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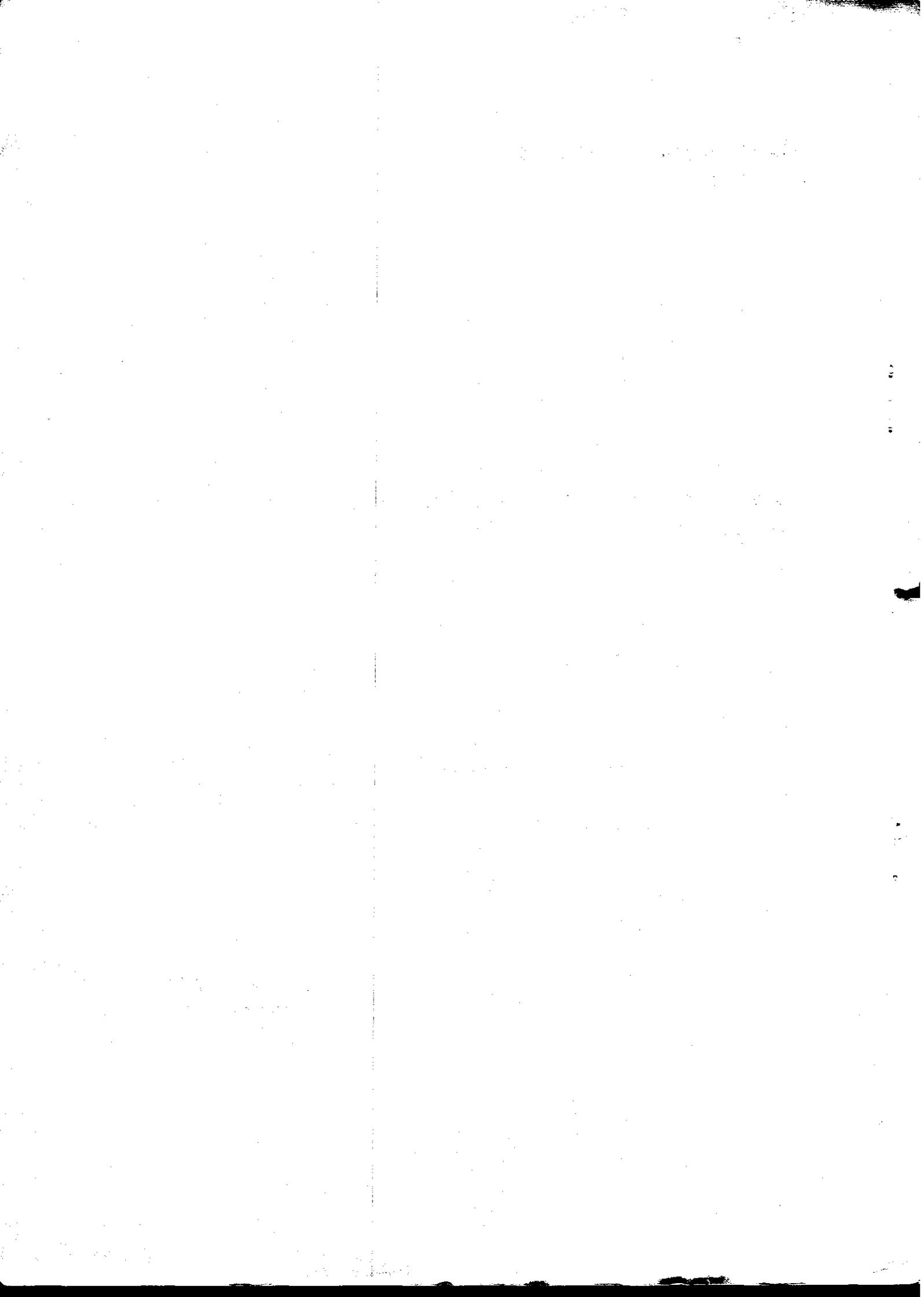
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THE SUCROSE INVERSION METHOD OF MEASURING TEMPERATURE AND  
ITS APPLICATIONS

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## 1. INTRODUCTION

This paper has three main aims:

- a) To summarize use of the sucrose inversion method by ourselves and by other authors, particularly those not mentioned in the brief review by Jones (1972).
- b) To discuss the advantages and disadvantages of the method. In existing relevant literature, the former tend to be given little attention whilst discussion of the disadvantages concentrates on the nature of the mean temperature being measured.
- c) To describe the method used at Merlewood Research Station placing emphasis on (i) practical details which tend to be excluded from formal scientific papers but which are essential for the easy and successful use of the method (ii) our modification of the versions of the method used by other authors (iii) accuracy, precision and sources of error of the method.

## 2. PRINCIPLES OF THE METHOD

The sucrose inversion method depends on the hydrolysis of sucrose to glucose and fructose in acid solution. All three sugars involved are optically active but sucrose and glucose are dextrorotatory whereas fructose is laevorotatory, the actual observed rotation values depending on sugar concentration (Gibb, 1942). As hydrolysis proceeds, the observed rotation changes from a positive value for uninverted sucrose to a negative value, the mean of the rotations for glucose and fructose, hence the reaction is called inversion. For the sucrose concentration which we use, 38.62% as in Berthet (1960), initial and final rotations are about  $51.3^\circ$  and  $-18.2^\circ$  angular (20 cm polarimeter tube, sodium D light,  $20^\circ\text{C}$ ).

The rate of inversion varies directly with hydrogen ion concentration,  $[\text{H}^+]$ , of the sugar solution and with temperature. For temperature measurement,  $[\text{H}^+]$  is kept constant using a buffer solution, hence the extent of inversion reflects the accumulated temperature effect and, by taking exposure time into account, may be expressed as an exponential mean temperature. This is always higher than the corresponding arithmetic mean if the temperature range is more than zero.

The temperature quotient,  $Q_{10}$ , for sucrose inversion, varies from 3.8 to 5.5 depending on the  $10^\circ$  range selected between  $0^\circ$  and  $40^\circ$  and on the sucrose concentration (Leininger and Kilpatrick 1938). This compares with quotients of 2-6 for various types of biological activity (Lee, 1969). According to the law of Arrhenius, the velocity constant for a chemical reaction

$$K_T = Ce^{-\frac{a}{T}} \quad (1)$$

where C and a are constants, C being dependent on  $[H^+]$  but independent of sucrose concentration, and a independent of  $[H^+]$  but dependent on sucrose concentration. T is temperature in kelvins, numerically equivalent to  $^{\circ}C$  above absolute temperature, and e is the base of natural logarithms.

For sucrose inversion

$$K_T = \frac{1}{[H^+] \cdot t} \cdot \log \left( \frac{R_0 - R_{\infty}}{R_t - R_{\infty}} \right) \quad (2)$$

where  $K_T$  is the velocity constant from (1)

$R_0$  is angular rotation at time 0

$R_t$  is angular rotation at time t

$R_{\infty}$  is angular rotation when the sucrose is completely inverted

$[H^+]$  is the hydrogen ion concentration of the sucrose solution

t is time expressed in appropriate units.

Choice of time units depends upon convenience for computation, the number of significant figures used being adjusted to give the desired accuracy and precision in the final temperature estimates.

For a solution with a fixed pH, (2) becomes

$$K_T' = \frac{1}{t} \cdot \log \left( \frac{R_0 - R_{\infty}}{R_t - R_{\infty}} \right) \quad (3)$$

where  $K_T' = K_T [H^+]$

Equation (1) may be expressed as

$$\ln K_T' = \ln C - \frac{a}{T} \quad (4)$$

$$\text{or } \log K_T' = \log C - \frac{a'}{T} \quad (\text{Berthet, 1960}) \quad (5)$$

$$\text{from (5) } T = \frac{a'}{C' - \log K_T'} \quad (6)$$

Equation (6) provides a basis for determination of an unknown temperature  $T$ ,  $K_T$  being calculated using equation (3) and the inversion constants,  $a'$  and  $C'$ , from the results of inversion under controlled conditions in the laboratory (Appendix A.6). Time must be expressed in the same units in the calculation of both temperature and the inversion constants (Appendix A.6).

