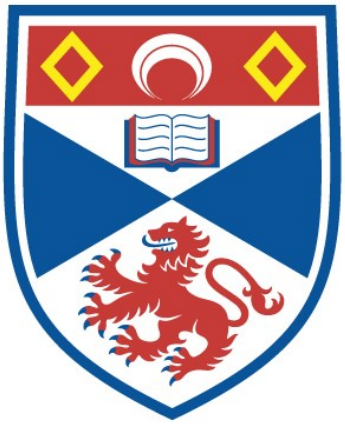
LOW TEMPERATURE ACCLIMATISATION IN THE ROWAN, SORBUS AUCUPARIA

Alan M. Barclay

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1979

DECLARATION

I hereby declare that this thesis has been composed by myself, and that it is a record of work which has been done by myself. This has not been accepted in any previous application for a degree. Any other sources of information have been specifically acknowledged.

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LOW TEMPERATURE ACCLIMATISATION IN THE ROWAN, (SORBUS AUCUPARIA)

A thesis presented for the degree of Ph.D at the University of St. Andrews 1979.

by

Alan M. Barclay, B.Sc.

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CERTIFICATE

I hereby certify that Alan M. Barclay has been engaged upon research from October 1976 onwards under my supervision to prepare the accompanying thesis for the degree of Doctor of Philosophy.

Signed

Prof. R.M.M. Crawford.

St. Andrews
August 1979.

STATEMENT

I, Alan M. Barclay, was admitted as a research student of the University of St. Andrews in October 1976 in accordance with Ordinance General No. 12 and the Resolution of the University Court, 1967, No. 1. The thesis was completed in August, 1979.

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INTRODUCTION

CHAPTER 1

LOW TEMPERATURE LIMITS TO TREE DISTRIBUTION

The research carried out for this thesis has been made possible by a Natural Environment Research Council training award for the study of low temperature metabolism in higher plants. Why then has the rowan, *Sorbus aucuparia* been the central theme of study?

Research on the rowan was prompted by the ability of this tree to grow successfully in low temperature environments. The rowan has a widespread distribution in Europe. Its range is from Iceland, Northern Norway and Russia to the mountains of Southern Europe. It probably grows at higher latitudes in the arboreal form than any other tree. Furthermore, the rowan grows at higher altitudes in the British Isles than any other tree species (Clapham *et al.* 1962) and has been found growing at over 900m (3000 ft.). Similarly, it grows at higher altitudes in the European Alps than any other tree (Huxley 1967). An attempt therefore has been made to uncover the metabolic strategies employed by this plant to overcome the difficulties in growing in such habitats.

Treeline or timberline have been the terms used to describe the more or less clearly marked line where tree growth ceases. It is thought of either as a latitudinal limit to tree growth such as the taiga-tundra boundary of North America, Scandinavia and Siberia or an altitudinal limit on mountain sides, marking the point where trees give way to low growing shrub vegetation. Inverted treelines can also be found where temperature inversions occur regularly. This can be due to land surface topography such as some valley basins where cold air is unable to drain away.

The terms treeline and timberline have been defined in various ways by different authors to describe the altitudinal limits of the tree growth. Central European ecologists have used the terms where timberline

(Waldgrenze) is the upper limit of tall erect tree growth occurring at forest densities, above which is the transition zone (Kampfzone). This refers to the zone between the timberline and the treeline (Baumgrenze), which is the line through the last few trees along the mountain slope. Many of the trees in the transition zone and for some distance above the treeline may be phenotypically dwarfed, twisted and prostrate and can be less than 1m in height. These environmentally determined "shrubby" specimens are known as "Krummholz". In some areas of the European Alps, the genetically dwarfed species, Pinus montana can be found growing at and above the treeline.

There is great taxonomic diversity amongst timberline trees (Wardle 1974). Many families of dicotyledons and five families of conifers are represented worldwide. This can be demonstrated by the many tree species which can form timberlines in Europe. Over much of this continent, the Pinaceae are the predominant trees. Genera include Pinus, Picea and Abies. Larix may replace these genera in the north of Europe. Betula species form arctic and alpine timberlines over wide stretches of Scandinavia. Other deciduous broadleaved species are to be found in central and southern Europe where Alnus species and Fagus sylvatica may be found at the timberline.

The many trees forming timberline in Europe suggests that the causal factors of timberline formation may differ from area to area.

In the European literature deciduous non-conifer trees such as Sorbus aucuparia and Alnus incana are often neglected in relation to treelines of high density forest trees such as Pinus species, although these broadleaved species may be found growing at higher altitudes. Similarly, these two species can be found growing at the northern edge of the boreal zone along with Populus tremulans, Betula and Salix species.

In Scotland it is hard to determine the climatic forest limit because

of the general scarcity of native forest. The lack of naturally growing trees has been caused by destruction of forest in the past and also to present land use practices which prevents regeneration of trees. This is discussed more fully in Chapter 6.

Ecologists have long agreed that climate has been the major factor determining the position of the timberline. Which climatic variable is responsible, however, has been in contention.

It has been shown (e.g. Köppen 1931; Daubenmire 1954) that climatic limits of timberlines coincide roughly with isotherms representing 10°C for the mean of the warmest month.

Michaelis (1934) was probably the first to suggest that the sharp cut-off point in tree altitudinal limits was due to low summer temperatures at high altitudes causing a reduction in the length of the growing season. This would result in new shoots being unable to mature sufficiently to withstand the rigours of winter.

A firm proponent of the hypothesis that woody plants are altitudinally limited by their ability to ripen their shoot tissues is Wardle (1971). He tentatively defines ripening as, 'At the morphological level, shoots are ripened when they have completed their seasonal length growth and production of leaves, and lost the succulent appearance imparted by high water content and incompletely lignified cell walls. Physiologically ripened shoots can withstand low temperatures and desiccation, and therefore can be expected to possess impermeable cuticles, cells which are not damaged when ice crystals form inter - or extracellularly, and protoplasts which tolerate loss of water.'

What are the rigours of winter for a tree shoot in a low temperature environment? Freezing temperatures and more importantly frost drought due to transpiration losses in shoot tips (and needles), without replenishment of water due to the slow uptake of water at low temperatures, or no uptake at all because of frozen soils therefore causing desiccation stress (Wardle

1968; Sakai 1970; Tranquillini 1976). Furthermore, with greater windspeeds found at high altitudes an increase in transpiration rate of plant tissues would be expected.

Workers in Europe (Tranquillini 1976; Baig and Tranquillini 1976) have followed up Michaelis suggestion and investigated maturation of conifer shoots at different altitudes. They also measured transpiration in Spruce and Pine shoots and needles. Under comparable conditions, cuticular transpiration rates were greater in samples from above timberline compared with samples from forest trees at lower altitudes. Needle cuticles and cutinised cell wall layers were much thinner in the high altitude trees. In the field during February and March, water content of krummholz needles dropped to a lethal level, whilst needles from lower altitudes retained relatively stable water contents.

Tranquillini and co-workers found experimentally that cuticle thickness in Picea abies depended on length of growing season (Tranquillini 1976). In addition shoot maturation and cuticle thickness depended on the mean monthly temperature of the growing season. They found a negative correlation between cuticle thickness and the rate of cuticular transpiration (Baig and Tranquillini 1976). After a short cool summer season, krummholz shoots were badly damaged by frost drought in winter.

Tranquillini (1976) was in no doubt that damage to twigs and needles was caused primarily by frost drought, as the frost resistance of twigs from natural stands at timberline reach well below winter temperatures. A study of many conifers and broadleaved tree species in North America by Sakai and Weiser (1973) on frost resistance of leaves and twigs showed that trees growing naturally in low temperature environments could survive temperatures down to -80°C, after low temperature pretreatment. In Japan, Sakai (1960) has shown that the twigs of various temperate deciduous tree species can survive temperatures as low as -196°C after being pretreated at

-30°C. It would appear therefore that trees growing naturally in a low energy environment can survive far lower temperatures than they would experience in nature.

Above, I have emphasised an explanation of tree limits which depends on a summer growing season which is long and warm enough to enable trees to ripen their shoots sufficiently to withstand desiccating conditions in late winter.

Another avenue of thought suggests that the balance between photosynthesis and respiration may limit tree growth in low temperature environments. Boysen-Jensen (1948) for example, noted the very narrow annual rings of Betula species at timberline, and concluded that trees were at a disadvantage compared with smaller plants at high altitudes as a large proportion of their photosynthates became locked up in the unproductive tissues of the trunk. The treeline resulted where net assimilation became too low to permit survival of the tree form.

The data of Schulze et al (1967) suggested a very delicate CO_2 balance in Pinus aristata at timberline in the White Mountains of California. The probability of having a negative yearly balance increased with altitude as the temperature decreased and the growing season became shorter. They concluded that the position of the upper treeline of P. aristata might in part be determined by a negative CO_2 balance.

Studies by Ungerson and Scherdin (1968) on Pinus sylvestris at the Arctic treeline throughout the year showed that under natural conditions this tree showed a net photosynthetic loss in August. This was due to a combination of cloud, shortening days and relatively high temperatures then. Only during the longer days of early summer and the clearer, cooler days of Autumn was a net photosynthetic gain found in these trees.

Bannister (1976) comments that this tenuous balance between photosynthesis and respiration may provide a physiological explanation for the occurrence of the Arctic treeline. He also stated that the data of

Poore and McVean (1956) could be explained by this CO_2 balance. Poore and McVean showed that the potential forest had higher altitudinal limits in the East of Scotland, which has a more severe winter climate than the West of Scotland with its milder more oceanic winter climate. Bannister suggested that in the West of Scotland, photosynthetic rates were limited by lower environmental temperatures and cloudiness at higher altitudes during summer, and the short day length and low light intensities of winter. Relatively high winter temperatures would encourage high respiration rates, thus leading to a depletion of carbohydrate reserves.

Other causal factors affecting the position of the treeline have been proposed. Griggs (1938; 1946) was a firm proponent that wind was a major factor affecting the position of the altitudinal treeline. This, however, has been widely questioned by many workers (e.g. Daubenmire 1954; Tranquillini 1964).

In many parts of the northern hemisphere, trees above the timberline are dwarfed and wind trained. This has led to the hypothesis that wind sets the upper limit of trees. This is supported by the fact that above the timberline, trees are often asymmetrically deformed in the direction of the prevailing wind. The last trees upslope are situated in depressions or equivalent microhabitats which offer a measure of protection from the wind. Timberlines may be found locally on exposed knobs or shoulders of hills, far below the average altitude of timberline in that area.

Daubenmire (1954) showed (with rather meagre data admittedly) that there was no correlation of altitudinal tree limits with wind speeds. This was in direct comparison with the conformity of timberline to isotherms representing the mean of the daily maximal air temperatures of the warmest month of the year. He also pointed out that there were many timberlines where there was no asymmetry in the last trees to be found up the mountain slope. Daubenmire concluded, however, that wind may alter the appearance and elevation of the timberline locally.

This local depression of altitudinal tree limits has been substantiated by Pears (1968) work in the Cairngorms in Scotland. His evidence of very high wind speeds in these mountains suggested that the natural timberline (Pinus sylvestris and Betula pubescens) was at a lower level than would be expected on consideration of summer temperatures alone.

Therefore, for trees to survive at high altitudes the ability for new shoots to grow and mature sufficiently in a short season in a low temperature environment is necessary, to avoid desiccation stress and/or for the tree to have the ability to tolerate that stress. In addition, it would appear that trees growing in a low energy environment must be able to capture, store and utilise energy economically to maintain a positive carbon dioxide balance.

The preceeding pages have summarised present thought on the factors limiting the growth of trees in low temperature habitats. As the reader must have realised, most research has been concentrated on high density forest conifers. This thesis may help redress this imbalance.

Research for this thesis has been concentrated on the twig and the shoot bud, the perennating organ of deciduous trees. Two major approaches have been made.

Firstly, metabolic processes of S. aucuparia have been compared with other tree species. In addition, a comparison has also been made between species having a northerly geographical range in Europe and which grow to high altitudes, with trees whose distribution is limited to more southerly latitudes. A comparison of several species which grow naturally in the low energy environment of high latitudes with their more southerly counterparts, should focus attention on any dichotomy of metabolic strategies which might be employed by the two groups.

As concluded previously, the yearly carbon dioxide balance of trees in low temperature environments is thought to be an important factor in determining tree limits. Experiments therefore were conducted into the

effect of temperature on dark respiration rates. This enabled a comparison of respiration rates to be made between different species over a range of temperatures as well as comparing the energy of activation of respiration rates. Further experiments were also made into the ability of different species to alter their respiration rates when subjected to a change in temperature i.e. temperature rate compensation. Although temperature compensation has been well studied in animals (see Hoar 1966) relatively few studies of this nature have been carried out on higher plants. For a tree growing in a low energy environment, the ability to adjust metabolic rates quickly in response to a change in temperature could result in the maximal use of the available energy.

As propounded earlier in this chapter, desiccation stress in winter time is thought to be another major factor affecting the ability of trees to survive at altitudinal tree limits. The effect of water deficits in tree buds and twigs on metabolic processes were therefore investigated.

Investigations included the quantitative measurement of tree bud and twig viability under increasing tissue water deficits.

Experiments were also carried out on the production of the hormone ethylene by tree buds after various periods of desiccation. This substance is a powerful plant growth regulator, and disruption in the production of this gas must have a great effect on the ability of buds to maintain a homeostasis of metabolic function.

In samples of tree buds subject to a standard desiccation treatment, soluble carbohydrate levels were monitored. The reasons for this were twofold. Firstly, to find out if bud tissues could maintain sufficient supplies of hexahydrates necessary for continued metabolism at low relative water contents. The second reason was that sugars have been proposed as possible membrane protectants in dehydrated tissues in plants under desiccation stress (Parker 1969). Therefore an increase in specific sugars

in bud tissues at high water deficits would indicate a possible tolerance mechanism to low relative water contents.

The second major approach utilised in the study of the rowan has been to study growth, metabolism and aspects of the water relations of shoot buds with increase in altitude of origin of this species. This necessitated both field observation, collection of samples and subsequent laboratory experimentation. As temperature decreases with increase of elevation, research into the effect of altitude on the growth and metabolism of the rowan allows these processes to be studied when this tree is subjected to a varying temperature regime. Differences in populations according to origin of elevation may become evident.

Research included the measurement of rowan growth rates with increase of altitude. An experiment on the dark respiration rates of rowan buds growing naturally at different elevations complemented comparative studies of tree bud respiration rates conducted with Botanic Garden material.

It was indicated earlier that the inability of plant tissues to 'harden' sufficiently during a short growing season prevented trees from withstanding the desiccating effects of late winter. An attempt was made therefore to guage the effect of altitude on the maturation of bud scales.

To what extent does winter frost drought affect the water relations of rowan shoot buds? This question prompted the measurement of water content of rowan buds from a range of altitudes throughout a six month winter period. Results were compared with birch (Betula pubescens).

For a tree species to maintain a viable population in a low temperature environment it must be able to reproduce successfully. Research by Wardle (1965) has shown that Nothofagus solandri, a deciduous tree species, produces seeds with very poor germination at the timberline. A small scale experiment was conducted therefore into the effect of altitude of origin on the germination of rowan seeds.

In this introductory chapter the distribution of S. aucuparia has been outlined. This showed that the rowan tree grows successfully in low energy environments. Present thought as to the possible causes of low temperature limits to tree growth was discussed and this identified two factors. First, the low summer temperatures found at high altitudes and at high latitudes, prevents the maturation of tree shoots. This produces increased tissue transpiration losses and results in lethal water deficits in late winter during periods of frost drought. Secondly, low temperature tree limits are the point at which trees can no longer maintain a positive carbon dioxide balance over a whole year. These two factors decided the direction of the research conducted for this thesis.

PART I

COMPARISON OF ROWAN AND OTHER TREE SPECIES

CHAPTER 2

DARK RESPIRATION RATES

2.1 Effect of Temperature on Respiration Rates

As outlined in Chapter 1, the carbon balance of trees growing in a low temperature environment may be a contributing factor limiting the distribution of trees. For deciduous trees in wintertime dark respiration is the major component operating in the carbon economy of the tree. One of the first series of experiments conducted therefore was to investigate the effect of temperature on dark respiration rates in tree buds.

The aim of these experiments was to compare respiration rates *per se* and the energy of activation of respiration rates in tree species having different geographical ranges in Europe. *Sorbus aucuparia* and *Betula pubescens* can grow in low energy environments. Their range extends further north and at higher altitudes in North Europe than both *Carpinus betulus* and *Quercus robur*. These four species are broad leaved deciduous trees. Rates were also measured for two evergreen coniferous species. *Pinus nigra* having a more southerly distribution than *P. sylvestris*. This work was carried out in November 1976 using plant material from the Botanic Gardens, St. Andrews.

Rates of respiration were measured with a Gilson Differential Respirometer, both oxygen uptake and carbon dioxide output being determined after Umbreit et al (1957). For each species about 500mg fresh weight of buds were placed in each flask containing a layer of thick filter paper and 2ml 0.10 M phosphate - 0.05 M citrate buffer pH 5.4. The buds therefore were in a vapour saturated environment but not totally immersed in buffer solution. To prevent photosynthesis taking place each flask was enclosed in tinfoil. For each species oxygen uptake was measured in 3 flasks by absorbing the carbon dioxide respired with 5% KOH placed in the central well of each flask. Change in gas volume equalled oxygen uptake.

In a further 3 flasks, net gas changes were measured. This allowed carbon dioxide output to be calculated by the difference. For each species the original temperature of the water bath was $2^{o}C$. After equilibration, gas volume changes were noted at 15 minute intervals for 1 hour. The temperature of the bath was increased 5 degrees centigrade and the flasks allowed to equilibrate at the new temperature for 45 mins. Hence, respiration rates could be calculated at $2^{o}C$, $7^{o}C$, $12^{o}C$, $17^{o}C$ and $22^{o}C$ for each species. Shaking rate of the sample flasks in the water bath was 100/min. Increase in shaking rate to 140/min. showed no increase in respiration rates at the highest temperature employed i.e. $22^{o}C$. This suggests that supply of oxygen was not a limiting factor in determining respiration rates.

After each experiment the buds were oven dried at $95^{o}C$ for 36 hours. Total organic nitrogen of the oven dried buds was determined by the Kjeldahl method (Allen 1974; Purvis et al 1966). Digestion took place in a round bottomed Kjeldahl flask to which were added 100mg oven-dried, ground plant material; 2 catalytic Kjeldahl tablets, B.D.H. Ltd. (each tablet contained 1g $Na_2 SO_4$ and the equivalent of 0.05g Se); 3ml c.$H_2 SO_4$ (Nitrogen free). The resulting pale green digest was introduced into a Markham still to which was added an excess of 50% NaOH. The NH_4^+ liberated was distilled into 5ml 2% Boric Acid, Two drops of indicator, a mixture of 6ml methyl red (0.16 per cent in 95 per cent alcohol); 12ml Bromo-cresol green (0.04 per cent in water); 6ml 95 per cent alcohol was added to the distillate and titrated against M/28 HCl. 1ml M/28 HCl $\equiv$ 0.5mg nitrogen.

For each species, 3 nitrogen determinations were carried out. The mean nitrogen content per dried weight of bud material could then be calculated. This allowed rates of respiration to be expressed as

μl gas/hr/mg N.

The respiration rate data is expressed in Table 2.1. There is no clear distinction between species with a northern distribution in Europe and the tree species with a more southerly range. Both B. pubescens, a species of high latitudes and C. betulus which grows at low latitudes, have low respiration rates at all temperatures when compared with other species. Q. robur has high respiration rates in comparison with the other deciduous species, especially at the higher temperatures. The two Pinus species have high respiration rates. S. aucuparia occupies an intermediate position with regards to respiratory rate values.

The energy of activation of respiration rate was calculated by plotting the natural logarithm of respiration rate versus the reciprocal of the absolute temperature. This resulted in a straight line plot for all species tested. Data and results of statistical analyses are tabulated in Table 2.2. The slope of the line is the energy of activation divided by the gas constant. Therefore the energy of activation of respiration rate was determined by multiplying the slope of the line by the gas constant.

For ease of comparison between species, the results are displayed graphically. Figure 2.1 shows an Arrhenius plot of respiration rates i.e. ln O_2 uptake versus the reciprocal of absolute temperature. The shallowest slope showing the lowest energy of activation.

It is interesting to note that S. aucuparia has the lowest energy of activation. The other three deciduous species have a higher and similar energy of activation. The two Pinus species show a similar but even higher value. Measurement of CO_2 output against temperature gave similar results (Figure 2.2) i.e. S. aucuparia again had the lowest energy of activation. It would appear that S. aucuparia was less affected by temperature than the other species tested.

Caution must be exercised here, however, in accepting the above data as representative of the species for the whole winter season. Bud

Table 2.1 Bud dark respiration rates, oxygen uptake and carbon dioxide output, of the six species tested at five temperatures.

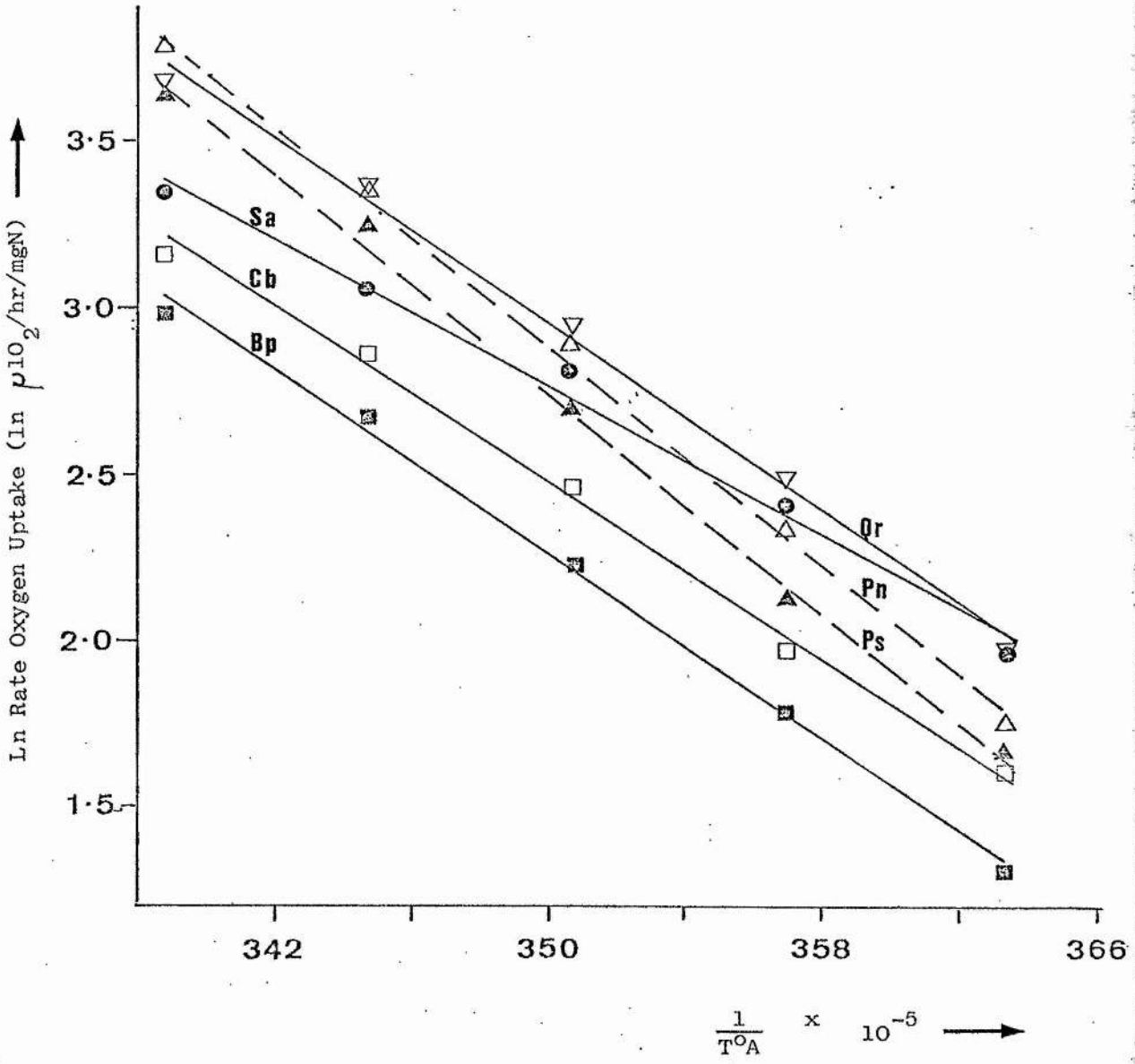
($Q^N_{O_2}$ = $\mu l O_2$ uptake/hr/mg Nitrogen)

($Q^N_{CO_2}$ = $\mu l CO_2$ output/hr/mg Nitrogen)

Species	Temperature (°C)	Respiration Rate	
		$Q^N_{O_2}$	$Q^N_{CO_2}$
Sorbus aucuparia	2	7.10	6.03
	7	11.17	9.41
	12	16.69	15.24
	17	21.60	21.14
	22	28.90	31.83
Betula pubescens	2	3.65	3.71
	7	5.93	5.83
	12	9.37	9.58
	17	14.68	15.87
	22	19.79	24.55
Carpinus betulus	2	4.95	4.01
	7	7.15	6.41
	12	11.67	10.96
	17	17.65	19.85
	22	24.22	28.84
Quercus robur	2	7.11	5.04
	7	12.06	8.01
	12	19.17	16.02
	17	29.35	29.71
	22	40.09	42.73
Pinus sylvestris	2	5.34	5.13
	7	8.59	8.05
	12	15.09	16.54
	17	26.26	32.36
	22	39.09	57.08
Pinus nigra	2	5.76	5.93
	7	10.26	7.81
	12	18.29	16.73
	17	29.00	30.20
	22	44.24	47.61

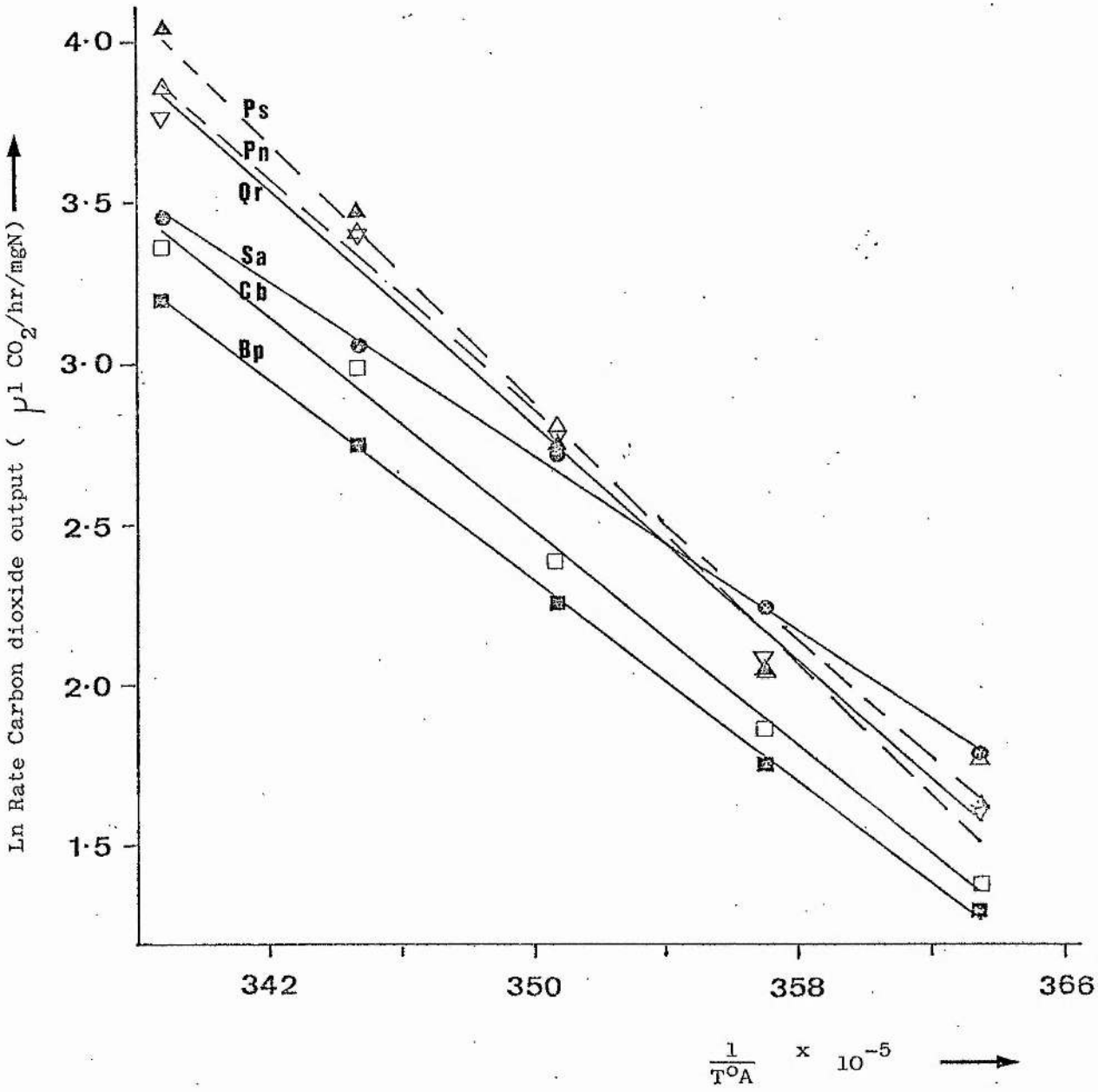
Table 2.2 Results and statistical analyses of a linear regression of the natural logarithm of respiration rate of tree buds versus the reciprocal of the absolute temperature.
Linear regression n = 15; $y = a_o + a_1 x$ ($\ln \text{rate} = A + \frac{Ea}{R} \cdot \frac{1}{T}$); where A = a constant; Ea = energy of activation; R = gas constant = 1.987 cal/degree; T = absolute temperature. r^2 = coefficient of determination; So = standard error of regression coefficient a_o; S_1 = standard error of regression coefficient a_1

Species	Rate O_2 or CO_2	a_o	a_1	Ea (kcal/degree)	r^2	S_o	S_1
Sorbus qucuparia	O_2	22.32	-5586.70	-11.10	0.93	1.5o	427.73
	CO_2	26.23	-6719.29	-13.35	0.99	0.74	212.09
Quercus robur	O_2	27.60	-7040.75	-13.99	0.97	1.12	319.25
	CO_2	34.70	-9112.28	-18.11	0.99	0.80	226.62
Pinus sylvestris	O_2	31.66	-8260.11	-16.41	0.93	2.27	646.54
	CO_2	38.24	-10102.67	-20.07	0.95	2.33	664.22
Pinus nigra	O_2	31.74	-8238.17	-16.37	0.97	1.39	394.60
	CO_2	34.18	-8948.34	-17.78	0.96	1.73	492.34
Betula pubescens	O_2	26.55	-6941.37	-13.79	0.99	0.74	212.09
	CO_2	29.53	-7772.79	-15.44	0.99	0.66	187.75
Carpinus betulas	O_2	25.67	-6628.83	-13.17	0.98	0.86	245.24
	CO_2	31.33	-8244.95	-16.38	0.99	0.66	188.81



Sa ●——●	Sorbus aucuparia	11.10
Cb □——□	Carpinus betulus	13.17
Bp ■——■	Betulu pubescens	13.79
Qr ▽——▽	Quercus robur	13.99
Ps ▲– –▲	Pinus sylvestris	16.41
Pn △– –△	Pinus nigra	16.37

Figure 2.1 Arrhenius plot of the bud respiration rates (oxygen uptake) of 6 tree species. Calculated values for the energy of activation are presented below.



