THE UNIVERSITY OF MANITOBA

# THERMAL ADAPTATION IN SOME FILAMENTOUS FUNGI

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## ABSTRACT

An investigation was made of adaptive changes in a group of fungi subjected to prolonged successive transfer at the upper and lower temperature limits for growth. Adaptation in the form of a limited increase in linear growth rate occurred in the first six weeks of transfer in some organisms. In most cases, the changes induced were reversible. It was found that prolonged transfer was only possible at the temperature extremes on a limited range of nutrient concentration; outside this range some fungi showed a marked degeneration that was not reversible.

The resistance of the fungi to thermal shock was markedly affected by the transfer temperature and by the sucrose concentration of the temperature bath.

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# INTRODUCTION

# INTRODUCTION

The effect of environmental conditions upon the vegetative growth of filamentous fungi has been a continuing interest in the Department of Botany of the University of Manitoba. Much of the work in this field has concerned the interaction of temperature and nutrient concentration on the linear growth rate of fungal hyphae, and it was during work in this area that the possibility that the cultures being used were behaving differently when taken from different temperatures was realized.

This hysteresis appeared similar to thermal adaptation that has been studied in animals, but which has not so far been demonstrated clearly in plants.

The purpose of this study was to investigate the reactions of a group of fungi to successive transfer at high and low temperatures on a range of nutrient concentrations with particular attention to any adaptive changes in growth rate and their ability to withstand thermal shock.

# LITERATURE REVIEW

## LITERATURE REVIEW

### I. GENERAL

The term 'Adaptation' has been widely used in biology in a broad sense, e.g., Went (1958) has applied the term to explain three different responses of higher plants to external stimuli. First, meaning 'adjustment to environmental conditions or change in the response mechanism', phototropic response to change in light or dark conditions would be included under this definition. Secondly, it could be defined as 'a modification of the organism so as to fit better to its environment'. There are two possible ways by which an organism can undergo such modification (i) physiological adaptation due to fluctuating environmental conditions and (ii) adaptation in an evolutionary sense. Thirdly, the word used to mean 'the ability of a plant species to cope with the conditions of its internal and external environment'.

Adaptation, in the broad sense, was alternatively defined by Prosser (1958) to mean any response or alteration in an organism useful for its survival at one stage in its life cycle. According to this author, the variation or response which is beneficial at one stage of the life cycle persists beyond the period of its 'usefulness'.

Interest in the problem of adaptation in the animal kingdom is primarily centered around the physiological response of the organism to changes in temperature, light or oxygen tension. For the past

fifty years, many reports on thermal adaptation in lower and higher organisms, have appeared in literature (e.g., Cottle and Carlson, 1954; Prosser, 1958; Edney, 1964; Barnett, 1965).

Edney (1964) investigated the behaviour of woodlice and found they could adapt themselves to different temperatures. The process required a week or two to reach the greatest degree of acclimatization to the different temperatures, after which the treated organisms showed different lethal temperatures depending on the treatment to which they had been exposed.

Barnett (1965) showed that mice could be adapted to below zero temperature viz. $-3^{\circ}C$. The adapted mice were shown to increase heat production and ate about twice as much food per unit body weight as compared to control mice kept at $21^{\circ}C$.

Evidence for thermal adaptation in higher plants was reported by Fulton (1954) and was subsequently confirmed by Lomagin (1961), Levitt _et al._, (1961) and Yardwood (1961, 1964). Yardwood (1964) used a two-leaf method to study the adaptation and sensitization of bean leaves to heat treatment and came to the conclusion that the occurrance of adaptation or sensitization was dependent on the conditions of the experiment. He treated one of the two leaflets attached to the same stalk at a certain temperature for a definite period of time by immersing it in a water bath. After this treatment both the leaves were given a challenge exposure at a certain temperature. These experimental results provided evidence that the heat treated

leaf showed less injury to the challenge dose than the control leaflet. Furthermore, the adaptation of leaves to heat was favoured by a lag of 24 hours after the first treatment with a challenge dose of $50^{\circ}C$/120 seconds.

The phenomenon of acquired tolerance to cold has been demonstrated by Levitt et al., (1961) and Parker (1963). Levitt et al., (1961) found that a rise in protein -SH was induced by exposing the plant to hardening conditions. The authors suggested that increase in the quantity of -SH groups, activated the plant from dormant to growing stage.

Since the beginning of the 20th century, a large amount of data on bacterial adaptation have been accumulated. Barer (1951) suggested that the mechanism of adaptation of "Bact. lactis aerogenes" to a given concentration of streptomycin was not due to the occurrence of mutation as proposed by Demerec (1948), but was the result of a direct interaction between the cell and the antibiotic. Further evidence on this line was substantiated by the work of Abraham (1953), Saz and Martinez (1956), Drabble and Hinshelwood (1961) and Susumu et al., (1965).

The adaptation of the bacterial cell to utilize new sources of carbon was clearly demonstrated by Ryan (1952), Hinshelwood and Jackson (1950), Dean (1957), Grant and Hinshelwood (1964). Hinshelwood and Jackson (1950) showed that Bact. lactis aerogenes would grow on d-arabinose after serial transfers. This bacterium has been reported to utilize lactose as the sole source of carbon after prolonged culturing in a lactose medium (Richard and Hinshelwood, 1962). Further-

more, it was also noted that the activity of the adaptive enzyme beta-galactosidase had increased as a result of lactose adaptation.

There have been several reports on the thermal adaptation in bacteria. Weil (1899) investigated the resistance of spores of *Bacillus anthracis* grown at 18° and 37°C respectively, to moist heat treatments. The experimental results suggested that the heat resistance of spores increased with increasing incubation temperatures of the organism. Similar observations on bacterial thermal adaptation were made by Williams (1929), Elliker and Frazier (1938) and Williams and Robertson (1954).

## II. THERMAL ADAPTATION IN FUNGI

Early work of Humphrey and Siggers (1933) and Middleton (1943) suggested that the optimum temperature for growth of some fungi of the same species showed variation depending on their geographic distribution. This finding initiated the interest of Mycologists, and further investigations on ecological adaptations of fungi were started. Brown and Wood (1953) observed a similar broad correlation for example, *Glomerella rufomaculans* cultures obtained from South America, had growth optima about 5 degrees higher than those from North America.

Yarwood et al., (1954) provided further evidence that *Erysiphe cichoracearum* isolated from cantaloupes from the hot Imperial Valley of California showed an optimum growth at 25° to 28°C, while the same

species of fungus isolated from squash grown in the cool Colma district showed a temperature optimum of 15°C.

Recently, Yarwood (1962, 1963) has shown the phenomenon of temperature adaptation in rust. It was found that bean leaves infected for 1-5 days with *Uromyces phaseoli* when heated at 45°C for 2 to 20 seconds could tolerate up to four times as much challenge heat as twin leaves not previously treated. Accordingly, *U. phaseoli* was maintained on the two primary leaves. At various intervals, one of the infected leaves was treated in a water bath for 2 to 20 seconds at 45°C. The treated leaf was termed 'Adapted leaf' while the untreated one was called 'Unadapted leaf'. Within 8 days of inoculation, both the leaves were subjected to 45°C for 20 to 100 seconds or to 50°C for 1 to 10 seconds. It was found that optimum heat adaptation was obtained in leaves receiving the initial heat treatment of 45°C/5 seconds. Similarly, the heat adaptation was clearly detected in leaves which were subjected to therapy heat treatment after an interval of 6 to 11 hours of adaptation treatment. These results suggested a slight adaptation of rust to heat.

Thermal adaptation in yeast has been studied extensively. Zakharov (1962) used a hybrid strain $D_3$ obtained by hybridization of *Saccharomyces cerevisiae* and *Saccharomyces globolus* and showed that the adaptation of yeast cells to lower or higher temperature was accompanied by a change in the frequency of chromosome aberration. The strain $D_3$ was maintained at three different temperatures 10°C, 30°C and 38°C respectively and the three sublines D-Za ad 10,

D-Za ad 30 and D-Za ad 38 obtained by spontaneous mutation at the given temperatures were used for further studies. It was observed that the adaptation of the mutants either to lower or higher temperature led to an increase in the rate of multiplication of the sublines at 30°C. Furthermore, the subline D-Za ad 38 developed chromosome aberrations and gave rise to white and red sectors. The hybrid $D_3$ strain was heterozygous with respect to the red pigmentation of the colonies.

Loginova and Guzheva (1961) reported that a thermotolerant strain XII of Saccharomyces cerevisiae when cultured in a liquid medium with high concentration of sugar and kept at 40°C for a long time, showed a decrease in the rate of respiration accompanied by a decrease in the amount of G-6-P dehydrogenase. The authors also found a decrease in cytochrome activity and suggested it to be due to insufficient oxygen in the medium. Further work on thermotolerant yeast strains by Loginova and Verhovtseva (1963) suggested the need of an exogenous supply of amino acids for growth. It was suggested that the enzyme system capable of synthesizing amino acids in the strain was unable to operate at the high temperature.

## III. THE CORRELATION BETWEEN THE HEAT RESISTANCE AND INCUBATION TEMPERATURE OF FUNGI

Although a lot of information is available on the heat resistance of fungal spores (Williams et al., 1941; Yarwood, et al., 1954; and Erikson, 1955), very little is known about the correlation between

the thermal death point and the incubation temperature of the fungal mycelium. Findlay (1934) stated that fungi grown at higher temperatures were more resistant to heat than when grown at lower temperatures. It was generally assumed that heat resistance of fungi, like many other characters, was influenced by the growing conditions which affected the physiological state of the organism. Ling and Yu (1941) investigated the influence of incubation temperature on thermal resistance of Colletotrichum conidia and found that conidia kept at the optimal temperature for growth showed the highest thermal resistance.

Wallace and Tanner (1931) found that the cultures of Rhizopus nigricans and Aspergillus niger showed a greater heat resistance when treated in sugar solution than in distilled water. This protective action of the sugar was not shown in the cases of Mucor mirus Trichothecium sp., Alternaria solani, Penicillium brevicaule or Oidium lactis. Fay (1934) reported that the heat resistance of several bacteria increased with the increase in the concentration of sucrose or dextrose in the solution. However, it was found that for equimolal concentrations of sucrose and dextrose, the sucrose gave greater protective action. In no case was the role of sucrose in this action explained.

Hull (1939) noted that the presence of sucrose in the heating medium influenced the heat resistance of asci by rendering them more resistant to heat. Erikson (1952) found that spores suspended either in 1% sucrose or nutrient broth could tolerate heat treatment

at 100°C for 45 minutes as compared to those suspended in distilled water which showed a lethal point at 85°C for 5 minutes. It was suggested by this author that the high degree of heat resistance in sucrose or broth medium was due to the protective effect of colloidal material in the suspending medium.

## IV. OTHER ASPECTS OF ADAPTATION

The adaptation of animal, bacteria and yeast to unfamiliar growth substrates has been reported to be due to the induction of enzymes necessary for the utilization of the substrates. This is termed as the 'physiological' or 'non-hereditary' adaptation (Prosser, 1958).

Gottlieb *et al.*, (1950) investigated the ability of certain white-rot fungi to utilize lignin as the sole source of carbon. They noted that cultures of *Polyporus abietinus* and *Poria subacida* after repeated transfers on a basal medium containing 0.5% lignin and 0.1% glucose, were able to grow on a medium containing lignin as the sole source of carbon. Further evidence on this line was provided by Goodman (1950). He found that there was an increase in the amylase content in cultures of *Aspergillus flavus*, *Aspergillus terrens* and *Penicillum notatum* when they were transferred from sucrose to starch medium.

Karasevich (1958) observed that prolonged exposure of yeast to arabinose-containing medium accelerated the adaptation, while

In order to minimize temperature changes during the measurement period cultures were measured at temperatures close to the treatment temperatures. Linear growth of each of the fungus tested was measured daily for almost six months.

A set of five tubes at each temperature-nutrient combination, was used to calculate the average daily growth increment to the nearest 0.1 mm for each of the cultures tested. The curve of linear growth against temperature was plotted from these measurements.

## V. THE EFFECT OF TEMPERATURE CHANGE UPON LINEAR GROWTH

In order to study the effect of temperature change on the rate of linear growth, cultures maintained at 6°C, 15°C and 30°C respectively for 4 months were transferred to higher or lower temperatures. Cultures growing at 6°C were transferred to 15°C and 30°C, the cultures at 15°C were changed to 6°C and 30°C, while the cultures growing at 30°C were changed to 6°C and 15°C.

Preliminary investigation showed that only the cultures of *Diplocladium* sp. and *S. sclerotiorum* gave an appreciable amount of growth at 6°C, while the other two cultures gave very poor growth at this temperature. Cultures of *Diplocladium* sp. and *S. sclerotiorum* were, therefore, selected for this test. Thermal adaptation was looked for in alteration in the duration of the lag period and in the rate of linear growth.

frequent subculturing to the same medium prevented the process.

Tikhomirova (1960) investigated the induction of amylase in cultures of *Aspergillus oryzae*. He found a 20-fold increase in the synthesis of amylase in cultures grown on starch media as compared to those grown on glycerol as the carbon source.

The physiological or enzyme adaptation of fungi to different carbon substrates, was further confirmed by Karasevich (1962). A strain $SD_5$ of *Canida tripicalis* was shown to grow on the synthetic medium containing d-ribose as the sole source of carbon, without any noticeable lag period. Moreover, the cultures growing at higher d-ribose concentration, when transferred to a medium containing reduced amount of d-ribose (0.5%) showed an increased growth rate only after a long lag period. These results clearly demonstrated that (i) the original culture contained all the enzyme system necessary for the utilization of d-ribose and (ii) the transfer of the culture from a medium containing a higher concentration of sugar to a medium with a lesser amount did not give any appreciable increase in the rate of adaptation to this sugar.

Investigations on the adaptive metabolism of d-galactose in *Aspergillus nidulans* (Roberts, 1963) showed that only the wild type strain *bil* $w_3$ was able to utilize d-galactose as the sole source of carbon. It was found that the mutants *bil* $w_3$ *gal I* to *gal 5* were able to utilize glucose normally but failed to adapt to galactose. These mutants showed a defect in their enzymatic mechanism for carbohydrate metabolism.

It is indicated from the review given above that adaptation of fungi to different carbon sources in the synthetic medium is due to the regulation of the enzyme systems by induction. It is also noted that a more or less prolonged induction period is required before increased activity of the enzyme in question is seen.

# MATERIALS AND METHODS

## MATERIALS AND METHODS

### I. FUNGI

The following fungi were used in the study:

*Rhizoctonia solani* ($R_3$)*

*Diplocladium* sp. ($D_4$)*

*Sclerotinia sclerotiorum* (Lib.) de Bary ($S_8$)*

*Poria cocos* (Schw.) Wolf. ($P_2$)*

*Stereum purpureum* (Pers. ex Fr) Fr. ($S_2$)*

Cultures of above four fungi were obtained from the stock culture collection of the Botany Department of the University of Manitoba. Stock cultures were stored on potato-carrot medium (Langeron,1945) in a culture room at 12 $\pm$ 2°C.

### II. CULTURE MEDIA

A culture medium with the following composition was prepared:

| | |
|---|---|
| Sucrose | 50.0 g |
| Bacteriological peptone | 25.0 g |
| $KH_2PO_4$ | 10.0 g |
| $MgSO_4 \cdot 7H_2O$ | 2.5 g |
| Agar | 15.0 g |

This concentration was known as 5-normal (5N) and was diluted with a plain agar solution containing 15 g agar/1000 ml water to give

* Index number of culture.

1N, 1/5N and 1/125N media as required.

The measurements of growth rate were made of cultures grown on 5, 1/5 and 1/125 Normal sucrose peptone media.

III. TEMPERATURE EFFECTS

Hot air incubators with a variation $\pm$ 1°C were used in the experiments. In order to study the effect of continuous high and low temperatures on the linear growth,cultures were maintained at 6°C, 15°C and 30°C by successive transfers at 2 week intervals for approximately 6 months.

A culture grown at 15°C was taken as a standard to compare the rate of linear growth of cultures grown at 6°C and 30°C respectively.

IV. MEASUREMENT OF LINEAR GROWTH

Ryan *et al*., (1943) measured the rate of linear growth of fungi grown in tubes and found it to be a convenient and precise method to study the quantitative response of the organism to various external and internal factors. This was further substantiated by the findings of Cowing and Kelman (1964) that radial growth measured in Petri dishes were more variable than linear growth measurements in growth tubes. It was, therefore, decided to measure the rate of linear growth of fungi cultures in tubes.

The growth tubes 152 x 23 x 11.5 mm as suggested by Tompkins

and Gardiner (1935) were used for growing the cultures. The tubes were prepared according to the method of Gow (1958). Accordingly, 3 ml molten medium was added to each tube, the tube was plugged with cotton and sterilized in the autoclave at a pressure of 18 pounds and 121°C for 20 mins. The agar was allowed to solidify while the tubes were held horizontally on the inoculation bench. After solidification the tubes were kept inverted in order to prevent any condensate from dropping onto the agar. The tubes were then ready for inoculation.

A. INOCULATION PROCEDURE

A number one cork borer was sterilized by flame, and discs 4 mm in diameter were cut out from near the margin of vigorously growing fungal cultures. The tubes were inoculated by placing one of these discs with the mycelial mat downwards on the agar surface at the edge of the lip. Following inoculation, the cotton-plugged cultures tubes were sealed with "parafilm" to check evaporation, and were incubated horizontally.

Cultures of *Diplocladium* sp., *S. sclerotiorum*, *P. cocos* and *S. purpureum* used for the inoculation procedure were respectively 3, 4 6 and 11 days old to allow for their different growth rates.

B. MEASUREMENT

Linear growth of hyphae was measured daily from the edge of the fungal disc to the hyphae front with the use of a millimeter scale.

## VI. THERMAL DEATH POINT TEST

The apparatus used in determining the thermal death points of mycelia consisted of a constant-temperature water bath.

### A. THERMAL TREATMENT

The standard method of Ame (1915) as used by Ling and Yu (1941) for testing the thermal death point of spores was modified as follows:

The fungal cultures maintained at 6°C, 15°C and 30°C respectively for certain period of time were used to provide inocula for the test. Cultures were grown in petri dishes and incubated at 6°C, 15°C and 25°C. Cultures of P. cocos were also incubated at 30°C in addition to the above mentioned temperatures. Discs 4 mm in diameter were cut from the actively growing margin of the culture with a No.1 cork borer. The agar part of the disc was removed with a sterile scalper to give a mycelial mat approximately 1 mm thick. The disc was held by a fine needle and placed in hot water, or different concentrations of sucrose solution (1M - 4M) in a beaker which was kept inside the temperature-constant water bath at the desired temperature, with a maximum deviation of ± 0.2°C during the course of one experiment. The discs as described above were treated at 40°C, 45°C and 50°C for times varying from 1/2 min. to 30 mins. After the treatment the disc was removed from the hot water bath and cooled as quickly as possible by immersion in cold water. The treated disc was placed in a Petri dish, containing 1N sucrose peptone medium. It

was incubated at 25°C. Sets of 5 Petri dishes (90 x 15 mm) were incubated for each culture and each experiment was run in duplicate.