Further information on the theoretical background to the method is given by Pallman et al (1940a, 1940b), Pallman and Frei (1943), Schmitz and Volkert (1959), Berthet (1960), Schmitz (1964), Lee (1969), Jones (1971, 1972).

### 3. USE OF THE METHOD

#### 3.1 Use by other workers

The sucrose inversion method has now been used in several different countries and habitats for measurement of the mean temperature of air, soils or water (Lee, 1969; Jones, 1971, 1972). At least two versions of the method described by Pallman et al (1940a, 1940b) are in use in Britain, the first by workers at Levington Research Station (S. Larsen, Pers. comm.) and in Aberdeen University (Adu, 1968; Jones, 1971, 1972), the second, Berthet's (1960) version of the original method, by several university zoologists and workers at Merlewood.

Lawton (1969), in a study of the energetics of damselfly larvae, measured mean pond temperature at two water depths for two to four week periods over 1 - 2 years whereas Lee and Samuel (1976) measured stream temperatures in relation to forest cutting in west Virginia. Coulson et al. (1976) measured mean temperature at 1 cm depth in upland soils in northern England for each month during 2 years in a study of inter-relations of crane fly life cycle and altitude. In the United States, Murdock and Rickard (1956) and Rickard and Murdock (1963) used the Murdock (1956) version of the original Pallman method in studies of the relationships of vegetation and soils. In the first study, mean temperatures for one month in spring were obtained for 10 cm soil depth in *Festuca* grasslands in Washington whereas in the second, similar estimates were obtained for 10, 30 and 50 cm depth under desert shrubs in Nevada. Wood and Lawton (1973) estimated means at the top and bottom of a woodland litter layer for approximately monthly periods over two years as background work to measurement of soil mite respiration in the laboratory.

#### 3.2 Use by the authors

##### 3.2.1. Detailed study of one site

This study aimed to explore microclimatic differences with microrelief created by site preparation prior to afforestation and to test the feasibility of using the sucrose inversion method in measurement of mean soil temperature over one year (Bocock et al., in preparation, a). It was carried out at 200-250 m altitude in the northern Pennines near Carlisle in a newly-planted hill forest. Site preparation prior to planting involved draining and turf ploughing. Sucrose inversion tubes were inserted a) at 5 cm depth in the

tops of the bare ridges of overturned turf and in the undisturbed flat areas alongside the ridges in four 15 m x 15 m<sup>2</sup> plots b) at various depths to 50 cm in two ride areas. Main conclusions relevant to the method are as follows:

- a) Exponential mean temperatures for the year for 50 cm soil depth, where diurnal temperature range was expected to be very low, were 7.1 - 8.6°C, almost identical with arithmetic means estimated for the area from independent meteorological data.
- b) Standard deviations for the means were 0.39° and 0.40°C for respectively all ridges and all flat areas (n = 40 for each).
- c) Duplicate means for the temperature profiles differed by 0.04° - 0.87°C (mean 0.44°C, n = 12) with no obvious relation between the difference and soil depth and hence apparently no relation to temperature range which is known to decrease with depth.
- d) The method is sufficiently sensitive to detect significant differences between means of as little as 0.3° - 0.4°C with modest replication (n = 8 for each treatment).
- e) Differences in exponential means which were found, between ridge and flat area (1.5°C), between flat areas on different sides of the ridge (0.36°C), and between the steepness in decrease of mean with soil depth in the two sides, can be explained by differences in arithmetic means and in temperature range. Differences in the latter are known to be related to differences in exposure, vegetation, soil composition and soil moisture content.
- f) All the 104 tubes placed on the site were recovered, one was broken by forestry operations and four others were damaged but the contained solutions were usable. Tubes became tightly incorporated in the soil during the year and, because of this, two were broken during removal.
- g) One set of sucrose tubes can be used to estimate mean soil temperature over periods as long as one year and to demonstrate small temperature differences arising from site treatment, but the effects on the mean of changes in the site during the measurement period need to be considered. In this study, the initially bare ridges tended to erode, flatten, spread and become partially vegetated during the year but the effects of these changes were not measured.

### 3.2.2. Comparison of forty-eight sites over one year

This use of the inversion method was part of a larger study in which variability between soil and other characteristics of woodlands in the Lake District, Cumbria, were examined and the use of the variables for site classification explored (Bocock et al., in preparation, b). Mean temperature for four-weekly periods over one year was estimated in duplicate for the top and bottom of the litter layer and for 5 cm depth on each site. Because of problems of logistics, sampling of sites had to be staggered so that only about 12 sites were visited per week. Data for each site were analyzed using harmonic analysis (Bocock et al., 1974) and estimated mean temperatures for

the same standard reference dates for all sites were read off each harmonic model. Comparable data sets were thus produced for all 48 sites. Harmonic analysis was used also to estimate the range and timing of the temperature curve for the year at the litter surface and at 5 cm soil depth and also differences between values for selected temperature variables at these extreme depths. The relations between derived temperature variables and other site and soil variables were also examined. Main conclusions from this study relevant to the sucrose inversion method are as follows:

- a) The range of variability over the 48 sites in exponential mean temperature for the year at 5 cm soil depth,  $8.3^{\circ} - 10.4^{\circ}\text{C}$ , was similar to the range for arithmetic means estimated from independent meteorological data.
- b) Over all sites and samplings, the mean difference between duplicate mean temperatures was  $0.27^{\circ}\text{C}$  at the litter surface but only  $0.08^{\circ}\text{C}$  at 5 cm. These differences varied considerably between sites and both they and their standard deviations varied seasonally, being greatest before the tree canopy came into leaf in spring (Fig. 2). This is the time when the greatest diurnal soil temperature range occurs near the soil surface in Cumbrian woods (Bocock et al., in preparation, c).
- c) Several results emphasize the effect of temperature range as well as arithmetic mean as a determinant of exponential mean temperature. For example, on average over the 48 woods, the mean for the year was  $0.67^{\circ}\text{C}$  higher at the litter surface than at 5 cm depth and was highly correlated with percentage canopy cover.
- d) The results in general were easily explained in an ecologically meaningful way. For example, temperature and site aspect were not related significantly and mean temperature lapse rates with altitude were only  $0.15^{\circ}\text{C}$  (litter surface) and  $0.21^{\circ}\text{C}$  (5 cm depth)  $100\text{ m}^{-1}$  compared with the expected  $0.55^{\circ}\text{C}$   $100\text{ m}^{-1}$  estimated from arithmetic means (Gloyne, 1971). Both results appear to be effects of the forest canopy.
- e) Of the 2496 sucrose tubes placed on the litter surface and at 5 cm depth, 63 (2.5%) were broken or missing after exposure for four weeks, 41 of these being from the litter surface. Both tubes at a soil depth were broken or missing on only 14 occasions, 11 of them for the litter surface. These figures are considered satisfactory in the light of the heavy public pressure on Cumbria for recreation, the easy access of the public to many of the 48 sites used and use of some sites by cattle which appeared to cause many of the tube breakages.
- f) In general, the sucrose inversion method can be used successfully to describe the soil temperature regime of a large number of sites for periods in a year, and over the year as a whole.