		Energy of Activation (kcal mol^{-1})
Sa ●——●	Sorbus aucuparia	13.25
Bp ■——■	Betula pubescens	15.44
Ps ▲– –▲	Pinus sylvestris	20.07
Pn △– –△	Pinus nigra	17.78
Qr ▽——▽	Quercus robur	18.11
Cb □——□	Carbinus betulus	16.38

Figure 2.2 Arrhenius plot of the bud respiration rates (carbon dioxide output) of 6 tree species. Calculated values for the energy of activation are presented below.

respiration rates and the energy of activation of respiration rate, may vary throughout the winter. Data from another experiment on S. aucuparia buds revealed that bud respiration rates increased from late winter to springtime - as would be expected as bud break approached. Furthermore, the Q10 of respiration rate also increased. Q10 is the increase in rate caused by a 10°C rise in temperature and is directly comparable to the energy of activation.

$$\text{Thus, } Q10 = \frac{kt + 10}{kt}$$

where kt is the rate at temperature t and kt + 10 the rate at 10°C higher.

Data is presented in Table 2.3 for 4 high altitude S. aucuparia trees growing at Glen Doll, NGR No. 240770. Values for the bud respiration rate of each tree is the mean of 3 replicates. Rates were measured as previously described, but only at 5°C and 15°C. This allowed the Q10 of respiration rate to be calculated between those two temperatures.

Results of a paired sample t-test on the data in Table 2.3 show that there is a high significant increase in respiration rate between the two dates sampled at both 5°C and 15°C (Table 2.4). In addition there is a high significant increase in Q10 of respiration rate.

Table 2.3 Bud respiration rates and Q10 of respiration rate of 4 S. aucuparia trees. The trees were growing at different altitudes and the respiration rates measured twice with an interval of 1 month

($Q_{O_2}^{5^oC}$ = $\mu l O_2$ uptake/hr/mgN at 5°C)

Altitude of Sample (m)	22.3.78			23.4.78		
	$Q_{O_2}^{5^oC}$	$Q_{O_2}^{15^oC}$	Q_{10}	$Q_{O_2}^{5^oC}$	$Q_{O_2}^{15^oC}$	Q_{10}
400	12.33	29.97	2.43	19.08	52.89	2.77
510	6.75	16.06	2.38	19.08	51.28	2.69
580	14.07	31.99	2.27	22.17	62.39	2.81
670	8.85	17.07	1.93	18.62	44.47	2.39

Table 2.4 Results of a paired sample t-test to test the difference in respiration rates and Q_{10} of respiration rate with time ($\bar{D}$ = mean change)

	$\bar{D}$	t	p
Respiration rate at 5°C	-9.24	-7.69	< 0.01
Respiration rate at 15°C	-28.99	-11.21	< 0.01
Q_{10} of respiration rate	-0.41	-7.72	< 0.01

2.2 Respiration Rate Compensation

In the previous experiment (Chapter 2.1) the immediate effect of change in temperature on tree bud dark respiration rates was illustrated. The experiment reported in this sub-chapter show the effect of a change in temperature on respiration rates over 25 hours. Experiments were designed to determine how quickly tree buds could alter their respiration rates when subject to an increase or decrease in temperature i.e. how fast could respiration rate compensation take place.

Again a comparative approach was employed, utilising six European tree species, two species from each of three genera. Within each of the three genera, one species had a more northerly distribution than the other. Species used are indicated in Table 2.5 and all were mature trees growing in the Botanic Gardens, St. Andrews.

Table 2.5 Tree species used in the measurement of respiration rate compensation.

Alnus incana	(Northerly)
A. glutinosa	
Prunus padus	(Northerly)
P. avium	
Sorbus aucuparia	(Northerly)
S. aria	

Twigs containing buds were cut from two trees of each species growing in the Botanic Gardens, St. Andrews. The twigs were quickly transported to the laboratory and recut to 15cm and placed in beakers containing distilled water to a depth of 2cm. Each beaker was then enclosed loosely in a clear polythene bag. These samples were then placed in a glass fronted cold cabinet subject to diffuse daylight and a temperature of 12^{o}C for 4 days. This was the standard pre-treatment employed.

After 4 days the buds were excised. Two 1g replicates of buds from each species were utilised for dark respiration rate measurements.

Respiration rates (μl O_2/hr/g dry weight) were measured in the Gilson Differential Respirometer method as previously described in Chapter 2.1. A more concentrated solution of KOH (20%), however, was placed in the centre well of each flask and throughout the experiment each flask was vented regularly with air and the KOH solution renewed. These precautions ensured that an adequate supply of oxygen for bud respiration and sufficient KOH to absorb the carbon dioxide respired was available. The precautions were necessary as respiration rates were measured over periods as long as 42 hours.

Oxygen uptake was measured for a number of hours at 12°C i.e. the same temperature as the 4 day pre-treatment. The temperature of the respirometer water bath was then altered to either 22°C or 2°C. After allowing 1 hour for gas equilibration at the new temperature, oxygen uptake rates were monitored over a period up to 25 hours. The results of bud respiration rates versus time were plotted graphically. Each point on the graph was the mean rate for two replicates and was the average rate for 1 hour plotted on the graph at the start of the hour.

The first experiment of this type was carried out in November 1978. Figures 2.3, 2.4 and 2.5 show the change in oxygen uptake rates for Alnus, Prunus, and Sorbus sp. respectively when temperature was increased to 22°C from 12°C. It can be seen that there is a sharp increase in respiration rate with increase in temperature. With the exception of S. aucuparia the rates continue to increase for a number of hours before a gradual reduction in rate is apparent i.e. there is an overshoot or hysteresis effect. After the initial increase of respiration rate with increase of temperature, S. aucuparia buds alone exhibit an immediate and continuing decrease in respiration rates to compensate for the new higher temperatures.

A further experiment was carried out in January 1979. Temperature change in the respirometer, however, was a reduction from 12°C to 2°C

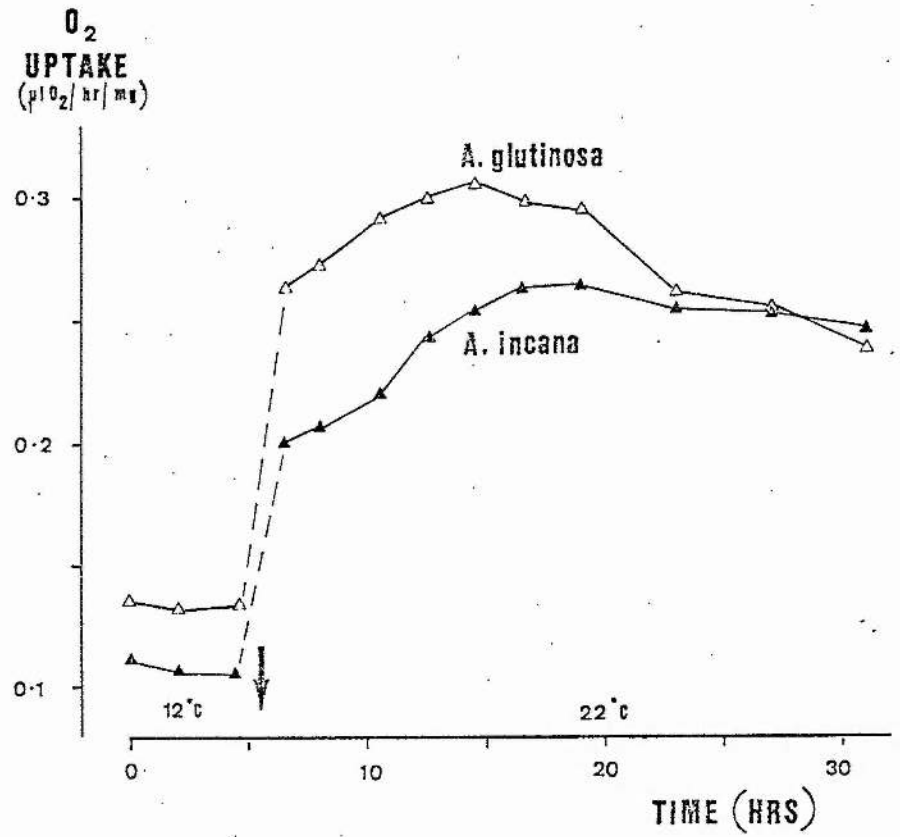


Figure 2.3 Change in respiration rates (oxygen uptake) of Alnus incana and A. glutinosa buds with time, when subjected to an increase in temperature regime from 12°C to 22°C. Arrow indicates time of temperature increase.

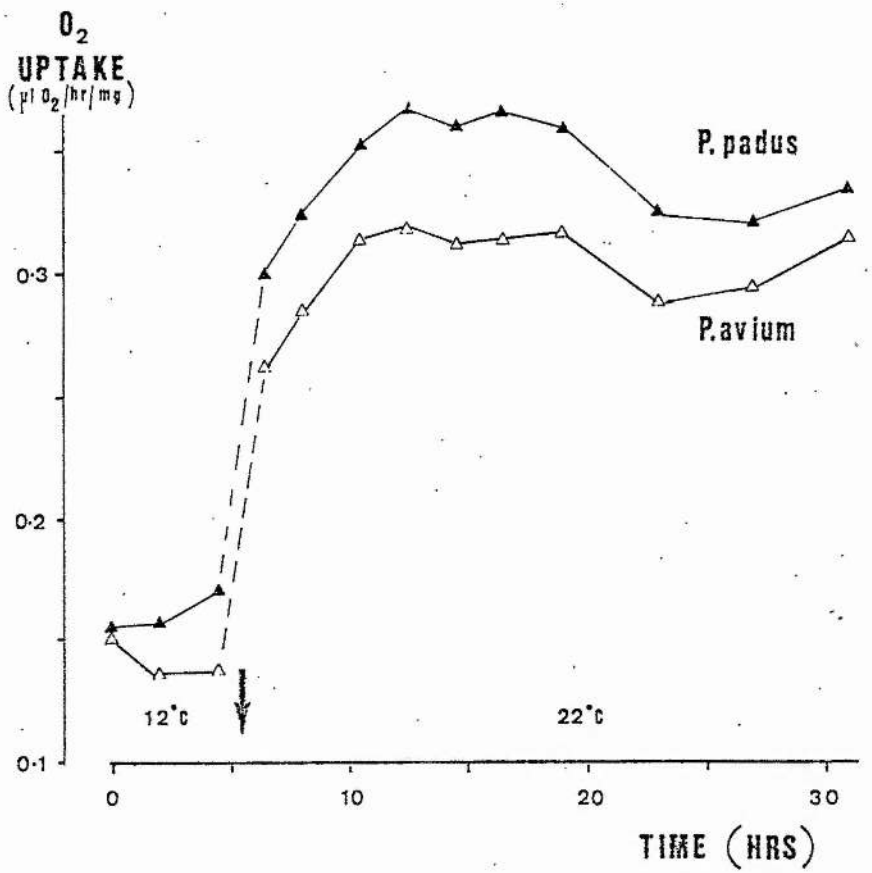


Figure 2.4 Change in respiration rates (oxygen uptake) of Prunus padus and P. avium buds with time, when subjected to an increase in temperature regime from 12°C to 22°C. Arrow indicates time of temperature increase.

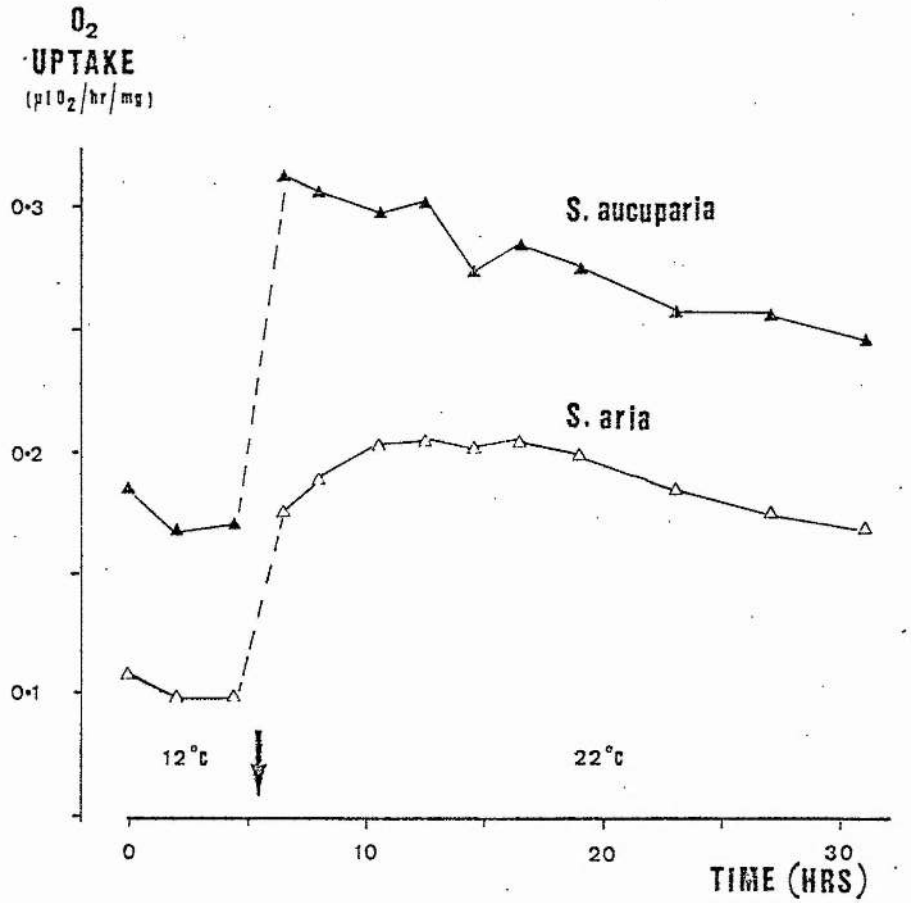


Figure 2.5 Change in respiration rates (oxygen uptake) of Sorbus aucuparia and buds with time, when subjected to an increase in temperature regime from 12°C to (12°C to) 22°C. Arrow indicates time of temperature increase.

instead of the increase to 22°C. With the decrease in temperature to 2°C, all six species showed a sharp decrease in respiration rates. This new low rate remained steady for the duration of the experiment. No hysteresis effect was observed and no increase in rate to compensate for the low temperature was noted. Results are shown graphically for S. aucuparia only, in Figure 2.6.

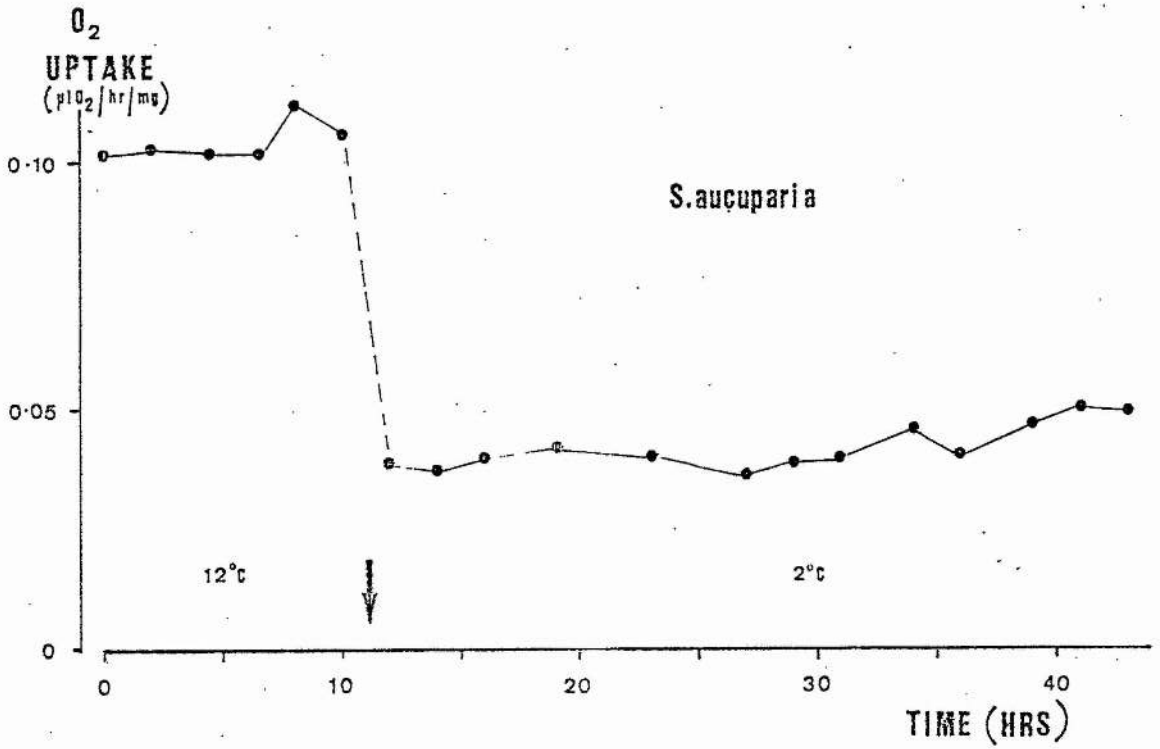


Figure 2.6 Change in respiration rate (oxygen uptake) of S. aucuparia buds with time, when subjected to a decrease in temperature regime from 12°C to 2°C. Arrow indicates time of temperature decrease.

2.3 Discussion

Measurement of bud respiration rates show that there is no apparent correlation between the latitudinal natural distribution of the tree species used and respiratory rates. Furthermore, data presented in this chapter has shown that there is no general pattern in the energy of activation of bud respiration rates with regard to the ability of a tree species to grow in low temperature habitats. The rates for S. aucuparia buds had the lowest value of energy of activation. Values for B. pubescens and P. sylvestris, the two other species found growing in low temperature environments were similar to the species which have a distribution limited to lower latitudes.

Attempts have long been made to correlate the value of energy of activation or the Q10 of biological processes with the temperature ecology of a species. There has been disagreement as to the adaptational significance of the changes in Q10 or energy of activation. Alexandrov (1977) discusses the arguments put forward to explain values for energy of activation and Q10 in organisms adapted to low temperatures. If for organisms adapted to low temperatures the above parameters were found to decrease, it was suggested that the organisms had a reduced temperature dependence. Conversely if the biological processes of an organism adapted to low temperatures showed an increased energy of activation the processes were regarded to have the ability to enhance their activity with only a small increase in temperature.

It can be said that the low value of energy of activation of bud respiration rates found in S. aucuparia would be advantageous in that respiration rates show a reduced temperature dependence. A high energy of activation would be disadvantageous in that a small increase in temperature would enhance metabolic rates during the winter time, a time unsuitable for growth. Increased metabolic rates would reduce carbohydrate reserves. The ability to conserve carbohydrate reserves must be a

prerequisite of a tree species capable of growing in a low energy environment.

A note of caution. The data put forward portraying the low energy of activation of dark respiration rates of S. aucuparia may in part be due to the conditions of the experiment. As shown in Section 2.2, the buds of this species have the ability to reduce respiration rates when subject to high temperatures. This, of course, would reduce the values for the energy of activation of dark respiration rates.

The measurement of the energy of activation of bud dark respiration rates over a whole winter season in the above 6 species might have been a useful exercise. Results from that could possibly increase our understanding of the ecological importance of this parameter. Lack of time, however, precluded such measurements being made.

Billings (1974) states that 'the key to successful adaptation of plants to low temperature habitats is the development and operation of a metabolic system which can capture, store and utilise energy at low temperatures in a short summer season.' The experiments on the energy of activation and on temperature compensation of respiratory rates have, however, been carried out on the perrenating organ of deciduous tree species. During the winter time little growth takes place in the bud. A large proportion of the energy needed must be for cellular maintenance. Increase of temperature causing an increase in respiration rates would squander carbohydrate reserves necessary for growth and metabolism in late spring. Buds which can reduce their respiration rate quickly in response to an increased temperature would therefore be at an advantage in a low energy environment.

In the experiments on temperature compensation of respiration rates reported in this chapter, all species tested showed an immediate rise in rate when subjected to an increase of temperature from 12°C to 22°C (see Figures 2.3, 2.4 and 2.5). When kept at the higher temperature, all

species with the exception of S. aucuparia further increased their respiration rates for several hours before a gradual reduction in rate was apparent. S. aucuparia alone showed an immediate decrease in rate. This may be an adaptation favouring the success of this species in low temperature habitats.

Respiratory acclimatisation has been studied and observed in plants. Rook (1969) studied the influence of growing temperature on the photosynthesis and respiration of Pinus radiata seedlings. Plants pre-treated under a day/night temperature regime of $15^{o}C/10^{o}C$ and transferred to a $33^{o}C/28^{o}C$ regime decreased their respiration rates by almost 100% in a few days. Conversely, seedlings transferred from a warm to a cold environment showed a reverse of this modification in respiratory rates. Adjustments in respiration rates were much more dramatic than the changes in photosynthesis.

In a study of the alpine flora of Sierra Nevada, Chabot and Billings (1972) found that many plants from their study area, grown under different thermal regimes had different respiration rates when measured under the same conditions. Plants grown at low temperatures had higher respiration rates than those grown at high temperatures.

They also measured the time necessary to adjust to a new temperature environment. When temperatures were raised, respiration rates initially increased, then after a variable lag phase, the rates decreased and soon levelled off. The rate of decline and also the length of the lag period to some extent was influenced by the previous temperature regime pre-treatment. A greater temperature shift caused a faster decline in respiratory rate and in some cases a reduction in the lag phase. Evidence indicated that acclimatisation occurred faster in plants from higher altitudes. This was evident between different species and also within a species.

The plant Chabot and Billings found to be most successful at reducing its dark respiration rates when subjected to an increase in temperature was Oxyria digyna. This plant, which has a circumboreal distribution, is restricted to high elevations in the low latitudes of the Sierra Nevada. When grown under a day/night temperature regime of 25°C/20°C and transferred to a temperature of 32°C, it showed an initial sharp increase in respiration rate. Within 10 hours, however, it had reduced this rate by 32%. Encelia virginensis, a plant restricted to lower elevations only reduced its respiration rate by 2% in this period. This plant and other "slow" species took 1-2 days to reduce their rates appreciably.

In contrast to the above results acclimatisation of dark respiration rates to low temperatures took 7 to 14 days in all species tested.

It would appear therefore that fast reduction in dark respiration rates when subjected to raised temperatures is a strategy employed by plants growing in low temperature habitats.

CHAPTER 3

DESICCATION STRESS IN TREE BUDS AND TWIGS

3.1 Introduction

In the introductory Chapter 1 it was suggested that desiccation stress may be a contributory factor in determining the low temperature limits of tree growth. In this chapter, the effect of desiccation stress on various aspects of the metabolism of tree buds and twigs are reported and discussed. A comparative approach has been employed.

Seven tree species found growing in various habitats and having different geographical ranges in Europe have been the experimental material.

Three of the species were of the genus Sorbus, S. aucuparia, S. aria and S. intermedia. The distribution of S. aucuparia in Europe and also its ability to grow in low temperature habitats has already been discussed in Chapter 1. This species is subject to physiological drought due to low temperatures in wintertime. Evidence for this is presented in Chapter 5. S. aucuparia is wind resistant. It occurs in heath communities along the North Sea coast in continental Europe. There it is found on dry sand dunes and subject to much wind and where no other pioneers of forest are to be found (Ellenberg 1963). S. aria is a tree of more southerly distribution in Europe. It is found growing in dry habitats and is known to be drought tolerant (Ellenberg 1963). Similarly, S. intermedia is also a tree of dry habitats although its natural distribution is limited to the Baltic area (Fitter 1978). It has been planted in the Orkneys as a shelter tree on these wind swept isles.

Alnus incana is a circumboreal species found growing on river gravels in mountainous districts (Strasburger 1965). This species would undoubtedly be subject to physiological drought in wintertime due to low temperatures. The main centre of distribution of Fagus sylvatica is

central Europe especially at moderate altitudes, but also in the lowlands where the soil is not too poor and dry (Ellenberg 1963). In Britain F. sylvatica is native in south England where it is dominant on chalk and often dominant on well drained loam and sands (Clapham et al 1962). Quercus robur is found over the greater part of Europe, especially in the lowlands in a great variety of habitats (Strasburger 1965). In the British Isles, this tree is rarely found over 300m in altitude. It is the dominant tree of heavy and especially basic soils of south, east and central England. Carpinus betulus has a latitudinal range in Europe from the Pyrenees in the south to South Sweden in the north. In Britain it is native to south-east England (Mitchell 1974). This tree tends to grow on drier soils (Fitter 1978).

3.2 Desiccation Stress and Viability

Twigs with attached buds were subjected to a standard desiccation treatment. The ability of the twigs to contain water losses under desiccation was measured. By treating a further sub-sample similarly, the effect of desiccation on the viability of the buds and twigs were determined.

Viability was determined by a vital staining technique. Triphenyl tetrazolium chloride is a colourless, water soluble compound which can be converted to a coloured water insolubl'e formazan by dehydrogenase enzyme system in biological tissues. Often the Triphenyl tetrazolium chloride (T.T.C.) test has been used in the past as a qualitative response. If the red formazan derivative was produced, the tissue was considered to be viable. This technique has often been used for testing viability of seeds (e.g. Mackay 1972). The technique utilised here, however, is a quantitative one based on a method by Steponkus and Lanphear (1967). These authors extracted the red formazan derivative from tissues and measured the extract colourimetri-cally. They used this method to evaluate cold injury in woody plants.

Buds and twigs from the various species tested were obtained from the Botanic Gardens, St. Andrews between 1 and 4 November 1977.

Tree species used were:-

Sorbus intermedia (Ehrh.) Pers.

S. aria (L.) Crantz

S. aucuparia L.

Alnus incana (L.) Moench

Quercus robur L.

Fagus sylvatica L.

From each species, ten twigs containing buds, from current year's growth were cut to about 7cm in length. They were placed in agitated

aerated distilled water for 24 hours. Twigs were then quickly dried with paper tissue and the cut ends lightly smeared with a small amount of petroleum jelly and weighed. This weight was the saturated weight of the plant tissues at time 0.

The twigs were then placed in glass desiccators containing $CaCl_2$, and housed in a glass fronted cold cabinet at $10^{o}C$. The cabinet was subject to diffuse daylight. Plant tissues were weighed at intervals to give the fresh weight. At the end of the experiment the twigs were dried at $95^{o}C$ for 36 hours and weighed to give the dry weight. Relative water content of the twigs was calculated from the above data.

$$\text{Relative Water Content} = \frac{\text{Fresh weight - Dry weight}}{\text{Saturated weight - Dry weight}} \times \frac{100}{1}$$

Due to respiratory losses, some error would be expected in relative water content determinations, as the dry weights were measured at the end, and fresh weights measured throughout the experiment. Each species, however, would be subject to some respiratory losses. This would help cancel out any error in interspecific relative water content comparisons.

Determination of viability of buds and twigs at different relative water contents were measured utilising parallel samples treated as above.

Viability was determined by a refined Triphenyl tetrazolium chloride (T.T.C.) test after Steponkus and Lanphear (1967). Stem sections, 10 replicates, were sliced to a thickness of about 1mm. Ten buds were cut longitudinally likewise. Samples were weighed (fresh weight) to give approximately 25mg residual dry weight after ethanol extraction. These tissue samples were placed in thick-walled test tubes containing 3ml of 0.6% W/V 2, 3, 5 Triphenyl tetrazolium chloride in 0.05 M Na_2HPO_4 - KH_2PO_4 buffer (pH 6.9) 0.05% V/V wetting agent - Teepol. The samples were infiltrated under vacuum for 10 minutes. After sealing the test

tubes with serum stoppers, the tissues were incubated for 20 hrs in the dark at 30°C.

The T.T.C. solution was drained and the sample tissues rinsed once in distilled water. Samples were then extracted 5 times in 4 ml 95% ethanol for 5 minutes. Extraction in water bath at 90°C. Extracts were filtered through Whatman No. 1 filter paper and made up to 10 ml with 95% ethanol. The absorbance was recorded at 530 nm, reference 95% ethanol. Whilst the reduced T.T.C. solution had a λ maximum at about 490nm, there was interference from endogenous plant pigments at this wavelength. Therefore, 530nm a wavelength still in the region of high absorption by the reduced T.T.C. but minimum absorption by tissue pigments, was chosen for assaying the formazan derivative. To compare results between different species, absorbance of the tissues after various periods of desiccation was calculated as a percentage of absorbance at day 0 for each species.

Illustrated in Figures 3.1 - 3.6 is the decrease in values of relative water content and tissue viability against length of desiccation treatment time for each of the six species tested. Inspection of these figures reveals that large differences are evident between species.

Figures 3.1 and 3.2, depicting results for Q. robur and F. sylvatica respectively show that, in these species, relative water content dropped quickly to about 20% within 5 days. This was paralleled by a large reduction in tissue viability. Viability decreased to a minimum in Q. robur buds and twigs within 5 days of desiccation treatment and in F. sylvatica tissues within 10 days.

These results contrast markedly with that of S. intermedia (Figure 3.3). A relative water content of 20% was not reached until about 12 days of desiccation treatment. A high value of viability of tissues was retained

for 10 days and a minimum was not recorded till 20 days. Water content and viability curves of S. aria and S. aucuparia (Figures 3.4 and 3.5) most closely resemble the S. intermedia type curves (Figure 3.3).

Data for A. incana is shown in Figure 3.6. Results for this species show that the relative water content decreased to a low level quickly i.e. to 20% within 7 days desiccation treatment time. Tissue viability did not reach a minimum until after 15 days treatment. Results for this species therefore are intermediate in character between the extreme types of curves produced by Q. robur and S. intermedia.

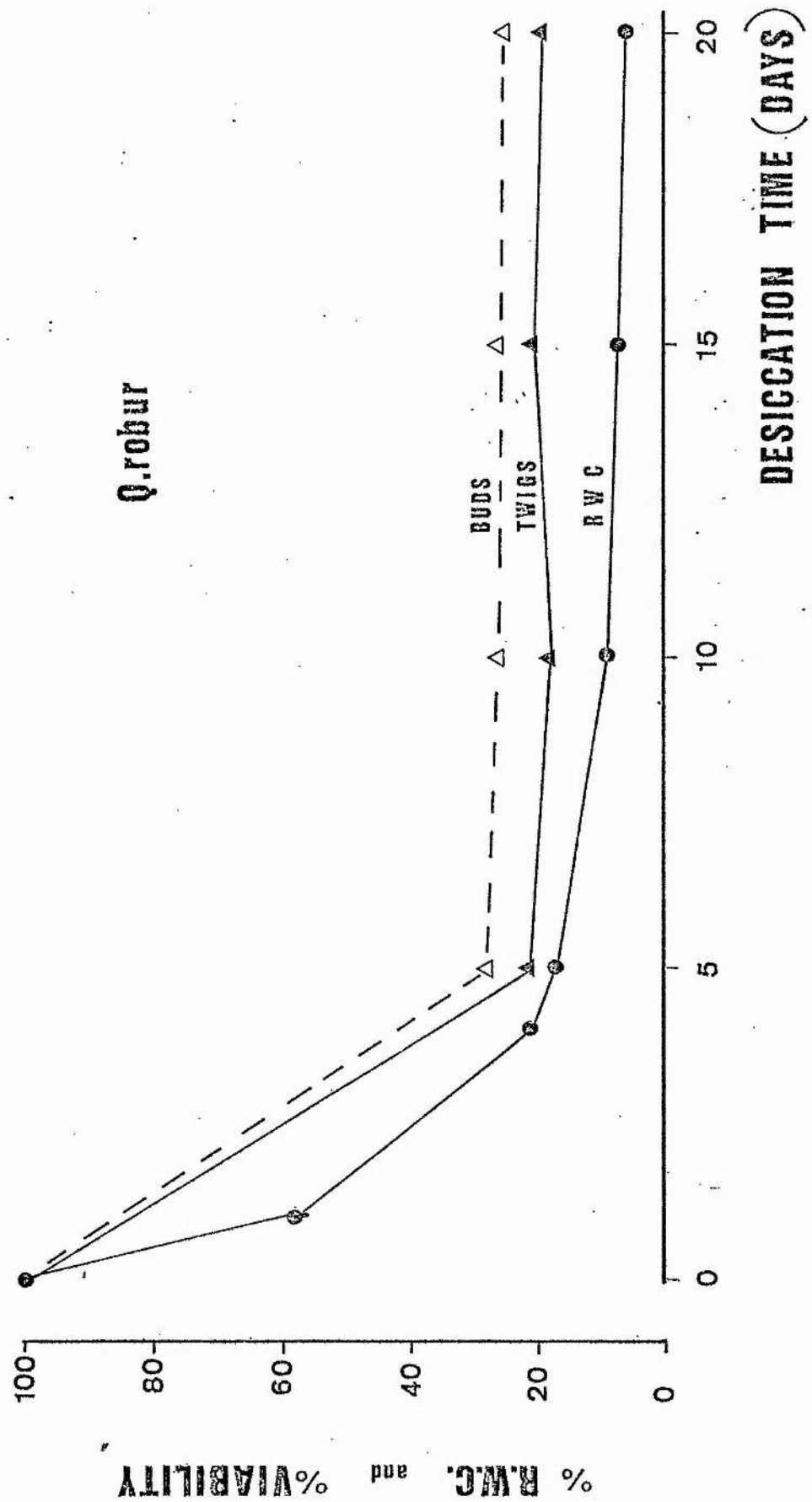


Figure 3.1 Decrease in percentage relative water content (●——●) and percentage viability of Quercus robur buds (△— — —△) and twigs (▲——▲) with length of desiccation treatment time.

