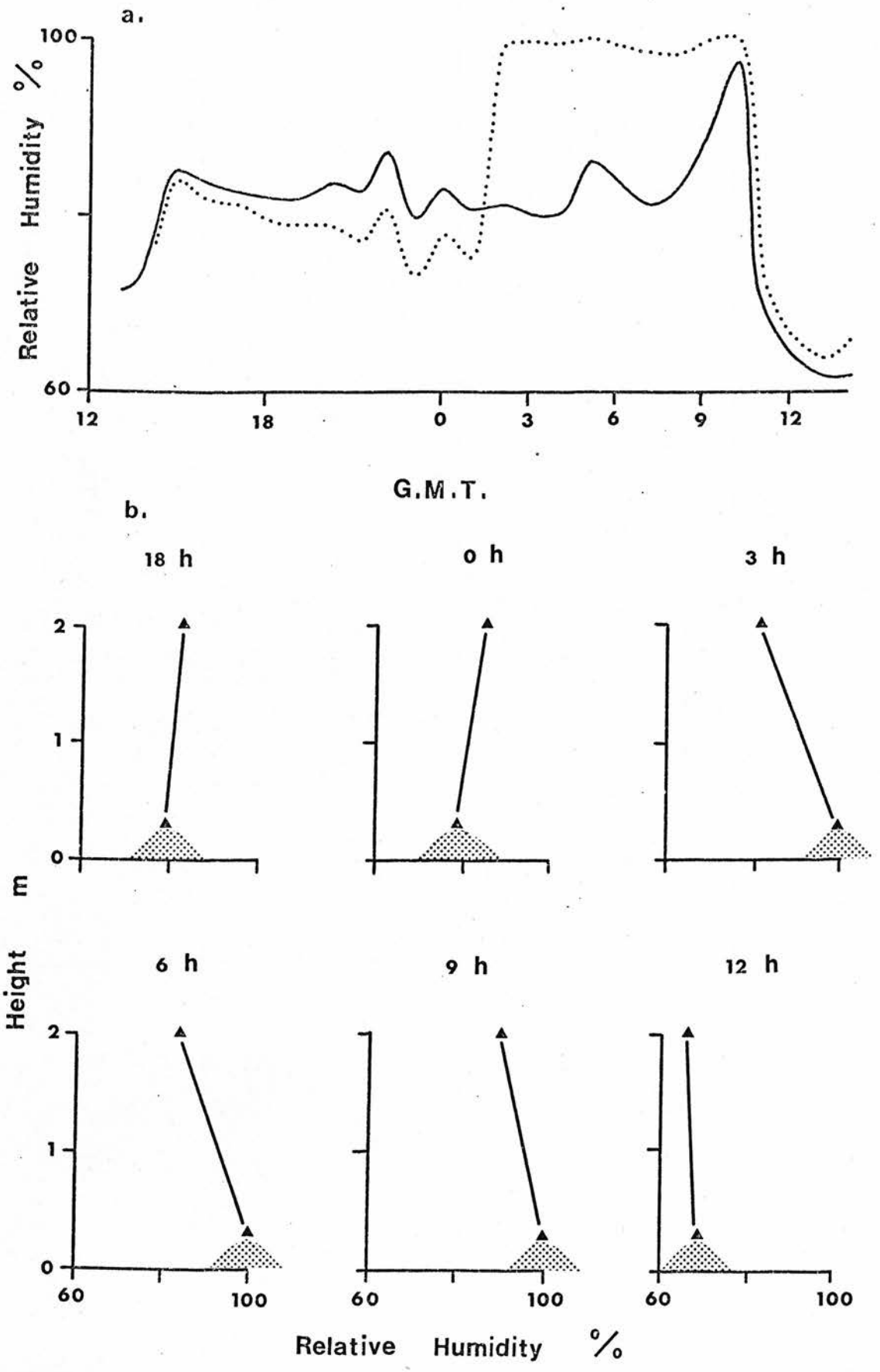
Swath temperature gradients were greatest during the afternoon (14.00 h) with a maximum at the surface, a minimum at ground level and a 12° difference between them. During the evening the gradient decreased, and the minimum moved upwards through the swath until it reached the surface (03.00 h) when the gradient became reversed with the maximum at ground level. After sun-rise, the minimum and maximum exchanged positions once more so that by 09.00 h the maximum was re-established at the swath surface.

By contrast, under adverse weather conditions (i.e. squally showers, zero vpd, overcast and cool, 12 June, Table 1.3) the diurnal pattern and gradients in temperature were quite different. There was little variation in swath or ambient temperatures throughout the 24 h wilting period with only a 2° difference between day and night values. Temperature gradients were much reduced, the swath surface temperature being 1 - 2° higher than ambient and ground level temperatures during the afternoon, or zero overnight.

(ii) Relative humidity

Fig. 1.3 shows the diurnal pattern of ambient and swath surface relative humidity throughout the second 24-h wilting period, 11 June.

FIG. 1.3 Relative humidity: a. diurnal pattern and b. gradients



24 h wilting period, H K, 11 June. solid line, ambient; dotted line, swath surface; ▲, gradient; stippled shading, swath

The ambient R H fluctuated irregularly around 80% during the evening and overnight, then fell steeply the following morning; whereas the swath surface R H showed a rapid increase to saturation about 02.00 h until 10.00 h before falling steeply with the ambient R H. The difference between ambient and surface values was up to 20% units overnight.

The R H gradient generally decreased from the swath surface upwards during the morning, although by noon the gradient was much smaller. During the evening the R H gradient was reversed (18.00 h), until saturation developed at the surface (02.00 h).

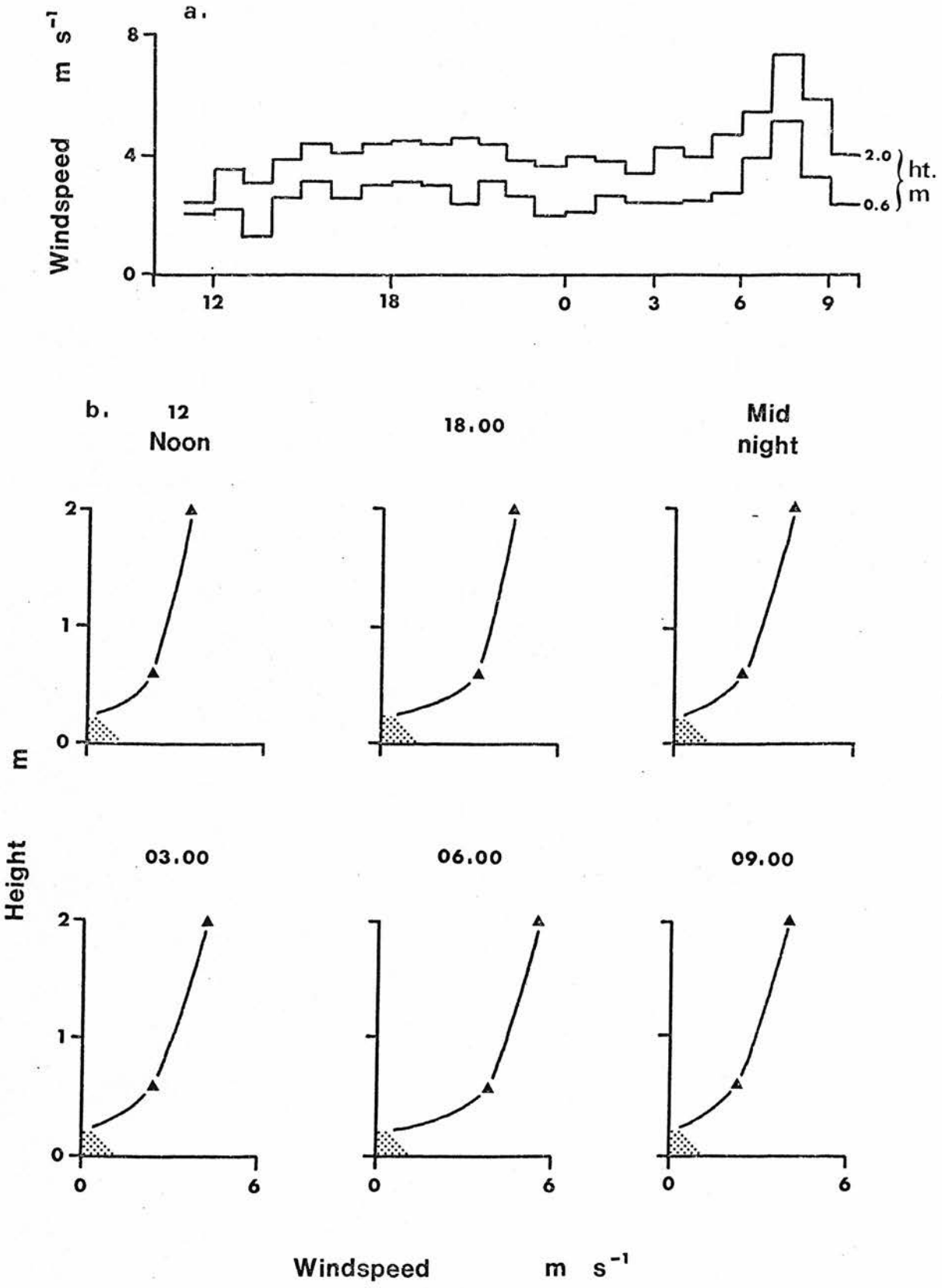
Under adverse weather conditions (12 June), ambient R H fluctuated irregularly between 85 and 100%, while conditions were uniformly saturating (100%) at the swath surface. There was no marked diurnal trend, and gradients were in general small and variable.

(iii) Windspeed

Fig. 1.4 shows the diurnal pattern in windspeed at 2 and 0.6 m above ground level, and wind profiles, on 10 June when the total run-of-wind was 150 miles (Table 1.3). The histogram shows mean hourly values of windspeed, which in general varied around 4 and 2 m s^{-1} at 2 and 0.6 m respectively throughout the day, apart from a particularly windy spell between 06.00 h and 09.00 h when windspeeds increased to maxima of 7.7 and 5.25 m s^{-1} respectively.

Wind profiles were logarithmic in form above the zero plane which was assumed to be at the swath surface.

FIG. 1.4 Windspeed: a. diurnal pattern and b. profiles



24 h wilting period, H K, 10 June. ▲, gradient; stippled shading, swath

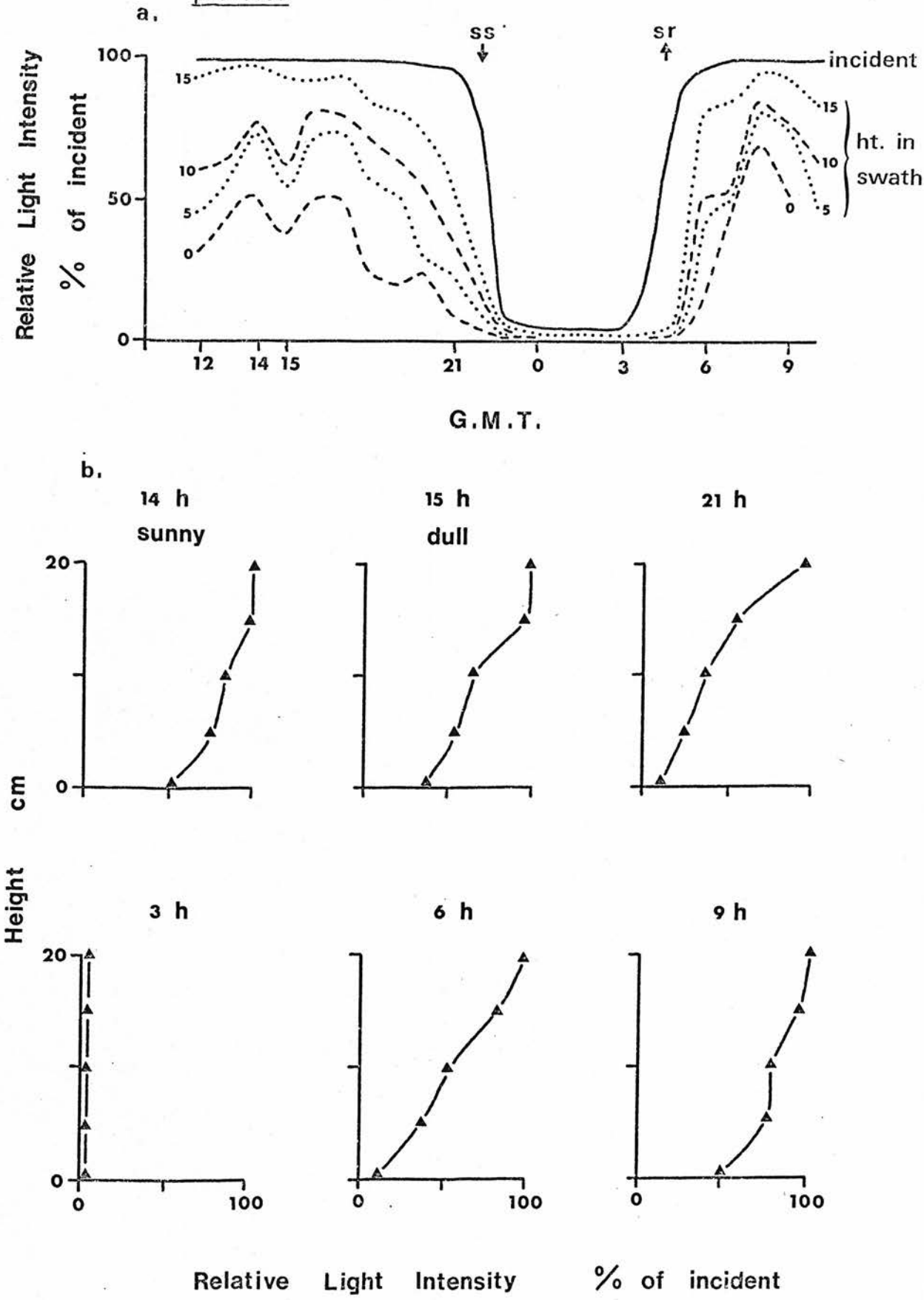
On a squally, gusty day of adverse weather conditions (12 June, Table 1.3) the total run-of-wind was 282 miles, the diurnal pattern was more irregular with occasional gusts of up to 11.2 and 9.2 m s^{-1} at 2 and 0.6 m respectively.

(iv) Light penetration

Fig. 1.5 shows the diurnal pattern of incident (ambient) light intensity and the relative light intensities at 15, 10, 5 cm and ground level inside the swath, and profiles of light penetration during the day (10 June). Incident light intensity followed the expected pattern, changing sharply from 100% to zero within 1 - 2 h at sunset and in reverse at sun-rise. Relative light intensity at 15 cm remained close to incident during the afternoon but began a gradual decrease to zero about 18.00 h. Relative light intensities at 10, 5 and 0 cm fluctuated around 70%, 60% and 40% of incident respectively following a similar pattern, before decreasing gradually to zero after 17.00 h. After sun-rise, the swath relative light intensities showed a 1 - 2 h lag phase before increasing, after which the 15 cm layer increased in intensity most rapidly to the highest relative value, followed by the lower layers in order down to ground level, attaining maximum values of 95%, 85%, 80%, 70% of incident respectively.

The profiles of relative light intensity or light penetration were in general sigmoid during the day under both sunny and dull conditions, 14.00 h and 15.00 h respectively. There was little diminution in intensity in the top 5 cm of swath; a rapid extinction through the next 5 cm (10 - 15 cm ht) from 90% to 60% for example; a more gradual decrease between 5 and 10 cm ht, then a further rapid extinction down to ground level. The gradients were particularly steep just before sun-set and just after sun-rise, 21.00 h and 06.00 h respectively.

FIG. 1.5 Light intensity: a. diurnal pattern and b. extinction profiles



24 h wilting period, H K, 10 June

▲, gradient; ss, sunset; sr, sunrise

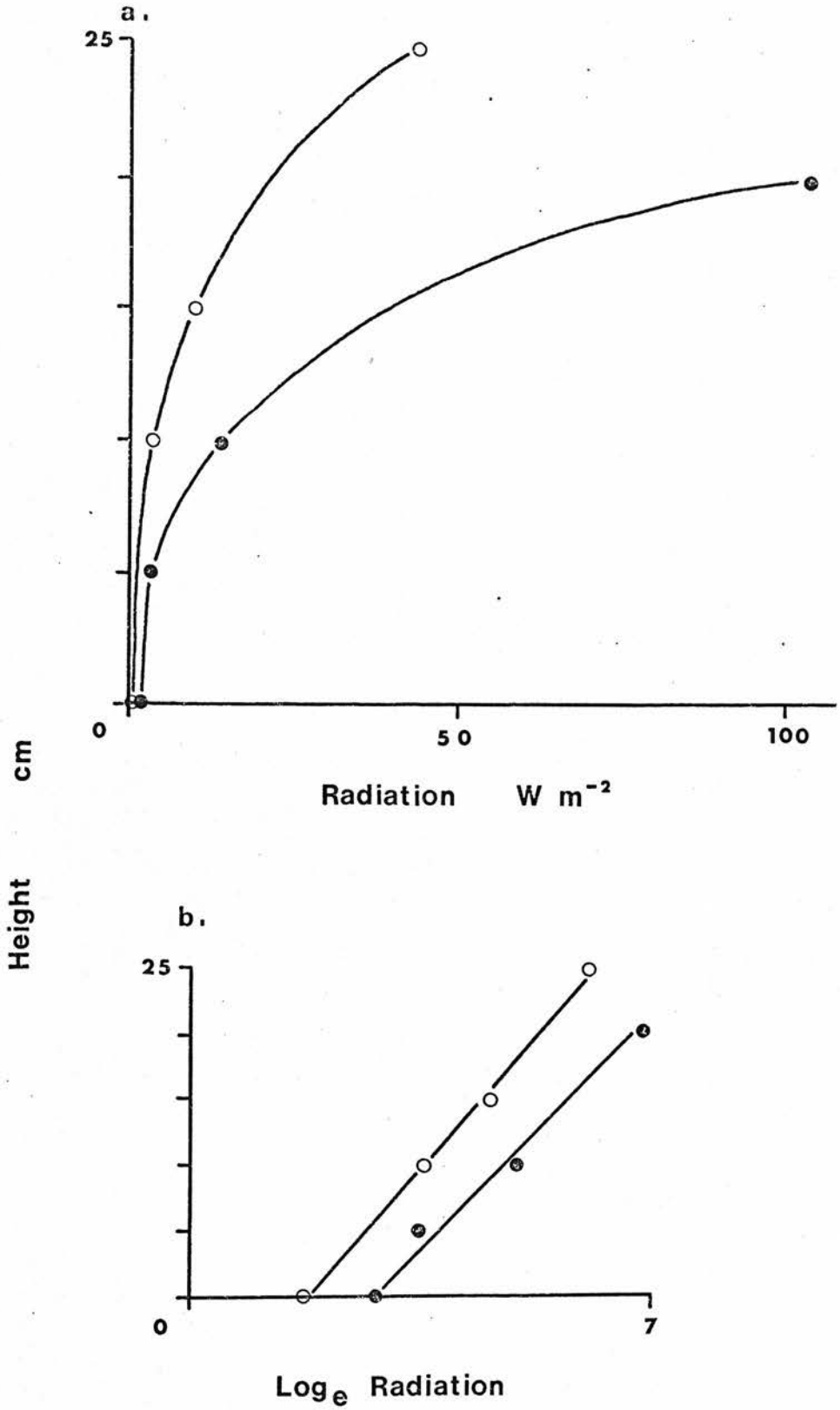


FIG. 1.6 Solar radiation: a. profiles, linear scale; b. profiles, log scale

24 h wilting period, H K, 15 July

● , sunny; ○ , dull

TABLE 1.5 Swath Albedo Values

$$\text{Albedo} = \frac{\text{Reflected Solar radiation}}{\text{Total incoming solar radiation}}$$

measured with paired tube solarimeters in W m^{-2} (integrated over the solar spectrum from 0.3 - 3.0 μm).

Position of Solarimeter Pair	Height cm	Mean Albedo	S.E. of mean
Swath surface	20	0.37	0.0028
Inside swath	15	0.43	0.0099
Inside swath	10	0.92	0.028
Inside swath at ground level	0	0.40	0.031
In stubble between two swaths	5	0.31	0.0019

Mean albedo values were based on at least 30 measurements, made between 13.00 and 17.00 h on a sunny afternoon with occasional cloudy intervals, 15 June 1973.

Under adverse weather conditions (12, 14 June, Table 1.3), the overall pattern of diurnal changes and profiles of light intensity was similar, but the absolute values of light intensity were lower.

(v) Solar radiation

Fig. 1.6 shows two profiles of solar radiation on the swath from 25 cm down to ground level under sunny and dull conditions on 15 July. The exponential decrease in solar radiation from the swath surface down to ground level was more rapid in sunny than dull conditions. Surface values were 100 and 40 W m^{-2} respectively, and ground level values were less than 3 W m^{-2}.

(vi) Albedo values

Table 1.5 gives swath albedo values at the surface, 15, 10 cm and at ground level, the proportion of reflected solar radiation varying from 0.37 at the surface, to 0.92 at 10 cm inside the swath where the fluxes of upward and downward radiation were almost equal.

SWATH DRYING

Figs. 1.7 - 1.20 illustrate the swath drying pattern on six consecutive 24-h wilting periods in June, and two 48-h wilting periods, one in July the other in August 1973. Figs. 1.7 - 1.12, 1.15 and 1.18 show a. the swath F W decline curves, b. mean D M content and c. stratified D M content curves during these respective wilting periods.

a. Swath F W decline curves

When harvesting occurred in the early morning (10, 11, 15 June; Figs. 1.7, 1.8, 1.12 respectively) and wilting conditions were relatively good (Table 1.3), there was a rapid, steady decline in swath F W until

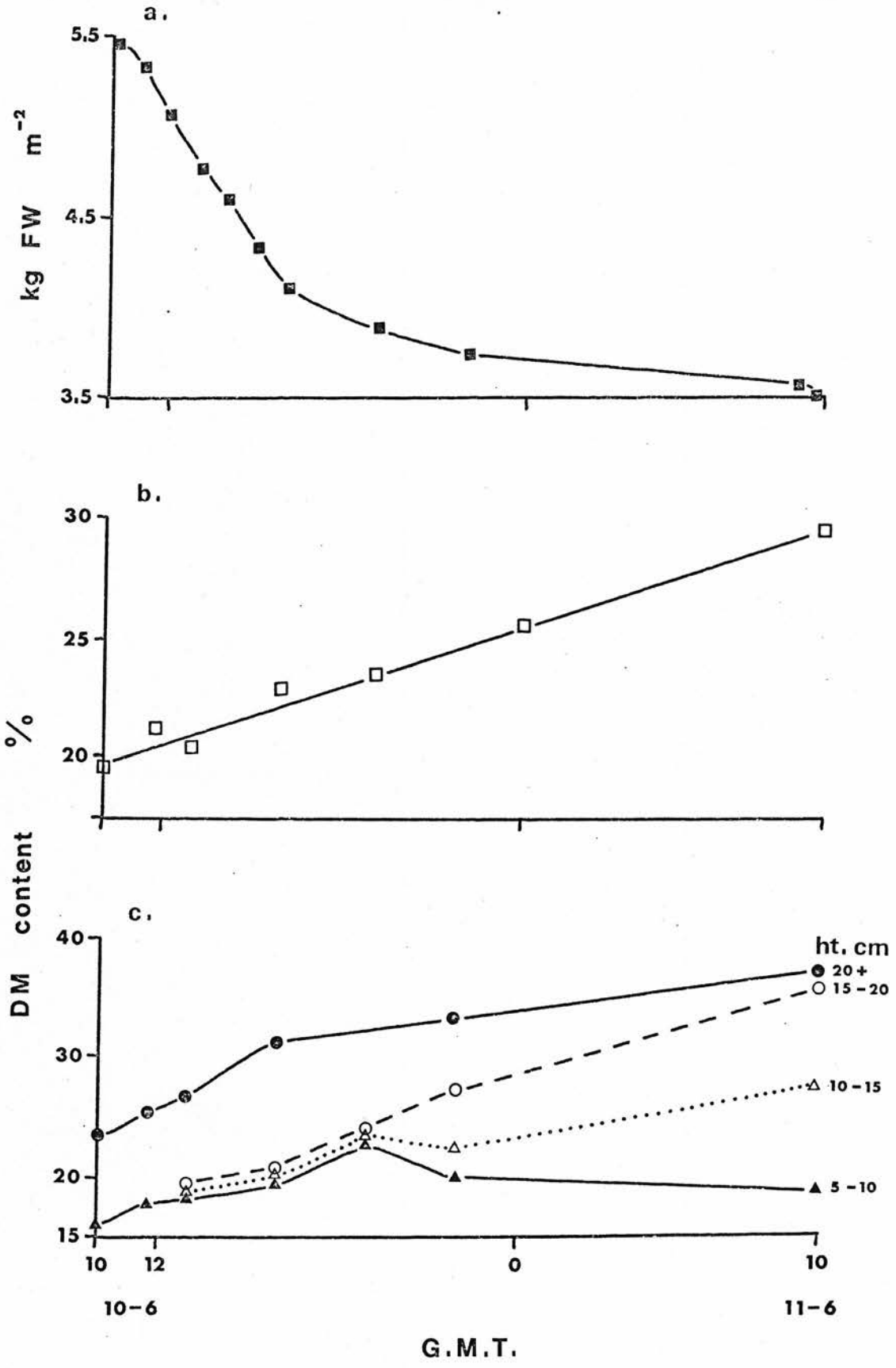


FIG. 1.7 Swath wilting on 10 June 1973, H K

a. Swath F W decline curve

b. Swath mean D M content

c. Swath stratified D M content

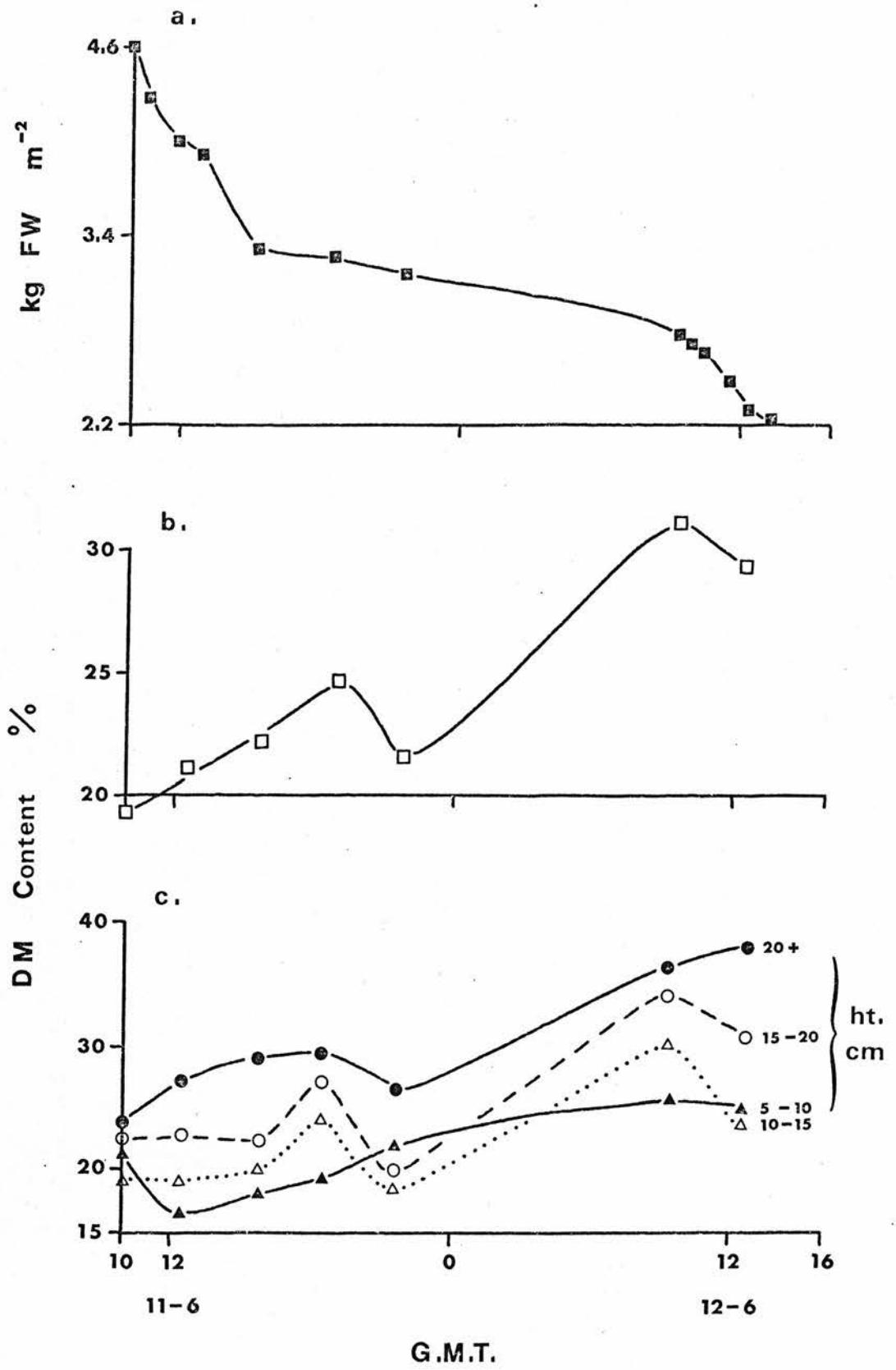


FIG. 1.8 Swath wilting on 11 June 1973, HK

a. Swath F W decline curve

b. Swath mean D M content

c. Swath stratified D M content

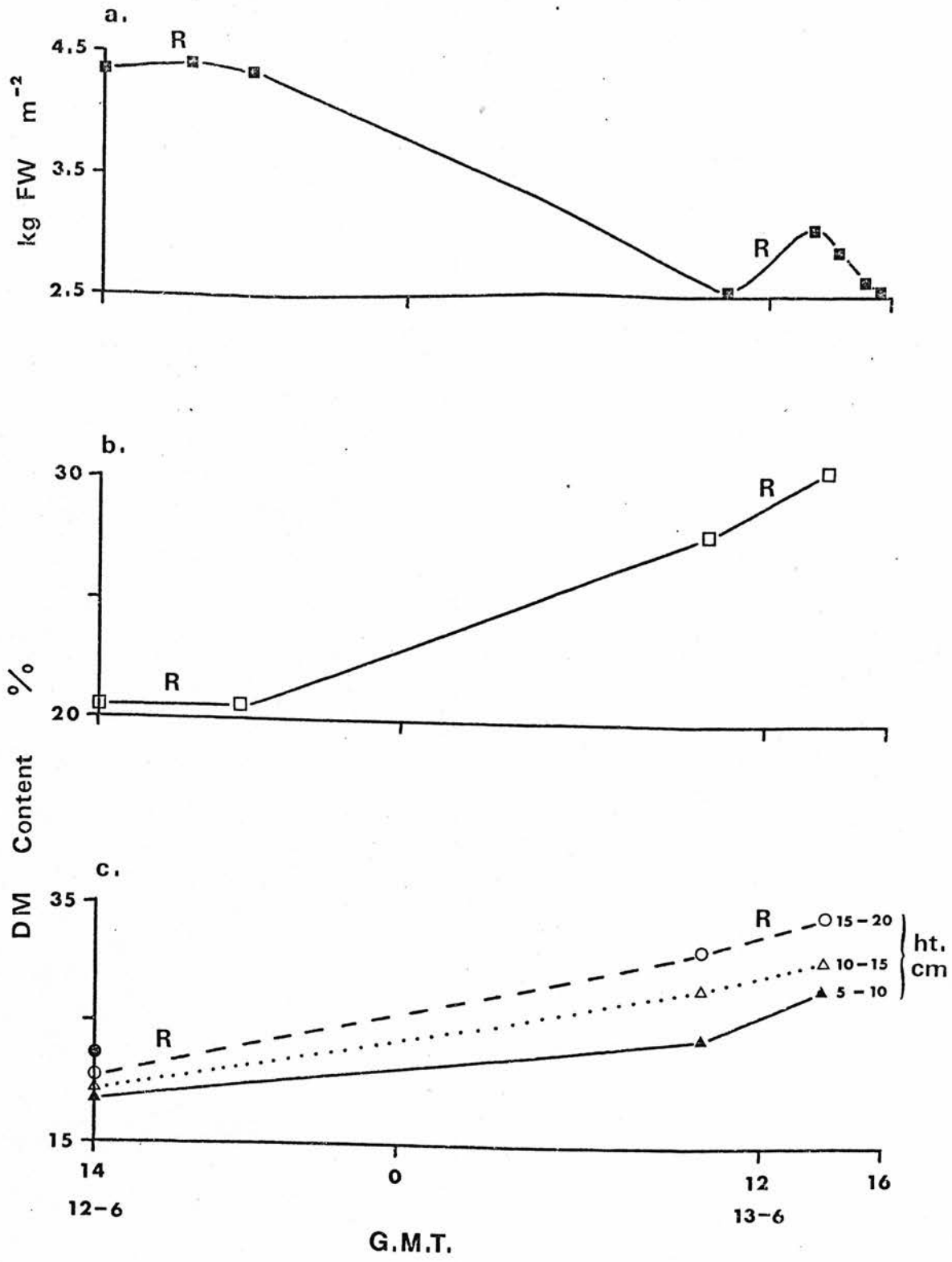


FIG. 1.9 Swath wilting on 12 June 1973, H K

a. Swath F W decline curve

b. Swath mean D M content

c. Swath stratified D M content

R, Rain

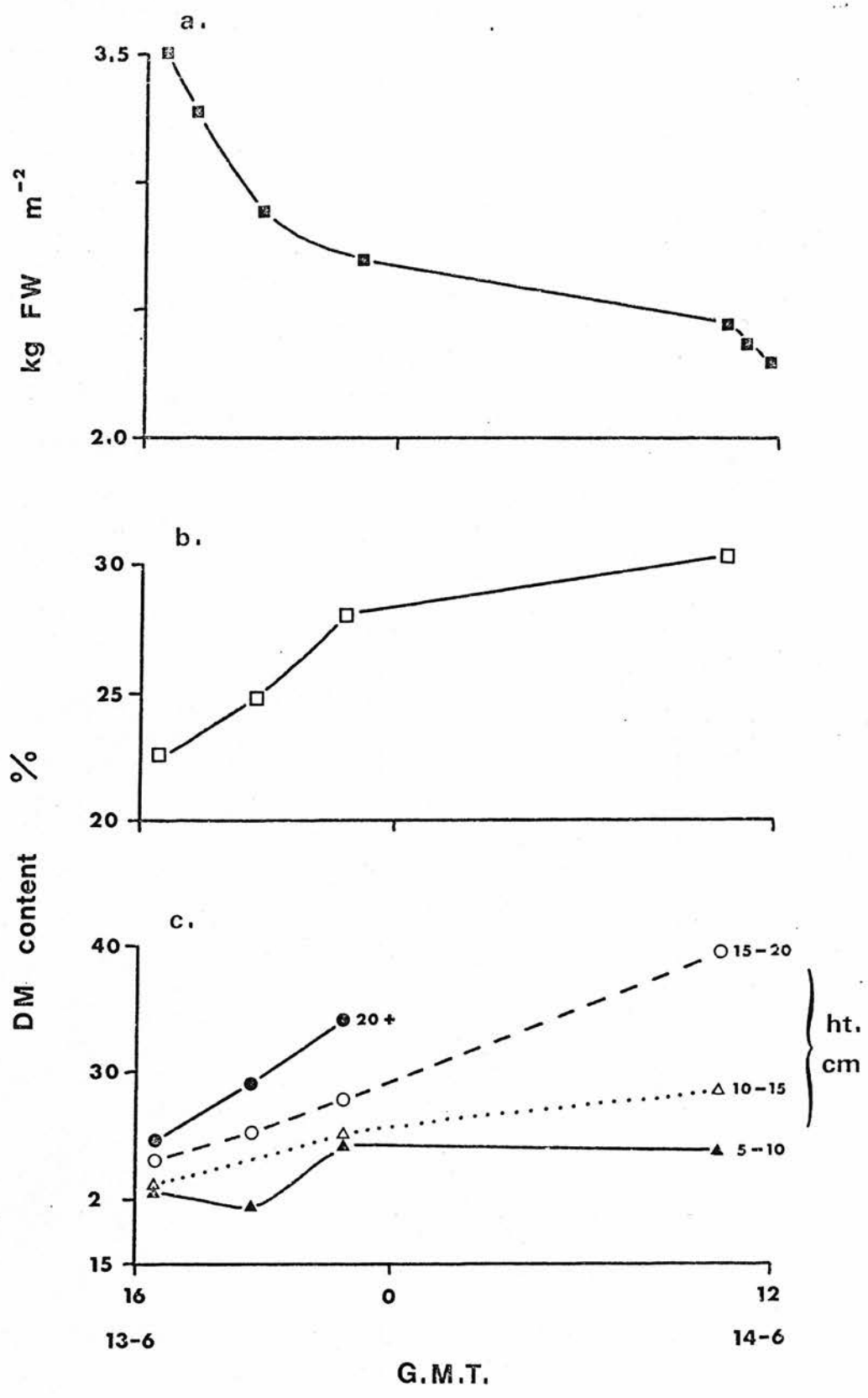


FIG. 1.10 Swath wilting on 13 June 1973, L F

a. Swath F W decline curve

b. Swath mean D M content

c. Swath stratified D M content

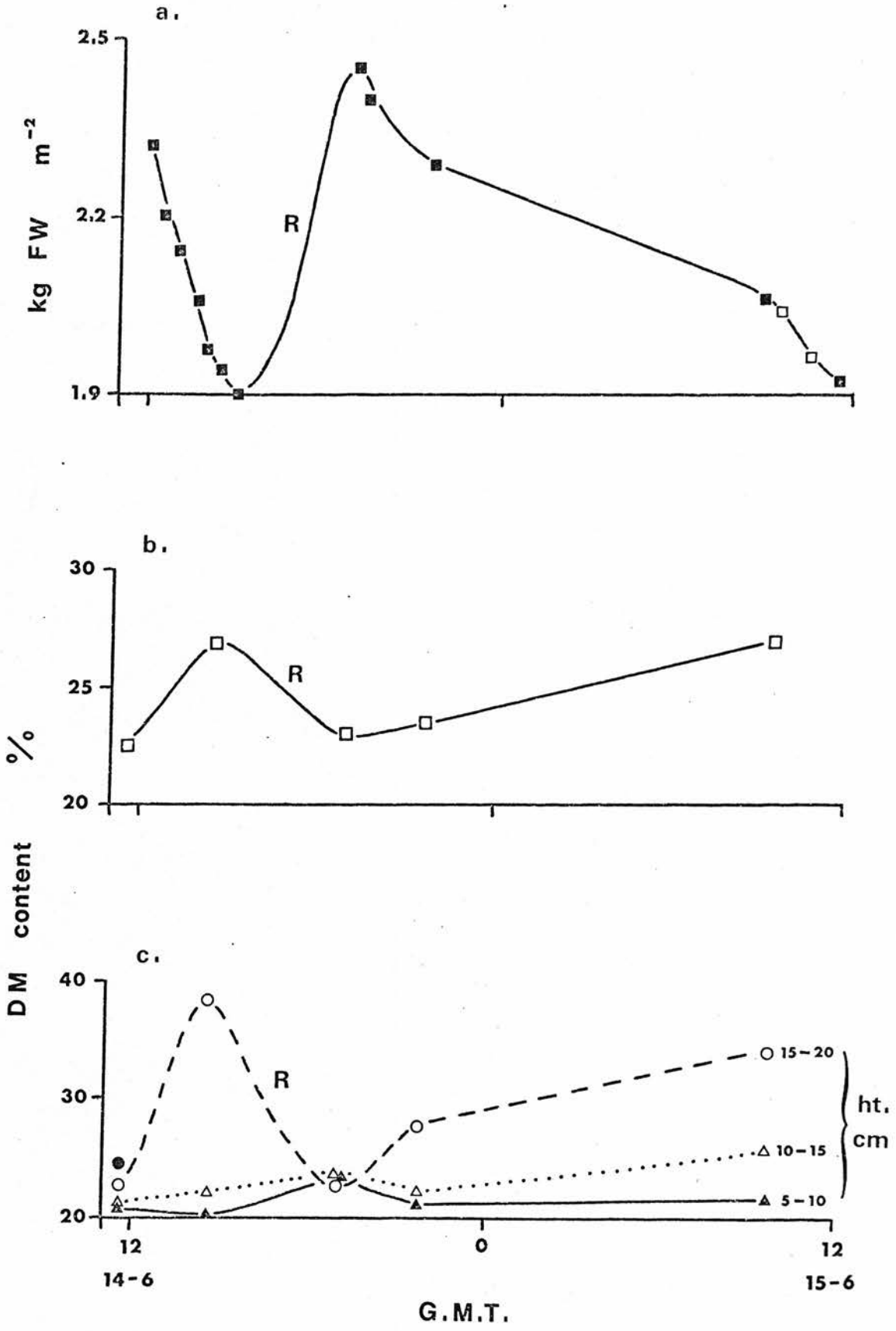


FIG. 1.11 Swath wilting on 14 June 1973, L F

a. Swath F W decline curve

b. Swath mean D M content

c. Swath stratified D M content R, rain

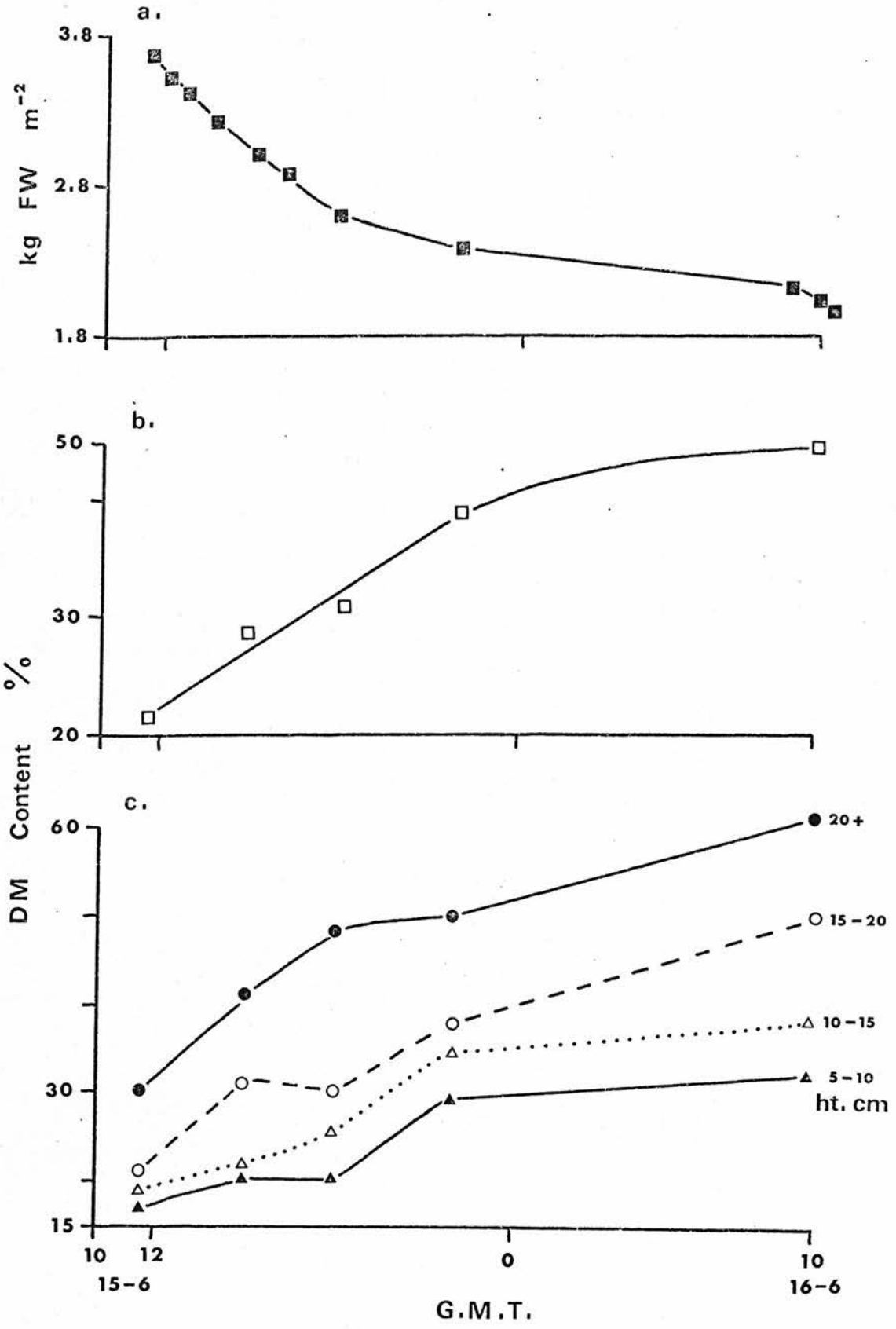


FIG. 1.12 Swath wilting on 15 June 1973, L F

a. Swath F W decline curve

b. Swath mean D M content

about 15.00 h after which F W declined more slowly until 18.00 h; thereafter F W declined very slowly until about 21.00 h after which there was little further weight loss until 09.00 h the following morning. After 09.00 h on the second day, swath F W declined once more but less rapidly than on the first morning after harvest. After noon on the second day of wilting, the swath F W fell more slowly, Fig. 1.8

When harvesting occurred in the late afternoon (13 June; Fig. 1.10) under good drying conditions (Table 1.3), the swath F W decline curve had a similar pattern except that the initial period of rapid weight loss continued until about 20.00 h on the first day.

When harvesting occurred around noon (12, 14 June; Figs. 1.9, 1.11) and subsequent wilting conditions were relatively poor with occasional showers (Table 1.3), the pattern of swath F W changes was variable. Swath F W either remained constant (Fig. 1.9) or increased (Figs. 1.9, 1.11) during periods of rain. When weather conditons improved, swath F W decreased as usual.

When harvesting occurred during the afternoon and wilting was extended to 48 h in July and August (Figs. 1.15, 1.18), the swath F W decline followed the characteristic pattern for good weather conditions. On the second day, weight loss slowed down after 15.00 h reaching little or no measurable loss after 18.00 h, and overnight. On the third day, weight loss recommenced and continued slowly after 09.00 h.

b. Swath Mean D M content

During the 24-h wilting periods in June, swath mean D M content generally increased from about 20% at harvest to about 30% at the end of

the wilting period, apart from 15 June (Fig. 1.12) when the final D M content exceeded 50%. D M levels of 23%, 24% and 31% were attained by 18.00 h after wilting, 09.00 - 18.00 h, under favourable conditions (10, 11, 15 June; Figs. 1.7, 1.8, 1.12); D M levels of 21%, 24% and 24% were attained by 18.00 h after an afternoon's wilting, 12.00 - 18.00 h, under less favourable conditions (12, 13, 14 June; Figs. 1.9, 1.10, 1.11). When cutting was delayed until 16.30 (13 June, Fig. 1.10), D M content continued to increase until 22.00 h, reaching 28%. During periods of rain either no net change or a decrease in D M was observed (12, 14 June; Figs. 1.9, 1.11) sufficient to suppress the final D M content to 27% in the latter case.

During the 48 h wilting period in July, the mean swath D M content increased from 19% at harvest to 28% after 24 h and to 38% after 48 h of wilting (Fig. 1.15). The corresponding figures for August when cutting for hay were 28%, 56% and 75% respectively (Fig. 1.18). D M levels of 22% and 32% were recorded at 18.00 h after one and two days' wilting for silage in July (Fig. 1.15).

Mean swath D M values of 30% were attained after 5 h (15 June; Fig. 1.12), 21 h (13 June; Fig. 1.10), 24 h (10, 11, 12 June; Figs. 1.7, 1.8, 1.9), 27 h (23 July; Fig. 1.15) or more (14 June; Fig. 1.11) of wilting. Of those days when a 30% D M level was attained in 24 h or less, cutting time was variable (early morning on 3 days; early afternoon on 1 day; late afternoon on 2 days) and weather conditions were variable, ranging from warm and dry to showery.

c. Swath Stratified D M content

The marked stratification in swath D M content, increasing from ground level to the swath surface, was evident at harvest and persisted throughout the wilting period. Under favourable weather conditions,

the surface layer (20 + cm) increased rapidly in D M content reaching 30% within a few hours of harvest, from 0 h (Fig. 1.12) to 8 h (Fig. 1.8), and then attaining high final D M levels of 35% (Fig. 1.7) to 60% (Fig. 1.12) after 24 h of wilting. In general the lower layers showed a lag phase of about 6 h during which their D M content remained constant (Fig. 1.8) or increased slowly (Fig. 1.7). The second layer (15 - 20 cm) then increased rapidly in D M approaching the high surface values after 24 h (Fig. 1.7). The ground layer (5 - 10 cm, above the stubble) showed an overall slow increase in D M with extended periods of no change or even net decrease in D M content (Figs. 1.10, 1.12, 1.15), so that after 24 h of wilting its D M level was generally between 19% (Fig. 1.7, 1.15) and 25% (Fig. 1.9). The stratification was still evident after 48 h of wilting for silage (Fig. 1.15) but had almost disappeared at the high D M levels associated with wilting for hay (Fig. 1.18).

Figs. 1.13 and 1.14 show a. the swath evaporation rate, E, b. swath resistance to evaporation, r_{st}, and c. ambient vapour pressure deficit, vpd, during the six 24-h wilting periods in June. Figs. 1.16 and 1.19 show a., b. as above and c. the climatological resistance r_I during the 48-h wilting periods in July and August.

a. Swath evaporation rate, E

The swath evaporation rate was derived by taking the tangents to the F W decline curves (a. in Figs. 1.7 to 1.12, 1.15, 1.18) throughout each wilting period.

Under favourable weather conditions (10, 11, 13, 15 June; July and August; Figs. 1.13, 1.14, 1.16, 1.19) a high sometimes rising (10 June, Fig. 1.13) swath evaporation rate was recorded immediately

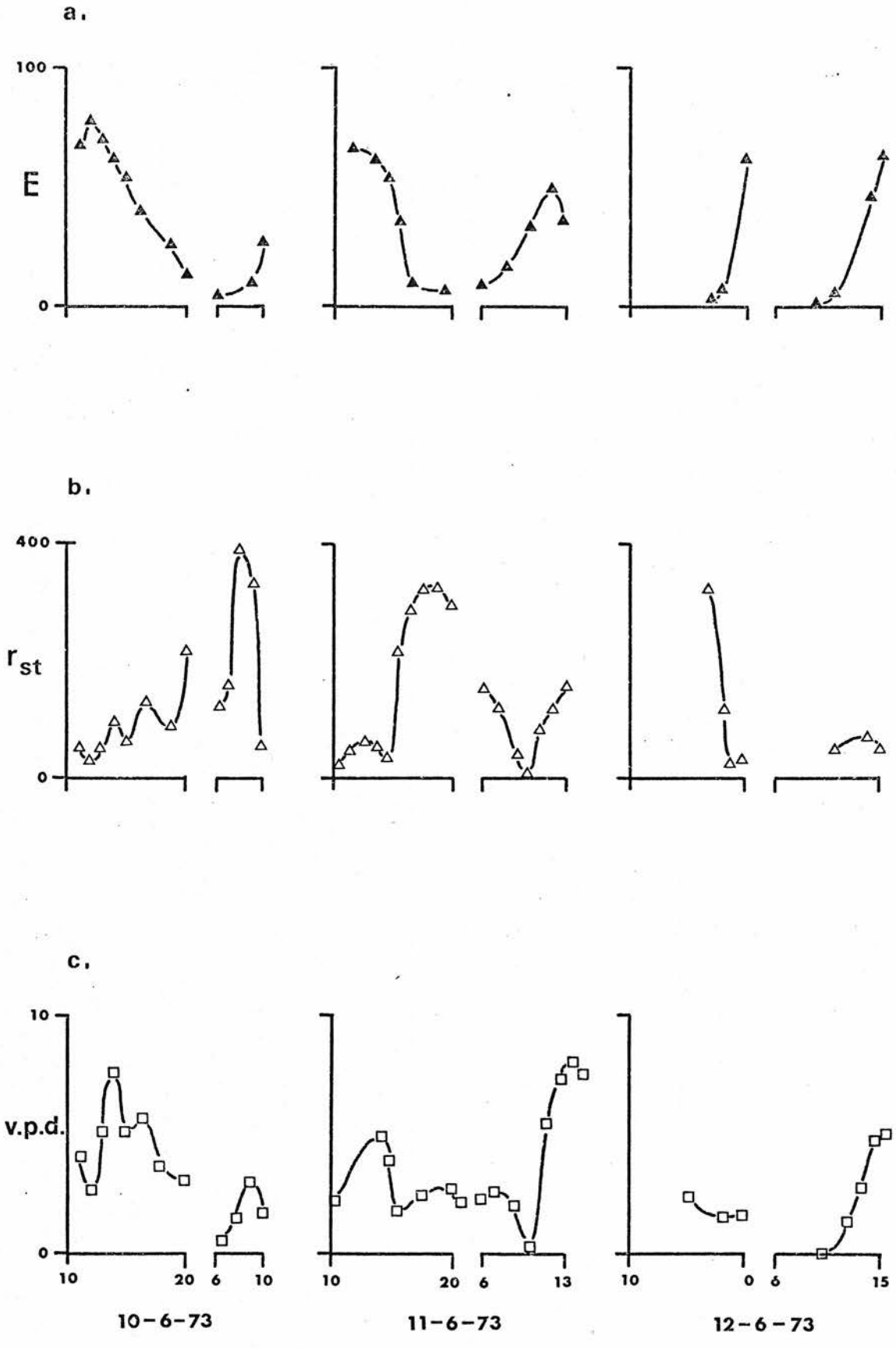


FIG. 1.13 Swath wilting on 10, 11 and 12 June 1973, H K

a. Swath evaporation rate, E, 10^{-6} kg H_2O m^{-2} s^{-1}

b. Swath resistance to evaporation, r_{st}, s m^{-1}

c. Ambient vapour pressure deficit, v.p.d., mb

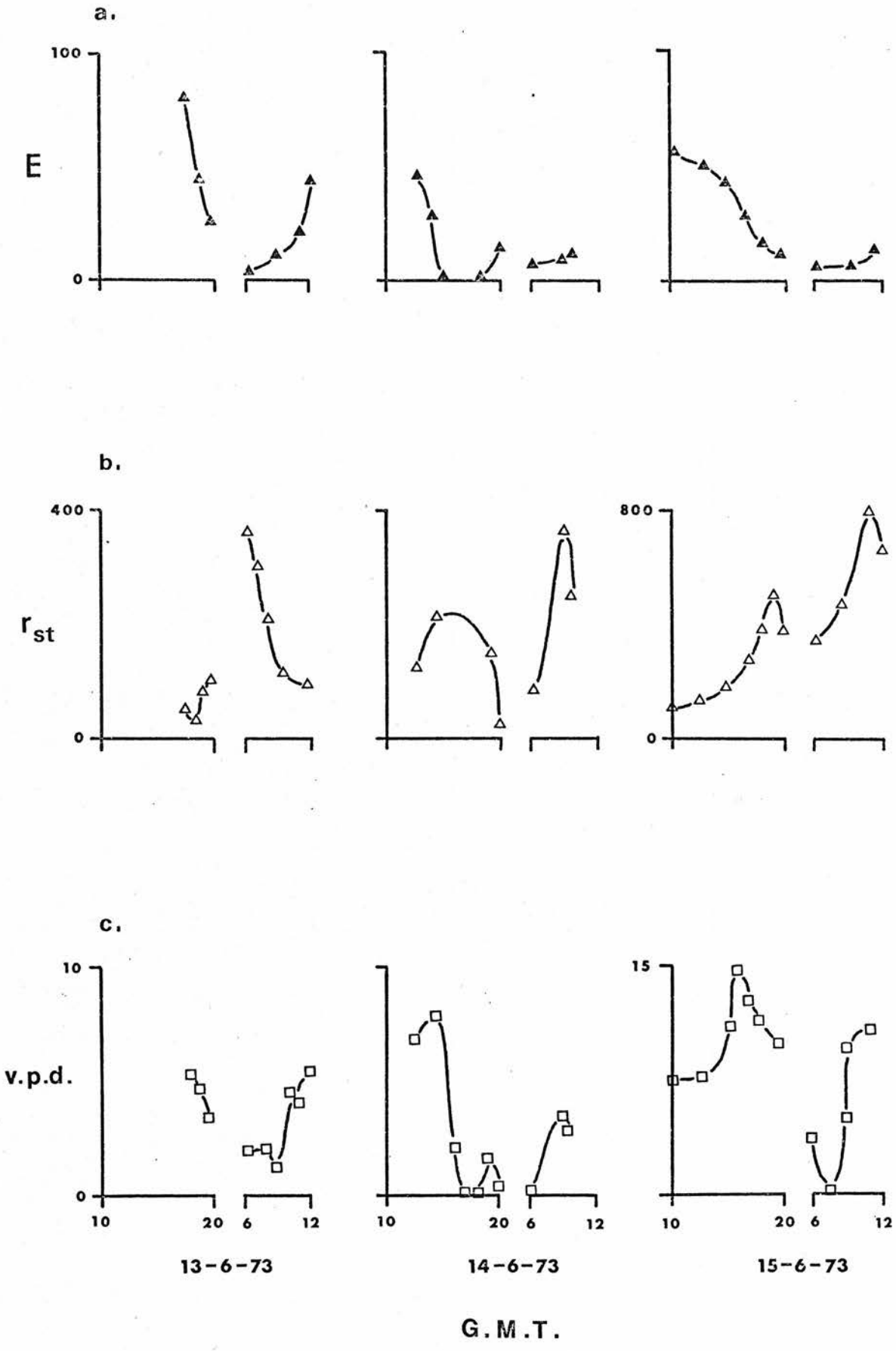


FIG. 1.14 Swath wilting on 13, 14 and 15 June 1973, L F

a. Swath evaporation rate, E, 10^{-6} kg H_2O m^{-2} s^{-1}

b. Swath resistance to evaporation, r_{st}, s m^{-1}

c. Ambient vapour pressure deficit, v.p.d., mb

after harvest. Thereafter E fell progressively during the course of the first day reaching a low level by 20.00 h. On the second day, E began to increase slowly after 06.00 h and rapidly after 09.00 h to a peak around noon (11 June, Fig. 1.13; August, Fig. 1.19); then E decreased progressively reaching a low level before 18.00 h (July, Fig. 1.16; August, Fig. 1.19). On the third day of wilting for silage, a high evaporation rate was again recorded at 09.00 h but thereafter fell steeply to a minimum at noon (July, Fig. 1.16).