### 3.2.3. Comparison of laboratory incubation areas

We estimated mean temperatures in each of three consecutive months amongst microbiological culture flasks in two incubation areas with partial temperature control. These areas were chosen to give temperatures varying around  $12^{\circ}$  and  $25^{\circ}\text{C}$  but, because of power cuts, temperature range was far greater than expected. In spite of this, mean temperatures were obtained which

were realistic in the light of knowledge of the frequency and duration of cuts and of the recorded maximum and minimum temperatures (Table 2). Variation between triplicate means averaged  $0.25^{\circ}\text{C}$  with a maximum of  $0.5^{\circ}\text{C}$ .

### 3.3 Accuracy, precision, sources of error

Much information on these topics can be found in work referred to elsewhere in this paper especially in Lee (1969) and Jones (1972).

In the literature, the term accuracy is often used as the difference between exponential mean and arithmetic mean temperatures. This appears undesirable as, according to theory, exponential means must be different from arithmetic means except where temperature is absolutely constant. Ideally, accuracy of the exponential mean should therefore be assessed by exposing sucrose solutions to temperature fluctuating in some standard way so that exponential means calculated from sucrose inversion and from an independent set of temperature measurements can be compared. This does not appear to have been done. An apparently less satisfactory alternative is to expose a solution to two constant temperatures consecutively for the same length of time and to compare exponential means calculated from theory with the measured means. This has been done by several workers, some details being given by Lee (1969) and Jones (1972). The results are difficult to assess because of differences in temperatures used and in calculation methods but it is clear that similar tests carried out by different workers can give mean temperatures which differ by more than  $1^{\circ}\text{C}$  and calculated and measured temperatures can differ by up to  $4^{\circ}\text{C}$ . The highest inaccuracies, as we have defined the term, occur where temperature range is around  $30^{\circ} - 35^{\circ}\text{C}$ . With the more prevalent field ranges of up to  $20^{\circ}\text{C}$ , maximum inaccuracies of about  $1.5^{\circ}\text{C}$  are possible. Accuracy of the method in constant temperature tests is commonly said to be about  $0.3^{\circ} - 0.5^{\circ}\text{C}$  (Lee, 1969; Jones, 1972). In our check of reproducibility of the constant C' (Appendix A.6) insertion of the first and lowest of the 11 C' estimates in equation (6) together with a  $K_T$  value for each of the 10 simultaneous runs gave incubator temperature estimates ranging from  $20.03^{\circ}$  to  $20.15^{\circ}\text{C}$  compared with  $19.98^{\circ}\text{C}$  measured using a mercury-in-glass thermometer.

Precision in exponential mean values depends to some extent on variation in temperature range in the location being examined. When the range is small, as in the soil or other situations well-buffered against temperature change, difference between duplicate mean temperatures averages less than  $0.5^{\circ}\text{C}$  and often less than  $0.1^{\circ}\text{C}$  and standard deviations of large samples are of the same order (Pallman and Frei, 1943; Lee, 1969; Lee and Samuel, 1976; Sections 3.2.1. and 3.2.2. above).

Some sources of error in the sucrose inversion method are mentioned elsewhere in this paper and some errors are discussed by other workers particularly Schmitz (1964); Lee (1969) and Jones (1972). Three interlinked aspects need particular emphasis:

- i) In our experience, the most important and easiest way of minimizing errors is by maintaining very strictly to standard procedures in all parts of the method. Simple routine checks for example, on timings of operations during polarimetry or on the temperature of thermos flasks, storage facilities or the polarimetry room reveal deviations from the standard.

- ii) Ideally, control solutions, which provide the  $R_0$  value for equation (6), should be treated exactly the same as solutions which are exposed in the field, except for the exposure, and sucrose tubes should be at field temperature when exposure begins and inversion should be stopped immediately at the end of the exposure period. Our procedures approach this ideal but assume that change in the control and field solutions is the same during each field visit, a reasonable assumption in the light of the degree of standardization adopted.
- iii) Other authors appear to use the rotation angle of each freshly made up buffered sucrose solution as the  $R_0$  value and to regard this as a constant throughout the life of the solution. This approach ignores the inversion of sucrose during a) warming up and transport of the solution to and from the field and b) storage at low temperature. These errors were examined in our multi-site study.

The rotation angle of the buffered sucrose solution was, on average,  $0.284^\circ$  higher before treatment a) than after it, however, use of the higher angle as  $R_0$  incurred an appreciable error in temperature estimates only if solutions were close to 10% inverted (Table 2). To test change in solutions during storage at  $-18^\circ\text{C}$ , we assumed that the control samples of any solution always inverted to the same extent during preparation and use of tubes in the field, and fitted regressions to mean rotation angle on time for the five solutions which were used longest (Fig. 3). All the regression coefficients were significantly different from zero ( $P < 0.05$ ). Two regressions were just significantly curved ( $F = 7.92$  with 1 and 4 d.f. and  $F = 6.04$  with 1 and 6 d.f. for departure from linear regression,  $0.01 < P < 0.05$ ), the other three showed no significant curvature. Linear regression coefficients ranged from  $-0.022$  to  $-0.043$  with no clear, strong relation with solution pH. Examination of errors caused by ignoring this change for a 28-day storage period (Table 3) indicates that the error is of importance only with solutions which are less than 50% inverted.

#### 4. ADVANTAGES AND DISADVANTAGES OF THE SUCROSE INVERSION METHOD

Many methods of measuring climatic variables such as temperature, involve keeping expensive and sophisticated equipment in the field. Complete protection of such equipment from the weather and from the effects of Man and other biological agents can be difficult and very expensive, particularly on exposed sites or on sites where the general public and animals have unrestricted access. Moreover, the use of protective measures may lead to alteration of site characteristics such as vegetation and hence affect the climatic variables which are being measured. Fencing is a good example of this. Such problems are avoided by using a method such as sucrose inversion which involves placing cheap, expendable, unprotected and relatively unobtrusive sensors in the field with minimum disturbance, whilst the associated capital equipment, the polarimeter, is used in the laboratory under ideal operating conditions for both instrument and operator. Polarimeters, suitable for use in the inversion method described here, cost no more than the cheapest temperature recorder. With care, these instruments can be used in measurements on less than 1 ml of sucrose solution which allows use of very small field tubes of low heat capacity (Appendix A.3). However, more expensive automatic polarimeters appear preferable for small volumes of solution (Jones, 1972).

Use of the sucrose inversion method in the laboratory or field avoids taking frequent temperature readings or tediously extracting and checking data from strip-charts. It also reduces the amount of expensive computer time used compared with that which is necessary when using strip-chart recorders and magnetic tape or punched paper tape loggers (Bocock, 1973). However, because the method integrates temperature, information which is of possible ecological value is lost. For example, it provides no details of periods of time with temperatures above or below certain critical values for organisms nor of the extreme temperatures reached. The inversion method is therefore complementary to some extent to the recording of temperature in detail or as maxima and minima.

The range of temperature experienced is not indicated by the method but, as we have stressed above (Section 3.2), the means from sucrose inversion are strongly dependent on range as well as on arithmetic mean temperature. By measuring the mean temperature for several sub-divisions of the year, as in our multi-site study (Section 3.2.2), a range estimate which relates to site exposure and soil thermal characteristics can be obtained.

Because the mean temperatures from sucrose inversion are exponential, relating these means simply to other meteorological data may be difficult. Murdock (1956) advises against such exercises suggesting instead that the exponential means ought to be related directly to values for other ecological variables. Other workers (Lee, 1969; Coulson et al., 1976) have successfully related exponential and arithmetic mean temperatures either using simple regressions or by assuming that diurnal temperature change is modelled well by a sine curve. Difficulties in interpretation and use may also occur if exponential means from, say, different soil depths or different sites are compared. Two exponential means may be exactly the same but based on different temperature curves, one having a high arithmetic mean but small range, the other a low arithmetic mean but a large range. Conversely, two exponential means may be different when their corresponding arithmetic means are the same but their ranges different (Section 3.2.1).