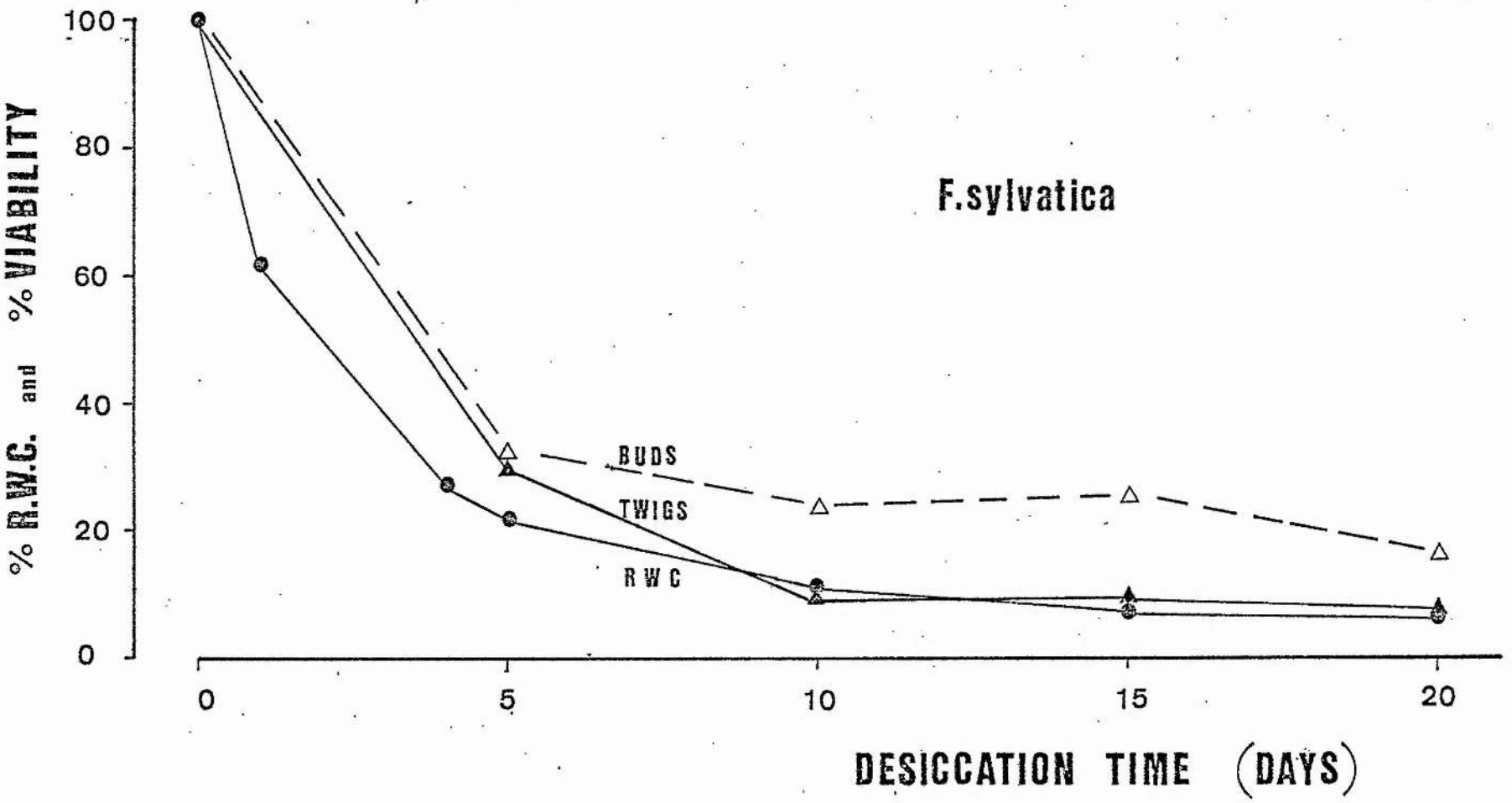


Figure 3.2 Decrease in percentage relative water content (●——●) and percentage viability of Fagus sylvatica buds (△— — —△) and twigs (▲——▲) with length of desiccation treatment time.

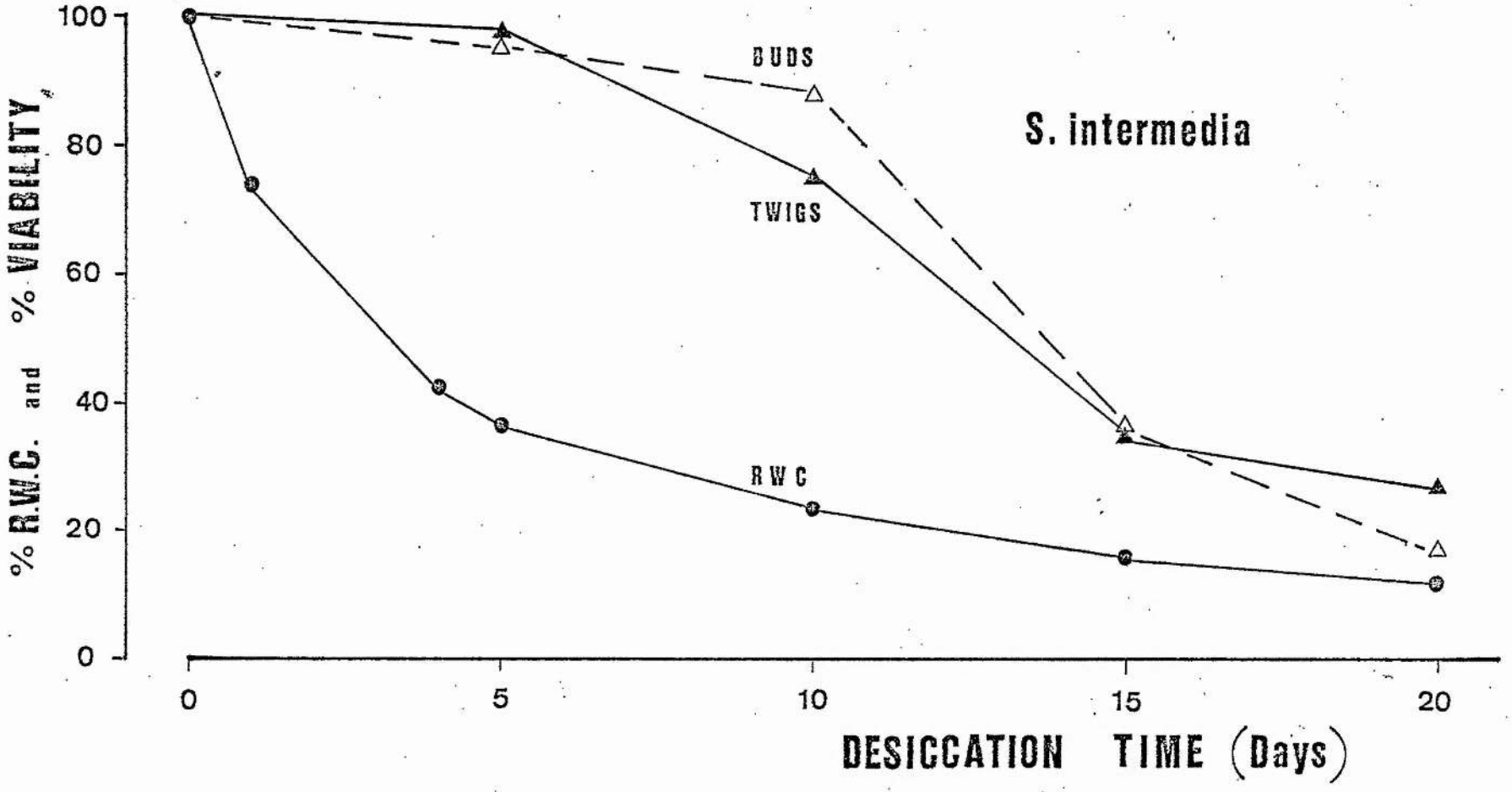


Figure 3.3 Decrease in percentage relative water content (●——●) and percentage viability of Sorbus intermedia buds (△— — -△) and twigs (▲——▲) with length of desiccation treatment time.

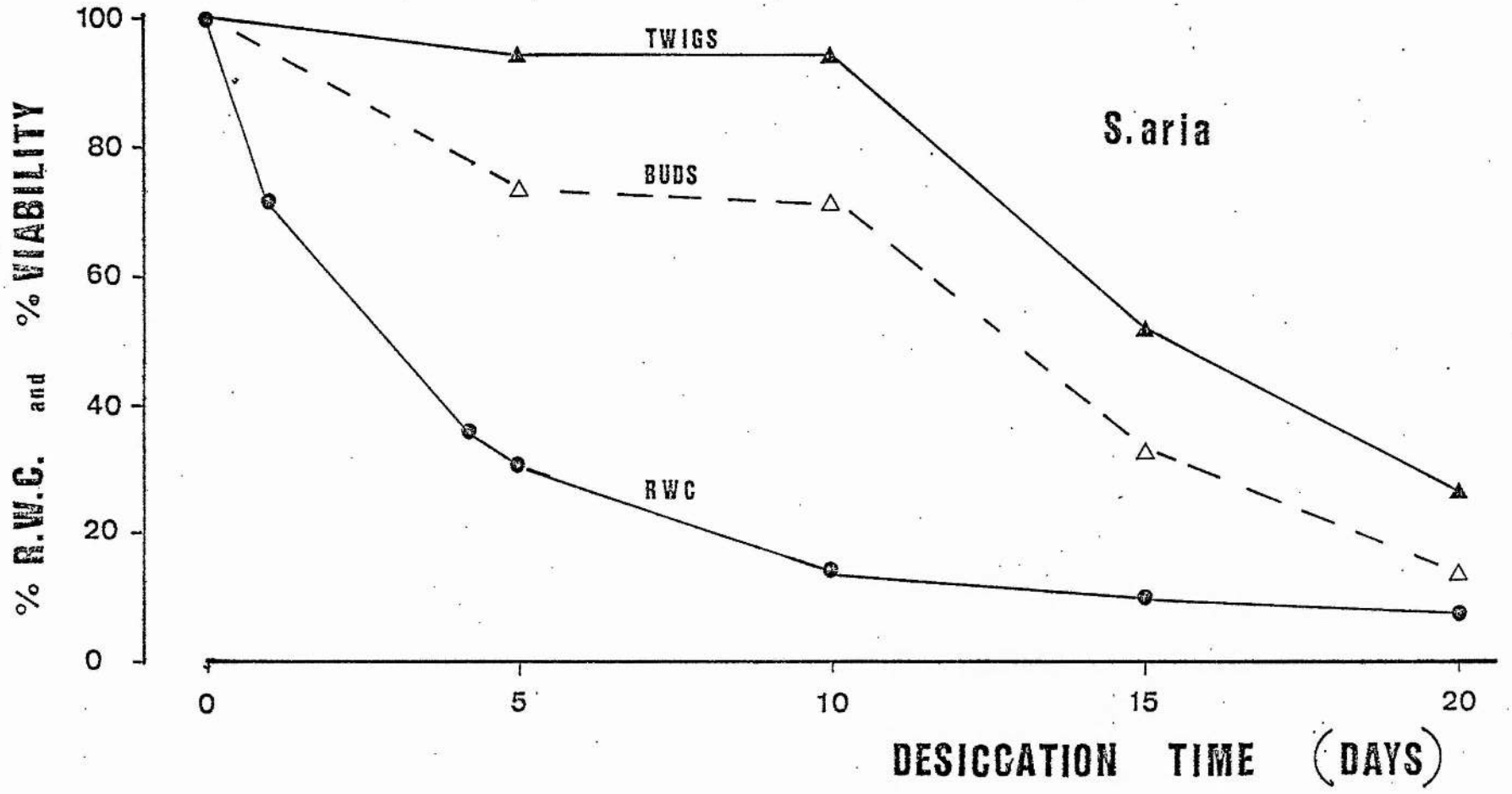


Figure 3.4 Decrease in percentage relative water content (●——●) and percentage viability of Sorbus aria buds (△— —△) and twigs (▲——▲) with length of desiccation treatment time.

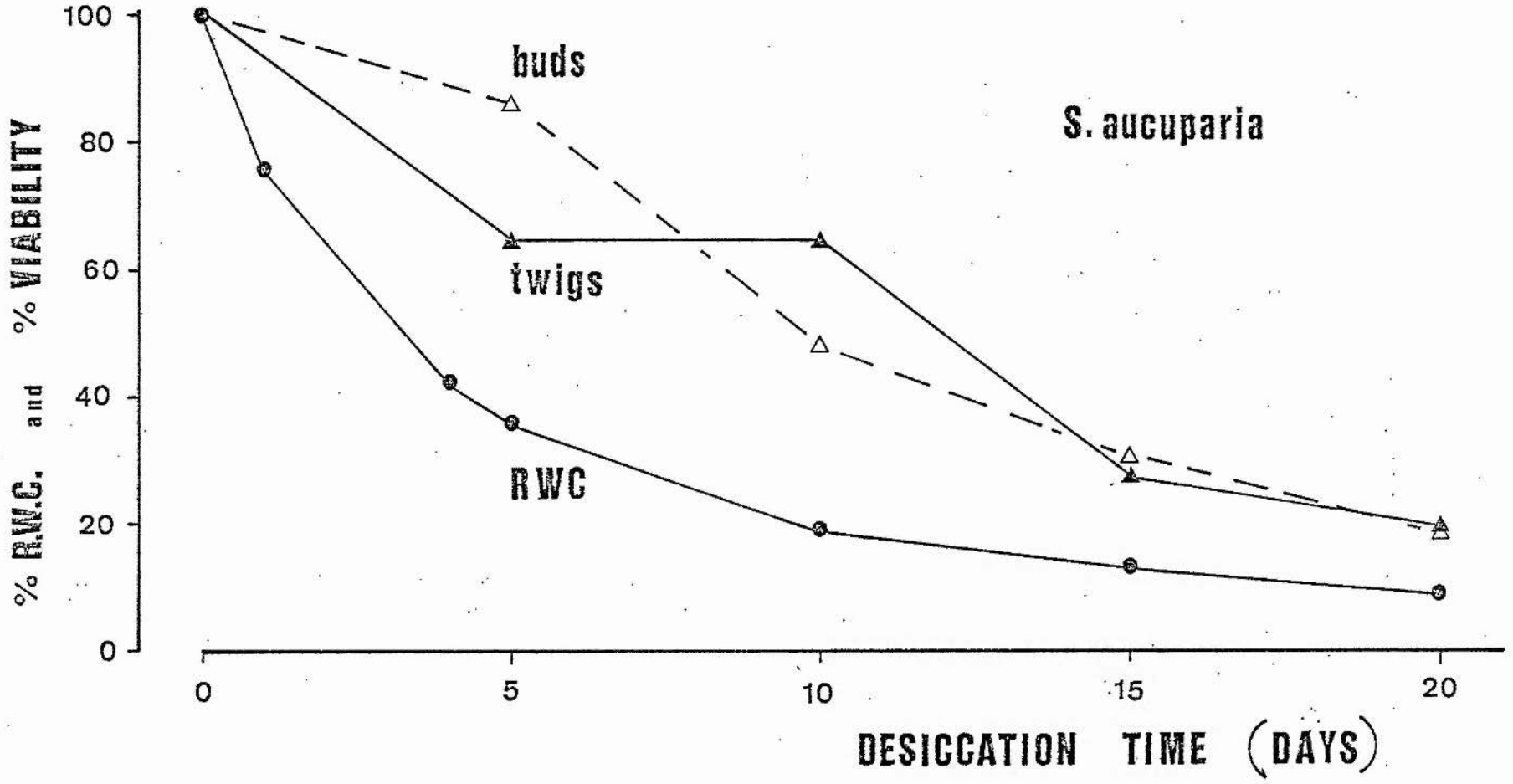


Figure 3.5 Decrease in percentage relative water content (●——●) and percentage viability of Sorbus aucuparia buds (△— —△) and twigs (▲——▲) with length of desiccation treatment time.

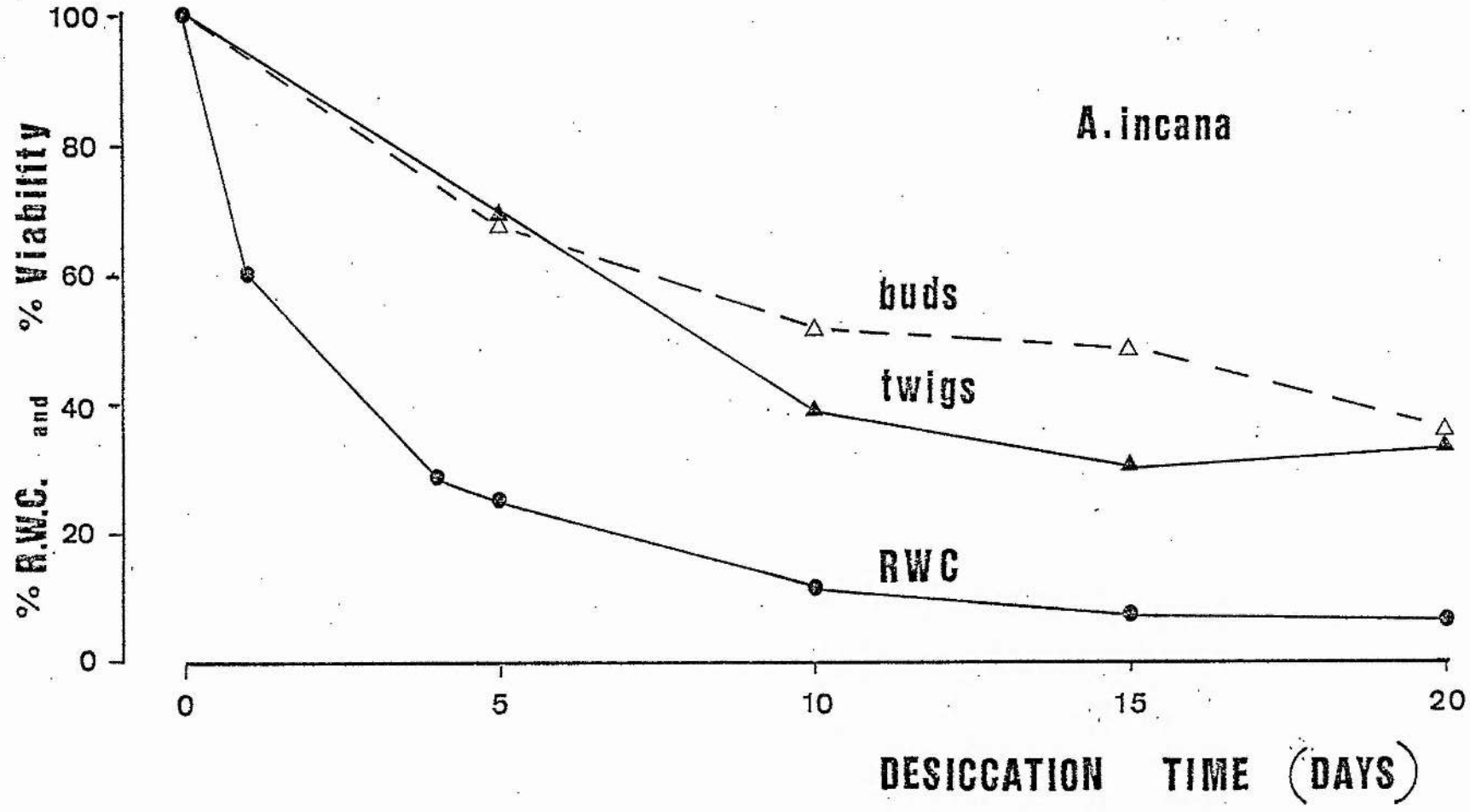


Figure 3.6 Decrease in percentage relative water content (●——●) and percentage viability of Alnus incana buds (△ — — △) and twigs (▲——▲) with length of desiccation treatment time.

3.3 Desiccation Stress and Ethylene Production

Buds from six tree species were desiccated under standard conditions. The decline in water content of the buds was monitored as was the change in bud tissue ethylene (ethene) production. The aim of the experiment was to determine the ability of bud tissues to maintain steady levels of ethylene production under desiccation stress i.e. internal homeostasis. The same species were used as in the viability studies on tree buds (Chapter 3.2). The species tested were therefore:-

Sorbus aria (L.) Crantz

S. intermedia (Ehrh.) Pers

S. aucuparia L.

Alnus incana (L.) Moench

Quercus robur L.

Fagus sylvetica L.

Branches from the above 6 species were cut from trees growing in the Botanic Gardens, St. Andrews. All material was cut the same day, 22 September 1977. The leaves were still attached and green in all species. Buds were cut from the branches and placed in aerated, agitated, distilled water for 24 hours. For each species, buds were dried with paper tissue and divided into 12 1.5g samples (saturated weight). The samples were put in glass desiccators containing $CaCl_2$ which were housed in a glass fronted cold cabinet at 10°C and subject to diffuse daylight. 3 samples of buds were removed at intervals of 0, 1, 3 and 7 days. On removal, the buds were weighed to give the fresh weight at that time.

Samples were then placed in 18ml test tubes fitted with serum stoppers, for about 48 hours in the dark at 20°C. 2ml aliquots of gas were removed from the test tubes by syringe for ethylene analysis. The buds were dried for 24 hours to give the dry weight. Relative water content for each sample of buds at time of removal from desiccator was

calculated as

$$\text{Relative water content} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Saturated weight} - \text{Dry weight}} \times \frac{100}{1}$$

The gas samples were analysed by gas - solid chromatography after Hollis (1966). 2ml gas samples were injected into a gas chromatograph (Pye unicam, series 104) fitted with a 1.52m glass column, diameter 4mm and a flame ionisation detector. Column packing was Poropak Q, mesh size 100/120 and temperature used was 40°C. Ethylene production was calculated as nl/g dry weight/hr.

The mean rates of ethylene production after different periods of desiccation are illustrated in Figures 3.7 - 3.12 for all six species. The numbers superimposed on these figures beside each point denote the mean relative water content of the bud sample.

There was variability in the amount of ethylene produced at the start of the experiment by the 6 species. A. incana buds (Figure 3.7) produced 2.35nl/g/hr. This compared with a range of between 0.30 to 0.85nl/g/hr produced by the other 5 species (Figures 3.8 - 3.12). The standard error of the mean of the ethylene production tended to increase over the first 3 days as the water deficits in the buds increased.

Only A. incana buds maintained a steady production of ethylene over 3 days.drying stress. This was not due to the ability of A. incana buds to maintain a high water content in the buds. After 3 days desiccation the buds of this tree species had a relative water content of only 40%.

The buds of the other species exhibited a variable response in the production of this substance. S. aria (Figure 3.8) and Q. robur (Figure 3.11) buds produced a large increase of ethylene within 3 days. Production in S. aria buds increased from 0.6 to 1.4nl/g/hr and Q. robur buds from 0.3 to 0.75nl/g/hr. S. intermedia (Figure 3.9) buds showed a

large decrease in production from 0.6 to 0.35nl/g/hr after 3 days desiccation stress. Ethylene production in S. aucuparia buds showed a reduction after 1 day desiccation treatment (Figure 3.12) from 0.85 to 0.35nl/g/hr then an increase after 3 days to 1.1nl/g/hr. The standard error of the mean of the 3 samples at 3 days was very large. F. sylvatica buds after desiccation reduced production of the plant hormone after 1 day to 0.4nl/g/hr from 0.7nl/g/hr (Figure 3.10). After 3 days desiccation treatment ethylene production had returned to 0.7nl/g/hr however, although the standard error of the mean of the 3 samples increased considerably.

In all species, ethylene production had decreased to a very low level after 7 days of desiccation stress. Production then was less than 0.05nl/g/hr in all species with the exception of S. aria buds which were producing 0.23nl/g/hr.

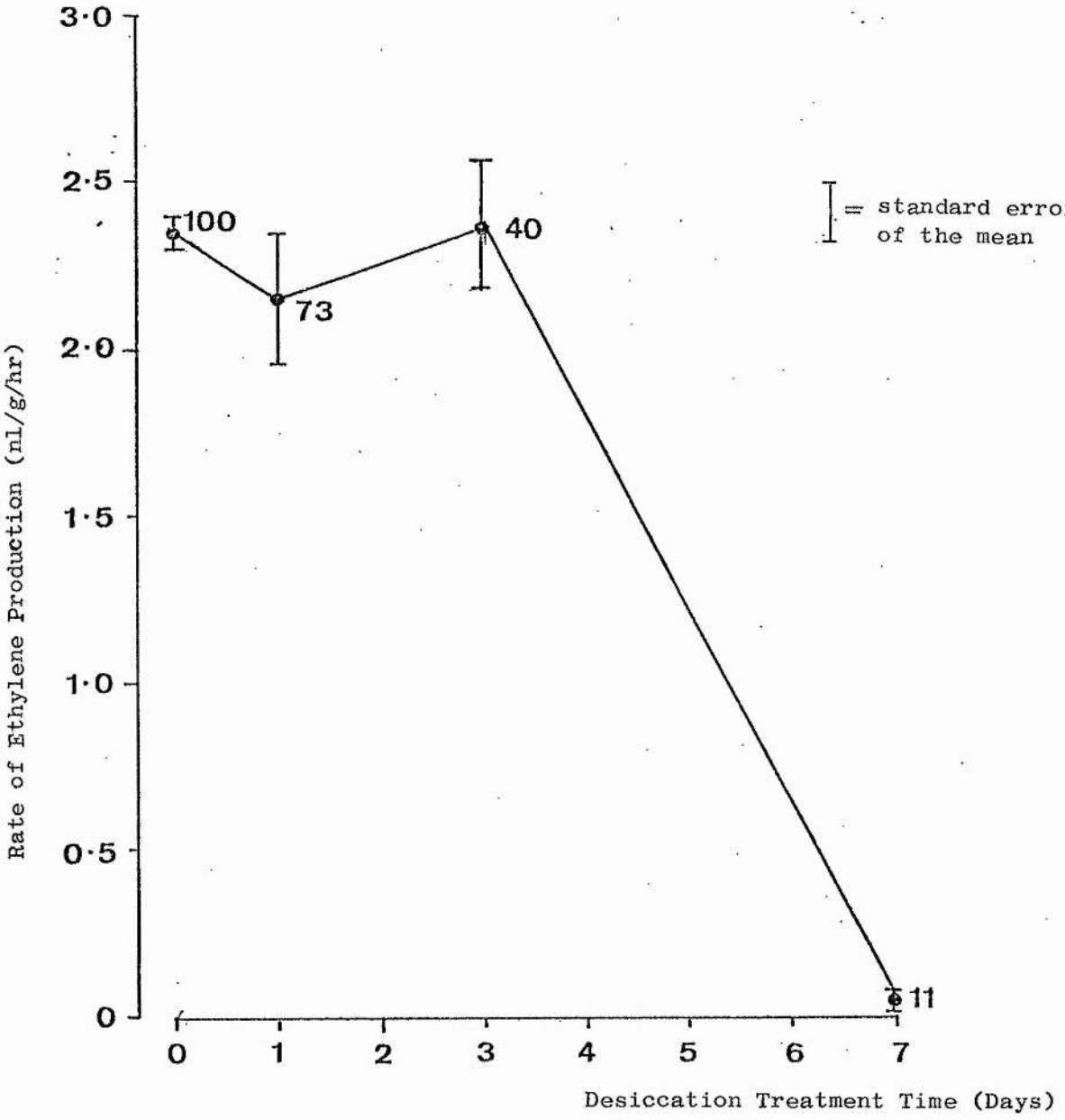


Figure 3.7 Rate of ethylene production in Alnus incana buds subjected to a standard desiccation treatment. Numerical values beside points are the mean relative water content (%) of the bud samples.

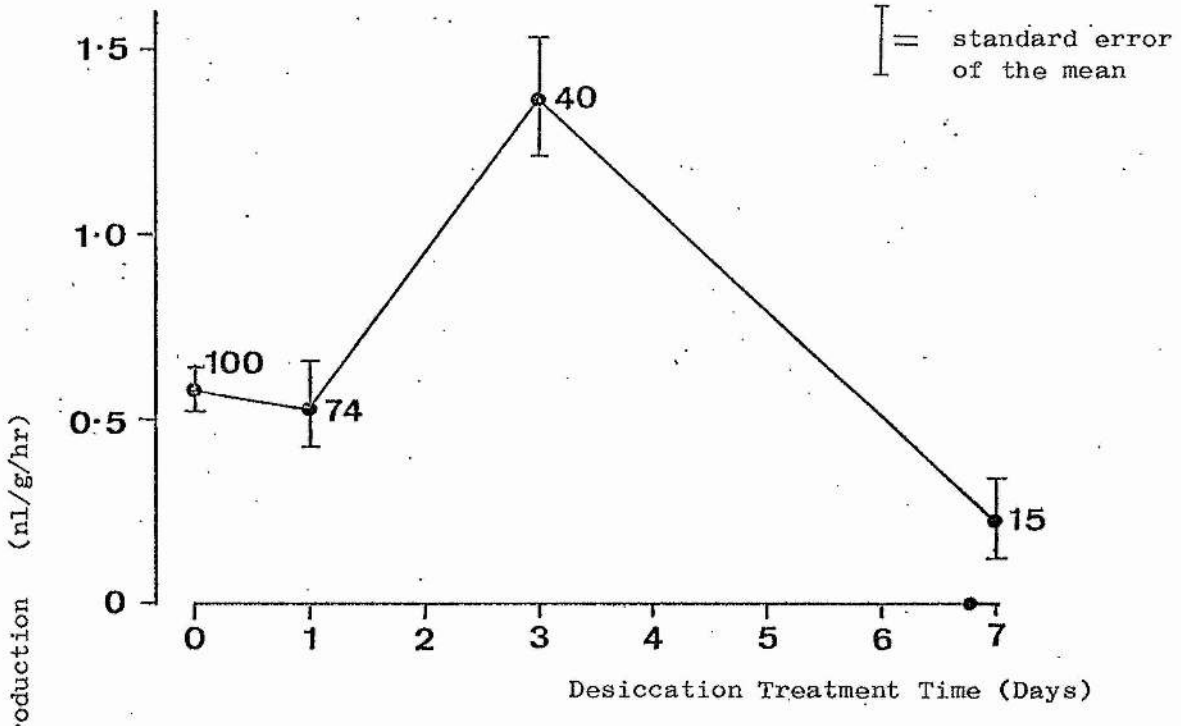


Figure 3.8 Rate of ethylene production in Sorbus aria buds subjected to a standard desiccation treatment. Numerical values beside points are the mean relative water content (%) of the bud samples.