B. MEASUREMENT OF DIAMETER OF THE COLONY

The thermally shocked cultures were observed for a period of 3 weeks. A measurement of the diameter (mm) of the culture after 10 days was taken as the criterion of growth,was suggested by Ling and Yu (1941), but in the experiment under study, it was found more suitable to measure the diameter of the cultures after 3-7 days, depending on the initial growth rate of the species. The final measurement was taken 3 weeks after the first measurement to reveal any stunted growth due to the thermal treatment.

The mean of each set of measurements from the dishes was calculated and plotted against the treatment temperature. Since the effect of sub lethal thermal shock was to increase the incubation period and subsequent growth was normal the linear growth occurring in a standard time could be used as a measure of the degree of shock sustained. In a few cases stunting of the culture was still evident after 3 weeks and this was noted separately.

# EXPERIMENTAL RESULTS

## EXPERIMENTAL RESULTS

### I. THERMAL ADAPTATION

#### A. CHANGES IN LINEAR GROWTH RATE IN SERIAL TRANSFER AT DIFFERENT TEMPERATURES AND NUTRIENT CONCENTRATION

Experiments were carried out to study the thermal adaptation of filamentous fungi on the basis of linear growth. Although linear growth of filamentous fungi represents only the elongation of the apex, it is still considered as a convenient and uniform way for the study of the successive growth on one culture.

To study the effect of successive transfers at low and high temperatures and at different concentrations of sucrose peptone medium on the linear growth, each culture was grown on 5 N, 1/5 N, and 1/125 N sucrose peptone medium at $6^{o}$C., $15^{o}$C. and $30^{o}$C. The linear growth of the cultures at each temperature-nutrient combination was calculated as the average of the growth increment in a set of five tubes. The linear growth rate in mms/100 hrs of various organisms was calculated from the last 100 hours during the first week from the inoculation and are presented in Tables I - V.

1. *Rhizotonia solani* (Table I)

From Table I it can be seen that each of the cultures at $6^{o}$C show a marked increase in growth rate during the first four weeks but further development of this trend was prevented by the deterioration of the cultures to a point where no further growth occurred. The

process occurred in 2 months on 1/5 and 5 N media and took 3 months on the 1/125 N medium. It was noticed that this lethal deterioration was accompanied by a change in colour of the culture to a reddish brown (Fig. 1).

The linear growth rate of the cultures on media of different concentrations at 15°C is shown in Table I (columns 3-6). It will be noted that the phenomenon of deterioration appeared abruptly in 2-3 months as at 6°C.

The cultures grown on a 5 N medium at 30°C showed delay of deterioration as compared to those kept at lower temperatures. Some replicate lines stopped growth in 3-4 months and the mycelia turned reddish-brown, and by the 4th month all lines on the 5 N medium showed the same phenomenon. Cultures grown on 1/5 and 1/125 medium survived for 5-6 months without showing any sign of deterioration. The linear growth of the cultures maintained on 1/5 N medium slowed down during the first 4 months, but its growth rate later increased to the original level. There is no indication of adaptation as revealed by increasing growth rate.

2. *Diplocladium* sp. (Table II)

It will be seen from Table II that this fungus grew under these conditions of successive transfer at all combinations of nutrient concentration and temperature tested without showing any consistent change in growth rate.

It was noted that the form of the growth curves of cultures

growing on different nutrient concentrations at 15°C differed considerably (Fig.3). The rate at which the culture reached its maximum growth rate was proportional to the concentration of the medium.

3. *Sclerotinia sclerotiorum* (Table III).

The cultures growing on 5 N media at both 6° and 30°C showed marked increases (3- and 5-fold respectively) in linear growth rate during the first five weeks, and the increases were maintained during further transfers. The very slow growth and deterioration in the lines maintained on 1/125 N and 1/5 N media at 30°C was the exact reverse of the pattern of deterioration shown by *R. solani* (*cf.* Tables I and III).

It was observed that the color of the mycelium of the strain growing at 6°C on the 5 N concentration changed during the experiment to a greenish-yellow (Fig. 2). When studied under the dissecting microscope it could be seen that the colour was concentrated in enlarged, submerged hyphae that formed a diffuse form of appressorium on the base of the culture tube.

4. *Poria cocos* (Table IV).

There was virtually no growth of this fungus at 6°C. Only the cultures maintained on 5 N medium showed 1-2 mm of growth after 2-3 months. From the data shown in Table IV, it will be seen that *P. cocos* could not maintain on 1/125 N sucrose peptone medium at 15°C or 30°C for more than two months.

The cultures grown on 5 N medium showed a steady growth rate at both 15° and 30°C, whereas the 1/5 N line slowed down and eventually ceased linear growth in 5-6 months at 15°C.

The 1/5 line at 30°C maintained a fairly steady growth rate during the course of the experiment.

5. Stereum purpureum (Table V)

The growth rate of S. purpeum at 6°C on 1/125 N medium showed an adaptation in the first five weeks of transfers involving a 2- to 3-fold increase. No similar change was seen at other temperatures or nutrient concentrations. At the same concentration (1/125 N) at 30°C there was a rapid deterioration, with no growth occurring after 4 months, although initially the maximum growth rate at 30°C was on this concentration. A similar lack of correlation between initial growth rate and subsequent behaviour is seen on the 5 N concentration at 30°C where the culture with lowest growth rate persisted longest in serial transfer.

B. THE EFFECT UPON THE LINEAR GROWTH RATES OF PREVIOUSLY CONDITIONED CULTURES OF TRANSFER TO DIFFERENT TEMPERATURES.

In this experiment cultures that had been conditioned by serial transfer at various temperatures for approximately five months (described in the previous experiment) were transferred to other temperatures. The subsequent growth rates were compared with those of cultures that had been maintained continuously at those temperatures. Owing to

the very large number of permutations possible cultures of two fungi only were studied and of these only the most actively growing line at each temperature was used. The results for Diplocladium sp. are shown in Figures 4-15 and for S. sclerotiorum in Figures 16-24.

Diplocladium sp.: At 6°C the transferred cultures both showed a markedly slower growth rates than the cultures adapted to 6°C. The culture transferred from 30°C showed a very low growth rate during all the eleven weeks of the experiment. The culture transferred from 15°C had an initial rate of about half the adapted line but was approximately equal to it after three weeks of transfer.

When cultures were transferred from 6°C to 15°C (Figs. 8-11) the growth rate in successive weeks differed only erratically from that of the controls kept at 15°C, however, the culture transferred from 30°C was markedly slower than the untreated culture even after 11 weeks of successive transfer.

The results of transferring cultures adapted to 6°C and 15°C to 30°C confirmed the results of the first experiment where it was seen that the cultures grown on these concentrations (1/125 and 1/5 N) grew poorly at this temperature.

S. sclerotiorum : In Figure 16 it is seen that cultures transferred from 15°C and 30°C to 6°C showed a markedly reduced growth rate compared to those cultures that had been transferred continuously at that temperature. This difference had disappeared after six weeks of subculturing at 6°C (Fig. 17).

The results with this fungus of transferring cultures from 6°C and 30°C to 15°C (Figs. 19 and 21) are difficult to understand because in each case the transferred cultures showed a higher growth rate than the cultures that had been maintained continuously at that temperature, and the difference was still as marked after eleven weeks of subculturing.

At 30°C, the growth rate of the transferred cultures (Figs. 22-24) were low and there was no significant difference between the lines transferred from 6°C and 15°C

## II. THE EFFECT OF INCUBATION TEMPERATURE ON HIGH TEMPERATURE TOLERANCE

### A. THE RELATION BETWEEN HEAT RESISTANCE OF THE FUNGI AND INCUBATION TEMPERATURES AND TIME

Since the preliminary experiments did not show very marked effects of the adaptation of the fungi tested to low or high temperatures on the basis of linear growth, further experiments were conducted to investigate further aspects of thermal adaptation.

The influence of the incubation temperature on the heat resistance of the fungi were studied by subjecting inocula to hot water at different temperatures and for various durations of exposure as described previously. In order to reduce the factor of heat penetration to a practical minimum, small inocula 4 mm in diameter and 0.5 - 1 mm in thickness were used. During heat period, distilled water was stirred

by a fine glass rod to keep the temperature homogeneous. The particular concentration of sucrose peptone medium on which the fungi had shown the best growth in the earlier experiments was chosen for this study. S. sclerotiorum and S. purpureum were grown on 1/125 N medium; P. cocos on 5 N medium; and Diplocladium sp. on 1/5 N medium. As there was very little growth, some nutrient-temperature combinations were not tested in this experiment.

1. Diplocladium sp. (Table VI a-d, Figs. 25,26).

It will be seen from the results that prolonged transfer (4 months) at 6°C lowered the resistance of this fungus to thermal shock. There was no significant change in cultures that had been kept at 6°C for 2 months. Except in one case noted (Table VIa), the effect of the heat treatment was to increase the subsequent incubation period without affecting the rate of growth of the cultures.

2. S. sclerotiorum (Table VII a-d, Figs.27,28).

The results with this fungus are more uniform than those with Diplocladium sp. but otherwise similar. Compared with this fungus S. sclerotiorum was less affected by treatment at 40°C, but more sensitive at 45°C. In no case was there any after effect (retarded growth) seen in those cultures that survived the treatment.

3. P. cocos (Table VIII a-d, Figs.29-31).

Previous experiments showed this fungus to have a higher optimum temperature for growth than the others tested and this is reflected

in the fact that inocula could tolerate short (1 min) exposures to 50°C, and those pretreated at 30°C were stimulated by treatment at 40°C. The after effects noted in cultures that had been pre-treated at 30°C for 5 months are difficult to understand in view of the fact that no such effect was seen in those kept at this temperature for a shorter period.

4. *S. purpureum* (Table IX a-d, Figs. 32-34).

*S. purpureum* showed the highest temperature tolerance of the species tested, and like *P. cocos* the inocula taken from cultures adapted to the high temperature were stimulated by treatment at 40°C and 45°C. The ability to withstand short exposures to 50°C was also similar.

## B. HIGH TEMPERATURE TOLERANCE IN CONCENTRATED SUCROSE SOLUTIONS.

In view of the findings described in the literature review that some micororganisms showed an increased heat resistance in concentrated sugar solutions, preliminary experiments were conducted to see whether the addition of sucrose to the temperature bath used in experiments described in the previous section would alter the findings.

Two fungi only were studied and cultures of these that had been growing at the control temperature, 15°C, were treated in 0, 1M, 2M and 3M sucrose solutions. In addition, cultures of *Diplocladium* sp. that had been transferred continuously at 6° and 15°C were treated

in a saturated solution of sucrose (conc. > 3M).

The results are presented in Tables X a-c and XI a-c and in Figures 35-43.

Diplocladium sp.: The protective effect of the addition of sucrose is very marked and clearly seen in Figures 35 and 36. It is not clear why short exposures at 50°C should be more inhibitory in 3M sucrose than lower concentrations (Fig. 37).

The difference in response between cultures conditioned at 6° and 15°C to heat treatment in saturated sucrose solution is irregular. At the sub-lethal temperature of 40°C prolonging the treatment appears to annul the initial difference between the cultures. At 45°C there is no apparent difference, while at 50°C the difference in conditioning is again seen in the greater sensitivity of the culture previously maintained at 6°C.

S. sclerotiorum: This fungus appears to be more sensitive to heat treatment than Diplocladium sp., but the effect of increasing the sucrose concentration is just as marked. The lowest concentration used (1M) had little protective effect, but the 2M concentration was almost as effective as the highest used (3M).

TABLE I. Linear Growth Rate of Rhizotonia solani (mm./100 hrs.)

| Temp. and Nut. Conc. | Time (Weeks) of Successive Transfers | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 4 | 7 | 10 | 13 | 16 | 20 | 23 |
| $6^{\circ}$ C. | | | | | | | | |
| 1/125 N | 0.5 | 7 | 2 | 2 | X | | | |
| 1/5 N | 1 | 4.5 | 3 | X | | | | |
| 5 N | 0 | 4.5 | X | | | | | |
| $15^{\circ}$ C. | | | | | | | | |
| 1/125 N | 15.5 | 9.5 | 10.5 | 2.5 | X | | | |
| 1/5 N | 17.5 | 16.5 | 32.5 | X | | | | |
| 5 N | 20.5 | 29.5 | 23 | X | | | | |
| $30^{\circ}$ C. | | | | | | | | |
| 1/125 N | 34 | 28 | 40.5 | 38 | 33 | 35 | 37.5 | 47.5 |
| 1/5 N | 46.5 | 33 | 22.5 | 23.5 | 23 | 19.5 | 37.5 | 33.5 |
| 5 N | 43.5 | 46.5 | 41.5 | 37 | 32 | X | | |

X indicates culture deterioration.

TABLE II. Linear Growth Rate of *Diplocladium sp.* (mm./100 hrs.)

| Temp. and Nut. Conc. | Time (Weeks) of Successive Transfers | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 5 | 9 | 12 | 15 | 22 |
| 6° C. | | | | | | |
| 1/125 N | 4.5 | 0 | 1 | 3 | 0.5 | 1.5 |
| 1/5 N | 3 | 4.8 | 7 | 8.5 | 3 | 3.3 |
| 5 N | 1.5 | 0.5 | 1 | 2.3 | 1.5 | 2.5 |
| 15° C. | | | | | | |
| 1/125 N | 57.5 | 14 | 56.5 | 61.5 | 58.5 | 51.5 |
| 1/5 N | 70.5 | 68 | 44 | 68.5 | 66 | 72 |
| 5 N | 23.3 | 17.5 | 17.8 | 16.8 | 21 | 25.5 |
| 30° C. | | | | | | |
| 1/125 N | 1 | 0 | 5 | 4 | 10 | 4.5 |
| 1/5 N | 1 | 1.5 | 3 | 5 | 6.5 | 0.5 |
| 5 N | 10.3 | 4 | 4.5 | 4 | 18 | 10 |

TABLE III. Linear Growth Rate of Sclerotinia sclerotiorum (mm./100 hrs.)

| Temp. and Nut. Conc. | Time (Weeks) of Successive Transfers | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 5 | 9 | 13 | 19 | 21 |
| 6° C. | | | | | | |
| 1/125 N | 11.3 | 11.5 | 14 | 13 | 10.5 | 13.3 |
| 1/5 N | 13 | 11 | 19.5 | 8.5 | 6.8 | 7.5 |
| 5 N | 4 | 9.5 | 10.5 | 12.8 | 12 | 11.3 |
| 15° C. | | | | | | |
| 1/125 N | 22.5 | 24 | 32 | 31.5 | 33 | 32.8 |
| 1/5 N | 22.5 | 23 | 30 | 11.5 | 11.8 | 11.5 |
| 5 N | 21.5 | 30 | 21.5 | 27 | 26 | 17.5 |
| 30° C. | | | | | | |
| 1/125 N | 0.5 | 1.3 | 0.3 | 2.5 | - | - |
| 1/5 N | 0 | 1.3 | 0 | 2 | - | - |
| 5 N | 2.5 | 14.8 | 18.8 | 38 | 17 | 11.3 |

\- indicates growth rate of < 0.5 mm./100 hrs. and no recovery.

TABLE IV. Linear Growth Rate of *Poria cocos* (mm./100 hrs.)

| Temp. and Nut. Conc. | Time (Weeks) of Successive Transfers | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 5 | 9 | 14 | 21 | 23 |
| 15° C. | | | | | | |
| 1/125 N | 5.5 | 0 | 0 | | | |
| 1/5 N | 3.8 | 5.5 | 3 | 3 | 1.5 | 0 |
| 5 N | 5 | 7.5 | 11.5 | 7.3 | 7 | 4 |
| 30° C. | | | | | | |
| 1/125 N | 19 | 8 | 0 | 0 | | |
| 1/5 N | 30 | 39 | 29.5 | 28.8 | 24.5 | 26.5 |
| 5 N | 28.5 | 39.5 | 36 | 39 | 44.5 | 37.5 |

TABLE V. Linear Growth Rate of *Stereum purpureum* (mm./100 hrs.)

| Temp. and Nut. Conc. | Time (Weeks) of Successive Transfers | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 5 | 9 | 13 | 15 | 17 |
| 6° C. | | | | | | |
| 1/125 N | 2.5 | 4 | 6 | 7 | 7 | 6 |
| 1/5 N | 2.5 | 4 | 3 | 2 | 2.5 | 3.5 |
| 5 N | 0.8 | 0.5 | 0.5 | 1 | 0.5 | 0.5 |
| 15° C. | | | | | | |
| 1/125 N | 11 | 10.5 | 20.5 | 16.5 | 8.5 | 16.3 |
| 1/5 N | 7 | 7.5 | 5 | 4.5 | 6 | 11 |
| 5 N | 6 | 2.8 | 4.5 | 6 | 2.5 | 3.5 |
| 30° C. | | | | | | |
| 1/125 N | 10.5 | 4 | 0.5 | 3.5 | - | - |
| 1/5 N | 8 | 0 | 0.5 | 1 | 3.5 | 3.5 |
| 5 N | 5.3 | 1 | 2.5 | 5.3 | 6 | 3.5 |

- indicates no linear growth measurement taken.
(Very little growth after long time).

TABLE VI. The growth of mycelia of <u>Diplocladium</u> <u>sp</u>. after thermal shock at different temperatures and times of treatment. The data in the tables show the mean diameters of the mycelia in mm. (5 replicates) incubated at 25°C.
- indicates no test done, 0 indicates no growth.

(a). Prior treatment of culture - 2 months at 6°C.
Treatment solution - distilled water.

| Treatment Time (mins.) | Treatment Temperature °C. | | |
|---|---|---|---|
| | 40 | 45 | 50 |
| 0.5 | - | 17 | 0 |
| 1 | - | 13 | 0 |
| 2 | - | 11 | 0 |
| 3 | - | 1* | 0 |
| 4 | - | 0 | 0 |
| 5 | 26 | 0 | 0 |
| 10 | 24 | - | - |
| 15 | 16 | - | - |
| 20 | 14 | - | - |
| 25 | 10 | - | - |
| 30 | 4 | - | - |

* growth retarded, 8 mm. diameter in 3 weeks.

(b). Prior treatment of culture - 4 months at 6°C.
Treatment solution - distilled water.

| Treatment Time (mins.) | Treatment Temperature °C. | | |
|---|---|---|---|
| | 40 | 45 | 50 |
| 0.5 | - | 21 | 0 |
| 1 | - | 0 | 0 |
| 2 | - | 0 | 0 |
| 3 | - | 0 | 0 |
| 4 | - | 0 | 0 |
| 5 | 25 | 0 | 0 |
| 10 | 4 | - | - |
| 15 | 1* | - | - |
| 20 | 0 | - | - |
| 25 | 0 | - | - |
| 30 | 0 | - | - |

* growth retarded.