Disadvantages of the method indicated above are counterbalanced by three points. First, because the exponential mean reflects the temperature range as well as the mean, and range is affected by site exposure, vegetation cover and soil characteristics, it may be generally useful in classification of sites and soils (Bocock et al. in preparation, b) or in studies of the effects of land-use changes (Bocock et al. in preparation a). Second, the suggestion has been made by several authors (Pallman et al., 1940b; Deschusses, 1953; Berthet, 1960) that the exponential mean temperature may be a more appropriate measure than the arithmetic mean in ecological studies because rates of biological processes are related exponentially to temperature. The validity of this suggestion does not appear to have been tested thoroughly but correlations between exponential mean and plant growth have been reported (Adu, 1968; Jones, 1971). Third, any method of temperature measurement has some disadvantages or difficulties associated with its use. For the sucrose inversion method, we suggest that the advantages far outweigh the disadvantages.

## 6. SUMMARY

The general principles of the sucrose inversion method of measuring temperature are described briefly. The version of the method used at Merlewood Research Station is described very fully with emphasis on practical details necessary for its successful use.

Use of the method by other workers is summarized with emphasis on applications which are not reviewed by other authors. Use by the authors is outlined, including examination of differences in mean temperature for a year with microrelief and soil depth in a newly planted hill-forest, assessment of variation in temperature with site, soil depth and season in 48 woodlands, and measurement of mean temperature in laboratory studies where temperature varies. Advantages and disadvantages of the method, compared with other methods of measuring temperature, are discussed.

Our studies confirm that an accuracy of  $0.3^{\circ} - 0.5^{\circ}\text{C}$  can be obtained when temperature is virtually constant. Sensitivity is high. Significant differences of  $0.3^{\circ} - 0.5^{\circ}\text{C}$  between annual means were detected in one of our studies. As sucrose inversion responds exponentially to temperature, the means estimated are dependent on temperature range as well as arithmetic mean temperature and the method is therefore useful for summarizing temperature changes resulting from changes in land use and management.

## 7. ACKNOWLEDGEMENTS

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## APPENDIX - VERSION OF THE SUCROSE INVERSION METHOD USED AT MERLEWOOD

This version is a modification of the method described by Berthet (1960).

### A.1 Stock solutions

Four basic solutions are used, freshly distilled water being used for making up and for subsequent dilutions.

#### a) Sucrose - formaldehyde

Dissolve 750 g A.R. sucrose in 480 ml of distilled water with stirring and gentle warming to 30° - 40°C to encourage rapid solution. Cool to room temperature and add 20 ml of approximately 37% (w/v) A.R. formaldehyde solution as a preservative. The final volume is about 971 ml. Berthet (1960) recommended filtration of the sucrose-formaldehyde solution, presumably to remove any precipitated formaldehyde polymer. This filtration is time-consuming and messy because of the high viscosity of the solution. It may be avoided by using either fresh 37% formaldehyde solution showing no signs of polymerization or slightly polymerized 37% solution after filtration on Whatman number 1 or similar filter paper in a fume cupboard. Slightly polymerized formaldehyde solution is usable because the recommended formaldehyde concentration is more than three times that required to prevent microbial activity in the buffered sucrose solution (Sykes, 1958).

#### b) 0.5 M or M hydrochloric acid.

Conveniently made by diluting the contents (c. 50 ml) of one ampoule of BDH concentrated volumetric solution containing 36.46% (w/v) hydrochloric acid and 0.01% (w/v) mercuric chloride to 1 l (0.5 M) or 0.5 l (1M)

#### c) 0.2 M potassium chloride

14.91 g A.R. potassium chloride made up to 1 l.

#### d) M sodium acetate

82.04 g anhydrous A.R. sodium acetate made up to 1 l.

The sucrose-formaldehyde solution (a) is made up in lots of 2 - 4 l, the other solutions in 1 - 2 l lots. All four solutions are stored in the dark at 1°C.

### A.2 Buffered sucrose solution

The pH of the buffered sucrose solution is selected using a) the likely temperature mean and range b) the period of exposure c) the known rates of inversion of sucrose. For a), air and earth temperatures for the nearest Meteorological Office sites (Mochlinski, 1969, 1970; Meteorological Office, 1975a, 1975b), or for other neighbouring sites provide a useful guide if allowances are made for between-site differences in site exposure, altitude, aspect, vegetation, height or depth of measurement and soil

characteristics, all of which can affect temperature (Smith et al, 1964; Mochlinski, 1969, 1970; Meteorological Office, 1975b). For b) and c), curves given by Berthet (1960) relating half-life of sucrose to pH for solutions with pHs from 1.2 - 3.0 and temperatures at 5°C intervals from 0° - 40°C, are particularly useful.

In general, a pH should be used which leads to about 50% inversion of the sucrose during the chosen exposure period. Working well within the range of 10 - 90% inversion as suggested by Berthet (1960) and Jones (1972) avoids the low accuracy and precision of temperature estimates at one extreme when the difference between  $R_0$  and  $R_t$  (Equation 3) is small and, at the other, when  $R_t$  is changing slowly with temperature. In addition, the effect of temperature of the sucrose solution during polarimetry becomes increasingly important as a source of error as sucrose inversion approaches completion (Schmitz, 1964).

Although the inversion is extremely sensitive to pH, the pH of the buffered sucrose solution needs to be measured with a maximum accuracy of only  $\pm 0.05$  unit because it is used only to guide selection of an appropriate solution and not, as in Lee, (1969) or Jones (1971, 1972), in calculation of  $K_T$ , the velocity constant for each solution. Our calculation of this constant is described in Appendix A.6.

We have used two types of buffer solution, both at double the concentration recommended by the originators to allow for subsequent dilution with sucrose-formaldehyde solution:

- a) Potassium chloride - hydrochloric acid mixtures with pHs of 1.0 - 2.2 (Bower and Bates, 1955; Gomori, 1955) were used in our multi-site survey and in laboratory tests (Sections 3.2.1 and 3.2.2). The recommended 0.2 M stock solutions contain too much water to give pHs of below 1.3 in the buffered sucrose solution so we use 0.5 M or M stock hydrochloric acid.
- b) An acetic acid-sodium acetate-sodium chloride mixture (Walpole, 1914) was used in the single-site survey. This contained 400 ml M sodium acetate and 400 ml M hydrochloric acid  $l^{-1}$  and had a pH of about 2.5 before dilution with sucrose solution.

Other buffer mixtures with perhaps superior buffering capacity may be used, if their effects on sucrose inversion, other than maintenance of pH (Jones, 1972; Lee, 1969) are understood.

Following the practice of previous authors (Pallman et al., 1940a, 1940b; Berthet, 1960), we make our buffered sucrose solution from equal volumes of sucrose-formaldehyde and buffer solutions. To simplify calculation of temperature from the constants and polarimetric readings, sufficient buffered sucrose is made to allow the same solution to be used a) in the whole of a study, as in our single site survey (Section 3.2.1) or b) in a discrete part of study, for example in up to three of the monthly periods of our multi-site survey (Section 3.2.2).

The concentration of buffer components in our buffered sucrose solutions is that suggested by the originators of the buffer but, because the sucrose solution is very concentrated, less water is present than is

indicated in the original recipe. Mixture of double concentration buffer with sucrose-formaldehyde solution lowers its pH, only slightly for the acetate buffer but by up to 0.3 unit for the chloride buffer. This reduction could be largely avoided and achievement of a desired pH facilitated by reducing the sucrose concentration from the 38.62% (w/v) used in the present studies. Jones (1972) was able to use concentrations as low as 5.6% (w/v) by using a highly sensitive automatic polarimeter.