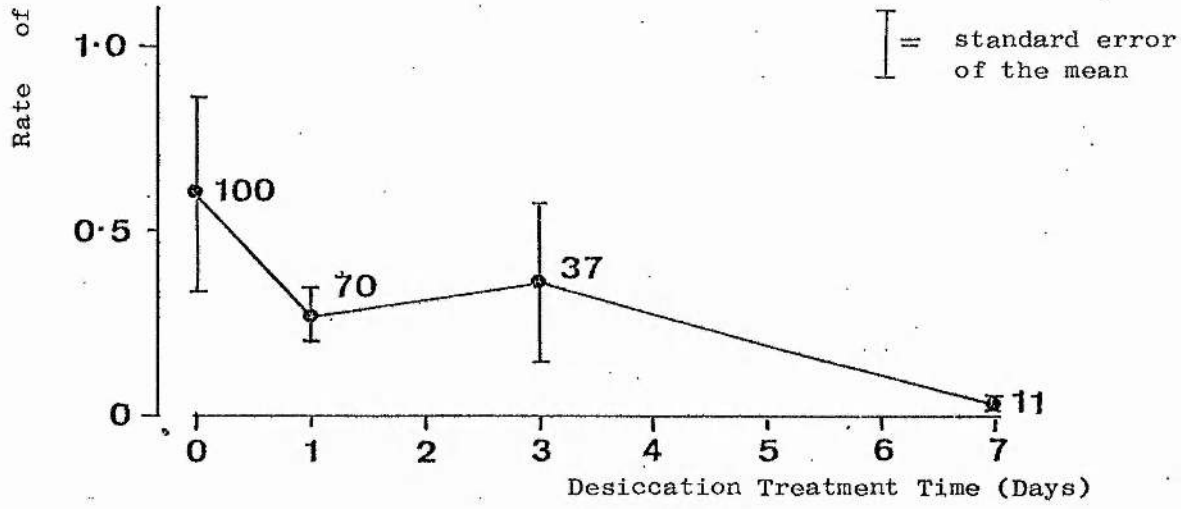


Figure 3.9 Rate of ethylene production in Sorbus intermedia buds subjected to a standard desiccation treatment. Numerical values beside points are the mean relative water content (%) of the bud samples.

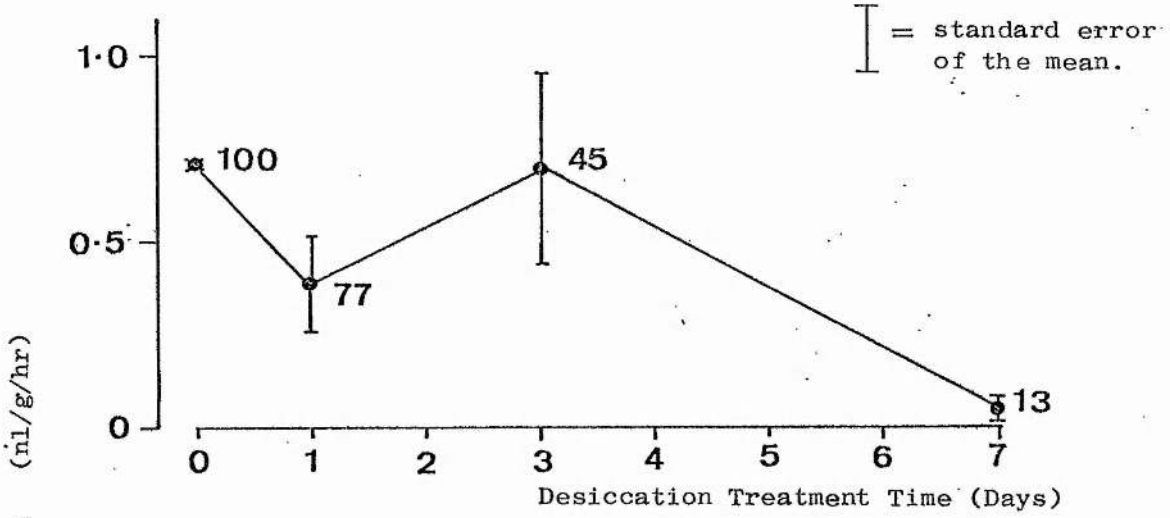


Figure 3.10 Rate of ethylene production in Fagus sylvatica buds subjected to a standard desiccation treatment. Numerical values beside points are the mean relative water content (%) of the bud samples.

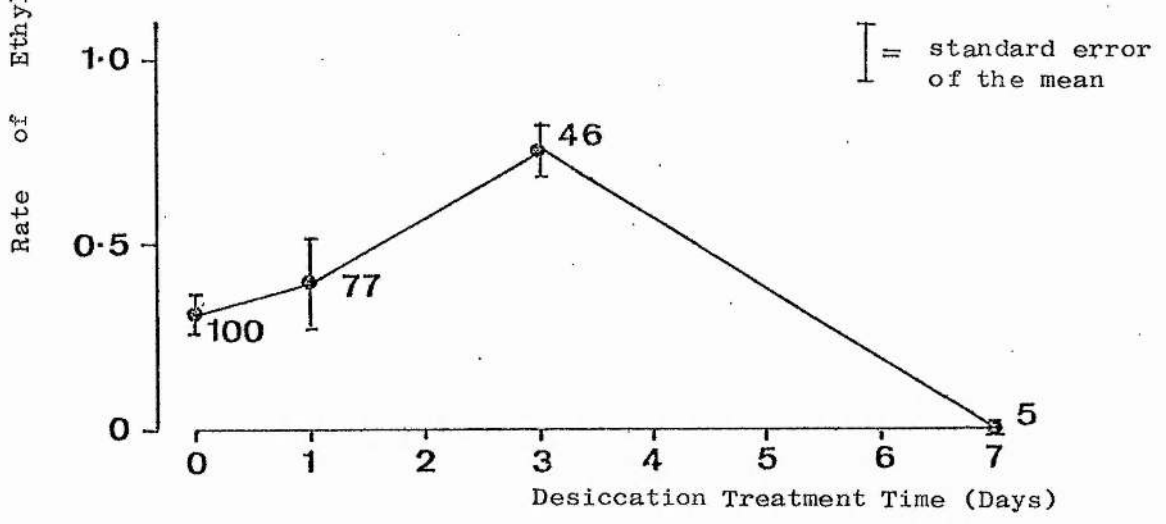


Figure 3.11 Rate of ethylene production in Quercus robur buds subjected to a standard desiccation treatment. Numerical values beside points are the mean relative water content (%) of the bud samples.

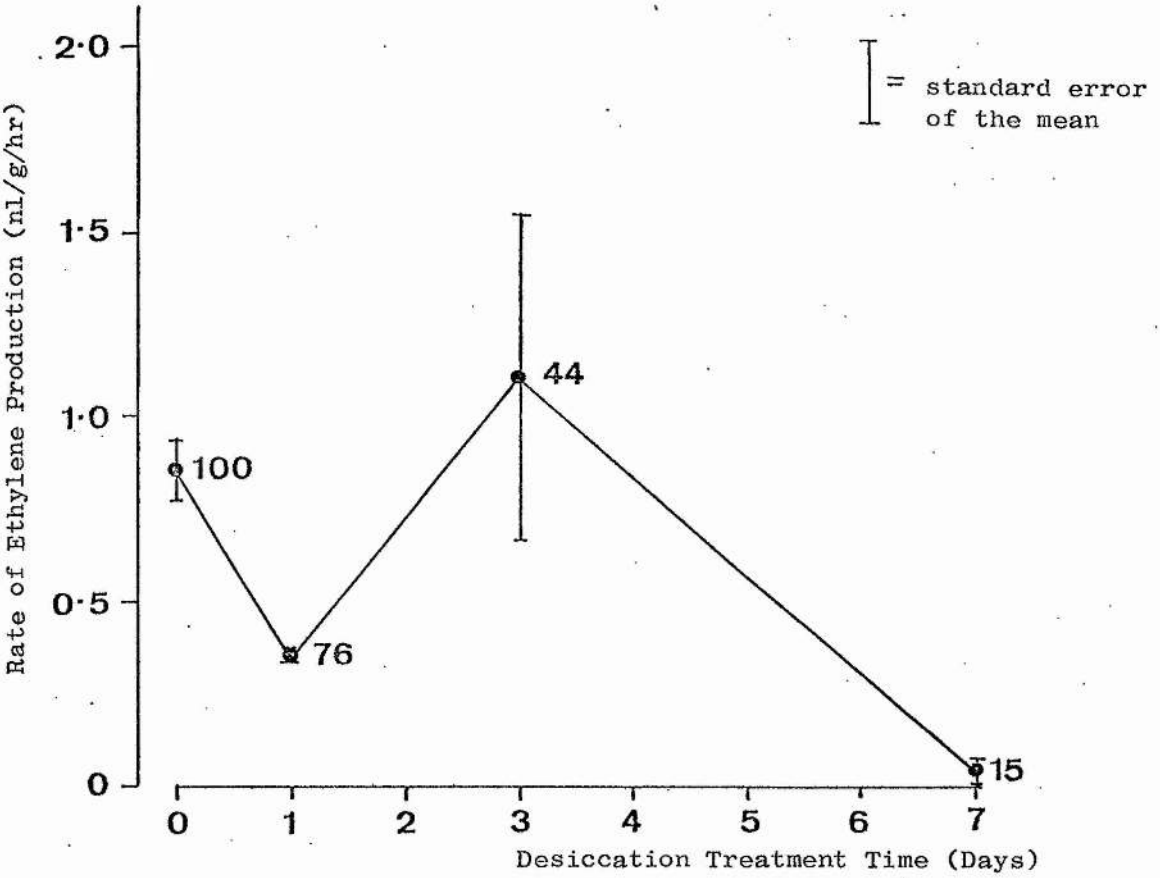


Figure 3.12 Rate of ethylene production in Sorbus aucuparia buds subjected to a standard desiccation treatment. Numerical values beside points are the mean relative water content (%) of the bud samples.

3.4 Desiccation Stress and Carbohydrate Levels

As in previous desiccation experiments, tree species with different geographical ranges and ecological affinities were used. The species were:-

Sorbus aria (L.) Crantz

S. intermedia (Ehrh.) Pers

S. aucuparia L.

Carpinus betulus L.

Quercus robur L.

Alnus incana (L.) Moench

Plant material was obtained from the Botanic Gardens, St. Andrews between 2 and 8 February 1977. From each species, 50 buds were cut from the branches. These buds were placed in agitated aerated distilled water for 24 hours to ensure full saturation of the buds. Individual buds were then dried with paper tissue and weighed. This weight was the saturated weight of the bud. The buds were then placed in glass desiccators containing $CaCl_2$. The desiccators were put in a glass-fronted cold cabinet, subject to diffuse daylight. Temperature was kept at $10^{\circ}C$. Ten buds of each species were removed at weekly intervals. These tissues were weighed individually to find the fresh weight at that time. They were then dried at $95^{\circ}C$ for 24 hrs to find the dry weight. The relative water content of the buds at each time interval could then be calculated where

$$\text{Relative Water Content} = \frac{\text{Fresh weight - Dry weight}}{\text{Saturated weight - Dry weight}} \times \frac{100}{1}$$

Parallel bud samples necessary for carbohydrate analysis received a similar desiccation treatment as above. Samples of 10 buds from each species were removed from the desiccators at time 0, 1, 2, 3 and 4 weeks. A further fresh bud sample was obtained from the Botanic Garden at time = 4 weeks. This extra tissue sample was analysed in an attempt to

differentiate between changes in carbohydrate levels over the 4 weeks of the desiccation treatment as opposed to changes in carbohydrates due to advance in the season i.e. phenological changes.

Bud samples were immersed in liquid nitrogen and ground in a mortar and immediately transferred to a freeze drier. The ground tissue was dried for 24 hours to 0.05mm Hg. Metabolic sugars were extracted from 100mg freeze dried samples with 2ml boiling 80% V/V ethanol for 5 minutes, three times and 2ml 60% V/V ethanol three times. The combined extracts were evaporated to dryness *in vacuo* at 50°C in a vortex evaporimeter. These dried extracts were then stored over phosphorus pentoxide (P_2O_5) in desiccators until required for the gas - liquid chromatography, which followed.

The analysis of soluble carbohydrates was determined by a method developed by Sweeley *et al* (1963) and Ellis (1969). It involved the conversion of the carbohydrates to the trimethylsilyl (TMS) ethers of carbohydrates. Each dried sugar extract was dissolved in 1ml dimethyl sulphoxide (DMSO). A 0.2ml aliquot of redissolved extract was pipetted into a 1ml flask which had a graduated neck. The walls of the flask were washed down with 0.2ml of DMSO. To the flask was added 0.2ml 1,1,1,3,3,3 - trimethylchlorosilane (TMCS). The flask was then stoppered to prevent contamination by moisture in the air, and shaken on a mechanical shaker for 90s. The flasks were stored overnight in a phosphorus pentoxide desiccator. This allowed separation of the solution into 2 phases. The upper phase was hexamethyldisiloxane (HMDSO) in which TMS ethers of carbohydrates exhibit a high partition. DMSO was injected into the lower phase displacing the upper phase into the calibrated neck of the flask. This allowed the volume of the upper phase to be measured accurately.

Sugar derivatives in the upper phase were analysed in a gas chromatograph (Pye Unicam Series 104) fitted with a 1.52m glass column, diameter 4mm and a flame ionisation detector. Column support was Diatomite C.Q., 60-70 mesh, coated with 1% Methyl Phenyl Silicone Gum (E52) stationary phase. Carrier gas was oxygen free nitrogen with a flow rate of 40ml/min. A temperature programme of 2 min. at 100°C was followed by an increase of 6°C per min. to 210°C. This programme was suitable for the analysis of monosaccharides as well as the disaccharide sucrose.

The TMS sugars were identified by preparing derivatives of pure sugars in a similar manner to the plant extracts. These samples were run with samples of the six species used until most peaks on the gas liquid chromatography trace were identified.

The same pure sugars were used to relate peak area to concentration of sugar. Calibration curves for each sugar were drawn by assaying different concentrations of pure sugars against area of the peaks formed. Peak area was calculated by multiplying peak height by the width at half height. A linear regression of sugar concentration versus peak area was obtained. The actual weight of each sugar in a plant sample could then be estimated from the regression line.

Sugar derivatives producing a peak area on the gas liquid chromatography trace of $< 4\%$ of total peak area were identified and quantified, with the exception of a sugar derivative from Q. robur tissues. This sugar had a relative retention time of 0.33 relative to sucrose. Table 3.1 lists the retention times relative to sucrose of the sugars found and for which data is presented.

Table 3.1

Retention times relative to sucrose of the sugars found and for which data is presented. Sucrose had a retention time of 25.61 minutes.

Compound	Relative Retention Time
Fructose	0.38
α Glucose	0.48
Hexitols	0.54
β Glucose	0.59
Sucrose	1.00

Under the conditions and temperature programme used in the gas-liquid chromatography analyses, hexitols produced a single peak. As several of the plant species tested had a large peak for hexitol, samples were analysed enzymatically for sorbitol. Subsequent comparison of the amounts of sorbitol found by enzymatic analyses and gas-liquid chromatography results revealed that sorbitol accounted for about 90% of the hexitol peak area produced on the gas-liquid chromatography traces.

Sorbitol was measured enzymatically by the method of Boehringer (1973) utilising sorbitol dehydrogenase (SDH). In the enzymatic analysis sorbitol was oxidised by nicotinamide adenine dinucleotide (NAD) to fructose.

$$\text{Sorbitol} + \text{NAD}^{+} \rightleftharpoons \text{fructose} + \text{NADH}$$

Under the test conditions employed the equilibrium of the reaction was completely on the fructose and reduced nicotinamide adenine dinucleotide (NADH) side. The amount of NADH formed in the above reaction was equivalent to the amount of sorbitol. The increase of NADH was determined on the basis of its absorption at 340nm.

Sorbitol was extracted from 100mg freeze dried bud samples 3 times with 2ml of water at 80°C for 5 minutes. Extracts were filtered and made up to 10ml and de-proteinised with ice cold IN perchloric acid in a ratio of 1 + 1 and centrifuged at 3,900g at 4°C for 15 minutes.

Into a 1cm light path glass cuvette was pipetted 2.50ml 0.1 M pyrophosphate buffer pH 9.5, 0.10ml 30mM NAD solution and 0.20ml sample extract (diluted as necessary). The assay temperature was 25°C and the absorbance measured against air. The above solution was mixed and the absorbance read (E_1) at 340nm wavelength. 0.05ml of SDH (4mg protein/ml) was added. The reaction had stopped after 70mins., therefore the absorbance (E_2) was read again at this time. Concentration of sorbitol was calculated as

$$c = E \times 0.417$$

where c = concentration of sorbitol

E = change in absorbance i.e. $E_2 - E_1$

0.417 = extinction coefficient for NADH at 340nm wavelength.

Unfortunately there was a major fault in the design of this experiment. Insufficient plant tissue material was retained for carbohydrate analyses. Therefore, all control buds and desiccation treatment buds were analysed once only. There was not enough material for replicates to be assayed. This greatly decreases the value of the results obtained in this experiment. Notwithstanding the lack of replicates the results were interesting and warrant inclusion in this thesis.

Figures 3.13 - 3.18 show the change in relative water content in the buds of 6 species which were subjected to the standard desiccation treatment. Differences can be seen in the rate of decrease of water content with time. In Q. robur (Figure 3.14) and C. betulus (Figure 3.13) buds, the relative water content had dropped to 11% and 16% respectively within 1 week. The water content values for S. aucuparia buds had also decreased to a low value, to 19%, within 1 week (Figure 3.15). The relative water content of A. incana, S. aria and S. intermedia buds

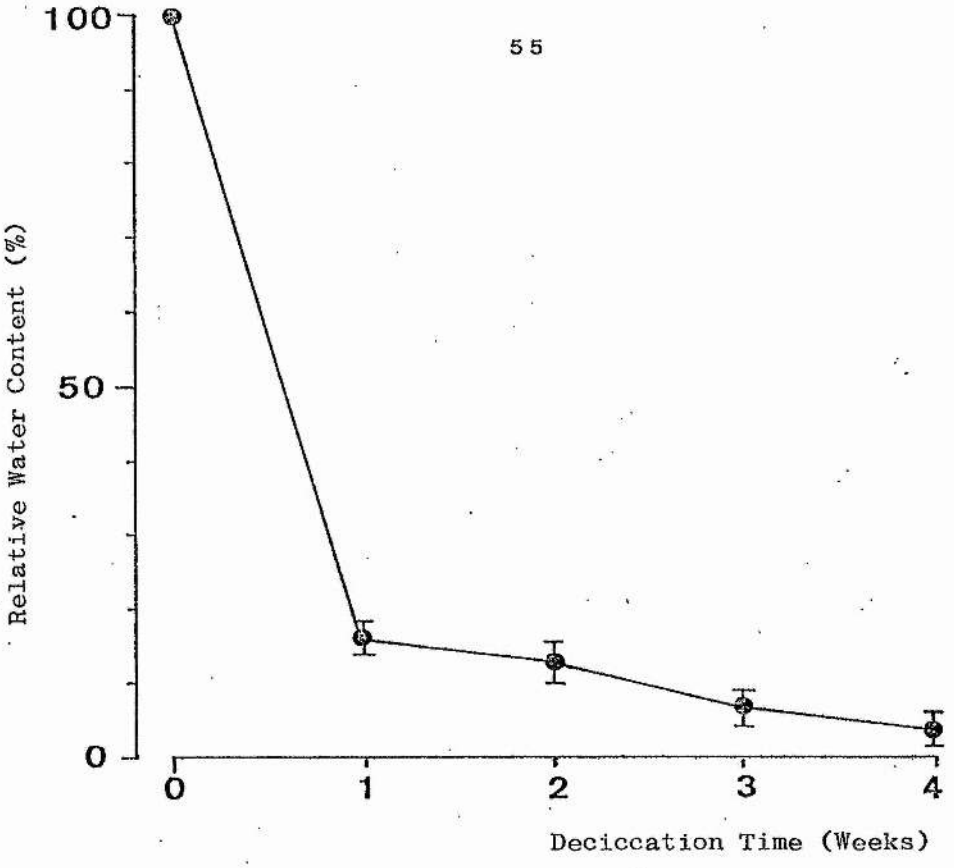


Figure 3.13 Decrease in relative water content of C. betulus buds with length of desiccation treatment time. Vertical lines represent standard errors of the means.

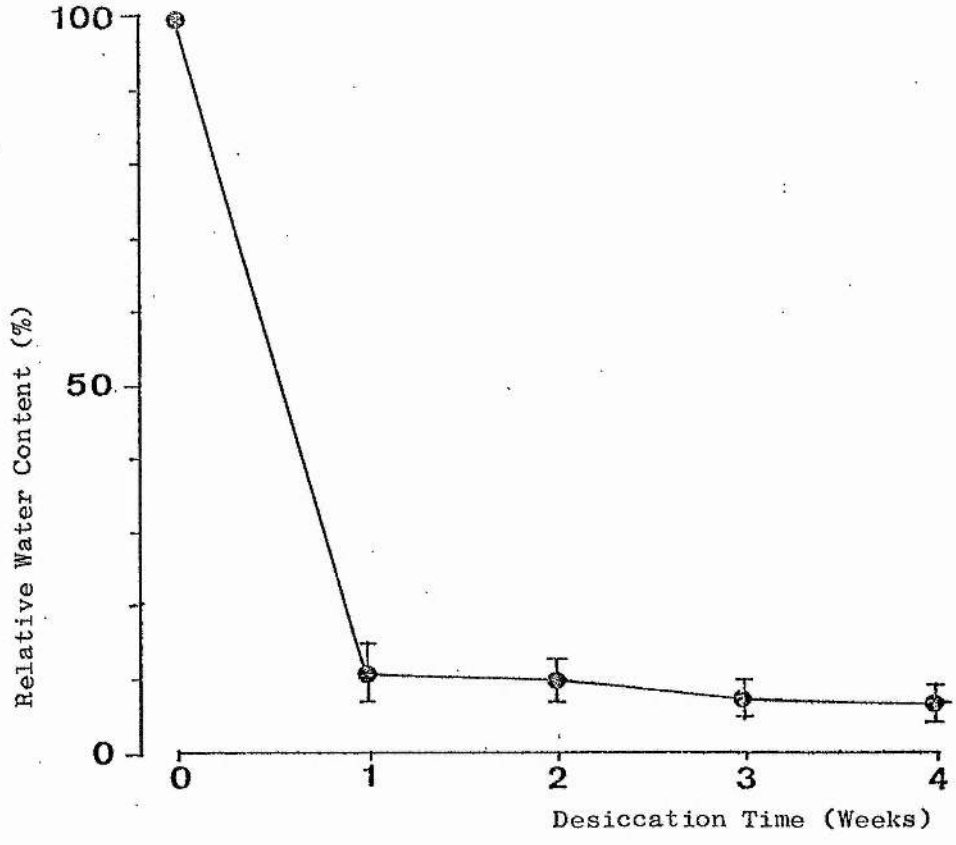


Figure 3.14 Decrease in relative water content of Q. robur buds with length of desiccation treatment time. Vertical lines represent standard errors of the means.

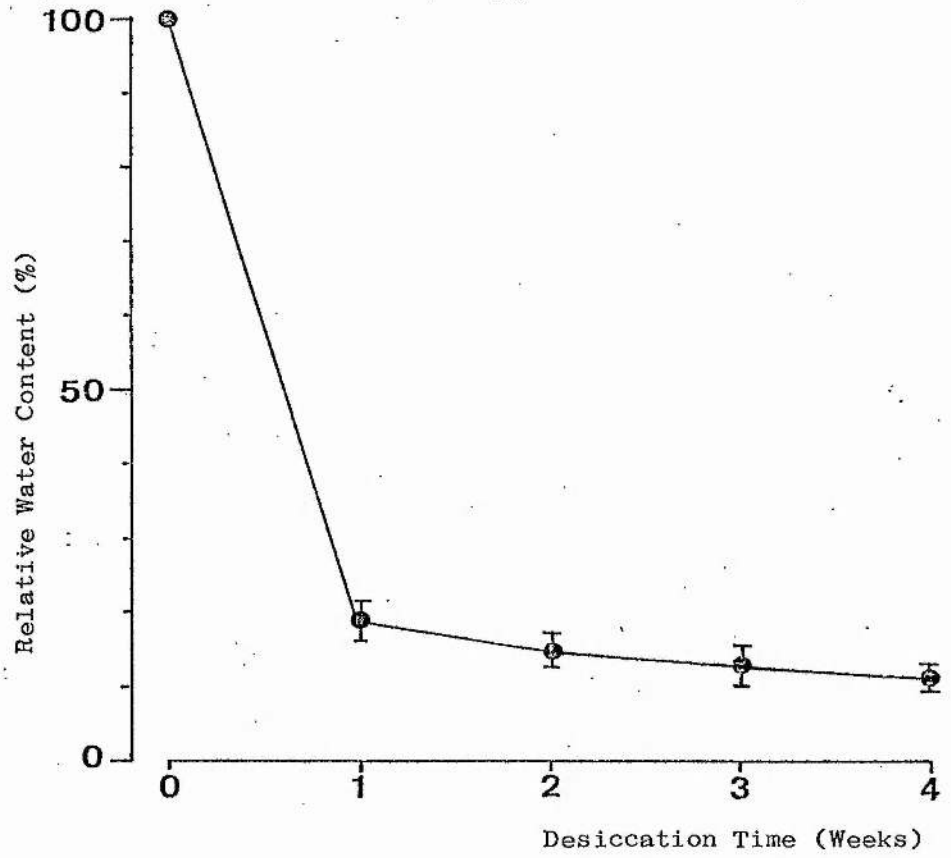


Figure 3.15 Decrease in relative water content of S. aucuparia buds with length of desiccation treatment time. Vertical lines represent standard errors of the means.

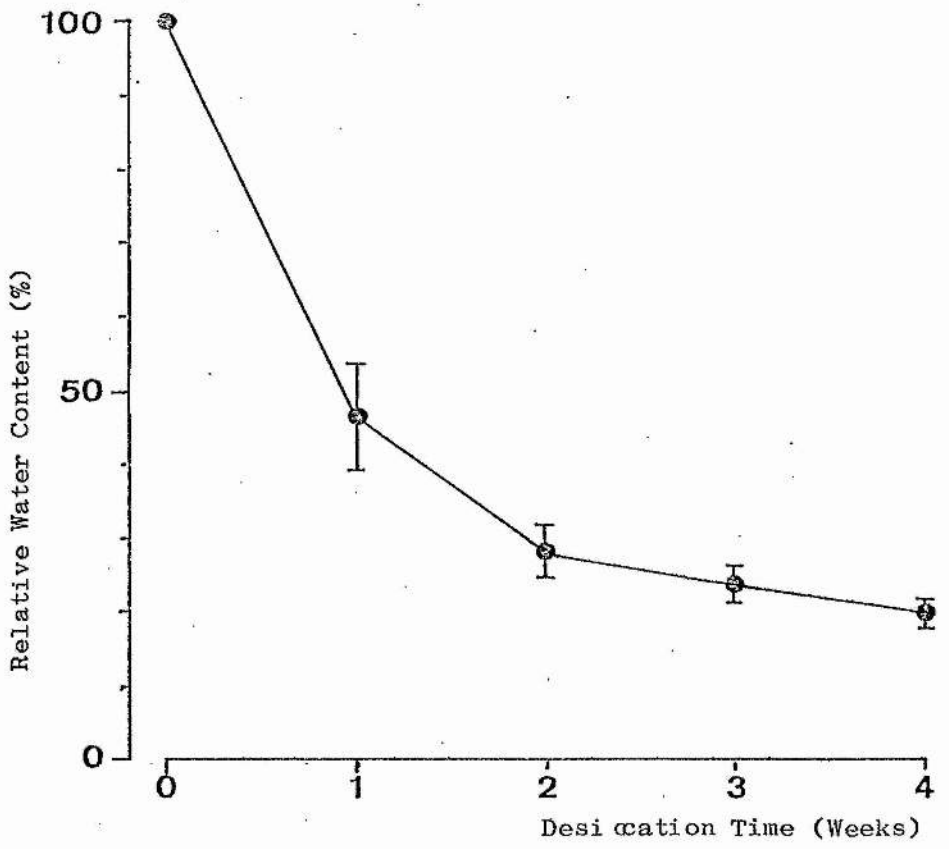


Figure 3.16 Decrease in relative water content of A. incana buds with length of desiccation treatment time. Vertical lines represent standard errors of the means.

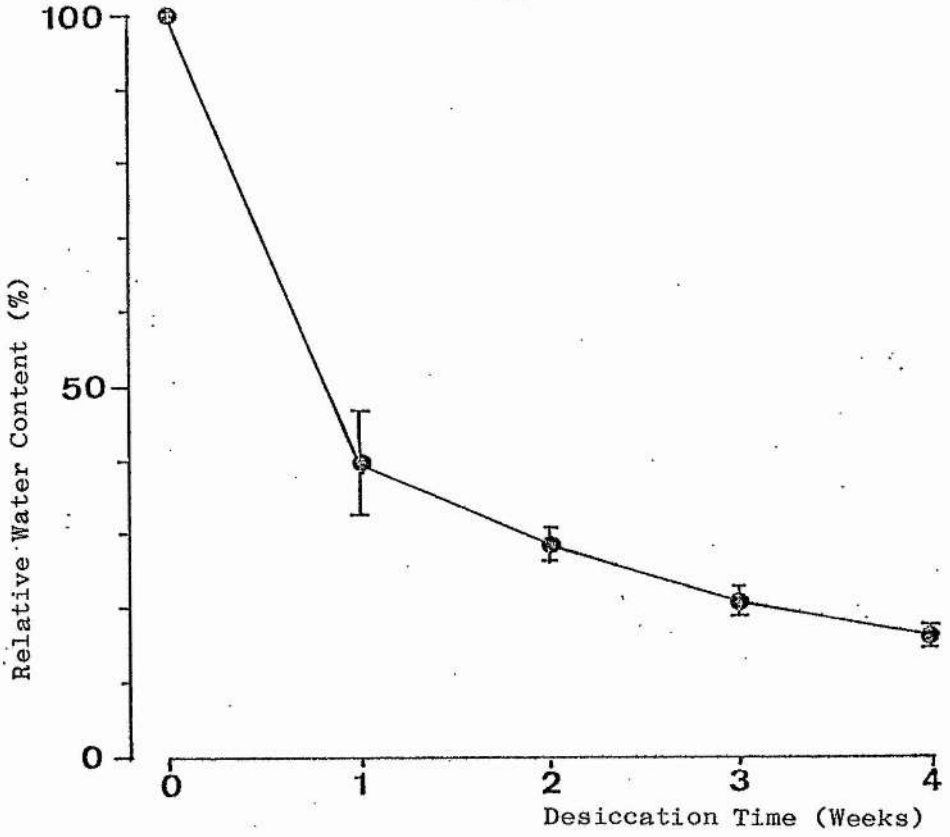


Figure 3.17 Decrease in relative water content of S. aria buds with length of desiccation treatment time. Vertical lines represent standard errors of the means.