Under poor weather conditions (12, 14 June, Figs. 1.13, 1.14) swath evaporation rate was occasionally reduced to zero, but increased rapidly in intervening periods reaching high levels comparable with the values recorded during the initial stages of evaporation on fine days.

b. Swath resistance to evaporation, r_{st}

In general, the swath resistance to evaporation curves are the inverse of the swath evaporation rate curves. r_{st} tended to increase during the course of each day (e.g. 15 June, Fig. 1.14) and throughout the length of the wilting period (e.g. July, Fig. 1.16). However r_{st} sometimes decreased during the early morning (e.g. 13 June, Fig. 1.14) or the late evening (e.g. 12 June, Fig. 1.13). Maximum r_{st} values of between 300 and 400 s m^{-1} were recorded during the 24-h wilting periods in June, apart from a high value of 800 s m^{-1} on June 15, (Fig. 1.14); maximum r_{st} values of 700 s m^{-1} and 1800 s m^{-1} occurred during the 48 h wilting periods in July and August (Figs. 1.16 and 1.19) when silage-making and hay-making respectively. Minimum r_{st} values of between 50 and 100 s m^{-1} were recorded, usually during the initial or final stages of evaporation each day (e.g. 10 June, Fig. 1.13, 13 June, Fig. 1.14). Under poor wilting conditions, low values of r_{st} were sometimes found (12 June, Fig. 1.13), but values comparable with those of fine days were also recorded (14 June, Fig. 1.14).

c. Climatological resistance, r_I

The climatological resistance had minimum values of about 50 s m^{-1} recorded during the period of 6 - 9 h straddling noon, rising to maximum values of about 200 s m^{-1} and 400 s m^{-1} at sunset (21.00 h) on each wilting day in July and August (Figs. 1.16 and 1.19) respectively.

Figs. 1.17 and 1.20 show a. the net radiation, R_n and the latent heat flux, λE on the swath and b. the vapour pressure deficit, vpd, of the air and the swath surface, during each wilting day in July and August.

a. Net radiation, R_n and latent heat flux, λE

The net radiation on the swath showed a maximum between noon and 15.00 h, and fell below zero before 07.00 h and after 21.00 h on each wilting day. Maximum R_n values never exceeded 400 W m^{-2}. Daily totals of R_n were 2328 and 1522 W m^{-2} on 24 July and 16 August (Fig. 1.17, 1.20) respectively.

The latent heat flux, λE, showed a maximum during the early stages of evaporation and thereafter fell progressively on each wilting day. Maximum λE values were generally less than half of the corresponding R_n values, never exceeding 200 W m^{-2}. The fall-off in latent heat flux generally occurred before the afternoon decrease in R_n, being particularly marked on the second and third days of wilting. Latent heat flux was zero before 09.00 h and after 21.00 h each day.

b. Vapour pressure deficit, vpd, of the air and the swath surface

The ambient vpd was generally low (<3 mb) overnight between 21.00 h and 06.00 h, then increased rapidly after 09.00 h reaching high

values of about 8 to 10 mb by noon on fine days. The ambient vpd remained high during the afternoon then fell rapidly after 18.00 h to the low night level (e.g. 10, 13 June, 24 July, 16 August; Figs. 1.13, 1.14, 1.17, 1.20). Under favourable weather conditions, the swath surface vpd followed a similar pattern except that the swath night values were lower than the ambient night values, and the swath afternoon maxima were greater than the ambient maxima (July and August; Figs. 1.17 and 1.20).

Under poor weather conditions, the ambient vpd was variable and irregular showing periods of zero vpd and also values comparable with fine days (June 12, 14; Figs. 1.13, 1.14).

Although the diurnal R_n and vpd patterns were similar, the swath latent heat flux decreased during the afternoon before the vpd values decreased (e.g. 24 July, 16 August; Figs. 1.17, 1.20).

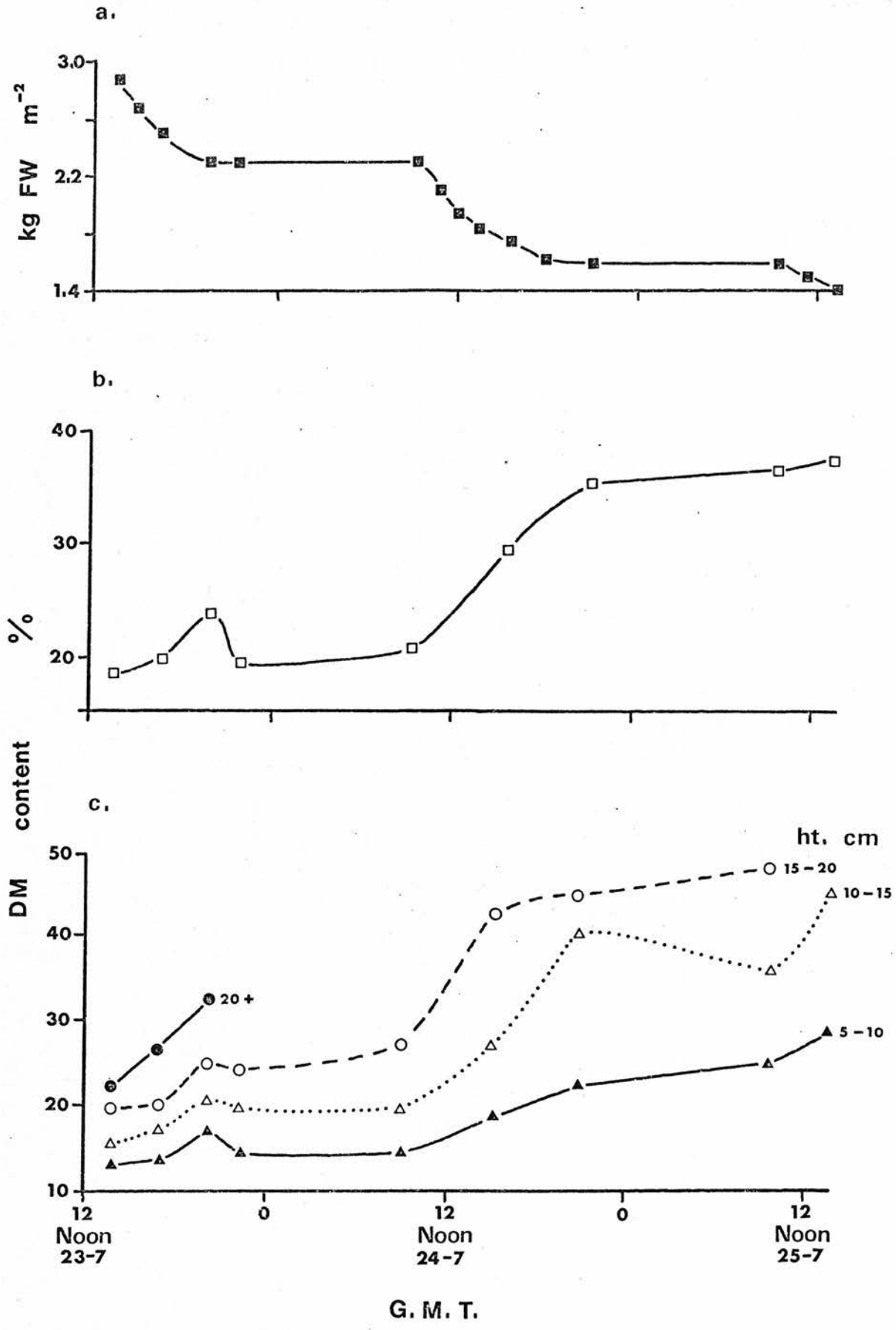
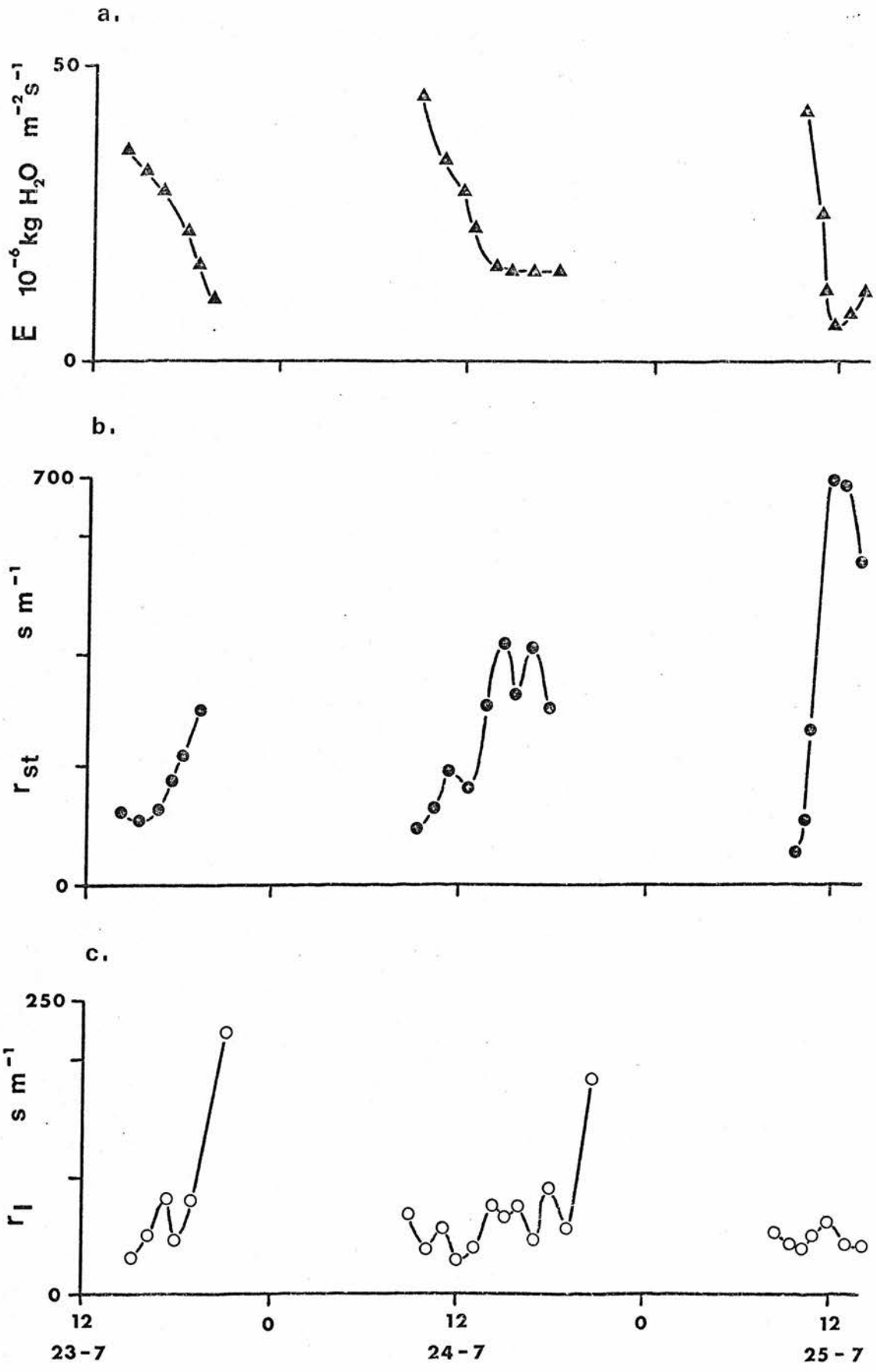


FIG. 1.15 Swath wilting on 23 - 25 July 1973, H K

a. Swath F W decline curve
b. Swath mean D M content
c. Swath stratified D M content

FIG. 1.16 Swath wilting on 23 - 25 July 1973, H K



a. Swath evaporation rate, E.

b. Swath resistance to evaporation, r_{st}

c. Climatological resistance, r_l

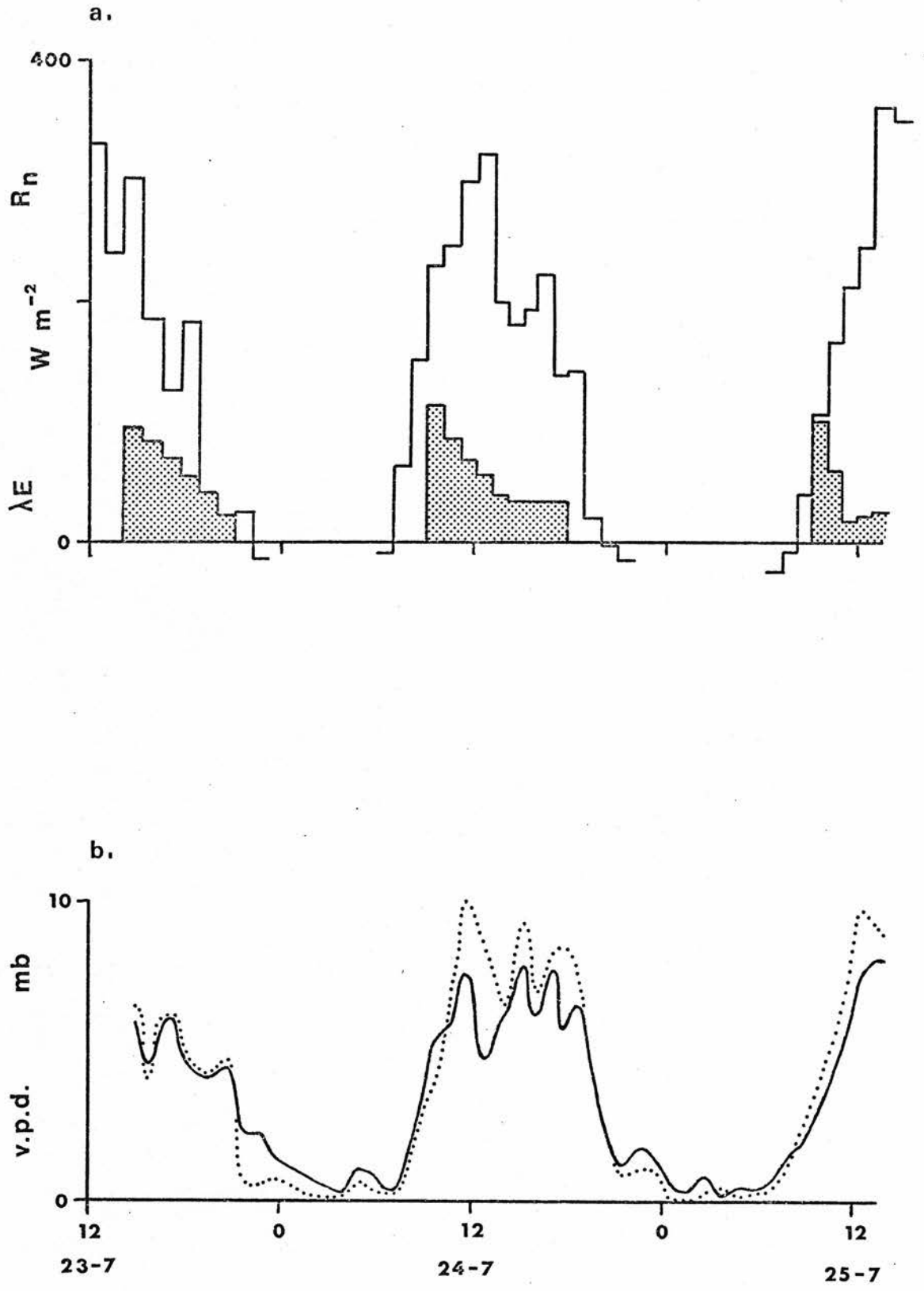


FIG. 1.17 Swath wilting on 23 - 25 July 1973, H K

a. Swath net radiation, R_n, and latent heat flux, λE; blank histogram, R_n; stippled histogram, λE

b. Swath and ambient vapour pressure deficit, v.p.d.; dotted line, swath; solid line, ambient

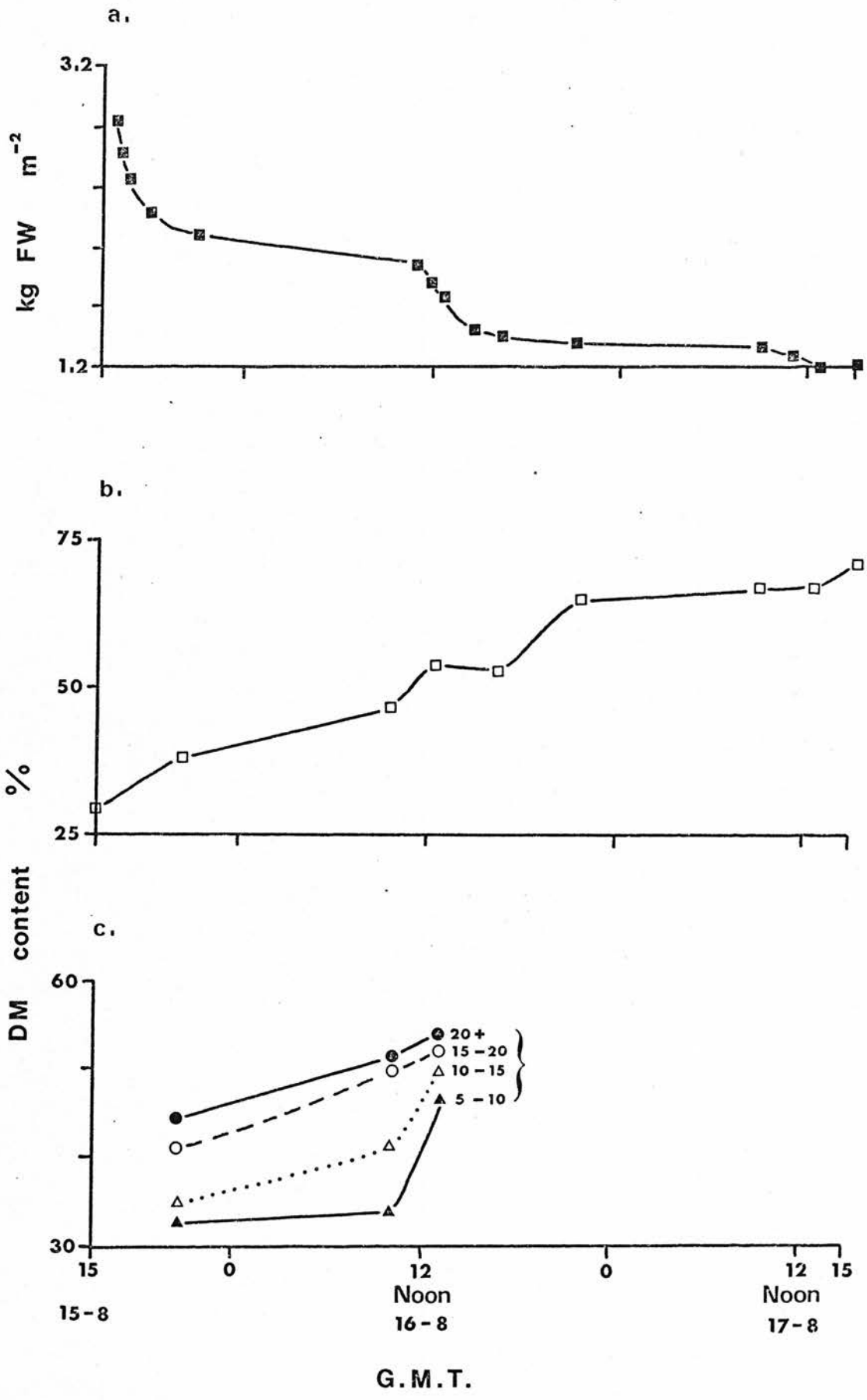
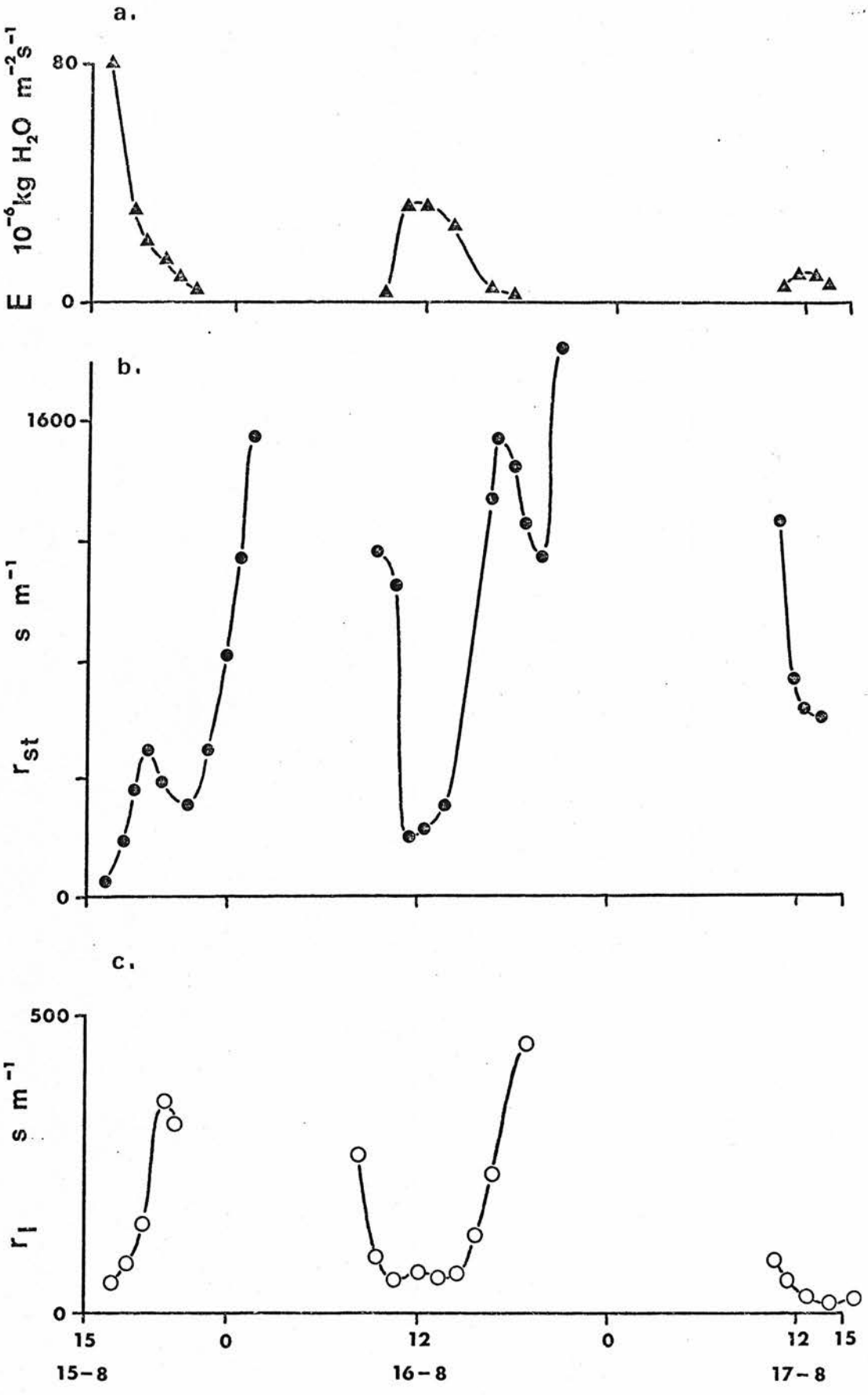


FIG. 1.18 Swath wilting on 15 - 17 August 1973, L F

a. Swath F W decline curve
b. Swath mean D M content
c. Swath stratified D M content

FIG. 1.19 Swath wilting on 15 - 17 August 1973, L F



a. Swath evaporation rate, E.

b. Swath resistance to evaporation, r_{st}

c. Climatological resistance, r_l

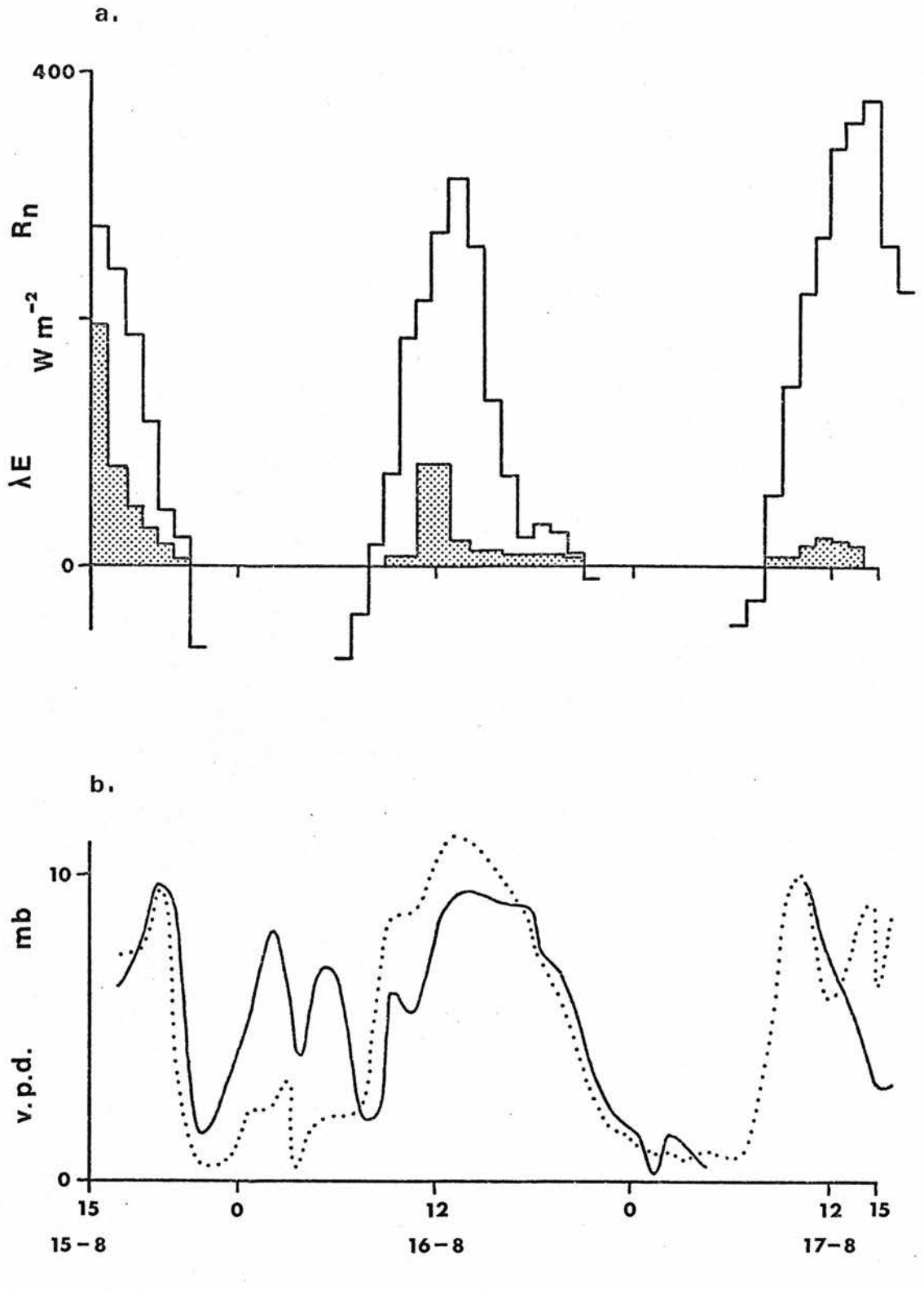


FIG. 1.20 Swath wilting on 15 - 17 August 1973, L F

a. Swath net radiation, R_n, and latent heat flux, λE; blank histogram, R_n; stippled histogram, λE

b. Swath and ambient vapour pressure deficit, v.p.d.; dotted line, swath; solid line, ambient

SECTION 1: DISCUSSION

PILOT PROGRAMME 1972

Considerable differences were found in swath structure according to the harvesting method used, and also from one region to another of the same swath, causing local differences in temperature, humidity and light penetration gradients. Such variations in microclimate would be expected to result in differences in drying rates and associated physiological and biochemical processes in the plant material.

The swath-air relative humidity gradient represents the water vapour concentration gradient which in part determines the swath evaporation rate and resistances to evaporation (see pp. 44-45). The large gradient found immediately after harvesting indicates a high potential flux of water vapour from the fresh plant material. So rapid evaporation may be expected following harvest if environmental conditions (swath radiation balance; windspeed, etc.) are suitable. The reduction in gradient with time after harvesting indicates increasing difficulty in evaporating water from the swath. The fact that conditions were near saturating inside the swath at ground level suggests that water vapour transfer is limited to slow molecular diffusion inside the swath, at least in the early stages of wilting.

The great reduction in light intensity within the first few cm of the swath surface indicates that photosynthetic activity is restricted to the surface layers, if it occurs at all, after harvest, as suggested by Pizarro and James (1972).

Small swaths may be expected to have the fastest drying rates since the path-length of molecular diffusion including the boundary layer must be reduced and concentration gradients must be steeper. Those pilot programme results strongly suggested that swath structure causes local differences in microclimate and associated drying rates.

FIELD PROGRAMME 1973

Swath structure

The characteristic bimodal shape of the mown grass swath indicates that considerable differences in microclimate and drying rates must also occur across one swath: the edges and central trough would be expected to dry faster than the main thick swath mass.

The large amount of spreading and subsidence recorded during wilting suggests that the structure of mown swaths was not resistant to the effects of wind, rain, etc. Klinner and Harris (1972) stated that the mechanical treatment of the crop at harvest should aim to form wide, uniformly dense, well-set-up swaths of large surface area and high resistance to settling. The 4-drum rotary mower clearly failed to do this.

Previous work cited by Wood *et al.* (1972) showed that crop treatment at and after cutting can significantly affect drying rate, yield and quality of the harvested crop. Wood *et al.* (1972) found that conditioned crops lost moisture more rapidly than unconditioned ones but also absorbed it more rapidly during periods of rain. A rotary mower did little to increase crop drying rate compared with the fingerbar control, but a crimper was very effective.

Swath structure is evidently of great importance. In order to obtain information specifically on the effects of swath density and shape, Wood *et al.* (1972) cut duplicate sets of plots with six different machines twice in one year. One set of plots was spread evenly immediately after cutting and in the other the swaths were preserved as long as possible. On average in the two crops, the control (fingerbar) derived the greatest benefit from being spread during the wilting period. Up to 40% D M, the 4-drum rotary mown crops dried more quickly after spreading by 10 - 15 h, but up to 65% D M there was no significant improvement in drying after spreading. In conclusion, the conformation of the swaths left by rotary mowers is critical for the early stages of drying but unimportant in the later stages. There may be a D M threshold above which drying is limited by internal plant factors primarily (Thaine 1967). Artificially spread Italian ryegrass swaths also showed greater moisture uptake than intact swaths due to either a limited conditioning effect or the greater crop surface area exposed (Wood *et al.* 1972).

In the light of these studies, it is suggested that natural spreading and subsidence during wilting may have large effects on drying rates, leading to significant improvements in the early stages under good drying conditions, but greater absorption of water and greater losses under poor drying conditions. Spreading and subsidence are often accelerated by adverse weather conditions. Klinner and Harris' (1972) recommendations for achieving swaths resistant to settling could be very important in this context. In trials with experimental machines, Klinner and Harris (1972) aimed to give a diffential treatment directed primarily at the lower parts of the crop where stems are thickest and most moisture is present; upper parts of

the crop, where damage can cause losses, were treated more gently. The crop was mixed so that stemmy and leafy parts were exposed to the atmosphere. They found that the swaths produced by the experimental machines settled much less quickly than those produced by commercial machines, and the swaths dried to a greater depth.

Swath microclimate

Information about the response of the swath to its environment can be derived from the nature of its microclimate - the regime of radiation, temperature, humidity, carbon dioxide and wind between the top of the swath and the soil surface. Many measurements of microclimate have been published for different types of growing vegetation ranging from forests (Stewart and Thom 1973) to cereal crops (Elderen *et al.* 1972; Catsky *et al.*1973), but there is very little information about cut crops.

Salient features of swath microclimate are illustrated in Figs. 1.2 - 1.6 and Table 1.5 where the series of profiles represent conditions within the swath of height $h = 20 - 25$ cm with most of the cut plant material between h/2 and ground level.

Temperature . The slope of the temperature profiles is a direct consequence of the heat balance of the system and indicates the direction of heat flow at each level (Monteith 1973). There was a temperature maximum at the swath surface during the day (between 09.00 h and 18.00 h) under favourable weather conditions, indicating an upward flux of sensible heat from the swath surface into the air and a downward flux from the swath surface to ground level. The less marked temperature minimum at the swath surface overnight indicated the reversal

of heat flow patterns. The complete lack of temperature gradients under adverse weather conditions suggested that heat flow was zero and the swath was at thermal equilibrium with the environment. The direction and magnitude of heat flow affects the water balance of the swath:

Vapour pressure. The shape of the vapour pressure and relative humidity profiles, like the temperature profiles, is a consequence of the heat and water balance of the system and reveals the direction of water vapour flux (Monteith 1973). During the day the vapour pressure usually decreases from the soil surface upwards. The decrease in vapour pressure or relative humidity from the swath surface upwards during the morning under favourable weather conditions indicated water vapour flux into the air. The reduction in water vapour concentration gradient during the afternoon suggested that the potential for evaporation from the swath decreased in the course of the day, to zero after 18.00 h. The minimum vapour pressure at the swath surface in the early hours of the morning occurred when dew was forming. The small and variable nature of the relative humidity gradients under adverse weather conditions indicated that the potential for evaporation and actual water vapour flux was low.

Windspeed. Windspeed is a logarithmic function of height above the zero plane which is assumed to be at the swath surface (Thom 1973). This assumption may be invalid for open, crimped swaths but is reasonable for dense, compact, mown swaths. Gaseous transfer inside the swath must therefore be limited to simple diffusion and is independent of windspeed. Gaseous transfer through the swath boundary layer (p.44)

also occurs by diffusion. The size of this boundary layer is highly dependent on swath structure and windspeed; for example, an increase in windspeed would reduce the height of the boundary layer, thereby decreasing the length of the vapour diffusion path, increasing the vapour concentration gradient, and increasing the upward water vapour flux. Windspeed may be expected to affect swath evaporation rate in this way.

Radiation. Monsi and Saeki (1953) suggested that the attenuation of light in plant stands may be described by an expression analagous to Beer's Law and it has been shown that the extinction of net radiation can be similarly described (e.g. Begg *et al.* 1964). Solar radiation is absorbed rapidly by foliage and the relation between irradiance and cumulative leaf area is almost exponential (Monteith 1973). Because leaves absorb visible light strongly but transmit long wave radiation between 0.7 and 1 μm, visible radiation is attenuated more rapidly than solar radiation, and beneath a mature crop is often reduced to 5 - 10% of the external irradiance. The leaf area index needed to produce 95% attenuation of visible radiation is c. 3 for stands with horizontal leaves (Monteith 1973). Measurements of swath visible radiation and total solar radiation profiles were in agreement with these results for standing crops. Rapid extinction occurred with successive accumulation of layers of horizontal leaves. The light inside the swath was both decreased in amount and changed in quality; absorption of photosynthetically active radiation occurred within the surface layers, so that persistance of photosynthetic activity in all but the top few cm of the swath would be most unlikely (Pizarro and James 1972).

The albedo or reflection coefficient of the swath depends on its geometry and on the angle of the sun as well as on the radiative properties of the component leaves. In general maximum values recorded over relatively smooth surfaces such as closely cut lawns are near to 0.25 (Monteith 1973). The high values of c. 0.37 found for the swath surface probably resulted from the large amount of reflection from the close parallel leaf arrangement in horizontal layers, together with increasing reflection from leaves bleached with progressive drying. Albedo values for the stubble, of vertical more widely-spaced components, were more typical.

The shape of profiles within a canopy can be used to determine the distribution of sources and sinks of heat, water vapour, etc. The interpretation of profiles in this way is valid only for horizontally homogeneous stands of vegetation in which the processes of exchange are dominated by vertical fluxes. In small swaths, microclimates may be determined by the rate at which horizontal fluxes change with distance from the edge as well as the rate at which vertical fluxes change with height (Monteith 1973). Horizontal profiles were not measured but may resemble the vertical profiles in the hemispherical bimodal swaths.

Swath Drying

The changes in swath F W and D M content in the course of wilting may be explained in terms of the swath microclimate and intrinsic plant factors. The changes which occur depend directly on the swath evaporation rate or latent heat flux, i.e. the response of the swath to its potential evaporation environment.

λE is a function of the swath net radiation or available energy but the diurnal pattern of λE did not correspond closely to that of R_n.

It has been shown theoretically that λE is the ratio of ambient vpd, i.e. the evaporating power of the air, to the swath resistance to evaporation (pp. 44-45). Therefore, the pattern of changes in swath latent heat flux may be analysed in terms of changes in these two components.

The diurnal trends of λE and ambient vpd were not in close agreement, so that changes in the evaporating power of the air alone cannot account for the changes in λE. Hence, microclimate factors, such as vpd and R_n, cannot in isolation explain the observed swath evaporation pattern.

Plant factors are also involved and may be most important in controlling evaporation rates. In general, the pattern of changes in swath resistance was the inverse of the changes in swath evaporation rate, suggesting an effect of internal swath factors.

The diurnal pattern of swath latent heat flux, on a fine day of good drying conditions, may be interpreted as follows:-

Between sun-rise and 09.00 h on each wilting day, available energy is relatively low (c. 0.25 of midday values) and is used entirely for sensible heating of the swath mass. The ambient vpd is zero or very low. Swath resistance is also low. Therefore, during this early phase, swath evaporation is limited by the poor evaporating power of the air.

Between 09.00 and 12.00 h, available energy increases to maximum values, and a high proportion (c. 0.5) of this available energy is used for latent heat giving a high swath evaporation rate which includes the

evaporation of external surface water (dew and/or rain) and internal plant water. During this phase, vpd increases to maximum values. Swath resistence is low but begins to increase slowly. Therefore, during the morning, the evaporating power of the atmosphere is sufficiently high and the swath resistance sufficiently low to produce rapid rates of water loss.

Between 12.00 and 18.00 h, the available energy decreases and the proportion used for latent heat of evaporation also decreases markedly indicating a shift in the swath energy balance in favour of sensible heat. The vpd of the air remains high. The swath resistance increases sharply. Therefore, during this phase, swath evaporation rate is reduced by the decrease in available energy and the increase in swath resistance.

Between 18.00 h and sunset, available energy is low and swath evaporation is kept near zero largely because of the very high swath resistances.

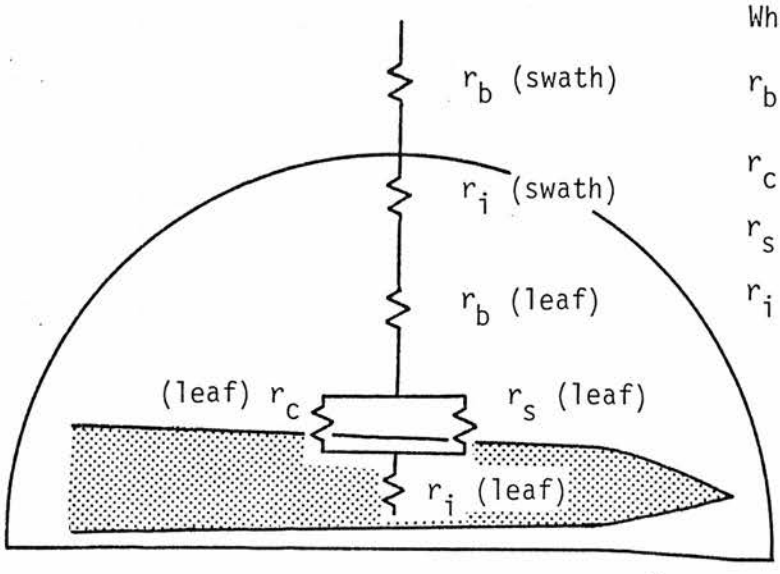
The pattern of latent heat flux throughout the wilting period may be interpreted in a similar way. The available energy and the vpd curves follow the usual diurnal patterns, but the swath resistance shows a marked and progressive increase reaching successively higher maxima on each wilting day (e.g. Fig. 1.16). This increase in resistance is sufficient to depress the swath evaporation rate in the converse manner.

Thus the swath evaporation rate during wilting depends upon the balance of environmental factors (R_n, vpd) and plant factors (r_{ST}). The swath resistance term, r_{ST}, in fact represents the sum of many resistance components at the levels of the individual leaf and the swath

mass. r_{ST} includes the sum of the boundary layer, stomatal, cuticular and internal resistances to water vapour transfer of all the individual leaves (Holmgren *et al.* 1965; Lewis 1972), and also the boundary layer and internal resistances of the swath itself. r_{ST} is likely to be low immediately after cutting since the important variable resistance due to the stomata (Jarvis and Slatyer 1970) is small until stomatal closure commences, and resistance to the evaporation of surface moisture liberated during harvesting is zero. r_{ST} is also low between sun-rise and 09.00 h on each wilting day because of zero resistance to the evaporation of surface moisture from dew or rain. Swath resistance is likely to increase in the course of each day and throughout the whole wilting period as a result of large increases in the stomatal, cuticular and internal resistances, and also the boundary layer resistances, as a result of desiccation.

The hypothetical resistance networks of a swath may be represented as follows:-

1. Immediately after harvest (open stomata)



Where,

r_b = boundary layer resistance

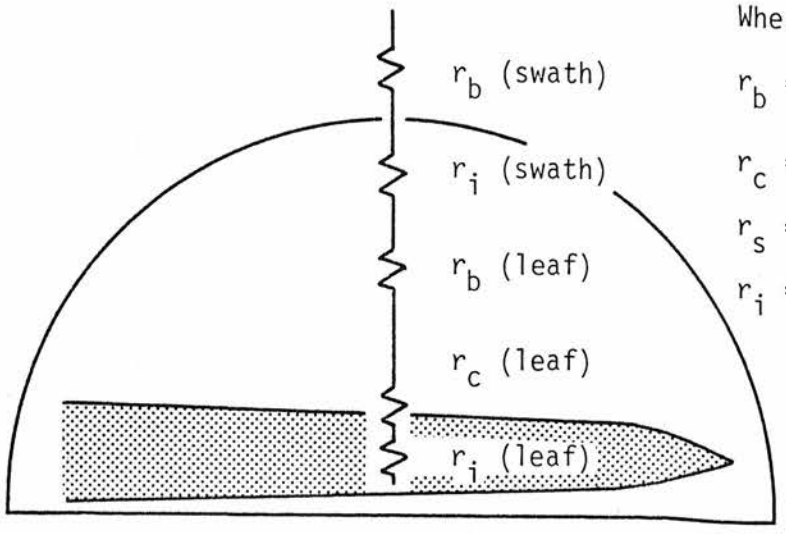
r_c = cuticular "

r_s = stomatal "

r_i = internal "

$$r_{ST} = r_b \text{ (swath)} + r_i \text{ (swath)} + \Sigma r_b \text{ (leaf)} + \Sigma \frac{1}{r_c} \text{ (leaf)} + \Sigma \frac{1}{r_s} \text{ (leaf)} + \Sigma r_i \text{ (leaf)}$$

2. After a period of Wilting (closed stomata)



Where,

r_b = boundary layer resistance

r_c = cuticular "

r_s = stomatal "

r_i = internal "

$$r_{ST} = r_b \text{ (swath)} + r_i \text{ (swath)} + \Sigma r_b \text{ (leaf)} + \Sigma r_c \text{ (leaf)} + \Sigma r_i \text{ (leaf)}$$

The relative importance of the leaf resistances, in particular r_c and r_i, probably increases with drying time as a result of desiccation effects on the plant cuticle, plant cell walls and membranes (Jarvis and Slatyer 1970; Lewis 1972).

These changes in the swath latent heat flux, brought about by alterations in the balance of vpd and swath resistance, produce the observed changes in swath F W and D M content. The swath stratified D M content reflects the microclimate gradients and the fundamental nature of the drying process, i.e. the diffusion of water vapour molecules from the saturated swath interior along a gradient of increasing vpd into the ambient air. This stratification is apparent immediately after harvesting, through unequal distribution of high M C stemmy plant material which is concentrated in the lower layers of the swath.

During the early phase of drying, the surface layers increase in D M content most rapidly, as a result of direct exposure to solar radiation providing the energy required for evaporation, and the relatively short resistance pathway through a few layers of leaves and the swath

boundary layer. The slow drying of the lower layers of the swath may be similarly explained by the low levels of available radiation, and the long and tortuous resistance pathway from the insides of individual leaves and stems, through the interior of the swath containing many successive layers of horizontal leaves, and thence into the ambient air.

As drying progresses, the D M content of the surface layer reaches a threshold level indicating that no further evaporation can occur because of high plant resistances. Then the D M content of the second layer continues to increase and approaches the D M threshold. Finally the D M content of the lowest layers increases slowly until the plant resistances also become limiting.

The swath D M at any stage of wilting is the mean of widely varying D M contents of the different layers. In order to provide rapid and uniform drying, special treatments are necessary, such as selective mechanical harvesting and conditioning followed by turning of the swath (Klinner and Harris 1972); steam treatment of the standing crop (Schukking 1972; Tetlow 1973); or possibly the use of chemicals to modify the cuticle structure (Thaine and Harris 1973), or to keep the stomata open after harvest (Turner 1970; Meidner 1974), thereby reducing the plant resistances to evaporation.

SECTION 2

CONTROLLED ENVIRONMENT STUDIES

SECTION 2: INTRODUCTION

In order to overcome problems of limited field equipment and restricted field techniques for detailed measurements, and the great variability of field conditions regarding both plant material and environmental factors, a supplementary programme of wilting experiments was carried out under controlled conditions. A controlled environnent plant growth cabinet was used for the cultivation of plant material and for experiments. Aspects of the physiology and biochemistry of wilting, in relation to plant and environmental factors, were investigated at a range of organisational levels, the intact swath, whole single leaves, leaf segments, plant cells and plant biochemical processes.

The experimental programme described in this section is divided into the following:-

Plant Material: nature and cultivation conditions.

Swath Simulation Studies.

Single Leaf Studies: plant physiological responses to cutting.

Leaf Segment Studies: changes in water saturation deficit, photosynthetic and respiratory capacity of plant tissue during wilting.

Stomatal Studies: nature and response to cutting.

Biochemistry of Wilting: timecourse of changes in leaf biochemical constituents during wilting.

SECTION 2: MATERIALS AND METHODS

PLANT MATERIAL

Lolium multiflorum Lam., R.v.P. var., a fast-growing Belgian variety of Italian ryegrass, was used as the experimental plant material in Section 2, apart from the Single Leaf Studies carried out in France where this variety was not available. The seed sample had a purity of 99.4% and a germination success of 87% (Official Seed Testing Station for Scotland report).

Lolium multiflorum Lam., Westervold Barenza var., which also has a very short vegetative cycle was used for the experiments carried out in France.

Cultivation conditions

The plant material was grown under controlled conditions in a plant growth cabinet (S.E. Scotland) and a growth room (N. France) and also under field conditions on a small experimental plot (S.E. Scotland) in order to ensure an all-year-round supply.

Growth cabinet. A Sherer controlled environment plant growth cabinet (Sherer-Gillett Co., Marshall, Michigan; model CEL-225-6) was used to raise ryegrass plants from seed. The seeds were sown in 30 x 60 cm plastic trays containing Levington compost, at a depth of 2 cm, using a 3 cm grid pattern of spacing. Germination and cultivation under controlled conditions produced a homogeneous population of plants in a reproducible manner. Illumination was provided by a bank of 8 fluorescent tubes (Lifeline, Sylvania, U.S.A., F48T12-CW-HO) giving a

radiant flux density of 120 W m^{-2} and illumination of 11,000 lumens m^{-2} at shelf level. A 16 h photoperiod from 05.00 h to 21.00 h was provided automatically by the light time switches. A synchronous temperature cycle of 15 ± 1° day and 10 ± 1° night temperature inside the cabinet was provided automatically by the temperature time switches. The air temperature was thermostatically controlled by means of heating coils and a refrigeration system. Continuous temperature recordings were made during the growing period and all growth cabinet experiments using a thermograph. Circulation of air of normal CO_2 concentration (0.03% or 300 µl l^{-1}) was provided by a fan intake and internal circulating fans, giving a complete change of air every 2 min. Relative humidity was controlled between the approximate limits of 60 and 70% by the automatic humidifying/dehumidifying system. These growth cabinet conditions were chosen to simulate early summer weather in S.E. Scotland. After germination, the plants were watered twice weekly. A balanced plant nutrient solution was used when they were more than six weeks old as a supplementary mineral supply.

Growth room. A controlled environment plant growth room was used to cultivate *L. multiflorum*, Westervold Barenza var., by hydroponic techniques (Prioul 1971). Radiant flux density was 100 W m^{-2} during a 12 h photoperiod; day and night temperatures were 18° and 13° respectively; relative humidity was 72 - 75%. Good homogeneous individuals are obtained in this fashion.

Plot. *L. multiflorum*, R.v.P. var., was grown on a 2 x 1 m experimental plot under field conditions to provide a larger supply of plant material. The plot was prepared by mixing Levington compost with the top soil. Seed was sown at a depth of c. 1 cm in mid-April and a top-dressing of

nitrochalk fertiliser was applied. In general, germination took 2 - 3 weeks and thereafter growth-rate was exponential for 7 - 8 weeks but decreased as the grass matured. The date of flowering was noted and subsequently the reproductive stage of the plants was expressed as percent ear emergence (percentage of tillers with flower-heads visible).

Harvesting technique

Plants cultivated in the growth cabinet and on the plot for use in Section 2 experiments (apart from the Single Leaf Studies) were scissors harvested at c. 09.00 h, cutting the stems at c. 2 cm above ground level. The cut plants were immediately transferred to a large polythene bag on ice in order to minimise water loss and enzymic changes. Disposable plastic gloves were worn in order to reduce microbial contamination. The harvested plant material was used for experiment as soon as possible.

Plants cultivated in the growth room were collected intact for use in the Single Leaf experiments.

Plant growth stage

For each experiment, the growth stage of the harvested plants was characterised by recording,

1. Age in weeks since germination.
2. Growth stage: young and vegetative (exponential growth) = Y V
   or mature (slower growth) = M V
   or reproductive = R, with % ear emergence = E E
   or senescent (net loss in D M) = S
3. Mean number of tillers and living leaves per plant.

SWATH SIMULATION STUDIES

Simulated swaths of harvested plant material, resembling field swaths as closely as possible, were used to measure swath evaporation rate and resistance to water loss under controlled environmental conditions. Changes in swath D M content were followed at the same time. The effect of plant factors, and of various mechanical treatments chosen to simulate harvesting and conditioning methods used in practice, was assessed.

Plant material

L. multiflorum Lam., R.v.P. var., was used to produce the swaths. For Expt. 1 (see below), the plant material was cultivated in controlled growth cabinet conditions, aged 8 weeks, and at the young and leafy stage of growth. For Expt. 2, plot-grown plant material aged 14 weeks and at 50% ear emergence was used.

Procedure

The plants were harvested as described above. Simulated swaths were produced from c. 50 g of fresh plant material, given various experimental treatments, then exposed to controlled wilting conditions in two experiments, as follows:-

Expt. 1. The effect of chopping on the swath drying pattern was investigated. Three simulated swaths were used, a control swath of whole intact plant leaves and stems, and two swaths of plant material pre-chopped into 5 cm and 2 cm sections respectively.

Expt. 2. The effect of crushing injury on the swath drying pattern was investigated, using a control swath as above, and two swaths of

plant material subjected to mild and severe crushing treatments respectively. A mild 'crimped' simulation treatment was produced by bending the plant material, thereby breaking the epidermis at c. 5 cm intervals and liberating a little sap. A severe 'crushed' treatment was produced by hammering the plant material at c. 2 cm intervals causing cell breakage and much sap liberation.

The 50 g samples of control or treated plant material were transferred onto pre-weighed wire gauze trays. The plant material was distributed evenly over the tray area giving a final depth of c. 10 cm. All swaths had similar weight:area ratios irrespective of the treatment.