(c). Prior treatment of culture - at 15°C.
Treatment solution - distilled water.

| Treatment Time (mins.) | Treatment Temperature °C. | | |
|---|---|---|---|
| | 40 | 45 | 50 |
| 0.5 | - | 18 | 0 |
| 1 | - | 13 | 0 |
| 2 | - | 12 | 0 |
| 3 | - | 1 | 0 |
| 4 | - | 0 | 0 |
| 5 | 28 | 0 | 0 |
| 10 | 14 | - | - |
| 15 | 12 | - | - |
| 20 | 4 | - | - |
| 25 | 1 | - | - |
| 30 | 1 | - | - |

(d). Prior treatment of culture - 3 days at 25°C.
Treatment solution - distilled water.

| Treatment Time (mins.) | Treatment Temperature °C. | | | |
|---|---|---|---|---|
| | 40 | 45 | 50 | 55 |
| 0.5 | - | 27 | 0 | 0 |
| 1 | - | 19 | 0 | 0 |
| 2 | - | 18 | 0 | 0 |
| 3 | - | 1 | 0 | 0 |
| 4 | - | 0 | 0 | 0 |
| 5 | 40 | 0 | 0 | 0 |
| 10 | 2 | - | - | - |
| 15 | 18 | - | - | - |
| 20 | 6 | - | - | - |
| 25 | 2 | - | - | - |
| 30 | 2 | - | - | - |

TABLE VII The growth of mycelia of Sclerotinia sclerotiorum after thermal shock at different temperatures and times of treatment. The data in the tables show the mean diameters of the mycelia in mm. (5 replicates) incubated at 25°C.
- indicates no test done, 0 indicates no growth.

(a). Prior treatment of culture - 2 months at 6°C.
Treatment solution - distilled water.

| Treatment Time (mins.) | Treatment Temperature °C. | | |
|---|---|---|---|
| | 40 | 45 | 50 |
| 0.5 | - | 15 | 0 |
| 1 | - | 8 | 0 |
| 2 | - | 0 | 0 |
| 3 | - | 0 | 0 |
| 4 | - | 0 | 0 |
| 5 | 20 | 0 | 0 |
| 10 | 16 | - | - |
| 15 | 15 | - | - |
| 20 | 12 | - | - |
| 25 | 10 | - | - |
| 30 | 8 | - | - |

(b). Prior treatment of culture - 5 months at 6°C.
Treatment solution - distilled water.

| Treatment Time (mins.) | Treatment Temperature °C. | | |
|---|---|---|---|
| | 40 | 45 | 50 |
| 0.5 | - | 13 | 0 |
| 1 | - | 7 | 0 |
| 2 | - | 0 | 0 |
| 3 | - | 0 | 0 |
| 4 | - | 0 | 0 |
| 5 | 15 | 0 | 0 |
| 10 | 10 | - | - |
| 15 | 8 | - | - |
| 20 | 6 | - | - |
| 25 | 5 | - | - |
| 30 | 0 | - | - |

(c). Prior treatment of culture - at 15°C.
Treatment solution - distilled water.

| Treatment Time (mins.) | Treatment Temperature °C. | | |
|---|---|---|---|
| | 40 | 45 | 50 |
| 0.5 | - | 27 | 0 |
| 1 | - | 22 | 0 |
| 2 | - | 0 | 0 |
| 3 | - | 0 | 0 |
| 4 | - | 0 | 0 |
| 5 | 26 | 0 | 0 |
| 10 | 24 | - | - |
| 15 | 22 | - | - |
| 20 | 22 | - | - |
| 25 | 15 | - | - |
| 30 | 13 | - | - |

(d). Prior treatment of culture - 3 days at 25°C.
Treatment solution - distilled water.

| Treatment Time (mins.) | Treatment Temperature °C. | | | |
|---|---|---|---|---|
| | 40 | 45 | 50 | 55 |
| 0.5 | - | 27 | 0 | 0 |
| 1 | - | 21 | 0 | 0 |
| 2 | - | 0 | 0 | 0 |
| 3 | - | 0 | 0 | 0 |
| 4 | - | 0 | 0 | 0 |
| 5 | 27 | 0 | 0 | 0 |
| 10 | 24 | - | - | - |
| 15 | 23 | - | - | - |
| 20 | 21 | - | - | - |
| 25 | 17 | - | - | - |
| 30 | 12 | - | - | - |

TABLE VIII. The growth of mycelia of Poria cocos after thermal shock at different temperatures and times of treatment. The data in the tables show the mean diameters of the mycelia in mm. (5 replicates) incubated at 25°C.
- indicates no test done, 0 indicates no growth.

(a). Prior treatment of culture - at 15°C.
Treatment solution - distilled water.

| Treatment Time (mins.) | Treatment Temperature °C. | | |
|---|---|---|---|
| | 40 | 45 | 50 |
| 0.5 | - | - | 1 |
| 1 | - | - | 0 |
| 2 | - | - | 0 |
| 3 | - | - | 0 |
| 4 | - | - | 0 |
| 5 | 7 | 3 | 0 |
| 10 | 2 | 2 | - |
| 15 | 1 | 0 | - |
| 20 | 1 | 0 | - |
| 25 | 2 | 0 | - |
| 30 | 3 | 0 | - |

(b). Prior treatment of culture - 7 days at 25°C.
Treatment solution - distilled water.

| Treatment Time (mins.) | Treatment Temperature °C. | | | |
|---|---|---|---|---|
| | 40 | 45 | 50 | 55 |
| 0.5 | - | - | 1 | 0 |
| 1 | - | - | 0 | 0 |
| 2 | - | - | 0 | 0 |
| 3 | - | - | 0 | 0 |
| 4 | - | - | 0 | 0 |
| 5 | 13 | 4 | 0 | 0 |
| 10 | 8 | 2 | - | - |
| 15 | 7 | 0 | - | - |
| 20 | 2 | 0 | - | - |
| 25 | 2 | 0 | - | - |
| 30 | 1 | 0 | - | - |

(c). Prior treatment of culture – 3 months at 30°C.
Treatment solution – distilled water.

| Treatment Time (mins.) | Treatment Temperature °C. | | |
|---|---|---|---|
| | 40 | 45 | 50 |
| 0.5 | - | - | 12 |
| 1 | - | - | 8 |
| 2 | - | - | 0 |
| 3 | - | - | 0 |
| 4 | - | - | 0 |
| 5 | 27 | 12 | 0 |
| 10 | 31 | 3 | - |
| 15 | 37 | 0 | - |
| 20 | 36 | 0 | - |
| 25 | 35 | 0 | - |
| 30 | 18 | 0 | - |

(d). Prior treatment of culture – 5 months at 30°C.
Treatment solution – distilled water.

| Treatment Time (mins.) | Treatment Temperature °C. | | |
|---|---|---|---|
| | 40 | 45 | 50 |
| 0.5 | - | - | 5* |
| 1 | - | - | 1* |
| 2 | - | - | 0 |
| 3 | - | - | 0 |
| 4 | - | - | 0 |
| 5 | 24 | 4* | 0 |
| 10 | 29 | 1* | - |
| 15 | 30 | 0 | - |
| 20 | 24 | 0 | - |
| 25 | 24 | 0 | - |
| 30 | 20 | 0 | - |

* indicates diameter measurement taken 10 days after the treatment. These cultures showed retarded growth.

TABLE IX. The growth of mycelia of Stereum purpureum after thermal shock at different temperatures and times of treatment. The data in the tables show the mean diameters of the mycelia in mm. (5 replicates) incubated at 25°C.
- indicates no test done, 0 indicates no growth.

(a). Prior treatment of culture - 2 months at 6°C.
Treatment solution - distilled water.

| Treatment Time (mins.) | Treatment Temperature °C. | | |
|---|---|---|---|
| | 40 | 45 | 50 |
| 0.5 | - | - | 16 |
| 1 | - | - | 10 |
| 2 | - | - | 0 |
| 3 | - | - | 0 |
| 4 | - | - | 0 |
| 5 | 23 | 15 | 0 |
| 10 | 20 | 4 | - |
| 15 | 19 | 0 | - |
| 20 | 16 | 0 | - |
| 25 | 12 | 0 | - |
| 30 | 10 | 0 | - |

(b). Prior treatment of culture - 5 months at 6°C.
Treatment solution - distilled water.

| Treatment Time (mins.) | Treatment Temperature °C. | | |
|---|---|---|---|
| | 40 | 45 | 50 |
| 0.5 | - | - | 18 |
| 1 | - | - | 15 |
| 2 | - | - | 0 |
| 3 | - | - | 0 |
| 4 | - | - | 0 |
| 5 | 25 | 20 | 0 |
| 10 | 19 | 6 | - |
| 15 | 15 | 0 | - |
| 20 | 16 | 0 | - |
| 25 | 13 | 0 | - |
| 30 | 9 | 0 | - |

(c). Prior treatment of culture - at 15°C.
Treatment solution.

| Treatment Time (mins.) | Treatment Temperature °C. | | |
|---|---|---|---|
| | 40 | 45 | 50 |
| 0.5 | - | - | 18 |
| 1 | - | - | 15 |
| 2 | - | - | 0 |
| 3 | - | - | 0 |
| 4 | - | - | 0 |
| 5 | 20 | 14 | 0 |
| 10 | 20 | 8 | - |
| 15 | 16 | 0 | - |
| 20 | 15 | 0 | - |
| 25 | 8 | 0 | - |
| 30 | 7 | 0 | - |

(d). Prior treatment of culture - 10 days at 25°C.
Treatment solution - distilled water.

| Treatment Time (mins.) | Treatment Temperature °C. | | | |
|---|---|---|---|---|
| | 40 | 45 | 50 | 55 |
| 0.5 | - | - | 23 | 0 |
| 1 | - | - | 23 | 0 |
| 2 | - | - | 16 | 0 |
| 3 | - | - | 15 | 0 |
| 4 | - | - | 15 | 0 |
| 5 | 25 | 23 | 13 | 0 |
| 10 | 26 | 27 | - | - |
| 15 | 28 | 27 | - | - |
| 20 | 30 | 31 | - | - |
| 25 | 32 | 24 | - | - |
| 30 | 32 | 22 | - | - |

TABLE X. The growth of mycelia of *Diplocladium sp.* after thermal shock at different temperatures and times of treatment. The data in the tables show the mean diameters of the mycelia in mm. (5 replicates) incubated at 25°C.
- indicates no test done, 0 indicates no growth.

(a). Prior treatment of culture - 5 months at 15°C.
Treatment solution - 1 M sucrose solution.

| Treatment Time (mins.) | Treatment Temperature °C. | | |
|---|---|---|---|
| | 40 | 45 | 50 |
| 0.5 | - | - | 38 |
| 1 | - | - | 28 |
| 2 | - | - | 0 |
| 3 | - | - | 0 |
| 4 | - | - | 0 |
| 5 | 36 | 20 | 0 |
| 10 | 34 | 16 | - |
| 15 | 34 | 0 | - |
| 20 | 35 | 0 | - |
| 25 | 32 | 0 | - |
| 30 | 28 | 0 | - |

(b). Prior treatment of culture - 5 months at 15°C.
Treatment solution - 2 M sucrose solution.

| Treatment Time (mins.) | Treatment Temperature °C. | | |
|---|---|---|---|
| | 40 | 45 | 50 |
| 0.5 | - | - | 40 |
| 1 | - | - | 31 |
| 2 | - | - | 0 |
| 3 | - | - | 0 |
| 4 | - | - | 0 |
| 5 | 40 | 28 | 0 |
| 10 | 38 | 16 | - |
| 15 | 38 | 0 | - |
| 20 | 35 | 0 | - |
| 25 | 34 | 0 | - |
| 30 | 28 | 0 | - |

(c). Prior treatment of culture - 5 months at 15°C.
Treatment solution - 3 M sucrose solution.

| Treatment Time (mins.) | Treatment Temperature °C. | | |
|---|---|---|---|
| | 40 | 45 | 50 |
| 0.5 | - | - | 17 |
| 1 | - | - | 15 |
| 2 | - | - | 10 |
| 3 | - | - | 8 |
| 4 | - | - | 8 |
| 5 | 40 | 30 | 2 |
| 10 | 40 | 28 | - |
| 15 | 35 | 27 | - |
| 20 | 33 | 28 | - |
| 25 | 34 | 25 | - |
| 30 | 30 | 24 | - |

TABLE XI. The growth of mycelia of Sclerotinia sclerotiorum after thermal shock at different temperatures and times of treatment. The data in the tables show the mean diameters of the mycelia in mm. (5 replicates) incubated at 25°C.
- indicates no test done, 0 indicates no growth.

(a). Prior treatment of culture - 6 months at 15°C.
Treatment solution - 1 M sucrose solution.

| Treatment Time (mins.) | Treatment Temperature °C. | | |
|---|---|---|---|
| | 40 | 45 | 50 |
| 0.5 | - | 30 | 0 |
| 1 | - | 28 | 0 |
| 2 | - | 5 | 0 |
| 3 | - | 0 | 0 |
| 4 | - | 0 | 0 |
| 5 | 29 | 0 | 0 |
| 10 | 28 | - | - |
| 15 | 28 | - | - |
| 20 | 20 | - | - |
| 25 | 19 | - | - |
| 30 | 15 | - | - |

(b). Prior treatment of culture - 6 months at 15°C.
Treatment solution - 2 M sucrose solution.

| Treatment Time (mins.) | Treatment Temperature °C. | | |
|---|---|---|---|
| | 40 | 45 | 50 |
| 0.5 | - | 38 | 30 |
| 1 | - | 36 | 14 |
| 2 | - | 36 | 0 |
| 3 | - | 24 | 0 |
| 4 | - | 20 | 0 |
| 5 | 40 | 15 | 0 |
| 10 | 39 | - | - |
| 15 | 39 | - | - |
| 20 | 38 | - | - |
| 25 | 38 | - | - |
| 30 | 38 | - | - |

(c). Prior treatment of culture - 6 months at 15°C.
Treatment solution - 3 M sucrose solution.

| Treatment Time (mins.) | Treatment Temperature °C. | | |
|---|---|---|---|
| | 40 | 45 | 50 |
| 0.5 | - | 40 | 36 |
| 1 | - | 38 | 26 |
| 2 | - | 38 | 0 |
| 3 | - | 32 | 0 |
| 4 | - | 31 | 0 |
| 5 | 43 | 26 | 0 |
| 10 | 43 | - | - |
| 15 | 40 | - | - |
| 20 | 41 | - | - |
| 25 | 38 | - | - |
| 30 | 36 | - | - |

TABLE XII. The growth of mycelia of Diplocladium sp. after thermal shock at different temperatures and times of treatment. The data in the tables show the mean diameters of the mycelia in mm. (5 replicates) incubated at 25°C.
- indicates no test done, 0 indicates no growth.

(a). Prior treatment of culture - 3 months at 15°C.
Treatment solution - saturated sucrose solution, (concentration = < 3 M).

| Treatment Time (mins.) | Treatment Temperature °C. | | |
|---|---|---|---|
| | 40 | 45 | 50 |
| 0.5 | - | - | 36 |
| 1 | - | - | 35 |
| 2 | - | - | 34 |
| 3 | - | - | 24 |
| 4 | - | - | 18 |
| 5 | 25 | 31 | 17 |
| 10 | 28 | 30 | - |
| 15 | 28 | 24 | - |
| 20 | 31 | 21 | - |
| 25 | 30 | 21 | - |
| 30 | 32 | 19 | - |

(b). Prior treatment of culture - 2 months and 19 days at 6°C.
Treatment solution - saturated sucrose solution, (concentration = < 3 M).

| Treatment Time (mins.) | Treatment Temperature °C. | | |
|---|---|---|---|
| | 40 | 45 | 50 |
| 0.5 | - | - | 17 |
| 1 | - | - | 12 |
| 2 | - | - | 10 |
| 3 | - | - | 10 |
| 4 | - | - | 6 |
| 5 | 44 | 30 | 4 |
| 10 | 42 | 29 | - |
| 15 | 42 | 25 | - |
| 20 | 36 | 25 | - |
| 25 | 34 | 24 | - |
| 30 | 32 | 25 | - |

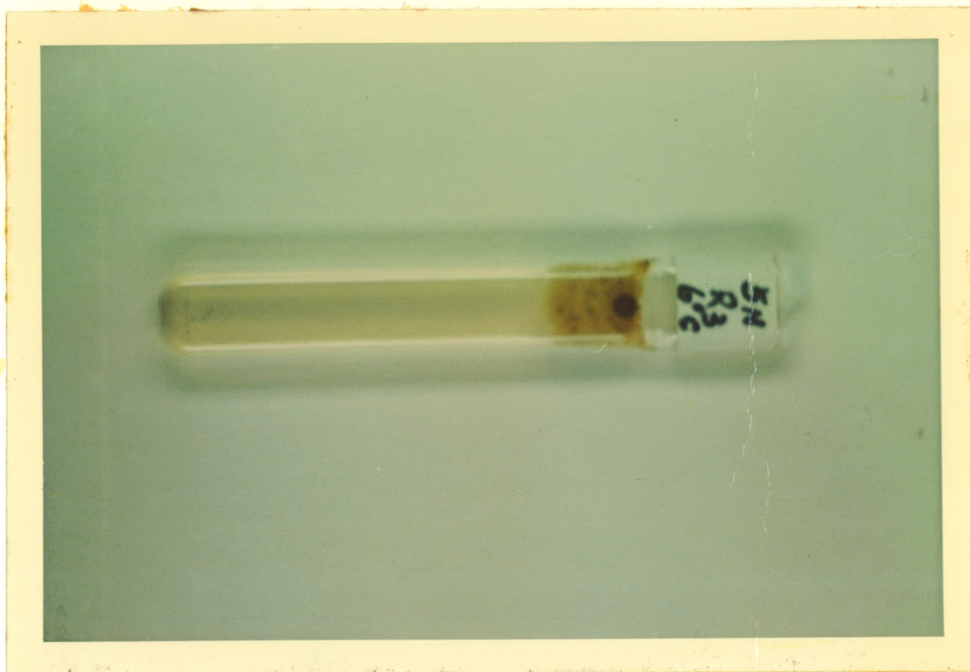


FIGURE 1. The deteriorated culture of *Rhizoctonia solani* at 6°C on 5 N sucrose peptone medium.