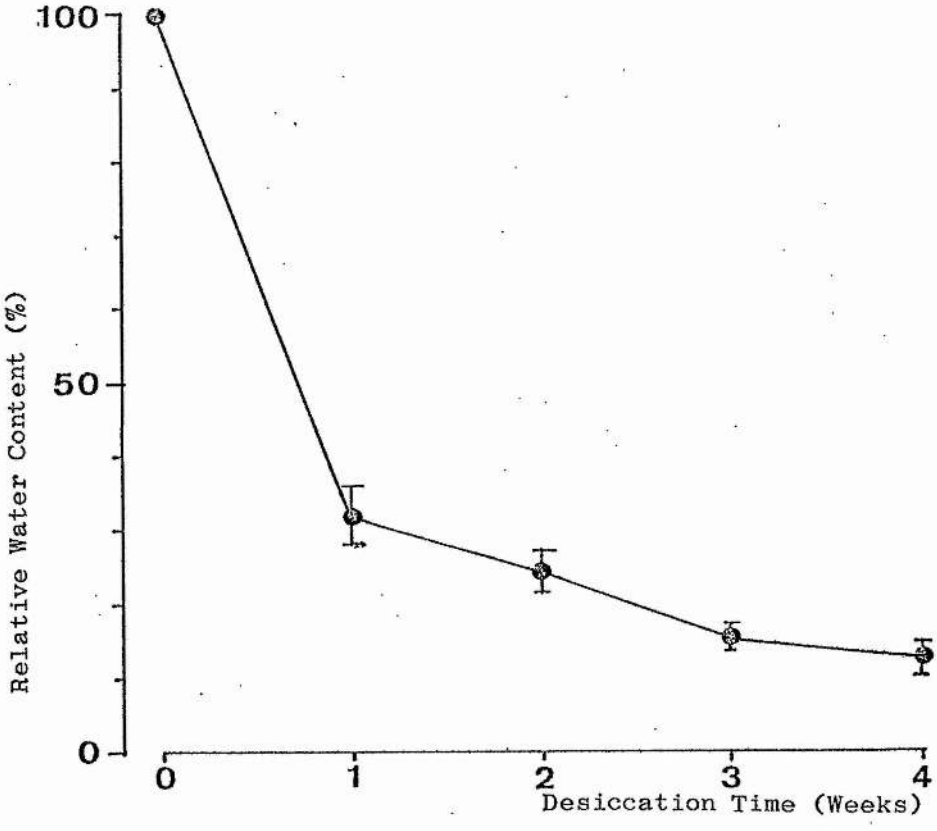


Figure 3.18 Decrease in relative water content of S. intermedia buds with length of desiccation treatment time. Vertical lines represent standard errors of the means.

decreased more slowly (Figures 3.16, 3.17 and 3.18). After 1 week desiccation treatment water content in the buds of these 3 species were 47%, 39% and 32% respectively.

Figures 3.19 - 3.24 illustrate the sugars found. Quantitative results are given for sucrose, glucose and fructose in all six species. In addition, data for sorbitol is shown. This sugar alcohol was found in the Sorbus species only. Values are given for buds desiccated for 1, 2, 3 and 4 weeks as well as control buds collected fresh and analysed at time = 0 and 4 weeks.

Sugar concentrations in S. aucuparia buds are illustrated in Figure 3.19. In the control buds, the concentration of sucrose increased from 8mg/g dry weight to 19mg/g dry weight whilst concentration of sorbitol glucose and fructose remained at similar levels. In the buds subject to desiccation treatment, sucrose levels were higher compared to the controls although the sample with 4 weeks desiccation treatment did not show this trend. The amount of sorbitol in desiccated buds was similar to the controls. Both glucose and fructose concentrations were much reduced in the treated buds compared to the controls, showing a decrease from 3-4mg/g to 1-2mg/g.

Sugar levels of S. aria buds are shown in Figure 3.20. Control buds showed no change in sucrose concentrations over the 4 week period, whilst the treated buds exhibited an increase in sucrose levels from 17mg/g to 22-26mg/g. There was a slight decrease in sorbitol concentrations of both control and treated buds from 16mg/g to 14mg/g. Both glucose and fructose levels decreased in the control buds. After 4 weeks desiccation treatment, glucose and fructose concentrations were only slightly lower in the treated buds in comparison to the control buds at that time.

Figure 3.21 illustrates the results of the carbohydrate analyses for S. intermedia. Sucrose levels increase in the treated buds from 20mg/g

to 31mg/g. This, however, is paralled in the control sample. Sorbitol concentrations remain at the same level throughout the experiment, both in control and treated buds. The amount of glucose in the control buds decreases from 6.5mg/g to 4mg/g. In the desiccated tissues although there is an initial large decrease in glucose concentration, after 4 weeks desiccation the glucose levels are similar to the control. Fructose levels increased in both control and treated buds.

Concentrations of sugars in C. betulus buds are depicted in Figure 3.22. Sucrose concentrations increase in the control buds from 21mg/g to 40mg/g over the 4 week period. This increase in the amount of sucrose is, however, exceeded in the desiccated buds whose concentration ranges between 43 and 55mg/g. A large decrease in glucose concentration is evident under desiccation stress as levels decrease from 6mg/g to 1mg/g, whilst glucose levels in the control buds remain at about 5.5mg/g. The amount of fructose decreases from 10mg/g to 8.5mg/g in the control samples. This contrasts with a large decrease of fructose in desiccated buds from 10mg/g to about 3mg/g.

A. incana sugar concentrations are illustrated in Figure 3.23. Sucrose concentrations increase in both control and treated buds although the largest increase is shown in the control buds. Glucose levels remain constant at 3.5mg/g over the 4 week period in the control buds. This contrasts with a decrease from 3.5mg/g to 2mg glucose/g in the desiccation stressed buds. In the control samples the fructose levels decreased to 1.5mg/g from 2.5mg/g. By comparison fructose concentrations decreased to the low value of 0.5mg/g in the desiccated buds.

Figure 3.24 illustrates the amount of sugar found in Q. robur buds. A small increase in sucrose levels is evident in control samples over the 4 week period. It is hard to draw any conclusions as to the effect of desiccation on sucrose concentrations in this species, as the results

show a variable level of this sugar although levels are approximately similar to controls. Glucose concentrations remain steady at 1-2mg/g in both control and treated samples. Fructose levels remained the same in both control and treated buds.

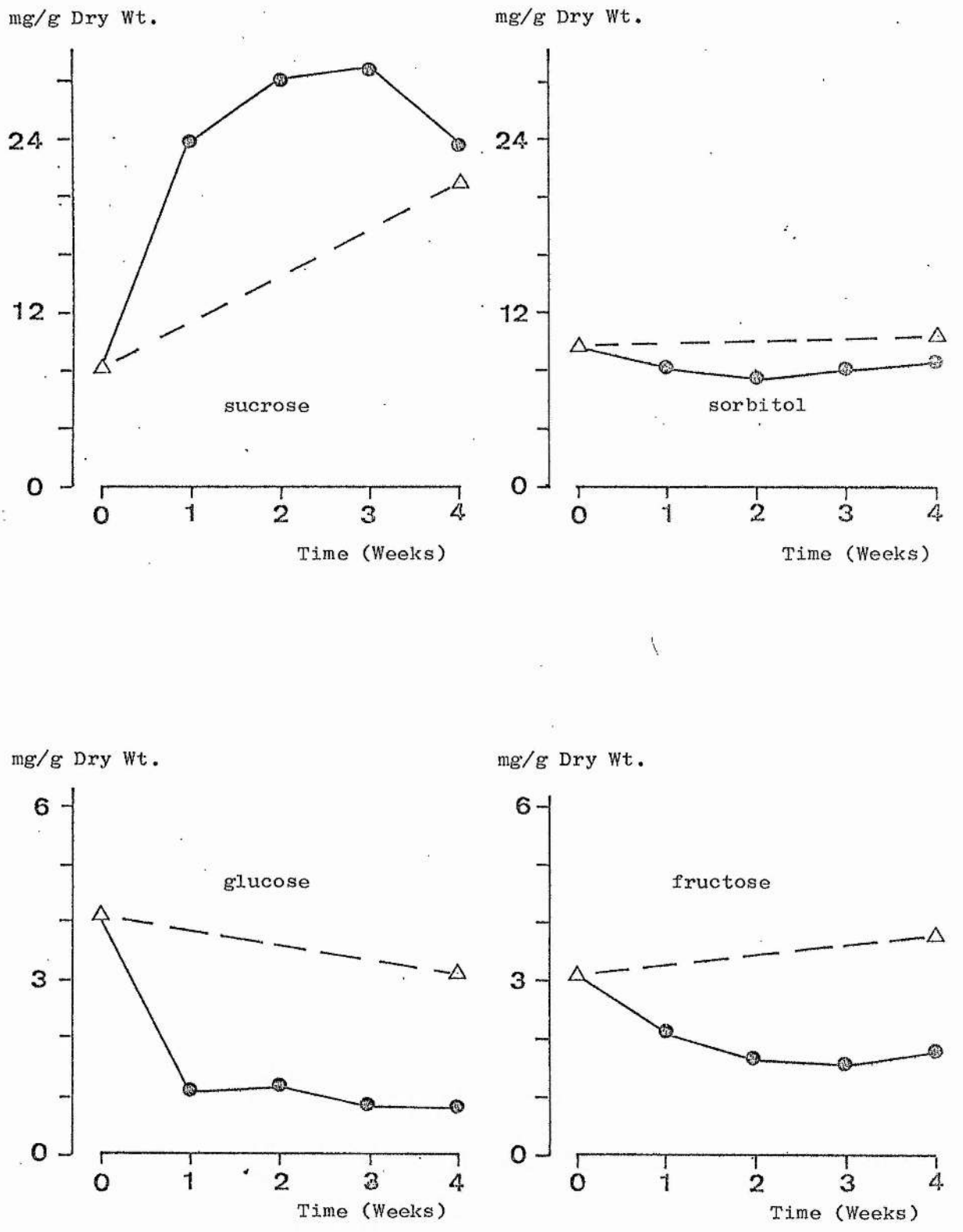


Figure 3.19 Sugar concentrations (mg sugar/g Dry Weight) in S. aucuparia buds with length of desiccation treatment time. Control buds △– — △ and desiccated buds ●——●

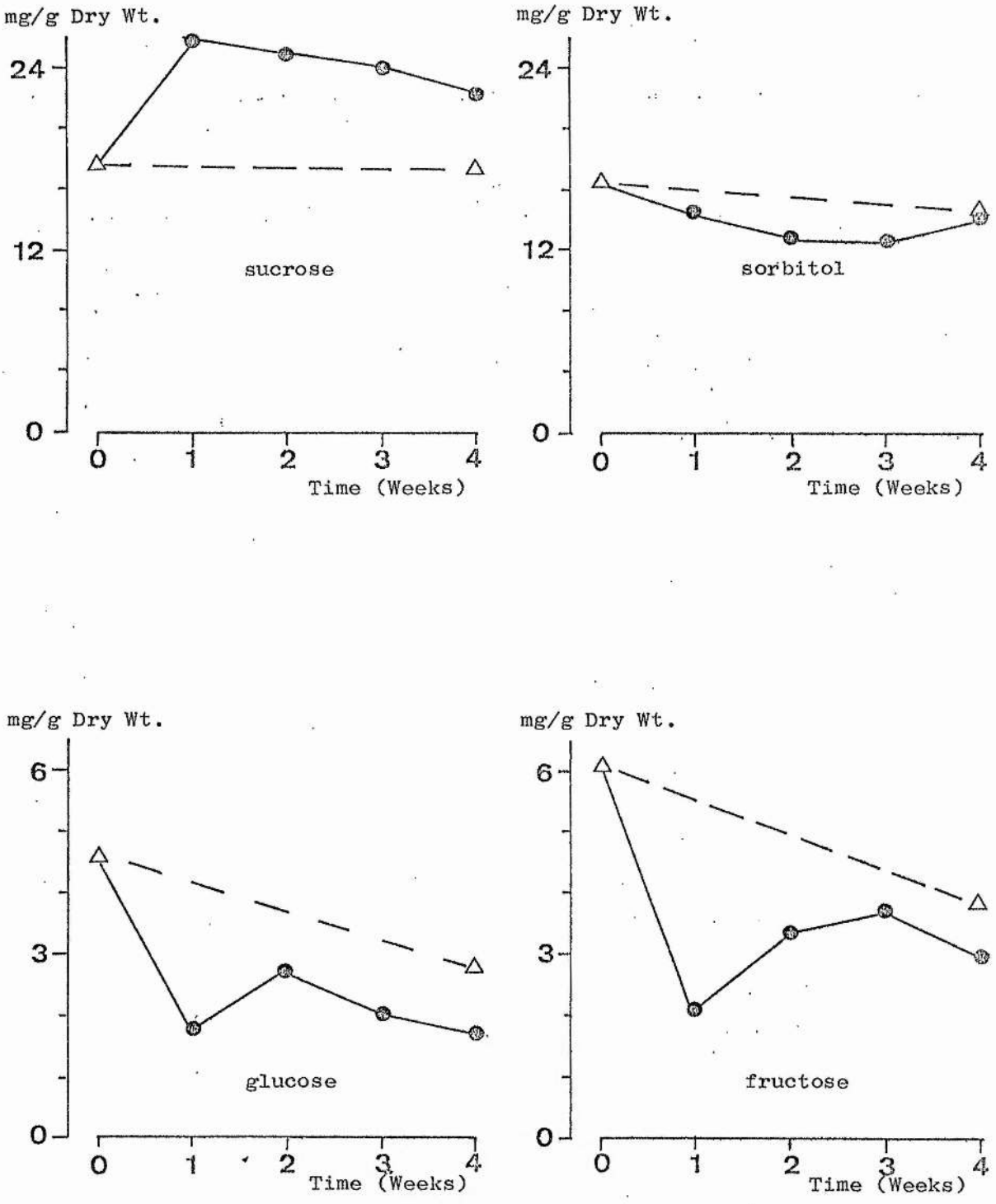


Figure 3.20 Sugar concentrations (mg sugar/g Dry Weight) in S. aria buds with length of desiccation treatment time. Control buds △—△ and desiccated buds ●—●

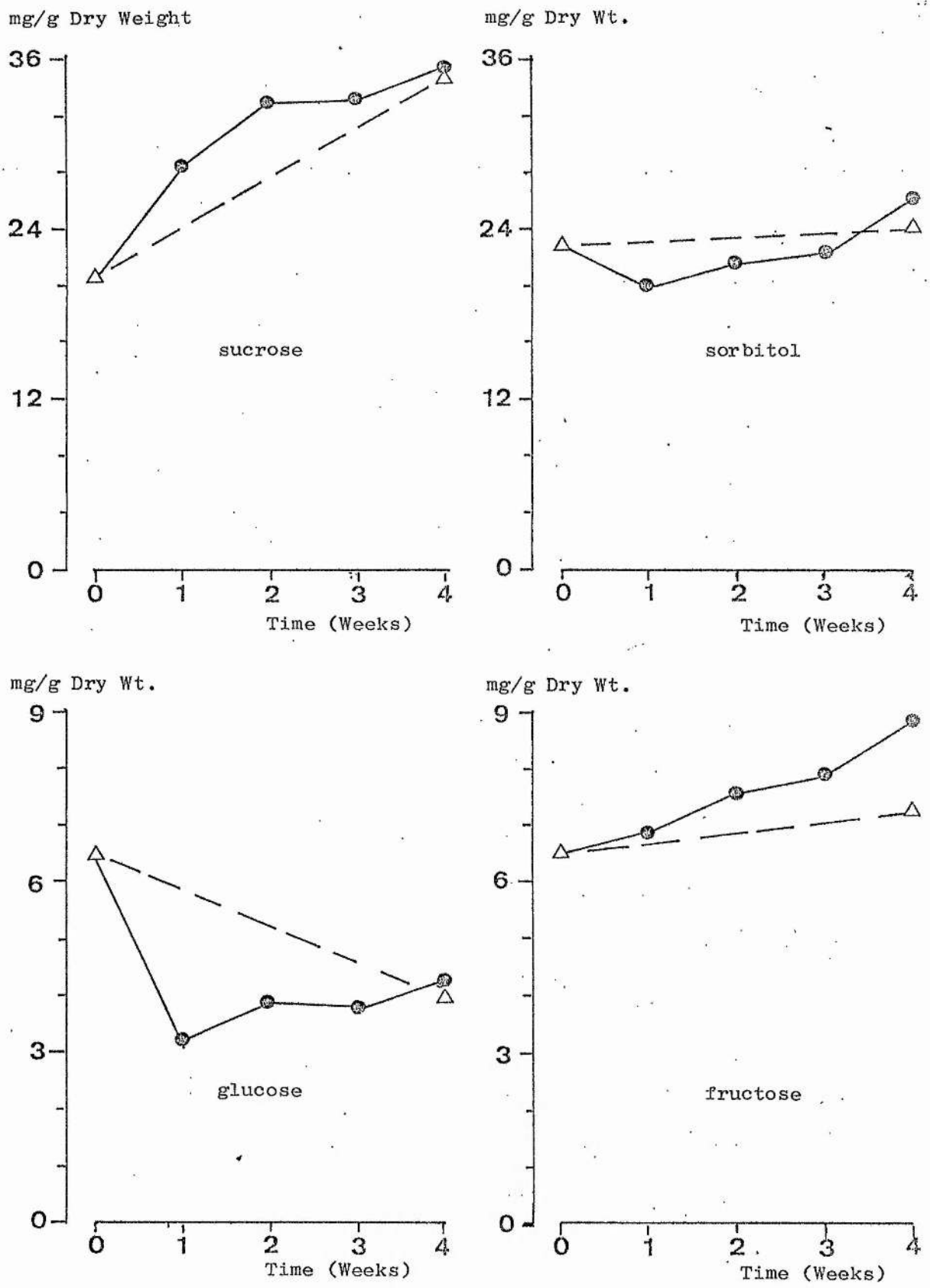


Figure 3.21 Sugar concentrations (mg sugar/g Dry Weight) in S. intermedia buds with length of desiccation treatment time. Control buds △— —△ and desiccated buds ●——●

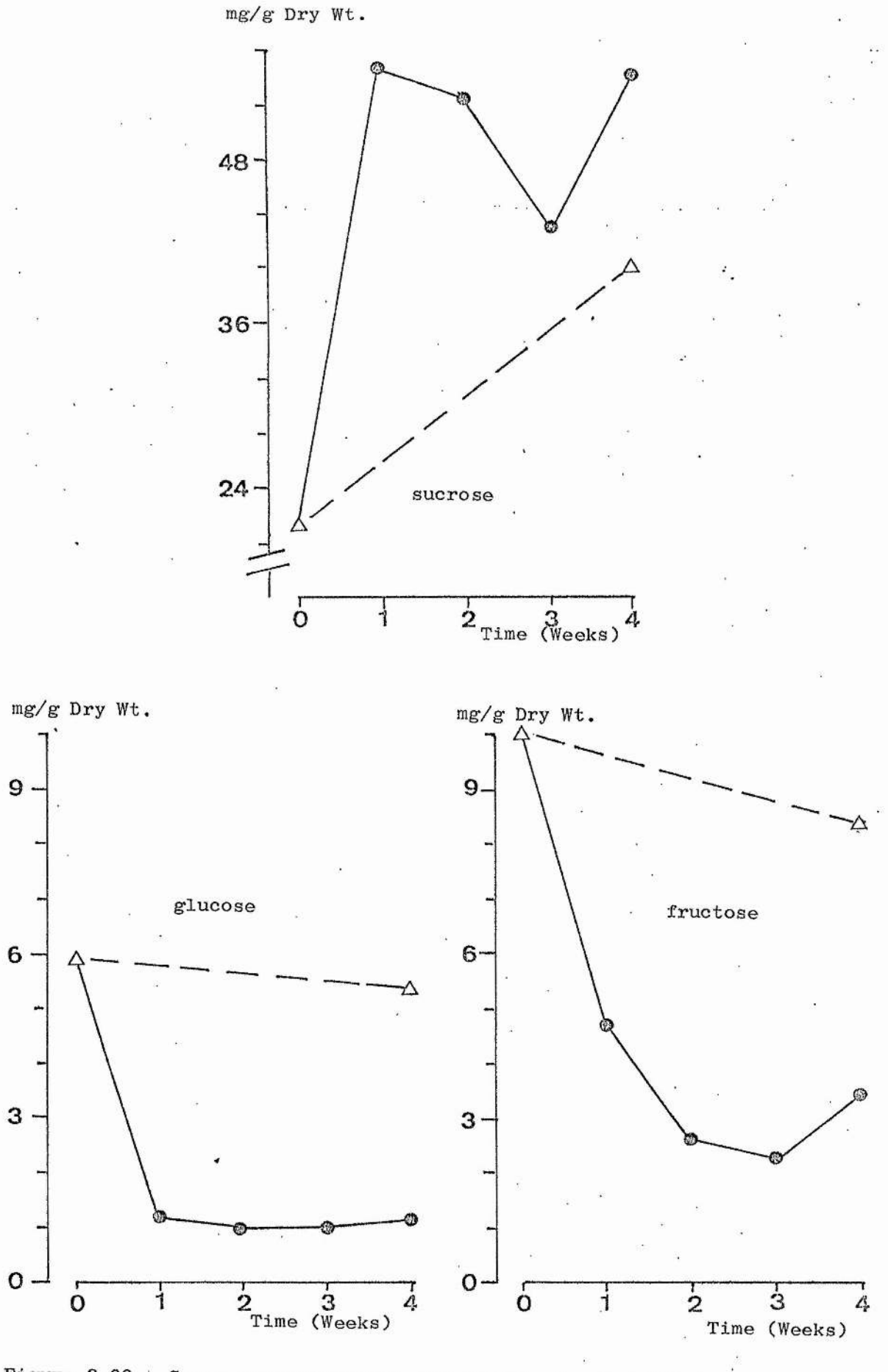


Figure 3.22 Sugar concentrations (mg sugar/g Dry Weight) in C. betulus buds with length of desiccation treatment time. Control buds △— —△ and desiccated buds ●——●

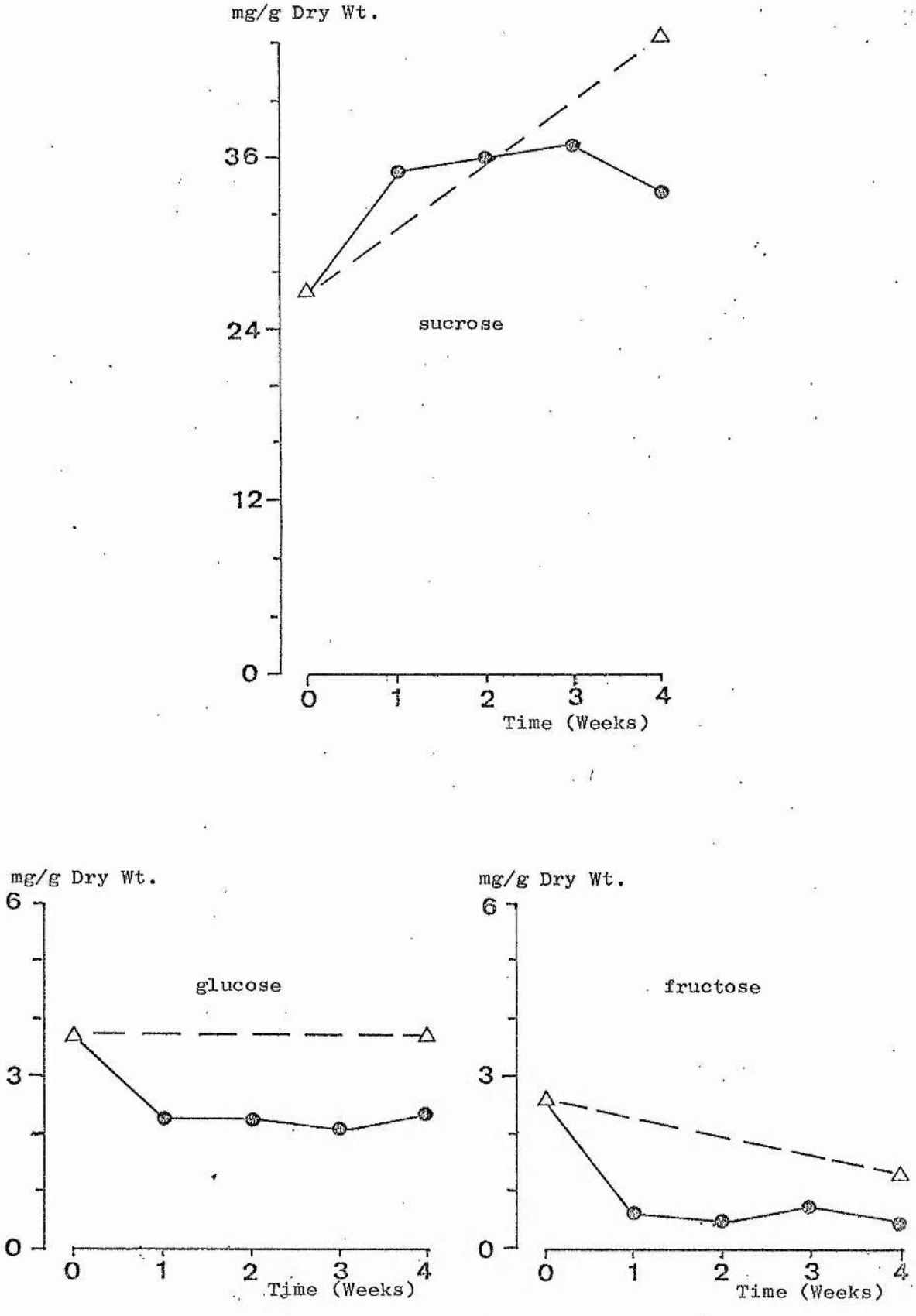


Figure 3.23 Sugar concentrations (mg sugar/g Dry Weight) in A. incana buds with length of desiccation treatment time. Control buds △— —△ and desiccated buds ●——●

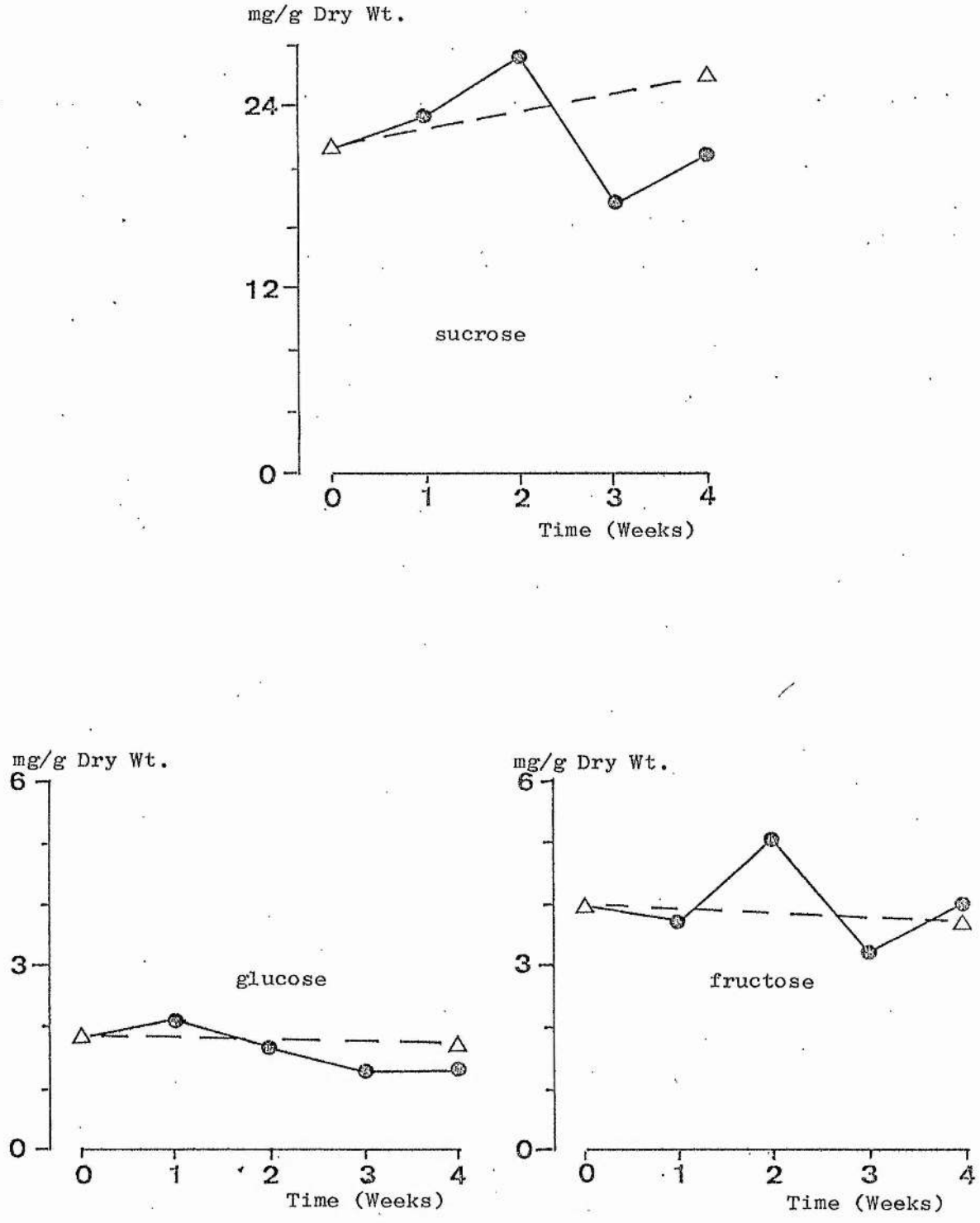


Figure 3.24 Sugar concentrations (mg sugar/g Dry Weight) in Q. robur buds with length of desiccation treatment time. Control buds △— —△ and desiccated buds ●——●

3.5 Discussion

A comparison has been made of the decrease in relative water content and the change in viability of the plant tissues under desiccation stress between the 6 species tested. It is apparent that at high water deficits, the Sorbus species, and to a lesser extent A. incana, can maintain a higher value for viability than can be explained by their slightly slower drying rates.

A decrease in tetrazolium reduction to the formazan derivative is not necessarily due to the inactivation of the dehydrogenase enzyme systems. Lack of tetrazolium reduction may be due to co-factor and, substrate limitations. This could be due to inactivation of other enzymes necessary for synthesis of the co-factors and substrates (Steponkus and Lanphear 1967). Furthermore, desiccation stress is known to have a disruptive effect on membrane systems (Simon and Raja Harun 1972).

No attempt has been made to correlate the amount of tetrazolium reduction compatable with the ability of the buds and twigs to survive. Steponkus and Lanphear (1967), however, found a high correlation between tetrazolium reduction and survival in stems and leaves of a woody plant Hedera helix, after cold injury. The best correlation with the viability of seeds has been found to be the activity of enzymes reacting with redox dyes such as tetrazolium (Mayer and Poljakoff-Mayber 1975).

The ability therefore of Sorbus buds and twigs to convert tetrazolium to the formazan derivative is probably a real indication of these buds and twigs to tolerate desiccation.

The production of ethylene in tree buds was disrupted when the buds were desiccated. A. incana buds alone produced this plant growth regulator at a steady rate to high water deficits i.e. a homeostasis of ethylene production when subjected to desiccation stress. Ethylene can have a

powerful effect on many plant processes e.g. Enzyme synthesis, respiration, disease susceptibility and pigmentation (Abeles 1973).

Here I think we may have a similar situation as Jackson et al (1978) found with an environmental stress affecting the shoot apex of tomato plants. They discovered that placing the roots in an anaerobic environment increased the rate of ethylene produced by the shoot apex.

The ability to regulate ethylene production under stress may be one factor allowing A. incana to grow in low temperature environments. There it would undoubtably be subject to physiological drought stress.

Some trends are evident in the sugar analyses of buds subjected to desiccation treatment. Sucrose concentrations increased initially in the buds of all species tested except Q. robur. Again with the exception of Q. robur, all bud glucose concentrations decreased initially with desiccation stress. S. intermedia buds, however, then showed an increased level of glucose, and similar levels of this sugar were found in desiccated buds as in the control buds at the fourth week.

The sugar concentrations in treated buds of Q. robur were approximately similar to the control buds. In this species, water content decreased at a fast rate (Figure 3.14). Viability studies of desiccated buds of this species indicated that the buds lost their viability rapidly when desiccated (Figure 3.1). It is possible that the buds were killed quickly on desiccation before changes in sugar concentrations could take place.

Paradoxically, there was little difference in the various sugar concentrations of desiccated and control buds of S. intermedia (Figure 3.21). In this species, however, buds maintained a high value for viability when desiccated (Figure 3.3), in direct contrast to Q. robur buds. Sugar levels in S. intermedia buds may have been maintained under desiccation stress by the ability of life processes to continue at high water deficits.