Wilting was performed under controlled environmental conditions representative of summer weather, in the growth cabinet. A 48 h wilting period was used in both experiments. The environmental conditions were monitored continuously; swath temperature was recorded regularly using the sensitive thermistor of the Light/ Temperature Meter.

Swath F W decline was followed by weighing each tray at intervals, 1 - 3 hourly during the early stages to 6 - 12 hourly intervals during the later stages of the wilting period. Swath evaporation rates were derived from these F W decline curves (p. 73).

Swath D M content was determined concurrently, by sampling from duplicate swaths prepared in identical fashion and wilted alongside the swaths used for evaporation rate determination.

Swath resistance to evaporation throughout the wilting period was derived from the evaporation rate and vapour pressure deficit, as for the field experiments (p.45).

Thus the drying pattern of each swath type under controlled conditions may be compared with that of field swaths under variable weather conditions in Section 1.

SINGLE LEAF STUDIES

Plant physiological responses to cutting. a. Preliminary studies

A preliminary investigation of the changes in leaf transpiration rates and leaf resistances to water loss after cutting was carried out using a simple quick-weighing method on single leaves, leaf pieces and filter paper leaf replicas (Hygen 1951, Gaastra 1959, Baron 1967). From the data obtained it is possible to calculate the transpiration rate (T), leaf boundary layer resistance (r_a or r_b) and leaf transpirational resistances, the cuticular resistance (r_c) and the stomatal resistance (r_s or r_{st}), to water loss after cutting (Gaastra 1959, Milthorpe 1961, Jarvis 1963, Holmgren *et al.* 1965, Lewis 1972). The decline in fresh weight (F W) of the leaf after excision reflects the leaf transpiration behaviour and follows a characteristic pattern from which it is possible to infer the nature of stomatal control of transpiration and the point of stomatal closure (Hygen 1951, Baron 1967).

Plant material

Lolium multiflorum Lam., R.v.P. var., was cultivated in the growth cabinet, aged 8 weeks and at the young and leafy stage of growth. Both the youngest fully expanded leaf (Type a, Fig. M.5; L1) and the second youngest leaf (L2) on a tiller were used.

Procedure

Two experiments, designed to measure leaf transpiration rate and resistances under various conditions, were carried out as follows:-

Preliminary Expt.: Measurements were made on whole leaves and their replicas under laboratory conditions of natural daylight, c. 20° C and c. 70% relative humidity.

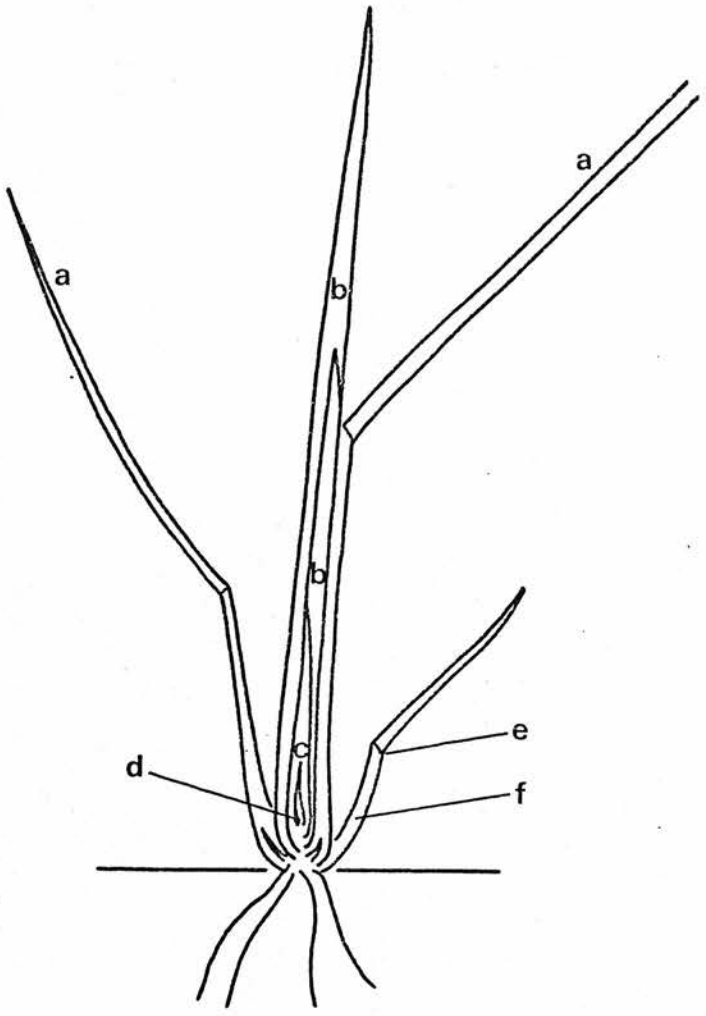


FIG. M.5 Diagram of the growth of a grass tiller

a = fully expanded leaf laminae

b = expanding leaves visible

c = expanding leaves enclosed

d = shoot apex

e = ligule

f = leaf sheath

Main Expt.: In this experiment, designed to relate to the Swath Simulation Studies, measurements were made on whole leaves, 5 cm and 2 cm leaf segments and their replicas under controlled environmental conditions in the growth cabinet. Irradiance was 120 W m^{-2}, air temperature 20° C and relative humidity c. 75%.

In both experiments, ryegrass plants were given a pretreatment in order to eliminate water saturation deficits and ensure stomatal opening. A tray of *L. multiflorum* plants was thoroughly watered and kept in darkness and high humidity (inside a large polythene bag) for several hours to optimise saturation of the leaves, and then exposed to high irradiance for 30 min after which the stomata would be fully open.

After the half-hour equilibration period, a healthy fully expanded (type a, Fig. M.5) leaf was excised at the ligule, and rapidly suspended using a loop of thin wire from the arm of an accurate analytical balance. The leaf weight to the nearest 0.1 mg was recorded immediately giving the initial leaf F W at zero time (F W_o). Then the leaf was rapidly placed, ridged adaxial surface with stomata uppermost, on a horizontal rack beneath the light source. It was reweighed at 2 min intervals and replaced on the rack between all weighings, continuing the procedure for some 40 min until the transpiration rate declined to a steady low level. The leaf and air temperatures were recorded at regular intervals throughout the experiment using the sensitive thermistor of the Light/Temperature Meter.

The evaporation rates of filter paper replicas of all leaves and leaf segments were determined under the same environmental conditions, as follows. An exact replica of each leaf was cut out from filter paper

and the area (one surface only) was measured using graph paper. The replica was saturated with distilled water, the excess drained off, then it was suspended from the balance arm and weighed (F W_o). The weight decline was followed as before, replacing the damp replica on the rack between weighings, continuing for about 10 min until a steady evaporation rate was obtained. The replica and air temperatures were recorded as before.

Calculation of results

1. Evaporation rate (E). The evaporation rate of each replica was obtained from its weight decline curve and area and expressed in kg m^{-2} s^{-1}.

2. Leaf transpiration rate (T). If an illuminated leaf with saturated tissues is cut from a plant and hung up to dry, its weight decline curve shows three distinct phases due to the stomatal control of transpiration (Hygen 1951, Baron 1967, Bannister 1964, Lewis 1971):

Phase I, the 'stomatal phase', of rapid weight decline immediately after cutting due to water loss through the stomata and upper and lower cuticles;

Phase II, the 'closing phase', of changing rates during stomatal closure;

Phase III, the 'cuticular phase', of upper and lower cuticular water loss only, producing a slow steady weight decline.

The leaf transpiration rate at the time of cutting may be safely obtained by extrapolation of the F W values during the first 3 min. This method is useful for accurate Phase I T measurements. Phase I and Phase III transpiration rates were derived from their respective parts of the weight decline curve, and leaf area, and expressed in kg m^{-2} s^{-1}.

3. Leaf boundary layer resistance (r_a or r_b). The leaf boundary layer is a thin skin of air surrounding the leaf through which gaseous transfer is limited by simple diffusion. It presents a resistance to evaporation which varies in magnitude with leaf dimensions and environmental parameters particularly windspeed (Gaastra 1959, Milthorpe 1961, Jarvis 1963, Holmgren *et al.* 1965, Lewis 1972). r_a may be calculated in several ways, the most usual method being from the evaporation rate of a saturated leaf replica at known temperature and water vapour concentration, where

$$E = \frac{\Delta\, c_{H_2O} \quad \text{(replica surface-air)}}{r_a}$$

and

$$r_{al} = r_{au} = 2\, r_a$$

The value of r_a for the leaf approximates closely to that of the saturated replica under the same conditions. However r_a probably has a relatively small effect on T and P_N as its value is typically low compared with the other components of the resistance network (Lewis 1971, 1972).

4. Transpirational resistances. The transpirational stream of water vapour from the evaporating sites inside the leaf, i.e. the mesophyll cell walls around the intercellular spaces and the substomatal cavity, through the stomata and cuticle into the ambient air encounters additional resistances, r_s and r_c respectively, which are in parallel.

Total leaf resistance, R, may be calculated from the transpiration rate of the leaf at known temperature and water vapour concentration,

$$T = \frac{\Delta\ c_{H_2O}\ \text{(leaf-air)}}{R}$$

R incorporates r_a, and all the internal leaf resistances up to the mesophyll cell walls sometimes collectively referred to as r_s (Chartier *et al.* 1970, Prioul 1971, Lewis 1972). When used in this sense, r_s may be derived directly since

$$R = r_a + r_s$$

However, strictly speaking, values for the cuticular, intercellular and true stomatal (pore) resistance should be calculated separately.

The cuticular resistance, r_c, may be obtained from the leaf cuticular transpiration rate (Phase III), and the known values of r_a, leaf temperature and water vapour gradient,

$$T_l = \frac{\Delta\ c_{H_2O}\ \text{(leaf-air)}}{R_l}$$

and as the lower surface (l) r_a and r_c are in series,

$$R_l = r_{al} + r_{cl}$$

$$\text{and } r_{cl} = r_{cu} = 2\ r_c \qquad \text{(Lewis 1972).}$$

The true stomatal resistance, r_s, may be obtained from the leaf stomatal transpiration rate (Phase I) and the known values of r_a, r_c, leaf temperature and water vapour gradient,

$$T_u = \frac{\Delta\ c_{H_2O}\ \text{(leaf-air)}}{R_u}$$

and as the upper surface (u) r_c and r_s are in parallel,

$$\frac{1}{R_u - r_{au}} = \frac{1}{r_s} + \frac{1}{r_{cu}}$$

It is rather unsatisfactory to assume that $r_{cu} = r_{cl}$ but since their values are so large in relation to r_s, the resultant error in r_s will not be particularly great.

Plant physiological responses to cutting. b. Detailed studies

A detailed investigation of the changes in leaf transpiration rates, net photosynthesis and leaf resistances to water loss after cutting was carried out using continuous infra-red gas analysis and dew-point measurements (Chartier *et al.* 1970, Prioul 1971).

Procedure

Four experiments were carried out, the first at the 'Laboratoire Structure et Métabolisme des Plantes', Université de Paris-Sud, Orsay, and the other three experiments at the 'Station de Bioclimatologie', I.N.R.A., Versailles in September and October 1973. For each experiment, a batch of 20 plants was selected randomly, each plant being removed carefully from the gravel with its roots intact and transferred to a flask containing nutrient solution.

For Expt. 1, the largest fully expanded leaf (Type a, Fig. M.5) of 6-week-old plants was used, this being the 5th leaf produced by the plant. For Expts. 2, 3 and 4, the largest fully expanded leaf of 4-week-old plants was used, this being the 3rd leaf produced on the main shoot. The leaves selected were in all cases healthy, well-developed and representative of the foliar apparatus.

Measurement of gas exchange during photosynthesis and transpiration was carried out by continuous infra-red gas analysis using the experimental system of Prioul (1971) at Orsay for Expt. 1, and Chartier (1970) at Versailles for Expts. 2, 3 and 4. The experimental system was similar to that of Bjorkman and Holmgren (1963), and Bierhuizen and Slatyer (1964), who developed the technique of I.R.G.A., and is shown diagrammatically, Fig. M.6

For Expts. 1 and 2, 12 leaves were used; for Expts. 3 and 4, 18 leaves were used. The leaves were placed very carefully in a horizontal position, adaxial surface uppermost, in the assimilation chamber, still remaining attached to their roots through their sheaths which passed across a gas-tight partition. The roots were placed in a small bath of nutrient solution to ensure continuous supply to the experimental leaves. The assimilation chambers both incorporated several ventilating fans, thermistors for air and leaf temperature measurements, a supporting grid of nylon threads for the leaves; that of Prioul (1971) had a miniature photocell, and a psychrometer with humidity thermocouple, modified after Bierhuizen and Slatyer (1964), in addition. The assimilation chambers were supplied with air of controlled CO_2 and water vapour concentration. The changes in composition of this air after passage over the leaves were recorded with a differential I.R.G.A., and also with two absolute I.R.G.A's in Expts. 2, 3 and 4. An electric dewpoint hygrometer was operated manually in Expt. 1 but automatically in the other three experiments, providing a measure of the water content of the chamber inflow and outflow airstream. The assimilation chamber was immersed in a thermostat water bath. Lighting was effected by a mercury vapour lamp (Philips HPL 2000 W, Expt. 1; Mazda MAF 1000 W, Expts. 2, 3 and 4) with paper and muslin screens used to produce light levels ranging from 34 - 168 W m^{-2}.

FIG. M.6 Infra-red gas analysis system for the measurement of CO_2 and water vapour exchange

IRGA abs. = Infra-red gas analyser for absolute measurements of CO_2 concentration

IRGA diff. = Infra-red gas analyser for differential measurements of CO_2 concentration

c_{CO_2} entry = CO_2 concentration at entry to assimilation chamber

c_{CO_2} exit = CO_2 concentration at exit from assimilation chamber

Δc_{CO_2} = CO_2 concentration gradient, entry/exit of assimilation chamber

Q = flow-meter; air-flow registered in $l\ h^{-1}$

Q_c = capillary flow-meter

Q_m = manometer flow-meter

T_d entry = dew-point temperature at entry to assimilation chamber

T_d exit = dew-point temperature at exit from assimilation chamber

T_l = leaf temperature in assimilation chamber

V_c = value for control of air-flow

V_p = value for pressure regulation

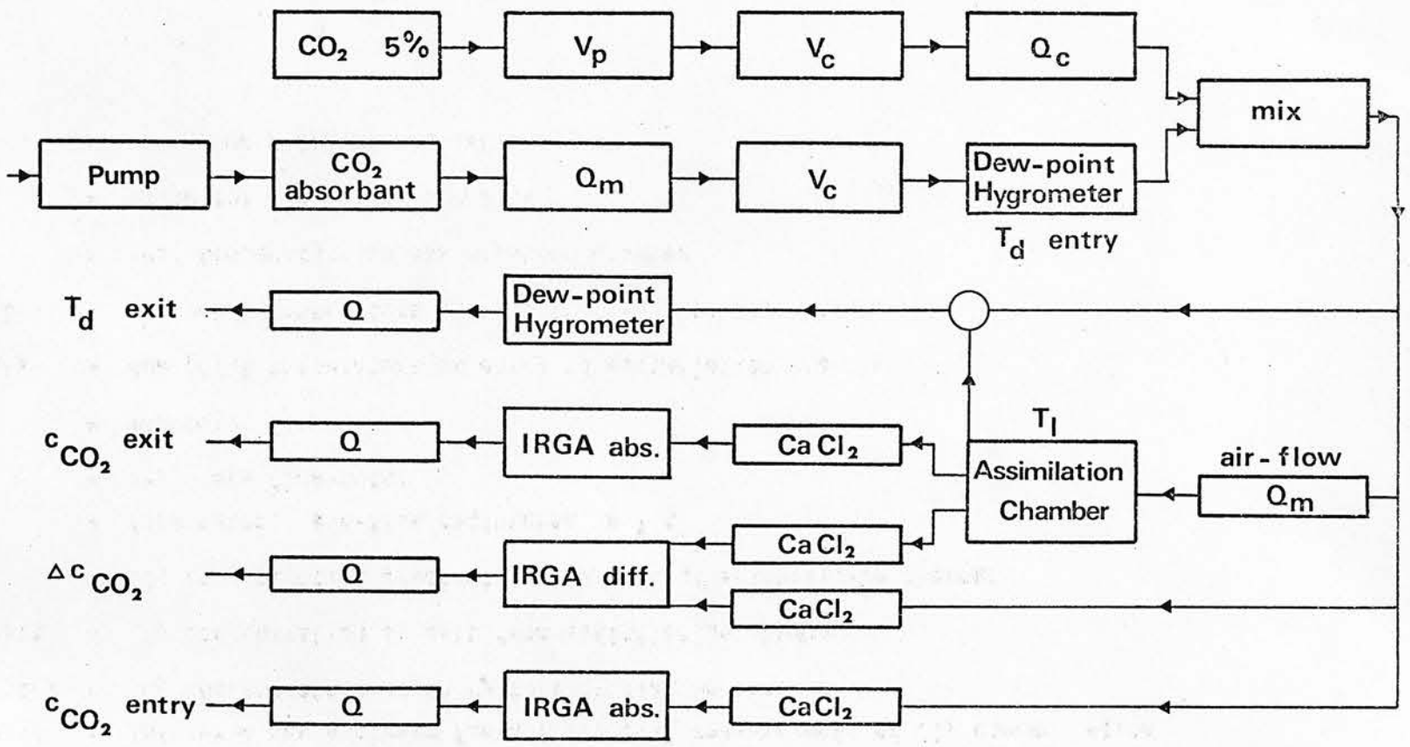


FIG. M.6 Infra-red gas analysis system for the measurement of CO_2 and water vapour exchange

A 16-channel mV recorder (Meci, France) gave continuous traces of CO_2 concentration, leaf and air temperatures, dew-point temperatures and light intensity, each reading being repeated at least once in a complete recording cycle and at 64 s intervals.

The plant leaves, still attached to their roots, were supplied with air of 300 µl l^{-1} CO_2 concentration and dew-point 18°. After a 1 h equilibration period in the dark, the leaves were brought to light saturation using successively higher light intensities of 34, 60, 94 and 126 W m^{-2} in Expt. 1; 100 and 168 W m^{-2} in Expt. 2. Beginning with the lowest intensity in each experiment, an equilibration period allowed the attainment of a plateau indicating steady states in P_N and T. Thus it was ascertained that the leaves were at light saturation at the highest irradiance. Therefore light and CO_2 were non-limiting.

When steady states in P_N and T were reached (under constant conditions of light and CO_2 saturation, leaf and air temperatures, humidity and air-flow) in the assimilation chamber, the leaves were rapidly severed from their roots by cutting through their sheaths just outside the assimilation chamber. Excision in this manner, removing the leaves' supply of water and nutrients, took c. 1 s. The resultant changes in P_N and T were monitored continuously for periods ranging from 2 h (Expt. 4) to 20 h (Expt. 2, overnight run).

Leaf D M content was determined after each experiment by oven-drying to constant weight at 105°. Total leaf area (one surface only, experimental leaves) was measured after each experiment by contact photography and planimetry (OTT graduated in mm^2).

Calculation of results

1. Net photosynthesis (P_N). The rate of net photosynthesis may be calculated from the change in CO_2 concentration (Δc_{CO_2}) of the air after passage through the assimilation chamber, the flow-rate and the leaf area. Δc_{CO_2} is obtained either directly, from the differential I.R.G.A. trace giving Δc_{CO_2} (measured); or indirectly, from the two absolute I.R.G.A. traces which represent the CO_2 concentrations of the chamber inflow and outflow air-streams, giving Δc_{CO_2} (calculated).

The chart readings in mV are converted into vpm (= ppm = $\mu l\ l^{-1}$) CO_2 from calibration curves previously prepared for each analyser using standard gas mixtures of known CO_2 concentration. Flow-rate was kept constant at 198 $l\ h^{-1}$. Leaf area was measured. A temperature-dependent conversion factor $\alpha(t)$ was used to convert P_N values into S.I. units. Now,

$$P_N = \frac{\Delta c_{CO_2} \times \text{flow-rate} \times \alpha(t) \quad 10^{-9} \text{ kg } CO_2\ m^{-2}\ s^{-1}}{\text{leaf area}}$$

2. Transpiration rate (T). The leaf transpiration rate, T, may be calculated from the change in water vapour concentration (Δc_{H_2O}) of the air after passage through the assimilation chamber, the flow-rate and the leaf area. Δc_{H_2O} is obtained from the dew-points of the chamber inflow and outflow air-streams. The chart temperature readings in mV are converted directly into degrees since the thermistors have temperature coefficients of 0.04 mV $^{o}C^{-1}$; then the dew-point temperatures are converted into the water vapour concentration of the air using standard tables. Hence the change in water vapour concentration may be calculated,

$$\Delta\, c_{H_2O}\,(T) = c_{H_2O}\,(\text{exit}) - c_{H_2O}\,(\text{entry}).$$

Flow-rate was constant and leaf area was measured. Now,

$$T = \frac{\Delta\, c_{H_2O}\,(T) \times \text{flow-rate} \quad 10^{-6} \text{ kg } H_2O\ m^{-2}\ s^{-1}}{\text{leaf area}}$$

3. Total leaf resistance to water vapour transfer $(r_a + r_s)_{H_2O}$.
In this case r_a is the leaf boundary layer resistance as before, and r_s is the resistance due to the stomata, the cuticle and the intercellular spaces up to the mesophyll cell walls (Chartier *et al.* 1970, Prioul 1971). The total leaf resistance may be calculated from the transpiration rate, the leaf temperature and the water vapour concentration gradient as before,

$$T = \frac{\Delta\, c_{H_2O}\,(R)}{(r_a + r_s)}$$

where $\Delta\, c_{H_2O}\,(R) = c_{H_2O}\,(\text{leaf}) - c_{H_2O}\,(\text{exit})$, and resistance units are $s\ m^{-1}$.

LEAF SEGMENT STUDIES

The physiology of wilting was studied in *L. multiflorum* leaves using leaf segment techniques to investigate changes in plant water relations, net photosynthetic capacity and respiratory capacity. Plant material was exposed to controlled wilting conditions in the growth cabinet, sampled after 0, 24 and 48 h and used for the physiological experiments. The effect of plant, environmental and methodological factors was investigated.

Wilting procedure

For each wilting experiment some 50 healthy ryegrass tillers were harvested. About a third of the plant material was reserved for the zero time physiological experiments whilst the remainder was subjected to controlled wilting. The plant material was spread in a layer c. 5 cm deep over the growth cabinet shelf, all the plants orientated in the same direction as in a mown grass swath in the field. Wilting conditions were representative of summer weather in S.E. Scotland, Table 2.1, and were monitored continuously throughout the wilting period. About half of the wilting plant material was removed from the simulated swath after 24 h, and the remainder after 48 h, and used for the physiological experiments.

Physiological experiments

Three kinds of experiments concerned with the physiological changes in wilting grass leaves were carried out:

1. Water relations studies. The degree of wilt was monitored by conventional D M determination, by following water uptake and by calculation of the water saturation deficit (WSD) in leaf tissue samples.

TABLE 2.1 Physiological experiments using leaf segments

Expt. No.	Plant Material			Wilting Conditions	Exposure Conditions	No. of Replicates	Parameters Measured
	Source	Age (Weeks)	Stage				
1	Growth-cabinet	6	YV	17.5° day 12.5° night 16 h photoperiod	6 h exposure, dark	6	water uptake W S D D M
2	Growth-cabinet	6	YV	-	6 h exposure, light:P_N dark:R_D	12	P_N R_D W S D D M
3	Growth-cabinet	8	MV	15° 16 h photoperiod	6 h exposure, light; 1 h pretreatment	6	P_N water uptake W S D D M
4	Growth-cabinet	10	MV	15° 16 h photoperiod	6 h exposure, dark; 1 h pretreatment	6	R_D water uptake W S D D M
5	Plot	10	R 5% EE	15° day 10° night 16 h photoperiod	6 h exposure, light	6	P_N D M
6	Plot	14	R 50% EE	20° day 15° night	6 h exposure, light	12	P_N D M
7	Plot	10	MV	20° day 15° night 16 h photoperiod	6 h exposure,	6	R_D D M

The D M content of the plant material was determined routinely using the control samples of leaf tissue in all experiments. Thus the wilting stage was characterised in terms of D M content of the plant material in order to relate to the field studies in Section I and to wilting studies reported in the literature.

The water uptake of leaf tissue samples was followed as their increase in F W during exposure to saturating conditions. Thus the extent of recovery from wilting could be assessed. The water saturation deficit (W S D) developed during wilting was calculated from the water uptake data.

2. Photosynthesis studies. The net photosynthetic capacity of fresh and wilting grass leaves was determined using resaturated leaf tissue samples as described below.

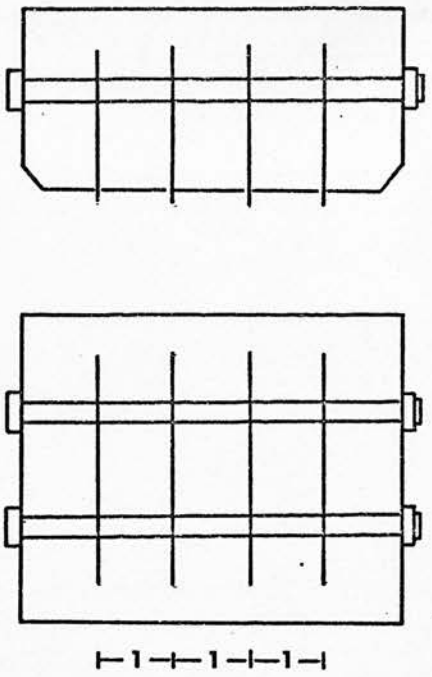
3. Respiration studies. The dark respiratory capacity of fresh and wilting grass leaves was determined similarly. The physiological experiments are summarised in Table 2.1

Leaf segment techniques

The use of leaf tissue samples for studying the physiological characteristics of plant leaves is a recognised technique (Setlik and Sestak 1971). Sets of 1 cm leaf segments were cut from fresh, 24 and 48 h wilted ryegrass leaves and used for the physiological experiments, as follows:-

Procedure. The leaf segments were cut rapidly from narrow monocot. grass leaves by means of the punch, Fig. M.7 a. A sampling pattern was

a.



b.

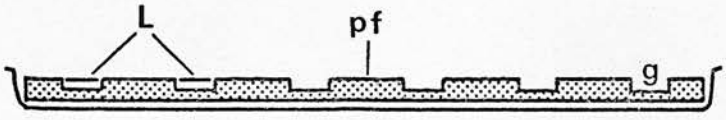


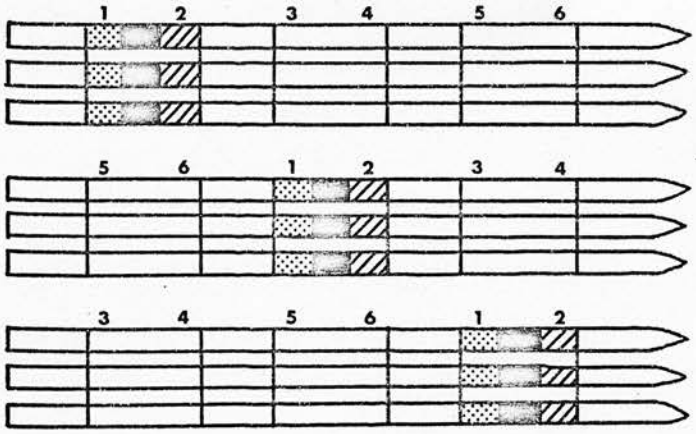
FIG. M.7 a Diagram of the punch for cutting leaf segments

Razor blades are fixed in the wooden block by means of screws. Dimensions in cm.

FIG. M.7 b Diagram of the polyurethane foam matrix in L.S.

Polyurethane foam matrix (pf) with grooves (g) for the support of leaf segments (L) during exposure.

a.



b.

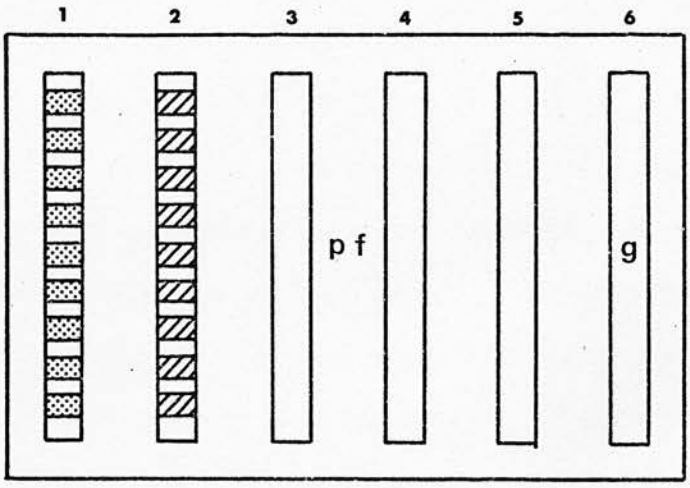


FIG. M.8 a Diagram of the sampling pattern for a population of ryegrass leaves

Each leaf belongs to the same insertion level on a different tiller. Heavy vertical bars represent the cutting positions of the leaf punch. Full shading, cross-hatching or stippling represents leaf segments of one replication. Full shading = control sample; cross-hatching or stippling = experimental samples which are put into parallel grooves in the polyurethane foam matrix. Numbers indicate the six replicate experimental samples.

FIG. M.8 b Polyurethane foam matrix with two experimental samples in position ready for exposure

Numbers indicate the six replicate experimental samples. pf = polyurethane foam; g = groove

devised to take account of the natural heterogeneity amongst leaves on a plant as well as within one leaf blade. The tissue samples were never chosen at random but so as to represent a mean value of the leaf and plant population, according to the plan illustrated in Fig. M.8. Thus the control and experimental samples were cut simultaneously in identical fashion and were as similar as possible in initial composition.

Each sample consisted of a set of twelve 1 cm leaf segments which may be weighed easily even when dry. Sets of 6 - 18 segments are recommended for standard physiological measurements (Setlik and Sestak 1971). On each sampling occasion, nine samples were obtained of which six were used as the experimental material and the remaining three as controls. Avratovscukova (1967) found it necessary to use 3 - 6 leaf disk samples to characterise the photosynthetic rate of a given leaf or plant, and 9 - 18 samples to represent a plant population. Therefore in all leaf segment experiments, 6 - 12 samples were used for determining each physiological characteristic, giving a value representative of the plant population and of the swath where a mixed population of leaves of different ages and physiological states is always found.

Twelve healthy non-senescing leaves were selected from the same relative position on twelve tillers and were arranged in parallel groups of three on a clean 'Benchkote' sheet. Samples were cut with the punch, according to the pre-determined pattern, rapidly and carefully. Segments were handled gently with forceps and a brush to avoid any further injury to the tissue, and were transferred onto labelled filter papers. The production of nine samples in this way took c. 10 min.

Immediately after cutting, all samples were weighed (FW_o). Then each experimental sample was inserted into a long rectangular groove in a water-saturated matrix of polyurethane foam, Fig. M.8 b. The segments were positioned with their long axes across the groove and their ridged adaxial surface with stomata uppermost, separated by c. 5 mm intervals. Each segment was placed at a depth of c. 1 mm below the foam surface, Fig. M.7 b, and its cut edges were pressed gently against the foam matrix to ensure good contact. Bartos *et al.* (1960) found the water demand of tissue samples to be fully satisfied under such conditions. This arrangement also allowed free circulation of CO_2 around the segments. Careful positioning of each experimental sample in this way was critical and took c. 5 min.

A period of equilibration of the samples in the foam matrix is recommended as a pre-treatment for physiological experiments (Natr 1970) in order to eliminate water saturation deficits which inevitably develop during sample preparation and wilting treatments. With high irradiance, the initial period of stomatal opening is decreased, which is important for photosynthesis measurements. Hence, both control and experimental samples were given a 1 h pre-exposure to light under controlled conditions in some of the experiments. The aim was to bring both fresh and wilted samples to similar physiological states of water balance, D M content and stomatal behaviour prior to making physiological measurements.

The experimental samples inserted in the foam matrix were used for the determination of initial water saturation deficit (W S D %), and rates of photosynthesis and respiration integrated over the whole exposure period. For all measurements, a 6 h sample exposure to a well-defined controlled environment was used, with or without the 1 h pre-treatment.

Exposure was carried out in the growth cabinet. The speed of the air input fan was increased in order to provide efficient ventilation and to eliminate possible increases in boundary layer resistance at the sample surfaces. Irradiance was 120 W m^{-2} at the level of the segments, apart from the respiration measurements made in darkness. Relative humidity was c. 70%. Temperature varied between 10° and 20° according to the experiment. Leaf temperatures were generally c. 1° below air temperatures.

The foam matrix must remain saturated throughout the exposure period. The addition of distilled water in the course of an experiment was occasionally necessary in order to compensate for evaporation from the matrix under conditions of high irradiance and temperature.

The gravimetric methods of W S D determination (Catsky 1965; Kramer and Brix 1965; Slatyer and Barrs 1965), photosynthesis determination (Bartos *et al.* 1960; Setlik *et al.* 1960; Natr 1970) and respiration measurement used in these experiments require very accurate determination of sample weights and areas.

All sample weighings were carried out using an analytical balance accurate to 0.01 mg. Fresh weights (F W) of all samples were recorded immediately after cutting and after the 1 h pretreatment when used. The F W of the experimental samples was recorded after the exposure period. After removal from the foam matrix, each sample was blotted lightly in order to remove any surface moisture before weighing. Sample dry weights (D W) were recorded after oven-drying to constant weight at 80° (c. 2 d). Samples were cooled in a desiccator, then the weight recorded at exactly 30 s after removal from the desiccator in order to

standardise the amount of water absorption occurring during the weighing process. Control sample D W's were determined either directly after sample cutting or after the 1 h pretreatment when used. Experimental sample D W's were determined after the exposure period.

The leaf area (A) of all experimental samples was determined by measuring the cumulative width of all the segments in each sample after removal from the foam matrix. A refers to the area of one surface only, which represents the effective photosynthetic surface of the epistomatous *L. multiflorum* leaves.

Calculation of results

1. Water saturation deficit (W S D)

$$\% \text{ W S D} = \frac{F W_6 - F W_o}{F W_6 - D W_6} \times 100 \quad \text{(Catsky 1965)}$$

where W S D = the water uptake expressed as a % of the saturation water content

and subscripts = exposure time in h.

An alternative method of expressing the physiological water status of plant tissue is to use the relative water content (sometimes inaccurately referred to as the relative turgidity),

$$\text{R W C \%} = 100 - \text{W S D} \quad \text{(Barrs 1968)}$$

where R W C = the actual initial water content expressed as a % of the saturation water content.

2. Net Photosynthesis (P_N). The rate of net photosynthesis may be calculated from the increase in sample D W representing the accumulation of photosynthates during the exposure period, the leaf area and the

exposure time (Bartos *et al.* 1960, Setlik *et al.* 1960, Natr 1970). The initial D W ($D\ W_o$) of each experimental sample is obtained from the initial F W ($F\ W_o$), and the initial D M content identical to that of the control sample, i.e.

$$D\ W_o = \frac{D\ M_o}{100} \times F\ W_o$$

The final D W was determined after the exposure period, leaf area was measured and exposure time was fixed. Therefore,

$$P_N = \frac{D\ W_6 - D\ W_o}{A \times h} \quad 10^{-9} \text{ kg D M } m^{-2}\ s^{-1}$$

Photosynthetic rates are generally expressed in terms of CO_2 flux rather than photosynthate accumulation. It may be assumed that the D W increase represents the synthesis of hexose sugars from CO_2 and water according to the classic summary equation for photosynthesis (Sestak *et al.* 1971). It is possible to calculate the weight of CO_2 assimilated in the formation of unit weight of hexose sugars, taking as a basis the average of the molecular weight of a fructan unit and of a fructose unit, 0.5 (162.14 + 180.16) = 171.15 . Thus conversion coefficients for the calculation of net photosynthesis in terms of CO_2 fixed at atmospheric pressure may be derived. The weight of CO_2 assimilated in the formation of unit weight of hexose sugars is 1.543; the weight of sugars formed per unit weight of CO_2 assimilated is 0.648 (Sestak *et al.* 1971).

3. Dark respiration (R_D). The dark respiration rate may be calculated in an analagous manner from the decrease in sample D W representing the dissimilation of photosynthates during the dark exposure period, the leaf area and the exposure time,

$$R_D = \frac{D\,W_o - D\,W_6}{A \times h} \quad 10^{-9} \text{ kg D M } m^{-2} \; s^{-1}$$

Assuming that the D W decrease represents the respiration of hexose sugars to CO_2 and water, a conversion factor may be derived to express the respiration rate in terms of CO_2 flux, as above. No attempt was made to measure photorespiration.

W S D, P_N and R_D were calculated separately for each sample. Mean values of each parameter, based on the six or twelve replicate samples, were derived. The results were analysed statistically.

STOMATAL STUDIES

The adaxial and abaxial epidermal surfaces of *L. multiflorum* leaves were examined using a range of techniques in order to find a satisfactory routine method for making stomatal observations. Good resolution is most important for assessing the condition of slit-type graminaceous stomata.

Light microscopy

Observations were made using a Vickers microscope with x10 and x40 objectives, giving magnifications of x100 and x400, for the leaves of *L. multiflorum*, R.v.P. var. An Ernst Wetzlar microscope with camera attachment and x11 and x22 objectives, giving magnifications of x140 and x275, was used for the leaves of Westervold Barenza var. (De Parcevaux 1973).

Direct observation of intact pieces of fresh leaf material mounted in water or glycerol; leaf material fixed and decolourised in absolute alcohol then mounted in glycerol; and leaf material fixed, decolourised and stained with a saturated solution of I / KI (for starch) then mounted in glycerol, was made using transmitted light.

Lloyds strips (1908) were prepared by tearing epidermal strips from fresh leaves, fixed immediately in absolute alcohol then mounted in glycerol and examined using transmitted light.

Leaf impressions were prepared by two methods:

1. Perspex-acetone impressions were made by dropping acetone onto a clean dry perspex slide, pressing a fresh leaf onto the liquefied perspex surface and holding it in position until the acetone had

evaporated and the perspex resolidified. The leaf tissue was carefully removed, leaving a permanent impression of the epidermal surface on the transparent slide which was then examined using transmitted light.

2. Silicone rubber impressions were made by the method of Sampson (1961) modified slightly. A thin layer of R S silicone rubber compound was spread over c. 1 cm of leaf blade and allowed to harden. When cured (c. 30 min), this white primary impression was peeled off the leaf and placed in a desiccator. Clear replicas of the impressed surface were made using amyl acetate. When quite hard (c. 10 min), these transparent secondary replicas were carefully peeled off the silicone rubber impressions using forceps. The replicas were mounted dry between two thin polished microscope slides then examined at an angle of 45° using transmitted light. This is a simple, rapid, reliable way of obtaining permanent records of epidermal surfaces.

Scanning electron microscopy

Observations were made of leaf surfaces using a Cambridge Stereoscan microscope, Institute of Tree Biology, Edinburgh, giving magnifications between x20 and x5000. Leaf tissue samples were prepared in several ways: fresh, coated, freeze-dried and critical point dried. Both epidermal surfaces were examined by conventional scanning electron microscope techniques (Heywood 1971). Further observations were made using a Jeol JSM-3 microscope, Université de Paris-Sud, with a special cryo-unit attachment to allow examination of leaf surfaces at the temperature of liquid nitrogen, -196° C (Couderc 1974). This technique is thought to reduce artefacts.

Experiments involving stomatal observations

The condition of the stomata was fixed and recorded permanently using leaf impressions and/or freezing in liquid nitrogen in the course of both field and laboratory experiments.

1. Field experiments. Impressions were made in triplicate of the adaxial and abaxial epidermal surfaces of ryegrass leaves wilting in the field. Leaves were selected randomly from the standing crop and from mown swaths and one impression was made per leaf in the mid-portion of the leaf-blade. The silicone rubber technique was used and, after curing, the impressions were peeled off the leaves and stored in sealed glass vials containing silica gel. Impressions were made of the standing crop, and of the cut crop immediately after harvest (0 - 30 s), and after 15, 30 min and 3 h of wilting.

2. Laboratory experiments. Impressions were made in the course of the Single Leaf Studies. During the determination of transpiration rate by the quick-weighing method impressions were made using silicone rubber before, and at 0 - 30 s, 10 and 30 min after leaf excision. In the course of the detailed experiments using infra-red gas analysis, impressions were made using the perspex-acetone method at 0 - 30 s, 15 and 30 min after leaf excision. On each sampling occasion the assimilation chamber was removed from the water-bath, opened and one leaf taken out to make the impressions, resealed and replaced. This procedure was carried out as rapidly as possible but disturbance of the system and a few s delay between leaf removal and impression formation was inevitable. At the same time, small pieces of leaf tissue were fixed by freezing very rapidly in liquid nitrogen and reserved for subsequent examination by scanning electron microscopy.

BIOCHEMISTRY OF WILTING

The biochemistry of wilting was studied in *L. multiflorum* leaves: the timecourse of changes in various leaf biochemical constituents was followed during 48 h of wilting under controlled environmental conditions in the growth cabinet. The effect of plant and environmental factors was investigated.

Wilting procedure

The plant material used was *L. multiflorum*, R.v.P. var., cultivated as described previously. For each wilting experiment, about 100 healthy ryegrass plants of known age and growth stage were harvested at c. 09.00 h. After selecting at random some twelve plants for analysis of the fresh material (zero time sample), the remainder was spread over the growth cabinet shelf in a simulated swath 5 - 10 cm deep and left to wilt under controlled conditions. Further samples of twelve plants were selected at random from the simulated swath after 3, 6, 9, 12, 24, 36 and 48 h of wilting.

On each sampling occasion, nine subsamples each of twelve leaf segments were prepared from healthy fully expanded leaves as in the Leaf Segment Studies. Each subsample was representative of the population of plant leaves; all subsamples were as far as possible identical. After recording the F W, each subsample was transferred to a small, sealed, labelled glass vial and stored temporarily on ice. The leaf material remaining after preparation of the subsamples was chopped, mixed and sampled in triplicate for D M determination.

Wilting conditions

Four wilting experiments were carried out using a range of temperature and light regimes and wilting atmospheres in the growth cabinet. Wilting conditions were as described previously, with the following modifications,

Expt. 1: 15° 16 h day; 10° night.

Expt. 2: 20° 16 h day; 15° night.

Expt. 3: continuous darkness.

Expt. 4: continuous darkness, nitrogen atmosphere, 100% relative humidity.

Expts. 1 and 2 simulated good field wilting conditions. Expt. 3 may be considered to represent either extended night wilting in the field or oxidative conditions during the very early stages of ensiling, thus providing information about the effect of light on the biochemical changes taking place. Expt. 4 was not in reality a wilting experiment since replicate samples of twelve plants were enclosed in large tubes under nitrogen, and successively opened on the sampling occasions. However, Expt. 4 was included in this section for the sake of completeness, to investigate the effect of wilting atmosphere and dehydration on the biochemical changes occurring in harvested plants, or alternatively to represent anaerobic conditions in the silo. Thus, Expts. 1 and 2 linked the field programme, Section 1, with the controlled environment programme, Section 2; Expts. 3 and 4 linked the wilting studies of the first two sections with the ensiling studies, Section 3.

Sample storage

In the course of one complete wilting experiment, 72 leaf tissue subsamples were obtained so that a storage period of several days to several weeks was inevitable before analysis. In order to reduce enzymic

changes in the plant material to a minimum, pre-weighed samples were stored at -20° at which temperature enzyme activity is inhibited. The samples for chlorophyll determination were given a pretreatment, (the addition of anhydrous acetone plus a small amount of $MgCO_3$ to each vial which was warmed to vapourise the acetone then rapidly sealed), before transfer to the deep freeze. This procedure prevents pigment destruction due to oxidation and acidity, and also softens the tissues to facilitate chlorophyll extraction. Losses of chlorophyll are < 3% and < 10% after 1 and 3 months respectively (Sestak 1959). A preliminary experiment designed to investigate the effects of freezing and storage on the plant biochemical composition showed that there was < 10% change in the contents of total soluble sugars, total soluble protein and chlorophyll after 16 weeks of storage under these conditions.

Sample extraction

All operations were carried out at ice temperature using precooled reagents (-5 to 0°), in order to minimise enzymic changes in leaf composition in the course of extraction resulting from the attack of enzymes on substrates previously inaccessible through cellular compartmentalisation. An all-glass Potter-Elvehjem type homogeniser was used to give a mild extraction treatment to the small leaf tissue samples, which were finely chopped then ground by hand for 1 - 5 min until the homogenate contained no visible leaf fragments. Glass-distilled water was used as the grinding medium for the extraction of soluble carbohydrates and nitrogenous components. Considerations of isotonicity and buffering capacity were unimportant as preservation of enzyme activity and structure in the homogenate was not required and was in fact undesirable. 80% acetone was used to extract chlorophyll from its lipo-protein complex *in vivo* . For the majority

of higher plants, maximum extraction is obtained with 80 - 85% acetone under cold and dark conditions (Sestak 1971). After cell disruption, the homogenate was filtered through double muslin to remove cell walls and any unbroken cells. The residue was washed with grinding medium then the filtrate or cell-wall-free homogenate was used for analysis.

Analytical methods

The biochemical changes in wilting ryegrass leaves were monitored by determining the amounts of various biochemical constituents on each sampling occasion during the wilting period. In all cases, analyses were performed in triplicate using conventional spectrophotometric methods. The results were expressed on a D M basis unless otherwise stated, taking the mean of the triplicate subsamples.

Soluble carbohydrates. The determination of total water-soluble carbohydrates, total glucose and total fructose in each leaf tissue sample was performed after acid hydrolysis of the protein-free filtrate and therefore was derived from the water-soluble poly-, oligo- and disaccharides as well as free monosaccharides.

Total water-soluble carbohydrates (W S C) were determined by the photometric method of Nelson (1944) modified by Somogyi (1945) which is suitable for the range 0.01 - 3.00 mg glucose (or reduction equivalent). Absorbance was read at 540 nm.

Glucose was determined by the highly specific glucose oxidase method as modified by Raabo and Terkildsen (1960). Absorbance was read at 450 nm.

Fructose was determined by Bacon and Bell's (1948) modification of Roe's method (1934) for the estimation of those substances giving the

Seliwanoff reaction (ketoses of which the most important is fructose). The effective range of the method is 0.02 - 0.10 mg fructose; glucose gives only c. 0.6% of the colour produced by fructose under the same conditions. Absorbance was read at 420 nm.

2. Nitrogenous components. A simple fractionation procedure was used to separate the soluble proteins from the non-protein amino compounds, (amino acids, amines and small peptides), thus allowing their separate estimation in the same leaf extract. The proteins were precipitated using trichloroacetic acid (T C A) to a final concentration of 5% (w/v); non-protein nitrogen compounds remain in the supernatent (Thurman and Boulter 1966).

Total soluble proteins (T C A - insoluble fraction) were determined by the sensitive spectrophotometric method of Lowry *et al.* (1951) modified after Folin and Ciocalteu (1927). This method can detect as little as 0.2 µg protein. Absorbance was read at 750 nm.

Total free amino compounds (T C A - soluble fraction) were estimated in a Technicon autoanalyser using T N B S (2, 4, 6 - trinitrobenzenesulphonic acid). The effective range of this method is 0.01 - 0.8 µ moles ml^{-1}. Absorbance was read automatically at 340 nm (Waring and Bolton 1967).

3. Chlorophyll. Total chlorophyll (*a* + *b*) was determined by the two wavelength method, based upon measurements of exact absorbance at the absorption peaks of chlorophylls *a* and *b*, 663 and 645 nm respectively (Sestak 1971).

Calibration curves for the spectrophotometric methods used were prepared routinely in the course of analysis. Typical calibration curves for carbohydrate and nitrogenous components are shown in Figs. M.9 and M.10

Calculation of results

Results were expressed directly on a D M basis. In order to calculate the protein:free amino group ratio, the total weight of free amino compounds was estimated, based on glycine.

For the calculation of chlorphyll amounts, the equations based on the specific absorption coefficients of Mackinney (1941) compiled by Arnon (1949) and checked by Bruinisma (1961) were used. For 80% acetone extracts,

$$\text{chlorophyll } (a + b) = 8.02\ A_{663} - 20.20\ A_{645}$$

A nomogram based on these equation, constructed after Sestak (1966), was used to speed up the routine calculation of results.

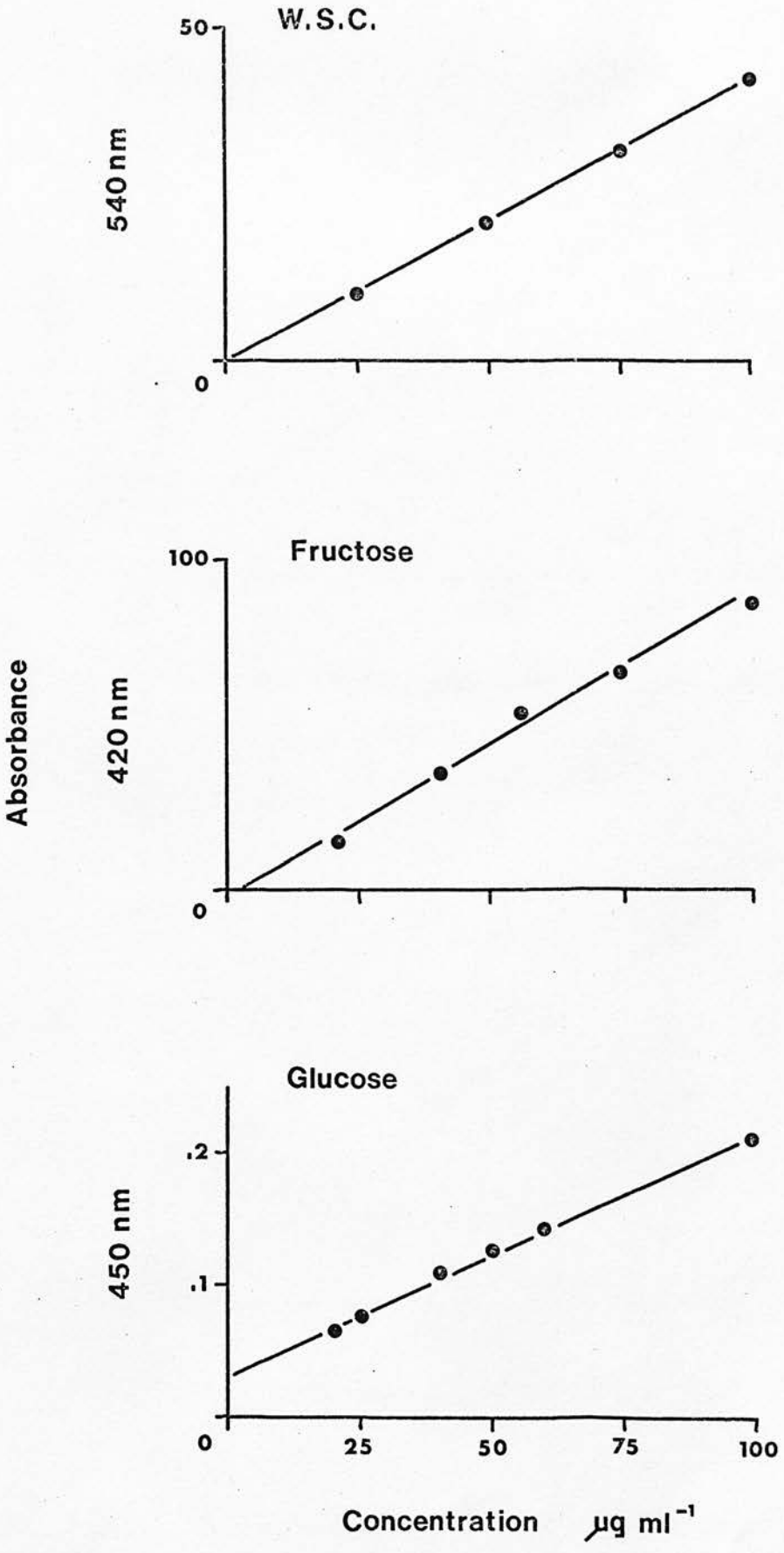


FIG. M.9 Calibration curves for total water-soluble carbohydrates (W.S.C.), Fructose and Glucose

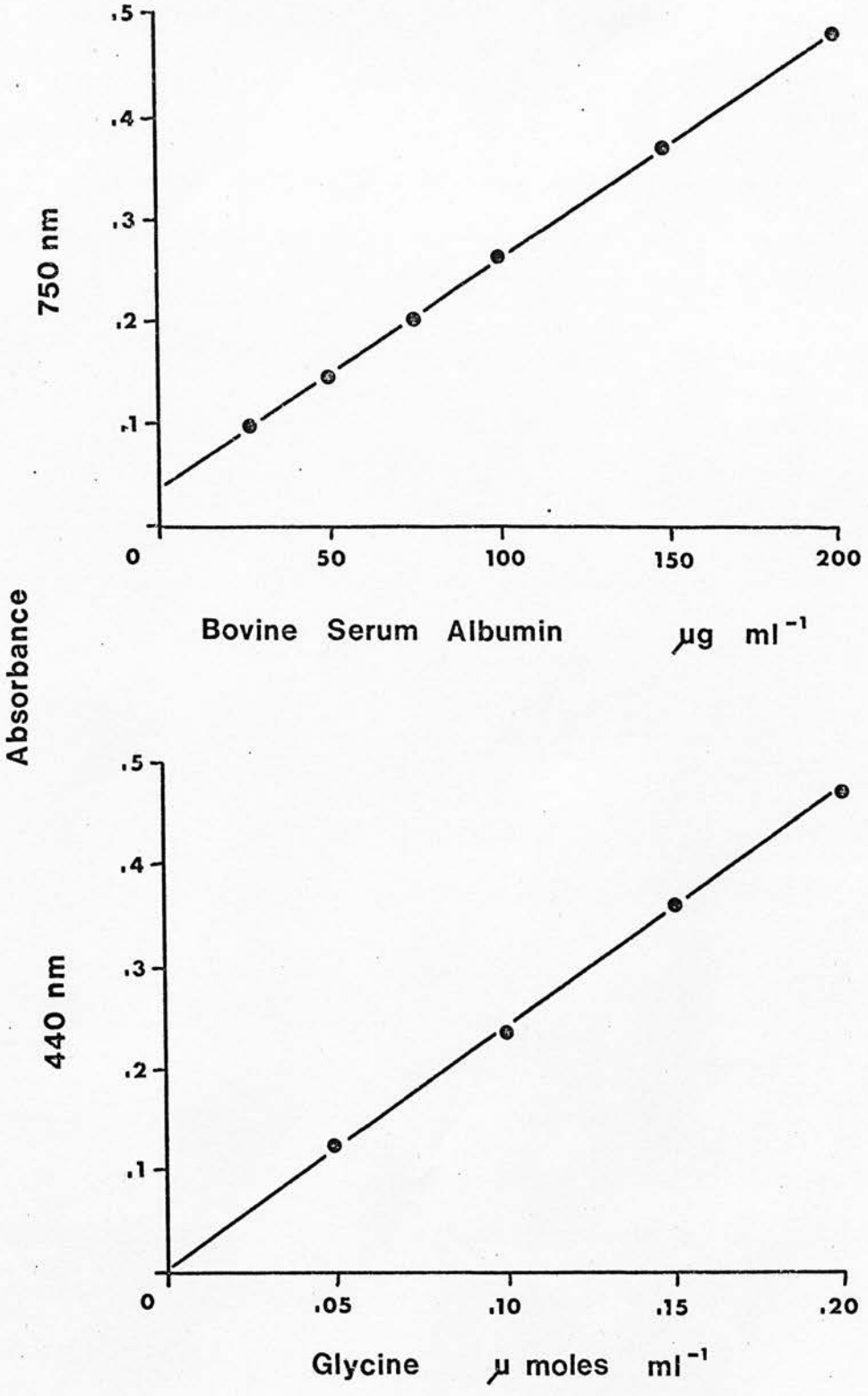


FIG. M.10 Calibration curves for total soluble protein (bovine serum albumin), and total free amino groups (glycine)

SECTION 2: RESULTS

SWATH SIMULATION STUDIES

Table 2.2 gives details of the growth-cabinet wilting conditions during the swath simulation studies:

TABLE 2.2 Wilting conditions during Swath Simulation Studies

	Radiant flux density $W\ m^{-2}$	Mean Temperature		Relative humidity %	vpd mb
		Ambient °C	Swath °C		
Expt. 1	120	15.2	14.7	60	6.4
Expt. 2	120	18.0	17.5	74	5.0

Figs. 2.1 and 2.2 show a. the swath F W decline curves and b. swath D M content during the 48 h wilting periods in Expts. 1 and 2 respectively.

a. Swath F W decline curves

In Expt. 1 using young grass, Fig. 2.1, F W declined most rapidly in the control swath during the first hour of wilting. Thereafter, the control swath and the 5 cm sections' swath followed a similar pattern of F W decline reaching final F W values of 47% and 44% respectively. The 2 cm sections' swath showed the most rapid weight decline after 2 h of wilting reaching a final value of 29% of $F W_o$ after 48 h.