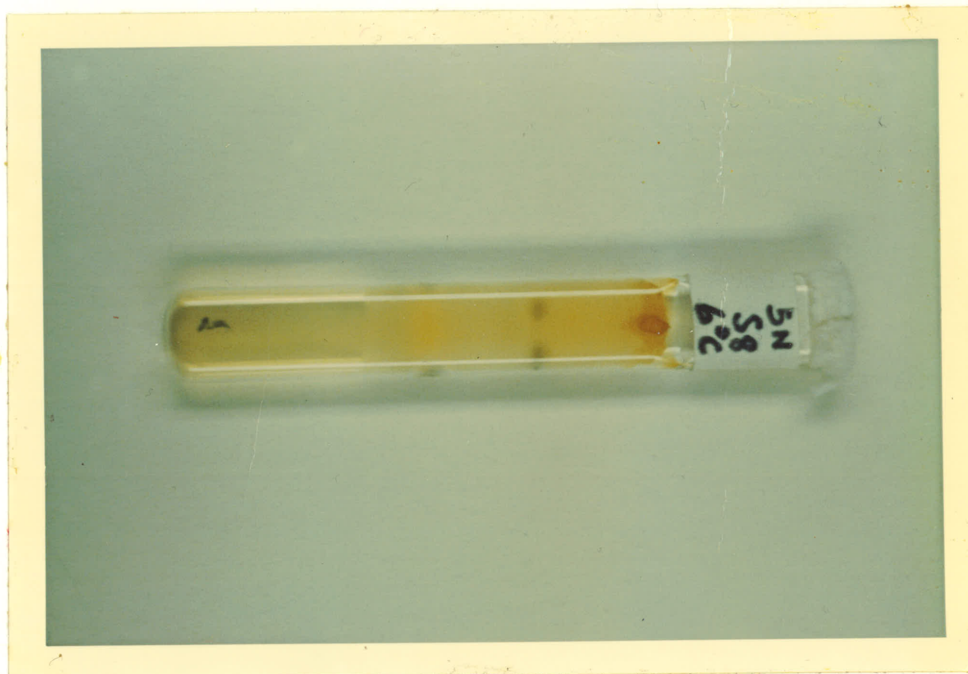


FIGURE 2. The old mycelia of *Sclerotinia sclerotiorum* changed to greenish-yellow at 6°C on 5 N sucrose peptone medium.

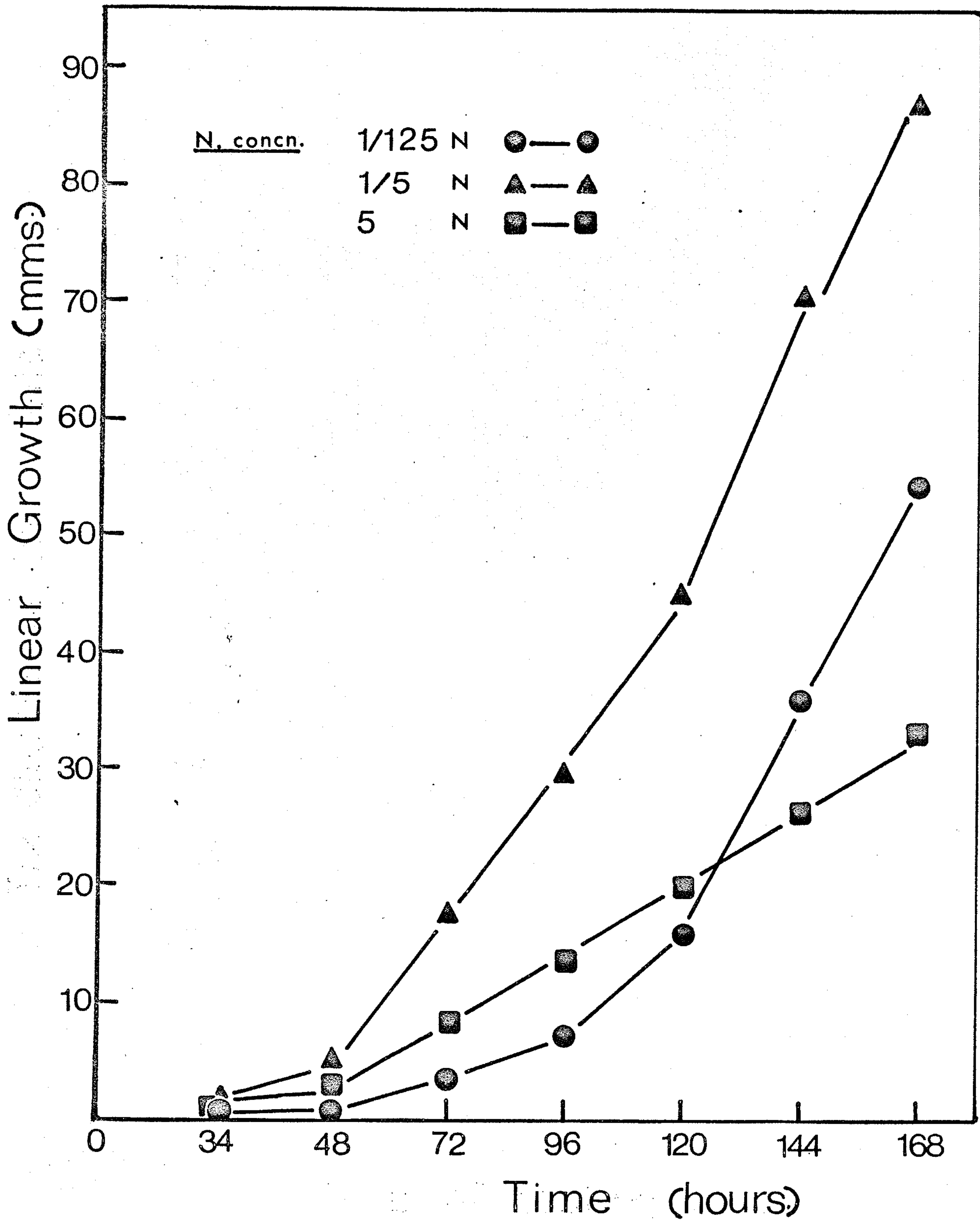
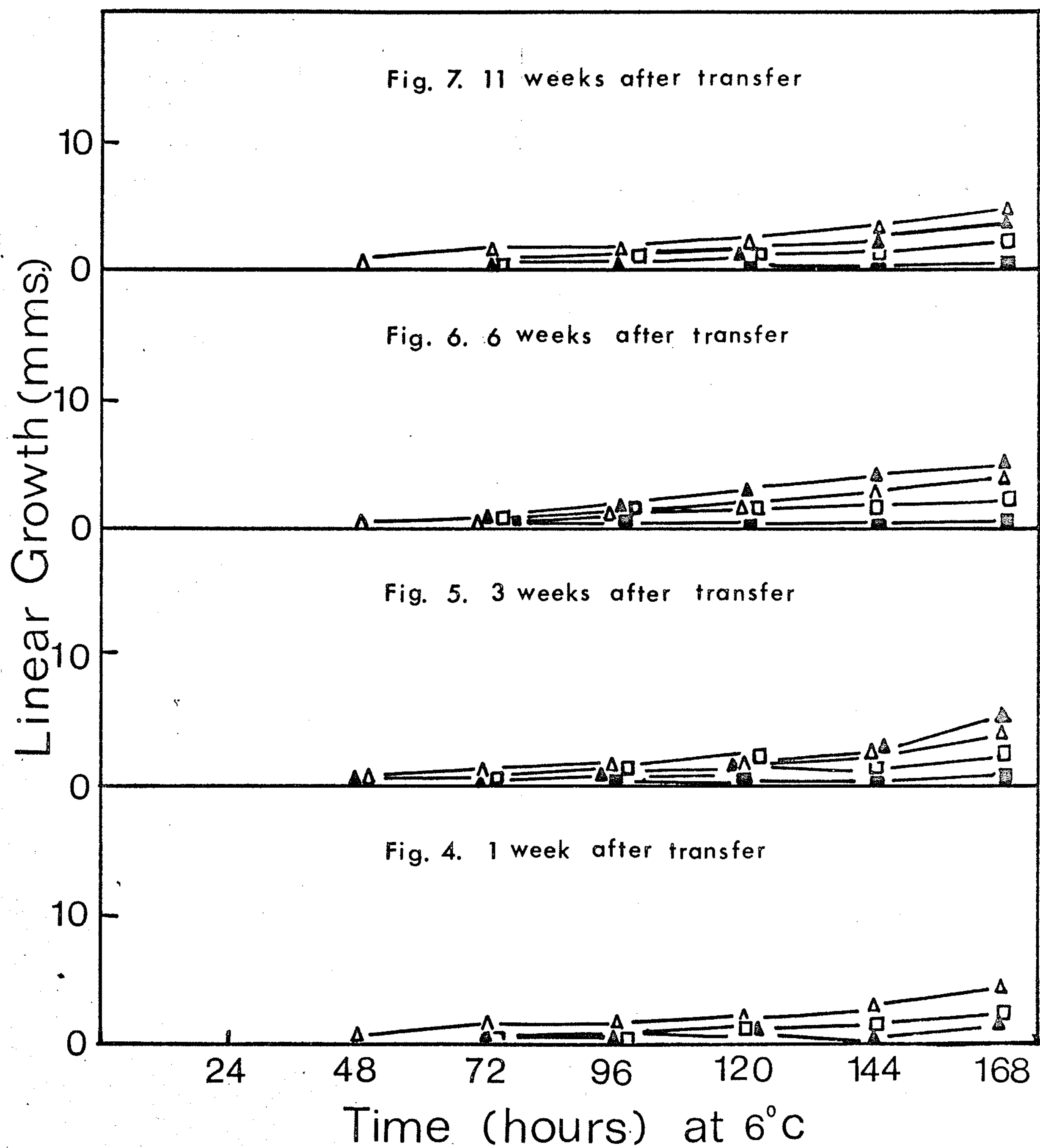



FIGURE 3. Graph of the linear growth of <u>Diplocladium</u> sp. on various nutrient concentrations of sucrose peptone at 15°C.

FIGURES 4 - 7. A comparison of linear growth rates of cultures of _Diplocladium_ sp. maintained previously at 15°C and 30°C and transferred to 6°C, with those of control cultures maintained at 6°C for 5 months.




1/5 N ▲—▲ (From 15°C)
5 N ■—■ (From 30°C)
1/5 N △—△ (Maintained at 6°C)
5 N □—□ (Maintained at 6°C)

FIGURES 8 - 11. A comparison of linear growth rates of cultures of *Diplocladium* sp. maintained previously at 6°C and 30°C and transferred to 15°C with those of control cultures maintained at 15°C for 5 months.