It has been proposed that sugars can help protect higher plants against drought stress (Parker 1969). Sugars usually increase in droughted

plants as a result of increased amylase activity (Vaadia et al 1961). When sugars do not increase, injury may have taken place preventing such a change (Levitt 1956).

No increase in sugar concentrations were found in S. intermedia buds in the experiment conducted here, although this species was very successful in maintaining viability when severely drought stressed (as measured by T.T.C. reducing ability). Both S. aria and S. aucuparia, 2 species also capable of retaining a high value for viability of buds when desiccated, increased their sucrose levels under stress. These species, however, showed decreased levels of glucose and fructose. However, only monosaccharides and the disaccharide sucrose were assayed. Increased levels of other disaccharides and trisaccharides may have taken place. No evidence is presented here therefore that an increase in sugar concentration helps to protect drought stressed tissues.

PART II

EFFECT OF ALTITUDE ON THE GROWTH AND METABOLISM OF THE ROWAN

CHAPTER 4

GROWTH, DARK RESPIRATION RATES AND GERMINATION

4.1 Climate versus Altitude

A study of the effects of altitude on the growth and metabolism of the rowan allows these processes to be compared under a varying temperature regime.

The latitudinal and longitudinal components of climate in Britain have been monitored in detail and are relatively well understood. In contrast, changes of climate with altitude have been studied little and are less well known, despite the fact that sharper gradients of climatic change occur over short distances with increasing elevation.

Temperature decreases with altitude. The Meteorological Office has adopted a standard lapse rate of 6.0°C per 1000m for mean temperatures. Lapse rates, however, have also been found to vary with latitude, longitude and aspect (Birse 1971; Taylor 1974), type of air mass, time of day, season and year. Furthermore, temperature lapse rates are probably exponential rather than linear and liable to reversals because of their sensitivity to variation in the local topography (Taylor 1976). The length of frost free season decreases generally with altitude. Above 600m minimum temperatures fall below freezing on most winter nights.

The concept of accumulated temperature, however, may be more pertinent to the effects of temperature on the growth of vegetation in respect to altitude. Accumulated temperature is defined by Shellard (1959) as "the integrated excess or deficiency of temperature with reference to a field datum, usually called the base temperature, over an extended period of time". The units used are usually day-degrees Celsius and the period is one year. The method used for calculating the average monthly accumulated temperature ($\bar{D}$) in day degrees above any base (b) was

described by Shellard (1959). It was based on an expression devised by Thom (1954). It is:-

$$\bar{D} = N (\bar{t} - b + l \sqrt{N\sigma_m})$$

where $\bar{D}$ = day degrees

N = number of days in the month

b = base temperature

$\bar{t}$ = the average monthly mean temperature in ^{o}C

l = a parameter obtained from a table of l plotted against h where $h = (\bar{t} - b) (\sqrt{N.\sigma_m})$

σ_m = the standard deviation of the monthly mean temperatures.

The base b is normally the temperature at which plant growth commences i.e. the growth threshold. For temperate crop plants a base of $5.6^{o}C$ is often used. However, for plants growing naturally in low temperature habitats a base several degrees lower would probably be more appropriate.

A complicating factor is the contrasting amplitude of maritime and continental type temperature curves and their seasonal variation with altitude. Figure 4.1 illustrates how the length of the growing season is shortened and temperature intensity weakened with increase in altitude. Changes with altitude are more gradual at the continental site where the greater intensity compensates for shortness of season at all altitudes.

Overlying the change of temperature with altitude, however, are other climatic variables. Gloyne (1967) has shown that windforces become greater and strong winds more persistent with increase in elevation. This has been substantiated by Pears (1967) data relating to the Cairngorms. Pearsall (1950) notes that the summit of Ben Nevis had an average of 261 gales of force greater than 50 m.p.h. compared with the figure of 40 gales at sea level.

Most studies in Britain have shown that average rainfall gradients have an approximately linear increase with altitude (Taylor 1976). Records from the Ben Nevis observatory (Pearsall 1950) show that the summit

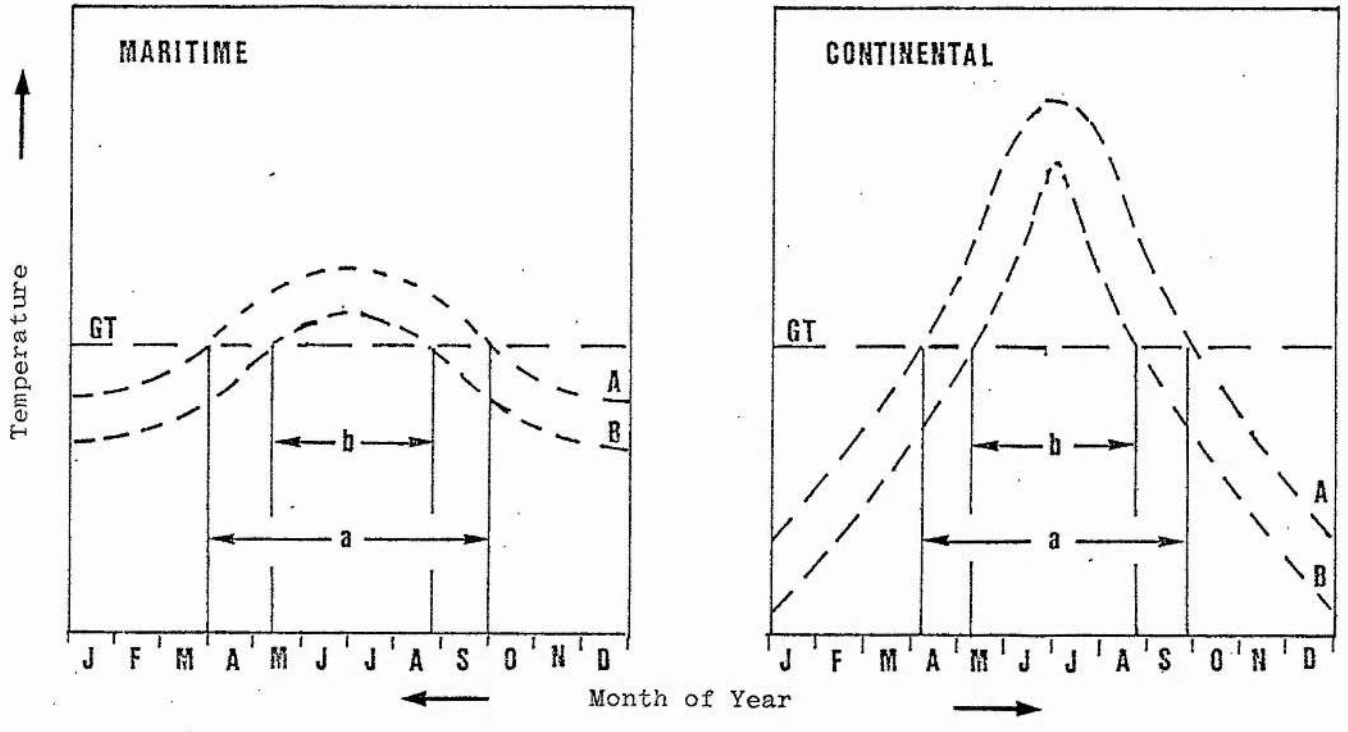


Figure 4.1 A generalised diagram illustrating the difference in the amplitude of annual curves for mean monthly temperatures with an increase in altitude between maritime and continental sites. GT = Growth threshold; A and B = low altitude and high altitude sites; a and b indicate length of growing season. (After Taylor 1976)

of Ben Nevis (1340m) received twice as much rainfall per annum as nearby Fort William at sea level. Increases in humidity and cloudcover also increases with altitude as does snow frequency and intensity.

It must be borne in mind therefore that not only temperature changes with elevation, but also a complex of climatic variables, all of which can affect plant growth.

4.2 Growth Rates

The effect of altitude on growth rates of the rowan was investigated by tree trunk core sampling and the subsequent measurement of radial increments of growth rings. Trees selected for sampling were growing naturally in North Angus and samples were taken during the summer of 1978. Tree trunk cores were taken from single bole trees at a height of 35cm from the ground with a Mattson tree corer no. 5. Diameter of core was 4mm. Altitude of sample was noted and cores were transported to the laboratory and radial increments of annual growth rings measured within the following two days. As the trees were of differing ages and in general, radial increments of growth rings decrease with age of tree (Fritts 1976), it was decided to measure a set number of growth rings from each core starting from the centre of the tree. This allowed a comparison of growth rates between trees of different ages. The cores did not strike the centre of the tree in a small percentage of the samples, therefore the first two growth rings could be difficult to interpret and could easily be overestimated. To overcome this difficulty, length of radial increment from growth ring 2 to 17, i.e. 15 years growth, was measured under a binocular microscope, magnification 50X to 0.5mm for all samples.

The tree wood-core samples were placed into 100m altitude classes and a histogram drawn for growth rate versus altitude of sample. Figure 4.2 illustrates the results for growth rates in North Angus. The unexpectedly sharp decline in growth rate above 500m was not merited by any other outward sign of poor growth. The trees above 500m, although having a much reduced growth rate, were still growing successfully.

It is hard to explain the sharp drop in growth rate at 500m. Many of the higher tree samples were taken from Corrie Fee (N.G.R. No. 250750). This corrie forms a bowl shaped depression and it is possible that the

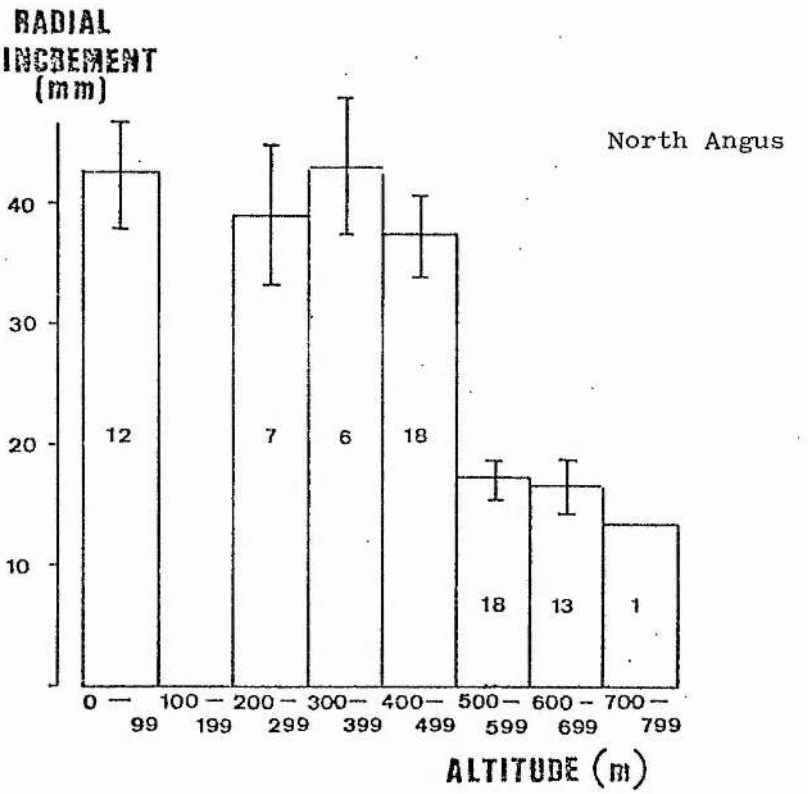


Figure 4.2 Growth rates of S. aucuparia as determined by tree trunk radial increments with respect to altitude of origin in North Angus. Vertical lines indicate standard errors of the means. Numbers superimposed on histogram denote number of samples per altitude class.

growth rate discontinuity could be due to temperature inversions occurring there. Inversions could be formed by cold air flowing down the surrounding hills into the corrie and collecting there. The floor of Corrie Fee is at an altitude of 440m.

An inspection of buds on the 14th May 1979 on rowan trees growing from the floor of the corrie at 440m to the top of the surrounding cliffs at 770m showed that bud break was furthest advanced at the bottom of the corrie. There was an obvious decrease in bud expansion with increase in altitude. This perhaps is evidence against temperature inversions occurring here at least in late springtime. If temperature inversions were a regular occurrence at this time of year it would be expected that bud opening would be retarded at the lower altitudes in the corrie due to lower temperatures. It is possible of course that temperature inversions might occur during the summer, the main growing season, when the greatest affect on growth ring increments would take place.

A further factor which could affect growth rates is the fact that samples were collected from trees growing in different soil types. Low altitude samples were mainly from trees growing at the side of minor roads i.e. in a mixture of soil types. Most high altitude trees were growing on north facing hillsides, parent rock substrate of mica-schist.

To determine if the above was the normal pattern of rowan tree growth with altitude, core samples were taken from another area. The site chosen was on the flanks of Ben Vair, Ballachulish (N.G.R. NN035595) on the west coast of Scotland. This site was on a north facing slope. It was ideal in many respects for looking at changes in growth rates with altitude as rowan trees were growing from sea level, with virtually their roots amongst the seaweed, to about 600m. It must be pointed out that altitudinal tree limits are lower on the west coast of Scotland compared with the east coast. Poore and McVean (1957) relate this difference in

tree limits to the more oceanic climate in the west and hence a shorter growing season there. The contrasting maritime and continental type temperature curves were illustrated in Figure 4.1. The rock substrate at the Ballachulish site is granite.

Figure 4.3 illustrates the same tree core data for Ballachulish as previously shown for North Angus. The results are different from North Angus and do not show a similar pattern. One factor which might obscure a trend due to altitude here is that rowan trees are found growing at higher densities along with birch (Betula pubescens). At lower elevations birch tends to be dominant and at high altitudes rowan is found in greater numbers. Rowan is sensitive to shading (Walter 1968) and when growing at high densities it would be expected that growth rates would be depressed in trees which were shaded.

To conclude, no clear pattern in growth rate as determined by annual growth ring increments versus increase in altitude has been found.

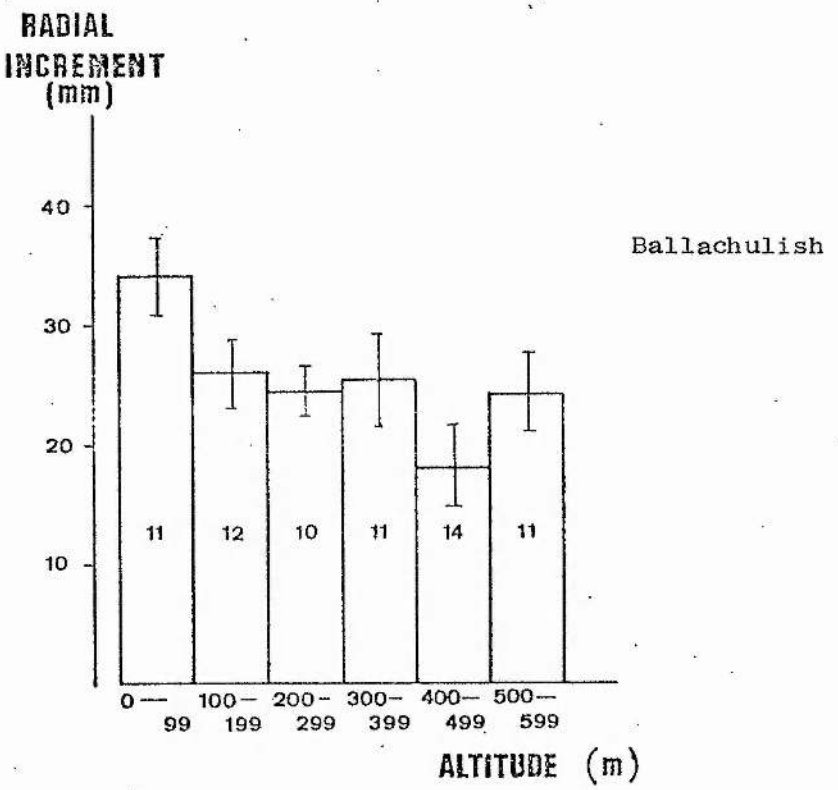


Figure 4.3 Growth rates of S. aucuparia as determined by tree trunk radial increments with respect to altitude of origin at Ballachulish. Vertical lines indicate standard errors of the means. Numbers superimposed on histogram denote number of samples per altitude class.

4.3 Dark Respiration Rates

The dark respiration rates of rowan tree buds were measured. Samples were collected from between 75m and 770m elevation. By measuring dark respiration rates, two questions could be answered.

1. Do rowan bud respiration rates alter to compensate for ambient temperatures found under natural conditions?
2. How do ambient temperatures affect the Q_{10} of dark respiration rates?

Rowan twigs containing buds were collected on the 14 and 16 January 1979 from North Angus. By measuring rates at this time of year it would be expected that the buds would be in the same phenological state. The cut twigs were placed in wetted polythene bags and brought back to the laboratory, the buds excised and respiration rates measured in the Gilson Differential Respirometer immediately. Method as in Chapter 2.1. Rates ($\mu l O_2$/hr/mg Dry weight) were measured at 5°C and 15°C. This allowed the Q_{10} of O_2 uptake to be calculated over this temperature range.

Figures 4.4 and 4.5 are graphs of oxygen uptake versus altitude of origin of bud samples. Points on the graphs are the mean rates of 2 replicates per tree. There is a very high significant linear correlation between respiration rate and altitude of sample. The effect of an increase in altitude on the Q_{10} of bud respiration rates is illustrated in Figure 4.6. There is no correlation between Q_{10} of oxygen uptake and altitude of origin of the sample.

A possible limit to bud respiration rates is the diffusion of oxygen into the bud tissues. From the results portrayed in Figures 4.4 and 4.5 one might predict that diffusion resistance e.g. bud scale thickness, is greatest at low altitudes and declines gradually with increase of elevation. Measurements made of bud scale cuticle thickness, however, made with

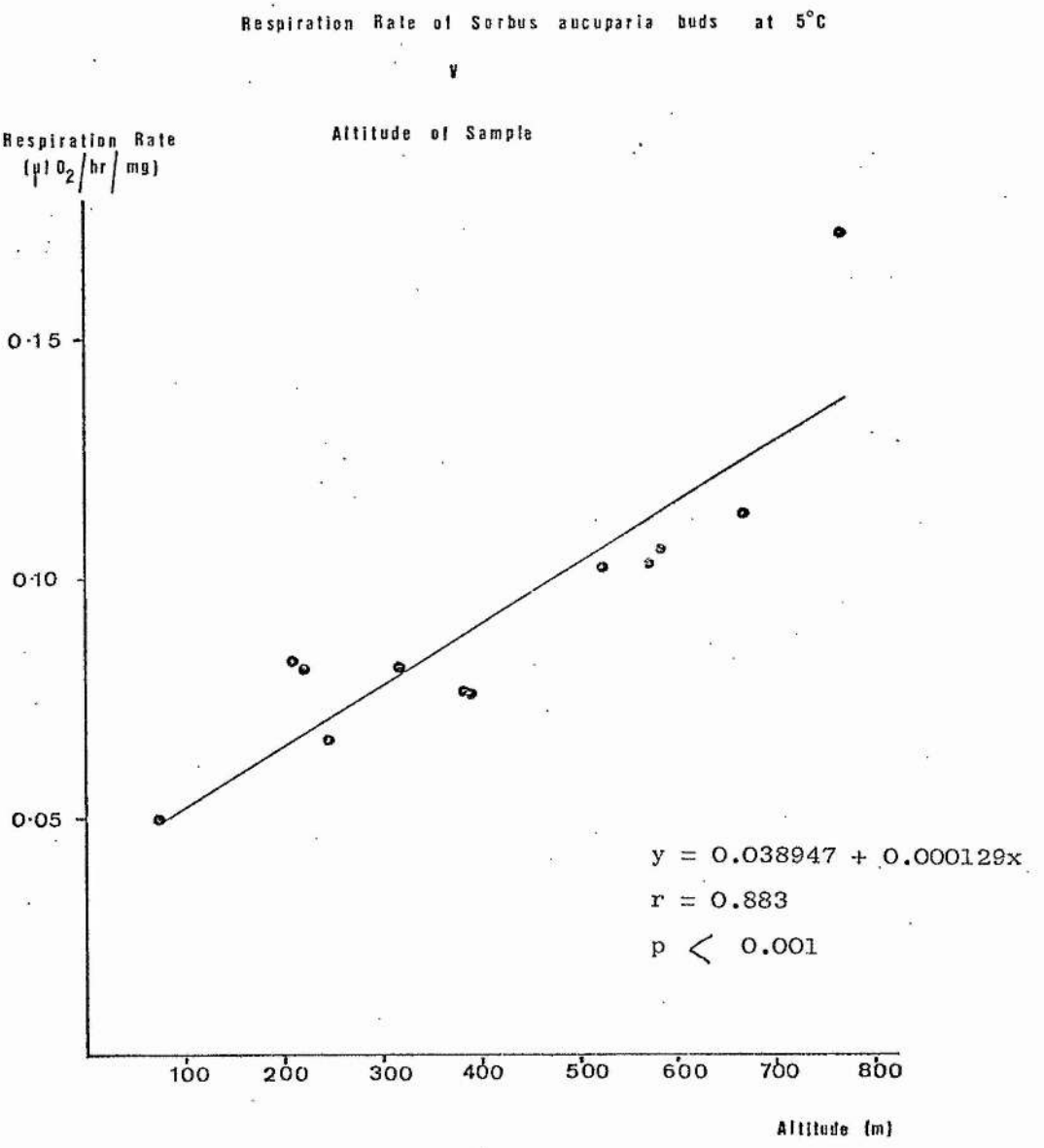


Figure 4.4 Relationship between respiration rate and altitude of origin of S. aucuparia buds. Rate measured at 5°C

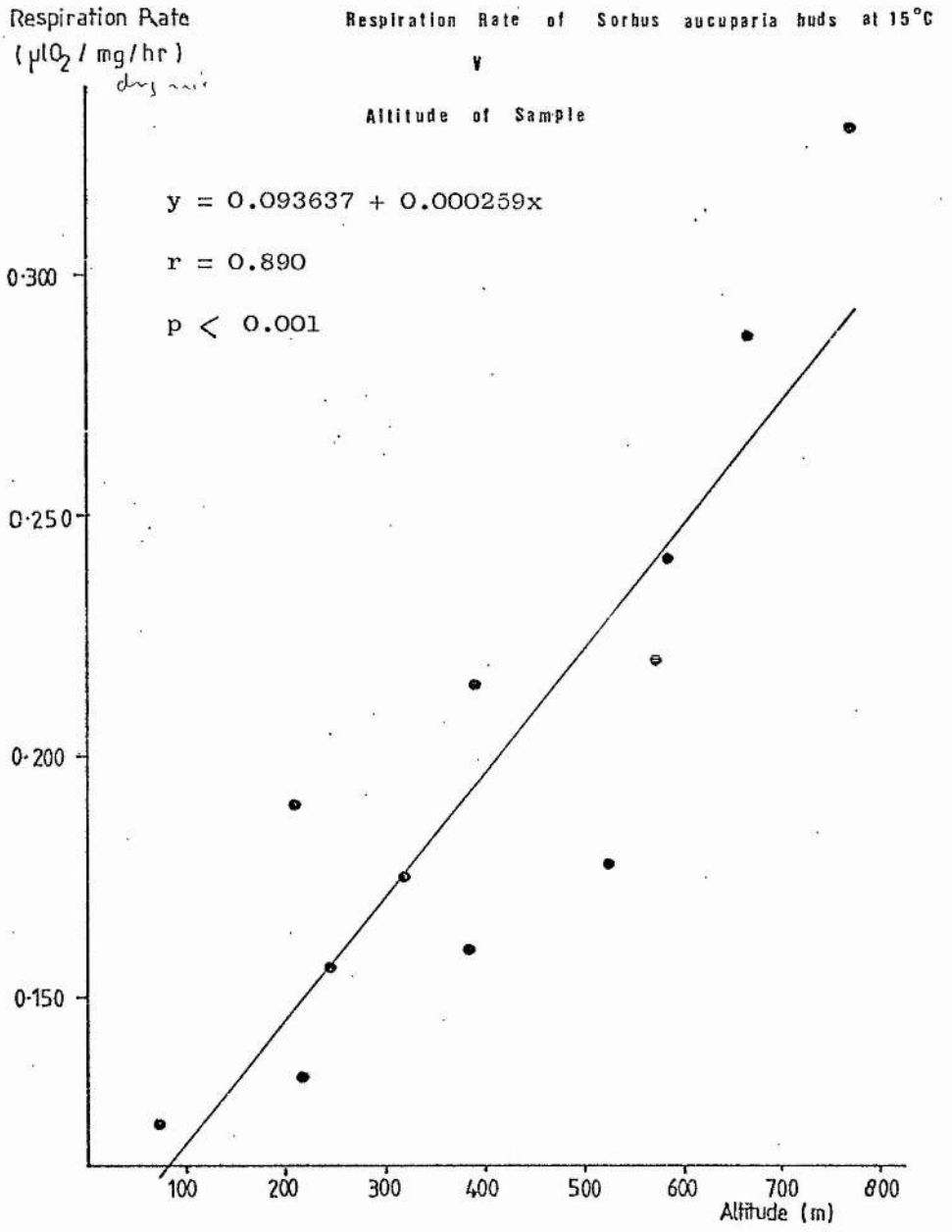


Figure 4.5 Relationship between respiration rate and altitude of origin of S. aucuparia buds. Rate measured at 15°C.

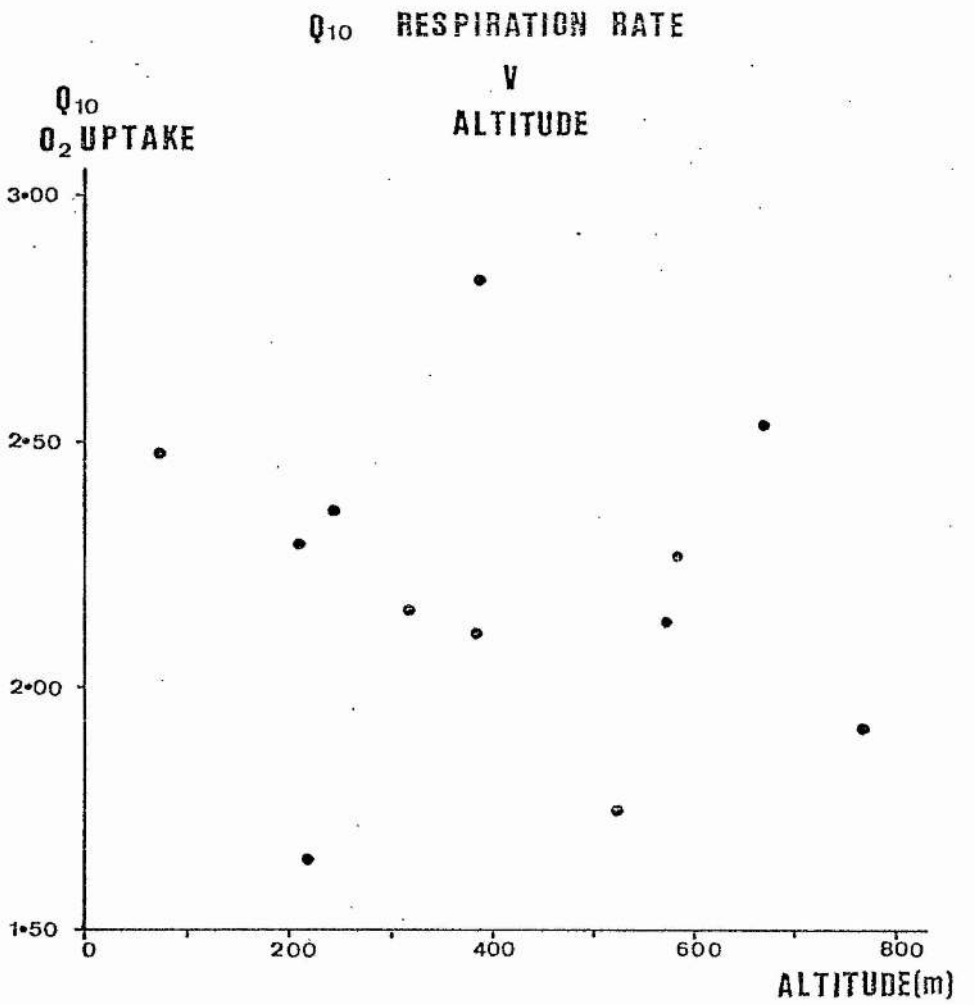


Figure 4.6 Relationship between Q10 of respiration rate (oxygen uptake) and altitude of origin. Rate measured at 5°C and 15°C.

material from another site (Figure 5.6) showed that cuticle thickness only decreased appreciably at the upper tree limit. There was no gradual decrease in cuticle thickness with altitude. Furthermore, as described in Chapter 2.1, when the shaking rate of the Gilson respirometer was increased from 100/min to 140/min no increase in respiration rate was noted with S. aucuparia buds in that experiment. It would appear therefore that the increase of respiration rates with altitude or origin is in fact an intrinsic increase in metabolic rate. There is respiratory rate compensation.

This data complements the results of the experiments on short term rate compensation conducted previously (Chapter 2.2). Adjustment of respiratory rates to suit ambient temperatures would prevent waste of carbohydrate reserves. This is especially useful in a tree which grows over a wide temperature range and is particularly successful in low energy environments.

4.4 Germination of Rowan Seeds

Subjective observations suggested that rowan trees at higher altitudes produced a smaller amount of berries. Could high altitude trees produce viable seeds? In the absence of the production of high altitude viable seeds, populations of rowan trees growing at high altitudes could be perpetuated by bird dispersed seeds from lower elevations. A small scale experiment was designed therefore to ascertain the affect of altitude of origin on the viability of rowan seeds.

Berries were collected at Ballachulish (N.G.R. NNO35595) on the 6 and 7 September 1978. They were collected from 4 trees at each of 5 different elevations. A further sample was collected from a high altitude tree at 580m. The altitudinal limits for rowan at Ballachulish is 600m. Altitude of sample was measured with a temperature compensated surveying aneroid altimeter and was correct to 10m.

After collection, berries were stored in the laboratory in open beakers for 4-5 months. Seeds were then extracted from the berries. In this seed germination experiment, 25 seeds from each tree were placed on wetted filter paper in separate petri dishes (only 20 seeds per tree from site C). Altitude of origin of samples is shown in Table 4.1. The dishes were placed in a refrigerator on the 16 February 1979 for 2 months at 3°C. This is the normal stratification treatment for S. aucuparia seeds (Hulme J.K. perscomm). Filter paper in the petri dishes were kept moist as necessary. No germination took place at this temperature. Dishes were removed to an incubator at 20°C on 17 April 1979.

After the change in temperature regime to 20°C, seeds were initially inspected daily then at longer intervals. Germination was defined by the appearance of the radicle protruding through the seed coat. A limited amount of germination took place within a few days at 20°C.

Table 4.1 Altitude of origin and germination data of S. aucuparia seeds collected at Ballachulish.

Site	Altitude (m)	No. of Seeds	No. of germinated seeds	Percentage Germination
A	10	100	1	1
B	170	100	10	10
C	350	80	6	7.5
D	430	100	14	14
E	520	100	21	21
F	580	25	5	20

The experiment was discontinued on the 25 May 1979 as the rate of germination had declined to a very low value. At this time, seeds had been subject to 38 days at 20°C with a stratification pretreatment of 2 months at 3°C. Figure 4.7 illustrates both amount and rate of germination. The total percentage germination is plotted against altitude of origin in Figure 4.8. There is a highly significant positive correlation between amount of germination and altitude under the conditions applied in this experiment.