In Expt. 2 using more mature grass, Fig. 2.2, F W declined the most rapidly in the control swath during the first 12 h; the F W decline of the crushed swath was intermediate and that of the crimped swath

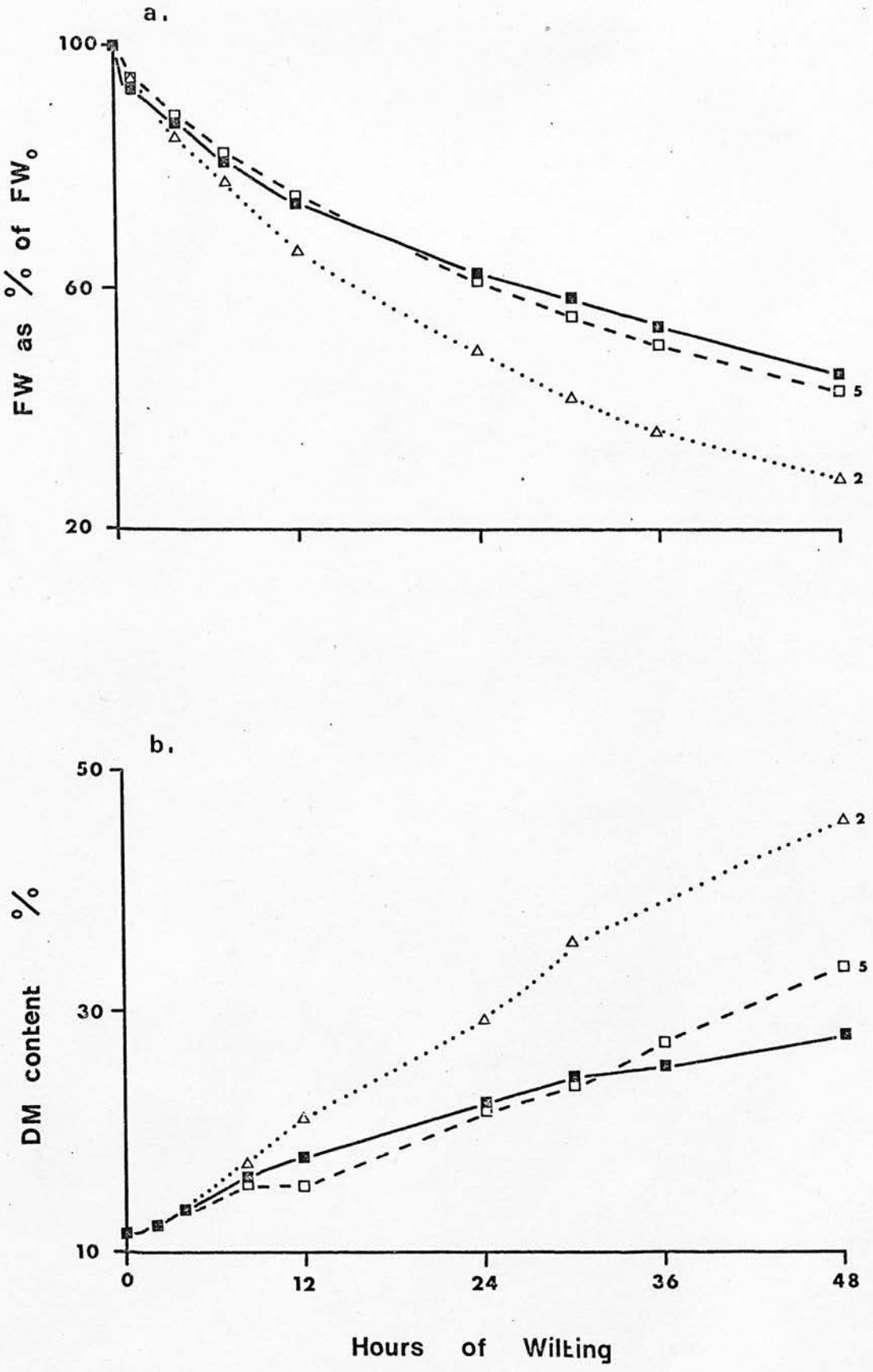


FIG. 2.1 Swath Simulation Studies: Expt. 1

a. Swath F W decline curves

b. Swath D M content

■ , control; □ , 5 cm sections; △ , 2 cm sections

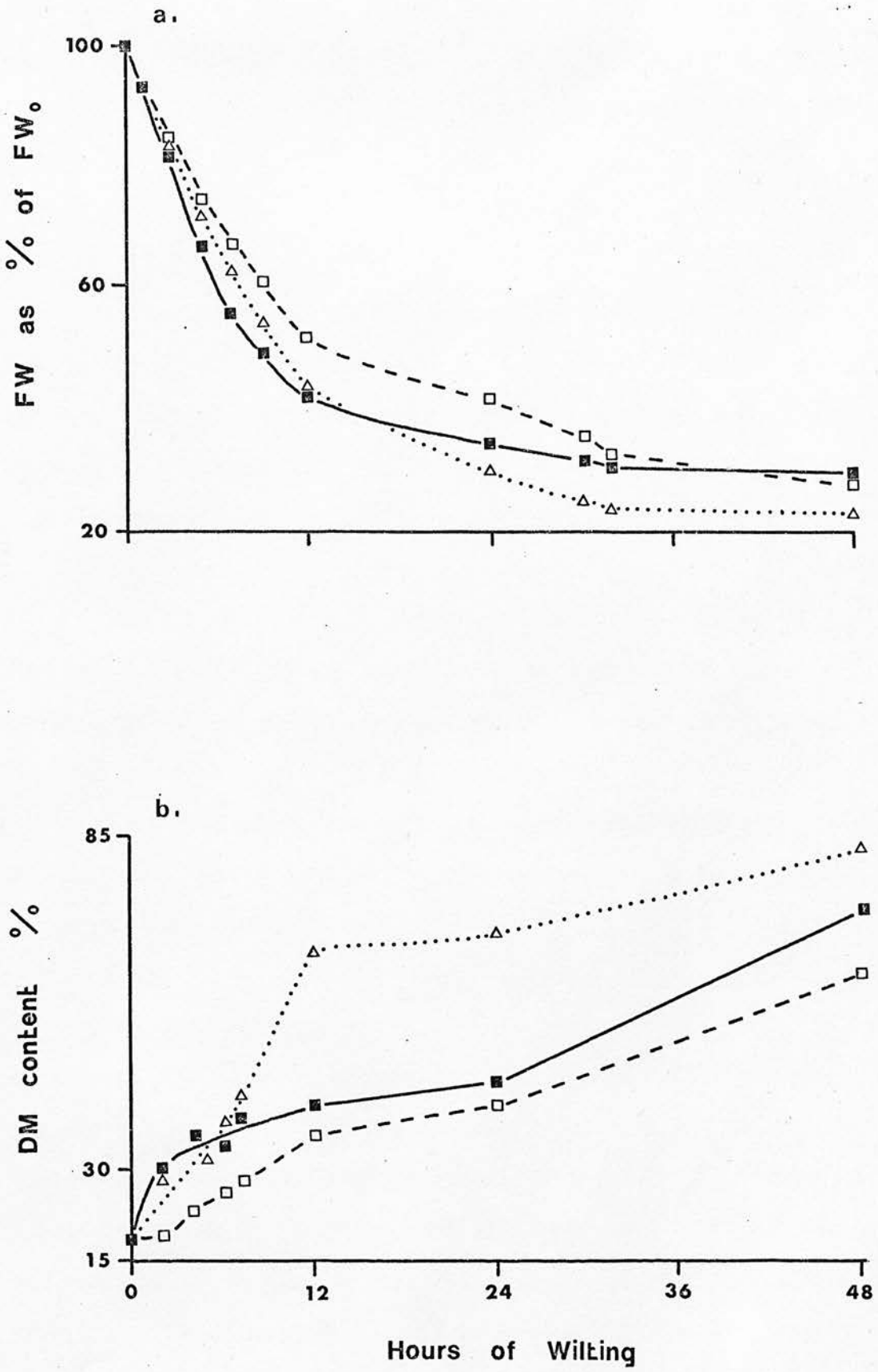


FIG. 2.2 Swath Simulation Studies: Expt. 2

a. Swath F W decline curves

b. Swath D M content

■ , control; □ , crimped; △ , crushed

slowest during this period. Thereafter swath F W declined more slowly in all treatments, to final F W values of 30%, 29% and 23% of $F W_o$ in the control, crimped and crushed swaths respectively after 48 h.

b. Swath D M curves

In general swath D M curves were the inverse of the swath F W decline curves. In Expt. 1, Fig. 2.1, the 30% D M level was attained after 25 h, 42 h and more than 48 h, and final D M levels after 48 h of wilting were 46%, 34% and 28% in the 2 cm sections, 5 cm sections and control swaths respectively.

In Expt. 2, Fig. 2.2, the 30% D M level was attained after 2, 4 and 9 h, and final D M levels after 48 h of wilting were 73%, 84% and 62% in the control, crushed and crimped swaths respectively.

Figs. 2.3 and 2.4 show a. the swath evaporation rate and b. the swath resistance to evaporation during the 48 h wilting periods in Expts. 1 and 2 respectively.

a. Swath evaporation rate, E

In Expt. 1, Fig. 2.3, evaporation rate was initially highest in the control swath, but followed the same pattern in all treatments: the early phase of rapid evaporation lasted for the first 3 - 6 h of wilting; the second phase of slower evaporation lasted until 15 - 18 h of wilting, and the final phase of slow evaporation persisted for the remaining period. Final values of E were between zero and 5 units in all treatments, the final control swath value being the highest, (for units refer to Figs.).

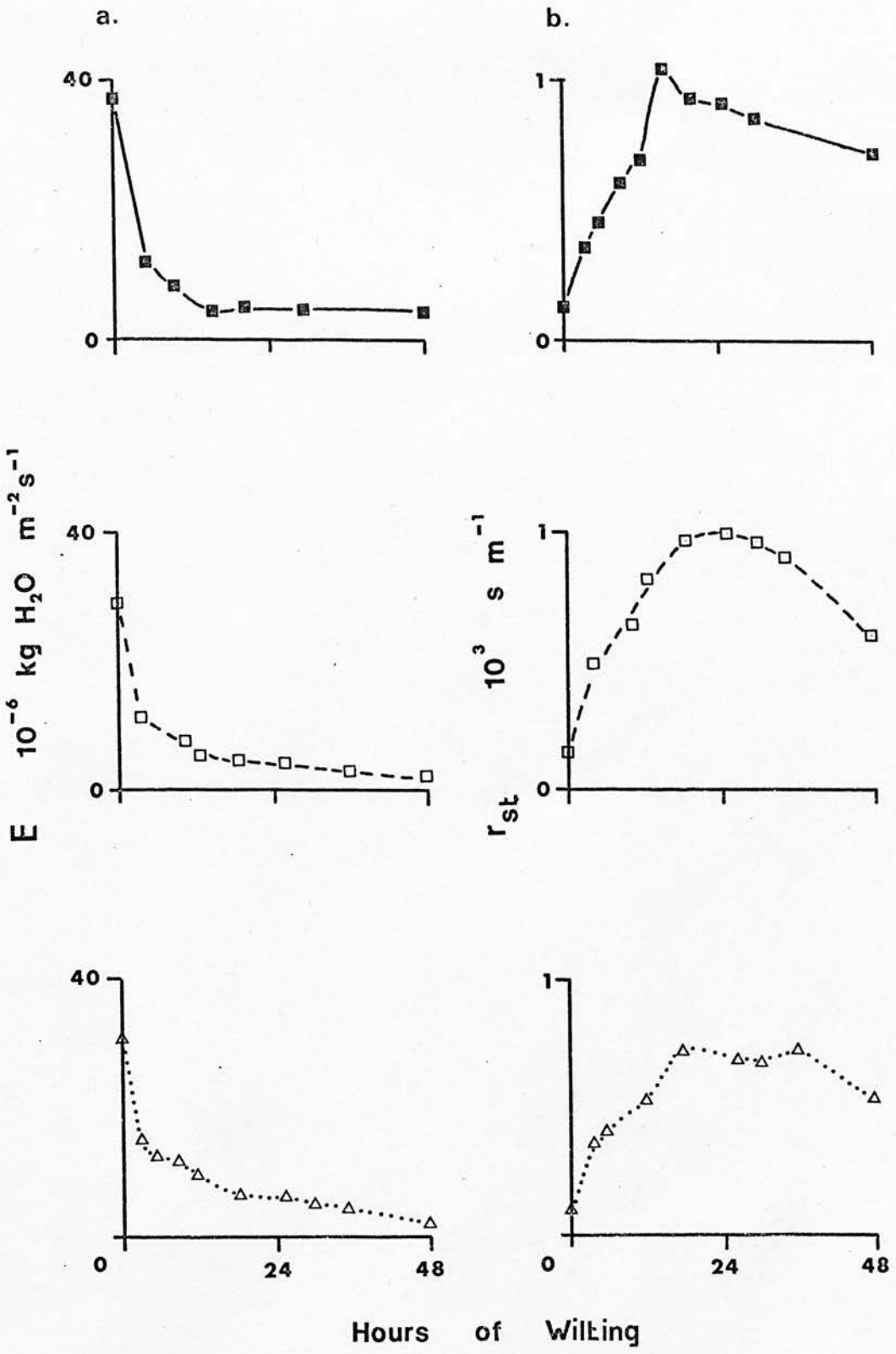


FIG. 2.3 Swath Simulation Studies: Expt. 1

a. Swath evaporation rate, E

b. Swath resistance to evaporation, r_{st}

■ , control; □ , 5 cm sections; △ , 2 cm sections

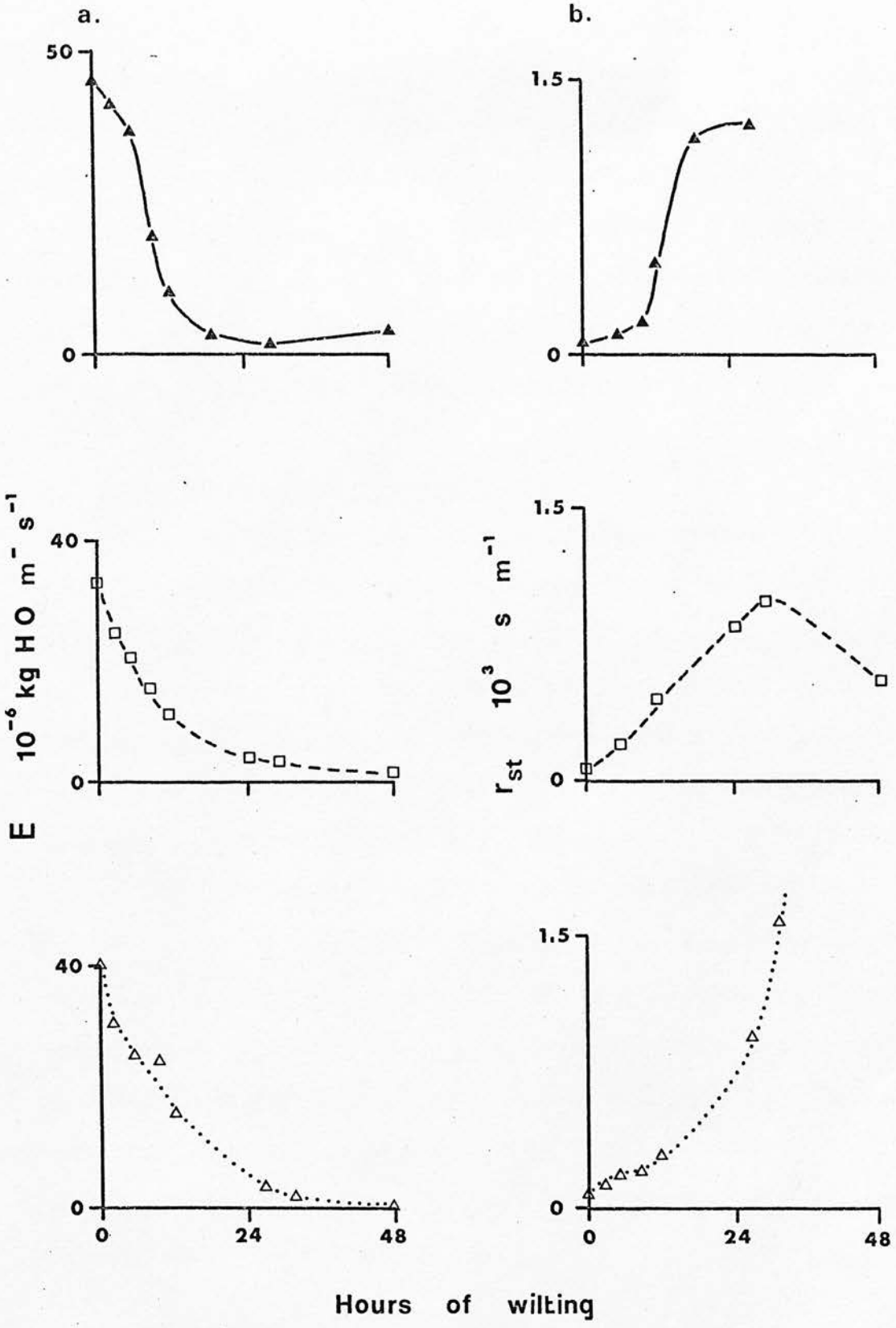


FIG. 2.4 Swath Simulation Studies: Expt. 2

a. Swath evaporation rate, E

b. Swath resistance to evaporation, r_{st}

▲ , control; □ , crimped; △ , crushed

In Expt. 2, Fig. 2.4, evaporation rate was initially highest in the control sample which showed a lag-phase of about 9 h before declining rapidly to a low level within the first 24 h of wilting. The crimped and crushed swaths showed the same overall pattern in E as in Expt. 1, but the second phase of slower evaporation persisted for more than 24 h before the final slow phase commenced, and final values of E were close to zero in both treatments.

b. Swath resistance to evaporation, r_{st}

In Expt. 1, Fig. 2.3, swath resistance to evaporation followed a similar pattern in all treatments, rising from a low initial level of 100 - 150 s m^{-1} to a peak after 12 - 24 h then falling off during the remaining 24 h of wilting. Maximum r_{st} values of the control and 5 cm sections swaths of c. 1000 s m^{-1} were 25% higher than that of the 2 cm sections swath. Final r_{st} values after 48 h of wilting were between 500 and 750 s m^{-1} in all treatments.

In Expt. 2, Fig. 2.4, r_{st} curves were variable in shape, rising from a low initial level of 50 - 75 s m^{-1} in all treatments. The control swath resistance showed a lag-phase of about 9 h before rising to a maximum of c. 1300 s m^{-1} after 24 h of wilting; the crimped swath resistance rose steadily to a peak of c. 1000 s m^{-1} after 27 h of wilting; the crushed swath resistance rose logarithmically approaching an asymptote after 27 h of wilting.

SINGLE LEAF STUDIES: RESULTS

Physiological Plant Responses to Cutting. a. Preliminary Studies

Fig. 2.5 shows transpiration curves of a. whole leaf, b. 5 cm segments and c. 2 cm segments. The F W decline curve of the whole leaf had three distinct phases, as indicated, whereas the curves of the leaf segments were less well-defined.

Table 2.3 gives leaf transpiration rates derived from the curves in Fig. 2.5. For whole leaves the Phase I transpiration rate was greater than the Phase III rate by a factor of ten, but this difference was reduced in the leaf segments to a factor of 2 - 4. The absolute values of T were generally higher in leaf 2 than in leaf 1. In addition Phase I T was highest in whole leaves and reduced by about half in leaf segments. By contrast, Phase III T was lowest in whole leaves and more than doubled in 2 cm segments.

Tables 2.4 and 2.5 give leaf resistances to water loss during Phases I and III in whole leaves and leaf segments. Boundary layer resistance (r_b) values of between 40 and 50 s m^{-1} were derived for both whole leaves and segments. A cuticular resistance, r_c value of c. 2300 s m^{-1} was found for whole leaves. The total resistance, r_Σ, the leaf resistance, r_l and the stomatal resistance, r_s, values derived varied with the leaf, (1 or 2) the transpiration phase (I or II) and the treatment (whole, 5 cm, 2 cm) considered. The largest difference recorded was between Phase I and II values in whole leaves, of up to 20-fold.

Plant physiological responses to cutting. b. Detailed Studies

Fig. 2.6 shows the light response curve for Expt. 1. Net assimilation rate increased with increasing light intensity between

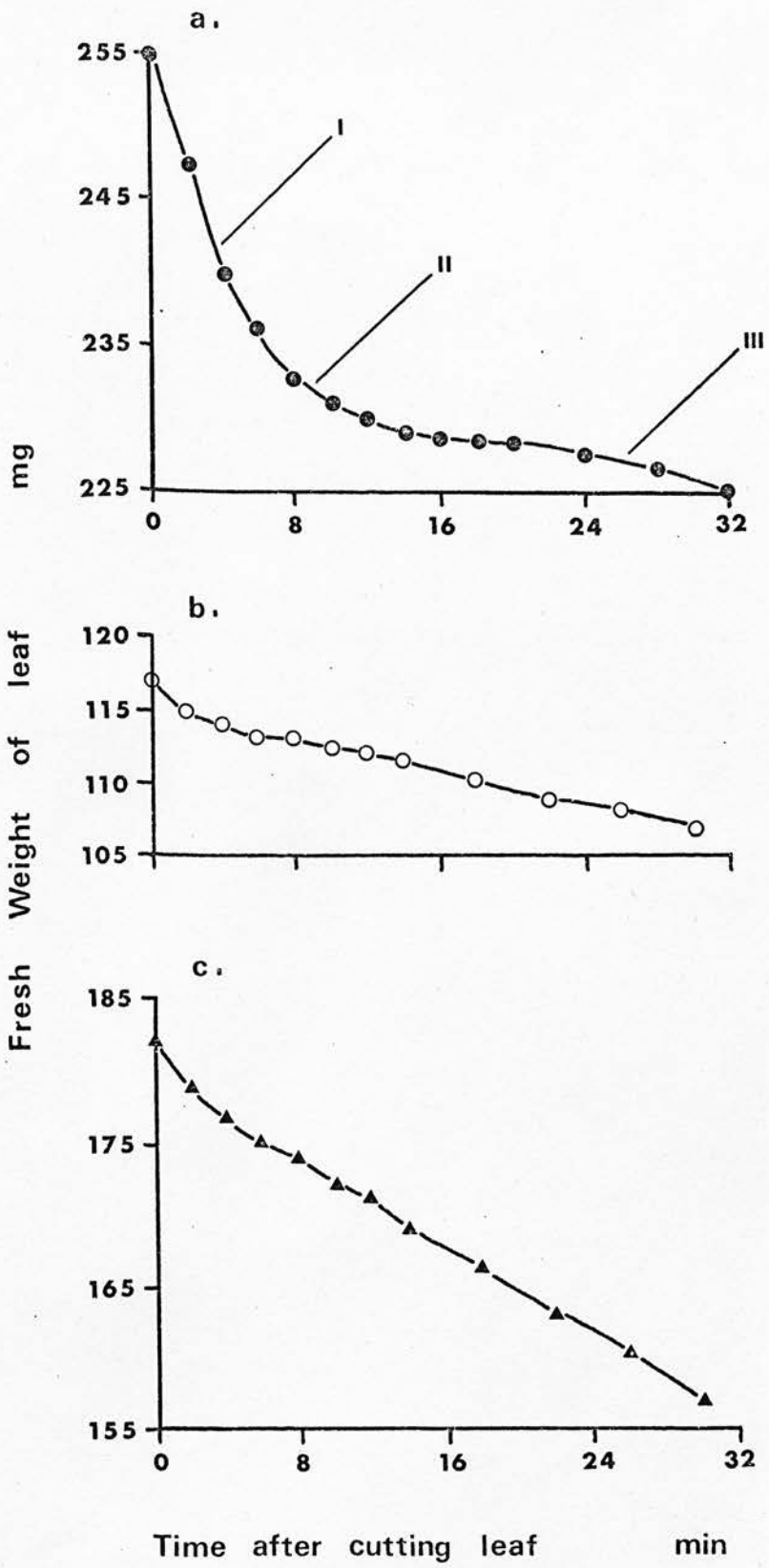


FIG. 2.5 Leaf transpiration curves

a. whole leaf, ● . b. 5 cm segments, O . c. 2 cm segments, ▲ .
I, II, and III correspond to the three separate phases of the transpiration curves

TABLE 2.3 Leaf Transpiration Rates (T in 10^{-6} kg m^{-2} s^{-1})

	Stomatal T Phase I	Cuticular T Phase III
Preliminary Experiment under laboratory conditions	91.9	10.9
	89.3	13.7
Growth Cabinet Experiments		
LEAF 1 whole leaf control	36.2	3.0
5 cm segments	9.9	5.0
2 cm segments	15.5	6.5
LEAF 2 whole leaf control	47.2	4.2
5 cm leaf segments	19.7	4.5
2 cm leaf segments	22.4	13.6

TABLE 2.4 Resistances to Evaporation of Whole leaves (r in $s\ m^{-1}$)

▒▒▒ = leaf

r_b = boundary layer resistance (subscripts U and L, resistance of upper and lower surfaces respectively)

r_c = cuticular resistance (subscripts U and L)

r_s = stomatal resistance

Growth Cabinet Experiments

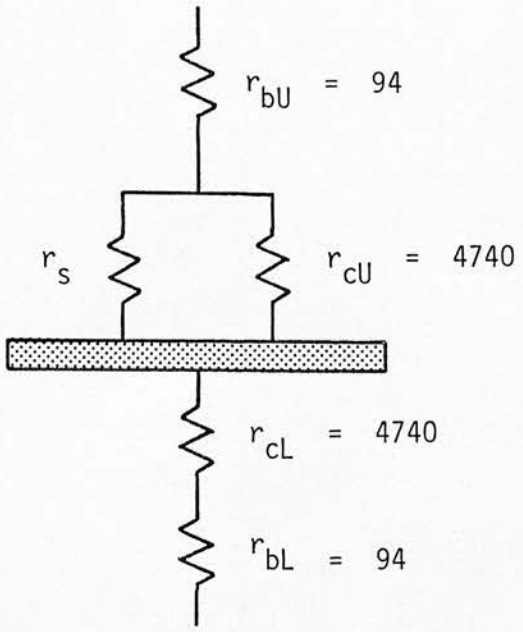
Diagrammatic representation of leaf resistances to evaporation

LEAF 1

r_b = 47

r_c = 2370

r_s = 113



LEAF 2

r_b = 50

r_c = 2338

r_s = 93

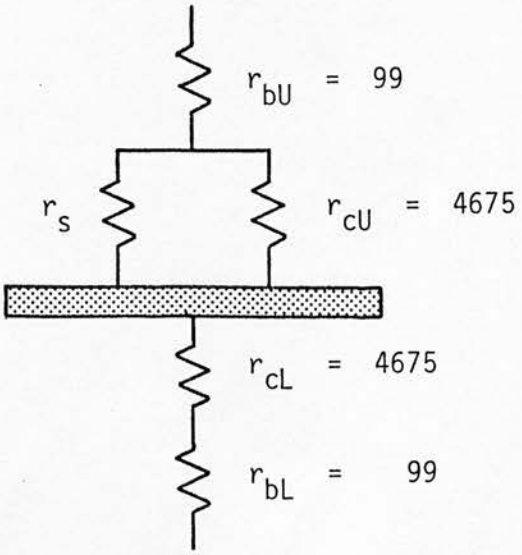


TABLE 2.5 Leaf Resistances to Evaporation:

whole leaves and leaf segments (r in s m^{-1})

r_b = leaf boundary layer resistance

r_Σ = total resistance

r_l = leaf resistance ($r_\Sigma - r_b$)

r_s = stomatal resistance estimate ($r_\Sigma - r_{b_1}$)

Growth Cabinet Experiments

			r_b	r_Σ	r_l	r_s
LEAF 1	whole	Phase I	47	196	149	102
		Phase III		2417	2370	2383
	5 cm	Phase I	55	591	537	481
		Phase III		1180	1115	1070
	2 cm	Phase I	41	417	376	335
		Phase III		991	950	909
LEAF 2	whole	Phase I	50	182	132	82
		Phase III		2387	2338	2287
	5 cm	Phase I	42	447	434	393
		Phase III		2124	2082	2040
	2 cm	Phase I	49	380	331	282
		Phase III		665	606	557

34 and 94 W m^{-2} then reached a plateau. Above 100 W m^{-2} further increase in light intensity had no effect on the net assimilation rate. *L. multiflorum* leaves were considered to be light saturated at intensities above 100 W m^{-2}.

Figs. 2.7 - 2.11 illustrate the plant physiological responses to cutting in Expts. 1 - 4. The four experiments showed the same overall pattern of changes in net photosynthesis, P_N, transpiration, T and resistances to water loss, $(r_b + r_s)$, following excision.

Initial net assimilation rates, P_N, before cutting were between 200 and 300 units (refer to Figs. for P_N units and T units) in Expts. 1 and 2 and c. 400 units in Expts. 3 and 4. In the first few minutes (3 - 5 min) following excision, P_N either increased slightly (Expts. 2 and 4, Figs. 2.8 and 2.11) or decreased slightly (Expts. 1 and 3, Figs. 2.7 and 2.10). After 5 min of isolation, P_N fell steeply to a rate below 100 units by the time 15 min had elapsed: Phase I. At c. 15 min after cutting, the rapid decline in P_N was suddenly checked producing the first break of slope. Then P_N declined more slowly: Phase II. A second break of slope occurred at c. 30 min after excision. Thereafter P_N declined very slowly to zero: Phase III. CO_2 uptake in net photosynthesis was zero after 20, 35, 400 and 100 min in Expts. 2, 1, 4 and 3 (Figs. 2.8, 2.7, 2.11 and 2.10) respectively, after which a net respiratory CO_2 output was found.

Initial transpiration rate, T, before cutting was c. 10 units in Expt. 1 and c. 25 units in Expts. 2, 3 and 4. The pattern of change in T following excision was similar in all experiments and corresponded precisely to the pattern of change in P_N. During the first few

minutes (3 - 5 min) after cutting, T increased slightly (Expts. 1 and 4, Figs. 2.7 and 2.11), remained the same (Expt. 3, Fig. 2.10) or decreased slightly (Expt. 2, Fig. 2.8). Thereafter, there was a rapid decline in T to the first break of slope after c. 15 min: Phase I. Then a slower decline in T occurred to a second break of slope after 30 - 50 min of isolation: Phase II. Finally T declined very slowly during Phase III reaching a low level of less than 5 units in three experiments (Figs. 2.7, 2.10 and 2.11) by the time 100 min had elapsed.

Initial values of $(r_b + r_s)$ before cutting were between 100 and 500 s m^{-1}. Following excision there was a short lag phase lasting up to 5 min before $(r_b + r_s)$ began to increase steadily, reaching 1000 s m^{-1} after 10, 15, 25 and 30 min of isolation in Expts. 2, 4, 3 and 1 (Figs. 2.8, 2.11, 2.10 and 2.7) respectively. Thereafter $(r_b + r_s)$ continued to increase to more then 3000 s m^{-1}, apart from in Expt. 2 when the resistance reached a plateau at about 1000 s m^{-1} after 40 min (Fig. 2.8). Three phases of increasing resistance, corresponding to the three phases of the photosynthesis and transpiration curves, may be distinguished.

Fig. 2.9 illustrates the pattern of T and $(r_b + r_s)$ during an extended 20 h drying period during which P_N was zero. During the first 5 h of desiccation, T declined relatively rapidly from 10 to 5 units, then there was a tendency for a further very slow decrease in T reaching c. 1 unit after 20 h. Values of $(r_b + r_s)$ continued to increase reaching a maximum of c. 6000 s m^{-1} after 17 h of isolation.

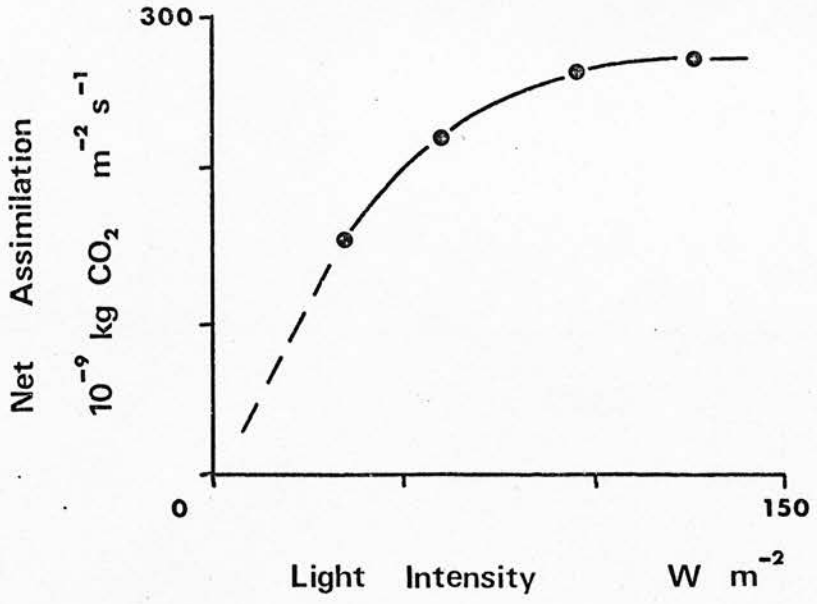


FIG. 2.6 Light saturation curve: Expt. 1

A batch of 12 leaves (leaf no. 5) was used. Each point corresponds to the steady state photosynthesis attained after c. 30 min at each light level

FIGS. 2.7 - 2.11 Physiological plant responses to cutting

b. Detailed studies using infra-red gas analysis, Expts. 1, 2, 3 and 4

a. Net photosynthesis, P_N, □

b. Transpiration, T, ■

c. Resistances to water vapour transfer, $r_b + r_s$, ○

r_b, boundary layer resistance

r_s, stomatal resistance

I, II, and III correspond to the three separate phases of the curves

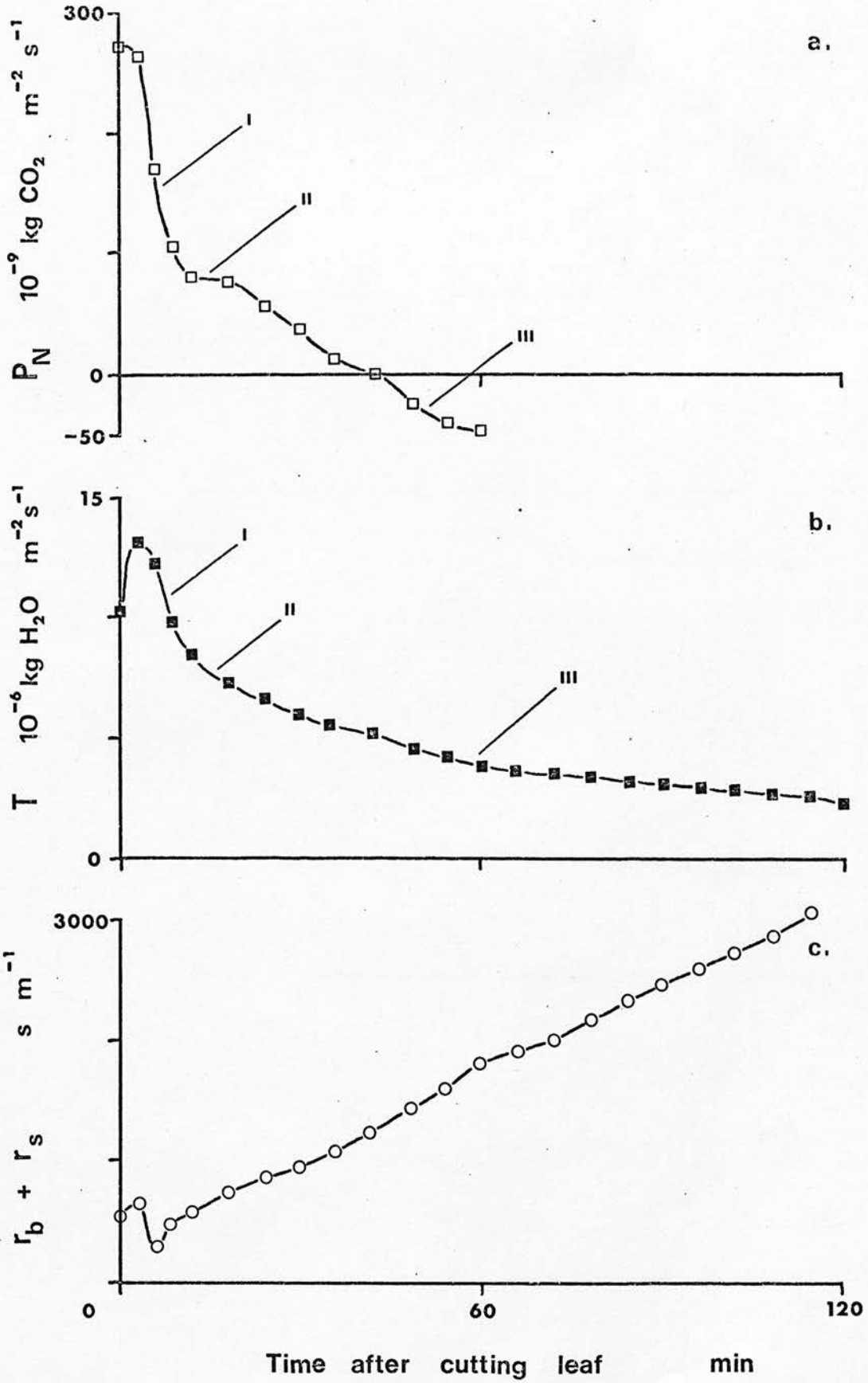


FIG. 2.7 Expt. 1. Changes in P_N, T and $(r_b + r_s)$ after cutting

A batch of 12 leaves (no. 5). 126 W m^{-2} radiant flux density

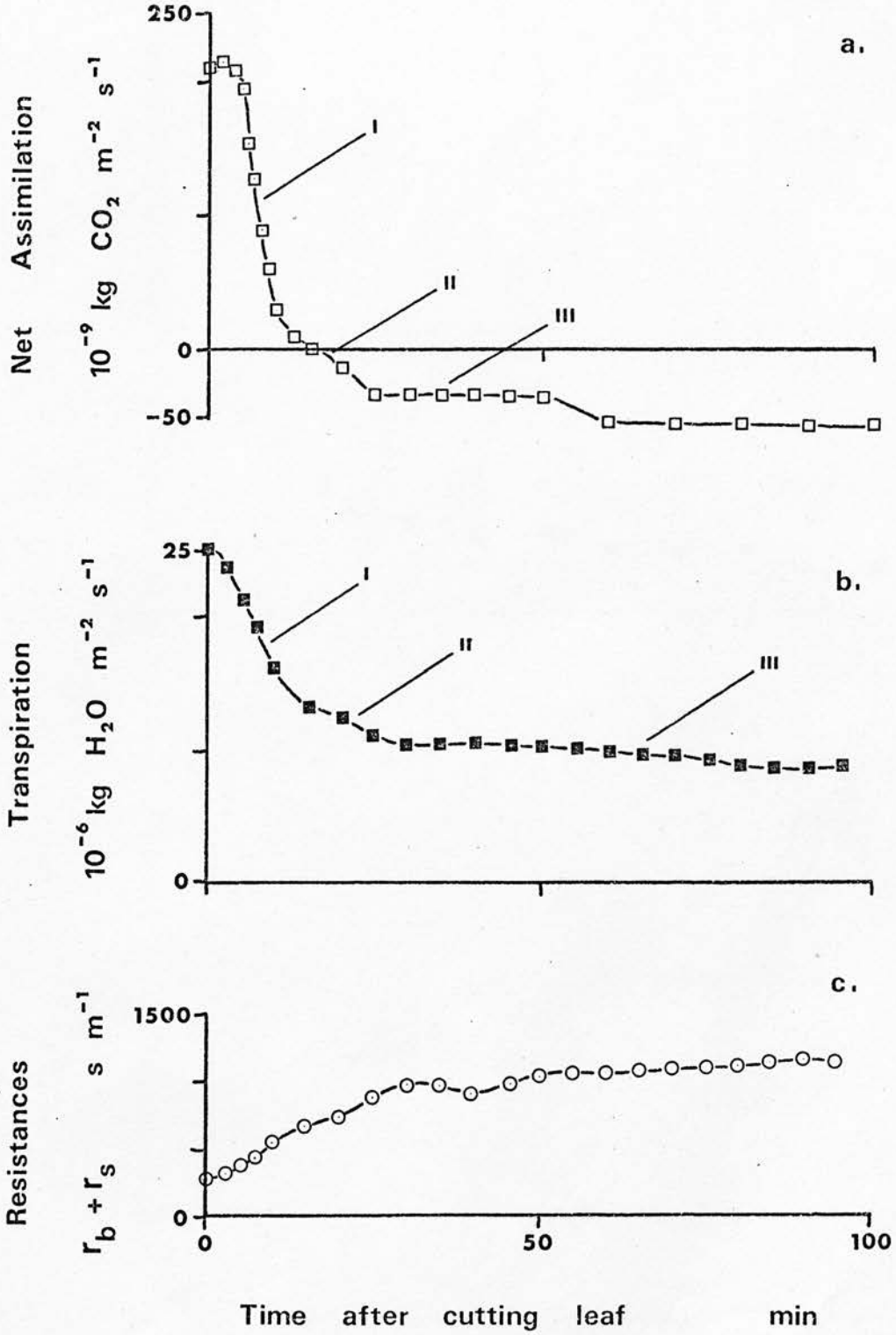


FIG. 2.8 Expt. 2. Changes in P_N, T and $(r_b + r_s)$ for the first 100 min after cutting

A batch of 12 leaves (no. 3) was used. 168 W m^{-2} radiant flux density

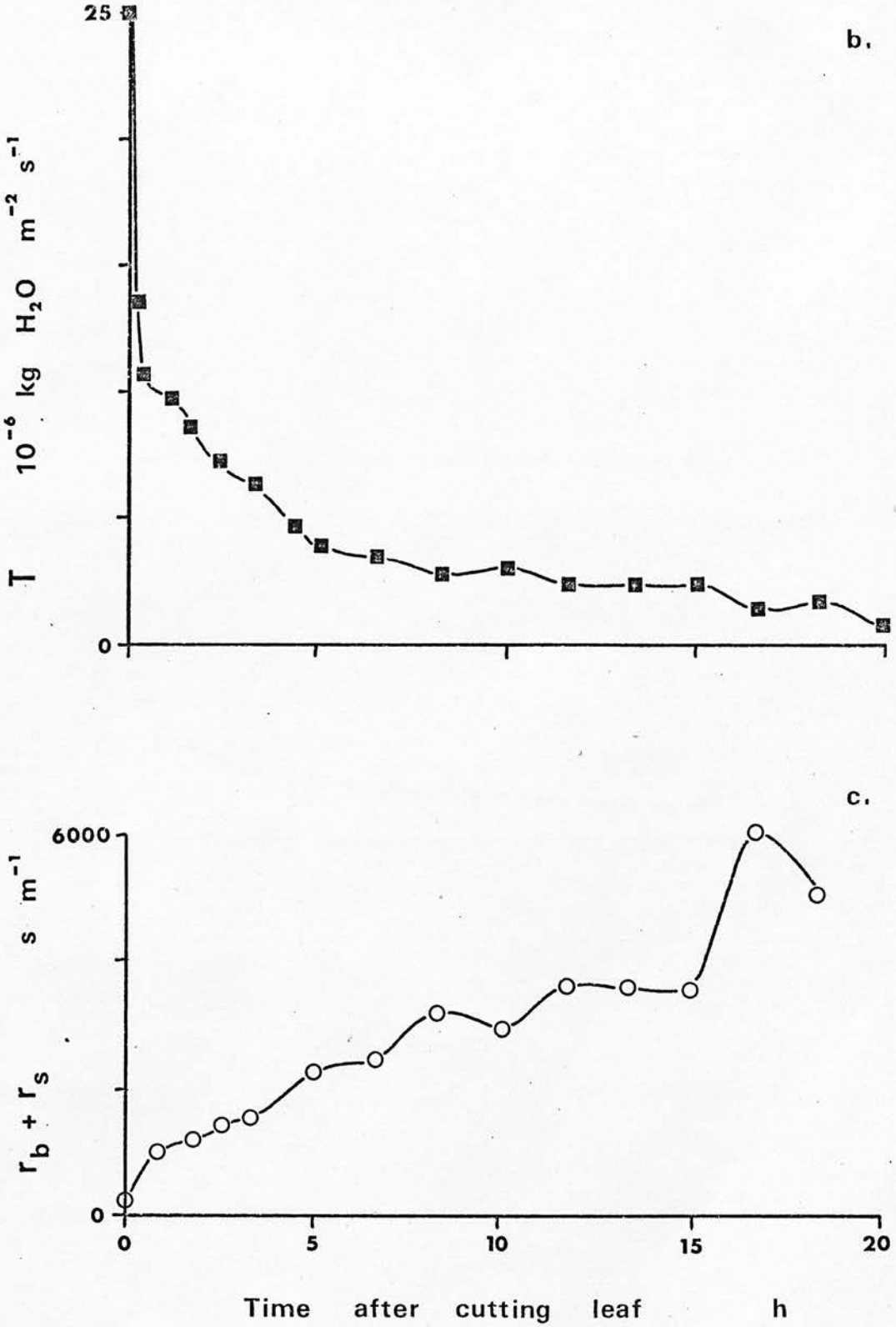
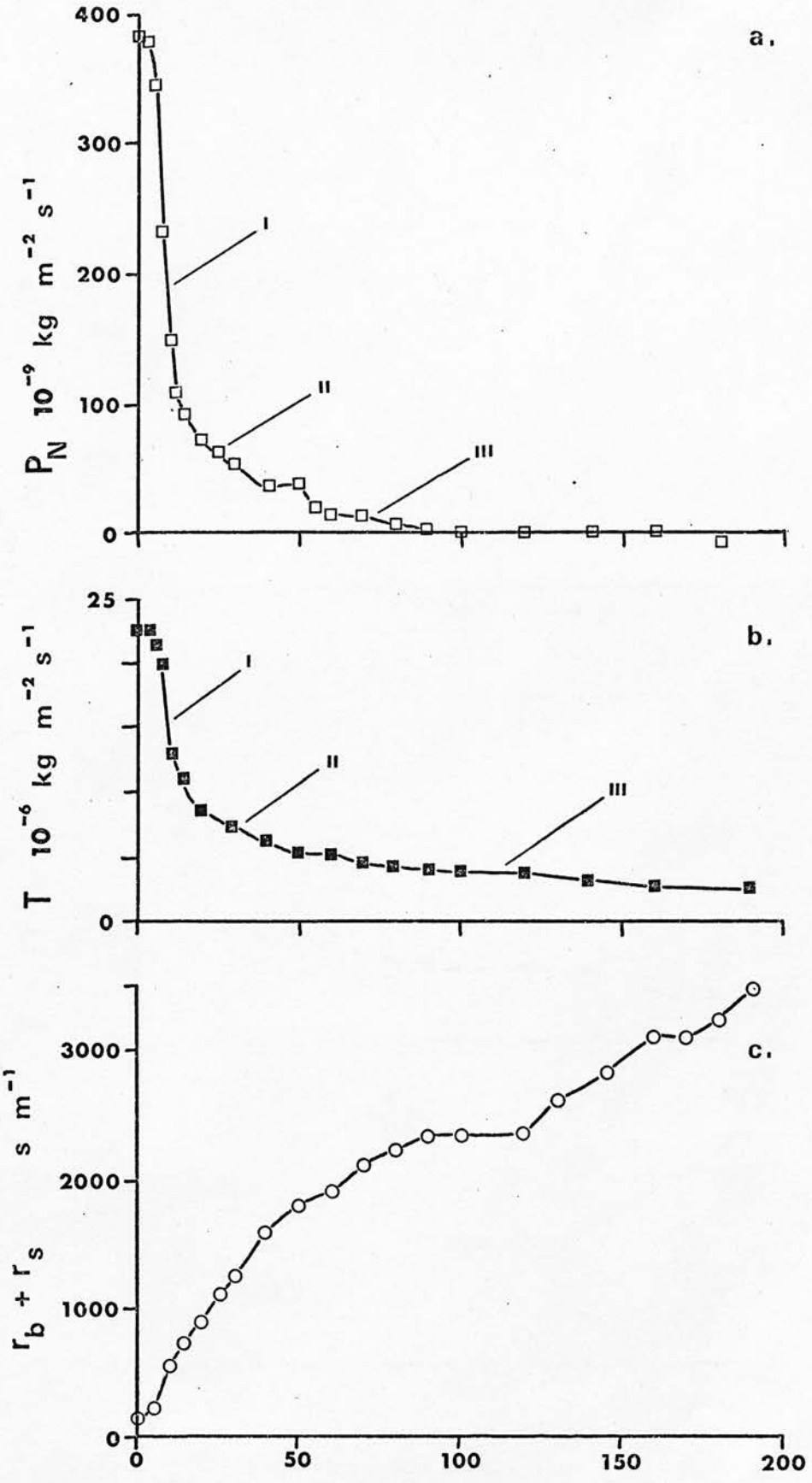


FIG. 2.9 Expt. 2. Changes in P_N and $(r_b + r_s)$ for the first 20 h after cutting

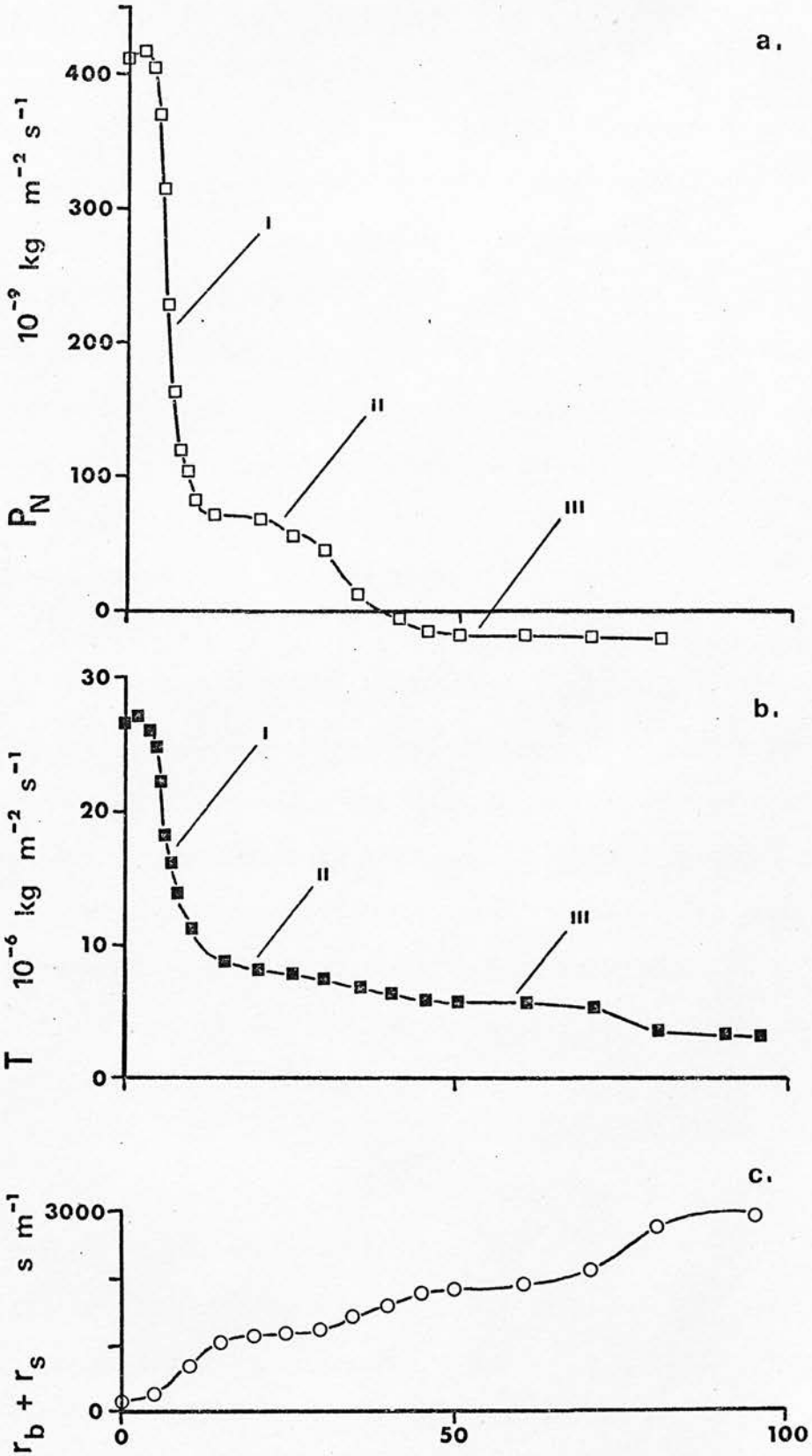
A batch of 12 leaves (no. 3) was used. 168 W m^{-2} radiant flux density

FIG. 2.10 Expt. 3. Changes in P_N, T and $(r_b + r_s)$ after cutting



A batch of 18 leaves (no. 3) was used. 168 W m^{-2} radiant flux density

FIG. 2.11 Expt. 4. Changes in P_N, T and $(r_b + r_s)$ after cutting



A batch of 18 leaves (no. 3) was used. 168 W m^{-2} radiant flux density

LEAF SEGMENT STUDIES: RESULTS

1. Water balance studies

Figs. 2.13, 2.14 and 2.17 show the changes in D M content during 48 h wilting under controlled conditions in Expts. 5, 6 and 7 respectively. During low temperature wilting (15° day, 10° night) with relative humidity 61% and vpd 6.4 mb, Expt. 5, DM content increased from 19% at harvest to 40% after 48 h wilting. During high temperature wilting (20° day, 15° night) with relative humidity 68% and vpd 6.1 mb, Expts. 6 and 7, D M content increased from c. 15% at harvest to between 45% and 75% after 48 h wilting. The 30% D M level was reached after 15 to 26 h wilting.