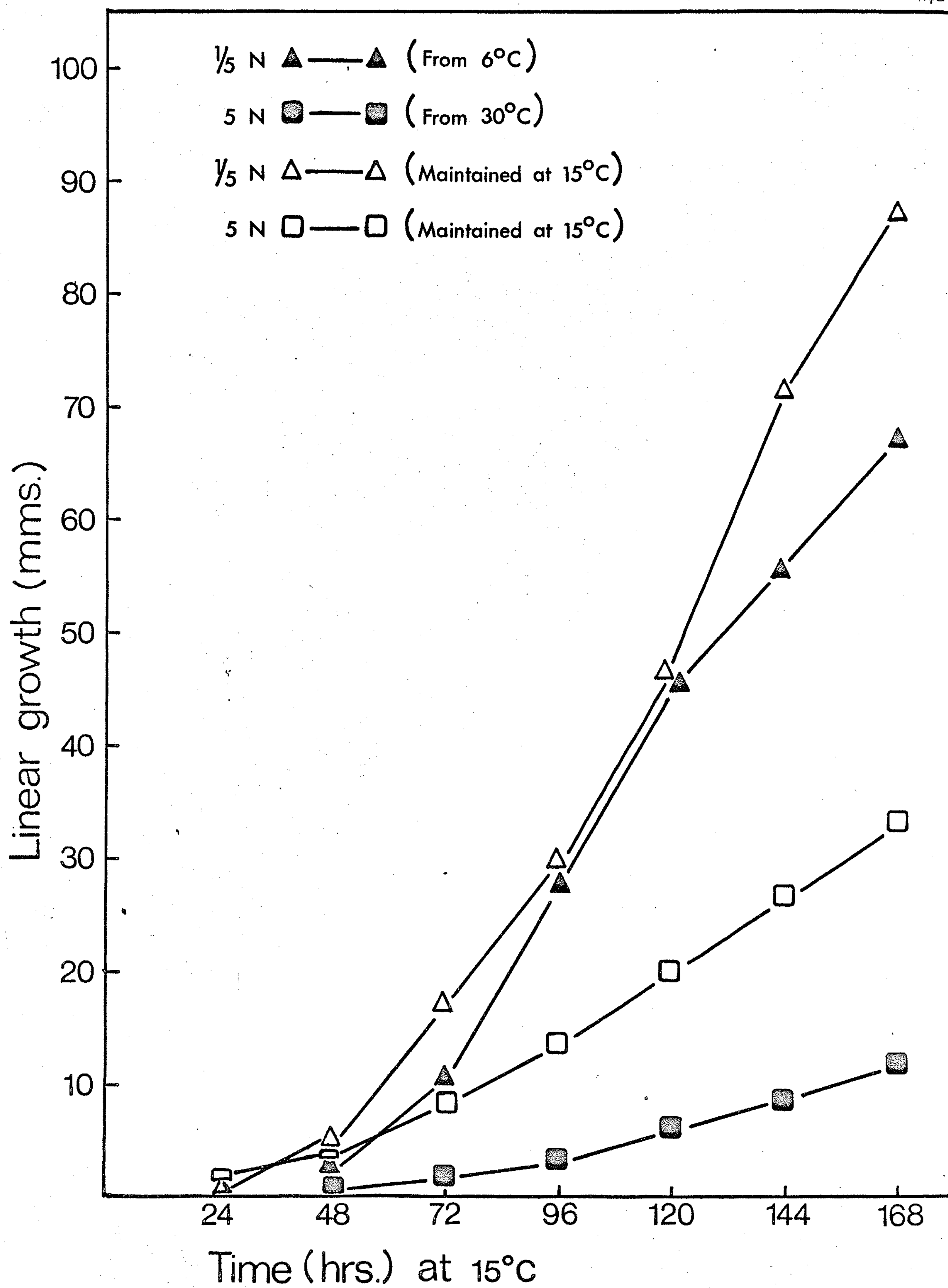



FIGURE 8

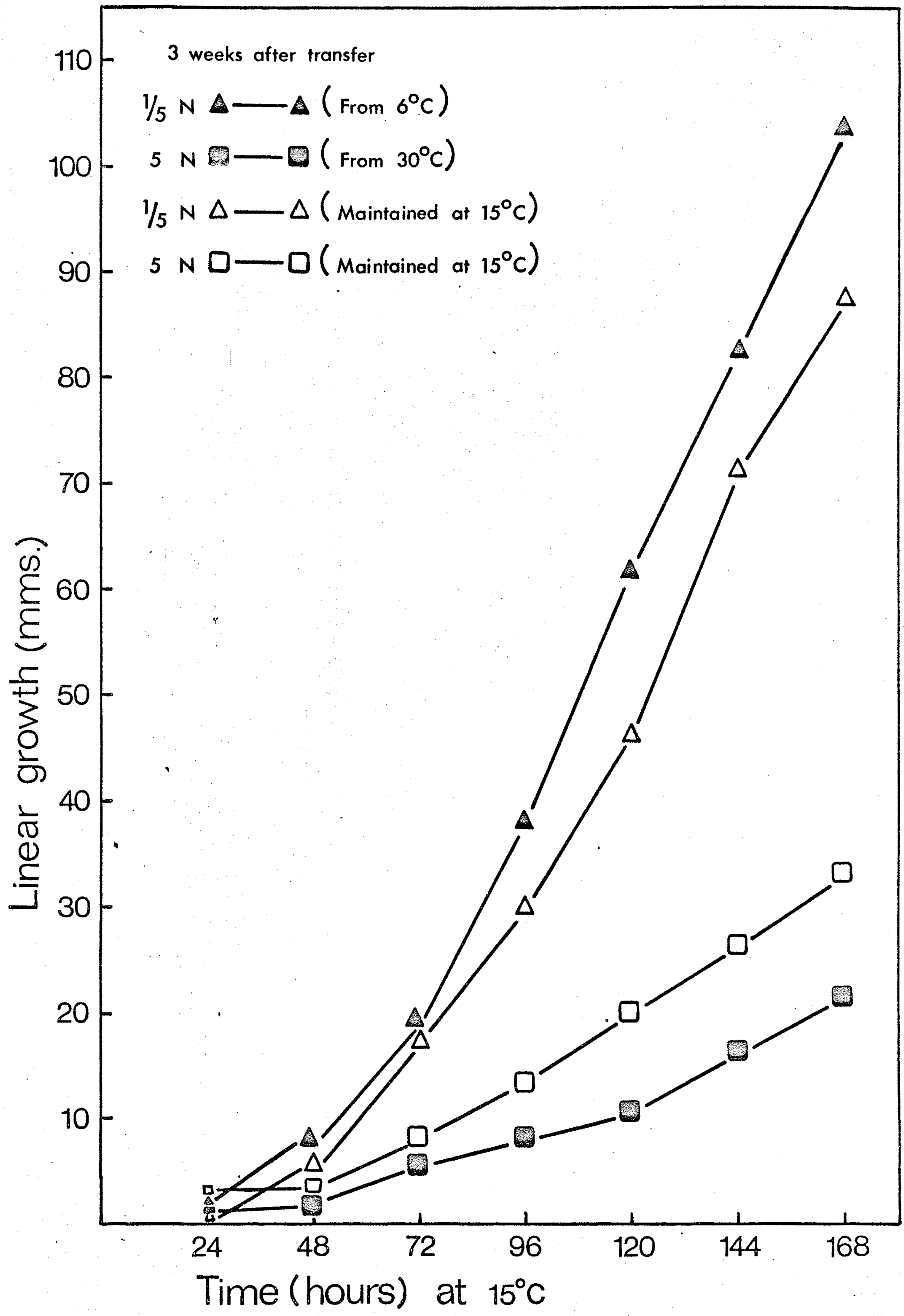



FIGURE 9

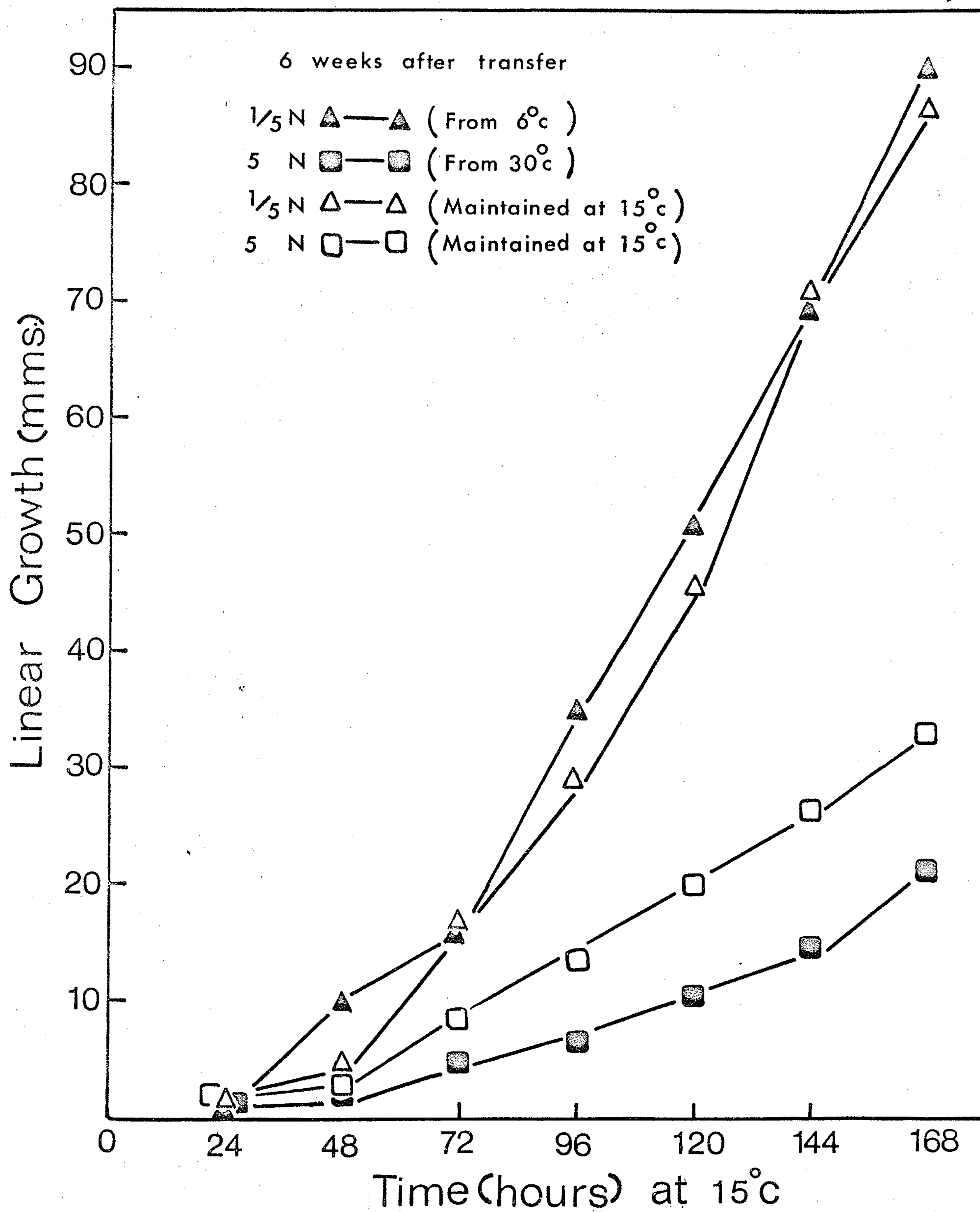



FIGURE 10

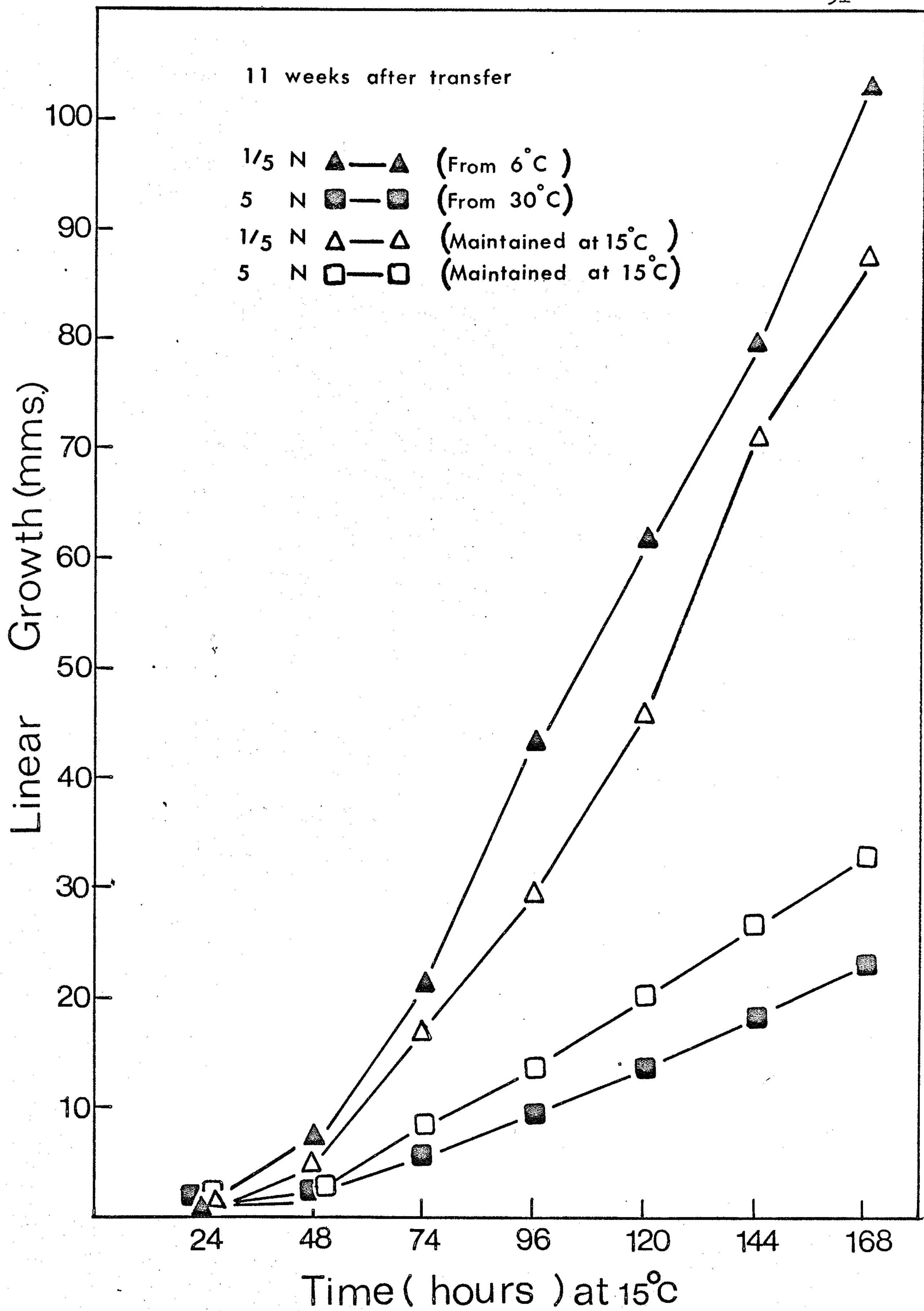
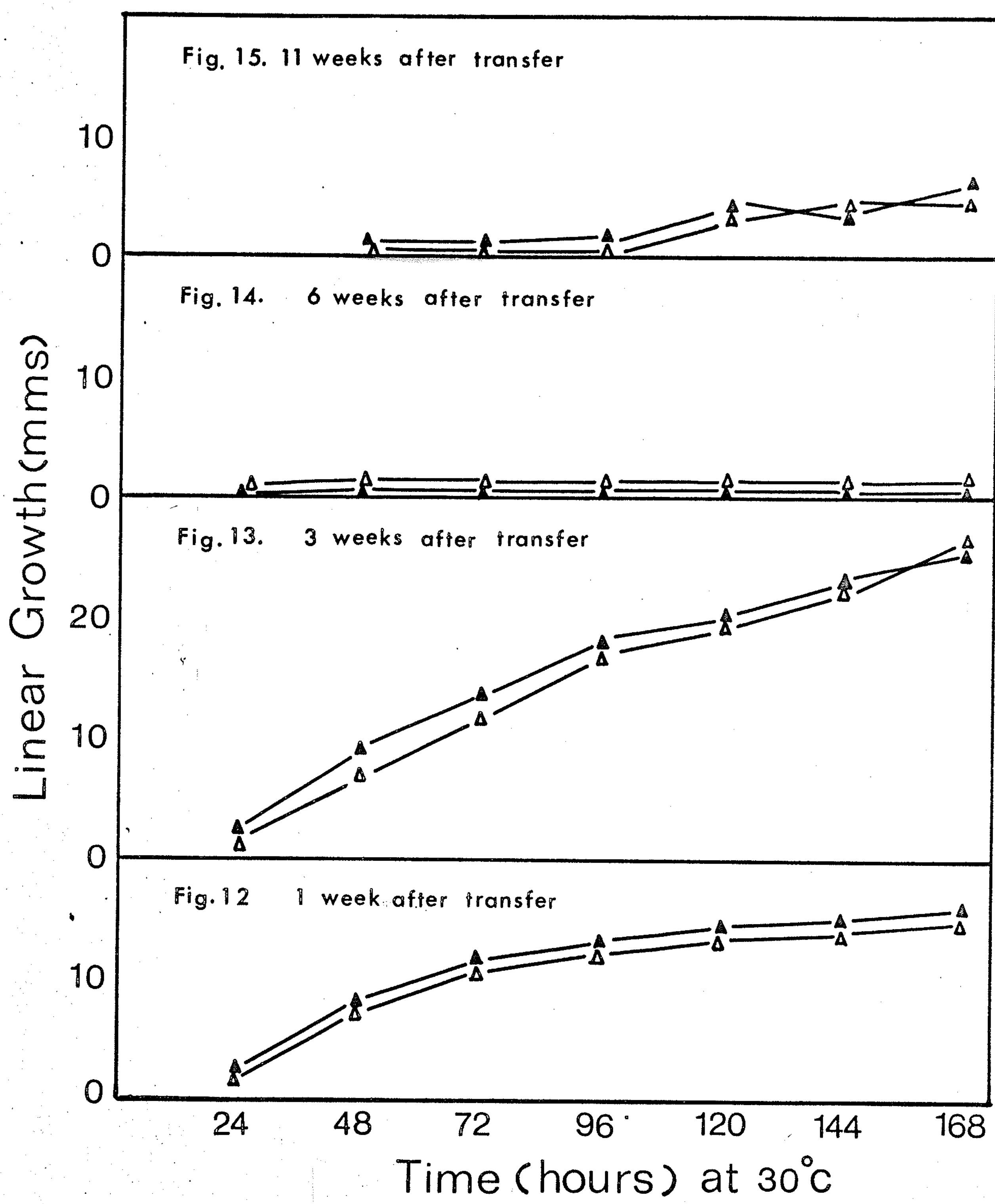



FIGURE 11

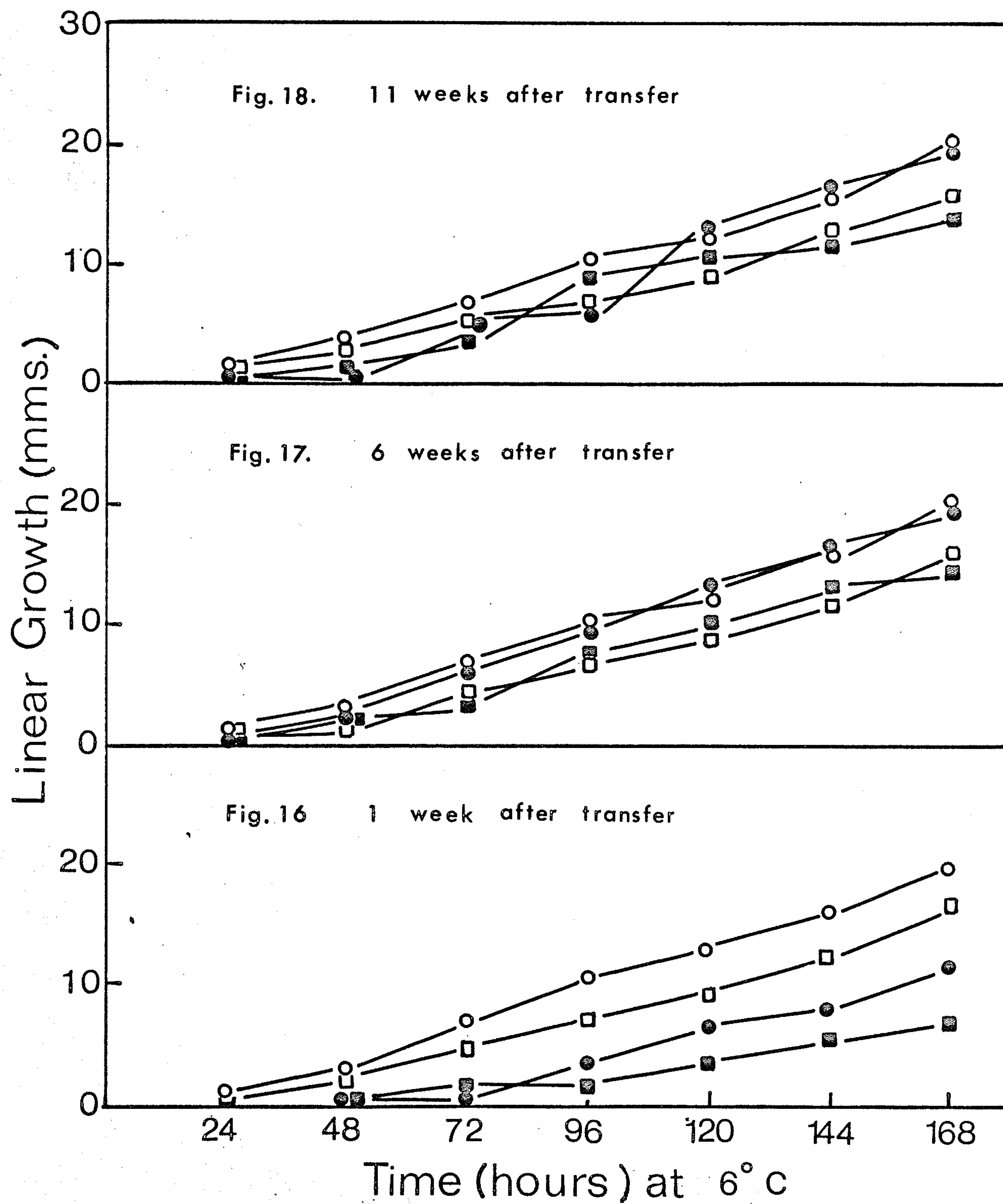
FIGURES 12-15. A comparison of linear growth rates of cultures of Diplocladium sp. maintained previously at 6°C and 15°C and transferred to 30°C with those of control cultures maintained at 30°C for 5 months.

Note: Linear growth for 1/5 N culture maintinaed at 30°C is 0.5 mm in 7 days.




1/5 N ▲——▲ (From 6°C)
1/5 N △——△ (From 15°C)

FIGURES 16 - 18. A comparison of linear growth rates of cultures of *S. sclerotiorum* maintained previously at 15°C and 30°C and transferred to 6°C with those of control cultures maintained at 6°C for 5 months.




1/125 N ●—● (From 15°C)
5 N ■—■ (From 30°C)
1/125 N ○—○ (Maintained at 6° C)
5 N □—□ (Maintained at 6° C)

FIGURES 19 - 21. A comparison of linear growth rates of cultures of S. sclerotiorum, maintained previously at 6°C and 30°C and transferred to 15°C with those of control cultures maintained at 15°C for 5 months.

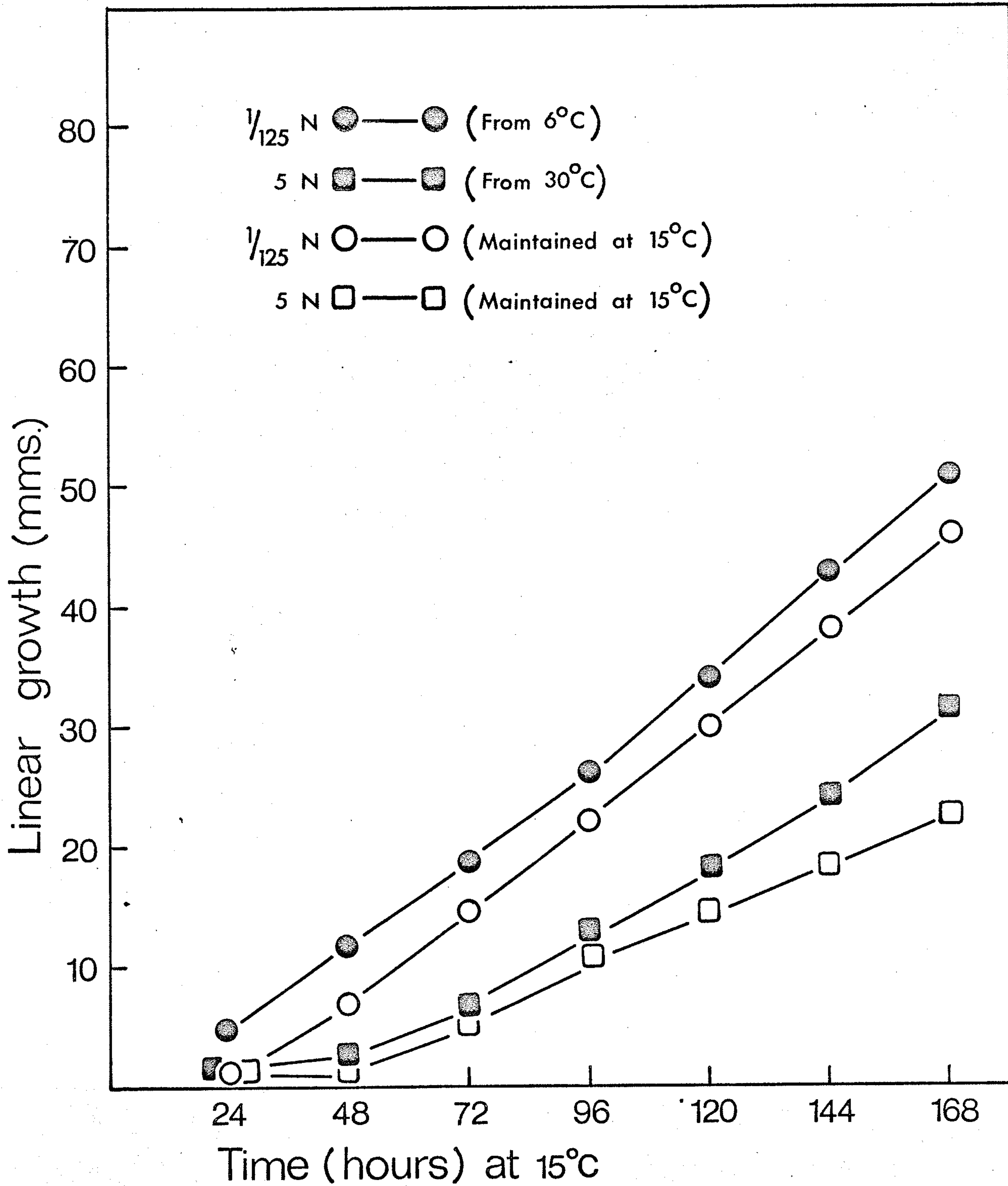



FIGURE 19.