### A.3 Preparation of tubes of buffered sucrose solution

Various sizes and types of tubes have been used by other workers. Glass tubes are commonly used but many of our sites have stony soils so we use the more robust Nuclon N8 polystyrene tissue culture tubes (12 cm long, maximum diameters with and without a cap of 1.7 and 1.4 cm) supplied by Sterilin Ltd., Richmond, Surrey. The cap supplied, which is rigid plastic with a slightly flexible plastic insert, tends to leak when subjected to the rapid changes of temperature associated with handling and storage, so all inserts have been replaced by discs of a special rubber (Code no. 3414/MOD) 2.5 mm in thickness, supplied by the GRG division of Dunlop Ltd., Manchester. This seals the tubes very effectively and has shown no signs of deterioration after use for more than two years. Several of the bodies of the tubes have cracked during prolonged and frequent use. Such damage is detected before tubes are re-used in the field using a method described below. Each tube is numbered permanently by scratching and by using a waterproof marking ink which reacts with the plastic of the tube.

10 ml of sucrose solution is placed in each tube, ample for two separate sub-samples for polarimetry (Appendix A.5). This volume can be reduced to as little as 2 - 3 ml if a modern automatic polarimeter, which requires very small volumes of sugar solution is available (Jones, 1972). Reduction in tube size also increases the speed of response of the sucrose to fluctuating temperatures (Jones, 1972) and allows temperature measurement in smaller and more precisely defined locations.

Sucrose-formaldehyde and buffer solutions at about 1°C are mixed immediately before use to minimize inversion. The plastic tubes are filled rapidly using a Zipette semi-automatic pipette and stored immediately at approximately -18°C in buckets of 50% (w/v) aqueous ethylene glycol stained strongly with amaranth, a water-soluble food-colouring agent. This colour diffuses or is drawn into a faulty tube through even the finest of cracks and is immediately obvious against the white frozen sucrose solution. The temperature used for tube storage is not critical but must be low (Jones, 1972). As inversion proceeds slowly even below -6.5°C, the freezing point of our buffered sucrose solution, control tubes should be taken whenever tubes are used in the field and the storage time of all solutions, whether designated as controls or used in the field, should be kept to a minimum (Section 3.3 for discussion).

### A.4 Exposure of tubes of buffered sucrose solution at unknown temperatures

This description refers primarily to use of the method in the field but the general principles involved apply also to laboratory use.

The tubes are transported to and from the field in wide-mouthed vacuum

(Thermos) flasks (volume 1700 ml). Flasks for the outward journey are each charged with 900 ml of tap water at approximately 3°C and stored at 3°C. On the evening before use in the field, sucrose tubes are removed from the coloured 50% ethylene glycol at -18°C, rinsed quickly with tap-water at 0°C, and checked visually for leaks. About 20 tubes are placed in each flask which is covered loosely and stored overnight at 3°C. Flasks for the return journey are stored in an open condition at -18°C. Within 24 h of use, each is half filled with uncoloured 50% ethylene glycol at -18°C. Both "outward" and "return" flasks are stoppered tightly on the morning of use.

After the above treatment, the tubes of buffered sucrose solution are approaching field temperature by the time they reach the field. They are removed from the "outward" flask, shaken well, wiped, inserted in the desired location, and the tube number and the time are recorded, the latter to the nearest five minutes for field exposures of one month. More accurate timing may be necessary for shorter exposure periods. Immediately before or after tube insertion, one or more tubes are removed from the "outward" flask, their numbers are noted and they are placed in the "return" flask as controls. The need for these controls is discussed later (Section 3.3). The number of controls taken and timing of their transfer will depend on the temperature sensitivity (pH) of the sucrose solution used and on the pattern of insertion of tubes. In our single-site study, two controls were taken before and after insertion of tubes in the soil on each experimental area, whereas in our multi-site study, one control per visit per wood sufficed. After field exposure, tubes are wiped and placed in a "return" flask and the time recorded. Back in the laboratory, control and experimental tubes are transferred to coloured 50% ethylene glycol at -18°C. If cold storage space in the laboratory or for transport of tubes to and from the field is limited, the contents of tubes being collected after exposure could be made alkaline in the field or immediately on return to the laboratory. This would stop inversion completely (Guggenheim and Wiseman, 1960). We have not yet needed to apply this method.

When soil temperature is being measured, as in two of our examples, tubes are always inserted horizontally in holes made with a sharp cork-borer or similar corer of the same diameter as the tubes. At least two replicate tubes per soil depth are used as an insurance against accidental loss or damage of tubes and to give some indication of within-soil depth variability. Surface tubes are pinned to the soil with light wire hooks. Sub-surface tubes are conveniently placed in a standard pattern in the walls of an access hole no less than about 12 cm in diameter. Access holes should always be filled to simulate the layering, composition and degree of compaction of the undisturbed soil and thus to preserve, as far as possible, the thermal characteristics of the soil near the tubes. Preservation of the surface sod or natural litter layer is advisable for a similar reason and also to reduce the risk of human interference.

### A.3 Polarimetry

All the procedures described here are carried out in a room controlled at  $20^{\circ} \pm 1^{\circ}\text{C}$ . Standard equipment includes a Bellingham and Stanley P3 manually operated, optical polarimeter reading to  $0.002^{\circ}$  angular, a sodium vapour lamp as a light source and four 4 ml unjacketted polarimeter tubes (length 20 cm, bore 5 cm). Tubes 5 cm in length with capacities as low as 0.04 ml are

available but we prefer the larger size because wider tubes are filled far more easily and use of longer tubes increases the accuracy and precision of rotation readings and hence the sensitivity of the inversion method.

For routine polarimetry, all equipment is equilibrated at 20°C for several hours, then tubes of buffered sucrose solution are removed from the -18°C store in pairs and warmed for 10 minutes in a vigorously stirred water-bath at 20°C. Each tube, which is then at 20°C, is shaken vigorously, dried superficially and 4 ml of solution is transferred to a polarimeter tube held vertically in a burette clamp. Trapped bubbles are largely avoided by running the solution down the sides of the tube from a fine-tipped dropper-type pipette. The tubes are closed and examined by eye through one of the end windows. This check reveals not only bubbles but also turbidity and any faint red colouration indicative of previously undetected slight leaks in field tubes (Appendix A.3). The polarimeter tube is then placed in the polarimeter or, if this is already loaded, placed horizontally near the instrument, care being taken to avoid bumping or rapid movement of the tube. When the polarimeter field becomes clear and successive readings agree to within 0.02° angular, three final readings are taken. Samples are identified using the field tube number, each filled polarimeter tube being kept with its relevant field tube until polarimetry is completed. Rotation readings are recorded on previously prepared data sheets which include field tube numbers.

About 30 minutes elapses between thawing the solutions and taking the final polarimetric readings. In future, we intend to increase this time to one hour as occasionally we have had to wait considerably longer than 30 minutes for readings to rise to a stable level. This phenomenon is probably due to thermal mutarotation, adjustment of proportions of sugar isomers after a temperature change (Schmidt, 1964). If mutarotation creates measurement problems or if difficulty is experienced in standardizing the timing of operations, the sucrose solutions may be made slightly alkaline. This stops inversion and causes almost instantaneous mutarotation (Guggenheim and Wiseman, 1960) but involves a correction for dilution caused by added alkali.

Polarimetry tubes are emptied as soon as possible after use and stood vertically on absorbent tissue for at least five minutes to drain. Rinsing of a tube with fresh solution prior to re-filling is unnecessary where successive solutions have similar rotation readings but it is always advisable where solutions of an unknown degree of inversion are being examined. Tube end glasses are always rinsed with distilled water and dried before re-use.

The above apparently complicated procedure is simple in practice if operations are overlapped in a standard way by a two-man team, one person warming field tubes and filling and emptying the polarimeter tubes and the other reading the polarimeter. 60 - 80 samples are usually processed in a normal working day.