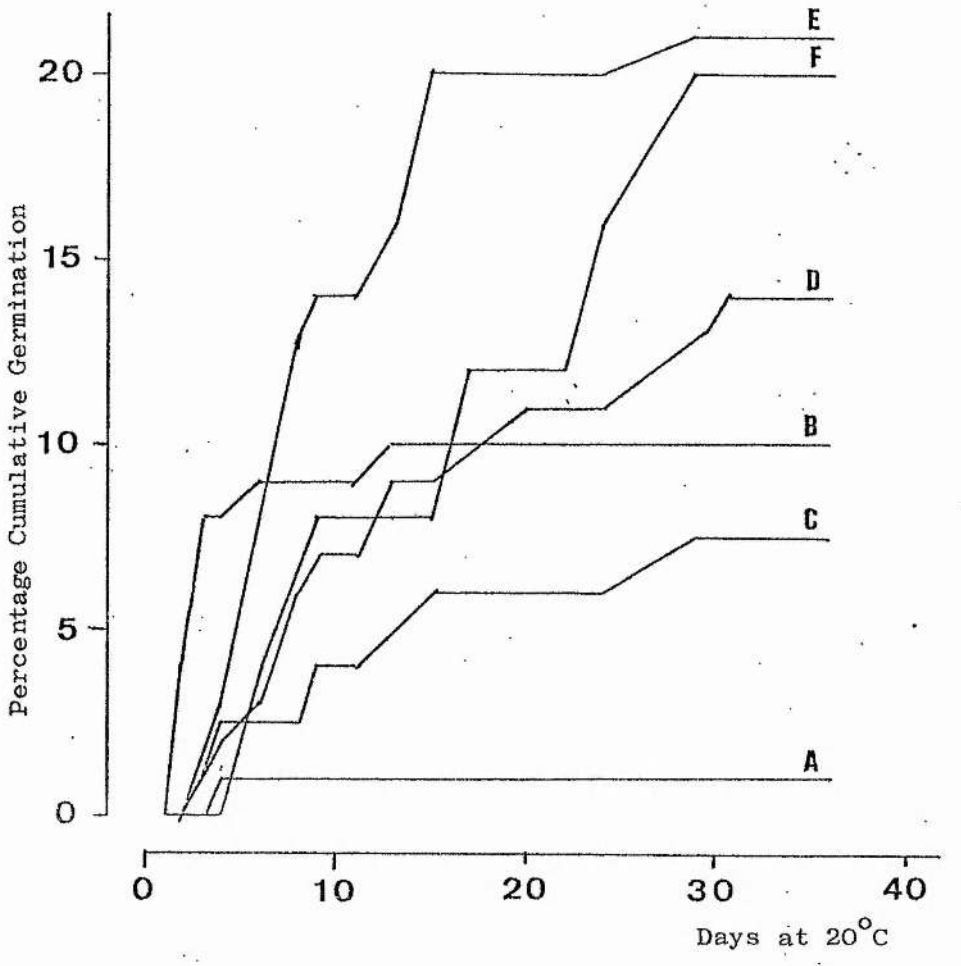


Figure 4.7 Rate and percentage cumulative germination of rowan seeds. Letters superimposed on the graph denote site and altitude of origin of samples. These sites are tabulated in Table 4.1.

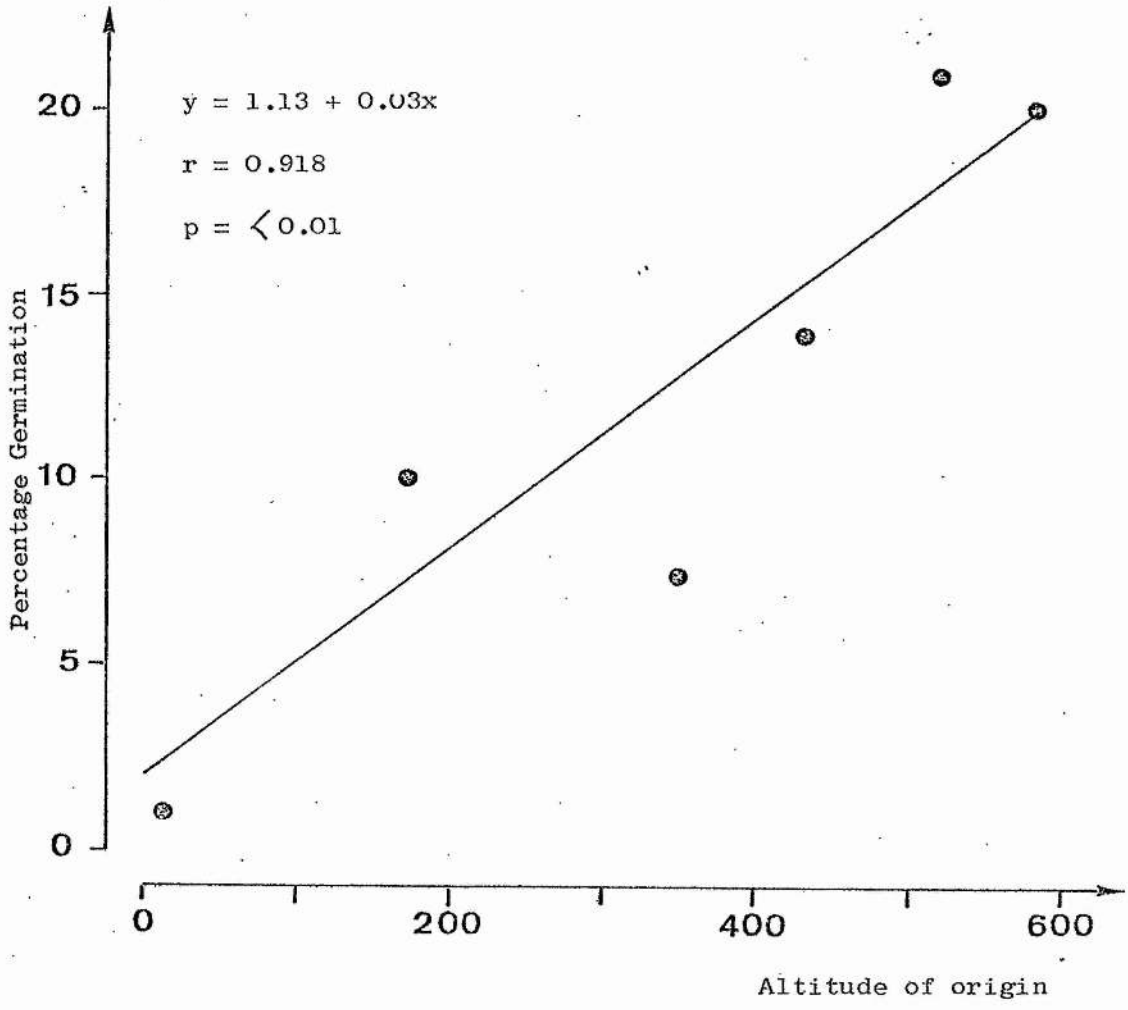


Figure 4.8 Relationship between percentage germination of S. aucuparia seeds and altitude of origin.

CHAPTER 5

WATER RELATION OF ROWAN AND BIRCH BUDS

5.1 Introduction

The research reported in this chapter was prompted by the work of Tranquillini and co-workers carried out in the Austrian Alps and discussed more fully in Chapter 1. They showed that the altitudinal limits of two coniferous species, Pinus cembra and Picea abies was determined by low summer temperatures at high altitudes. Low summer temperatures were responsible for the inability of new shoots and needles to mature sufficiently to withstand the desiccating conditions of winter.

Trees in low temperature habitats which produce mesomorphic leaves shed their leaves in winter. Only extremely xerophytic trees such as many conifers are capable of persisting through the winter frost period in a leafy condition. Deciduous trees enclose their delicate shoot buds during the winter period with a covering of bud scales. These scales can make an effective barrier against desiccation.

The aim of the work carried out here was to find:-

1. The extent of desiccation stress suffered by S. aucuparia buds in winter.
2. The effect of altitude on desiccation of buds.
3. The effect of altitude (and hence summer temperatures) on the maturation of bud scales.

The parameters investigated included monitoring the water content of buds in the field over a six month winter period as well as measuring the change in relative water content of buds under desiccating conditions in the laboratory. Number of bud scales per bud and cuticle thickness of bud scales were also measured.

The area chosen for this research was as in some previous experiments -

Ballachulish (N.G.R. NN 035595). Birch (Betula pubescens) also grew in profusion in this area although having lower altitudinal limits. Similar data therefore was also collected for that species. Five sample sites were chosen at Ballachulish, from sea level to 600m up a rather exposed north facing slope. The upper limit of rowan was an altitude of 600m but birch only grew to 510m. Samples therefore of birch were only obtained from four sites. Altitude of each site was measured with a surveying aneroid altimeter and is correct to 10m. Elevation of each site is tabulated in Table 5.1.

Table 5.1 Site numbers and altitudes at Ballachulish

Site	Altitude (m)	Species
1	10	S. aucuparia; B.pubescens
2	230	" "
3	425	" "
4	510	" "
5	600	S. aucuparia only

At each site, three trees of each species were selected for sampling (with the exception of site 5 which only contained rowan)

5.2 Bud Water Content Throughout the Winter

The water relations of S. aucuparia and B. pubescens buds were monitored over the course of 6 months of winter during 1978-79. The most useful criteria for measuring the water deficits of a plant tissue is by estimating the relative water content or the water potential of the tissue (Barrs 1968).

It was decided, however, that to measure either of these parameters was impractical. It is more convenient to determine relative water content or water potential of a tissue in the laboratory than attempt to make a direct measurement in the field. The site was 120 miles from the laboratory. Loss of water from the tree buds during transportation to the laboratory would result in inaccurate estimation of both relative water content and water potential. Processing of samples in the field would be hampered both by hazardous terrain underfoot and the bad weather conditions normally experienced at high altitudes in Scotland in wintertime. Therefore, water content per dry weight of bud was measured using the method described below.

To determine water content buds were sampled every few weeks throughout the winter. On each sampling date, for both S. aucuparia and B. pubescens, 10 buds were cut from the 3 trees of each species at the sites listed in Table 5.1. The buds were cut from twigs removed from all sides of the tree at a height between 1.5m and 2.5m from the ground. The buds were surface dried with paper tissue if necessary and placed in an airtight weighed container immediately (i.e. 10 buds/tree/container), and transported to the laboratory. There the container and buds were weighed. This allowed calculation of the fresh weight of the buds. The buds were then dried for 36 hrs at 95°C to obtain the bud dry weight. It was then possible to calculate the percentage water content of the

buds per dry weight where

$$\% \text{ Water Content} = \frac{\text{fresh weight - dry weight}}{\text{dry weight}} \times \frac{100}{1}$$

The inherent weakness in the use of this expression is that it is strongly influenced by changes in the dry weight component. In this experiment seasonal fluctuations in the dry weight factor may affect the apparent percentage water content value.

Daily temperature data for the 6 month sampling period was obtained from the Meteorological Office for a climatological station at Onich NGR : 27/028633, altitude 15m. This station is only 2km from site 1. Mean daily air temperatures were calculated from this data and defined as half the sum of the daily maximum and minimum temperature. The maximum air temperatures relate to the 24 hour period following 9 a.m. whilst the minimum temperatures relate to the 24 hour period ending at 9 a.m. on the date to which entered.

Figure 5.1 shows the mean daily air temperatures for sites 1 and 5 assuming a lapse rate of 0.6^{o}C per 100m increase in elevation.

Figure 5.2 illustrates the bud water content results obtained on 21 November 1978 for S. aucuparia and B. pubescens. At this time air temperatures were relatively mild and the soil unfrozen. It is assumed therefore that the buds would not be suffering from water stress. Water content of the bud increases with altitude in S. aucuparia i.e. the fresh weight : dry weight ratio increases with altitude. This trend is not so apparent in B. pubescens.

The data obtained for S. aucuparia over the six months winter period is presented in Figure 5.3. Samples from site 1, which is at sea level, have a similar water content throughout the winter. In contrast samples

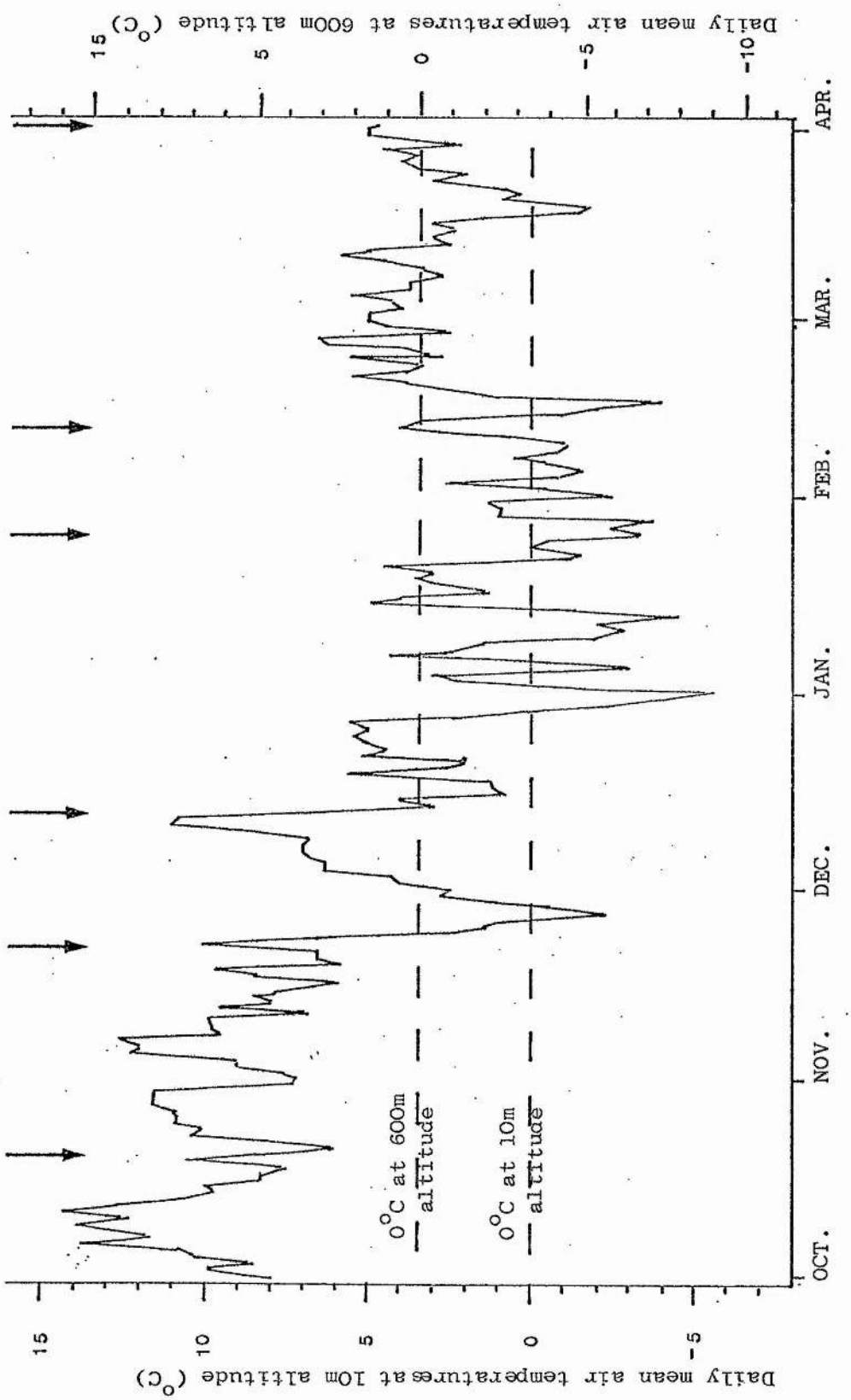


Figure 5.1 Daily mean air temperatures at Ballachulish, Winter 1978/9 at 10m (site 1) and 600m (site 5) altitude assuming a temperature lapse rate of $-0.6^{\circ}C/100m$ altitude. Arrows indicate dates on which bud samples were collected for bud water content determination (sub-chapter 5.2).

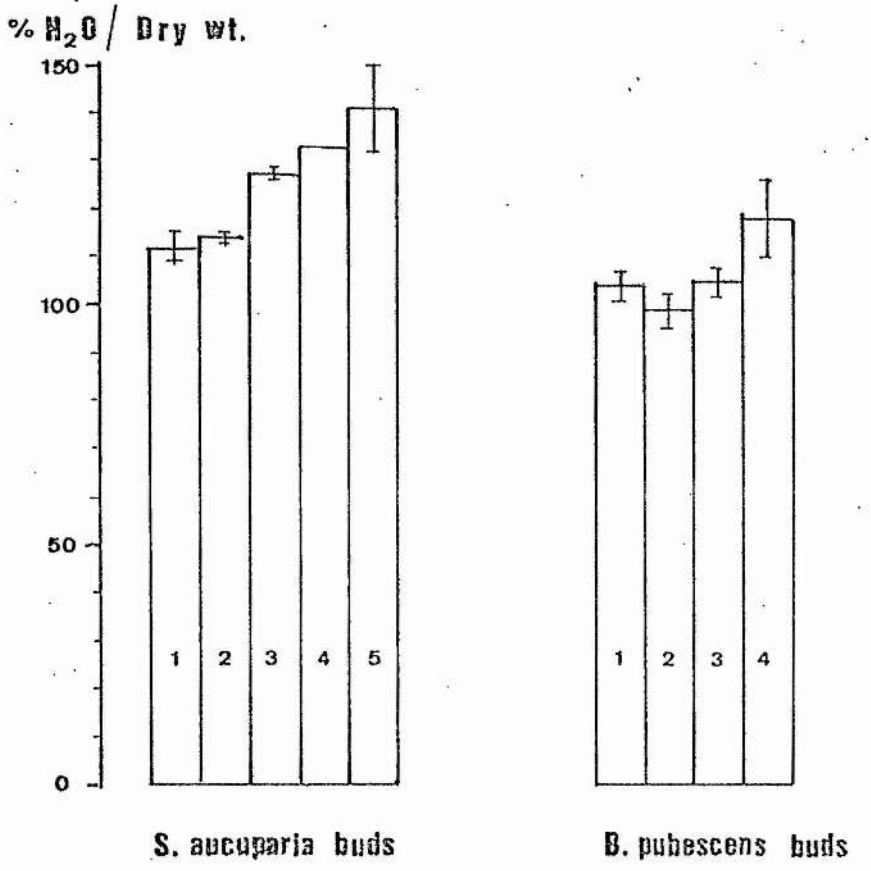


Figure 5.2 Water content of Sorbus aucuparia and Betula pubescens buds at Ballachulish on 21 November 1978. Site numbers are shown inside bars. Vertical lines represent standard errors of the means.

from site 5, the highest site at 600m, have a varying water status and have a very low water content in February. The February sample was collected after a very long cold spell. Water content of buds at site 5 in February was 83% compared with a value of 141% in November. This is proof indeed that rowan tree buds can suffer physiological drought in the winter time. When all five sites are considered, it is evident that there is a larger decrease in water content of buds with altitude on February 11 if a comparison is made with November 21 values. Apart from high altitude site 5, bud samples on April 1 have regained water contents approximately similar to November 21 values.

Similar data is shown in Figure 5.4 for B. pubescens. Buds from site 1 show a small percentage drop in water content in January and February compared with the November figures, whilst at the higher sites there is a large change in water status. With increasing elevation there is a larger percentage decrease in water content during the colder months of the year. Values for April 1 show that only buds from site 1 have regained water content values similar to November 21 figures.

It is useful to make a comparison between the two species of the decrease in bud water content during the colder part of the winter. B. pubescens buds at the high altitude site 4 show a larger decrease in water content between November 21 and February 11 than do S. aucuparia buds from site 5 which is at a higher elevation. Water content of B. pubescens buds decreases from 128% to 57%. This compares with the smaller decrease in water content of S. aucuparia bud samples from 141% to 83%.

On sampling date April 1, S. aucuparia twigs containing buds were cut from the three trees at sites 1 and 5, and B. pubescens twigs from sites 1 and 4. Twigs were transported in wetted polythene bags to the laboratory where the twigs were recut to 13cm, placed in 50ml beakers

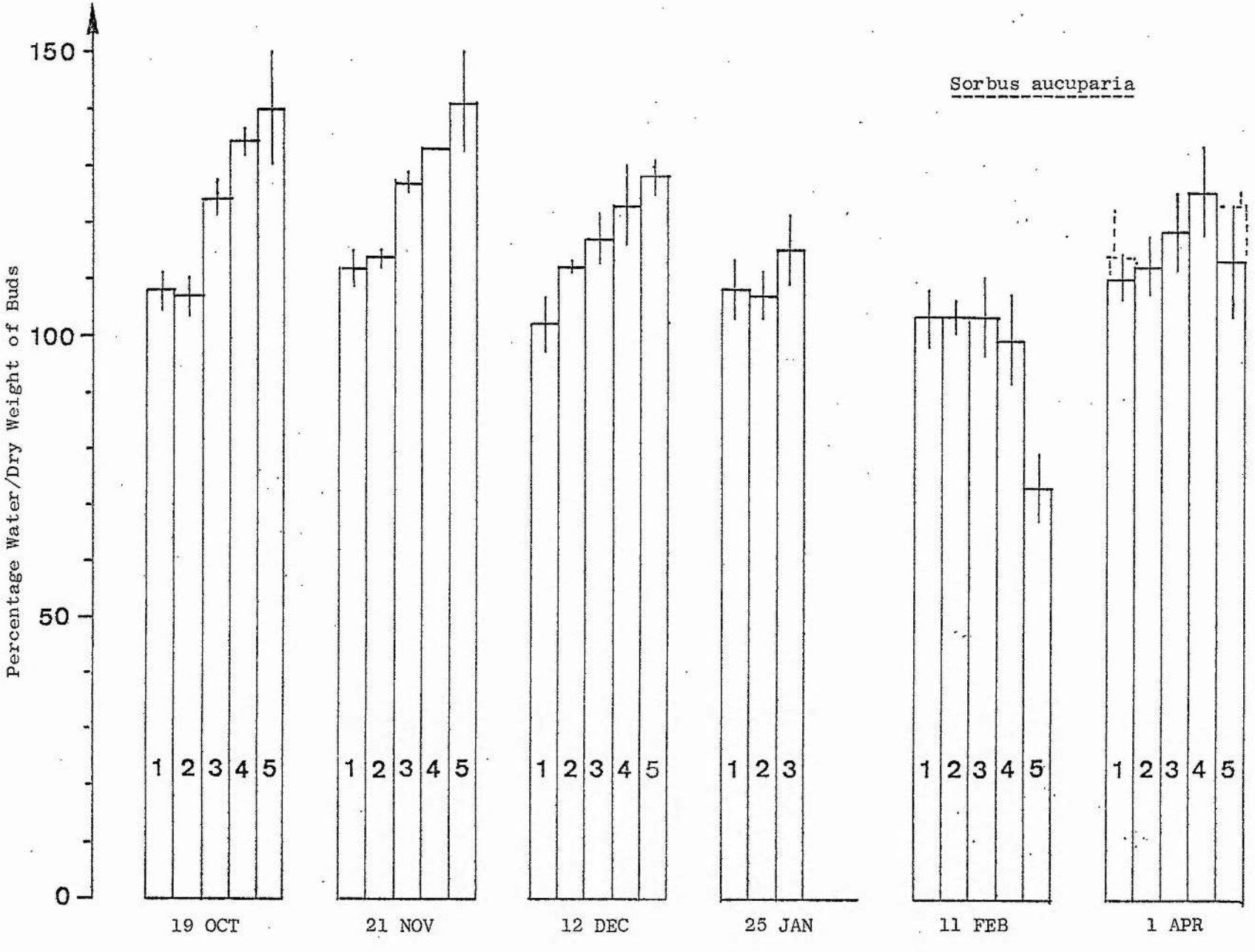


Figure 5.3 Field water content of S. aucuparia buds at Ballachulish throughout winter of 1978/9. Site numbers are shown inside bars. Dashed bars superimposed on April 1 samples show values for fully saturated buds. Vertical lines represent standard errors of the mean.

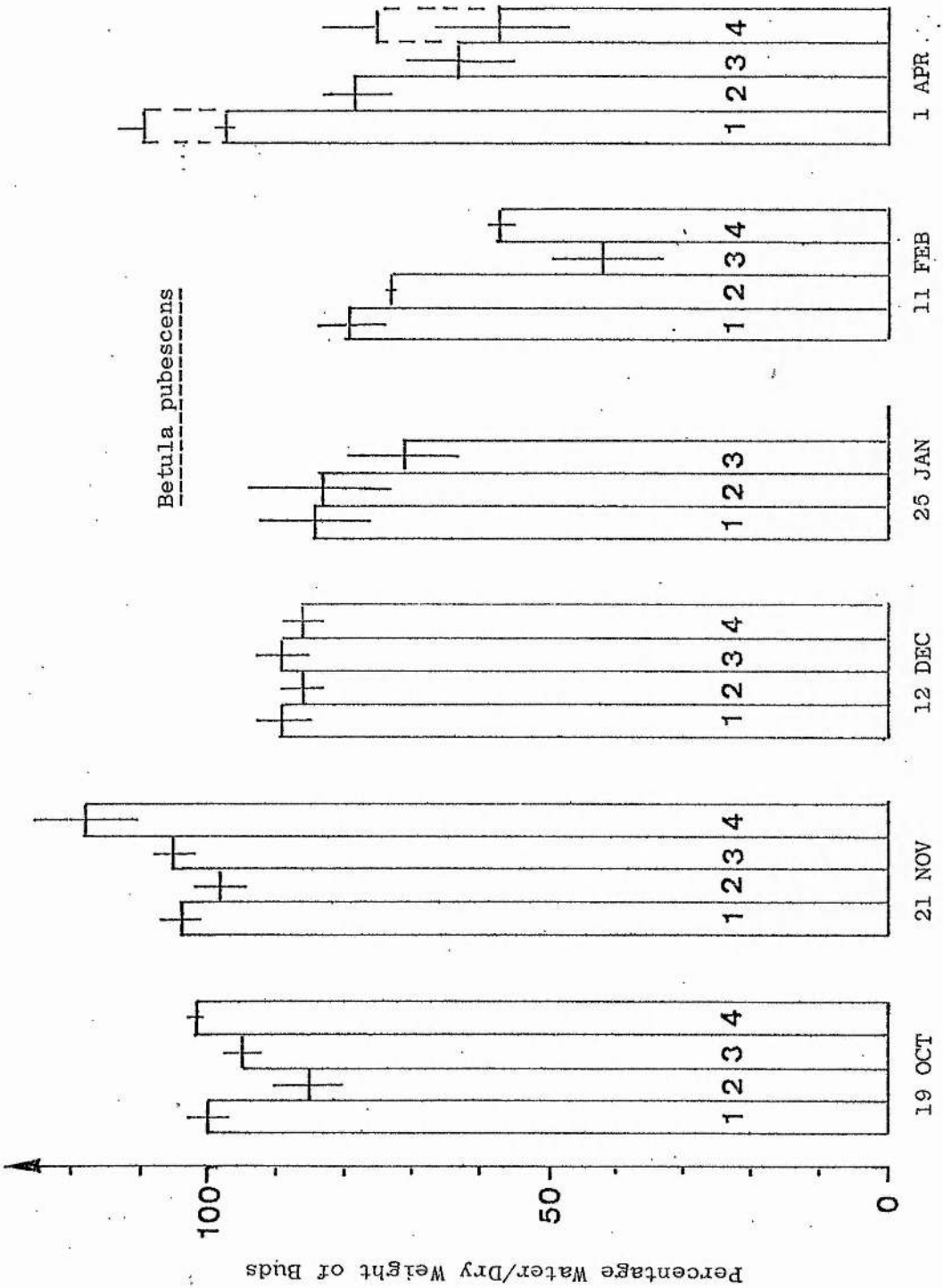


Figure 5.4 Field water content of B. pubescens buds at Ballachulish throughout winter of 1978/9. Site numbers are shown inside bars. Dashed bars superimposed on April 1 samples show values for fully saturated buds. Vertical lines represent standard errors of the means.

containing distilled water to a depth of 1cm. Beakers containing twigs were enclosed in polythene bags and placed on a laboratory bench at the window. There they were subject to diffuse daylight and a temperature of about 20°C. (Preliminary experiments had shown that 16 hrs was sufficient time to ensure full saturation of twigs and buds.) After 16 hrs, the saturated buds were cut from the twigs. As before bud samples contained 10 buds per tree. Each 10 bud samples were weighed to give the fully saturated weight. Buds were then oven dried at 95°C for 36 hrs to obtain the dry weight. The percentage water content of fully saturated buds could then be determined for this date.

This procedure allowed a comparison of the water content of fully saturated buds with the water content of buds in the field for the same date. Comparison could also be made with the water content of buds determined in the field in early winter. The dashed lines superimposed on the histograms of Figures 5.3 and 5.4 show the results of this exercise. Values are tabulated with standard errors of the mean in Table 5.2.

S. aucuparia buds, fully saturated in the laboratory, from site 1 on April 1, showed an insignificant increase in percentage water content to 114±8 (mean ± S.E.) compared with the water content of buds in the field i.e. 110 ± 4. This compares with a similar mean percentage water content of buds, 112 ± 3 from this site on November 21. In contrast there is a difference in water content between field and laboratory fully saturated buds from the high altitude site 5 (Table 5.2). Even when fully saturated, the buds from this site did not contain the higher water contents of the November 21 samples.

Table 5.2 Mean percentage water content per dry weight of buds in the field and when fully saturated in the laboratory.

Species	Site	NOV. 21 Field Water Content Mean ± S.E.	APR. 1 Field Water Content Mean ± S.E.	APR. 1 Saturated Water Content Mean ± S.E.
S. aucuparia	1	112 ± 3	110 ± 4	114 ± 8
S. aucuparia	5	141 ± 9	113 ± 7	124 ± 2
B. pubescens	1	104 ± 3	97 ± 1	109 ± 4
B. pubescens	4	118 ± 8	57 ± 10	75 ± 8

The data for B. pubescens illustrates the same trends as above with S. aucuparia but with larger differences between field water contents and laboratory fully saturated bud water contents. Fully saturated buds from the low altitude site, i.e. site 1, have a percentage water content of 109 ± 4 compared with a field water content of 97 ± 1. Field percentage water content of the high altitude site 4 buds was 57 ± 10. On saturation buds from this site had a percentage water content of 75 ± 8. Water contents of buds from this site showed a large decrease compared with values obtained on November 21 (Table 5.2). Buds from site 1 on this date have approximately similar values as recorded on November 21.

5.3 Bud Scales

Buds are formed in late summer, and bearing in mind that summer temperatures may be the proximal cause of altitudinal tree limits and winter frost drought the distal cause, morphological factors which would affect bud transpiration losses were investigated. This was done using bud material from the same trees and sites as in the previous investigation into bud water content and tabulated in Table 5.1.

Scales enclose the shoot buds in both S. aucuparia and B. pubescens. The number of scales per bud were counted in both species versus altitude. This was done in the laboratory after collecting 5 buds per tree, i.e. 15 buds per site for each species. Buds were collected on December 12 and were removed from different sides of each tree at a height of 1.5m to 2.5m from the ground.

In S. aucuparia the bud scales are probably derived from leaf bases and are quite thick. B. pubescens bud scales are stipulate. In this species the number of bud scales counted included the first pair of stipules which enclose the outermost leaf in the bud, as these stipules are similar in morphology and function to the scales.

Figure 5.5. is a graph of number of bud scales per bud against altitude for both species. There is a gradual decline in the number of bud scales versus altitude in B. pubescens. The trend is not quite so clear with S. aucuparia although the mean number of bud scales per bud is lower for the top two sites.