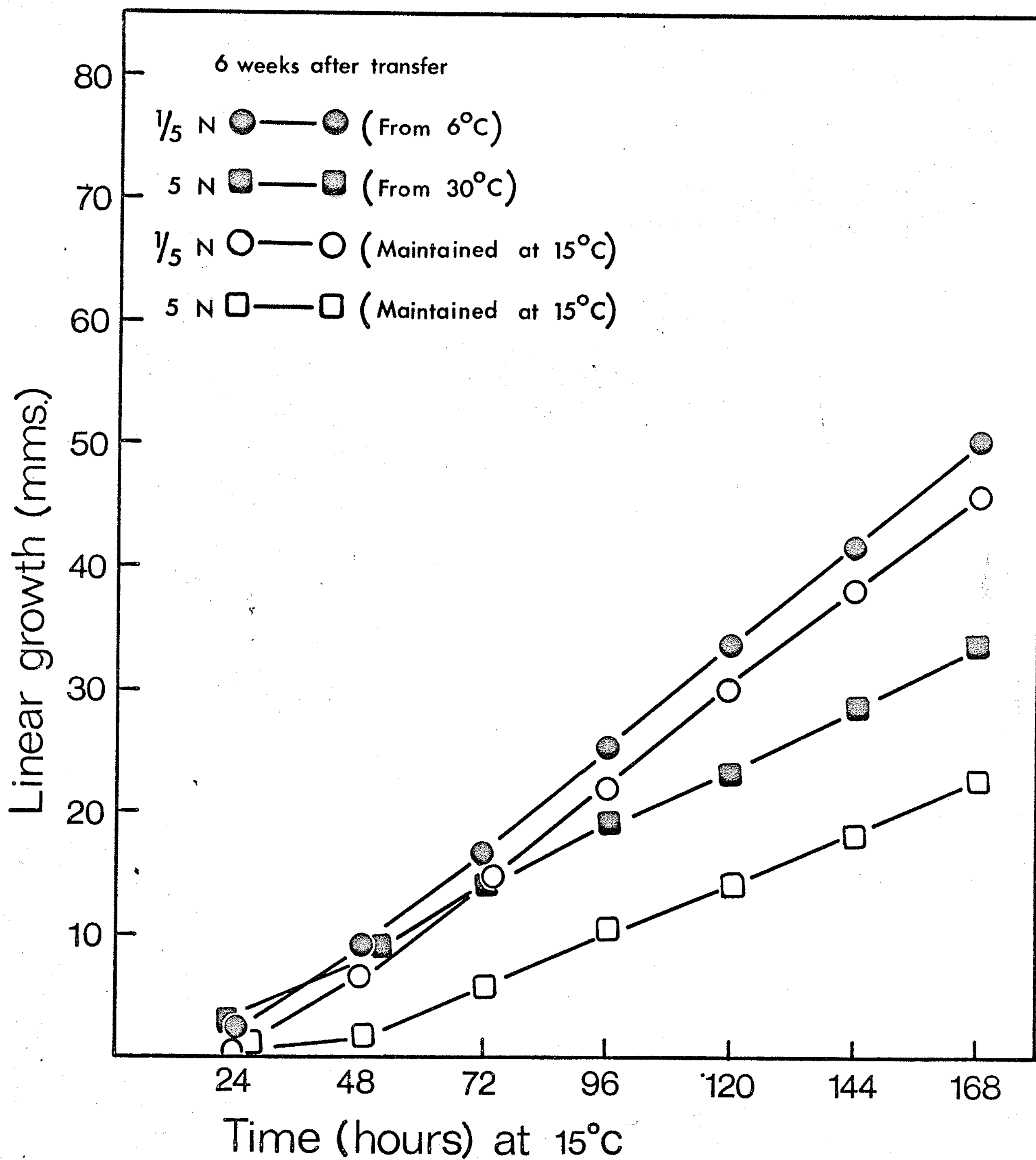



FIGURE 20.

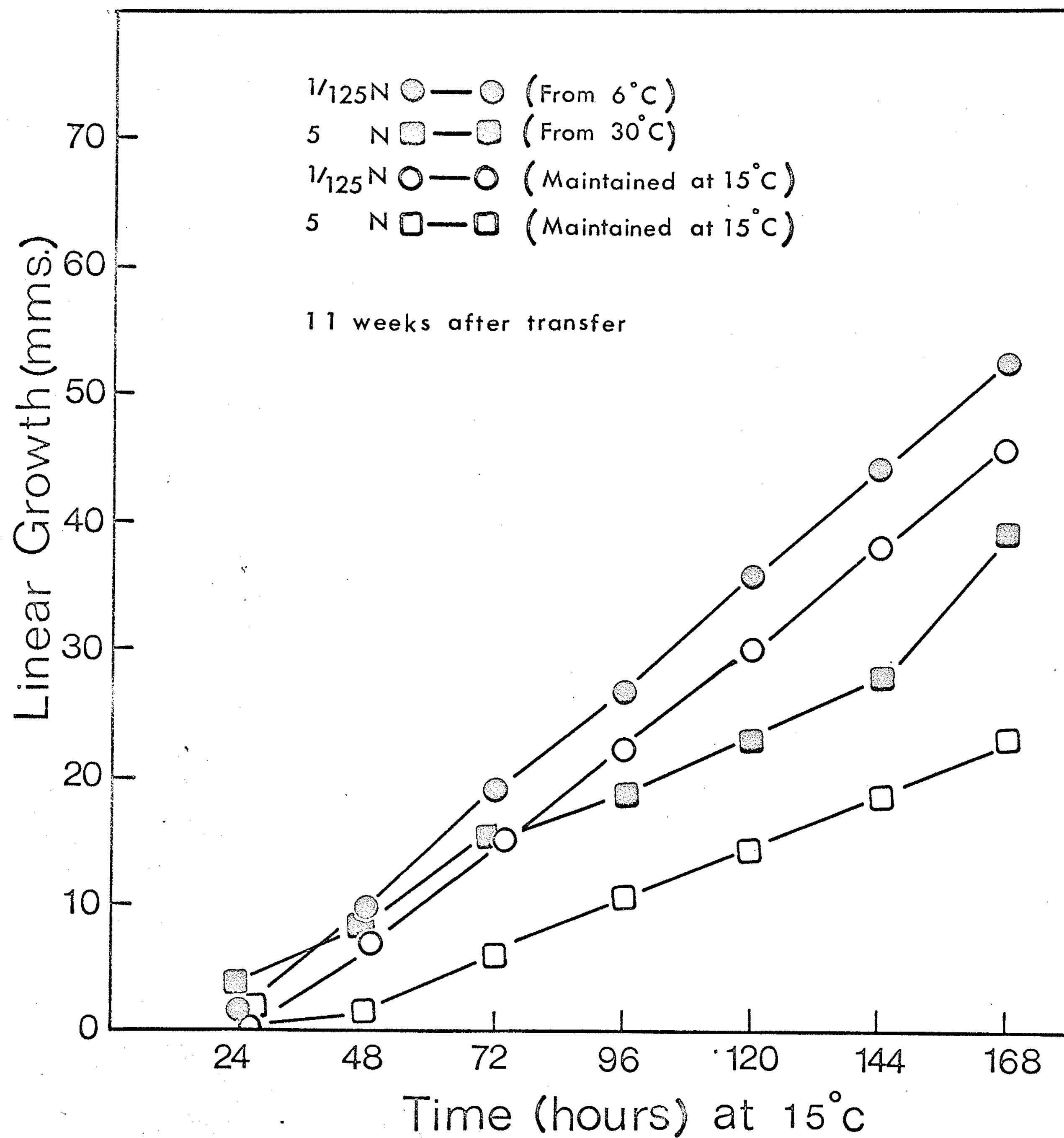
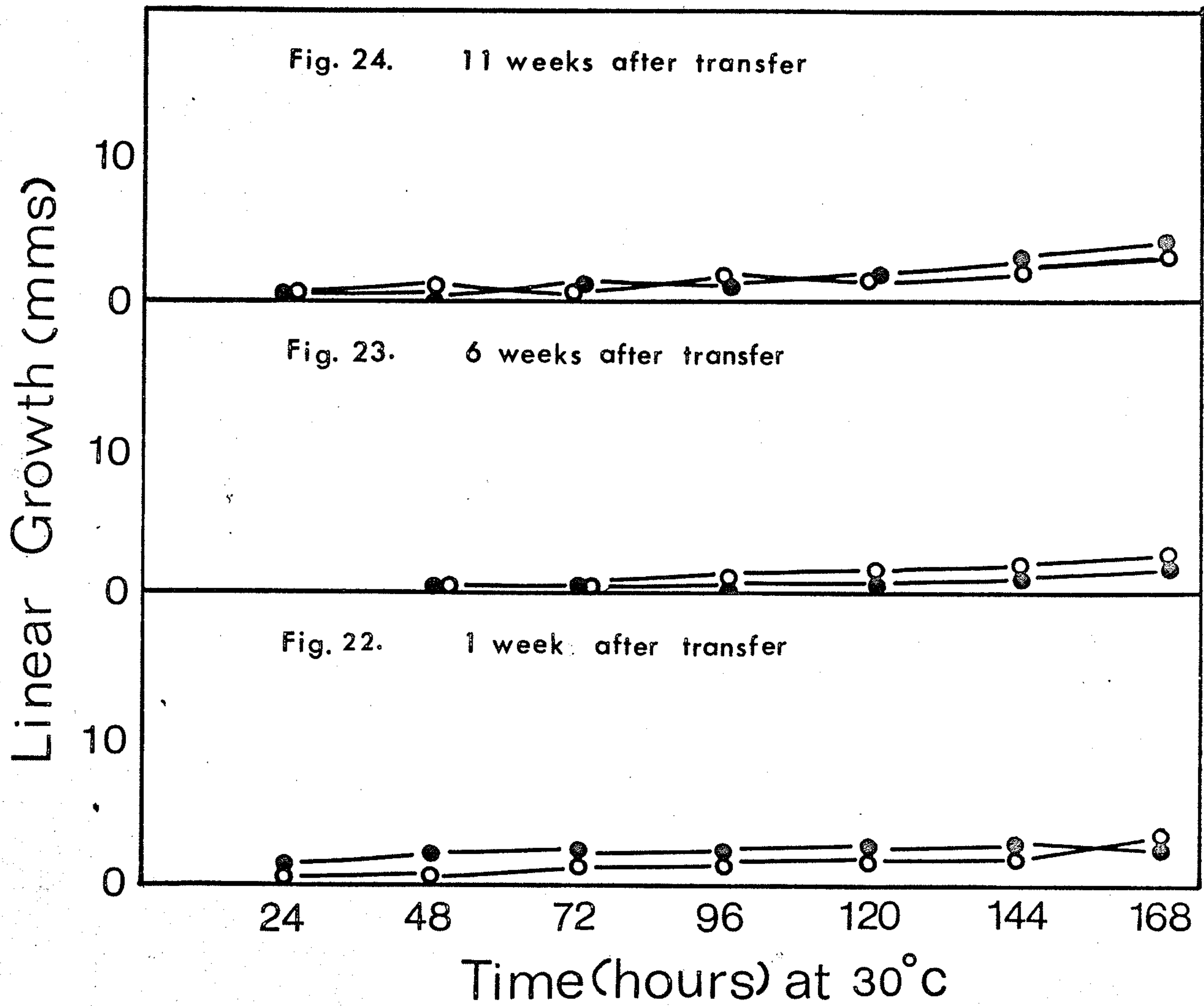



FIGURE 21.

FIGURES 22 - 24. The linear growth rates of cultures of *S. sclerotiorum* maintained previously at 6°C and 15°C and transferred to 30°C.

Note: No control cultures available.




1/125 N ●——● (From 6°)

1/125 N ○——○ (From 15°)

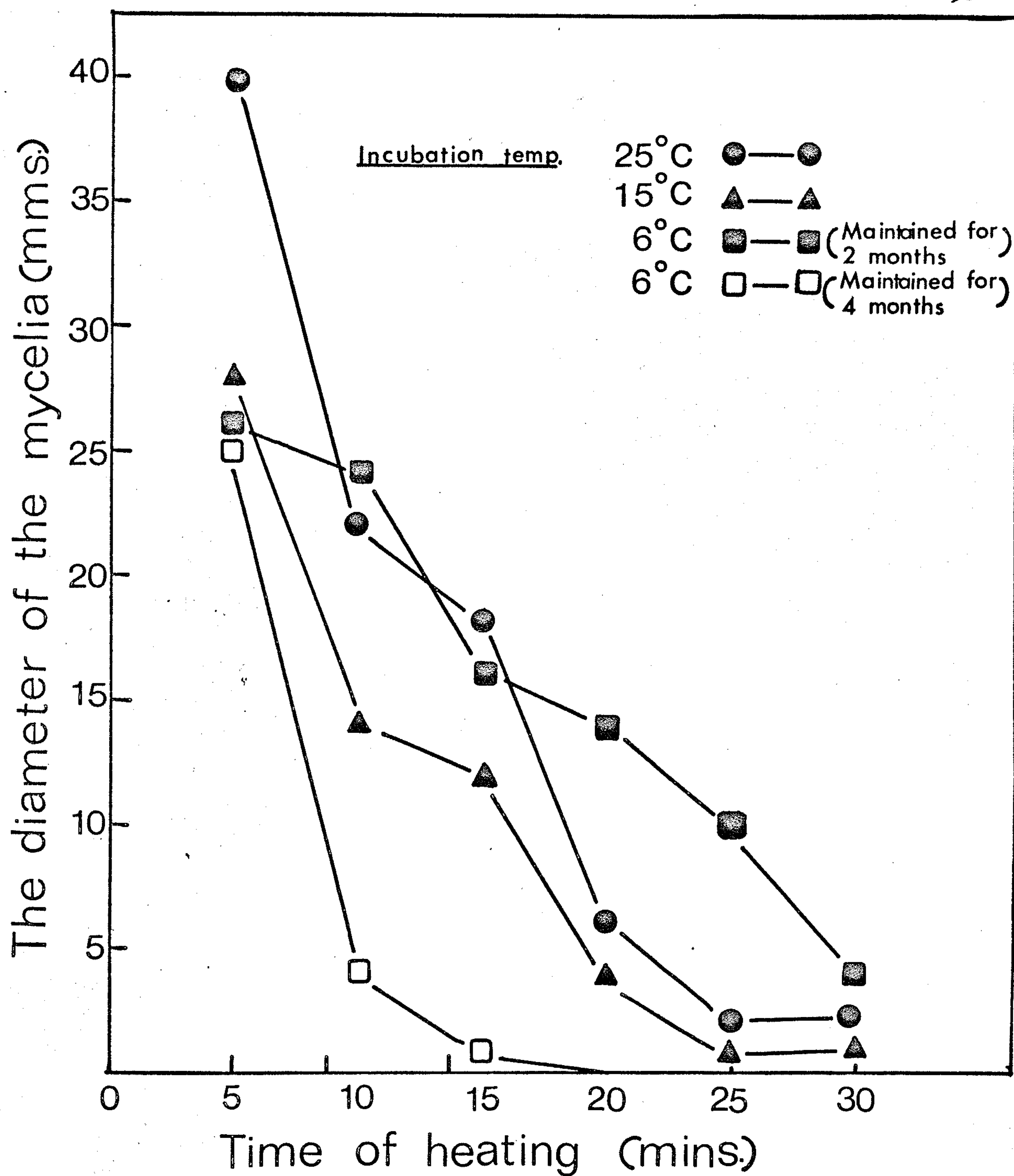



**FIGURE 25.** The effect of the incubation temperatures and time on thermal resistance of *Diplocladium* sp. at 40°C.

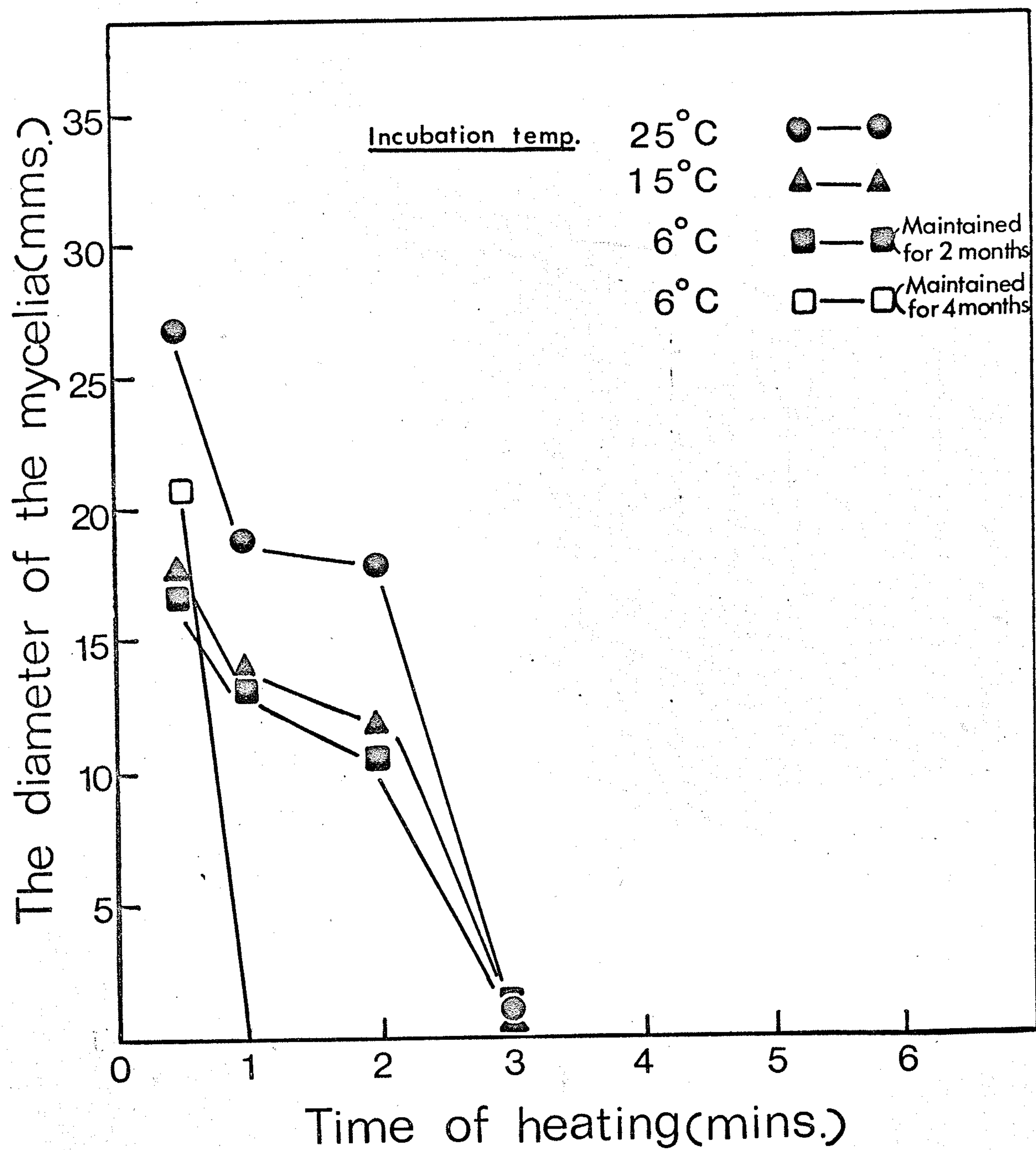



FIGURE 26. Similar to Figure 25 at 45°C.