#### A.6 Determination and use of the inversion constants for each buffered sucrose solution

Using solutions with approximately the same sucrose concentration but buffered at different pHs, Fallman et al. (1940a) and Berthet (1960) determined constant  $a'$  in equation (6) independently under controlled conditions in the laboratory. The two sets of results were used by Berthet

(1960) to give a weighted mean of 5854 for  $a'$ . We use this value in our calculations as we also use the same sucrose concentration as Berthet in our buffered sucrose solution.

Re-arrangement of equation (6) and insertion of Berthet's value for  $a'$  gives

$$C' = \frac{5854}{T} + \log K_T' \quad (7)$$

which allows determination of  $C'$  for any buffered sucrose solution at some standard convenient temperature using  $K_T'$  estimated as in equation (3). Berthet (1960) averaged values of  $K_T'$  for intervals between removal of tubes from the constant temperature. We determine  $K_T'$  as the regression coefficient in

$$\log (R_t - R_{\infty}) = \log (R_0 - R_{\infty}) - K_T' t \quad (8)$$

a re-expression of equation (3). We collect data for this by keeping 10 - 20 sucrose tubes immersed in 50% ethylene glycol in a standard microbiological incubator at  $20^{\circ} \pm 0.5^{\circ}\text{C}$ . A thermometer of known accuracy (e.g. NPL certified) is kept in air alongside the tubes and read to  $0.1^{\circ}\text{C}$  once or twice daily, always before opening the inner glass door of the incubator if tubes are being removed. One tube is removed, frozen at  $-18^{\circ}\text{C}$  and the time recorded to the nearest minute, once or twice daily, every 1 - 3 days or every 3 - 5 days for sucrose solution pH's of respectively 1.0 - 1.5, 1.5 - 2.2 and above 2.2. Polarimetry is carried out within a few days and data are fed into a computer with a program which calculates  $K_T'$ ,  $C'$  and also the solution pH using  $C'$  and  $C'$  for a pH 1.21 solution (Berthet, 1960). Comparison of calculated pH and pH determined using a bench meter and glass plus reference electrodes usually indicates fairly good agreement and provides a useful check on the  $C'$  determination (Fig. 1).

The above method of calculating  $C'$  avoids direct pH measurement, which may be inaccurate for a variety of reasons, and substitutes temperature measurement which, has potentially very high accuracy. In a test with a pH 1.6 solution, 11 replicate determinations, 10 of them simultaneous, gave a  $C'$  value of  $18.6793 \pm 0.0040$  (S.D.). When the extremes of the 11  $C'$  estimates were used in calculation of field temperatures in the range  $0^{\circ} - 20^{\circ}\text{C}$ , differences of  $0.17^{\circ} - 0.26^{\circ}\text{C}$  were found between extreme estimates. From data in Schmitz and Volkert (1959) and Lee (1969) an error of about  $0.3^{\circ}\text{C}$  in the temperature estimate is given by an error of 0.02 in pH measurement, a minimum error according to Berthet (1960) and also in our opinion after comparisons of measurements made with different meter/electrode assemblies and different standard buffers. Correlation coefficients between  $\log (R_t - R_{\infty})$  and  $\log (R_0 - R_{\infty})$  are always 0.999 or better even for data which give slightly but clearly curved lines when plotted. Significant curvature occurs only when the pH of buffered sucrose solutions is below about 1.2. This agrees with the findings of Schmitz (1964). To minimize errors introduced by non-linearity caused by slight changes in solution pH during inversion (Lee, 1969) or by other factors,  $C'$  is best determined using the range of inversion experienced by the solutions in the field.

In the determination of  $C'$ , the time 0 polarimetric readings refer to the newly prepared buffered sucrose solution.  $R_0$ , the angle of rotation at time 0 for the tubes used in the field is determined as the mean of three polarimetric readings on each control tube (Appendix A.5).

$R_{\infty}$ , the angle of rotation at complete inversion, depends on the final concentrations of glucose and fructose and hence on the initial concentration of sucrose. We always use the concentration of sucrose used by Berthet (1960) and also his value of  $-9.10^\circ$  for  $R_{\infty}$ . We made two unsuccessful attempts to determine the constant directly. Closed tubes of solution kept at  $100^\circ\text{C}$  for eight hours, as suggested by Pallman et al (1940b) produced a strongly browned solution with a polarimetric reading of about  $-7^\circ$ . Similar solutions kept at  $20^\circ\text{C}$  for five months gave readings of  $-8.00^\circ$  to  $8.5^\circ$  with no significant change after a further month. These results, taken with those of Saprnov (1968), suggest that direct determination of  $R_{\infty}$  is impracticable and that one of the regression procedures discussed by Berthet (1960) or Jones (1972) must be used.

Temperature is estimated for a particular site by inserting the above constants and polarimetric readings for control and experimental solutions in equations (3) and (6), the control rotation angles acting as  $R_0$  values. A computer program is used which calculates a mean temperature for each tube as well as a mean for each soil depth.

Table 1. Temperature ( $^{\circ}\text{C}$ ) in two laboratory incubation areas during three separate months a) exponential means from the sucrose inversion method b) minimum and maximum values from mercury-in-glass thermometers.

Area	Month 1		Month 2		Month 3	
	a	b	a	b	a	b
"12 $^{\circ}$ "	12.6		12.3		12.2	
	12.6	11.2	12.3	11.5	12.2	8.7
	13.1	13.0	12.5	12.9	12.5	12.8
"25 $^{\circ}$ "	25.5		26.2		25.4	
	25.6	24.3	26.3	24.6	25.3	20.8
	25.4	29.0	26.1	29.5	25.3	28.5

Table 2. Error in temperature estimates obtained using the sucrose inversion method as a result of a) inversion during preparation of tubes for field use and transport to and from the field b) inversion during storage of solutions at  $-18^{\circ}\text{C}$  for 28 days.

		Error $^{\circ}\text{C}$	
% inversion <sup>1</sup>		at pH 1.20	at pH 2.50
a)	10	0.21 <sup>2</sup>	0.24 <sup>2</sup>
	20	0.10	0.12
	50	0.03	0.04
	90	0.01	0.01
b)	10	0.48 - 0.97 <sup>3</sup>	0.54 - 1.10 <sup>3</sup>
	20	0.23 - 0.45	0.26 - 0.51
	30	0.14 - 0.28	0.16 - 0.32
	40	0.10 - 0.20	0.11 - 0.22
	50	0.08 - 0.15	0.09 - 0.17
	90	0.02 - 0.05	0.03 - 0.05

1. Inversion assumed to occur in sucrose tubes fixed for measurement of field temperature.
2. Difference between temperatures calculated using rotation angles of freshly made up sucrose solution and of the same solution after use in the field as a control sample.
3. Difference between temperatures calculated using rotation angles of freshly made up solution and of the same solution after cold storage. Extreme values are calculated using extreme rates of inversion found in the multi-site study (Fig. 3).

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Fig. 1. Comparison of pHs a) measured using glass and reference electrodes and b) calculated from our own and Berthet's (1960) determinations of the velocity constants for sucrose inversion. The two pHs are the same along the line indicated.