Fig. 2.12 shows the water uptake by leaf samples during exposure in the saturated foam matrix. Water uptake was most rapid during the first hour after which the D M content of the fresh and wilted samples was about the same. There was little further water uptake after the first hour in fresh and 24-h wilted samples, and after the first three hours in 48-h wilted samples. The capacity for water uptake increased approximately 1.5-fold from fresh to 24-h wilted to 48-h wilted samples.

Figs. 2.16 and 2.19 show the changes in water saturation deficit (W S D) during wilting at 15° in Expts. 3 and 4. W S D increased from c. 13% in fresh samples to c. 45% after 24 h and 55 - 65% after 48 h wilting. However, after a 1 h saturation pretreatment, W S D was reduced greatly to 15% or below in all samples.

2. Photosynthesis studies

Figs. 2.13, 2.14, 2.15 and 2.16 illustrate the changes in photosynthetic capacity during wilting under controlled conditions in Expts. 5, 6, 6 and 3 respectively. Net assimilation (P_N) was c. 150 units* in fresh leaf samples but fell to zero within first 12 - 24 h in Expts. 5 and 6. After 24 h wilting a net dissimilation of between 10 and 120 units was found; after 48 h wilting net dissimilation had increased to between 95 and 225 units in Expts. 5 and 6, in which a sample pretreatment was not used. However, the corresponding values of net assimilation were 188, 27 and 5 units in fresh, 24-h and 48-h wilted samples when a 1-h saturation pretreatment was given, Expt. 3 (Fig. 2.16). Net dissimilation was not found under these conditions.

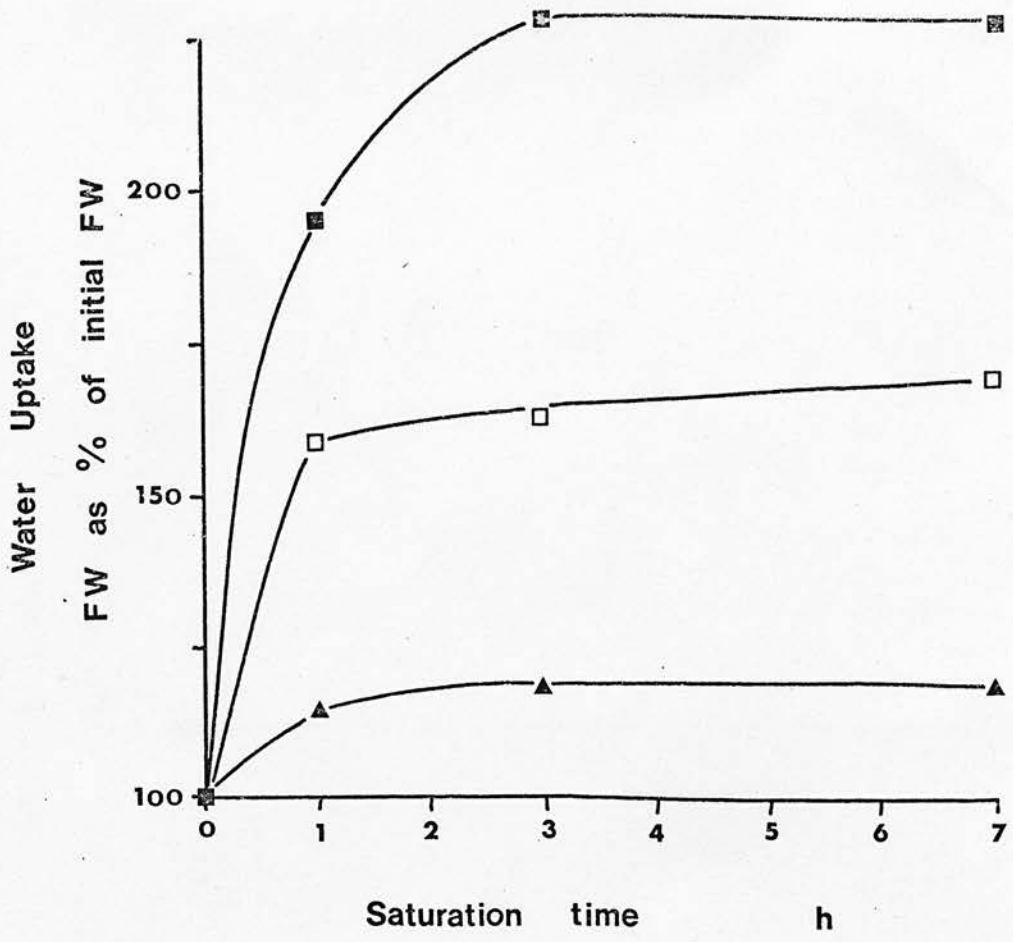
Fig. 2.15 shows the relationship between net assimilation and D M content in Expt. 6. A decrease in net photosynthetic capacity with increasing D M content was found. Net photosynthetic capacity was zero above 27% D M after which a net dissimilation was recorded.

3. Respiration studies

Figs. 2.17, 2.18 and 2.19 illustrate the changes in respiratory capacity during wilting under controlled conditions in Expts. 7, 7 and 4 respectively. Dark respiration was 18 units* in fresh samples rising to 73 units and 260 units after 24 h and 48 h wilting when a pretreatment of the leaf samples was not used, Expt. 7. However, the corresponding values were 2, 17 and 62 units when a 1-h saturation pretreatment was given, Expt. 4.

Fig. 2.18 shows the relationship between dark respiration and D M content, Expt. 7. An increase in respiratory capacity with increasing D M content was found.

*For units, refer to Figs.



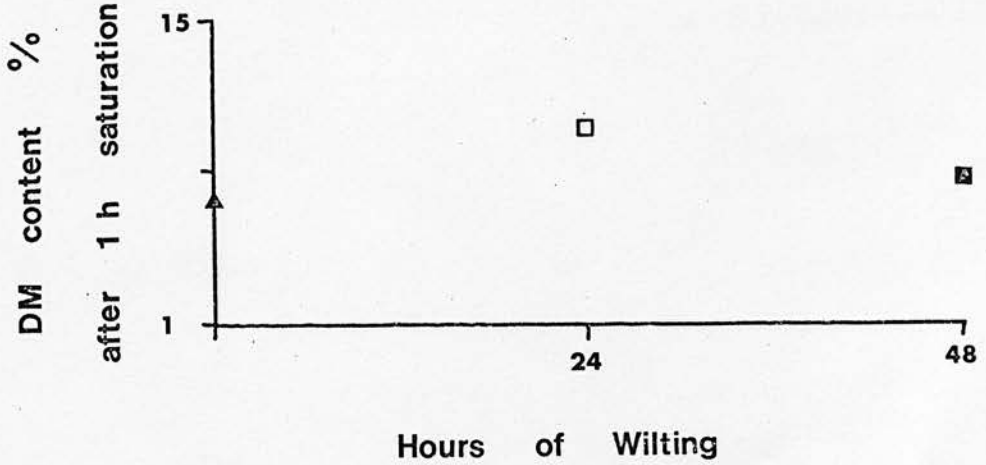


FIG. 2.12 The water relations of experimental samples during exposure. Expt. 3

a. Water uptake by leaf 1 samples during the exposure perio

b. D M content of leaf samples after 1 h saturation pre-treatment

▲, fresh samples; □, 24-h wilted samples; ■, 48-h wilted samples

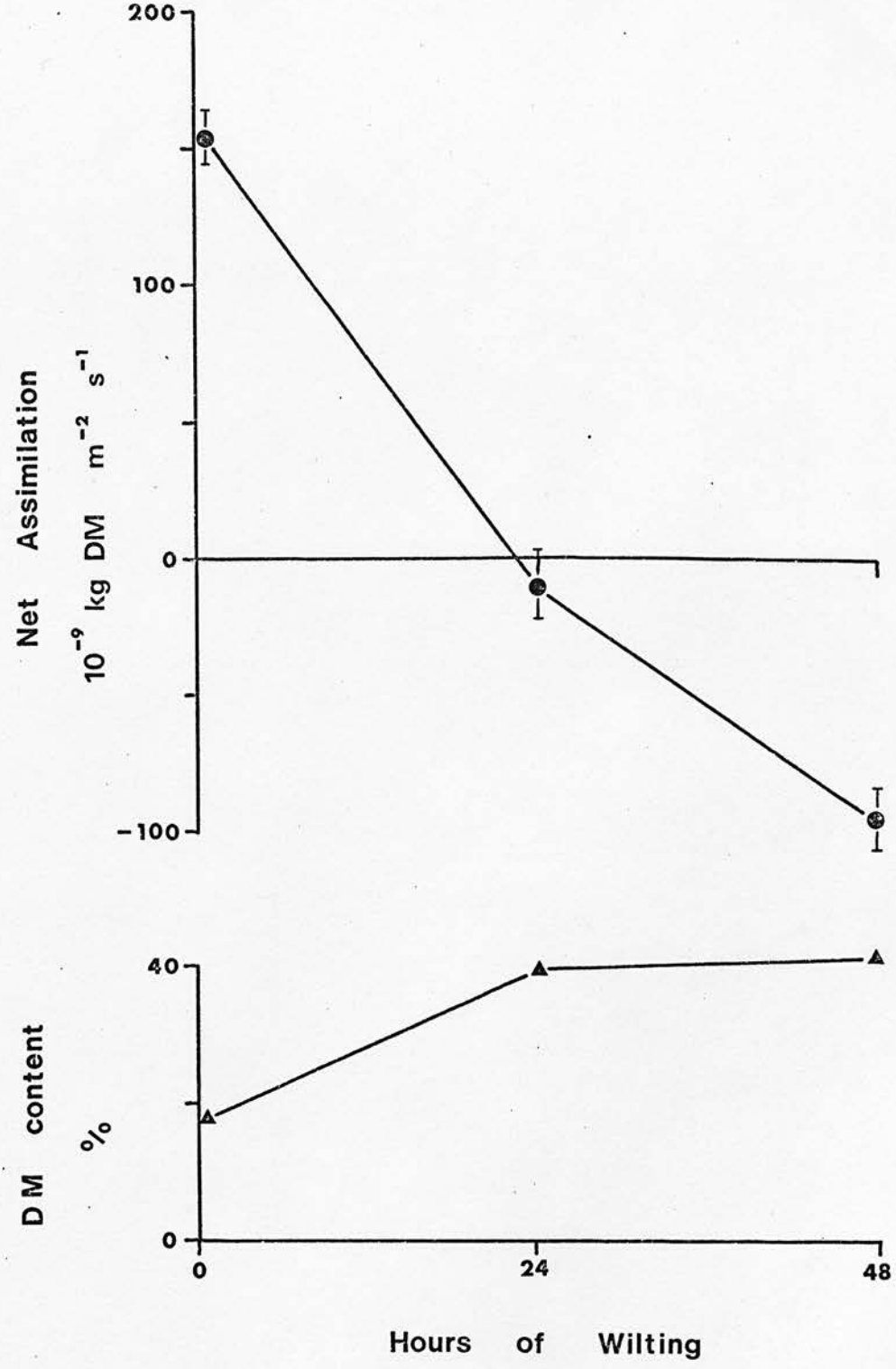


FIG. 2.13 Changes in photosynthetic capacity and D M content during 48 h wilting, Expt. 5

Bars indicate standard errors

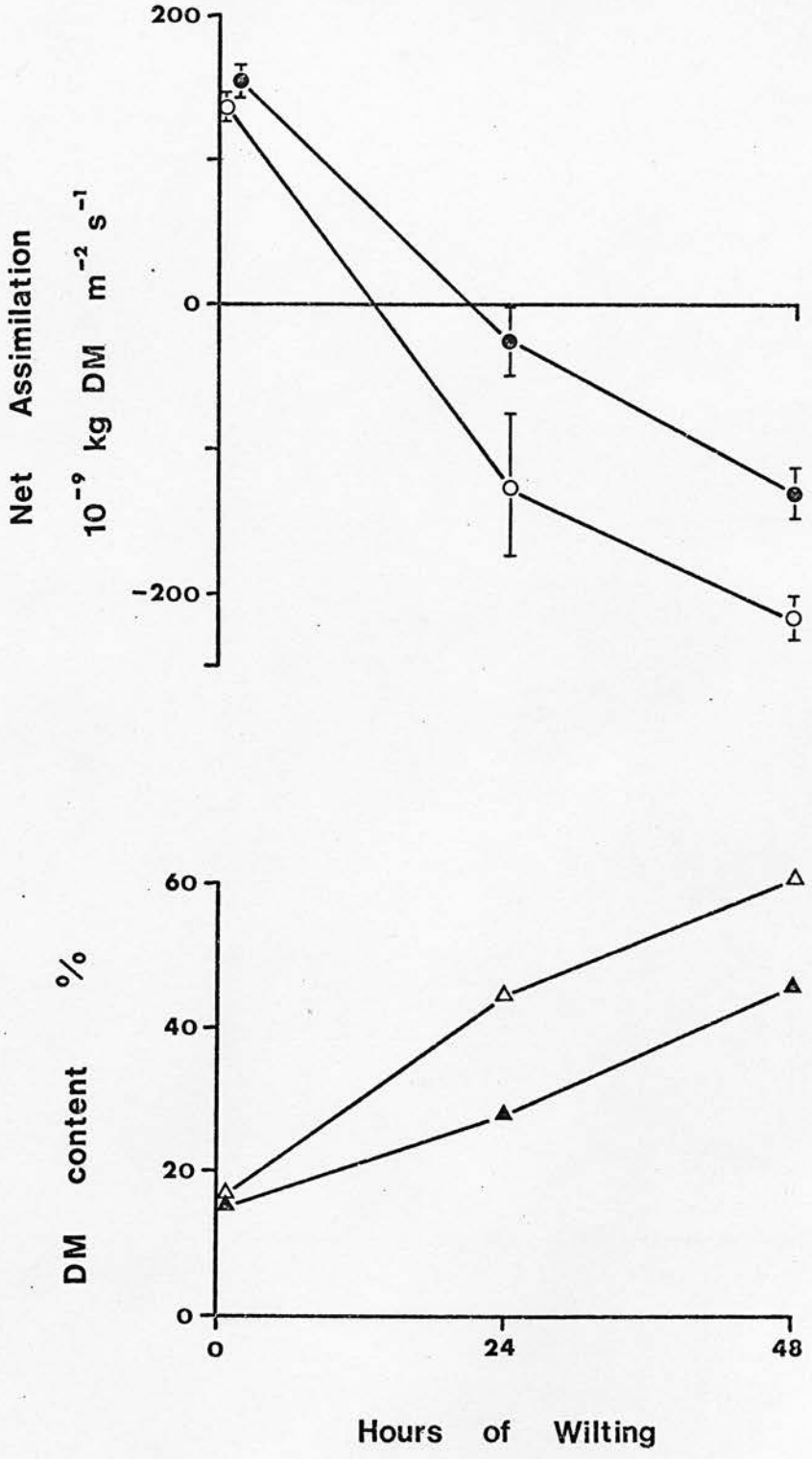


FIG. 2.14 Changes in photosynthetic capacity and D M content during 48 h wilting, Expt. 6

Bars indicate standard errors
Open and closed symbols indicate two sets of samples

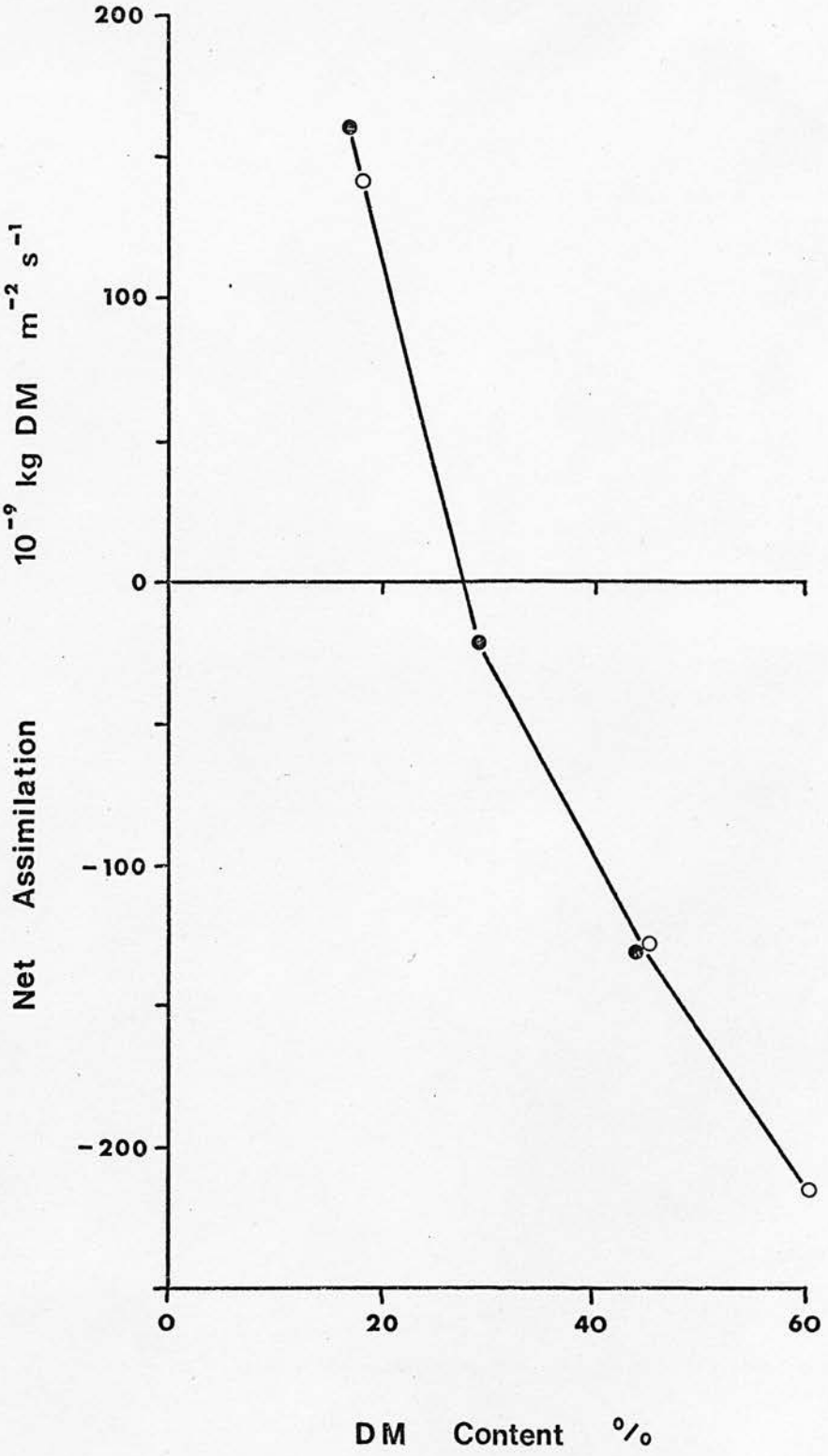


FIG. 2.15 Relationship between photosynthetic capacity and D M content during wilting, Expt. 6

Open and closed symbols indicate two sets of samples

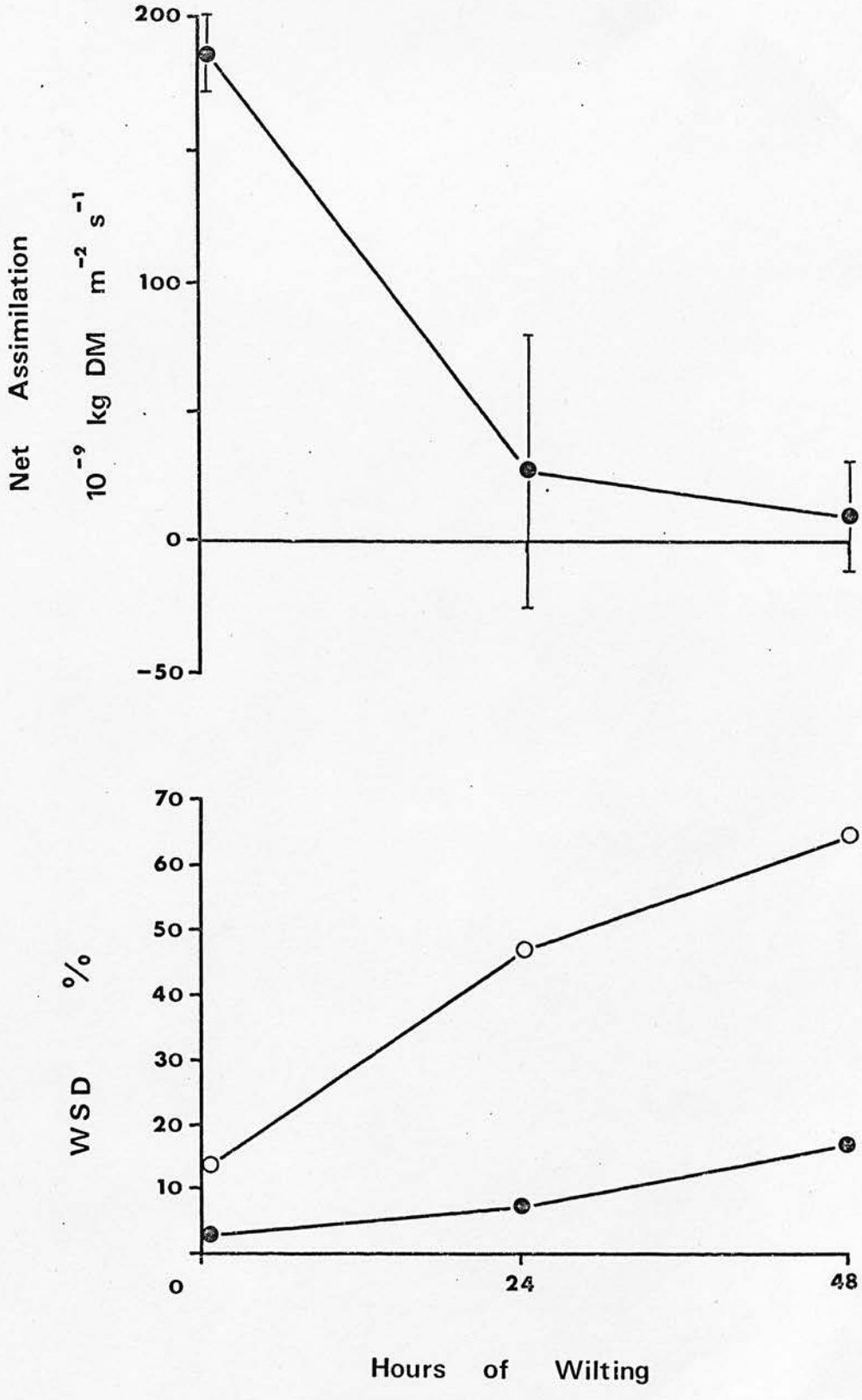


FIG. 2.16 Changes in photosynthetic capacity and W S D during 48 h wilting after 1 h saturation pre-treatment, Expt. 3

● , experimental samples given a 1 h saturation pre-treatme

O , experimental samples without a 1 h saturation pre-treat

Bars indicate standard errors

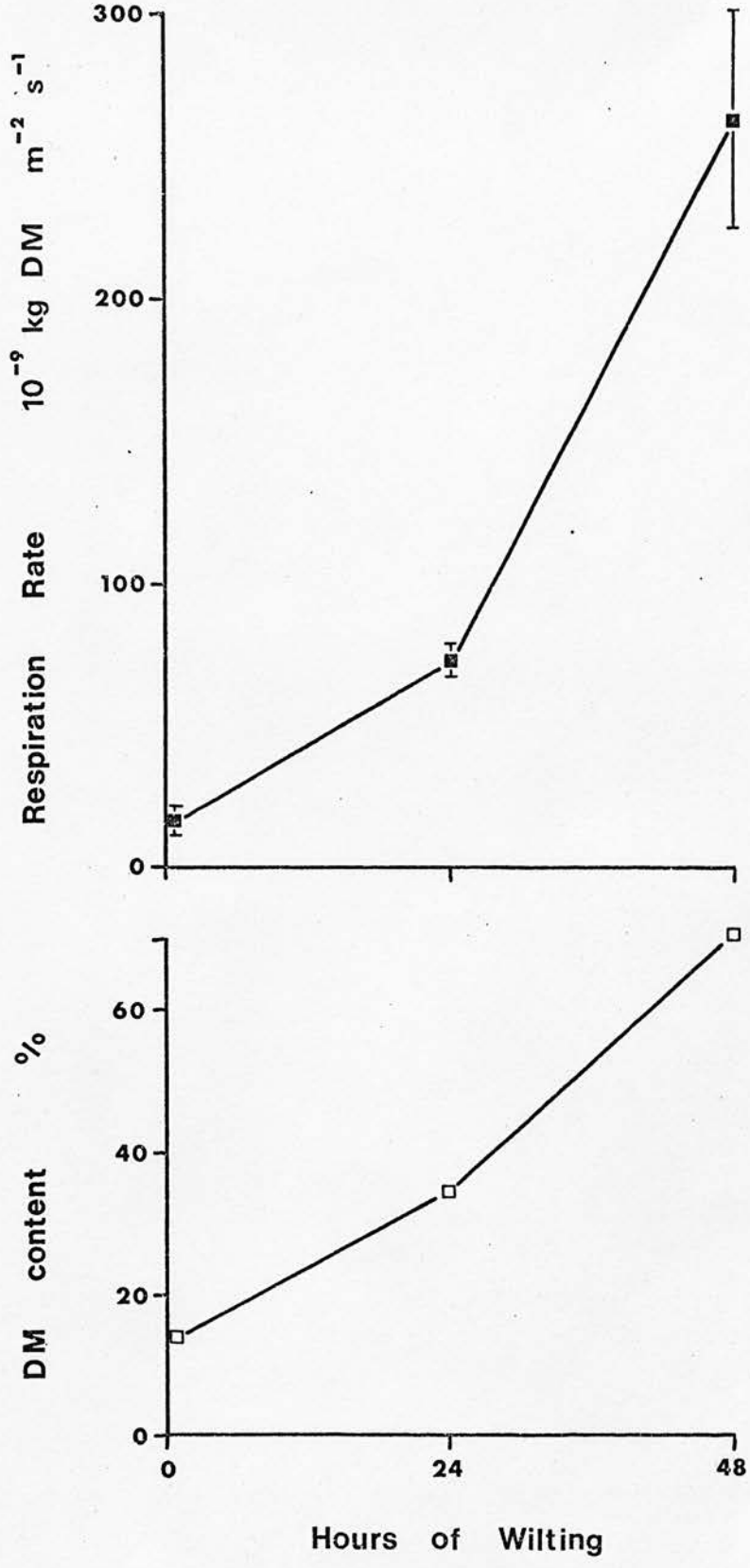


FIG. 2.17 Changes in respiratory capacity and D M content during 48 h wilting, Expt. 7

Bars indicate standard errors

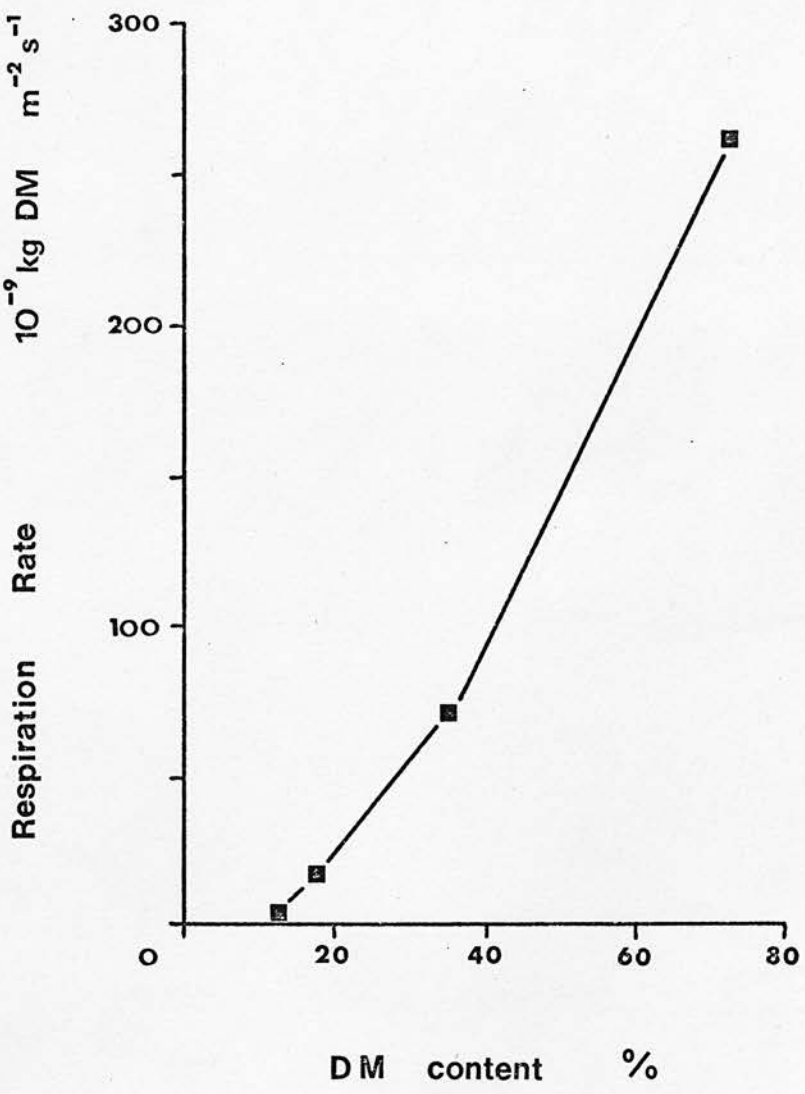


FIG. 2.18 Relationship between respiratory capacity and D M content during wilting, Expt. 7

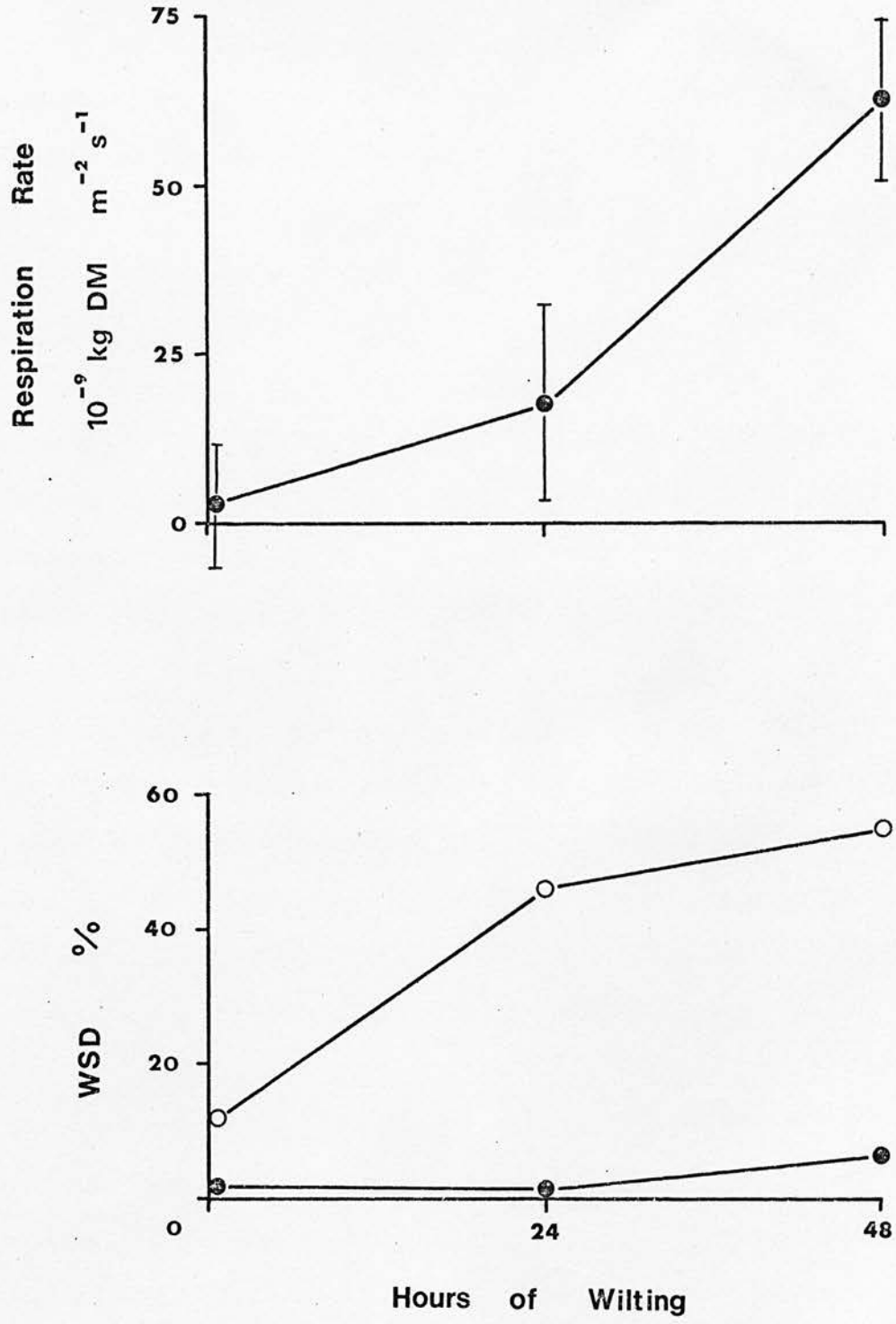


FIG. 2.19 Changes in respiratory capacity and W S D during 48 h wilting, after 1 h saturation pre-treatment, Expt. 4

● , experimental samples given a 1 h saturation pre-treatment

○ , experimental samples without a 1 h saturation pre-treatment

Bars indicate standard errors

STOMATAL STUDIES: RESULTS

a. Light microscopy

1. Direct observation. Direct observation of fresh leaf material mounted in water or glycerol revealed the presence of stomata on the adaxial surface and layers of mesophyll cells containing chloroplasts below. The stomata could not be studied in detail since the many cell layers visible caused interference. The network of epidermal cells and the arrangement of the stomata, in rows parallel to the long axis of the leaf, was much clearer in decolourised material. No stomata were found on the abaxial surface. However this method was unsatisfactory for the routine examination of the condition of the stomata. Treatment with I/KI solution resulted in no change in the appearance of the leaf: no evidence of starch was found.

2. Lloyd's method was unsuccessful because of the ridged nature of the adaxial surface and the adherence of the mesophyll to the abaxial surface. It was impossible to obtain epidermal strips containing stomata. Only long colourless sclerenchyma-type cells could be stripped off the adaxial surface ridges; and patchy areas of lower epidermis obscured by green cells were obtained from the abaxial surface.

3. Leaf impressions prepared by both the perspex/acetone and the silicone rubber methods produced clear replicas of the two epidermal surfaces. The abaxial epidermis consisted of a smooth layer of uniform rectangular or rhombic cells. There were no hairs and no stomata. The adaxial epidermis was deeply ridged, consisting of alternate ridges and furrows running parallel to the long axis of the leaf. The leaf was angled at the mid-rib and more flattened towards the edges. The pattern of cells was clearly distinguished and formed a regular arrangement along the ridges and furrows. The epidermal cells of the adaxial

surface were in general long and rectangular or hexagonal, those on the tops of the ridges and in the depths of the furrows being particularly long. Hairs of non-glandular character were present on the tops of the ridges. The stomata had a restricted distribution, occurring on the sloping sides of the ridges in fairly narrow longitudinal bands three to four cells thick.

The stomatal structure was typical of the Graminae with long thin dumbell-shaped guard-cells and ovoid accessory cells. Difficulty occurred in determining whether the stomata were open or closed, through their characteristic form and angular position. Adjustment of the depth of focus gave the illusion of the stomata opening and closing since the central slit-like pore appeared to widen and darken.

Stomatal behaviour on cutting leaf

a. Light microscopy. Leaf impressions were prepared by the perspex/acetone method from leaf samples taken from the I.R.G.A. assimilation chamber at 0, 15 and 30 min after cutting, Expt. 4, Fig. 2.11. The leaf impressions were examined by light microscopy and photographed. Plates 2.1, 2.2, 2.3 and 2.4 show the adaxial epidermis of the leaf after 0, 0, 15 and 30 min had elapsed.

The arrangement of the stomata was clear, but it was impossible to distinguish the details of stomatal behaviour by this method.

b. Scanning electron-microscopy. Leaf samples were taken from the assimilation chamber at 0, 15 and 30 min after cutting, Expt. 4, and fixed immediately using liquid nitrogen. The fixed leaf tissue samples were subsequently examined using conventional scanning-electron-microscopy techniques. Plates 2.5 - 2.9 show the appearance of the

stomata after cutting. Immediately after cutting, the stomata all appeared to be open, Plates 2.5, 2.6. After 15 min of isolation, the stomata all appeared to be closed, Plate 2.7. After 30 min all the stomata were tightly closed, except for one stoma near an injury to the lamina, Plates 2.8, 2.9.

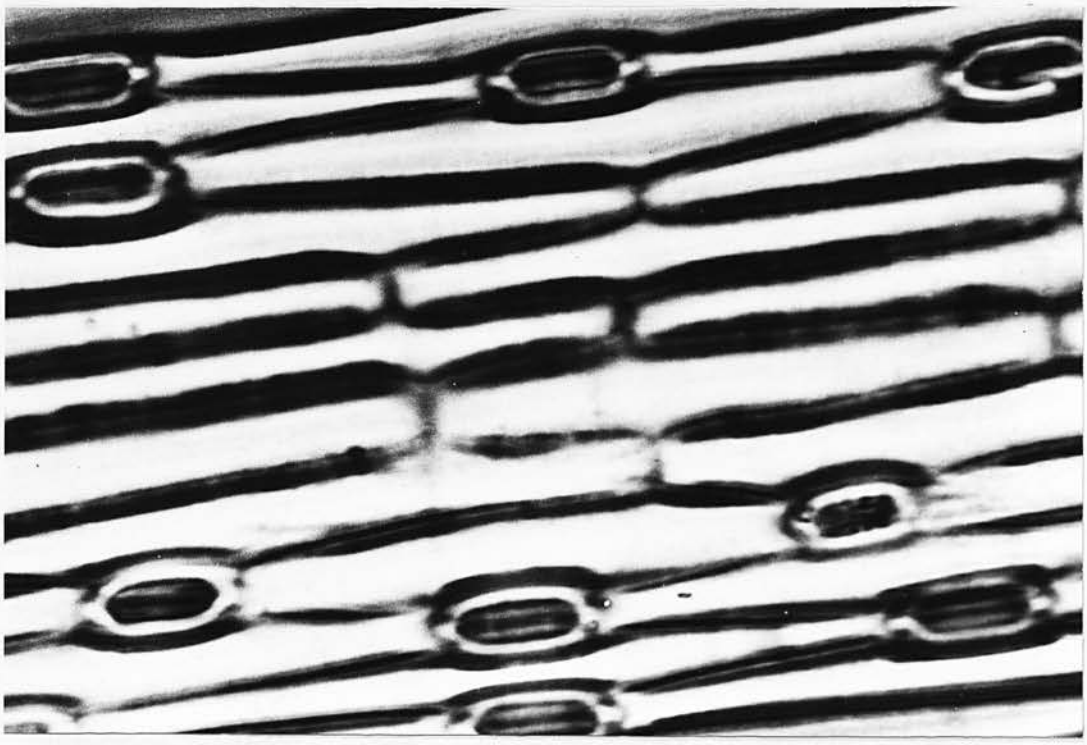


PLATE 2.1 **x 500**

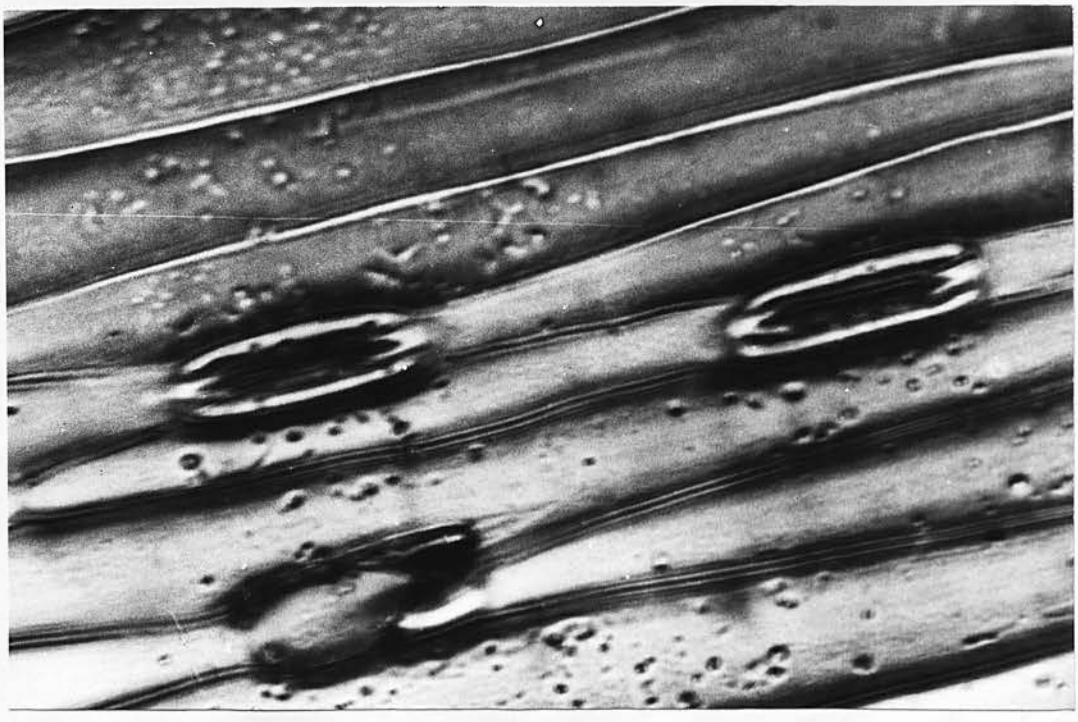


PLATE 2.2 **x 1000**

Perspex/acetone impressions of the adaxial epidermis of a Lolium multiflorum leaf at zero time

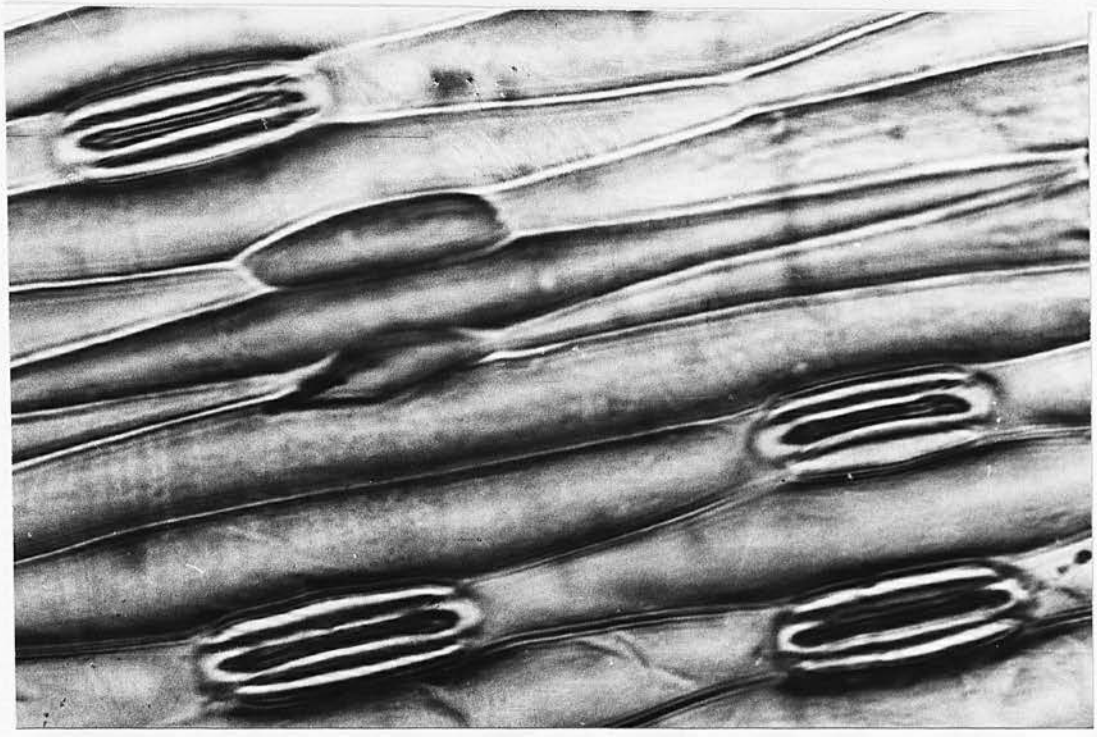


PLATE 2.3 **× 1000**

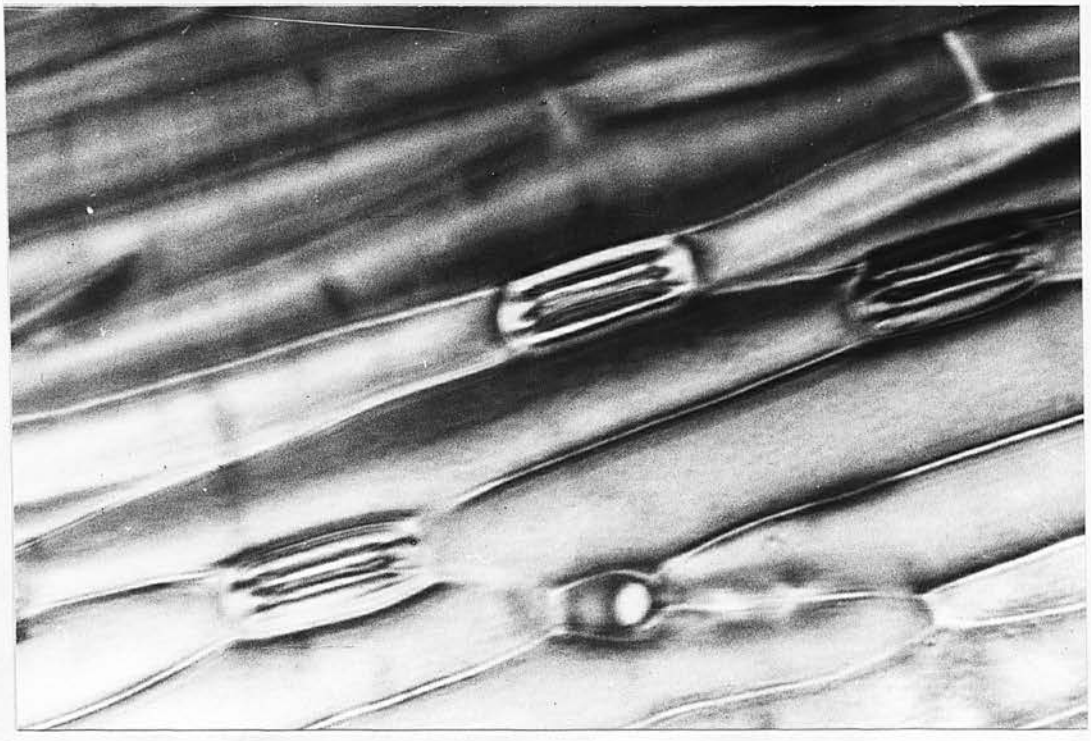
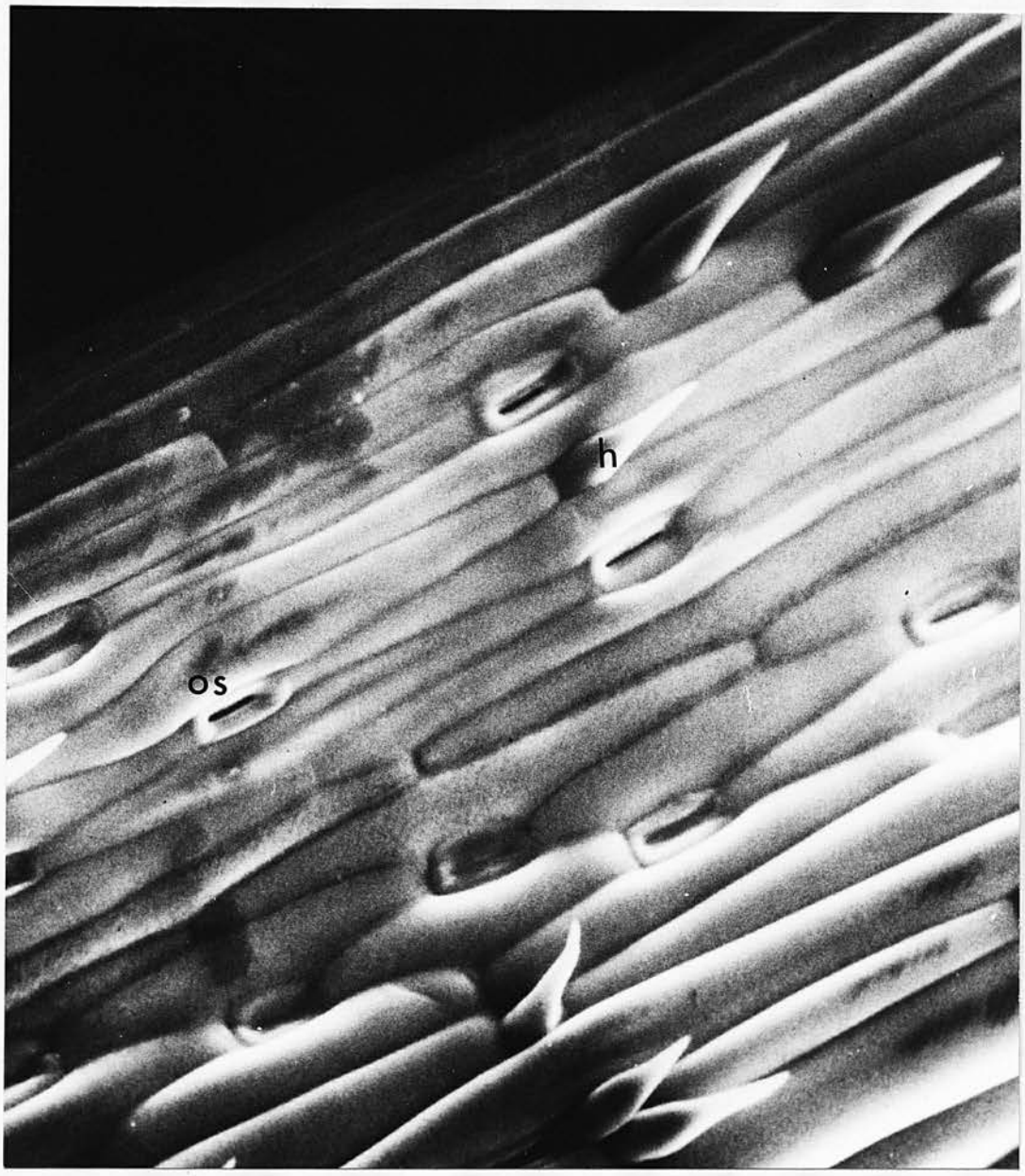


PLATE 2.4 **× 1000**

Perspex/acetone impressions of the adaxial epidermis of Lolium multiflorum leaves at 15 min, Plate 2.3, and 30 min, Plate 2.4, after cutting

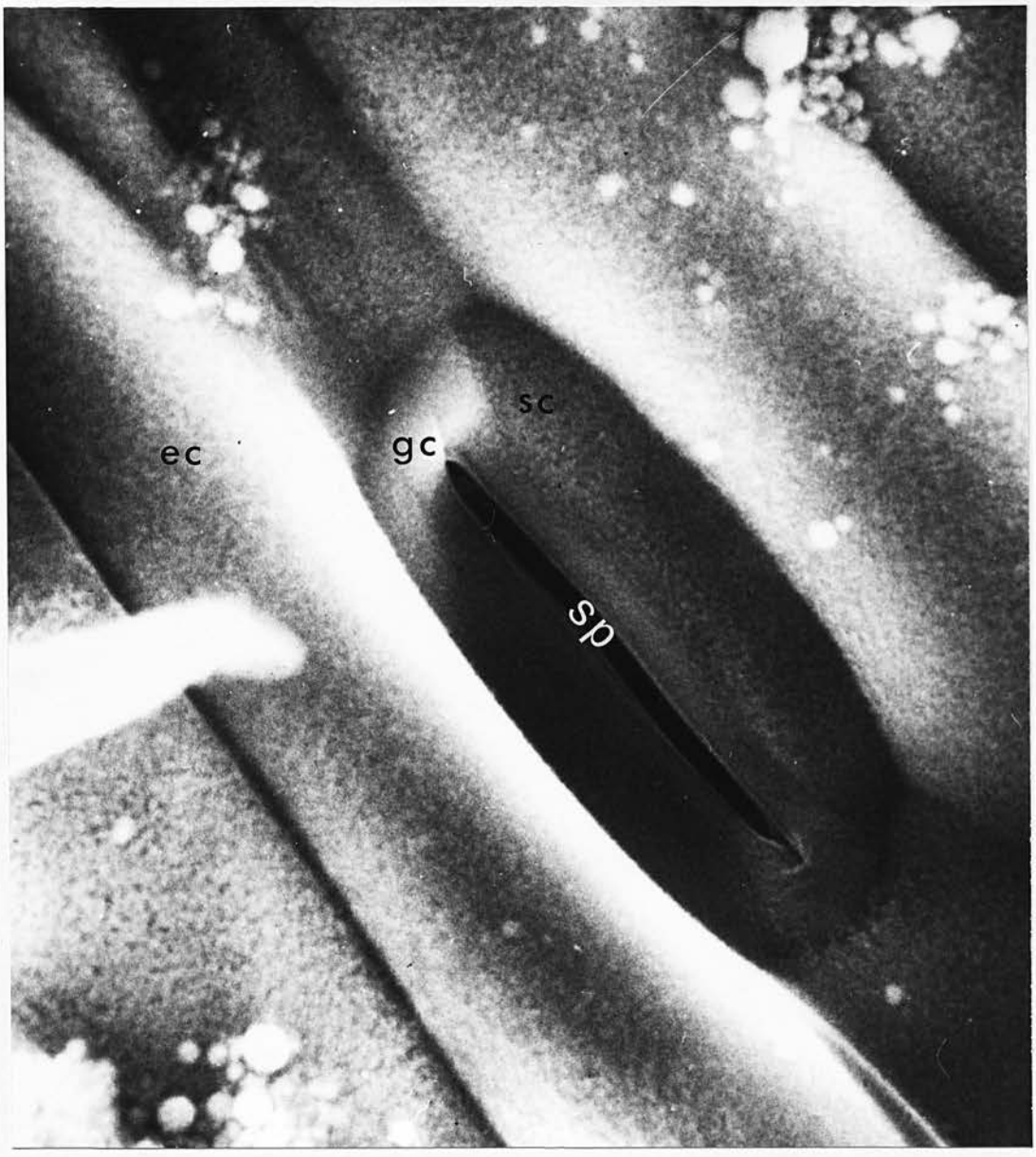


× 400

PLATE 2.5 Scanning electron micrograph of the adaxial epidermis of a Lolium multiflorum leaf at zero time

o s = open stoma

h = epidermal hair



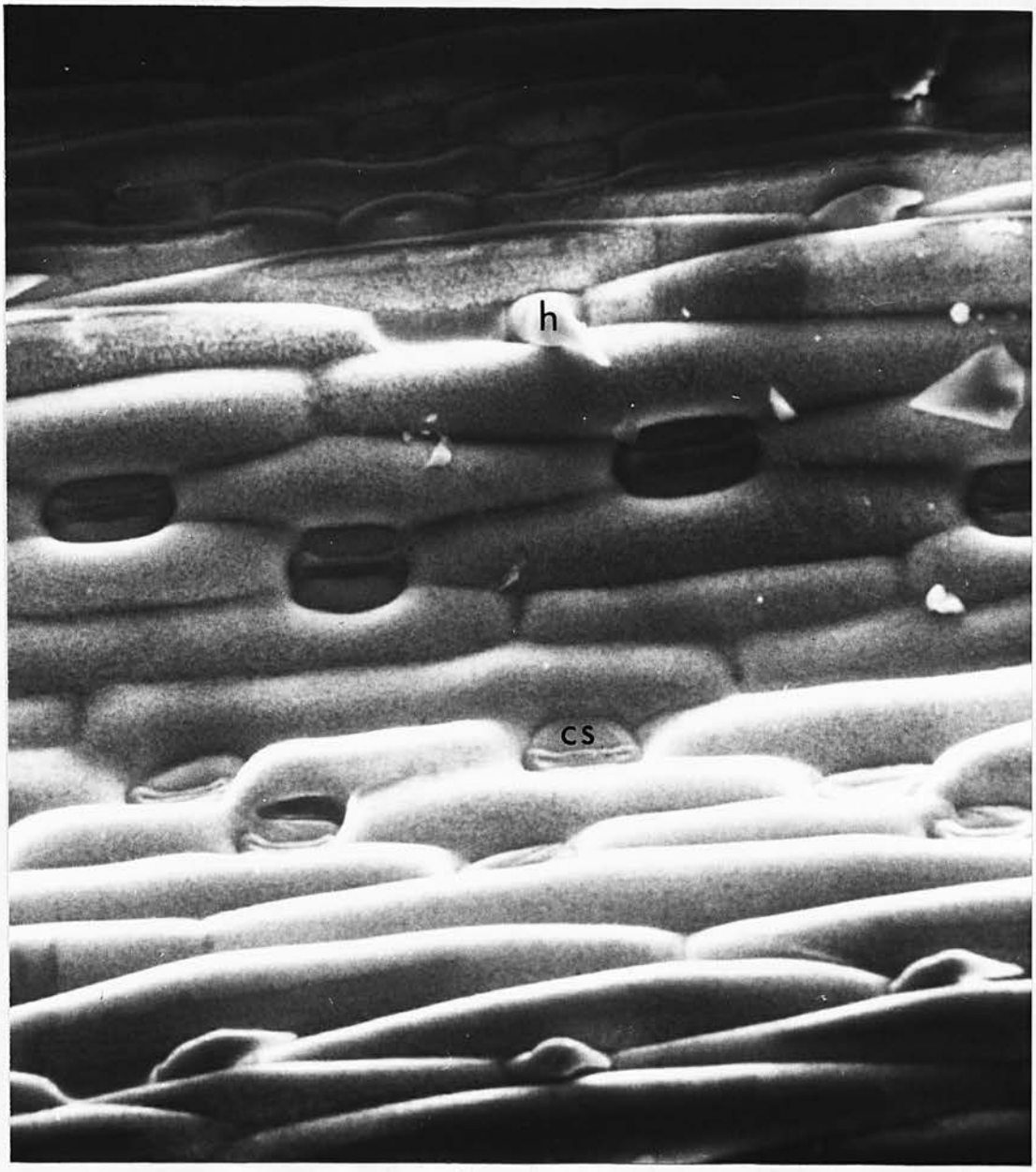
x 4500

PLATE 2.6 Scanning electron micrograph of a single stoma of Lolium multiflorum at zero time

g c = guard cell
s c = subsidiary cell
s p = stomatal pore
e c = epidermal cell

× 1250

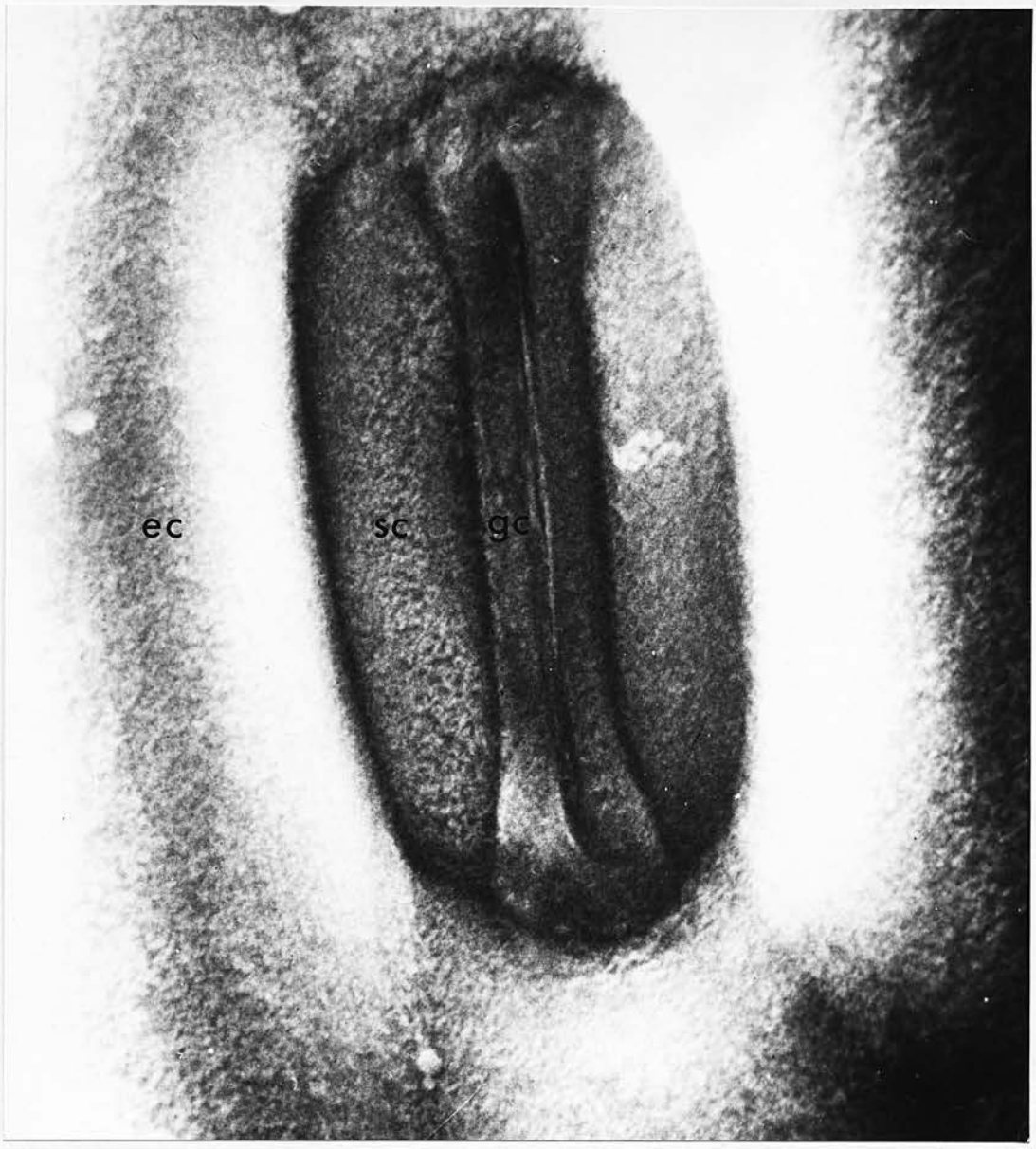
PLATE 2.7 Scanning electron micrograph of the adaxial epidermis of a *Lolium multiflorum* leaf at 15 min after cutting; c s = closed stoma.