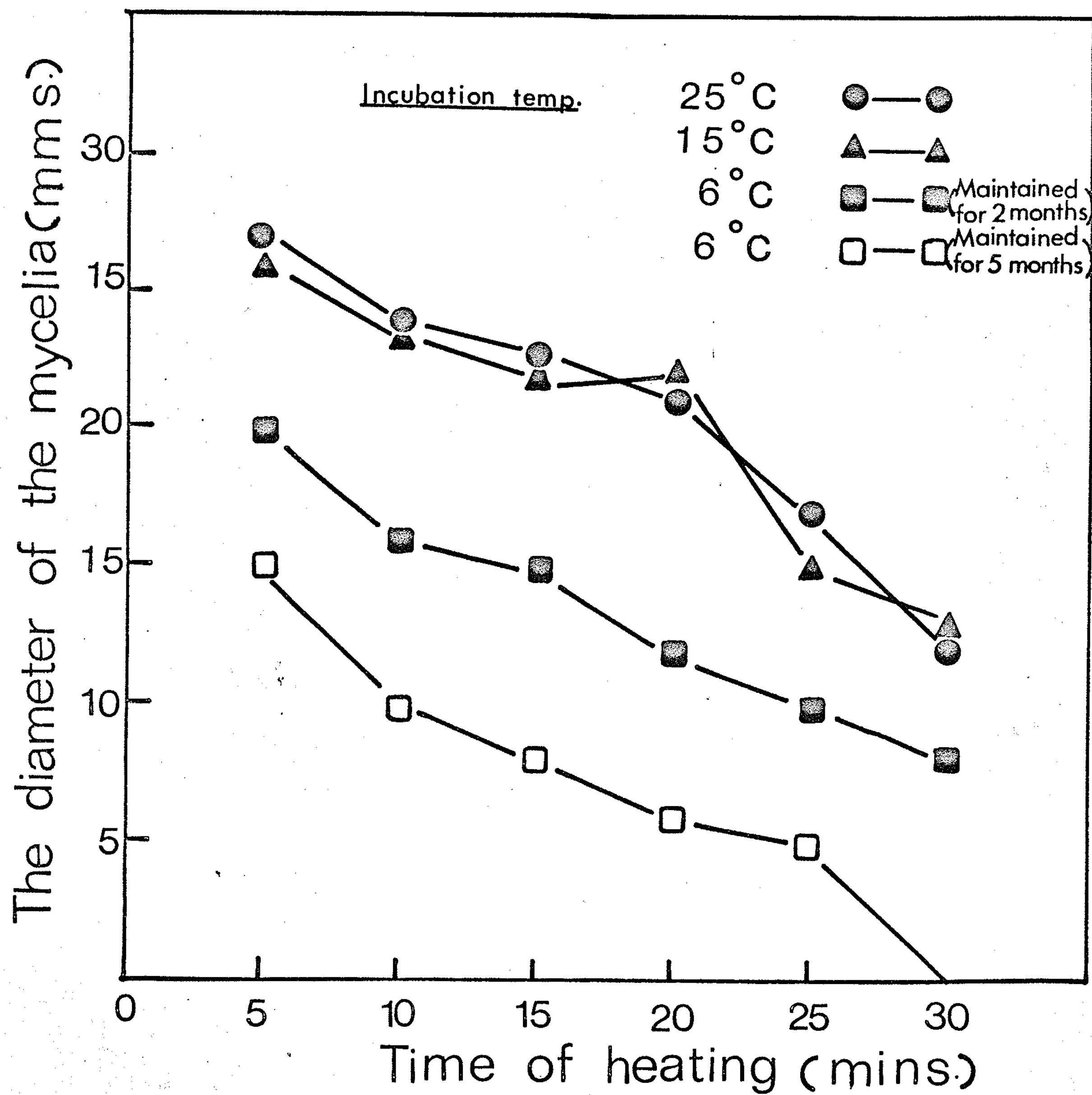



FIGURE 27. The effect of the incubation temperatures and time on thermal resistance of Sclerotinia sclerotiorum at 40°C.

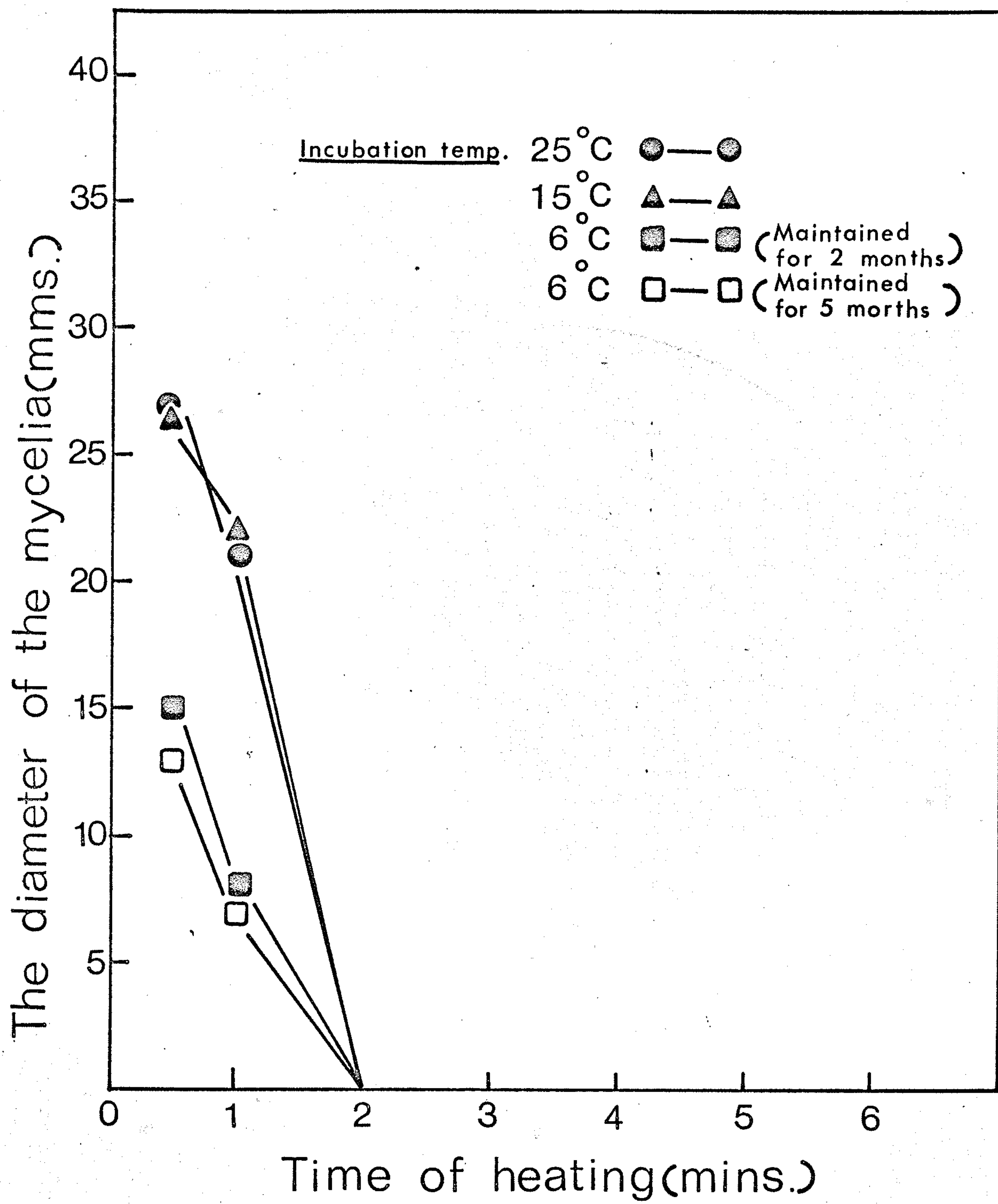



FIGURE 28. Similar to Figure 27 at 45°C.

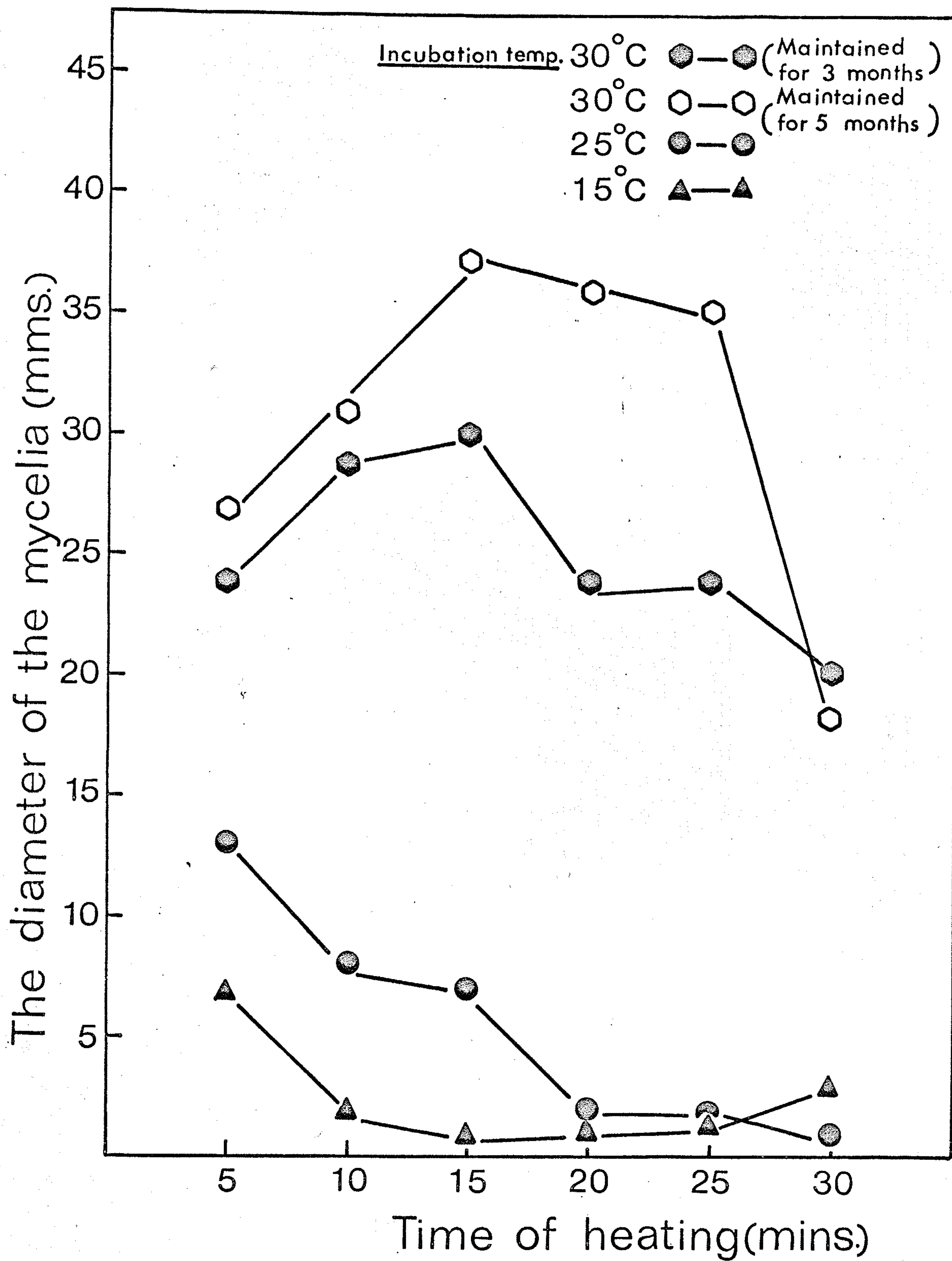



FIGURE 29. The effect of the incubation temperature and time on thermal resistance of *Poria cocos* at 40°C.

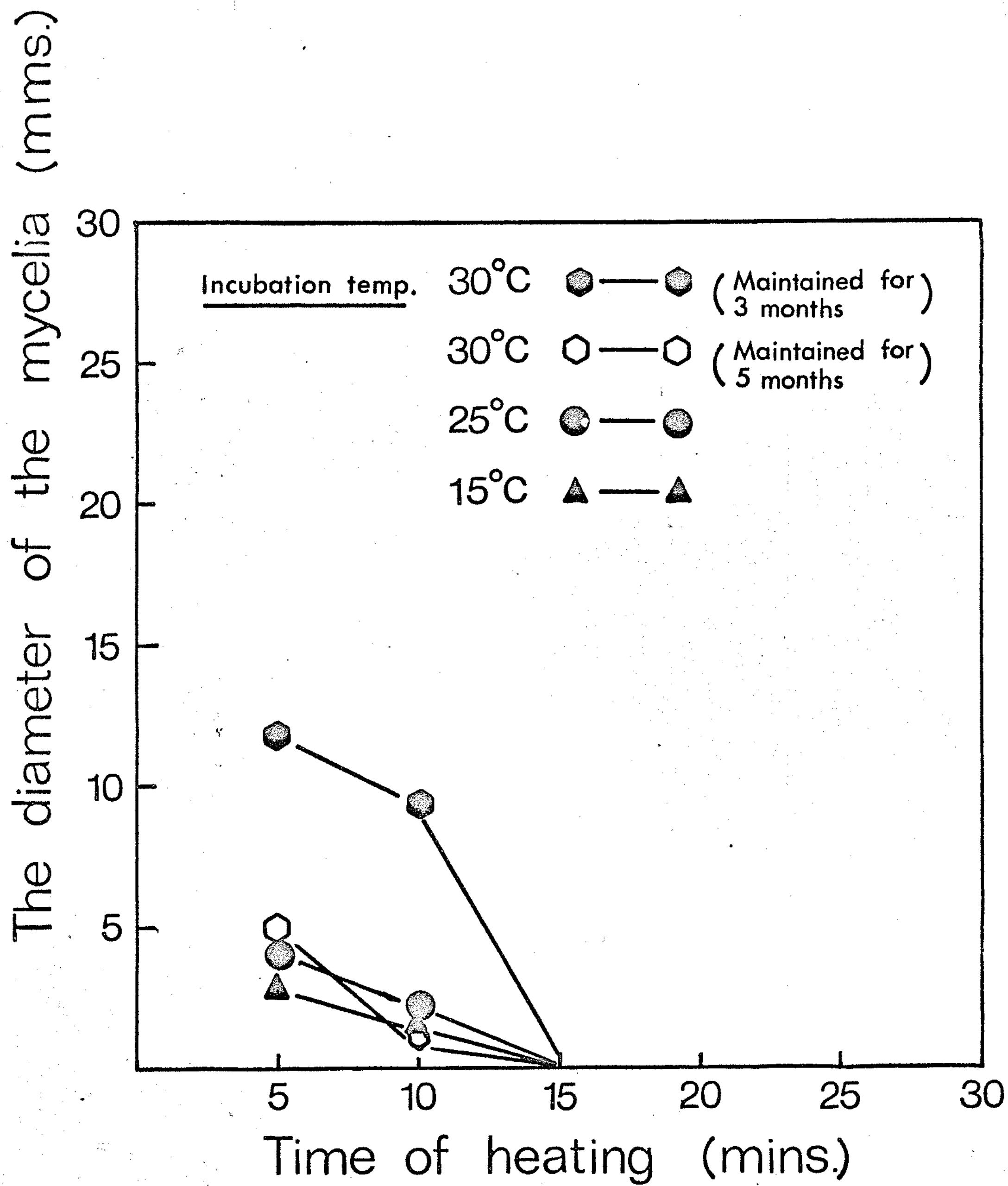



FIGURE 30. The effect of the incubation temperatures and time on thermal resistance of <u>Poria cocos</u> at 45°C.

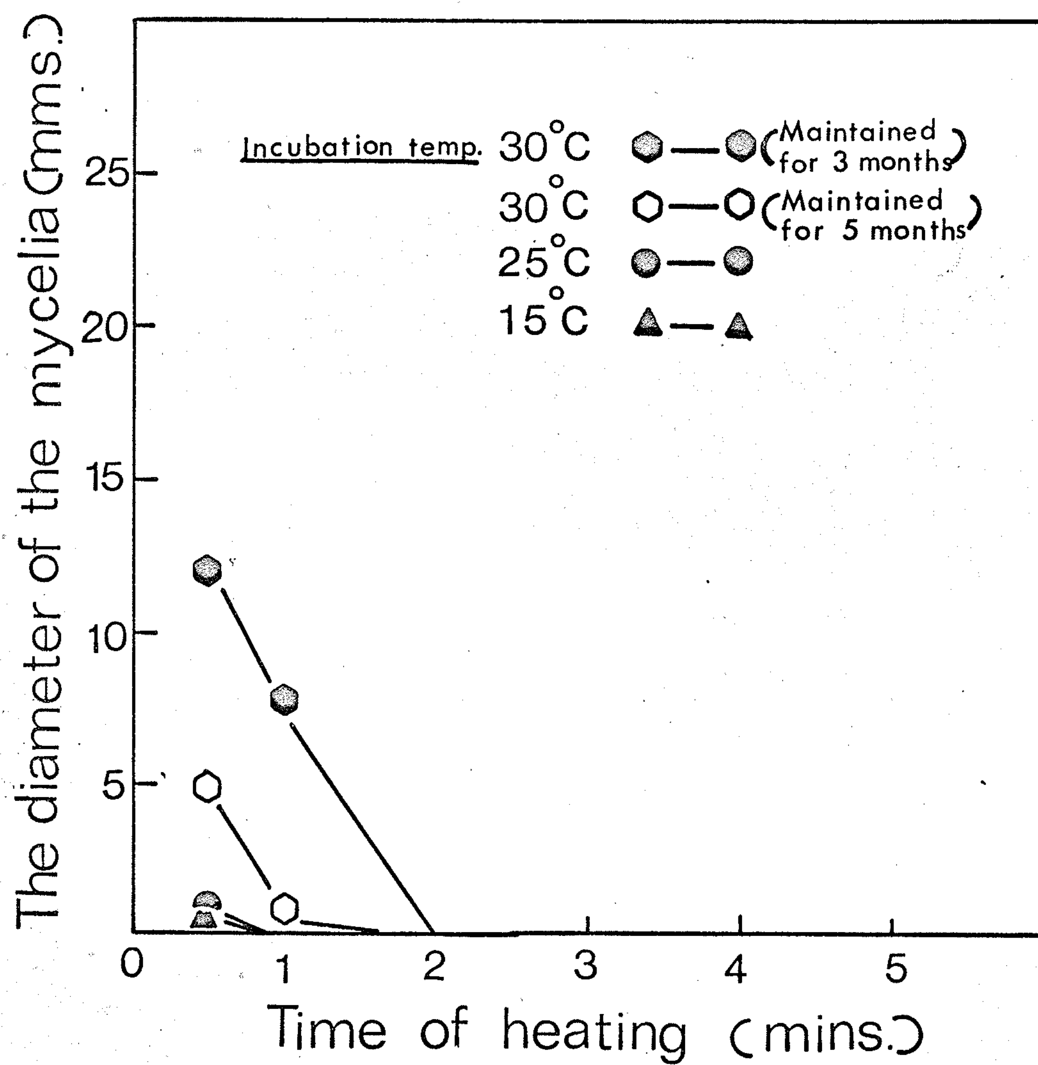



FIGURE 31. Similar to Figure 30 at 50°C.