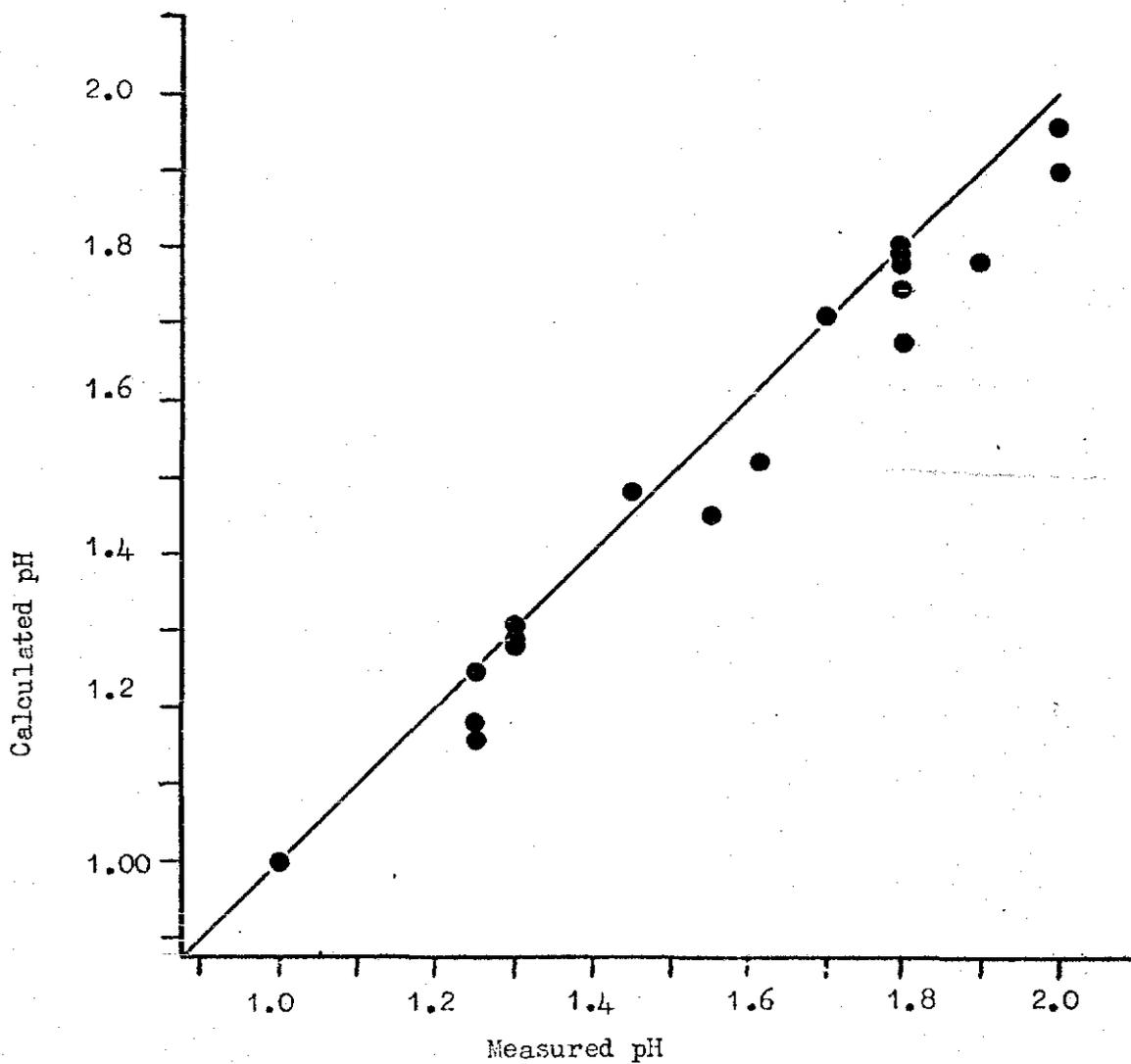
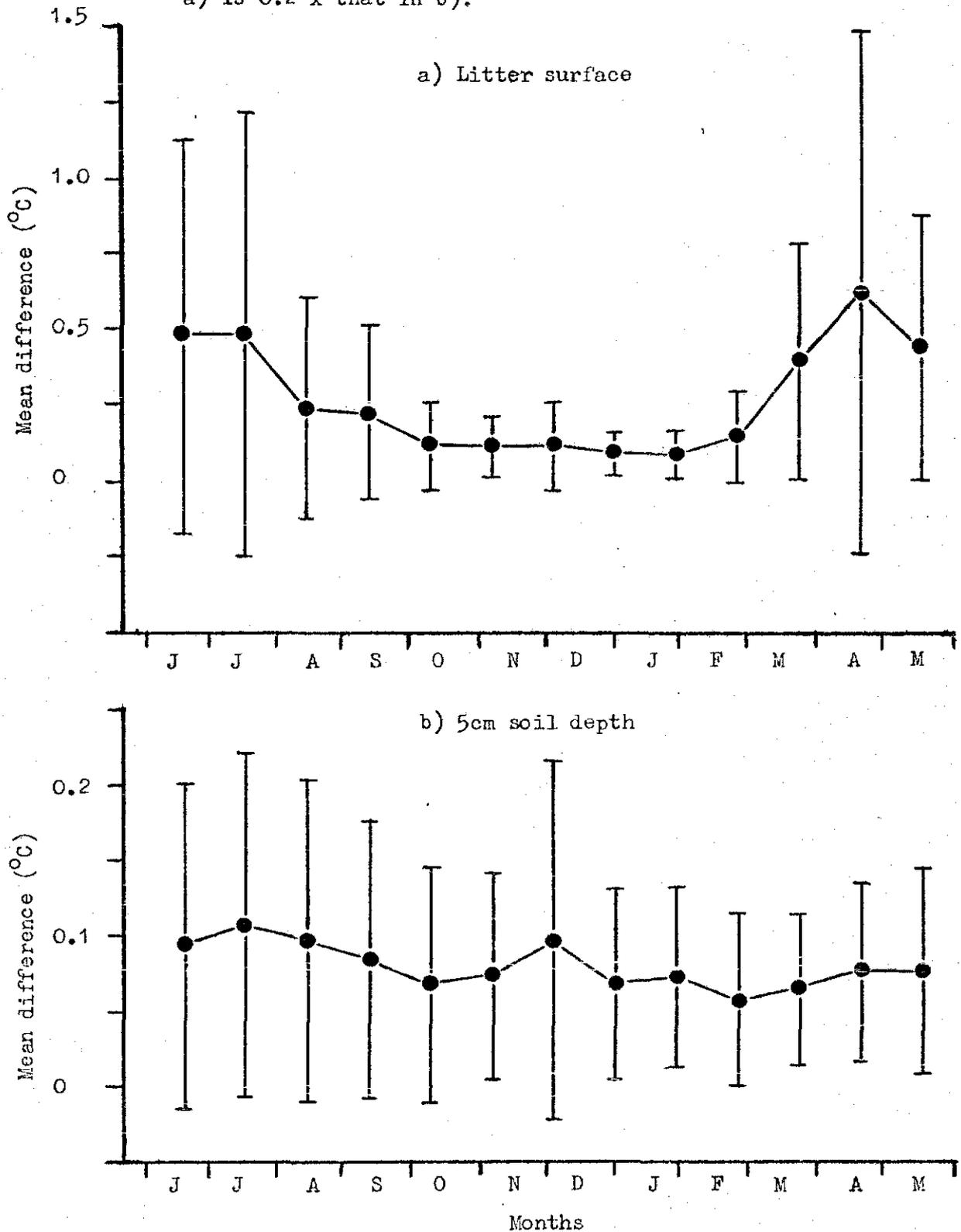




Fig. 2. Seasonal variation in the mean difference between duplicate exponential mean temperatures  $\pm$  S.D. Each mean is based on 43-47 differences and refers to the whole four weeks in which it is centred. Note that the vertical scale in a) is 0.2 x that in b).