× 900

PLATE 2.8 Scanning electron micrograph of the adaxial epidermis of a Lolium multiflorum leaf at 30 min after cutting

c s = closed stoma
h = epidermal hair



× 6000

PLATE 2.9 Scanning electron micrograph of a single stoma of Lolium multiflorum at 30 min after cutting the leaf

g c = guard cell
s c = subsidiary cell
e c = epidermal cell

BIOCHEMISTRY OF WILTING: RESULTS

Fig. 2.20 shows the changes in D M content of *Lolium multiflorum* leaves during wilting under controlled conditions. D M content increased from 18 - 23% in fresh grass to 45 - 60% after 48 h wilting, except when samples were enclosed under nitrogen when the D M content did not change.

Figs. 2.21 - 2.26 illustrate the changes in some biochemical constituents of grass leaves during wilting.

1. Water-soluble carbohydrates

Fig. 2.21 indicates that soluble carbohydrates tended to decrease over the wilting period although oscillations occurred. The changes in fructose levels followed the changes in total soluble carbohydrates, but the glucose levels varied independently and with less magnitude. Oscillations were particularly great during dark wilting in air, when changes in sugar levels of more than 50% in 12 h were recorded. In general, a decline in total W S C and fructose contents during the first 12 h was followed by an increase to a peak at 24 h, then a second decline and sometimes a second increase until 48 h of wilting had elapsed. Glucose levels showed a small decrease (light wilting) or increase (dark wilting) over the whole wilting period.

2. Nitrogenous components

During light wilting (Fig. 2.22) protein changes were irregular, but there was a 2- to 3-fold increase in free amino groups and a corresponding fall in the protein:free NH_2 ratio which was especially marked during the first 9 h. After 18 - 24 h wilting, there was little further

change in the protein:free NH_2 ratio. The initial rapid increase in free amino groups and the falling ratio were more pronounced at 20° than at 15° after 3 h of wilting had elapsed.

During dark wilting (Fig. 2.23) the changes in nitrogenous components were more irregular. There was an overall decrease in protein levels, oscillation in free amino groups and a general decrease in the protein:free NH_2 ratio. In the nitrogen treatment, the ratio followed a similar pattern to that found in light wilting, i.e. an early rapid decrease to a low level. The initial fall was not found during dark wilting in air when the ratio showed a peak during the first 12 h.

3. Chlorophyll

Fig. 2.24 shows the absorption spectra of the chlorophyll extracts before and after 48 h of wilting. There was little change after light wilting in air apart from a slight decrease in the main chlorophyll *a* peak, 663 nm. However, large changes in the chlorophyll absorption spectrum were found after dark wilting in nitrogen. The main peaks showed both a marked reduction in size and a shift in position, peak 1 from 663 to 665 nm; peak 2 from 620 to 615 nm, and peak 3 disappeared altogether.

Figs. 2.25 and 2.26 show the changes in chlorophyll content, expressed on a D M basis and a leaf area basis respectively, during wilting. When expressed on a D M basis, chlorophyll levels showed a general tendency to decrease over the 48-h wilting period. The decrease was greater at 20° than at 15° during light wilting, and greater in nitrogen than in air during dark wilting. The greatest loss was

recorded with the nitrogen treatment. However, when expressed on a leaf area basis, Fig. 2.26, the chlorophyll content appeared to increase or remain the same (light wilting) or decrease (dark wilting in nitrogen).

FIG. 2.20 Changes in grass D M content during wilting under controlled conditions

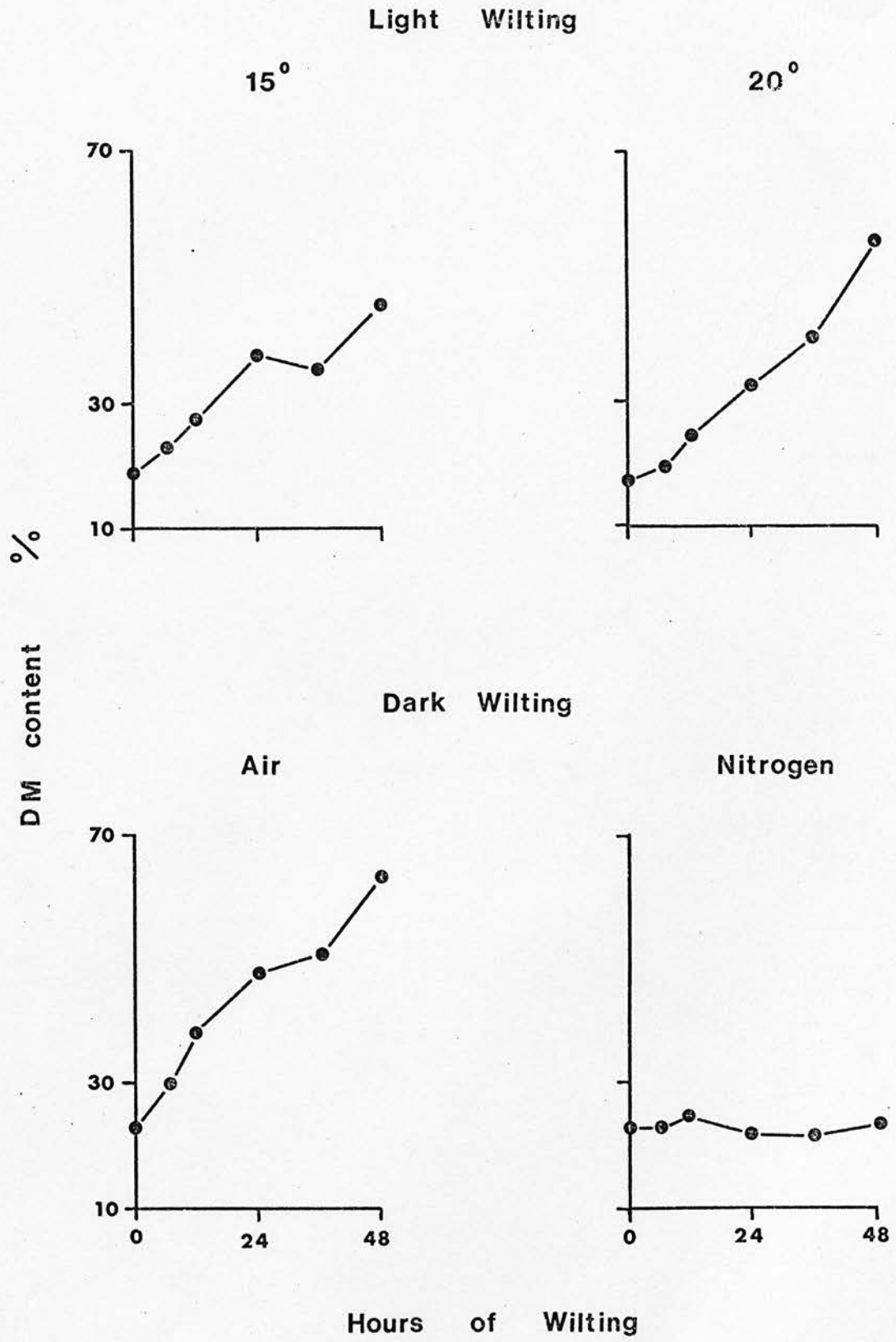
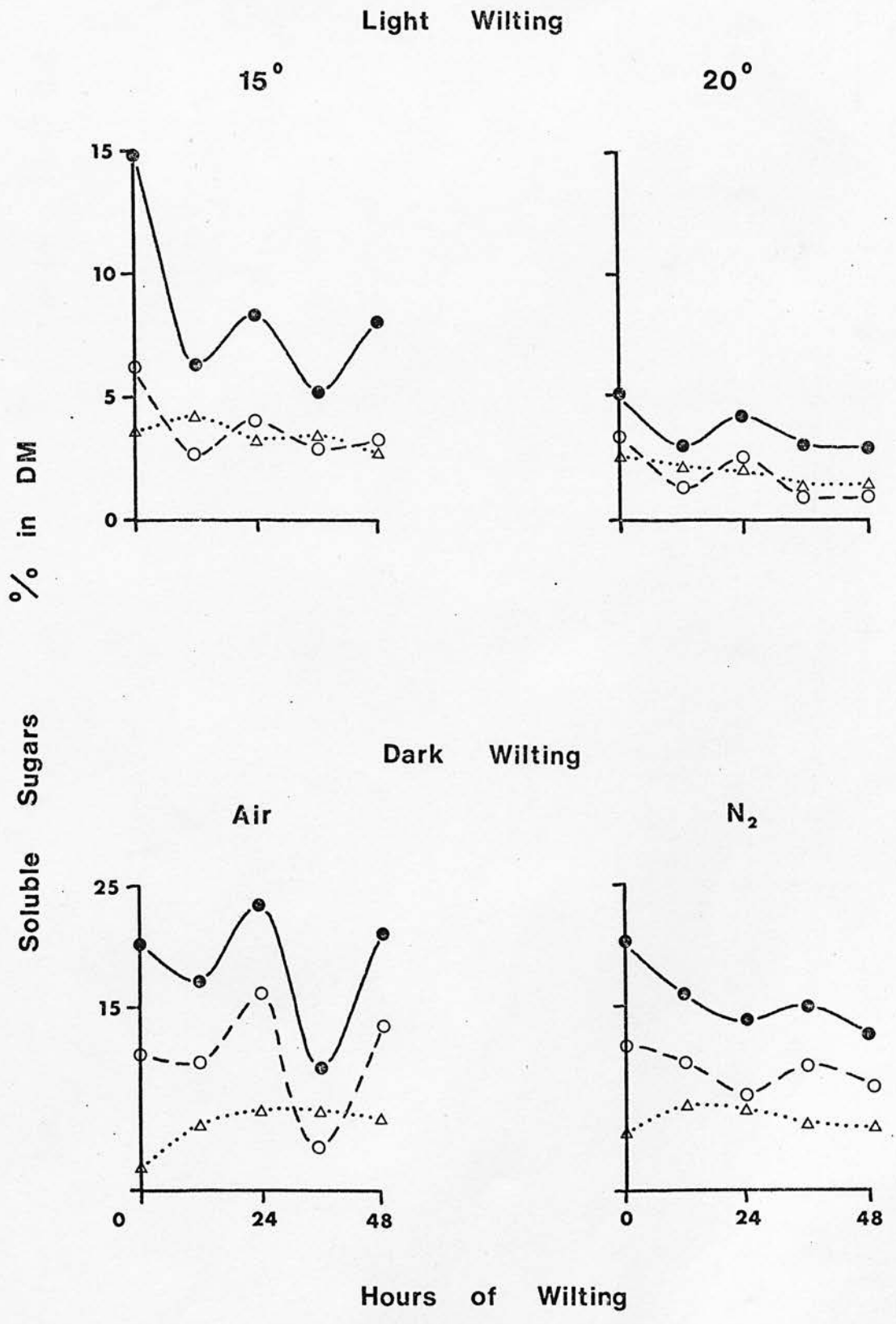
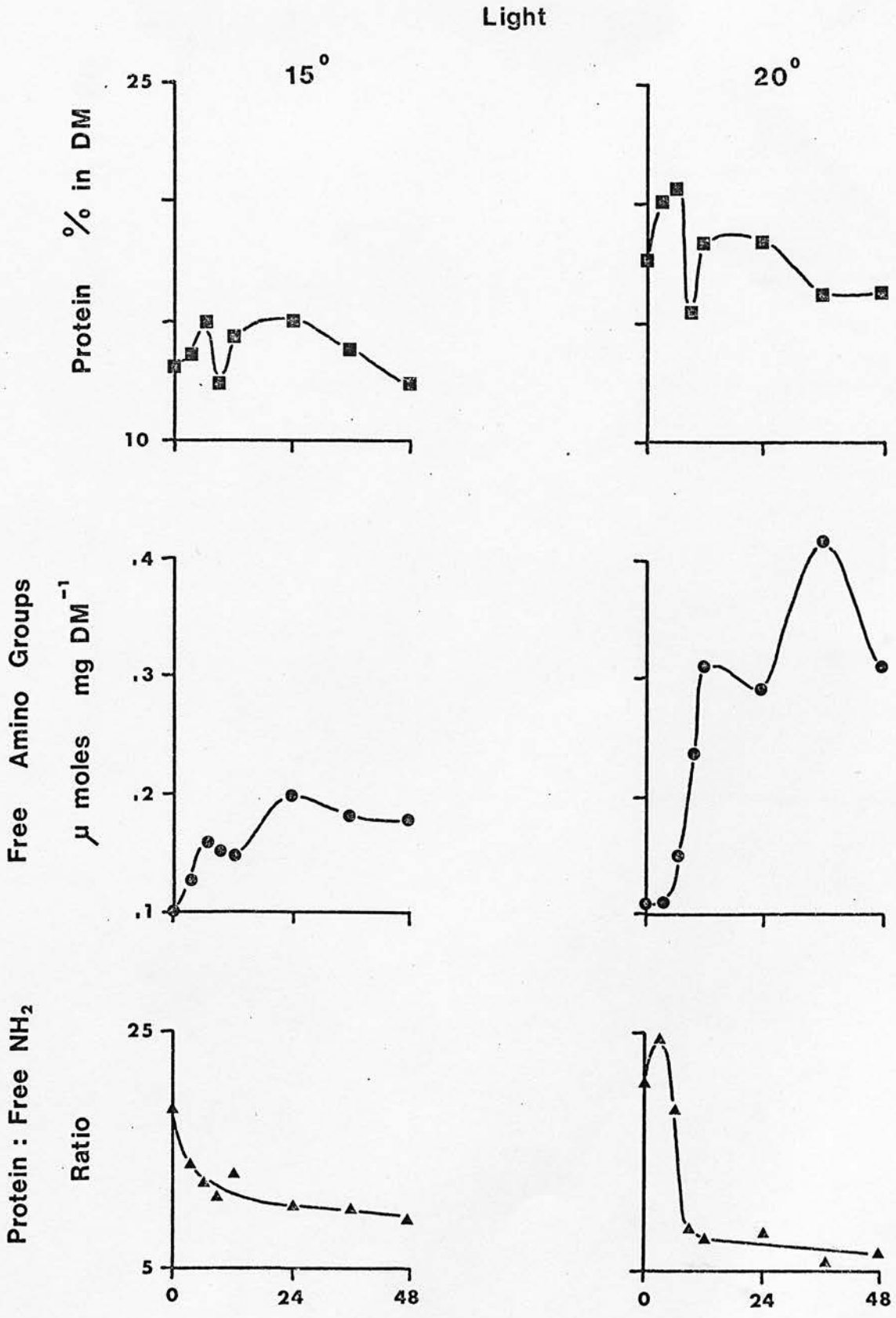


FIG. 2.21 Changes in soluble sugars during wilting under controlled conditions



● , total water-soluble carbohydrates; ○ , fructose;
△ , glucose: all determined following hydrolysis

FIG. 2.22 Changes in nitrogeneous components during wilting under controlled conditions in the light

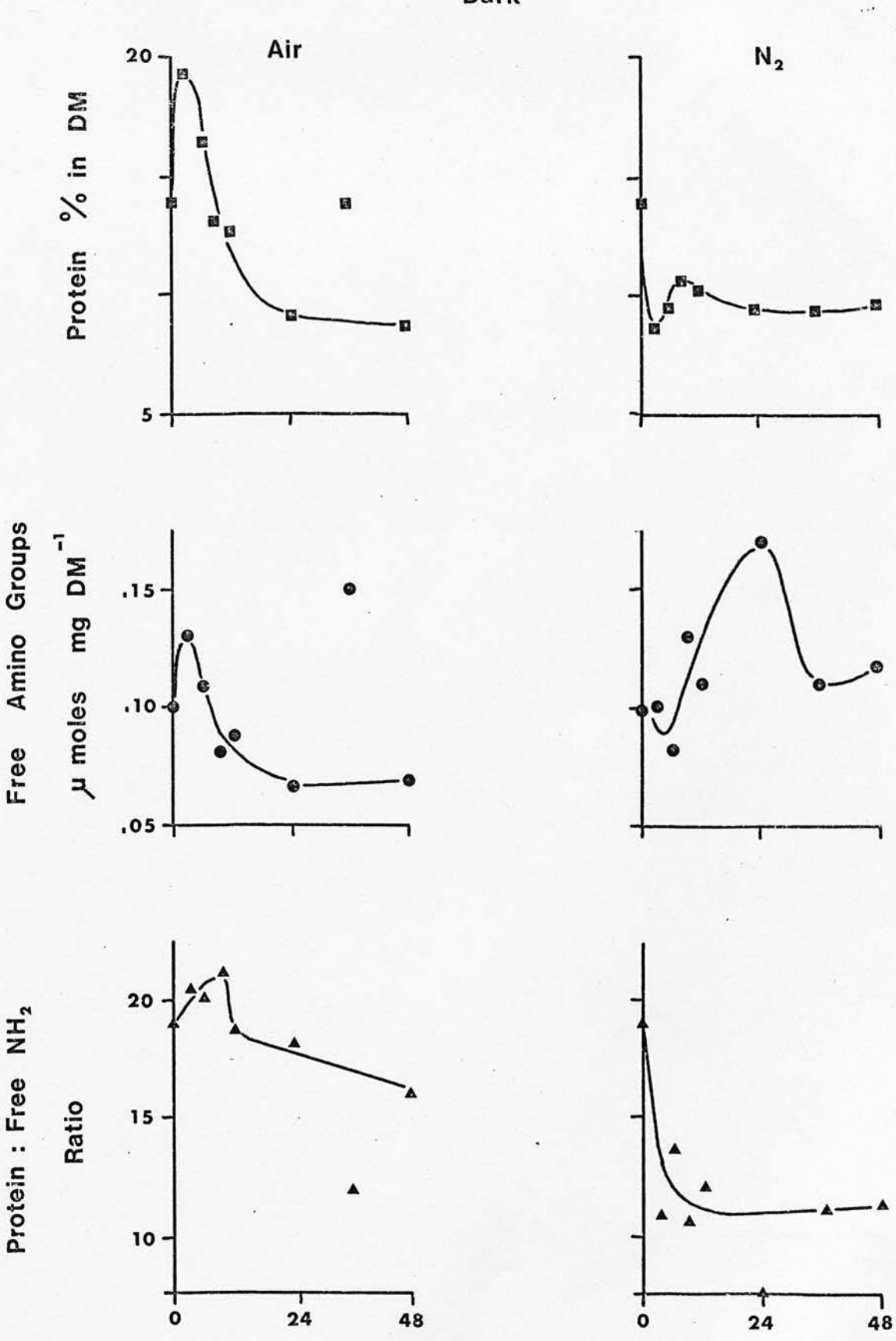


■ , total soluble protein (TCA - insoluble fraction);

● , total free amino groups (TCA - soluble fraction);

▲ , protein:free amino group ratio

FIG. 2.23 Changes in nitrogeneous components during wilting

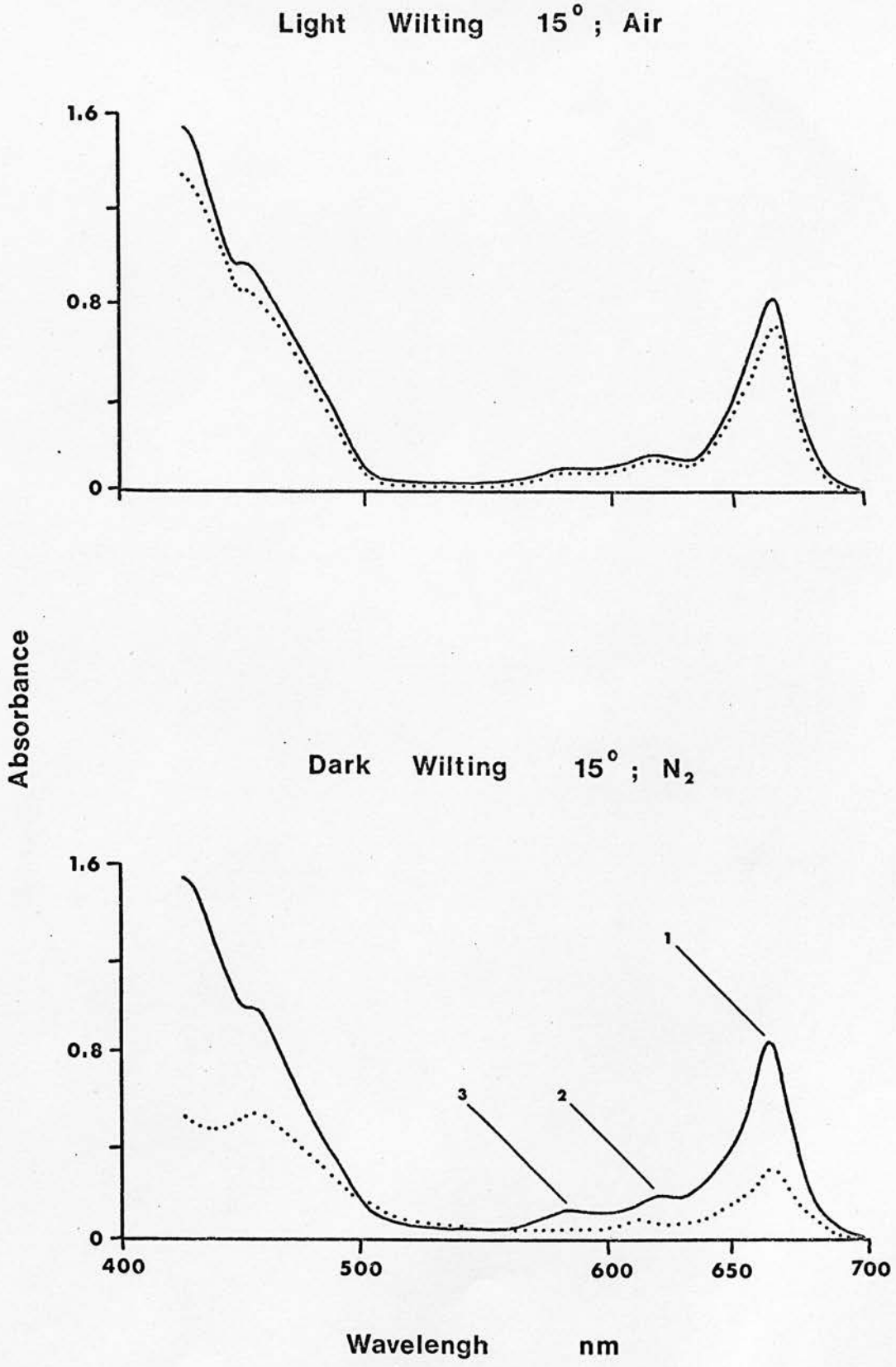


■ , total soluble protein (TCA - insoluble fraction);

● , total free amino groups (TCA - soluble fraction);

▲ , protein:free amino group ratio

FIG. 2.24 Changes in the absorption spectra of chlorophyll extracts during wilting under controlled conditions



solid line, fresh plant extract;
dotted line, 48-h wilted plant extract

FIG. 2.25 Changes in total chlorophyll content (on a D M basis) during wilting under controlled conditions

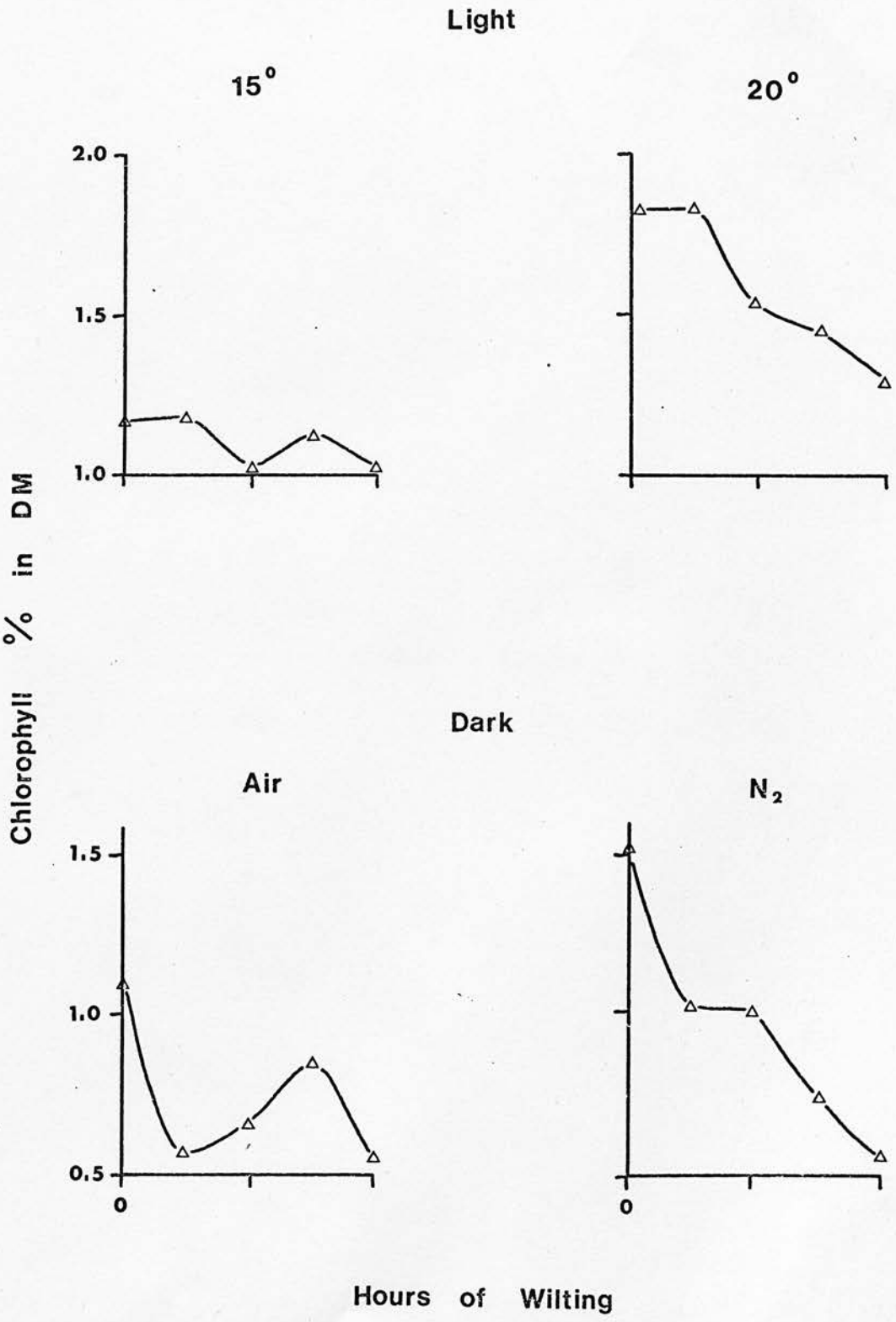
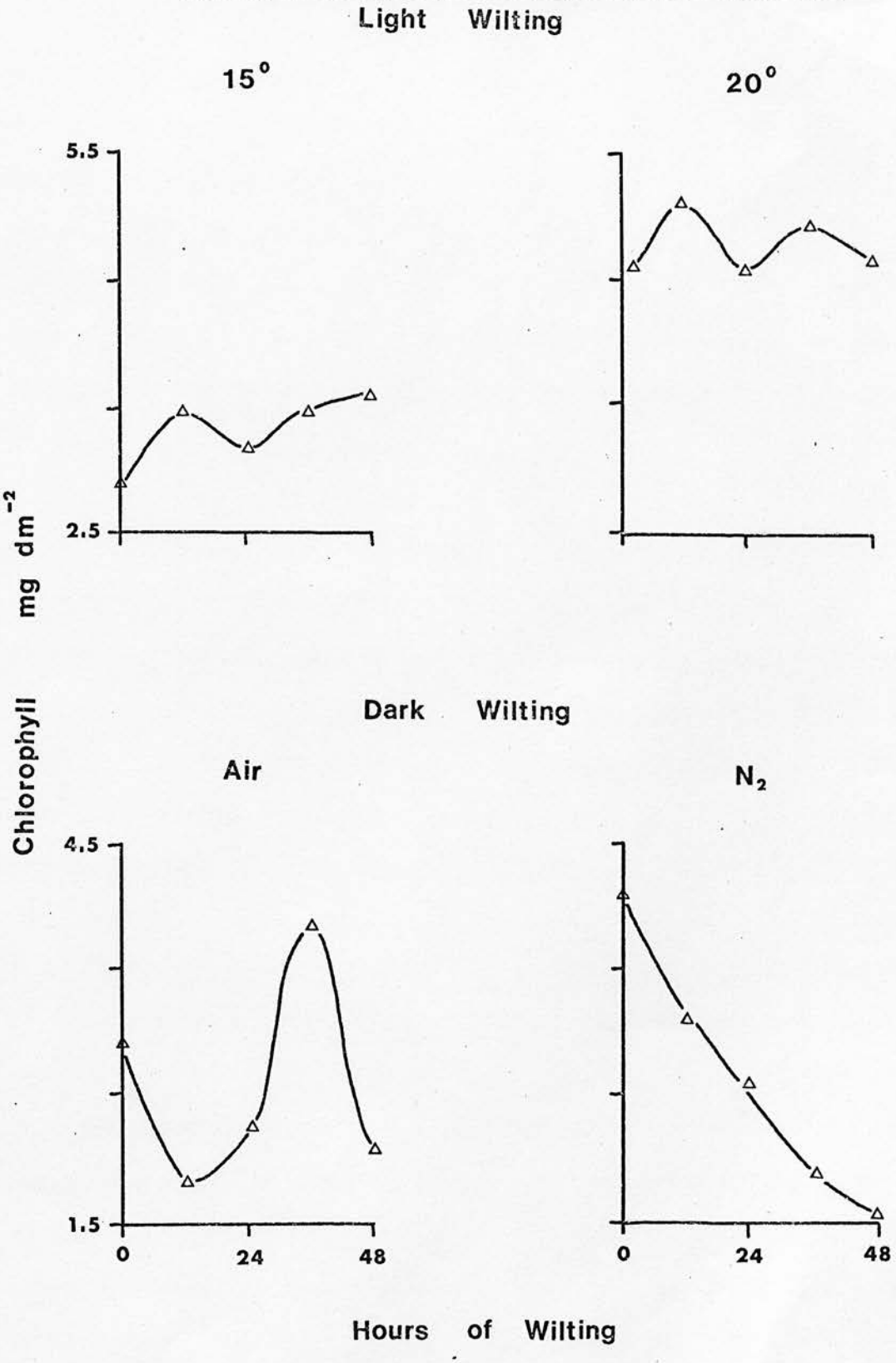


FIG. 2.26 Changes in total chlorophyll content (on a leaf area basis) during wilting under controlled conditions



SECTION 2: DISCUSSION

SWATH SIMULATION STUDIES

The swath simulation studies revealed some drying characteristics and the influence of some plant and mechanical factors on the drying process of swaths under controlled environmental conditions. The wilting conditions were chosen to represent the field situation but were generally more favourable in that continuous drying was possible (see F W decline curves), so caution is necessary in the extrapolation of the results.

The results of Expts. 1 and 2 are not strictly comparable since the nature of the plant material and the environmental conditions differed. However, comparison of the control swath curves from both experiments suggests an effect of plant age and maturity on the drying pattern. Under poorer evaporating conditions (lower vpd), the more mature plant material (50% ear emergence, Expt. 2) exhibited higher initial evaporation rates and lower resistances, rapidly attaining the 30% D M level. This suggests that initially the younger plant material (Expt. 1) showed greater physiological resistance to evaporation associated with a higher moisture content. However, after 12 h wilting, the resistance of the older plant material increased rapidly causing a sharp fall in evaporation whereas the younger plant material continued to lose water throughout the wilting period. The shape of the F W decline curve of Expt. 2 indicated that a threshold level of D M existed at c. 40%, above which further evaporation was very slow. Such a threshold was never reached in Expt. 1. Under favourable field wilting conditions,

herbage at c. 50% ear emergence would be expected to have higher initial but lower final evaporation rates (above the threshold) than young leafy herbage.

In both experiments, the control swaths had the highest initial evaporation rates which persisted at high levels for longer than in the mechanically treated swaths, indicating that early stomatal transpiration was greater in amount and duration in whole leaves than in treated leaves where premature stomatal closure was probably induced (see Single Leaf Studies and Stomatal Studies for details of stomatal behaviour after cutting). The evaporation curves exhibited three distinct phases. Swath evaporation rates were greatest during Phase I, the first 3 h after cutting which included the stomatal transpiration period; then declined during Phase II from 3 - 18 h when readily available water was evaporated through the cuticle (and any wounds or cut edges in the treated swaths); and finally evaporation approached zero after 24 h during extreme desiccation. These three phases of evaporation must reflect the physiological response of the plants to drying since the evaporating potential of the environment was unchanged. Physiological limitations on the swath evaporation rate were observed in both control and treated swaths. The two more extreme mechanical treatments (chopping into 2 cm segments, Expt. 1, and crushing, Expt. 2) caused large increases in the swath D M content but had little effect on the evaporation curve shape. Possibly the physiological limitations were sub-cellular in origin, reflecting the difficulty in evaporating protein-bound water at the higher D M levels; mechanical treatment would have little effect on subcellular structure and hydration of cell colloids (Henckel 1964; Kessler *et al.* 1964; see pp. 25 - 26).

The curves of bulk physiological resistance of the swath, r_{st}, also reflected the drying patterns found. In Expt. 1, the early rapid increase in resistance in all treatments corresponded to the steep decrease in evaporation, resulting from increases in stomatal resistance primarily, and other cellular resistances as drying progressed. The peak resistance values at 24 h corresponded to very low evaporation rates, probably after the evaporation of all readily available water leaving only the unavailable protoplasmic water. Thereafter resistance decreased and evaporation remained near zero. In Expt. 2, the resistance pattern showed more variation with the treatments. The early lag-phase in the control indicated prolonged stomatal opening, followed by a rapid increase in r_{st} during progressive exhaustion of available water. The exponential increase in resistance in the crushed treatment could have two different explanations: a physiological response to excessive injury, or liberation of all available water during crushing followed by rapid evaporation in the early stages accompanied by r_{st} increases.

Therefore, under controlled, constant evaporating conditions, the plant material itself exerts limitations on the swath evaporation rate, leading to increases in resistance and decreases in swath drying. Mechanical treatment has little effect on the evaporation pattern and may even increase the resistance. The use of chemical treatments such as formic acid or organic solvents has been suggested (Thaine and Harris 1973) in order to reduce the resistance to evaporation caused by the plant cuticle. Steam treatments which kill the plant cells and reduce the resistance to zero instantaneously, lead to very rapid herbage drying in the standing crop (Schukking 1972; Tetlow 1973). Such alternative treatments have potential value for the control of herbage drying, but must be assessed in relation to the fundamental process of evaporation under standard conditions.

SINGLE LEAF STUDIES

Physiological plant responses to cutting. a. Preliminary studies

Transpiration (T) from plants involves the evaporation of water from sites within the leaves and the subsequent diffusion of water vapour to the leaf surface then into the air beyond. Two main pathways for water movement are recognised, one associated with movement directly across the leaf cuticle, the other associated with movement through the stomatal pores. The cuticular pathway is relatively short but of high resistance and, when the stomata are open, carries only a small proportion of the total transpiration flux. The stomatal pathway involves evaporation of water from the outer surfaces of the mesophyll cells and its diffusion through the intercellular spaces and the stomatal pores. Although much longer, this route normally carries most of the transpiration but the presence of the stomata introduces a powerful, variable resistance into the pathway and, when the stomata are closed, stomatal transpiration can effectively cease (Jarvis and Slatyer 1970).

The transpiration curve for the whole leaf, Fig. 2.5 a. illustrates well the typical 3-phase pattern of decline in fresh weight which results from the stomatal control of transpiration. The period of stomatal transpiration, Phase I, lasted for only c. 8 min after cutting, followed by a period of changing transpiration rate during stomatal closure, Phase II, then finally an extended period of slow cuticular transpiration, Phase III. The transpiration curves for the leaf segments, Fig. 2.5 b. and c. have a similar pattern with an initial rapid stomatal phase and a final slower cuticular phase, indicating that stomatal control of transpiration still operates after chopping the leaf blade into segments; the three phases were less well defined however, possibly because of water loss from the cut edges superimposed upon the overall transpiration pattern.

The difference between leaf 1 and leaf 2 T values indicates an age effect on stomatal and cuticular water loss, the highest rates occurring in the slightly older, mature, fully expanded leaves. The Phase I stomatal T was highest in whole leaves; a shock and injury response to chopping may have affected leaf segment stomatal behaviour, possibly inducing premature closure, and thereby reducing T. However, the leaf segments exhibited higher Phase III cuticular T values than the whole leaves indicating that chopping reduced leaf resistances to cuticular water loss. Hence regulation of leaf transpiration by stomatal closure after cutting was more effective in whole leaves than in leaf segments.

The leaf resistance to water vapour transfer has three main components (Raschke 1958; Milthorpe 1961; Jarvis 1967): the boundary layer resistance (r_a or r_b), which is in series with the diffusive resistances of the stomata (r_s or r_{st}) and the cuticle (r_c) which are in parallel with one another. However, there is some confusion about resistance terminology, for example, the r_s term of Chartier *et al.* (1970) and Prioul (1971) incorporates the diffusive resistances of the stomata, the cuticle the substomatal cavity and connecting intercellular spaces up to the mesophyll cell walls (Prioul 1973), whereas other workers use r_s to represent the diffusive resistances of the stomata and cuticle or the stomata alone. In the latter case, the substomatal and intercellular resistances would be incorporated in the total internal resistance (r_i) or the mesophyll resistance (r_m) (Sutcliffe 1967; Jarvis and Slatyer 1970). Hence caution is necessary in the interpretation of the results and comparison of resistance values from such studies.

The calculation of leaf boundary layer resistance to water vapour transfer (r_a or r_b) from the evaporation rate of a filter paper leaf replica is an established technique (Gaastra 1959; Prioul 1971). The evaporation curves for *L. multiflorum* leaf replicas were always very similar, and r_b values of about 50 s m^{-1} were derived for whole leaves and leaf segments. This r_b value is in agreement with that of Prioul (1971) who reported a mean r_b value of 52 s m^{-1} for *L. multiflorum* leaves, and it lies within the range of 30 - 100 s m^{-1} expected for small leaves in light wind. Other workers have found r_b values of 20 s m^{-1} for grass leaves and 10 - 30 s m^{-1} for ryegrass (Monteith 1973). r_b depends upon both plant factors such as the nature of the leaf surface, and environmental factors, particularly windspeed which affects the length of the diffusion path (Heath 1969). In general, small narrow leaves have thinner boundary layers. The rather high r_b values for *L. multiflorum* compared with other grass leaves may in fact underestimate r_b as a result of the furrowed and hairy leaf surface. Shaving the hairs from soybean leaves with an electric razor demonstrated the common assertion that hairs can contribute significantly to r_b (Woolley 1964). An r_b value of 50 s m^{-1} may be regarded as characteristic of *L. multiflorum* leaves.

The cuticular resistance is relatively constant for a given leaf type but it may vary to some extent with leaf age and under extreme environmental conditions, through an effect on the cuticle structure and thickness (Sutcliffe 1967). r_c is by far the largest resistance term indicating that the cuticle presents the greatest barrier to water loss. The r_c values of 2370 and 2338 s m^{-1} for whole *L. multiflorum* leaves fall within the range of 2000 - 6000 s m^{-1} expected for mesophytes (Monteith 1973).

The stomatal resistance is variable through regulation of the stomatal pore size, and depends upon the physiological condition of the plant and environmental factors (Sutcliffe 1967; Jarvis 1967). The r_s values of 93 and 113 s m^{-1} found for whole *L. multiflorum* leaves are at the lower end of the range 100 - 200 s m^{-1} expected for mesophytes (Monteith 1973). All the resistance values derived for *L. multiflorum* are of the expected order of magnitude and relationship to one another. So long as the stomata remain open, the transfer of water vapour from the leaf cells into the air may be expected to follow the path of least resistance, namely via the stomata.

The effect of stomatal closure on the total resistance to diffusion of a leaf can be estimated, taking account of the stomatal distribution. For epistomatous leaves like *L. multiflorum* the resistances of the two surfaces can be combined in parallel giving the total resistance as $\left(\frac{1}{r_{bU} + r_s} + \frac{1}{r_{bL} + r_{cL}}\right)^{-1}$ which is approximately equal to $(r_{bU} + r_s)$ as r_c is very large. The total resistance calculated for the whole leaves corresponds very closely to this approximation. Hence r_s values for the leaf segments may be derived. It is interesting that the total resistance of whole leaves is much lower than that of the leaf segments during the stomatal phase, again indicating an effect of chopping on stomatal behaviour and overall transpiration pattern. However the total resistance during the cuticular phase is highest for whole leaves and substantially reduced in the leaf segments. Thus, chopping increases cuticular transpiration rate through an effect on the leaf resistance network. Since the values of r_i and r_c are not expected to vary greatly, the primary effect is probably on r_s, and the estimated values of r_s for whole leaves and segments support this hypothesis. The leaf segments had higher initial r_s values but much lower final r_s values than the whole leaves.

b. Detailed Studies

These experiments provided continuous information about the pattern of changes in transpiration rate, T, net photosynthesis, P_N, and total resistance to water vapour transfer ($r_a + r_s$), from the moment of cutting for periods ranging from 2 to 20 h. Immediately prior to cutting, the leaves were actively photosynthesising and transpiring under steady state conditions in the assimilation chamber, and the stomata were assumed to be fully open as the total diffusive resistance was low. The water deficit of the leaves under these conditions was also assumed to be low (10%) as they were kept supplied with nutrient solution continuously.

The sequence of events occurring immediately after cutting are of particular interest regarding the possible continuation of transpiration and photosynthesis after harvesting grass. The parallel changes observed in T and P_N suggest that a common factor regulates the rates of both after cutting. Brix (1962) and Boyer (1970) attributed the parallel decline in T and P_N in water-stressed plants to stomatal behaviour (see p. 24).

Many observations have been made which indicate that pronounced changes in the transpiration rate of leaves may occur when they are severed from the plant (Darwin 1878; Darwin and Pertz 1911; Laidlaw and Knight 1916; Andersson, Hertz and Rufelt 1954; Milthorpe and Spencer 1957; Rufelt 1963). Often there is a temporary increase in T (Heath and Meidner 1960) as found in Expts. 1 and 4, but under some conditions there is a decrease in T from the moment of excision Willis *et al*, 1963) as found in Expt. 2. The water deficit of the leaves at the time of excision affects the subsequent transpiration

response. When the water deficit is small, the increase in T is large but when water stress is severe at the time of cutting there is a progressive decrease in T (Willis *et al.* 1963). On these grounds, a temporary transpiration peak after excision would have been expected in all experiments; possibly the leaf water deficit under the experimental conditions used was close to a threshold value, thus accounting for the variable response found.

Two different explanations have been advanced to explain the increase in T resulting from leaf excision; Darwin and Pertz (1911) suggested a temporary stomatal opening which precedes closure in the later stages of wilting, a theory supported by the results of Willis *et al.* (1963) who found a close relationship between stomatal behaviour and transpiration in a range of plants under conditions resulting in a temporary widening of the stomatal pores. Hoever, Iwanoff (1928) regarded the release of water stress in the conducting system as of primary importance, leading to an increased water supply to the leaf. This theory was supported by the results of Rufelt (1963). It is probable that release of tension leads to turgor changes in the epidermal and guard cells and hence to changes in stomatal apertures, thus influencing transpiration rate. So a combination of both theories may be used to explain the transient increase in T after excision in *L. multiflorum* leaves.

The subsequent decline in T found in all experiments showed the typical 3-phase pattern traditionally associated with the stomatal control of transpiration (Bannister 1964; Baron 1967). The corresponding increase in $(r_b + r_s)$ was also primarily a function of the

powerful variable resistance r_s, as changes in r_b are likely to be relatively small. However, leaf shrinkage and wrinkling with progressive drying must affect the boundary layer resistance to some extent.

The sharp breaks of slope found at the ends of Phase I and Phase II of the transpiration curve, after 15 and 30 min of isolation, have not generally been observed by other workers. Furthermore, the progressive increase in total resistance continued during Phase III and became especially marked after 15 h of drying. Stomatal examinations using scanning electron-microscopy (see pp.132-3) revealed that all the stomata were fully open at the beginning of Phase I, zero time, and all the stomata were closed at the beginning of Phase II, 15 min after excision, and of Phase III, 30 min after excision. These observations indicate that the stomatal control of transpiration explains the initial transient increase followed by sharp decrease in T during Phase I, and the first break of slope at 15 min. Further explanations are necessary to account for the second break of slope at 30 min and the progressive increase in diffusive resistance during Phase III. The stomata may be described as 'optically closed' at the beginning of Phase II (15 min) but not 'physiologically closed' until the beginning of Phase III (30 min), since T persisted at a rate intermediate between 'stomatal' and 'cuticular' during Phase II. The stomata may appear 'optically closed' when viewed from above since the guard cells look in apposition, but may in fact permit water vapour transfer. The stomata of maize have longitudinal ridges on either wall of the stomatal pore, when viewed in transverse section using scanning electron microscopy (Chartier 1973; de Parcevaux 1973), and the graminaceous stomata of *L. multiflorum* are probably similar. It is proposed that such stomata are not 'physiologically closed' until these

longitudinal ridges are interlocked, thus preventing water vapour transfer through the pores and greatly increasing the stomatal resistance. This evidently happened by the end of Phase II (30 min) in *L. multiflorum*, after which transpiration was entirely cuticular.

It is generally accepted that variation in stomatal aperture is the main mechanism by which the plant exercises control over transpiration. From time to time, however, considerable controversy has arisen about the possible significance of other mechanisms and the issue is still an active one (Jarvis and Slatyer 1970). Two main mechanisms have been proposed to account for non-stomatal transpiration control, should it occur. The 'incipient drying' hypothesis (Livingston and Brown 1912) suggests that the evaporation sites may retreat from the outer surfaces of the mesophyll cells from which most evaporation occurs, into the walls themselves, thereby increasing the length and tortuosity of the vapour path, and hence the total diffusive resistance. The other hypothesis suggests that the vapour pressure at the mesophyll cell walls may be reduced significantly below saturation, thereby reducing the leaf-air vapour concentration gradient which provides the driving force for water vapour transport. This may occur as a direct result of the dehydration associated with water stress (Shimshi 1963; Rawlins 1963) or by accumulation of solutes carried to the evaporating surfaces in the transpiration stream (Boon-Long 1951). It could also arise from the presence of the internal cuticle which lines the mesophyll cell walls (Scott 1950, 1966). Should either or both of these mechanisms operate, they are probably of greatest significance during periods of limiting water supply or excessive evaporative demand (Jarvis and Slatyer 1970), and

could be particularly important afer excision. Jarvis and Slatyer (1970) obtained evidence of an extra resistance in the water vapour pathway associated with transport in the mesophyll cell walls of cotton leaves. This extra resistance appeared to be insignificant at low transpiration rates and in turgid leaves but increased with transpiration rate and dehydration. It was attributed to a reduction in the leaf-air vapour pressure gradient rather than to 'incipient drying'. The experiments were conducted with induced stomatal opening under normal environmental conditions. Stomatal closure would tend to reduce this extra resistance. However, under excessive water stress such as experienced by the *L. multiflorum* leaves several hours after excision, the increasing resistance observed may be explained by such mechanisms. Extremely high water deficits would be required to cause 'incipient drying' (Jarvis and Slatyer 1970), and were possibly attained after 10 - 20 h of exposure to severe drying under the experimental conditions used, thus explaining the marked increase in total diffusive resistance during Phase III.