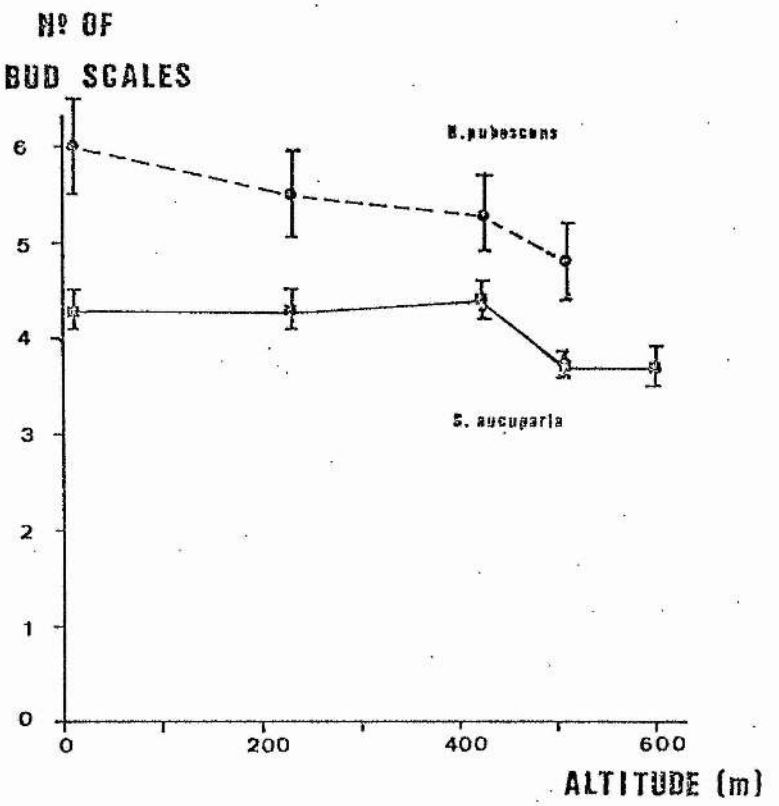


Figure 5.5 Number of bud scales per bud in S. aucuparia and B. pubescens with altitude of origin. Vertical lines indicate standard errors of the means.

5.4 Bud Scale Cuticles

Starting from the outside of both S. aucuparia and B. pubescens buds, bud scales increase in size towards the inside of the bud. To measure the outer (abaxial) cuticle thickness in bud scales of these species, the cuticle thickness of the first bud scale to enclose at least 7/8 of bud, was measured. This was the bud scale which had the largest area of cuticle exposed to the atmosphere. In S. aucuparia it was generally the third bud scale but in B. pubescens buds it varied and depended on the number of smaller outer scales. Only the outer cuticle thickness was measured. The inner cuticle of scales of both species were thin by comparison. Four buds per tree i.e. 12 buds per site, were collected, as in Chapter 5.3, on December 12.

Cross sections were cut across the middle of the bud scale using a freezing microtome. Sections 12 μm thick were cut, placed in 10% chromic acid for 10 minutes, washed in water and placed in Sudan IV stain for 1 hr. To remove excess stain, sections were washed in alcohol and then mounted in glycerine. Microscopic measurements were carried out under oil immersion using a calibrated graticule. Three measurements were made at $\frac{1}{4}$, $\frac{1}{2}$ and $\frac{3}{4}$ along the outer cuticle of a bud scale. These measurements were then averaged to get the mean value.

Figure 5.6 illustrates the results of the above investigation. In both species there is a drop in mean cuticle thickness at the highest altitude site.

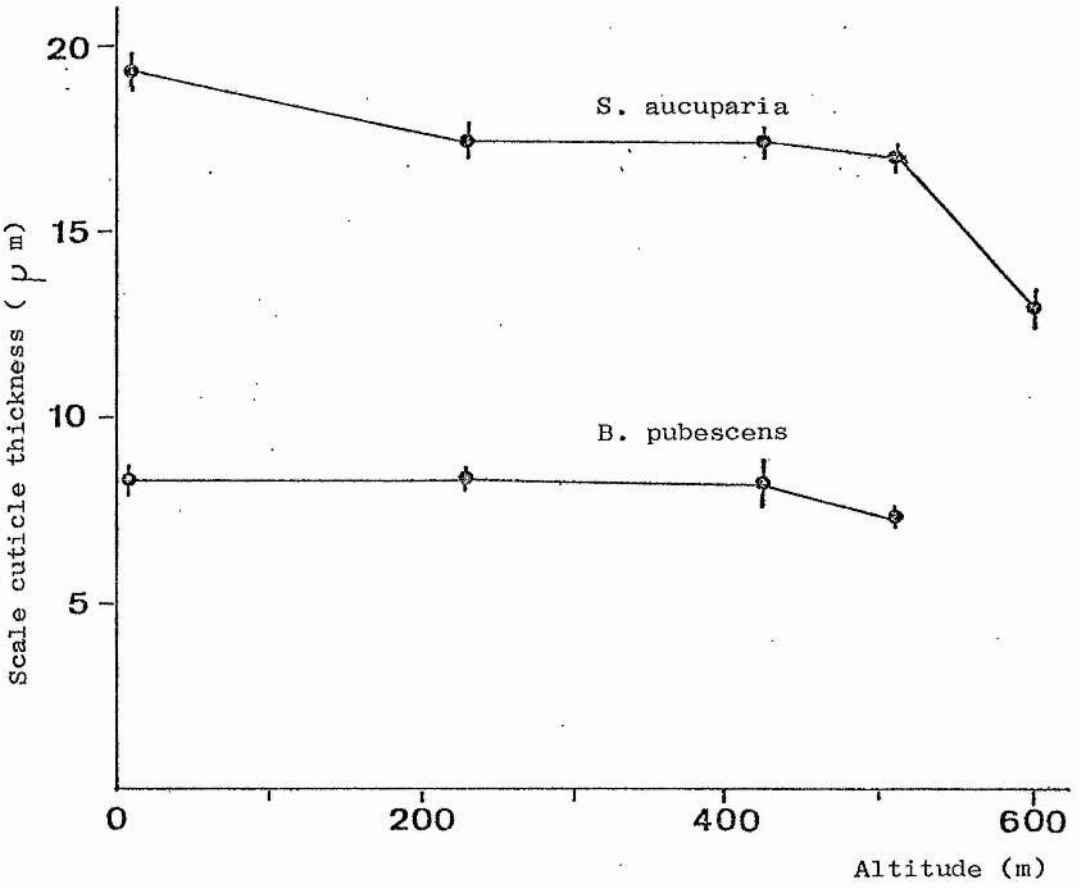


Figure 5.6 Scale cuticle thickness of S. aucuparia and B. pubescens buds with altitude of origin. Vertical lines indicate standard errors of the means.

5.5 Experimental Desiccation of Buds

In sub-chapters 5.3 and 5.4 it has been shown that S. aucuparia and B. pubescens buds from high altitude sites have a smaller number of bud scales compared with buds from lower altitudes. Furthermore, bud scale cuticles are thinner at the high altitude sites.

How do the above factors affect the water status of buds under desiccation stress? To answer this question, the effect of desiccation on fully saturated buds was investigated.

Twigs containing buds were collected from the Ballachulish site on April 1. They were cut from trees used throughout the series of experiments reported in this chapter. However, twigs of S. aucuparia were only collected from the sea level site 1 and site 5 which is the altitudinal limit of this species. Twigs of B. pubescens were collected from site 1 and from site 4, which is the highest site for this species. (Altitudes for each site are shown in Table 5.1.) Twigs containing buds were collected from the three trees at each of the above sites, placed in wetted polythene bags and transported to the laboratory. There the twigs were recut to 13cm and placed in 50ml beakers containing distilled water to a depth of 1cm. The beakers were enclosed in a polythene bag for 18 hrs at a temperature of about 20°C. After this treatment, the buds were fully saturated. The buds were cut from the twigs (5 buds from each tree i.e. 15 buds from each site) and the cut ends very lightly smeared with petroleum jelly, weighed and placed in desiccators containing $CaCl_2$. The buds were re-weighed daily and the relative water contents calculated as in Chapter 3.

A graph of relative water content of buds versus desiccation treatment time is shown in Figure 5.7. It can be seen that there is a greater decrease in relative water content with time in buds from the high altitude sites in both species.

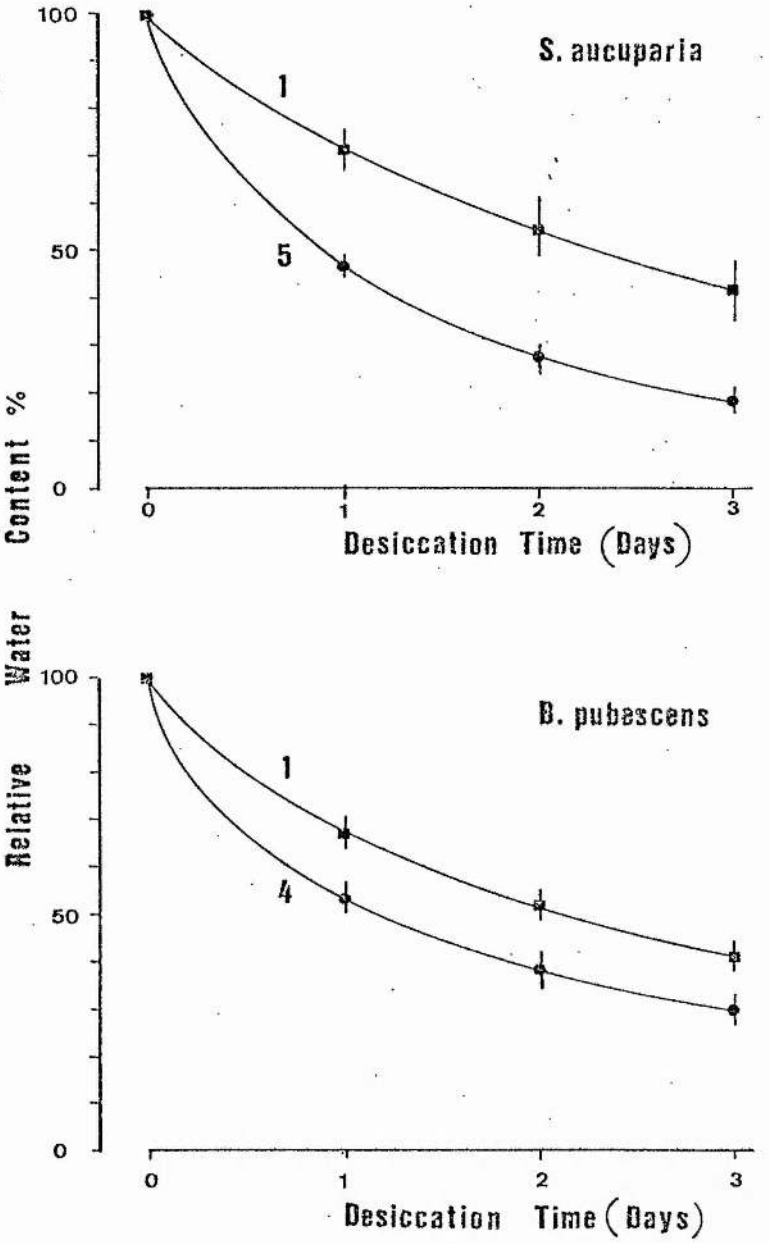


Figure 5.7 Decrease in relative water content of experimentally desiccated buds of S. aucuparia and B. pubescens from high altitude (Sites 4 and 5) and low altitude (site 1) trees. Numbers superimposed on graphs indicate site. Vertical lines are standard errors of the means.

The water content of S. aucuparia buds from the high altitude site 5 decreased to 19% with 3 days desiccation treatment. This compared with a smaller decrease in the low altitude samples to 42%. Similarly, with B. pubescens buds a decrease to 30% was evident in the high altitude sample after 3 days treatment. By comparison the water content of bud samples from the sea level site in this species showed a smaller decrease to 41%.

5.6 Discussion

The research carried out over the winter of 1978-79 at Ballachulish and reported in this chapter, has revealed that the buds of S. aucuparia and B. pubescens can suffer desiccation stress. This stress occurs at the coldest periods of the winter and increases with altitude.

The desiccation stress appears to affect S. aucuparia and B. pubescens differently. When milder spring conditions prevail, S. aucuparia buds can regain water contents similar to November values whilst B. pubescens buds are still stressed at this date.

When twigs are removed from the field in spring and the buds experimentally saturated in the laboratory, high and low altitude buds of S. aucuparia have a water content similar to bud water contents in the field in November. In contrast, although low altitude B. pubescens saturated buds are similar in water content to buds in the field in November, high altitude samples cannot regain the values found then.

The ability of plant tissues to resaturate after being drought stressed is frequently used as a means of assessing damage to the tissues (e.g. Bannister 1970). It is possible that the inability of high altitude buds to regain the high water contents of early winter may be due to damage caused by desiccation.

Summer temperatures appear to affect the maturation of both rowan and birch buds. Although the evidence presented in this chapter is not dramatic, with increasing elevation and hence decreased number of day-degrees, there is an increase in fresh weight : dry weight ratio in buds. This is more pronounced in the rowan data. A decrease in the number of bud scales per bud and bud scale cuticle thickness at the high altitude sites is also apparent. How much the decrease in number of bud scales and a reduction in the thickness of scale cuticles affect bud transpiration rates is hard to guage. The most important function of the cuticle is probably to supplement the action of the stomata in regulating the passage of water from within the plant to its environment

(Martin and Juniper 1970). As no stomata were present in the bud scales of S. aucuparia and B. pubescens, the thinner cuticles found in the bud scales at the altitudinal limits of both tree species must increase the rate of water lost from the buds. Other factors such as bud size and cell wall lignification, however, may also affect the rate of water loss from the buds. A clear difference in decrease of relative water content values between bud samples from low and high elevations under standard desiccating conditions in the laboratory has been demonstrated.

It must be pointed out that high altitude buds in the field would be subject to higher wind speeds than low altitude samples. This would increase transpiration rates. This effect would be compounded by the low temperatures at high altitudes which would slow down uptake and replenishment of water. Replenishment would be non-existant at sub-zero temperatures as explained in Chapter 1. The apparent higher transpiration rates of buds from high altitudes under standard conditions would therefore make these buds doubly vulnerable to desiccation stress.

CHAPTER 6

NON-CLIMATIC FACTORS AFFECTING THE DISTRIBUTION OF THE ROWAN IN THE HIGHLANDS OF SCOTLAND

The rowan has the potential ability to grow in the Highlands of Scotland from sea level to very high altitudes. Large tracts of the Scottish uplands are in fact treeless. This area of mountain and moorlands extends to 4.8 million hectares in Scotland and represents two-thirds of its land surface (Nicholson 1971). Why are the grass or heather (Calluna) clad hills not clothed instead by the rowan and other tree species?

The destruction of natural forest in Scotland has taken place since early times. The biggest effect man has exerted on the history of the Highlands of Scotland has been the destruction of the Caledonian Forest (Darling and Boyd 1964). Even in this century remnants of forest in Scotland have been destroyed during the two World Wars.

Land use practises in the Highlands today prevents much effective regeneration of trees. Moor burning is practised over much of hill and heathland in Scotland. This is done to partly or wholly destroy the existing vegetation and to encourage new growth of Calluna vulgaris which is a major food source of both sheep and red grouse (Lagopus lagopus). Controlled rotational burning of Calluna increases the productivity and nutrient value of this species for grazing. These measures therefore prevent trees from becoming established in many areas.

Grazing by red deer (Cervus elaphus), sheep and cattle also prevent regeneration of trees. In a study in Glen Feshie, Miller and Cummings (1974) found large populations of tree saplings. This included 14 rowan saplings per hectare. Nearly 90 per cent of the tree saplings had been browsed, however, and were all less than 50 cm. tall. In this case,

browsing damage was attributed to red deer. Red deer also browse mature trees. Mitchell et al (1977) have tabulated data from various sources regarding this animal's preferences for different trees. Where available, S. aucuparia appears to be a preferred tree species. Two of the commonest tree species in Scottish Highland woodland, Pinus sylvestris and Betula sp. are relatively unpalatable to red deer. The scarcity of rowan is probably due to previous grazing pressure. Data is also cited by Mitchell et al (1977) for the practise of bark stripping by red deer. Although most of the data put forward refers to forests in Europe, the rowan is ranked as being moderately susceptable to bark stripping.

Extensive damage to rowan by bark stripping by cattle has also been observed (Kinnaird et al in press). In one wood under study 45 per cent of the rowans growing there were killed by bark stripping after cattle had been allowed to graze there over two successive winters. This wood contained many tree species. Some trees other than rowan suffered damage but only slight in comparison. The authors point out that S. aucuparia is scarce in many woodlands used regularly for grazing, yet common in the same localities on roadsides and steep rocky ground. They concluded that rowan was utilised either as a nutrient, mineral or vitamin in short supply, or because it had the most pleasant tasting bark that was readily available.

The writer has observed in east Scottish glens that the bark of S. aucuparia and Salix sp. appear to be eaten preferentially to Betula sp. by rabbits (Oryctolagus cuniculus) when deep snow is present.

McVean and Ratcliffe (1962) state that pure rowan woods, and mixed woods with birch are not uncommon in the west Highlands. They think that these woods may have been more prevalent in the past and further point out (although without quantitative date) that rowan regeneration is

potentially more widespread than that of birch and more seedlings become established. These rowan seedlings, however, are heavily grazed by sheep and red deer on account of their palatability.

Rowan seeds are bird dispersed and the berries are readily eaten by many birds. Various species of the thrush family eat the berries of the rowan (Simms E. 1978). Even at high altitudes birds can be found which eat rowan berries. These bird species include the ring ouzel (Turdus torquatus), a bird which breeds to high altitudes in the Highlands and the fieldfare (T. pilaris) which can be found in large flocks up to and over 800m elevation during migration.

Evidence can be seen today of the rowan's potential wide distribution in the Highlands. Deer fences, erected to protect new forestry plantations, often enclose large populations of rowan. In the hills, outcrops of rocks and cliffs support large numbers of rowan (along with birch), out of reach of nimble footed herbivores. It is not unusual to see small wooded islands in Scottish lochs surrounded by tree-less countryside which is dominated by dwarf shrub or grassland communities. These communities are perpetuated by grazing or burning practices.

McVean and Ratcliffe (1962) show an attempted reconstruction of the dominant woodland types in the Scottish Highlands in the past. In the west Highlands, Oak (Quercus robur and Q. petraea) predominated along with birch (Betula pubescens and B. verrucosa), whilst in the central and east Highlands Pine (Pinus sylvestris) and birch were predominant. In the far north, and the western isles, the dominant tree was birch. Rowan was and still is, a subdominant in these different woodland types. These authors state that formerly rowan may have replaced oak to some extent both altitudinally and to the west on the more base rich soils.

The rowan appears to prefer drier sites than B. pubescens probably

the rowan's greatest competitor on upland sites. It is found growing mainly on lighter soils and is rare or absent on clays and soft limestone (Clapham et al 1962). In lowland high density woodland rowan would be a poor competitor as it is a shade intolerant tree (Walter H. 1968).

From the foregoing it can be seen that land use practices in much of the Highlands prevent regeneration and growth of the rowan. Although present in most woodland types at lower altitudes it is rarely the dominant tree species. S. aucuparia would be much more prevalent today at higher altitudes if more natural conditions prevailed.

CONCLUSIONS

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSION

Current thinking as to the causes for low temperature limits to tree growth was discussed in Chapter 1. This underlined the need for a tree shoot to 'harden' both morphologically and physiologically by the end of the summer growing season to withstand the desiccating conditions experienced during late winter due to frost drought. A further major requirement proposed for tree growth was the ability to maintain a positive carbon dioxide balance throughout the year in a habitat where energy was in short supply.

The distribution of the rowan was described. This showed that the rowan has the ability to grow to higher altitudes in the British Isles and the European Alps than other tree species. It grows to high latitudes where it is found growing along the northern edge of the boreal zone at northern tree limits. It is obviously capable of growing successfully in low temperature habitats.

The choice of experiments performed for this thesis, in the main, reflects the problems encountered by trees growing in low energy environments. Throughout much of this thesis a comparative approach has been employed where the metabolism of the rowan bud and twig has been compared with other trees.

The major questions raised in this thesis are as follows. Is the unique distribution of the rowan matched by differences in metabolism, when compared with other tree species? If so, what are these differences?

Metabolic differences between the rowan and other species have been found. To expand on this theme the results of experiments have been divided into two classes.

A. Where distinct differences in the metabolism of the rowan have been found.

1. The energy of activation of bud dark respiration rates.

2. Temperature respiratory rate compensation.

3. Water relations of high altitude shoot buds.

4. Tissue viability at high water deficits.

5. Seed germination.

B. Where no distinct differences in the metabolism of the rowan have been found.

1. Absolute values of dark respiration rates in tree buds.

2. Ethylene production in shoot buds under desiccation stress.

3. Soluble carbohydrate levels in buds at increasing water deficits.

The experimental results obtained from the measurement of dark respiration rates of tree buds and reported in Chapters 2 and 4 were interesting as differences were apparent between S. aucuparia and the other species used in these experiments. Although no general trend was noted in respiration rates per se with geographical range of a tree species, the calculation of the energy of activation of respiration rates measured between 2°C and 22°C showed that S. aucuparia had the lowest figure. Doubt, however, was expressed of the importance of these results as the calculation of the Q10 of respiration rates from naturally growing rowan tree buds showed that the Q10 (which is comparable with the energy of activation) of rowan bud dark respiration rates altered with time throughout the spring. It was suggested that the measurement of the energy of activation of dark respiration rates of the six species tested over the whole winter period might shed more light on the importance of this parameter.

There was a clear difference between S. aucuparia and the other species tested in the experiment of respiratory rate compensation. When subjected to an increase in temperature, S. aucuparia buds had the ability to reduce respiration rates quickly in comparison to the other species. A reduction in environmental temperature produced no change in respiratory rates in all species, at least over the time period tested i.e. 32 hours.

The mean air temperature data throughout the winter at Ballachulish (Figure 5.1) illustrates the extent to which daily mean temperatures can alter from day to day. Overlaying day to day changes are diurnal changes in temperature.

Tranquillini (1964) cites data collected in the European Alps showing that although average monthly plant tissue temperatures and air temperatures differed little from each other, the amplitude of tissue temperatures was much greater. During periods of high incoming radiation and cool air temperatures, plant tissue temperatures can far exceed ambient temperatures. He found that Pinus cembra needles experienced the greatest temperature differences in April, when the maximum daily amplitude was 34°C; that of air was only 13°C.

It is apparent therefore, a plant tissue which can regulate dark respiration rates quickly would achieve considerable savings in energy. S. aucuparia buds in the experiment on respiratory rate compensation (Chapter 2) were subjected to a pre-treatment of 12°C and the respiration rates (oxygen uptake) measured. Temperature was increased to 22°C and respiration rate measured at this temperature after 1 hour equilibration time. At this time, respiration rate was 0.312 $\mu l O_2$/hr/mg dry weight (Figure 2.5). After 24 hours at this temperature the rate had decreased at a steady rate to 0.246 $\mu l O_2$/hr/mg dry weight i.e. a decrease in rate of 0.00275 $\mu l O_2$/hr/mg dry weight. If respiration rates had remained at the higher value i.e. 0.312 $\mu l O_2$/hr/mg dry weight, the amount of glucose equivalents respired would have been 10.04 μg/mg dry weight over the 24 hour period (where 6 x 22.4 $\mu l O_2 \equiv$ 1 μmol glucose). With the decrease in oxygen uptake to 0.246 $\mu l O_2$/hr/mg dry weight only 8.93 μg/mg dry weight glucose equivalents had been respired. This resulted in 1.11 μg glucose/mg dry weight being conserved over the 24 hour period.

If the reader will permit some speculation, the above conservation of carbohydrate over a 24 hour period, taking place say four times a

month over a six month winter period, would result in a saving of 26.64 μg glucose equivalents/mg dry weight of bud tissue. A considerable amount.

Such "neat" temperature changes as in the above respiratory rate temperature compensation experiment, however, do not take place in nature. As inspection of Figure 5.1 shows, however, daily mean temperatures do vary widely from day to day, although air temperatures are lower than the 22°C used in the experiment. It was explained earlier in this chapter that plant tissues can often have higher temperatures than ambient, expecially during periods of insolation, therefore the value of 22°C may not be too unrealistic.

The measurement of respiration rates of buds of rowan trees growing naturally up an altitudinal gradient demonstrated a positive correlation between respiration rates and altitude of sample. This was interpreted as an adaptation to decrease in temperature with increase in altitude. This is further evidence of the ability of rowan bud tissues to alter respiration rates in response to environmental temperatures. There was no correlation between Q10 of respiration rate measured at 5°C and 15°C with altitude of sample. This perhaps further reinforces doubts as to the significance of the energy of activation and the Q10 of respiration rates of tree buds with respect to environmental temperatures.

It would appear therefore from the results given in this thesis that S. aucuparia has the ability to decrease its dark respiration rates quicker in response to an increase in temperature than the other species tested, and when growing naturally alters its respiration rates to suit ambient temperatures.

Increased metabolic rates would deplete carbohydrate reserves. The ability to reduce metabolic rates in response to an increase in temperature was interpreted as a means of conserving these carbohydrate

reserves. As emphasised before, this would be important for a tree growing in a low energy environment during the winter time when little or no active growth would be taking place.

The experiments on the carbon economy of tree buds were confined to dark respiration rates. No measurements were made of photosynthetic rates. Photosynthesis, however, must only play a minor but perhaps significant role in the carbon dioxide balance in deciduous tree buds in winter time. Bud scales which enclose deciduous tree buds do not normally have stomata (Ward 1904). This was certainly true in the case of S. aucuparia and Betula pubescens, the two species where scale morphology was studied in detail. Although chlorophyll was present in mesophyll cells in the bud scales of these species, no palisade cells were present. It is unlikely therefore that photosynthesis plays a large part in the carbon balance of the shoot buds in these two species. This is probably true for the buds of other species in which dark respiration rates were studied.

During the summer with the advent of leaf expansion photosynthesis must be the over-riding factor in the gas exchange budget of deciduous tree species. Bannister (1976) cites photosynthetic temperature optima for plant species from different climatic zones. Species from low temperature environments have lower temperature optima for photosynthesis than species from warmer climates. As he further points out, temperature optima for photosynthesis are not a species characteristic e.g. in central Europe, Betula pendula growing at 1900m altitude shows a photosynthetic temperature optima at 14°C although material from 600m shows an optimum at 17°C.

Chabot and Billings (1972) have shown experimentally that alpine plants responses are strongly influenced by the temperature regimes in which they are grown. Plants grown at low temperatures have photosynthetic peaks at lower temperatures than plants grown at higher temperatures.

A study in Deeside, Aberdeenshire by Forbes and Kenworthy (1973) on the distribution of two species of birch, Betula pendula and B. pubescens odorata, showed that the arborescent B. pendula ascended no higher than 305m, whilst the more shrubby B.p. odorata grew at higher altitudes. They concluded that the shrubbiness, greater leaf area index and leaf biomass in B. p. odorata was an adaptation to improve the efficiency of solar energy utilisation in the climate of high altitudes.

From the above it can be seen that differences and adaptations can therefore be expected in photosynthetic rates and photosynthetic temperature optima and also in life form strategies in trees.

Photosynthesis has been discussed briefly to remind the reader that whilst adaptation in respiration rates has been a major topic researched in this thesis, photosynthesis has an equally important part to play in the annual carbon dioxide budget of a tree. It is interesting to note, however, that studies by Rook (1969) on Pinus radiata seedlings showed that adjustments in respiration rates with change in environmental temperature were far more dramatic than the change in photosynthesis.

The measurement of the percentage water content of rowan and birch buds in the field produced data for this thesis which showed the extent to which tree buds can suffer water stress at different altitudes. Both rowan and birch buds from high altitude sites had very low water contents in late winter. By April 1, however, rowan buds proved more successful in regaining water contents similar to early winter samples. This may have been due to tissue damage in birch (Betula pubescens) buds due to high water deficits and may be the reason altitudinal limits of birch are lower than rowan.

The increase in freshweight : dry weight ratio of buds in early winter with increase in altitude, and the smaller number of bud scales and thinner scale cuticles found at high altitude sites in both rowan

and birch can be explained by the reliance of the tree form on summer temperatures to mature sufficiently to withstand winter conditions.

As was clearly demonstrated in the laboratory, under standard desiccating conditions, water loss was greater from high altitude bud samples in both species. The above morphological parameters must, at least in part, explain this phenomenon. The greater incidence of frost drought in winter at higher altitudes due to the decrease in temperature found with increase in altitude makes buds from high altitude trees more vulnerable to desiccation stress.

It is interesting to note at this point the work of White (1974). He studied tree height increments of S. aucuparia, B. pubescens and Pinus sylvestris during the growing season. These trees, 4 to 5 years old, were planted at an elevation of 580m near the altitudinal tree limits in the Pennines, North England. During the season studied (1960), growth started in all three species in mid-May. Growth finished in mid-August for both S. aucuparia and P. sylvestris but continued until the end of September in B. pubescens. He suggested that B. pubescens had insufficient time to harden to withstand air frosts which normally start at this site during mid-September. This conclusion was supported by the observation that practically all the B. pubescens died in September 1972. He also observed in 1972 that P. sylvestris trees were doing relatively badly compared with S. aucuparia.

The low water content of rowan and birch buds in late winter at high altitudes vindicated the emphasis placed on laboratory experiments concerning the effect of desiccation stress on tree buds. The effects of desiccation stress on the metabolism of tree buds and twigs was reported in Chapter 3. Perhaps the most illuminating data resulted from the drying effect of desiccation on the viability of buds and twigs. In this experiment large differences were noted between the species tested. The

Sorbus species were clearly superior in their ability comparison with the other species tested. This appeared to be due to tolerance of low relative water contents at the cellular level. This apparent tolerance of S. aucuparia buds and twigs at the cellular level must be a factor allowing this species to grow in situations where it is subject to physiological drought in winter time. Useful knowledge would perhaps have been gained if B. pubescens had been included in the species tested for viability under desiccating conditions in the laboratory.

Experimental germination of rowan seeds collected from different altitudes at Ballachulish produced a somewhat surprising result. Percentage germination increased with altitude under the conditions used. This contrasts markedly with seed germination studies of Wardle (1965). He found that the broadleaved Nothofagus solandri at the timberline in the Craigieburn Range, New Zealand produced seed with very poor germination. The quantity and proportion of sound seeds of this species decreased towards the timberline (Wardle 1970). This agrees with non-quantitative visual estimates of rowan berry production of the rowan in Scotland. High altitude rowan trees produce very few berries.

The investigation into the effect of altitude on the growth of the rowan, as determined by annual growth ring radial increments, failed to present a clear picture of changes in growth rate with altitude. The sharp decline in growth rates above 500m in the North Angus samples was not repeated in the data collected from the Ballachulish site. It was emphasised that although temperature decreases with altitude, differences in soil types in the North Angus samples and competition with birch at lower altitudes at Ballachulish would have perhaps obscured the results of the effect of temperature on the growth of the rowan.

One interesting fact, however, emerging from the growth rate data collected from both sites. There is no gradual decline in growth rates

to zero at tree limits. At the Ballachulish site, the data from the highest altitude class, (500-600m), shows that growth rates are similar to those from lower altitudes. This suggests that net carbon dioxide assimilation may not be a limiting factor determining altitudinal limits for S. aucuparia.

Little comment needs to be added to the results obtained in the experiment on ethylene production in tree buds at varying water deficits. Of the species tested, only Alnus incana continued, when under stress, with a steady production of this gas. The other species gave a variable response, showing either a large increase, decrease or both over the first 3 days of desiccation treatment. It was concluded that the ability of A. incana buds to produce a steady supply of this plant growth regulator under desiccation stress would be advantageous to a species which would be subject to desiccating conditions in winter time.

The effect of desiccation stress on sugar levels have already been fully discussed in Chapter 3 and needs no further comment here.

In conclusion, the rowan is able to grow successfully in low energy environments. To do this it must be able to grow, mature and harden both morphologically and physiologically in a short growing season to withstand desiccating conditions encountered in late winter. At this time of year environmental conditions induce physiological drought in the rowan resulting in high water deficits in the shoot buds. The apparent tolerance of rowan buds and twigs at the cellular level to low relative water contents may allow this species to withstand periods of physiological drought.

The success of the rowan in low energy habitats may in part be due to its ability to regulate dark respiration rates in shoot buds in winter time which would result in the conservation of carbohydrate reserves.

Rowan seed germination studies showed that high altitude trees can produce viable seed. This suggests that rowan populations at high elevations can be self perpetuating and not necessarily reliant on bird dispersed seed from lower altitudes.

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