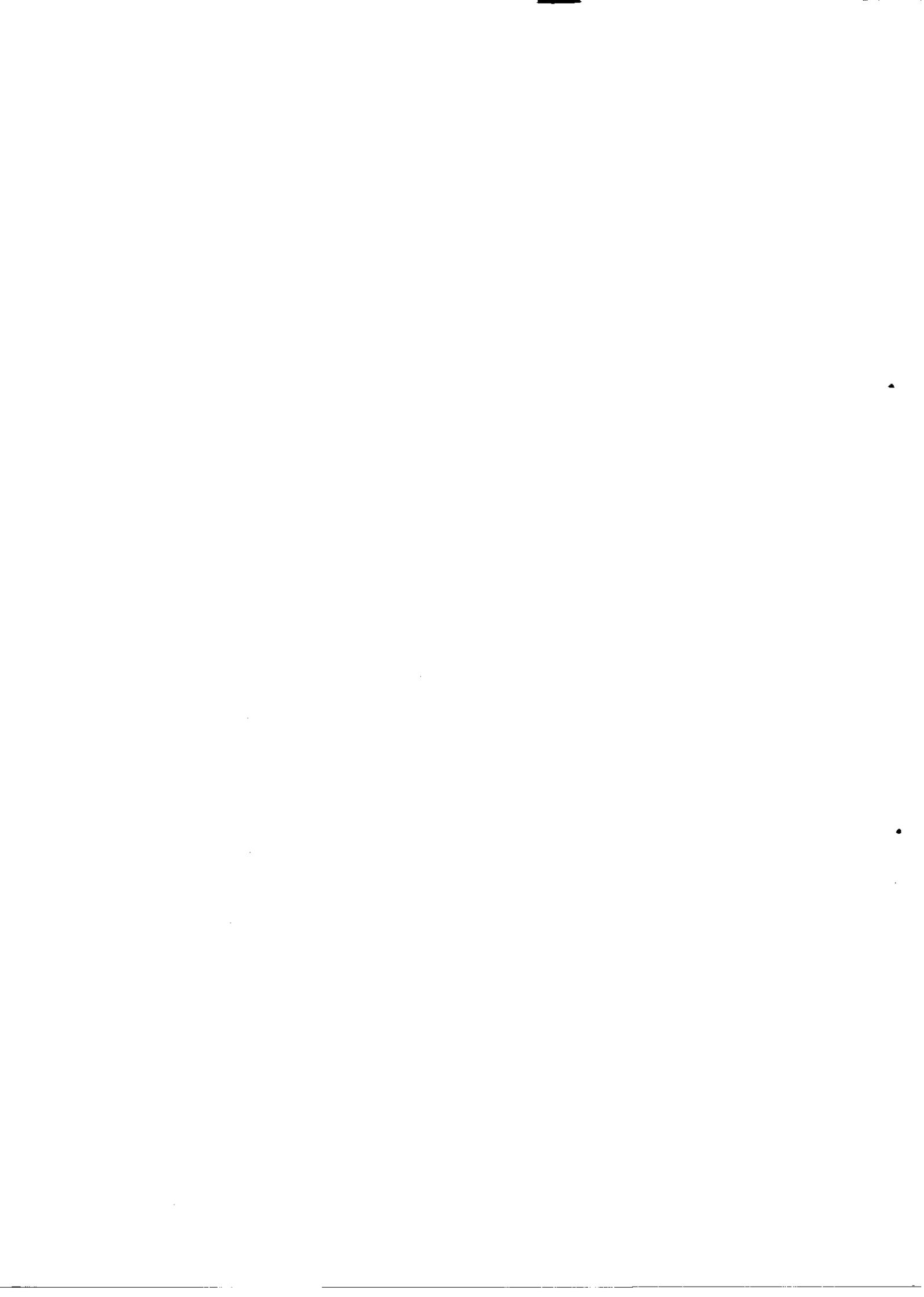
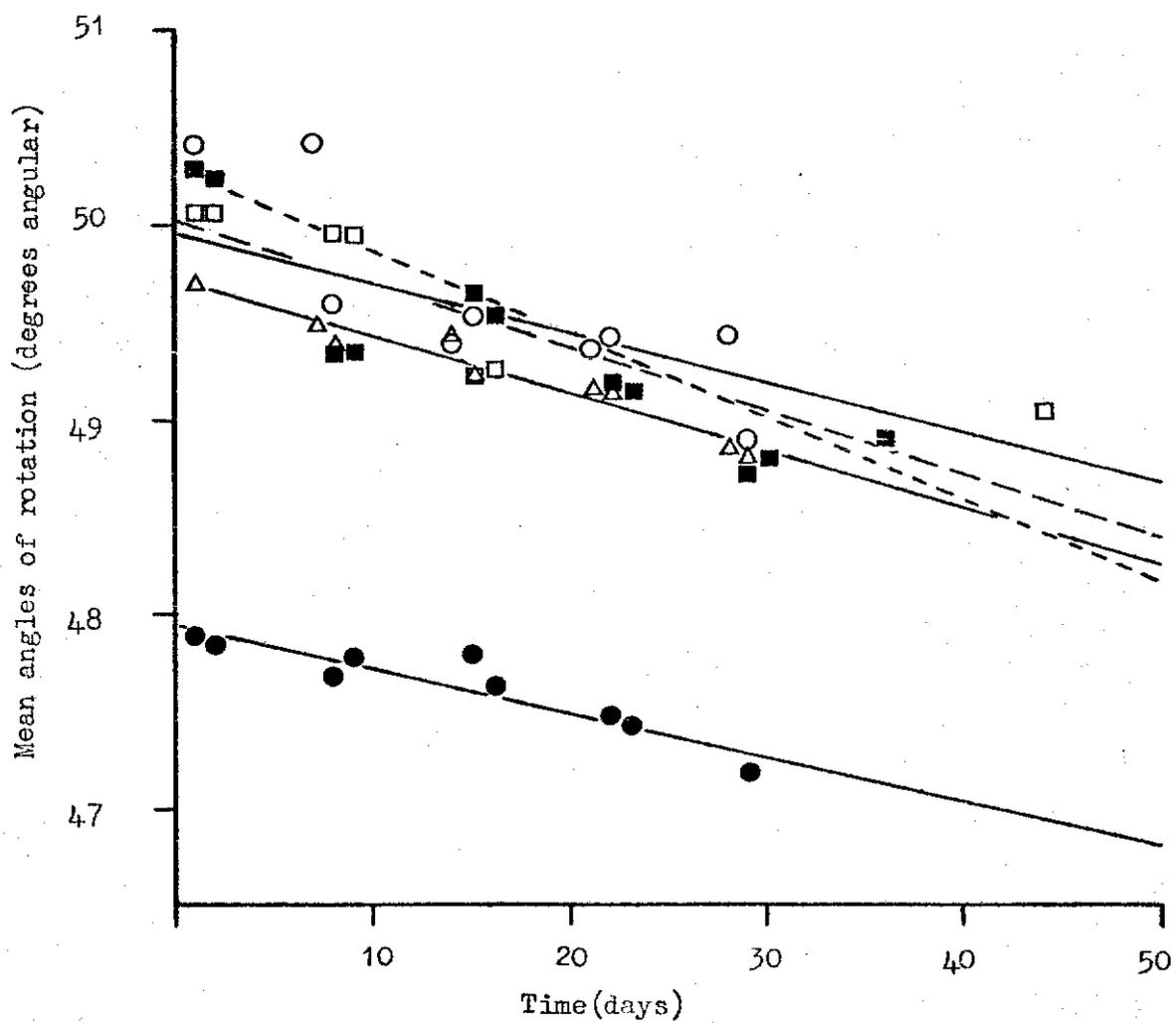


Fig. 3. Some examples of regression lines for the inversion of sucrose at  $-18^{\circ}\text{C}$  in solutions with the following pHs: 1.25 ( $\bullet$ — $\bullet$ ), 1.30 ( $\Delta$ — $\Delta$ ), 1.55 ( $\circ$ — $\circ$ ), 1.62 ( $\square$ — $\square$ ) and 1.90 ( $\blacksquare$ — $\blacksquare$ ).





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