The corresponding patterns of change in T and P_N during Phases I, II and III after excision indicates that stomatal control of both functions was operating. The temporary increase in P_N accompanied by stomatal opening immediately after excision has been reported by other workers in studies using detached leaves subjected to mild water deficits (Heath and Meidner 1961) or severe drying conditions (Scarth and Shaw 1951). Factors other than stomatal behaviour may also contribute to the transient initial increases in P_N, for example, Heath and Meidner suggested that light absorption may increase, perhaps due to wrinkling of the epidermis leading to

darker green leaves. Brilliant (1924) found that water deficits of 5 - 25% produced an increase in P_N in *Hedera helix*, but greater deficits caused an abrupt fall to zero. Removal of the epidermis did not affect the results, suggesting that stomatal movements were not entirely responsible for the P_N changes found.

Observations of reduction in P_N with wilting have been made by plant physiologists from the end of the last century onwards (Heath and Meidner 1961). The most obvious explanation is that the progressive increase in stomatal resistance accompanying stomatal closure limits the diffusion of carbon dioxide into the leaf, thus reducing photosynthetic fixation.

As shown by Stalfelt (1935) for oats, and by Pisek and Winkler (1956) for selected plants of different ecological types, absorption of CO_2, like stomatal transpiration, depends on stomatal aperture. Larcher (1960) found that stomatal closure resulting from increased saturation deficit finally brings any further assimilation to a stop. No uptake of CO_2 has been reported in plants with closed stomata, which at most reassimilate their own respiratory CO_2 (Larcher 1960). Therefore, zero net assimilation would be expected in *L. multiflorum* leaves after 'physiological closure' of the stomata at 30 min after excision, as found in Expts. 1 and 2.

However, under conditions of water stress, net photosynthesis is thought to be limited by additional factors such as protoplasmic dehydration, irrespective of stomatal behaviour (Slavik 1963; Brady 1973). Reductions in P_N could also result in part from increased respiration rates (Heath and Meidner 1961), but there is no doubt that

the principle mechanism regulating both CO_2 and water vapour transfer following excision in *L. multiflorum* leaves is that of stomatal control. With prolonged drying, other factors must assume importance.

In Expts. 1 to 4, *Lolium* leaves were subjected to severe drying conditions so that large water deficits occurred within 2 h of excision, the leaves rapidly losing turgor, shrinking and becoming darker in colour. Such extreme conditions are unlikely to be met with in the field where cut plants in a swath to some extent protect one another from desiccation. Hence the timecourse of changes in T, P_N and total diffusive resistance is probably extended under field conditions although the pattern of changes is expected to be similar in both circumstances. Net assimilation may persist for longer than 30 min after harvesting but it is most unlikely to occur for periods of several days as suggested by MacGregor (1966), since stomatal closure induced by water stress (or nightfall) is irreversible unless water is re-supplied, and light is insufficient in all but the surface layers of a swath (Pizarro and James 1972).

The changes in total diffusive resistance ($r_b + r_s$) both reflected and determined the changes in T and P_N discussed above. The initial lag phase (see p.128) corresponded to the transient stomatal opening leading to increases in T and P_N. The subsequent steady increase in resistance corresponded to stomatal closure and reduction in T and P_N. Hence changes in total diffusive resistance occurring during the first hour after excision were a function of changes in stomatal resistance. The final progressive increase in resistance resulted from alternative mechanisms such as 'incipient drying' during the later stages of desiccation.

LEAF SEGMENT STUDIES

Leaf segments provide an extremely useful and versatile experimental material since many replicate samples may be prepared rapidly according to a pre-determined pattern, then exposed to well-defined environmental conditions. Such leaf tissue samples represent a level of organisation intermediate between whole leaves and plant cells or organelles and are particularly suitable for physiological studies (Setlik and Sestak 1971). However the leaf segment technique has been criticised on the grounds that isolated tissue samples may differ in their physiology from whole leaves or plants, in part through the effects of injury during sample preparation. There is no conclusive evidence about injury-induced physiological changes in leaves, but there is evidence that the photosynthetic behaviour of leaf portions of *L. perenne* shows no significant difference from the behaviour of whole attached leaves (Wilson *et al.* 1969), and barley leaf segments continue to photosynthesise for remarkably long periods of up to 100 h (Natr 1967). Criticism of the use of leaf tissue samples is largely unjustified (Setlik and Sestak 1971). Moreover their use may be singularly appropriate for the study of cut leaf physiology during wilting, since possible injury and isolation effects on the leaf segments may also occur in whole detached leaves. The aim of using leaf tissue samples in Section 2 was to provide a reliable characterisation of their physiological behaviour under well-defined conditions, and to complement the studies on whole leaves.

The use of leaf tissue samples for the measurement of water stress in plants is an established technique (Catsky 1965; Kramer

and Brix 1965; Slatyer and Barrs 1965). The water saturation deficit (W S D) gives a good indication of a plant's water balance which controls its physiological and biochemical processes, in particular the intensity of photosynthesis. The water uptake curves of leaf segments are essentially similar to those of entire detached leaves, and show two distinct phases of water uptake: rapid uptake during the first 1 - 3 h indicating tissue saturation, followed by a slow second phase. The saturation of the 48 h wilted segments within 3 h indicated that some degree of recovery was possible even after extended wilting. W S D calculations from such water uptake curves gave values of > 10% in the fresh samples. Small water deficits were probably accumulated during sample preparations; deficits < 5% are never recorded in practice (Kramer and Brix 1965). Water deficits of > 40% were found after 24 h wilting. Such values are very high and rather rare under natural conditions (Catsky 1965). The extremely high W S D values recorded after 48 h wilting may have slightly overestimated the true values since infiltration of water may occur through the cut edges of the segments, but in any case indicate severe desiccation of the leaf tissue. After 1 h saturation periods however, W S D values were reduced below that of the fresh plant material indicating that deficits were effectively eliminated by such treatment. Moreoever, after 1 h saturation periods, the fresh and wilted samples all attained similar D M levels, giving samples under comparable conditions of water balance for the subsequent physiological measurements.

Leaf tissue samples have been used since the early 1960's in studies of photosynthesis (Bartos *et al.* 1960; Setlik *et al.* 1960; Natr and Spidla 1961; Natr 1967, 1968, 1970). The early experiments

showed that photosynthesis can proceed at an even and reproducible rate for several hours of leaf segment exposure to a rather high irradiance, but declines after 6 - 9 h through some deterioration processes in the samples. The accumulation of dry matter provides an excellent measure of net carbon fixation for use over such periods (Heath 1969), and is particularly relevant to wilting studies where D M changes are of direct practical interest. During the experimental procedure, samples were resaturated in order to induce stomatal opening and standardise their physiological condition. Hence, the levels of net assimilation recorded may indicate the maximum net photosynthetic capacity of *Lolium* leaves during wilting. These levels would not be attained in practice unless the resupply of water (dew, rain) were sufficient to resaturate the wilting leaves leading to stomatal opening.

Under similar conditions of light saturation and normal CO_2 concentration, P_N values for fresh ryegrass in the I.R.G.A. studies using whole attached leaves were in good agreement with those found in the leaf segment studies (after conversion of the D M accumulation figures to the amount of CO_2 fixed, see p.112). This indicates that the leaf tissue samples provided a good estimate of photosynthetic rate in fresh *Lolium* leaves; hence injury and isolation effects were probably negligible. P_N values for fresh presaturated segments (Fig. 2.16) and attached leaves (Fig. 2.6) with irradiance of 120 W m^{-2} were in very close agreement, at 270 and 275 units* of CO_2 fixed respectively.

*For units, refer to Figs.

The leaf segment studies showed that photosynthetic capacity decreased rapidly as D M content increased during wilting, and above the threshold level of 28% D M, net dissimilation was recorded. These findings suggest that the level of 28% D M and 24 h wilting generally recommended for silage-making (McDonald and Whittenbury 1973) have a physiological basis since, when exceeded, net losses are inevitable. It seems that MacGregor's (1966) claim of the continuation of photosynthesis for several days in harvested grass is most unlikely. Other reports of net gains during wilting due to photosynthetic activity (Nash 1959; Watson and Nash 1960; Kormos and Chestnutt 1967) also appear highly improbable under good drying conditions. Moreover, physiological studies have shown that net assimilation is reduced with increasing water stress through stomatal and other effects (see pp.149-150). The most rapid fall in photosynthetic capacity occurred during the first 24 h of wilting when both physiological and biochemical changes are expected to be greatest. Between 24 and 48 h, net dissimilation increased as D M content increased from 28 to 60%, but the changes were more gradual at the higher D M levels, possibly approaching a plateau above 60% D M representing maximum dissimilation under the conditions used.

However, further experiments showed that following a 1 h saturation pretreatment, as recommended by Natr (1970), a small capacity for net photosynthesis was maintained even after 48 h of wilting, presumably since the equilibration period allowed the elimination of the saturation deficit and stomatal opening to occur. Thus the samples had a longer exposure to conditions favouring photosynthesis. It appears that following complete resaturation of wilting grass, limited photosynthesis may be possible. Kocevyh (1962) claimed that

the peculiar weather conditions of the far north of Russia favour the continuation of photosynthesis in freshly cut herbage, which is frequently moistened by rain and dew. Day-length is extended and drying is prolonged. However, in the U.K. such photosynthetic activity, if it occurs, is likely to be of little importance and restricted to the surface layers of the swath (Pizarro and James 1972).

The estimates of dark respiration rate were carried out using resaturated leaf segments and the values obtained may be considered to represent maximum values of respiratory potential. No attempt was made to estimate photorespiration since suitable techniques do not yet exist but it is thought that light may cause a stimulation of respiration (Ludlow and Jarvis 1971). So true values of the respiratory potential during daylight hours may in fact be higher than the values given.

The capacity for dark respiration in harvested ryegrass increased with increasing D M content throughout the wilting period. The changes were relatively slow during the first 24 h, but above the 40% D M level, a very marked increase in respiratory capacity occurred during the second 24 h of wilting. These observations suggest that potential respiratory losses are particularly great above the 40% D M threshold after 24 h. So again, there is a physiological basis for the usual silage-making practice of restricted wilting to about 28% D M. Further evidence is provided for the common assertion that the degree of wilt is vitally important since losses are potentially much greater at the higher D M levels, both in the field, and in the silo through overheating.

Although respiratory losses may be relatively small in terms of D M, they nevertheless represent depletion of readily available carbohydrates (Pizarro and James 1972), which has serious implications for the subsequent ensilage process. The precise effects of water stress on plant respiration are not well established, but several studies have reported increased respiration rates with increasing water deficits. In other cases, respiration rates fell, for example in *Lolium* leaves, with reduced moisture content (see p. 25), but it is conceivable that the unexpressed potential for respiration increases even in these studies. The increasing respiratory capacity found in *L. multiflorum* leaves may represent a 'starvation' phenomenon which becomes particularly marked in the later stages of desiccation. It is possible that resaturation of the samples leads to complete exhaustion of readily available carbohydrates, followed by the use of alternative respiratory substrates such as structural polysaccharides and proteins under extreme conditions (see pp. 31-32). Such a succession of respiratory substrates would be analagous to the respiration drifts described by James (1953). Thus, it is suggested that the potential for dark respiration is high at all times and increases under the extreme conditions of desiccation and senescence during wilting. This potential for respiratory loss can only be expressed however if water supplies are sufficient to maintain metabolic processes. So losses may increase markedly after resaturation of the wilting herbage by rain or dew. These suggestions are in agreement with the observations that rainfall can result in serious losses usually attributed to leaching of mineral and inorganic ions which would however have only a small effect on the herbage D M content. Further evidence for this theory of high (but normally unexpressed) respiratory potential during wilting is provided by the persistance of active mitochondria in senescent and water-stressed cells (see p.33).

BIOCHEMISTRY OF WILTING

Biochemical changes recorded during wilting could in principle be caused by plant enzymes, bacterial enzymes or both. It is generally accepted that leaf surfaces possess a bacterial flora, the 'phylloplane bacteria', consisting mainly of aerobic chromogenic types (Stirling 1953; Austin 1974). Numbers vary from several thousands to several millions per g FW, for example, Austin (1974) found between 10^5 and 10^7 bacteria per g FW of ryegrass in the U.K. with maxima occurring in summer. This bacterial population is restricted to the surface, but micro-organisms have been seen in stomatal depressionsand may occur inside the plant tissues after damage (Stirling 1953).

Characteristically, isolates of phylloplane bacteria utilise only simple sugars such as glucose and fructose as single carbon sources. Macromolecules such as cellulose and starch are hydrolysed comparatively rarely. Cultures generally possess oxidative metabolism (Austin 1974). Under field conditions, surface exudates of the plants must provide the bacteria with their nutritional requirements. After harvesting, the stomata close relatively rapidly, so bacteria would have no access to plant cell substrates, except via cut ends and wounds. Conditioning, rather than simply mowing, would be expected to favour bacterial growth as greater damage to the plant material results.

The proliferation of bacteria on plant surfaces during wilting is considered most unlikely since the liquid media for bacterial growth, provided by exudates or liberation of cell sap through injury, would rapidly disappear by evaporation during the drying process. The proliferation of bacteria inside the plant material (inside the plant cells or in the intercellular spaces) is also considered improbable.

The redox potential in respiring plant tissue is low at harvest and during wilting after stomatal closure (Mann 1974; Ohyama 1974), so the development of aerobic species under such strongly reducing conditions would be suppressed. Furthermore, the multiplication rate of phylloplane bacteria is low so little growth in population within the 48 h wilting period would be expected.

It is concluded that leaf surface bacteria have a negligible effect on total plant respiration and biochemical changes during wilting.

Fungi can also grow on leaves and are thought to enhance the rate of leaf senescence (Skidmore 1974). However their growth rate is slow. Fungal spore tests have shown that germination and penetration of the leaf by fungal hyphae takes 2 - 4 d under favourable laboratory conditions (Skidmore 1974). So the proliferation of fungi resulting in biochemical changes in the plant material during wilting is also considered most unlikely.

It is concluded that the biochemical changes recorded during wilting are very largely, if not exclusively, plant enzyme mediated. The biochemical changes during wilting under controlled conditions will now be discussed in the light of this conclusion. The different wilting treatments used are not strictly comparable, apart from the two dark wilting treatments, since the source and physiological condition (age, growth stage) of the plant material varied.

(i) Soluble carbohydrates

The overall decrease in soluble carbohydrates during wilting probably resulted from the utilisation of these readily available carbon

sources as respiratory substrates by the plant enzymes. Furthermore, photorespiration is likely to increase under water stress, when the conditions of low CO_2 concentration, following stomatal closure, favour the diversion of ribulose diphosphate into the photorespiratory pathway (Kluge 1974). However, the similar decrease in soluble carbohydrates found in the nitrogen treatment suggests that factors other than aerobic respiration were involved. Lugg (1949) reported that when leaves are starved in an atmosphere of nitrogen, carbohydrate losses may be greater than in air.

The large fluctuations in soluble carbohydrate levels during wilting may represent alternate phases of polysaccharide hydrolysis and utilisation of the hydrolysis products. Possibly successive induction of hydrolytic and respiratory enzymes occurred under conditions of increasing water stress. There could be threshold levels of W S D for the induction of specific enzymes, but this is purely speculation. α-amylase increases during water stress producing hydrolysis of starch to glucose (Kluge 1974), and starch-sugar conversion is often found (Woodhams and Kozlowski 1954; Bornkamm 1974).

Fluctuations in soluble carbohydrates during wilting have been reported by other workers (Greenhill 1959; Melvin and Simpson 1963; Lanigan 1966; p.22). In studies on the senescence of detached leaves, James (1953) reported early rapid decrease in readily available carbohydrates through normal aerobic respiration, followed by occasional increases in the later stages due to hydrolysis of polysaccharides such as glycoside in cherry-laurel or hemicellulose in runner bean and maize (pp.31-32). So the hypothesis of alternate utilisation and hydrolysis of carbohydrates during wilting may be an explanation of the changes found in these studies.

The large contribution of fructose to the W S C levels, and the parallel changes in both components found during wilting, suggests that fructan changes were important. Fructans may accumulate in water-stressed tissues since their utilisation is suppressed (pp. 26-27), producing the peaks in total W S C and fructose observed after 24 h. Sucrose may also accumulate (pp. 26-27) leading to variation in glucose levels.

The persistance of peaks in the dark treatments indicates that photosynthetic fixation was not a contributory factor in the light treatments. In fact net photosynthesis would be most unlikely beyond 1 h after harvest under the favourable wilting conditions used since early stomatal closure prevents CO_2 uptake (see Single Leaf Studies, pp.132-133, 146).

(ii) Nitrogenous components

Many workers have reported a rapid breakdown of protein into non-protein forms of nitrogen, attributed to intense proteolytic activity, during wilting (e.g. Kemble and MacPherson 1954; Brady 1960; Singh 1962; Brady 1965; pp.23, 27-29) but factors other than proteolysis may also be involved.

The variations in soluble protein levels found between the four treatments, and in the course of each experiment, may be explained in part by the notorious variability in the protein content of temperate herbage grasses (p.19). Also, changes may occur in the soluble:insoluble protein ratio during wilting, contributing to the fluctuations found, although Wood *et al.* (1943) claim that proteins of

the soluble and insoluble fractions are broken down at much the same rate. Ohyama (1970) reported a 50% loss of protein in *L. multiflorum* leaves by the fifth day after cutting. Net protein losses, most obvious in the dark wilting treatments, may in principle result from failure of synthesis, enhanced proteolysis or both (Racusen and Aronoff 1954).

Many workers have found rapid increases in peptides, free amino acids and amides, also attributed to increased proteolysis during wilting. Changes in the levels of individual amino acids, in particular a great increase in proline, were usual (Kemble and Macpherson 1954; Brady 1965; Hegarty and Peterson 1973; see p.23). The rise in free amino groups, most obvious in the light wilting treatments, may similarly result from decreased protein synthesis, increased proteolysis, increased synthesis or decreased breakdown of amino acids, or a combination of these effects.

Considering the evidence of many plant physiological studies of changes in nitrogenous components during water stress and senescence, the relative importance of these possible causes of net protein loss and amino group increase is by no means resolved (pp.27-29, 33-34). However, it is generally agreed that protein synthesis is particularly susceptible to inhibition by water stress (pp.27-29), for example Racusen and Aronoff (1954) found decreasing ability for protein synthesis with increasing time after excision in soybean leaves. Woolhouse (1972) suggested that stomatal closure after excision prevents CO_2 transfer and fixation, thereby restricting amino acid and A T P supplies essential to maintain protein synthesis. Bornkamm (1974) and

Kluge (1974) reported changes in ribosomal fractions, in particular the breaking of polysomes giving single ribosomes, leading to a decrease in protein synthesis and the disappearance of short-lived enzymes such as nitrate reductase, under conditions of water stress. Failure of synthesis alone need not necessarily lead to a rapid loss of protein but could result in progressive decrease accompanied by accumulation of free amino groups. Other studies (Barnett and Naylor 1966; p.29) have shown that the synthesis of amino acids is less influenced by water stress and can apparently continue during drying, thus contributing to the increasing amino acid levels observed.

Hardwick *et al.* (1968) consider that the failure of protein synthesis is more important than increased proteolysis in explaining the net loss of protein from detached leaves. The silage literature contains many references to the role of plant proteases during wilting (e.g. Ohyama 1970; McDonald *et al.* 1968; Henderson *et al.* 1972; Gouet *et al*, 1973), but no unequivocal demonstration of proteolytic activity by such enzymes *in vitro* or *in vivo*. Physiological studies of plant responses to water stress and senescence similarly refer to the role of plant proteases in causing reductions in protein levels and concomitant increases in amino acid levels (Brady 1973). However, conclusive evidence that protease activity increases before or as net protein hydrolysis begins, is lacking. In some cases no increase in proteolytic activity has been found although protein content declined. It seems that the potential for protein hydrolysis is high at all times: the regulation of this activity is the critical factor (Brady 1973). Chibnall (1922) noted a decrease in protein content during low-temperature drying of runner bean leaves, and Tracey (1948) made a study of leaf

proteases. Singh (1962) investigated the proteolytic activity of leaf extracts from eleven plant species and found them to form a homogeneous group of enzymes of low activity, probably belonging to the S H - group, with optimum pH 5.2 - 5.7. Other workers (Tracey 1948; Brady 1961) reported optimum pH values between 5.0 and 6.3, a range characteristic of fresh plant extracts.

However no protease activity could be detected by the author in wilting *L. multiflorum* leaves using a casein digestion technique (Kunitz 1947; Umana 1968), which was used successfully by Beevers (1968) to follow proteolysis in leaf disks of nasturtium. It is possible that the ryegrass enzymes do not attack casein, so this negative result cannot be taken to indicate the absence of proteolysis in wilting ryegrass leaves. Ohyama (1970) studied protein loss and amino acid increase in *L. multiflorum* leaves and found that the protein:amino acid ratio decreased rapidly in whole plant leaves with closed stomata but not in chopped leaves. He attributed these results to the activity of plant proteases under reducing conditions in whole leaves, and a reduction in activity under more oxidative conditions in chopped leaves (Ohyama 1974). It seems that conditions are suitable for plant proteases in terms of pH, redox potential and available substrates during wilting, and a role for these enzymes in effecting changes in N-components is probable. Caution is necessary, however, in attributing all such changes to plant protease activity exclusively since their role is implicated and deduced rather than proven. It is possible that protease activity is suppressed through compartmentalisation in 'lysosome'-type bodies (De Duve 1959; Gahan 1965) in plant cells under normal conditions and only released under abnormal conditions of water stress and senescence during wilting.

This is pure speculation but, if justified, would suggest a lag-phase before the full expression of proteolytic activity after harvesting. Such a delay was found in two of the wilting experiments (light wilting at 20°; dark wilting in air).

It is concluded that the early rapid decline in the protein:free amino acid ratio found in the wilting experiments (apart from dark wilting in air) was caused in part by decreased ability for protein synthesis, in part by the persistance of amino acid synthesis, and in part by the activity of plant proteases leading to net protein hydrolysis, under conditions of water stress. The fall in the ratio was observed in an atmosphere of nitrogen indicating that anaerobic conditions support such changes, and in fact favour proteolysis. Protein synthesis is stimulated by light (Racusen and Aronoff 1954; Woolhouse 1972); proteolysis may also be enhanced by light. The unusual pattern of changes in dark wilting in air may have resulted from inhibition of protein hydrolysis together with a decrease in synthetic reactions under these conditions. The more rapid decline in protein:amino acid ratio at 20° than at 15° during light wilting indicates a temperature effect.

(iii) Pigments

Chlorophyll loss is a characteristic symptom of plant senescence (Yemm 1965; Beevers 1968; p.30) and may be expected under the stressful conditions of wilting. The chlorophyll absorption spectra indicated that chlorophyll loss occurred in all wilting treatments. Shifts in the chlorophyll peaks in the nitrogen treatment suggested that chlorophyll conversion and breakdown was greatest under dark anaerobic conditions.

Chlorophyll content was expressed on a D M and a leaf area basis as recommended by Sestak (1971). However, neither method of expression

is very suitable for wilting studies since the sample dry weights and leaf areas decrease during drying. There is no better alternative since fresh weight is more variable and levels of proteins and nucleic acids also decline (pp.27-28). Therefore, great caution is necessary in the interpretation of the results, and chlorophyll content cannot be used as a colour index of the biochemical changes taking place during wilting.

When expressed on a D M basis, the phases of most rapid chlorophyll loss occurred during darkness. Chlorophyll content in principle may decrease as a result of decreased synthesis, increased breakdown or both. Reduced synthesis is likely under wilting conditions since stomatal closure inhibits CO_2 fixation and thus restricts essential A T P supplies soon after harvest. Chlorophyll and chloroplast formation is prevented in darkness (Haber 1971). Hence, suppression of chlorophyll synthesis must assume importance during wilting, especially in the dark, leading to reduction in chlorophyll levels and, therefore, assimilation potential. Chloroplast disintegration can be equated with chlorophyll loss in the dark (Haber 1971), and is temperature dependent, as indicated by the difference between the two light wilting treatments.

Little is known about the biochemical processes involved in chlorophyll breakdown (Holden 1963). The acidic conversion of chlorophylls to pheophytins is unlikely to occur at the normal pH values of plant sap (c. pH 6.0 - 6.5). The enzyme chlorophyllase may be of considerable importance in catalysing the conversion of chlorophyll to chlorophyllide (by removal of the phytol group) under stress conditions, although the synthetic role of this enzyme is probably more important under normal conditions (Holden 1963). Oxidative conditions result in

the formation of green chlorophyll peroxides in plant extracts (Bruinsma 1963), but in the wilting experiments, the greatest chlorophyll losses were recorded under nitrogen. The general tendency for chlorophyll loss during wilting further suggests that net photosynthesis must decline.

SECTION 3

ENSILING STUDIES

SECTION 3: INTRODUCTION

In order to extend this investigation of the early stages of ensilage to the fermentation process itself, and to link the wilting studies with the events in the silo, a further programme of controlled experiments was carried out. Conventional laboratory microsilo techniques were used to investigate the effects of plant and microbial factors on the initial response to ensiling. An attempt was made to differentiate between the activities of the plant and microbial enzymes using a gamma-irradiation sterilisation technique. The biochemistry of the early stages of ensiling was monitored by following the timecourse of changes in plant biochemical constituents during the first 48 h in the silo.

SECTION 3: MATERIALS AND METHODS

PLANT MATERIAL

L. multiflorum Lam., R.v.P. var., used for the ensiling experiments, was cultivated under field, plot and greenhouse conditions. Field-grown ryegrass was obtained from Easter Howgate Farm (Stackyard Field) during 1972. Plot-grown ryegrass was obtained from 4 x 4 m experimental plots given different N P K fertiliser treatments and the two extremes of the range, designated 'high' and 'low' treatments, were selected for use in the ensiling experiments ('high' = 4.5 tonne ha^{-1} N P K + 1.125 tonne ha^{-1} nitrochalk; 'low' = 1.5 tonne ha^{-1} N P K + 0.375 tonne ha^{-1} nitrochalk). A greenhouse supply of ryegrass was obtained by cultivation of *L. multiflorum* in a Dutch-light greenhouse with supplementary lighting and heating to give a 16 h 17.5° day, 15° night and relative humidity of c. 70%.

Harvesting was carried out by hand as in Section 2, apart from the field-grown ryegrass which was harvested by a 4-drum rotary mower then conditioned by a Haymaster and wilted in the swath for 24 h. A fresh grass sample was collected immediately after mowing, a wilted sample after 24 h field wilting, by taking plant material from ten random swath locations on each sampling occasion. Each sample was stored temporarily in a polythene bag on ice until processing in the laboratory. The plant growth stage was characterised.

ENSILING EXPERIMENTS

The timecourse of biochemical changes during the first 48 h of ensilage was investigated in three experiments, summarised in Table 3.1 :-

Expt. 1: Ensilage of fresh plot-grown grass from the 'high' and 'low' fertiliser treatments.

Expt. 2: Ensilage of fresh and wilted field-grown grass.

Expt. 3: Ensilage of sterile and inoculated greenhouse grass.

TABLE 3.1 Ensiling experiments

		Expt. 1	Expt. 2	Expt. 3
Plant material	Source	Plot	Field	Greenhouse
	Age	16 weeks	14 weeks	13 weeks
	Stage	50% E E	50% E E	Y V
Wilting treatment		None	24 h field-wilting in swath	None
Initial D M		14.76% 'low' 14.47% 'high'	20%	11.22%
Final D M		-	35%	-
Sterilisation treatment		None	None	All samples apart from controls: 2.5 M rad dose over 11 h
Inoculation treatment		All samples: mixed population of L A bacteria	None	Half of samples: mixed population of L A bacteria

Thus the effects of plant factors such as previous cultivation method, growth stage, wilting treatment, and microbial factors such as inoculation of lactic acid bacteria and sterilisation treatment, could be assessed.

ENSILING PROCEDURE

The sample of plant material was chopped on a plastic tray into c. 1 cm pieces using sterile forceps, scissors and plastic gloves to minimise microbial contamination. 12 g amounts were rapidly weighed out into plastic bowls using a Mettler top-pan balance accurate to 2 dec. pls. Universal container bottles of 28 ml capacity, the microsilos, were filled using forceps and a plunger to transfer each sample and ensure even distribution and compaction, giving a final grass:air ratio of c. 1:2. After checking that there was no air space at the mouth, each bottle was sealed tightly with its screw cap. Fermentation locks and effluent outlets were considered unnecessary in this investigation of the very early stages of fermentation. When necessary, experimental silos were stored at -20°, as recommended by Gouet (1972, 1973).

STERILISATION TREATMENT

Sterile (axenic) samples of plant material were obtained by exposing sealed microsilos at ice temperature to gamma-irradiation. A 2.5 M rad dose was given over 11 h from a ^{60}CO source. Bousset *et al.* (1972) recommended a 1.2 - 2.5 M rad dose for the sterilisation of similar microsilos. Sterility checks were carried out routinely in the course of the experiments using axenic microsilos (pp.169,172).

INOCULATION PROCEDURE

An inoculum of a mixed population of lactic acid bacteria, *Lactobacillus plantarum* and *Streptococcus faecalis* (homofermenters), was grown in liquid culture, spun down and resuspended in distilled

water. The inoculum was prepared immediately before use to avoid lysis of the bacterial cells, diluted to give c. 10^6 cells ml^{-1} then a 0.1 ml aliquot used for each sample. Inoculation was performed either immediately before filling the silo, Expt. 1; or after sterilisation, storage at -20° and thawing, Expt. 3 (Gouet *et al.* 1972).

BIOCHEMICAL ANALYSES

Experimental silos were incubated at 30° for 48 h. Duplicate or triplicate silos were removed from the incubator after 3, 6, 9, 12, 24, 36 and 48 h and subjected to biochemical and microbiological analysis. A sample of the original plant material was analysed giving the biochemical status of the fresh (or wilted) plant material at zero time. When freezing or sterilisation treatments were used in the course of the experiments, additional frozen and axenic control silos were analysed at zero time.

Spectrophotometric methods of analysis were used to determine the same biochemical components as in Section 2 with the addition of pheophytin. All analyses were performed in triplicate on the contents of each silo. pH and D M content were also determined routinely. pH extracts were prepared by blending 6 g silage in 60 ml ice-cold distilled water for 30 s, filtered through double muslin, then the pH measured using a Pye Unicam pH meter. D M content was determined by oven-drying at 80° to constant weight.

Pheophytin. Vernon's (1960) method for the simultaneous determination of chlorophylls and pheophytins in silage extracts treats the aqueous acetone extracts as a 4-component mixture of chlorophylls *a* and *b*, and pheophytins *a* and *b*,

and can be used to estimate each component in the mixture. Absorbances were read at 666, 662, 655 and 645 nm prior to and after the addition of oxalic acid solution which converts the chlorophylls present to their respective pheophytins. Equations based on the absorbances at these four wavelengths are used to calculate pheophytin amounts (mg l^{-1}).

MICROBIOLOGICAL ANALYSES

Total counts. The kinetics of microbial growth during the early stages of ensilage was followed by assaying for total counts in fresh, frozen (zero time controls) and 24 h and 48 h ensiled plant material (Expt. 3). About 3 g plant material was transferred aseptically to a sterile round-bottomed flask, 10 ml sterile distilled water added, and the surface microflora dislodged by swirling at intervals for c. 30 min. The resultant microbial suspension was decanted, dilutions made down to 10^{-8}, and plated out in duplicate on yeast extract agar. After incubation at 30° for 3 - 5 d, the plates were counted and the results expressed as no. micro-organisms per g F W of plant material (Gouet 1973).

Sterility checks. The irradiated samples were checked for sterility by preparing an extract as above and plating out dilutions down to 10^{-4} on Y E agar; and also by plating out small pieces of irradiated plant materials directly. The first method was used initially on duplicate control irradiated silos; the second method was used routinely in the course of Expt. 3.

SECTION 3: RESULTS

EXPERIMENT 1

Figs. 3.1 - 3.6 illustrate the results of Expt. 1, the ensilage of fresh grass, grown with 'high' or 'low' fertiliser treatment, after inoculation with a mixed population of lactic acid bacteria.

Fig. 3.1 shows the pH and D M changes during the first 7 d of ensilage. A small transient pH peak in the first six h, followed by a plateau from 9 - 12 h, preceded the main phase of pH fall in both treatments. After 12 h of ensilage, the pH fell rapidly by c. 2.5 units to pH 4.5, and by c. 1 unit to pH 6.0, after 2 d of ensilage in the 'low' and 'high' fertiliser treatments respectively. Both treatments showed a transient secondary increase in pH of c. 0.5 unit after 3 d, followed by a decrease to levels of 4.2 and 5.7 in the 'low' and 'high' treatments respectively, after which little further change occurred.

The D M content fluctuated irregularly in both treatments but fell during the main phases of pH decline from c. 14.7% in fresh grass to 12% and 10.7% after 7 d in the 'low' and 'high' treatments respectively.

Fig. 3.2 shows the soluble carbohydrate changes during the first 7 d of ensilage. In general, soluble carbohydrate levels in the 'low' fertiliser treatment were about double those in the 'high' fertiliser treatment. In the former, a transient increase to a

peak in total soluble carbohydrates, fructose and glucose during the first six h, was followed by a rapid fall to a low level after 2 d. In the latter, changes in soluble carbohydrate levels were more irregular: a small peak during the first 3 h was followed by fluctuations until a steady low level was attained after 2 d (glucose) to 4 d (total soluble carbohydrates, fructose).

Figs. 3.3 and 3.4 show the changes in nitrogenous components during the first 7 d of ensilage in the 'low' and 'high' fertiliser treatments respectively. The overall pattern of changes was similar in both treatments. Protein levels were rather variable but showed a general tendency to decrease over the ensilage period. Free amino groups decreased slightly during an initial 6 - 12 h lag-phase, then increased to a peak between 3 and 4 d. The protein: free NH_2 ratio decreased rapidly during the first day (after an early temporary peak in the 'low' fertiliser treatment), reaching a low constant level after 36 h of ensilage. In general, protein and free amino group levels in the 'low' treatment were only 0.33 to 0.5 of those in the 'high' treatment, but the ratio values were about the same in both treatments.

Figs. 3.5 and 3.6 show the pigment changes during the first 7 d of ensilage in the 'low' and 'high' fertiliser treatments respectively. The overall pattern of changes was very similar in both treatments. Chlorophyll levels were rather variable but showed a general decrease over the ensilage period. Pheophytin levels decreased slightly during the first 6 h, then increased rapidly during the first 3 d to maximum values. The chlorophyll:pheophytin ratio showed a transient increase during the first 6 h then fell rapidly from 6 to 24 h reaching a steady low level after 2 d. Changes in the ratio were greatest in the 'low' fertiliser treatment.

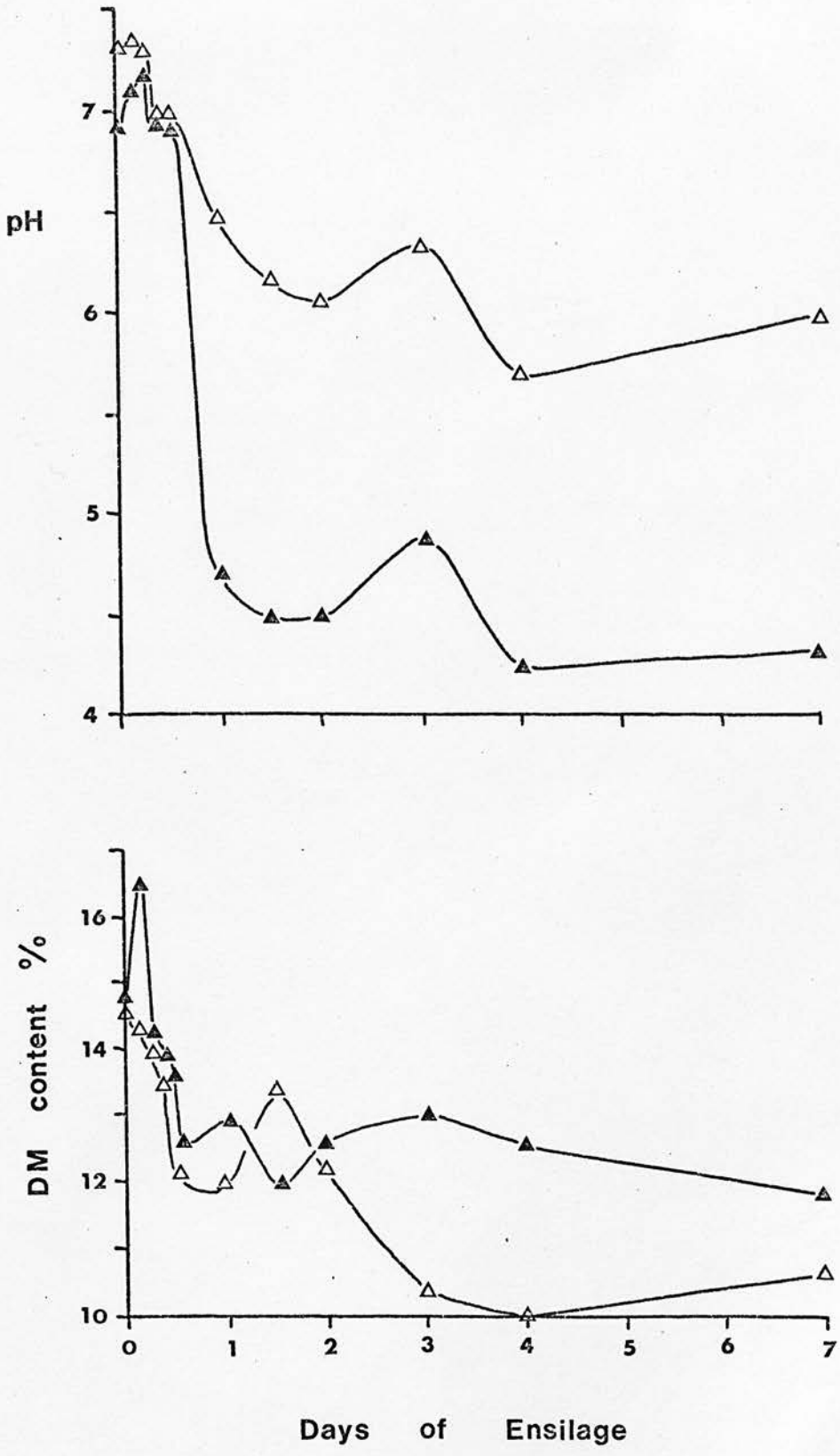


FIG. 3.1 Changes in pH and D M during the first 7 d of ensilage, Expt. 1

▲ , 'low' fertiliser treatment; △ , 'high' fertiliser treatment

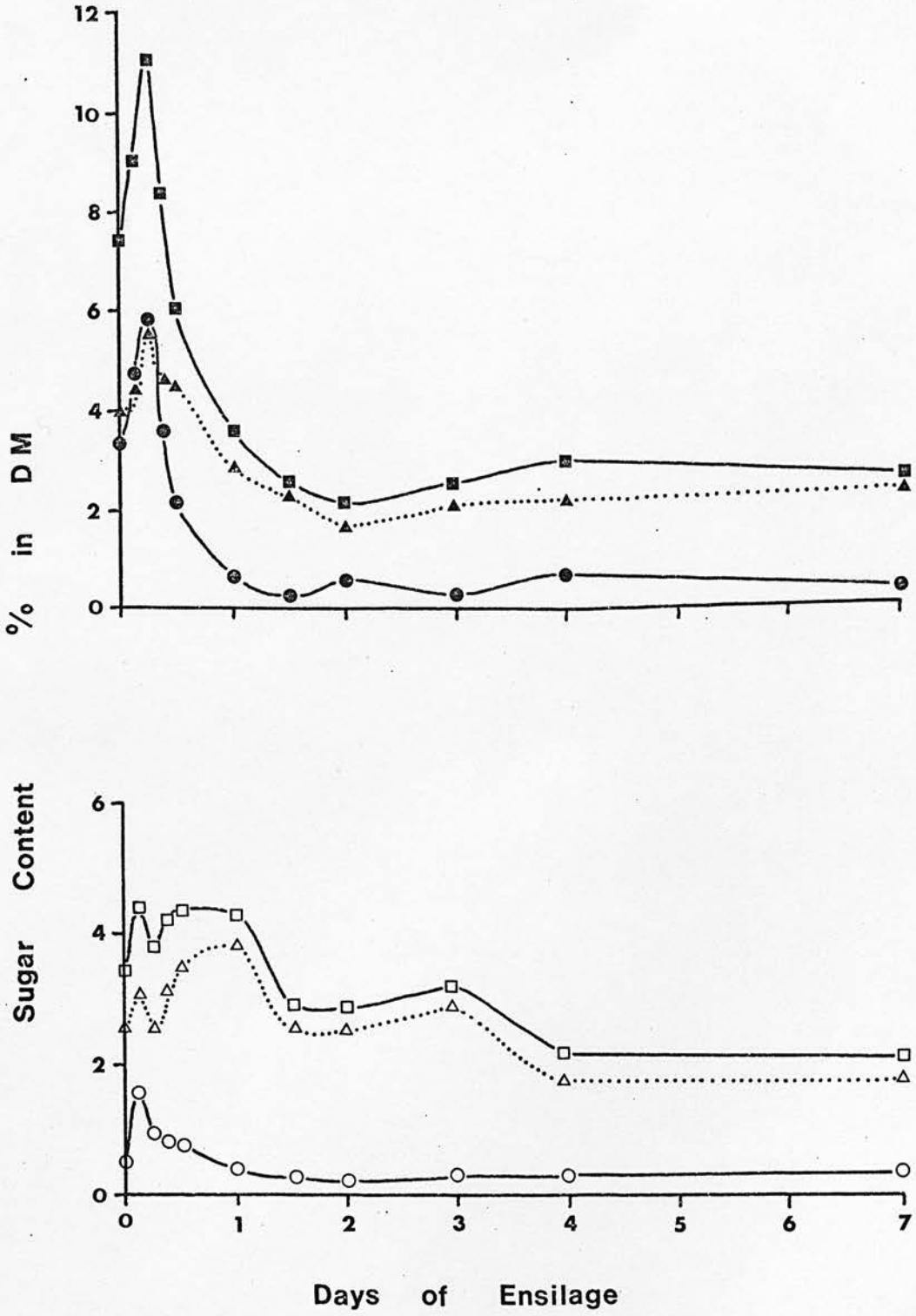


FIG. 3.2 Changes in soluble carbohydrates during the first 7 d of ensilage, Expt. 1

■ , □ , total water-soluble carbohydrates; ● , ○ , fructose; ▲ , △ , glucose (all determined after hydrolysis); filled symbols, 'low' fertiliser treatment; open symbols, 'high' fertiliser treatment

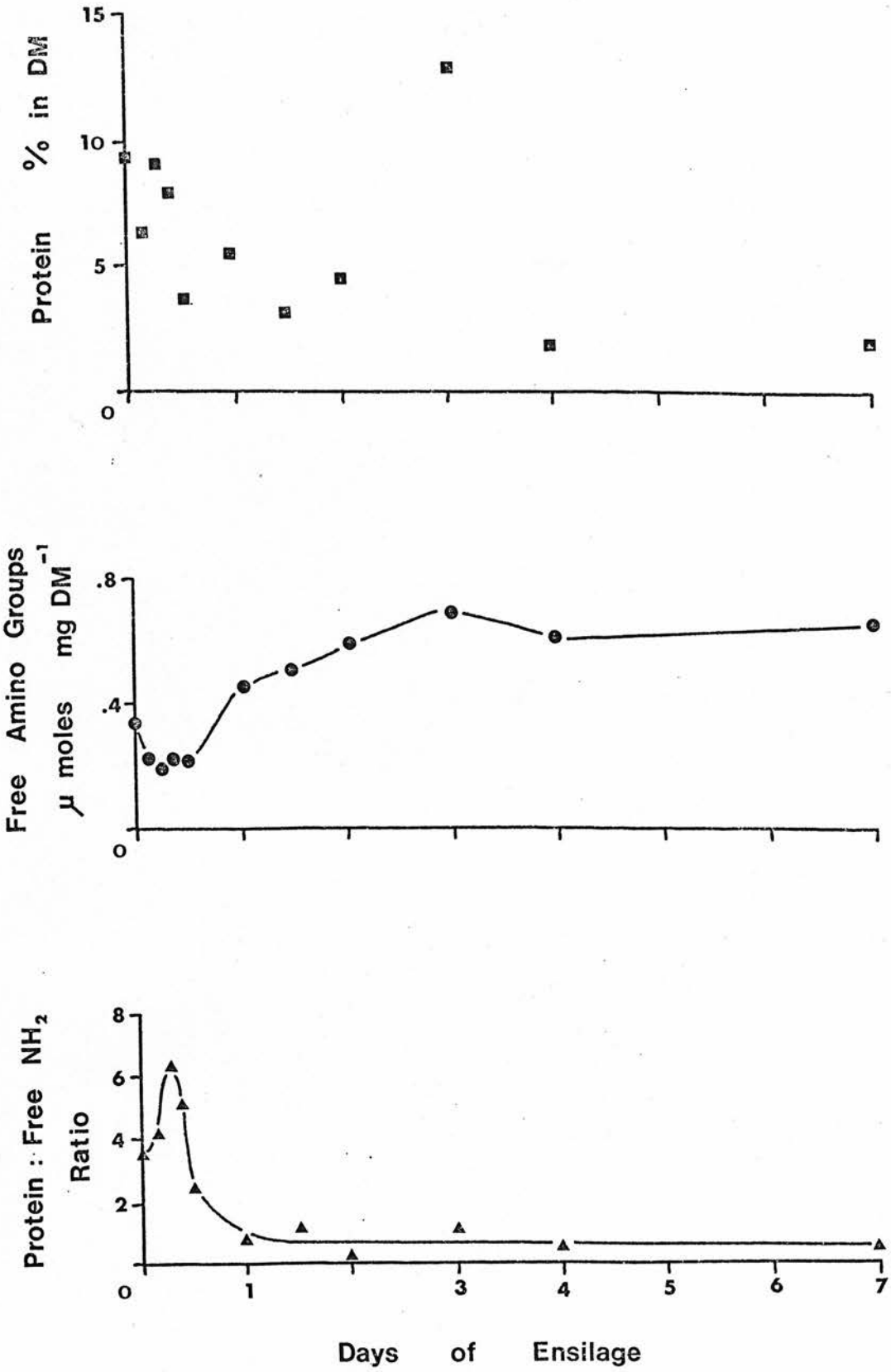


FIG. 3.3 Changes in nitrogenous components during the first 7 d of ensilage, 'low' fertiliser treatment, Expt. 1

■ , total soluble protein; ● , total free amino groups;
▲ , protein:free amino group ratio

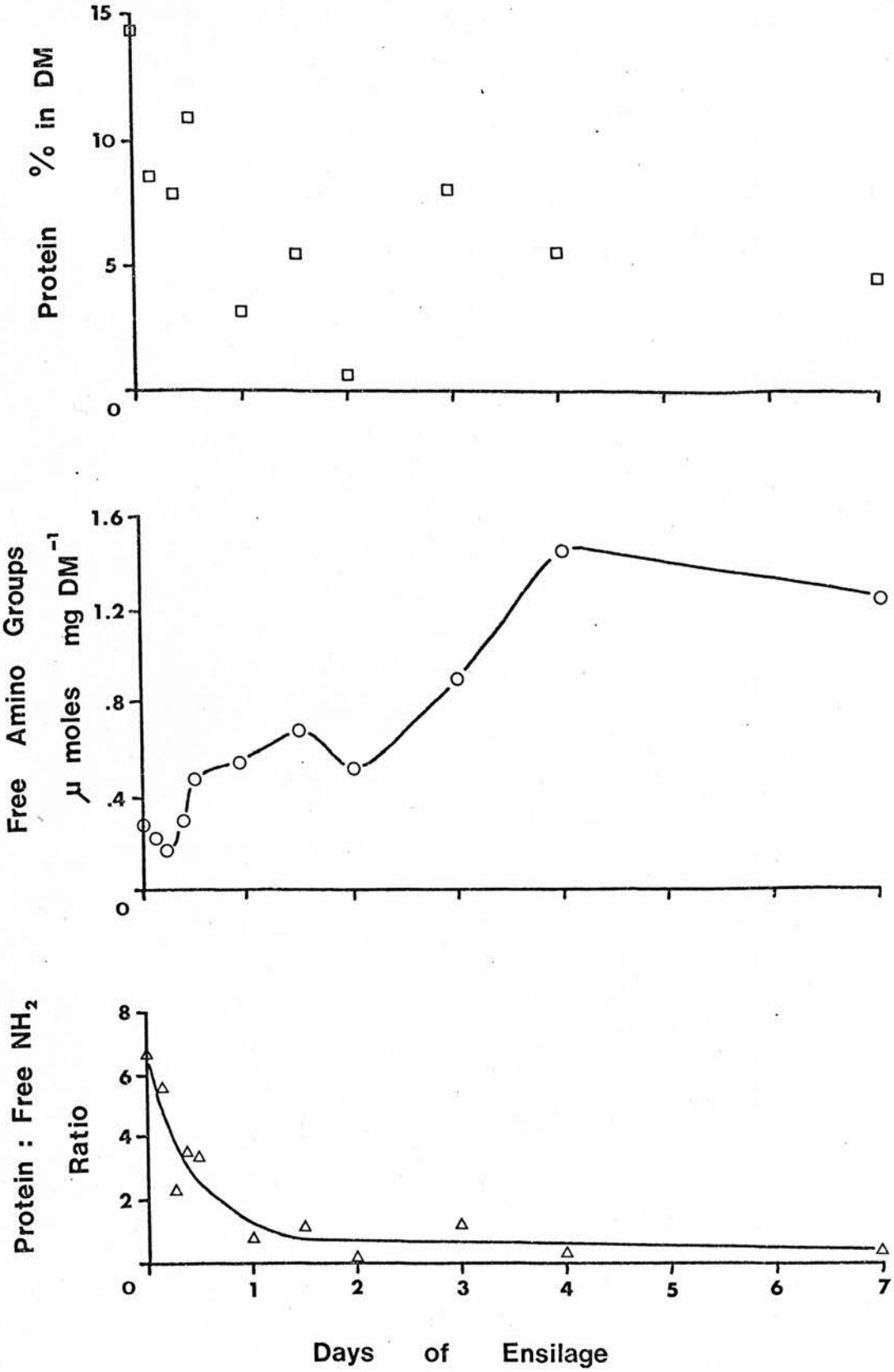


FIG. 3.4 Changes in nitrogenous components during the first 7 d of ensilage, 'high' fertiliser treatment, Expt. 1

□ , total soluble protein; ○ , total free amino groups;

△ , protein:free amino group ratio

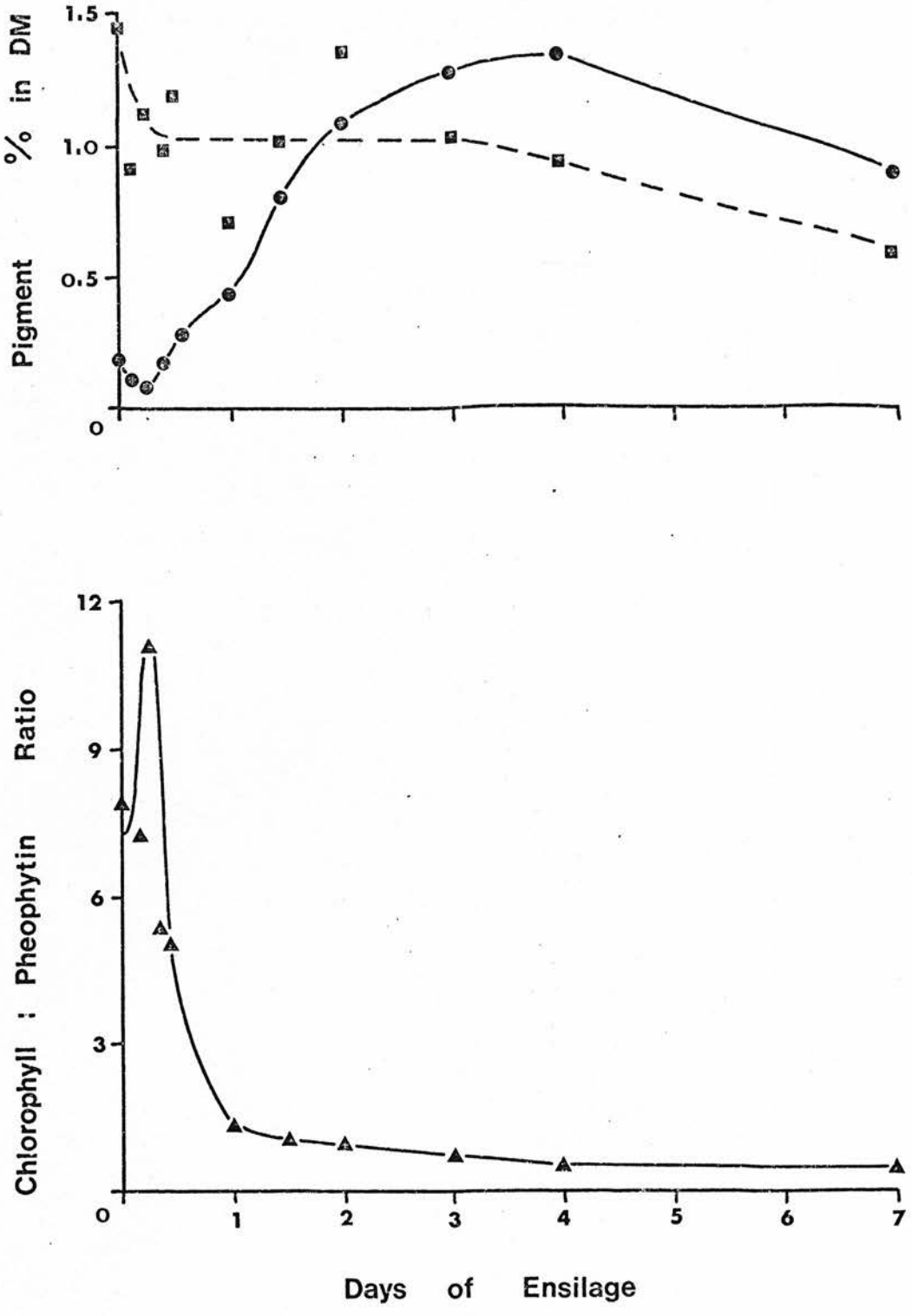


FIG. 3.5 Changes in pigments during the first 7 d of ensilage, 'low' fertiliser treatment, Expt. 1

■ , total chlorophyll a + b ;

● , total pheophytin a + b ;

▲ , chlorophyll:pheophytin ratio

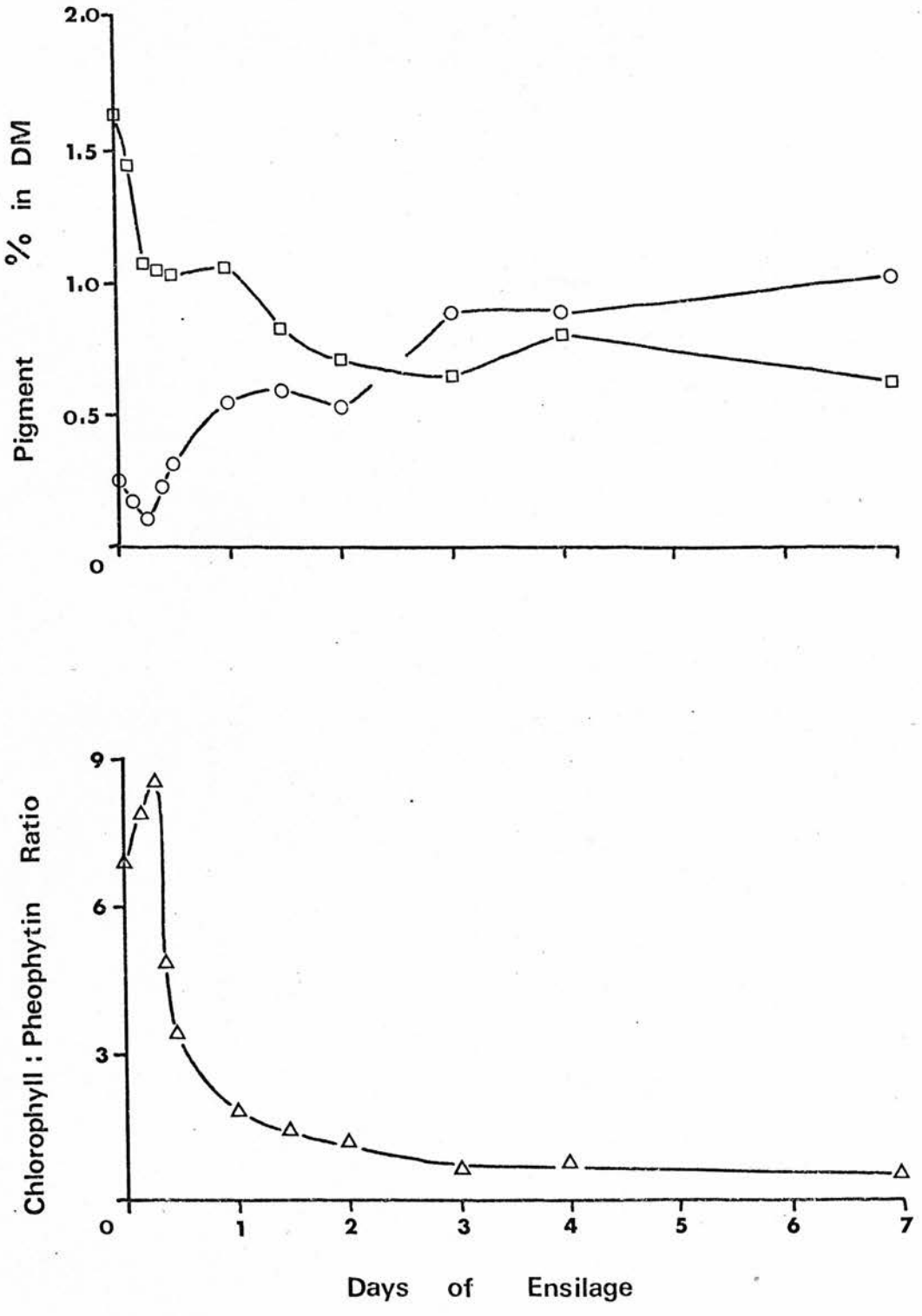


FIG. 3.6 Changes in pigments during the first 7 d of ensilage, 'high' fertiliser treatment, Expt. 1

□ , total chlorophyll a + b ; ○ , total pheophytin a + b;
△ , chlorophyll:pheophytin ratio

EXPERIMENT 2

Figs. 3.7 - 3.10 illustrate the results of Expt. 2, the ensilage of fresh and wilted field grass, without inoculation. Fig. 3.7 shows the pH and D M changes during the first 48 h of ensilage. A small transient pH peak during the first 3 - 6 h was followed by a plateau in the wilted treatment, and a decrease in the fresh treatment, between 6 and 12 h. Thereafter, the pH declined slowly throughout the 48 h ensiling period. The D M content showed a small overall decrease after 48 h in both treatments, from initial values of 36% and 20% to final values of 30% and 16% in the wilted and fresh silages respectively.

Fig. 3.8 shows the soluble carbohydrate changes during the first 48 h of ensilage. In both treatments, the levels of total soluble carbohydrates, fructose and glucose fluctuated irregularly throughout the ensiling period, showing initial peaks in the first 12 h, and secondary peaks after 36 h. There was little net change in sugar levels after 48 h of ensilage. In general, the soluble carbohydrate contents of the fresh silage were from a third to a half as high again as those of the wilted silage.

Fig. 3.9 shows the changes in nitrogenous components during the first 2 d of ensilage. Protein levels were rather variable but tended to decrease in the fresh treatment or remain the same in the wilted treatment. After an initial lag-phase of c. 12 h, free amino groups increased steadily. The protein:free NH_2 ratio showed a small initial peak in both treatments, then decreased rapidly to a constant low level attained after c. 12 h in the fresh treatment, but varied

around a fairly constant low value in the wilted treatment. In general, in the fresh silage, protein levels were lower, free amino groups higher and the ratio values lower than in the wilted silage, after the first 12 h had elapsed.

Fig. 3.10 shows the pigment changes during the first 2 d of ensilage. Chlorophyll levels were very variable; pheophytin levels were also very variable but tended to increase during the second day. The chlorophyll:pheophytin ratio fluctuated widely during the first 12 h but thereafter decreased steadily, most markedly in the fresh silage.

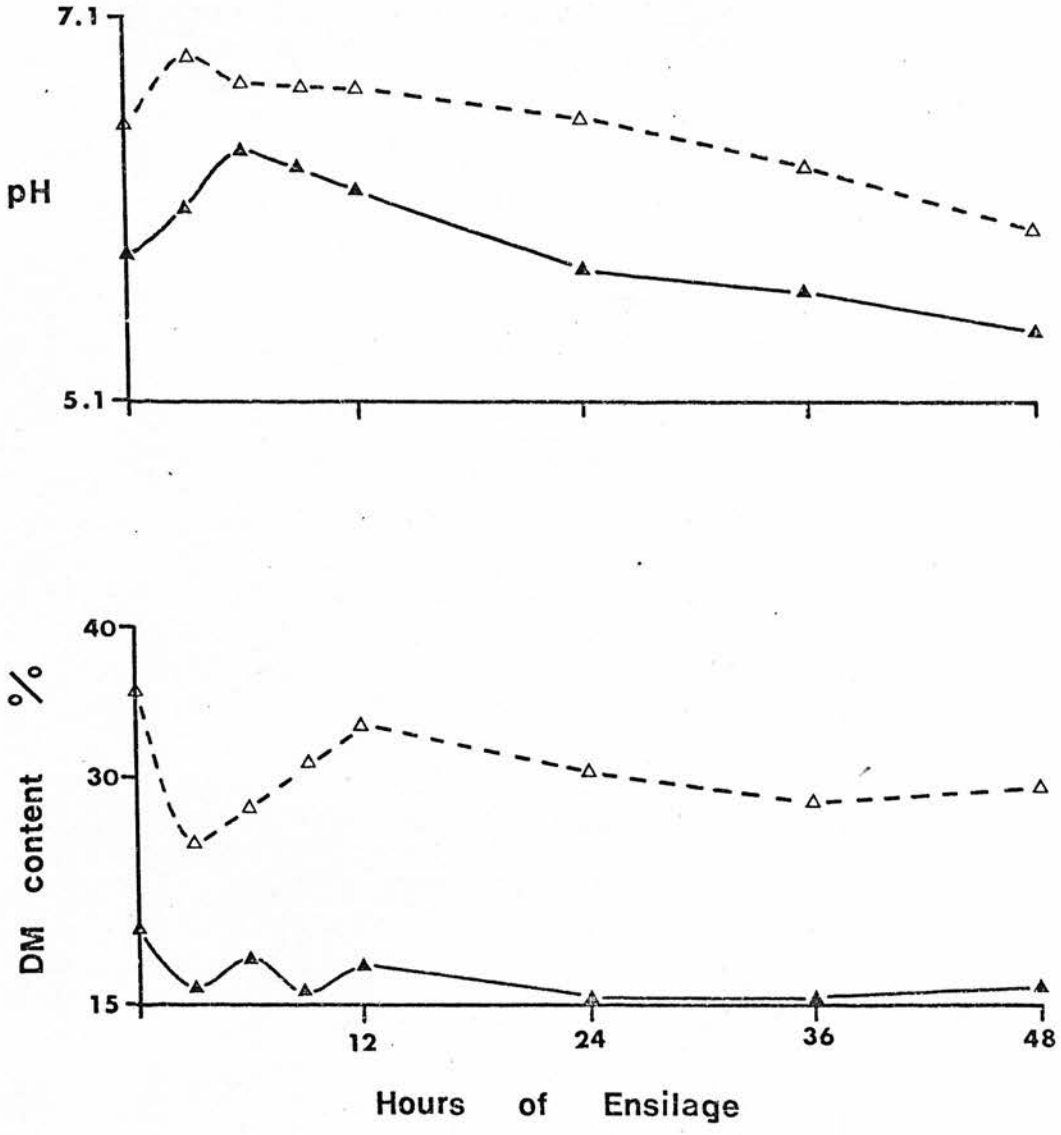


FIG. 3.7 Changes in pH and D M content during the first 2 d of ensilage, Expt. 2

▲ , fresh;

△ , wilted

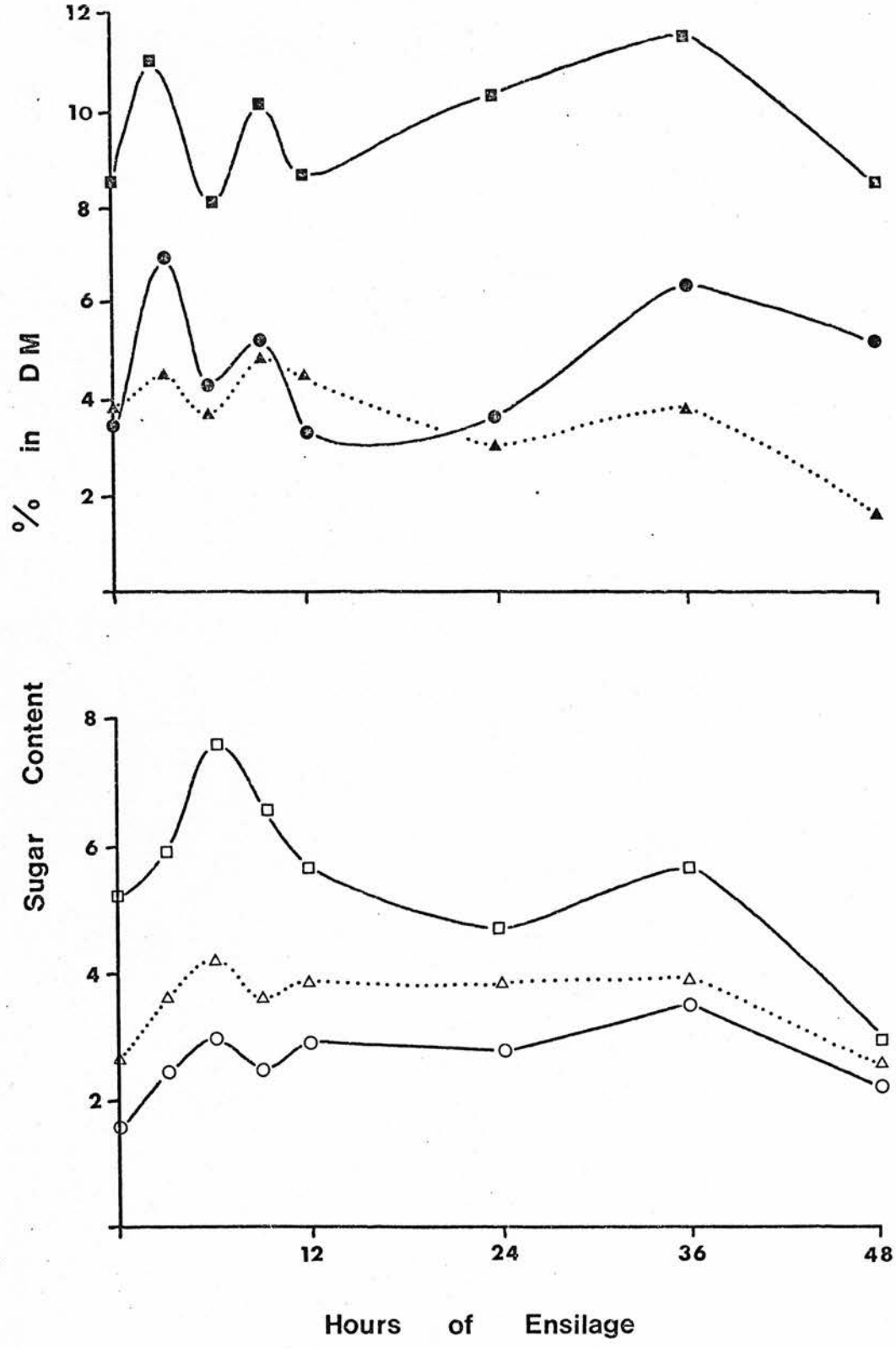
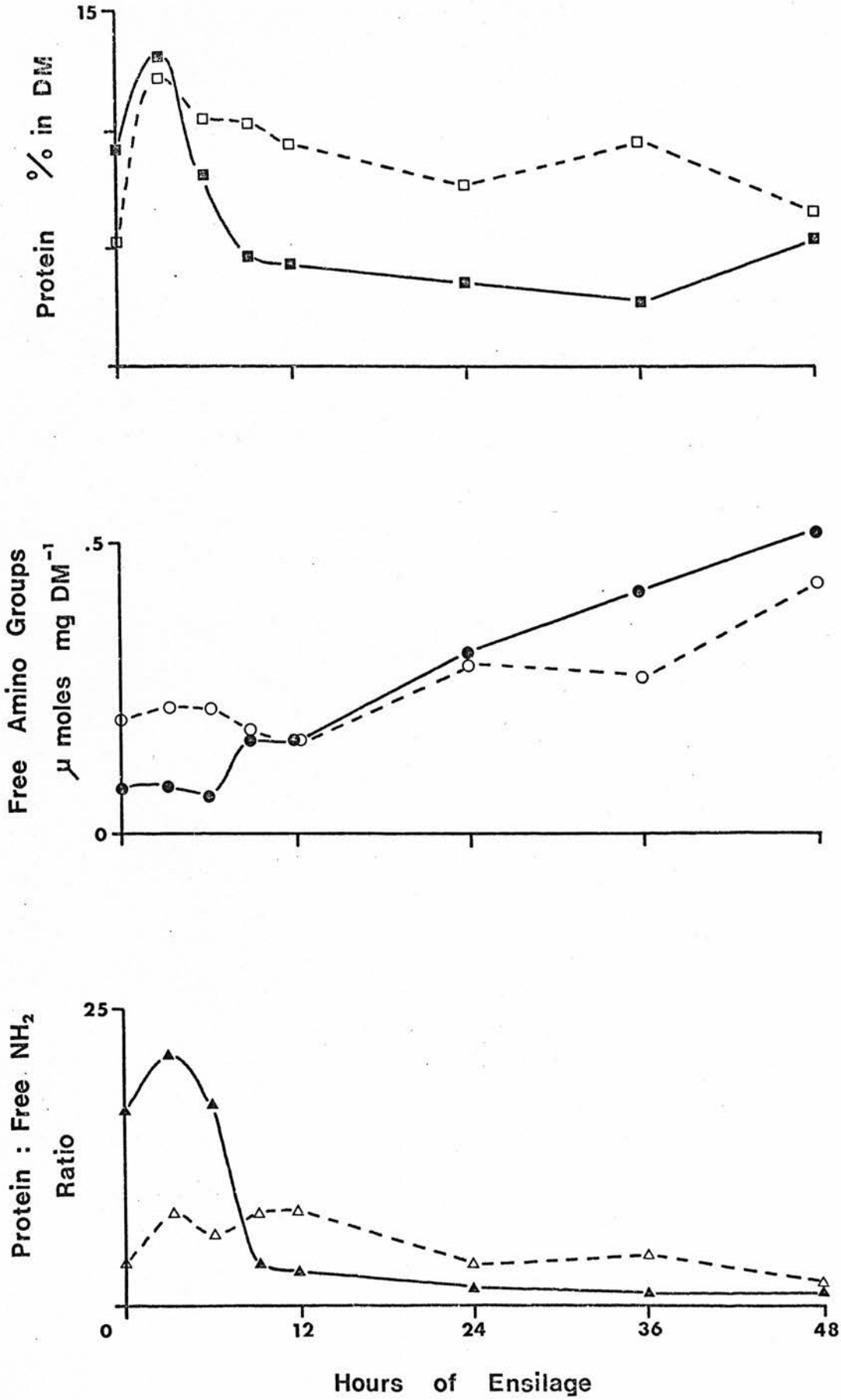


FIG. 3.8 Changes in soluble carbohydrates during the first 2 d of ensilage, Expt. 2

■ , □ , total water-soluble carbohydrates; ● , ○ , fructose, ▲ , △ , glucose (all determined after hydrolysis); filled symbols, fresh; open symbols, wilted

FIG. 3.9 Changes in nitrogenous components during the first 2 d of ensilage, Expt. 2



■ , □ , total soluble protein; ● , ○ , total free amino groups; ▲ , △ , protein:free amino group ratio; filled symbols, fresh; open symbols, wilted

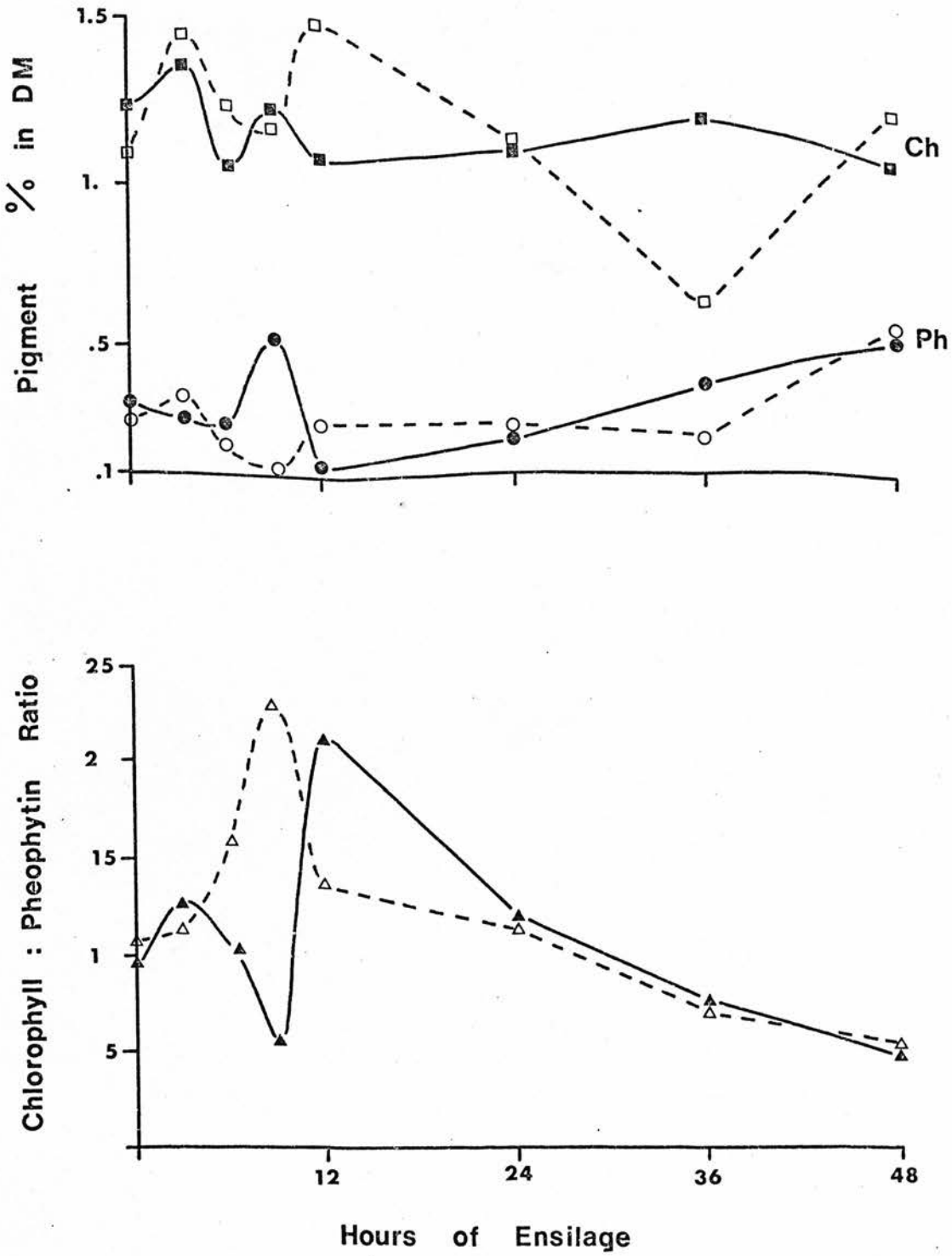


FIG. 3.10 Changes in pigments during the first 2 d of ensilage, Expt. 2

■ , □ , total chlorophyll a + b; ● , ○ , total pheophytin a + b; ▲ , △ , chlorophyll:pheophytin ratio; filled symbols, fresh; open symbols, wilted

EXPERIMENT 3

Fig. 3.11 illustrates the results of Expt. 3, the ensilage of γ-irradiated sterile and inoculated grass. The total count increased ten-fold over 7 d in the inoculated silos.

The pH fell slightly during the first 6 h of ensilage, then remained unchanged in the sterile treatment, but fell steadily after 1 d in the inoculated treatment. The total W S C content increased slightly during the first 6 h but thereafter remained the same in the sterile treatment; total W S C content increased rapidly to a peak after 3 h, then fell rapidly during the next 3 h and decreased further after 48 h of ensilage in the inoculated treatment. However, after 7 d, W S C levels were about the same in both treatments.

Protein levels decreased from 10% in the fresh control, to 5% in the frozen control to 1.3% after irradiation. After ensilage, the pattern of protein changes was the same in both treatments, i.e. a rapid initial fall to a fairly steady low level, attained after 3 - 6 h.

Sterility checks on the irradiated grass samples were consistently negative.

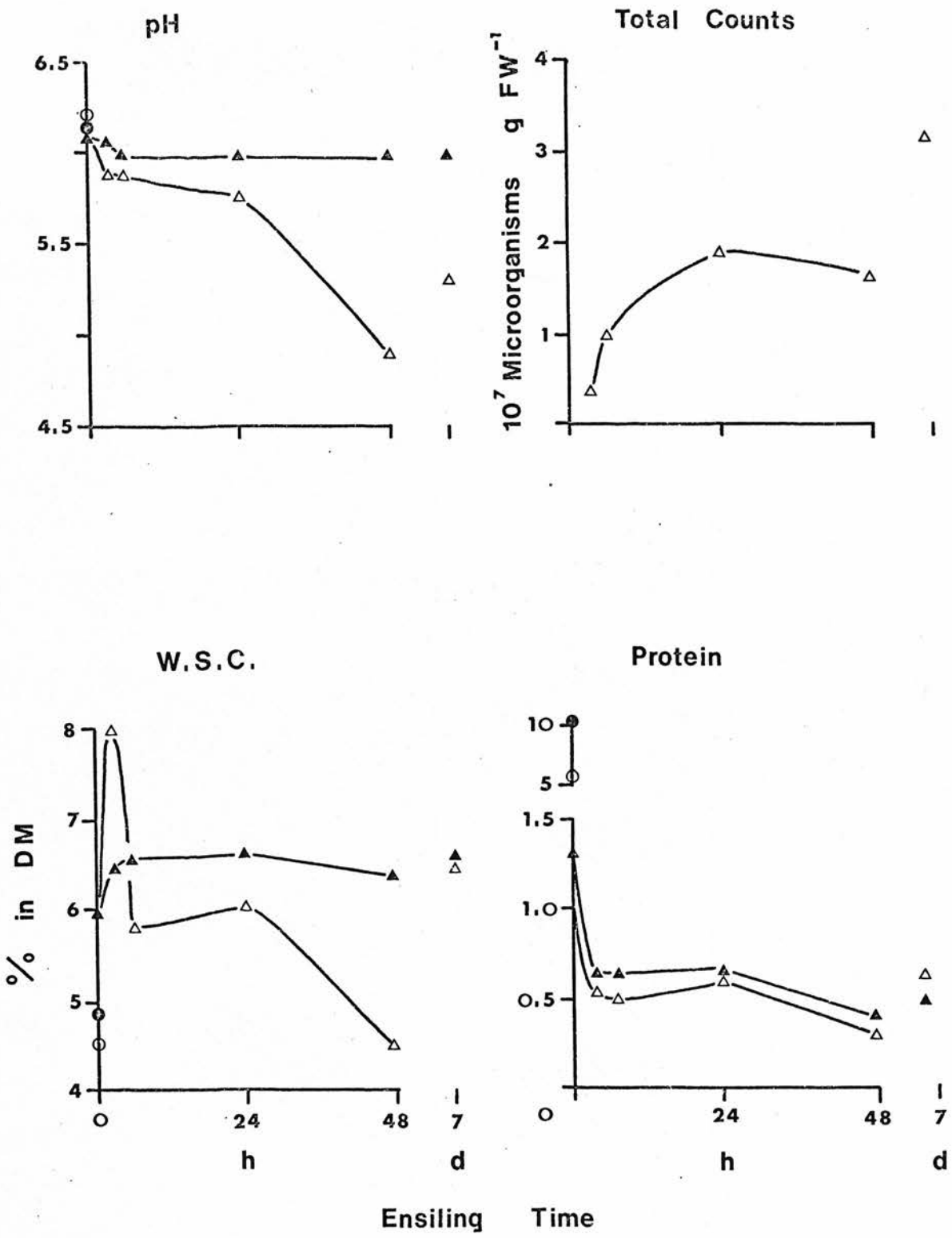


FIG. 3.11 Changes in axenic and inoculated silos during the first 7 d of ensilage, Expt. 3

⊕ , fresh control; ○ , frozen control;
▲ , axenic silo; △ , inoculated silo

SECTION 3: DISCUSSION

Small laboratory silos were used in all the experiments in order to investigate the timecourse of biochemical changes occurring during the very early stages of ensilage. It was not practicable to construct many large-scale silos for opening within the first few hours of ensilage, whereas it was possible to set up numerous small experimental silos rapidly after harvest, allowing for replication, and the minimisation of enzymic changes by cold storage during filling. Extensive use has been made of small laboratory silos in several countries (Pedersen *et al.* 1973) and in general the results obtained give a picture similar to that obtained from larger experimental silos (Stirling 1951; Wilson and Wilkins 1972; Pedersen *et al.* 1973). However, caution is necessary in extrapolating from the results of small laboratory silos to authentic farm silos since differences may occur both in the time-scale and in the magnitude of the changes involved. Bousset (1973) predicts lag-periods of 3 - 6 h in laboratory silos but 10 - 12 h in farm silos before 'plasmolysis' occurs, resulting in accessibility of substrates to enzymes and rapid fermentation changes. Greenhill (1964 a) detected plasmolysis within the first 8 - 10 h when ensiling ryegrass and lucerne in 100 g laboratory silos. Furthermore, edge-effects may be important in small experimental silos (Bousset 1973). A scale-up factor of about c. 1:4 may be necessary when comparing laboratory silos with farm silos (Woolford 1973). Therefore, although the overall pattern of changes found during the early stages of ensilage may be considered representative of ensilage under both laboratory and farm conditions, the relative time-scales and the magnitude of the changes involved may vary from one to the other.

EXPERIMENT 1

Expt. 1 provided information about a. the pattern of biochemical changes occurring during the first 7 d of ensilage of fresh inoculated grass, and b. the effect of grass nutrient status and biochemical composition on the subsequent ensilage process.

a. Pattern of biochemical changes

(i) pH The small transient pH peak observed during the first 6 - 9 h of ensilage coincided with a temporary increase in soluble carbohydrates; a temporary decrease in total free amino groups and marked changes in the protein:free NH_2 ratio; transient decreases in both chlorophyll and pheophytin and an increase in the pigment ratio.

An increase in pH during the very early stages of ensilage (first 1 - 2 d) has often been observed (Bousset *et al.* 1972; McDonald 1974; Ohyama 1974). Bousset *et al.*(1972) reported pH increases in silages inoculated with *Str. faecalis*. This may arise from decarboxylation reactions leading to a net increase in cations, for example, decarboxylation of di- and tri- carboxylic acids present as natural buffering constituents in fresh herbage (Playne and McDonald 1966), or decarboxylation of amino acids. The large gaseous losses of carbon dioxide frequently found during the early stages of ensilage (Daniel *et al.* 1970; McDonald 1974) depend on the fermentation substrate and the microbial spp. present (Bousset *et al.* 1972), and may result in part from such decarboxylation reactions. The net decrease in free amino groups at this stage could result from reduced synthesis or increased breakdown or both. Deamination reactions leading to ammonia formation and pH rise are thought to be unlikely at this early period however.

The main phase of pH reduction was delayed until 12 - 24 h after ensiling. The lactic acid bacteria used for the inoculum, *L. plantarum* and *Str. faecalis*, have a lag-phase of several hours before the onset of exponential growth in pure culture and a 12 - 18 h period is usual before a great increase in numbers and activity is observed (Mann 1974). The growth of lactic acid bacteria on grass substrates in experimental silos is analagous to microbial growth in pure culture. The 12 - 18 h period needed for the establishment of the lactic acid bacterial population in part explains the delay in the onset of pH reduction. Ohyama *et al.* (1973) followed the growth of *L. plantarum* in an extract of Italian ryegrass and found a similar delay of c. 24 h before the build-up of bacterial numbers and the onset of pH reduction. Greenhill (1964 a) reported a delay of c. 10 h before pH fall and concomitant lactic acid production was detected and c. 20 h before lactic acid was present in large amounts in ryegrass silage. Collapse of the silage mass and an increase in electrical conductivity after 8 - 10 h was assumed to indicate plant cell breakdown, protoplasmic injury and the release of plant juices (plasmolysis), a necessary pre-requisite for silage fermentation (Greenhill 1964 a). The main phase of pH reduction must succeed the establishment of conditions suitable for the growth of lactic acid bacteria, namely anaerobiosis (Greenhill 1964 b) and plasmolysis (Greenhill 1964 a), and their exponential growth phase. The delay in onset of fermentation may be much greater in farm silos especially if oxygen persists (Ruxton and McDonald 1974).

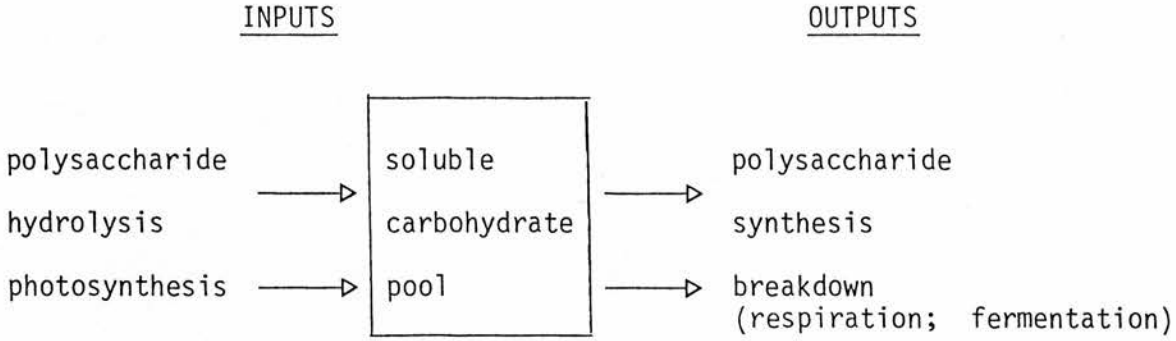
The temporary increase in pH after 3 d of ensilage may reflect microbial succession at the lower pH values (McDonald *et al.* 1966) as frequently occurs in experimental and authentic farm silos (Whittenbury 1968; Mann 1974). *Str. faecalis* metabolises very little sugar and

cannot lower the pH below 4.6, so *L. plantarum* probably becomes dominant at the lower pH values as this species can utilise sugars down to pH 3.8 in ryegrass (Bousset *et al.* 1972). Alternatively the secondary pH rise could indicate the onset of clostridial activity under anaerobic conditions in relatively high moisture plant material (McDonald *et al.* 1966).

(ii) D M The overall fall in D M content, with the periods of most rapid decline coinciding with periods of rapid pH fall, indicates that production of water or dry matter loss occurred during the early stages of ensilage. Production of water may occur during aerobic respiration and fermentation. Greenhill (1964 a) estimated that the oxygen trapped in small laboratory silos is consumed within 2 - 5 h, so the persistance of aerobic respiration beyond such a period is unlikely. Bousset *et al.* (1972) attributed a fall in D M content to water produced in various fermentations, lactic, acetic, alcoholic, etc. The D M losses associated with ensilage are mainly due to aerobic respiration; fermentation losses are relatively small and should not exceed 4 - 6% of the D M (McDonald and Whittenbury 1967). A close correlation between gaseous losses and the variations in D M content of the forage during conservation was reported (Bousset *et al.* 1972). These workers consider that the losses attributable to plant respiration have been overestimated and that gaseous losses are essentially due to bacterial fermentations. In the closed system studied here, net D M losses of about 15 - 25% of the initial D M occurred over 7 d. Since effluent losses were largely prevented, it is concluded that the D M reduction resulted in minor part from the formation of water and in major part from gaseous losses. Clostridial fermentation leads to especially high gaseous losses in poorly preserved high pH silage (McDonald and Dewar 1960).

The possible error arising from volatile losses on oven-drying of D M samples is very small at 3 - 5% (McDonald 1974) and unimportant in the early stages of ensilage.

(iii) Soluble carbohydrates. An increase in total W S C has often been observed during the very early stages of ensilage (Bousset 1973; McDonald 1974; Ohyama 1974). The size of the soluble carbohydrate pool in grass or silage depends on the balance of synthesis, breakdown and the hydrolysis of polysaccharides:-



Theoretically, an increase in pool size could result from increased synthesis, decreased breakdown of soluble carbohydrates or polysaccharide hydrolysis. Increased synthesis can be ruled out under ensiling conditions since photosynthesis would be impossible (lack of light and CO_2; irreversible changes in chloroplast and chlorophyll structure) and existing A T P supplies in the plant cells would be rapidly exhausted. Hydrolytic and degradative changes are likely to predominate in both plant and microbial cells. It is conceivable that the rate of sugar catabolism may fall after the trapped oxygen has been consumed in aerobic respiration but before fermentation is well established. This would be expected to occur at c. 6 - 12 h after ensiling, when the soluble carbohydrate peaks in fact appeared, so the decreased breakdown of sugars may be a contributory factor.

However, the explanation for soluble carbohydrate peaks generally offered is the hydrolysis of plant polysaccharides (Dewar *et al.* 1963; Bousset 1973; McDonald 1974; Ohyama 1974). The major non-structural polysaccharides in ryegrass are fructans (p.14) which are thought to be rapidly hydrolysed during ensilage and determined with the total W S C and fructose components (Whittenbury *et al.* 1967). Fructans are not usually considered when trying to account for net increases in soluble carbohydrates. However, the plant fructans may be partially protected from hydrolysis by cellular compartmentalisation in the very early stages of ensilage. It is possible that total extraction of fructose components from membrane complexes analagous to amyloplasts ('fructoplasts') may be prevented until after plasmolysis has occurred. This would explain the early fructose peaks which appeared between 3 and 12 h after ensiling.

The plant structural polysaccharides include cellulose, hemicelluloses, pectins and β-glucans which constitute the plant cell walls (pp. 16-17) and are separated from the cytoplasmic enzymes through cellular compartmentalisation. So under normal conditions, hydrolysis does not occur. Theoretically, hydrolysis of plant structural polysaccharides could occur following plasmolysis when the previously inaccessible substrates may be exposed to enzymic attack. However, since plasmolysis takes several hours after ensiling, the hydrolysis of plant structural components in this way must show a similar lag-phase.

Bousset *et al.* (1972) studied the carbohydrate metabolism of various silages, including ryegrass, in detail. They found a large reduction in cellulose levels in the presence of *L. brevis* or *Str. faecalis*, species known to metabolise glucose. They concluded that attack was made

on true cellulose, and that the breakdown of wall polysaccharides explained the considerable accumulation of fermentation products, particularly by *Str. faecalis* whose apparent utilisation of soluble sugars is very small. Since *Str. faecalis* was used in the inoculum for Expt. 1, cellulose hydrolysis *may in part* explain the early peaks in total W S C and glucose, and the high glucose concentrations found in the ryegrass silages studied. Another contributory factor *may be* the hydrolysis of starch reserves. Although rarely reported as a constituent of temperate forage grasses (p.15), chloroplasts unable to synthesise starch are unknown (Duffus 1974) and it is possible that starch or β-glucans may accumulate in the unusual conditions of ensilage. β-glucans have been reported (p.17) in fresh plant material and in silage (Bousset *et al.* 1972) where they are associated with the hemicelluloses. Hydrolysis of reserve glucans would contribute to the early glucose peak and the high glucose levels and provide readily available fermentation substrates, but is probably of secondary importance and is still a matter of speculation. So the hydrolysis of structural and reserve polysaccharides, such as cellulose and starch respectively, may be the source of the additional sugars. These polysaccharides would not be included in the normal soluble carbohydrate fraction.

As well as glucose and fructose, the presence of mannose, galactose, arabinose and xylose after conservation is reported (McDonald *et al.* 1968; Bousset *et al.* 1972) originating from the noncellulosic polysaccharides. Partial degradation of the plant hemicellulose component may occur. Dewar *et al.* (1963) demonstrated plant hemicellulase activity leading to the liberation of pentoses *in vitro* and Bousset *et al.* (1972) attempted to confirm these findings *in vivo*. They reported decreases in hemicellulose levels, a reduction in arabinose relative to

xylose, as found by Dewar *et al.* (1963), but were unable to demonstrate enzymic breakdown of non-cellulosic polysaccharides. They concluded that liberation of pentoses *in vivo* was not attributable to hemicellulase activity. Bousset (1973) considers that hemicellulases do exist but have a very low activity through compartmentalisation which could suppress and retard hydrolysis until the later stages of ensilage. Moreover, hemicellulases have a pH optimum about 6 (Dewar *et al.* 1963) so that their activity falls with pH during ensilage. Therefore it seems that the potential for hemicellulose breakdown is low at all times, initially through cellular compartmentalisation and later through acidification of the substrate. Hemicellulase activity appears most likely after plasmolysis but before establishment of the lactic acid bacteria, at c. 6 - 12 h of ensilage when the total W S C peaks occurred. So a minor hydrolytic role of hemicellulases is possible during the early stages of ensilage.

Bousset *et al.* (1972) suggest the possibility of pectinase activity, and consider that plant enzyme hydrolysis of non-cellulosic polysaccharides during ensilage should not be ruled out.

The rapid decrease in soluble carbohydrate components between 6 and 48 h of ensilage coincides with the main phase of pH decline, indicating the utilisation of sugar substrates in fermentation reactions, as traditionally found (McDonald *et al.* 1966; Whittenbury *et al.* 1967). The decline in glucose and fructose follows the decline in total W S C and indicates that both hexoses are used as fermentation substrates.

The small secondary peak in sugar levels after 3 - 4 d coincides with a secondary pH rise, and may suggest further polysaccharide hydrolysis

under slightly less acid conditions. After 7 d, fructose was reduced to a very low level but glucose and total W S C persisted at c. 2% of D M. Bousset *et al.* (1972) found that when acidification is rapid, enzyme activity is relatively limited and water-soluble sugars remain at higher levels than when acidification is slow or prevented when they disappear almost completely.

In summary, fructans are hydrolysed to fructose and, so long as the pH remains high other enzymes, possibly pectinases and/or hemicellulases, may hydrolyse the non-cellulosic polysaccharide fraction after plasmolysis has begun. It is conceivable that the hydrolysis of cellulose, β-glucans and possibly starch may contribute to the early sugar peaks. When the pH falls rapidly during fermentation, the soluble carbohydrates are utilised as substrates until inhibition of microbial enzymes at the lowest pH values.

(iv) Nitrogenous components. Many workers have reported a rapid breakdown of protein into non-protein forms during ensilage (Kemble 1956; Brady 1965; Macpherson and Violante 1966; Whittenbury *et al.* 1967; Ohyama and Masaki 1968, 1969, 1971). Even in well-preserved silage 50 - 60% of the protein is broken down and large amounts of amino acids are released and may be further metabolised (Whittenbury *et al.* 1967).

In the ensiling experiments, Section 3, the soluble protein determined comprises the cytoplasmic protein involved in plant cell metabolism, as distinct from the relatively inert structural proteins of the insoluble fraction (p.18). The soluble protein component is also an important constituent of the plant juice, liberated by plasmolysis,

which forms the liquid phase for microbial growth: the bacteria occur not inside the plant cells but in this external medium (Lanigan 1966). Therefore, variations in the soluble protein fraction will have an important bearing on ensilage.

In Expt. 1, more than 50% of the soluble protein was lost during the first 24 h of ensilage. The concomitant increase in free amino groups, and the decrease in the protein:free NH_2 ratio over the same period, indicates that early, rapid hydrolysis of plant proteins occurred. However, the 6 - 12 h lag before increase in free amino groups was detected suggests that proteolysis was delayed until after plasmolysis had occurred.

Many workers have attributed such changes in silage nitrogenous components to plant enzyme activity (Mabbitt 1951; Kemble 1956; Watson and Nash 1960; Gouet *et al.* 1969, 1970, 1973). The highest concentrations of non-protein nitrogen often occur in silage of relatively high pH, for example pH 5.3 - 6.3 (Gouet *et al.* 1973). This agrees well with the optimum pH of plant proteases observed by Tracey (1948) for leaves of various species, o pH 5.0 - 6.0; Singh (1962) for barley, o pH 5.2 - 5.7, and Brady (1961) for clover, o pH 5.9 - 6.3. These authors observed a dramatic fall in activity at acid pH values. From comparison of the rate of protein loss and the rate of fall of pH in grass sap, Macpherson (1952) concluded that attainment of pH 4.3 during ensilage prevents further proteolysis. Singh (1962) suggested that plant proteases belong to the S H - group of enzymes which require reducing conditions and Ohyama (1970, 1971) has demonstrated the suppression of proteolysis by oxidising conditions (aeration) during ensilage.

In summary, proteolysis occurs after 3 - 6 h of ensilage when the trapped oxygen has been consumed and plasmolysis has taken place, and continues until the pH falls to a low inhibitory level. Gouet *et al.* (1973) claim that plant proteases are active from the beginning and that high activity persists especially when acidification is slow. Comparatively little is known, however, about the mechanism of, and the factors affecting, proteolysis (Brady 1961).

(v) Pigments. The most obvious change during ensilage is in the colour of the herbage. The light brown colour of silage is caused by the action of organic acids on chlorophyll which results in the formation of the magnesium-free pigment pheophytin (Watson and Nash 1960). Therefore, the pigment changes must reflect the pH changes, as found. There was a 6 h lag-phase before increase in pheophytin and decrease in the pigment ratio could be detected, coinciding with the small initial pH peak and the delay in plasmolysis and development of microbial populations. The sharp decreases in chlorophyll:pheophytin ratio and in pH occurred simultaneously between 6 and 24 h after ensiling. Therefore the pigment ratio may be regarded as an index of pH fall.

The pigment ratio curves also follow exactly the same pattern as the protein:NH_2 ratio curves and are similar in shape to the soluble carbohydrate curves. Moreover, the timescales are almost identical. Thus, the chlorophyll:pheophytin ratio of silage may serve as a useful colour index of the biochemical changes taking place! The initial increase in pigment ratio indicates the time-lag in establishment of anaerobic conditions, and the plasmolysis of the plant cells; the subsequent fall in pigment ratio indicates a normal acidic fermentation with the associated biochemical changes such as sugar catabolism and proteolysis.

b. The effect of grass nutrient status and biochemical composition on the subsequent ensilage process

The application of nitrogen fertilisers increases the content of protein and especially of non-protein nitrogen compounds in grass (Ferguson and Terry 1956; Nowakowski *et al.* 1965; Goswami and Willcox 1969; Jones 1970) but decreases the content of non-structural carbohydrates (Waite 1957; Nowakowski and Cunningham 1966; Smith 1973) and structural carbohydrates (Waite 1970). Such responses to fertiliser application were found in fresh ryegrass and in the resultant silages in Expt. 1. The ensiling characteristics of herbage are modified by fertiliser treatment during growth. Jones (1970) found that grass silages were generally less well preserved after heavy fertilisation.

In Expt. 1, high fertiliser application increased the pH and decreased the D M content of ryegrass silage. Fox and Brown (1969) and Jones (1966, 1970) reported increases in silage pH when high levels of N were applied. Plant growth stimulation after fertiliser treatment results in herbage and silage of reduced D M content (Jones 1970). The conditions of high pH and high moisture content favour clostridial growth (McDonald *et al.* 1966) as found in the high fertiliser treatment, Expt. 1, where large D M loss of c. 25% occurred.

The reduction in soluble carbohydrate levels following fertiliser application has important implications for ensilage, since an adequate supply of available carbohydrate is an essential requirement for a satisfactory lactic acid fermentation (McDonald *et al.* 1966). The low sugar levels in the high fertiliser treatment were a limiting factor regarding the rate and extent of pH fall. A reduction in structural carbohydrate content at high levels of N application may indirectly affect the

supply of available carbohydrates through polysaccharide hydrolysis. However the extent of proteolysis, deduced from the protein:free NH_2 ratio, was apparently similar in both treatments.

In summary, the application of high fertiliser levels may adversely affect the fermentation process and the nutritional quality of the silage product through effects on the pH, D M, soluble carbohydrates and nitrogen components, producing conditions favouring clostridial growth. Increasing use of high levels of fertiliser is likely in the future if the high yields of herbage required for intensive systems of animal production are to be achieved. The conservation characteristics of such herbage should be considered and further investigated.

EXPERIMENT 2

Expt. 2 provided information about the pattern of biochemical changes occurring during the first 48 h of ensiling fresh and wilted grass with a natural microbial population. The overall pattern of biochemical changes was similar to that found in Expt. 1 in several respects but the onset of fermentation was delayed, probably attributable to the extended lag-phase needed for the buildup of the microbial population originating from the natural grass microflora. Lactic acid bacteria are relatively scarce on fresh grass, of the order of 100 per g (Henderson *et al*, 1972) which would give about 10^3 per silo in Expt. 2, compared with the inoculation of about 10^4 per g giving 10^5 per silo in Expt. 1. Establishment of the microbial population to about 10^8 per g would be expected to take at least twice as long (24 - 36 h) in Expt. 2, especially in the wilted treatment where plasmolysis may be delayed.

The small initial pH peak observed during the first 12 h of ensilage coincided with early soluble carbohydrate and protein peaks but little change in free amino groups. The general fall in D M content was most obvious during the first 3 h, probably due to the production of water in aerobic respiration and possibly also through volatile losses. The gradual decrease in D M and pH after 12 h may indicate the slow onset of fermentation reactions during plasmolysis and the establishment of lactic acid bacterial populations.

The initial oscillation in soluble carbohydrate levels and the secondary peak at 36 h may represent phases of polysaccharide hydrolysis by either plant or bacterial enzymes while the pH remained high, in fact optimal for hemicellulases(c. pH 6).

The initial increase in soluble protein may be an artefact resulting from the decrease in D M, or may represent the conversion of insoluble protein into a soluble form during the very early stages of ensilage. Little change in free amino groups occurred during this period. The subsequent fall in soluble protein and steady increase in free amino groups indicated the onset of hydrolysis in the fresh silos after anaerobic conditions were attained and plasmolysis had occurred. Proteolytic activity, inferred from the protein:free NH_2 ratio, was most intense between 3 and 9 h of ensilage in the fresh treatment.

The pigment changes were very irregular, but the steady decrease in the pigment ratio after 12 h of ensilage, coinciding with the gradual pH fall, indicated the onset of slow fermentation in both treatments.

The wilting treatment increased the pH of the herbage and the silage formed. Wilted silage is characterised by a higher final pH

than fresh silage since prewilting leads to restricted fermentation and decreased organic acid content (McDonald *et al.* 1968; Daniel 1970; Ohyama *et al.* 1970). The soluble carbohydrate levels were lower in the wilted herbage. Depletion of available carbohydrates is likely during wilting (pp.22,26,27). Wilted silage generally has higher residual sugars than fresh silage (Anderson and Jackson 1970; McDonald *et al.* 1968). In Expt. 2 soluble carbohydrate levels were higher in the fresh silage but fermentation was incomplete after 48 h of ensilage, so no conclusions are possible. Soluble protein levels were lower and free amino group content was higher in the wilted herbage as a result of the pretreatment (pp.175-6), but the pattern was reversed in the silages. The protein:free NH_2 ratio showed little overall change in the wilted silage during the 48 h period studied. This suggests that proteolysis was restricted following wilting, possibly because of enzyme inactivation during the pretreatment, the extended period of aerobic conditions and delay in plasmolysis likely with wilted material, or an unsuitably high pH, (pp.186-8) (Ohyama 1970; Ohyama and Masaki 1971). Brady (1965) found that the extent of protein hydrolysis, and the metabolism of the amino acids released, was less in wilted ryegrass silage than in fresh silage. Changes in the chlorophyll: pheophytin ratio after 12 h ensilage were similar in both treatments, but the rate of decrease was slower in the wilted silage indicating restriction of lactic acid fermentation.

EXPERIMENT 3

Expt. 3 provided information about the respective roles of plant and bacterial enzymes, differentiated by γ-irradiation sterilisation treatment, during the first 7 d of ensilage (Fig. 3.11). γ-irradiation resulted in a 20 - 25% increase in total W S C and a 75 - 90% decrease

in soluble protein compared with the controls. Gouet *et al.*(1970) found negligible changes in plant biochemical composition when using a dose of 1.2 Mrad and concluded that this sterilisation treatment had no significant effect on either biochemical composition or enzyme activity. However, important differences have sometimes been observed in the content and/or composition of soluble carbohydrates in herbage after sterilisation with 2 Mrad. Bousset *et al.* (1972) found slight losses of soluble sugars, attributed to sampling and analytical errors, but little effect of increasing the dose from 1 to 3 Mrad. In their studies, after irradiation, two new peaks appeared in the soluble sugar fraction; fructose was less degraded than the other components since it exists in the plant primarily as fructans which must first be hydrolysed. Hence the existence of plant enzyme activity during the sterilisation process is possible, particularly under the conditions used in Expt. 3: a 2.5 Mrad dose given over 11 h. So caution is necessary in interpreting the results of this experiment since alteration of the plant substrates may produce abnormal patterns of biochemical changes during ensilage. However, the changes in axenic and inoculated silos, subjected to the same sterilisation pretreatment, may be compared.

In Expt. 3, small initial pH peaks did not occur. Therefore such peaks cannot result from plant enzyme activity alone, but must be produced at least in part by bacterial enzyme activity during the very early stages. The lactic acid bacteria used for the inoculum, *L. plantarum* and *Str. faecalis*, cannot cause the early pH peaks which may result from the activities of aerobic species, involving respiratory catabolism and decarboxylation reactions leading to CO_2 evolution. The small pH fall during the first 6 h of ensilage in both axenic and inoculated treatments was probably a plant enzyme phenomenon since the populations of

lactic acid bacteria were not well established at this period. The maintenance of a constant pH level after 6 h in the sterile controls indicates that plant enzymes have no further effect on pH. By contrast, the fall in pH in the inoculated silos after 24 h represented the onset of fermentation which occurred after exhaustion of trapped air, plasmolysis of the plant cells and establishment of the lactic acid bacterial populations. The increase in total counts over the first 24 h supports this hypothesis.

The increase in soluble carbohydrates observed during the first 3 h was small in the sterile controls but marked in the inoculated silos, suggesting a minor role of plant enzymes and a major role of the microbial enzymes in the formation of early sugar peaks, possibly through polysaccharide hydrolysis at the higher pH values. Subsequently, the soluble sugar content of the axenic controls remained more or less constant indicating little sugar utilisation by plant enzymes under anaerobic conditions. However, the sugar content of the inoculated samples decreased markedly after 24 h indicating the utilisation of available sugars as fermentation substrates by the lactic acid bacteria (pp. 185). The marked increase in soluble carbohydrates after 48 h in the inoculated silos suggests a role of bacterial enzymes in the formation of secondary sugar peaks, possibly by further polysaccharide hydrolysis after the induction of suitable enzymes. Bousset *et al.* (1972) found an overall increase of 6% in total soluble sugars and a reduction in hemicellulose in axenic ryegrass controls. They concluded that the role of bacterial enzymes in the metabolism and fermentation of soluble carbohydrates is immeasurably greater than that of the plant enzymes. However, the plant enzymes catalyse a certain number of reactions, such as fructan hydrolysis and possibly non-cellulosic polysaccharide hydrolysis as well.

The early rapid fall in soluble protein levels in both treatments during the first 3 h of ensilage indicates proteolysis by plant enzymes at the pH optimum of the plant proteases (pp. 187). Apparently proteolysis does not require previous plasmolysis as it was initiated immediately after sealing the silos in this experiment. Gouet *et al.* (1973) also attempted to dissociate the respective roles of plant and bacterial enzymes in the N-metabolism of lucerne silage sterilised by γ-irradiation then inoculated. They found that the considerable hydrolysis of plant proteins and the concomitant accumulation of free amino acids was a result of plant enzyme activity. Hydrolysis was markedly reduced in the presence of all the lactic acid bacteria studied, the effect being greatest with the homofermenters. So the bacteria have an indirect role in proteolysis, through modification of the pH of the medium which affects plant protease activity. The bacteria were responsible for any amino acid breakdown detected during ensilage; the role of plant enzymes in the degradation and changes in amino acids and amides was minimal (Gouet *et al.* 1973).

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ABSTRACT OF PAPER PRESENTED

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Stomatal control of transpiration and photosynthesis after excision in *Lolium multiflorum* leaves

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When considering the nature of the physiological changes which occur in the leaves of forage grasses at harvest and during the subsequent 'wilting' period, a pretreatment for ensilage, it is necessary to determine the sequence of events which occur immediately after the cut. The kinetics of decline of photosynthesis and transpiration were followed in a batch of *Lolium multiflorum* leaves at light saturation in an assimilation chamber where air temperature, leaf temperature, carbon dioxide and water vapour concentrations were controlled. After excision of the leaves, photosynthesis declined to zero after 15 minutes, whilst transpiration rate increased or remained the same for 1 to 2 minutes then decreased slowly following a curve with two breaks of slope at 15 minutes and 30 minutes. In order to determine the degree of stomatal opening during this sequence of events, fragments of leaf were removed at regular intervals (and fixed by freezing in liquid nitrogen) then examined subsequently with the scanning electron microscope. It was found that the stomata were completely closed after 15 minutes.

This experiment indicates therefore that in the course of drying, the most rapid physiological response is the closure of the stomata and not, as is often stated, the desiccation of the mesophyll cells. Moreover, a strict correlation was observed between stomatal closure and the decline in photosynthesis. The mechanism of stomatal closure under these conditions is discussed.