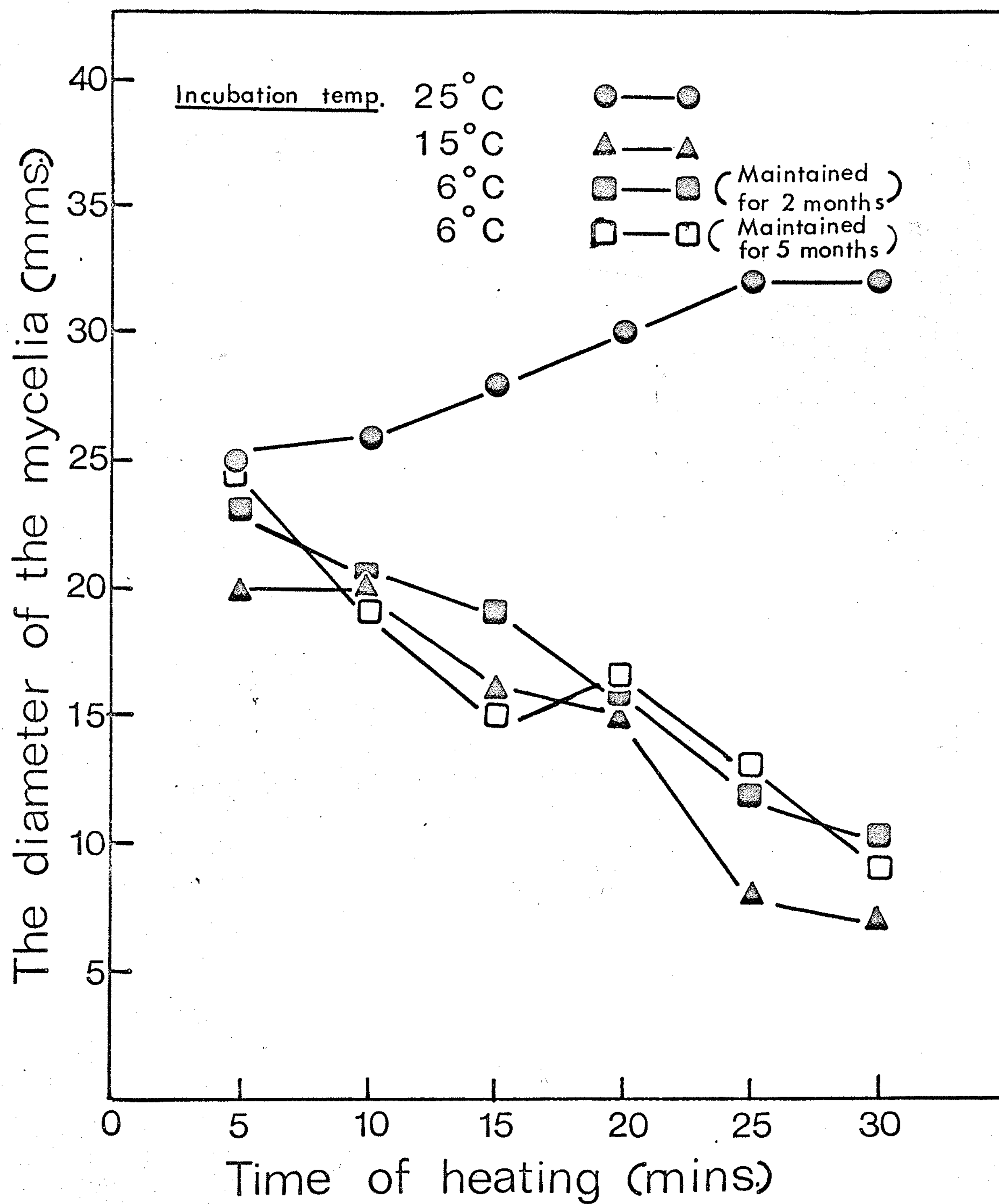



FIGURE 32. The effect of the incubation temperatures and time on thermal resistance of Stereum purpureum at 40°C.

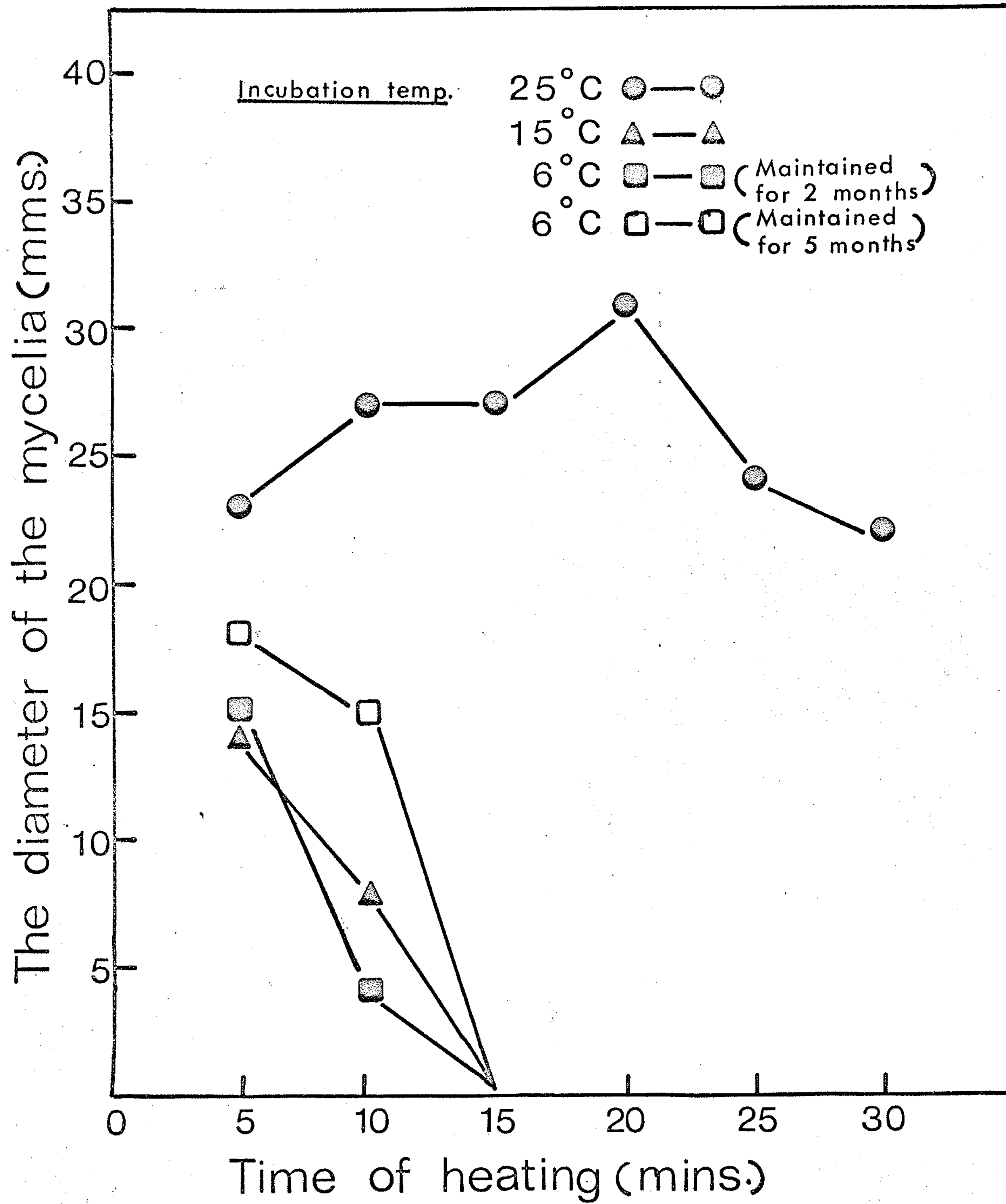



FIGURE 33. Similar to Figure 32 at 45°C.

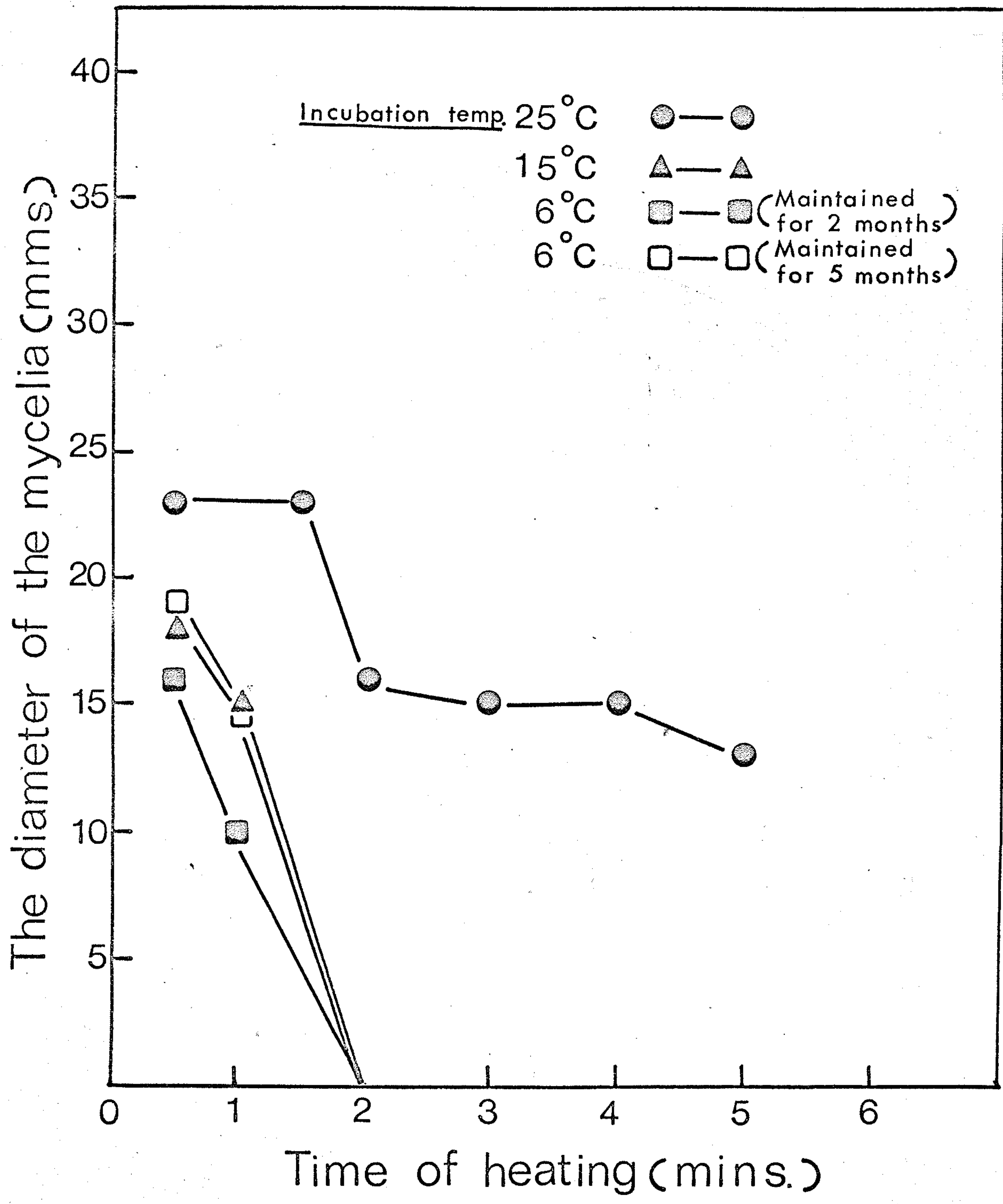



FIGURE 34. Similar to Figure 32 at 50°C.

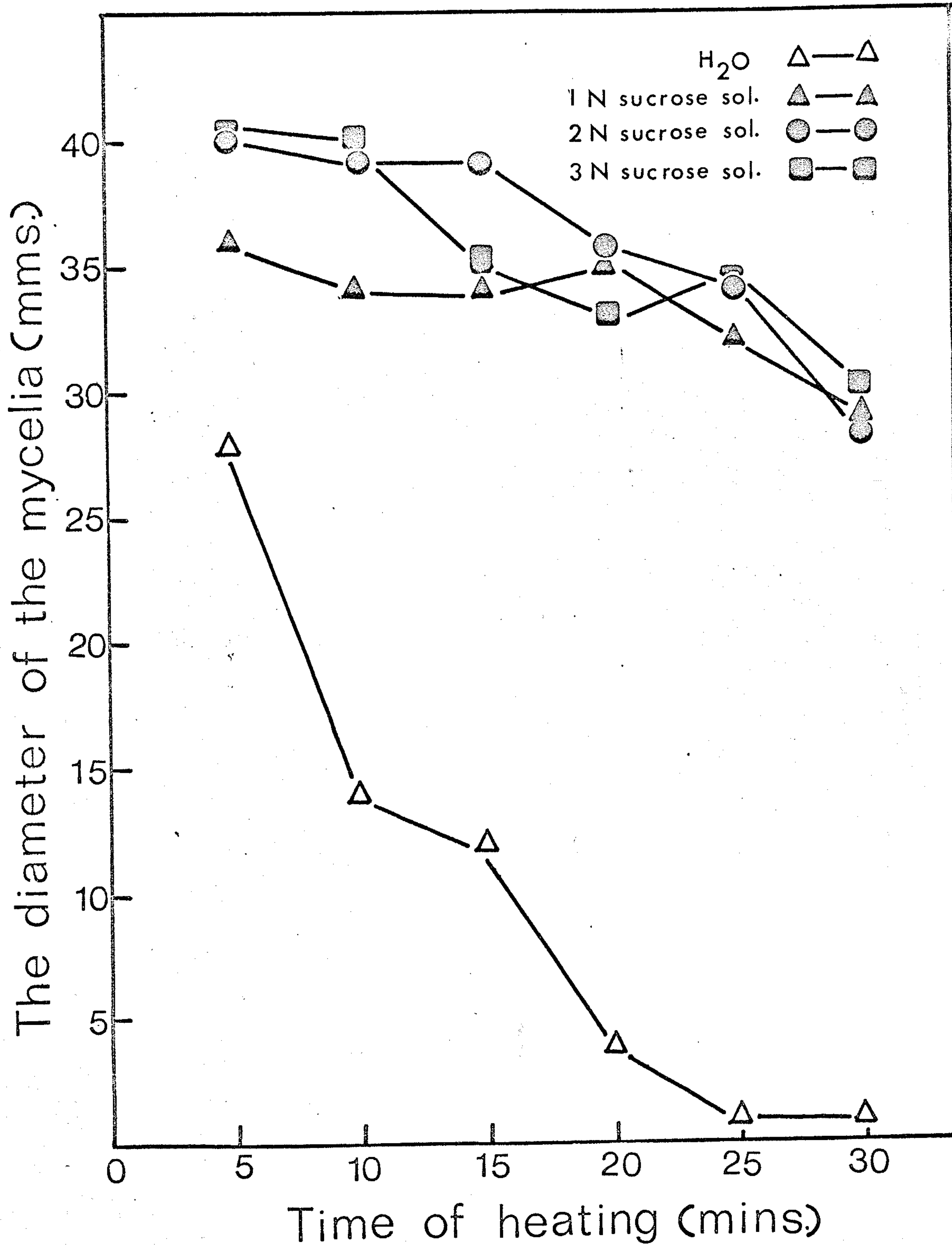



FIGURE 35. Graph comparing the thermal resistance of <u>Diplocladium</u> sp. after heating in distilled water and in various concentrations of sucrose solution at 40°C.

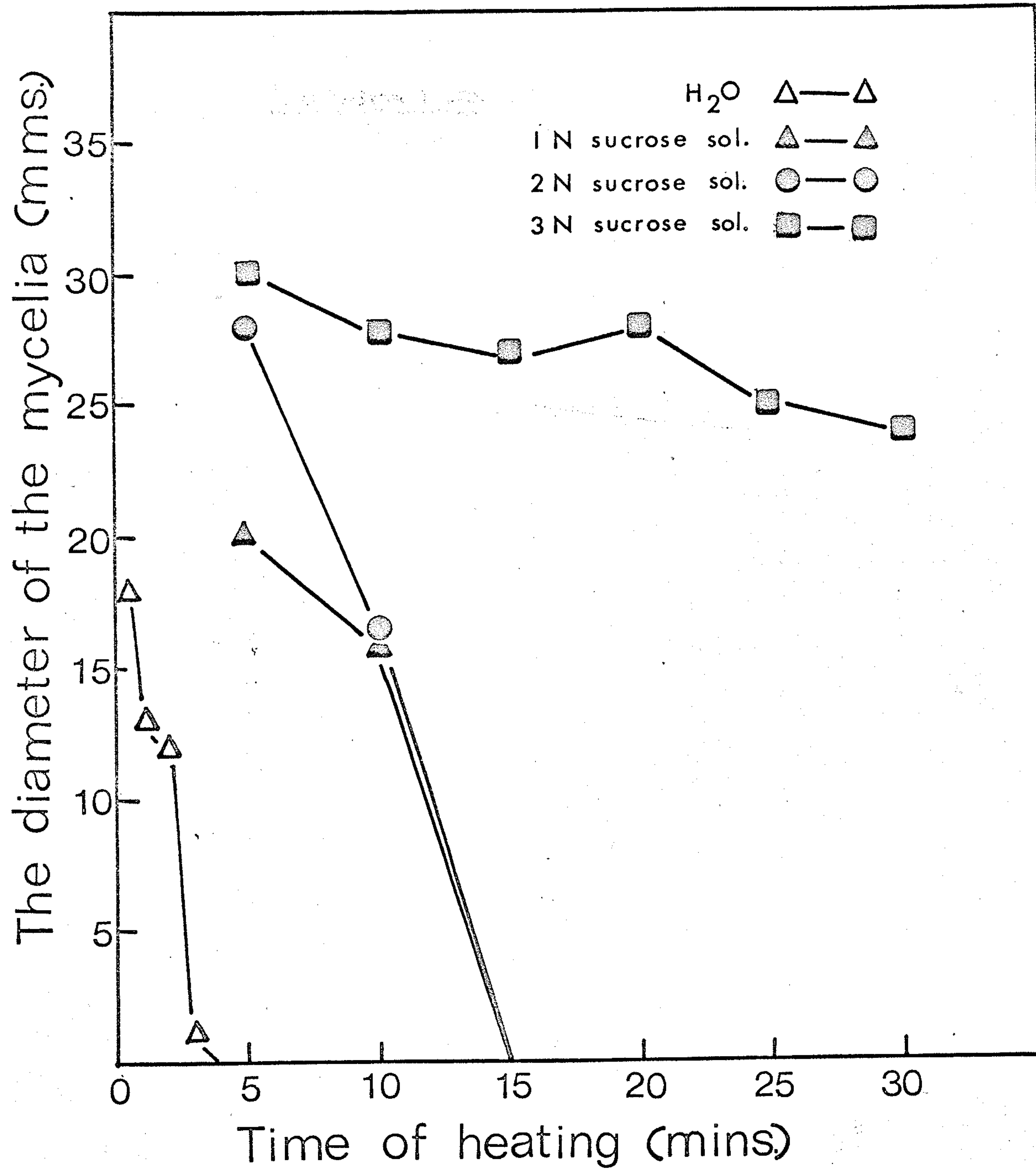



FIGURE 36. Similar to Figure 35 at 45°C.

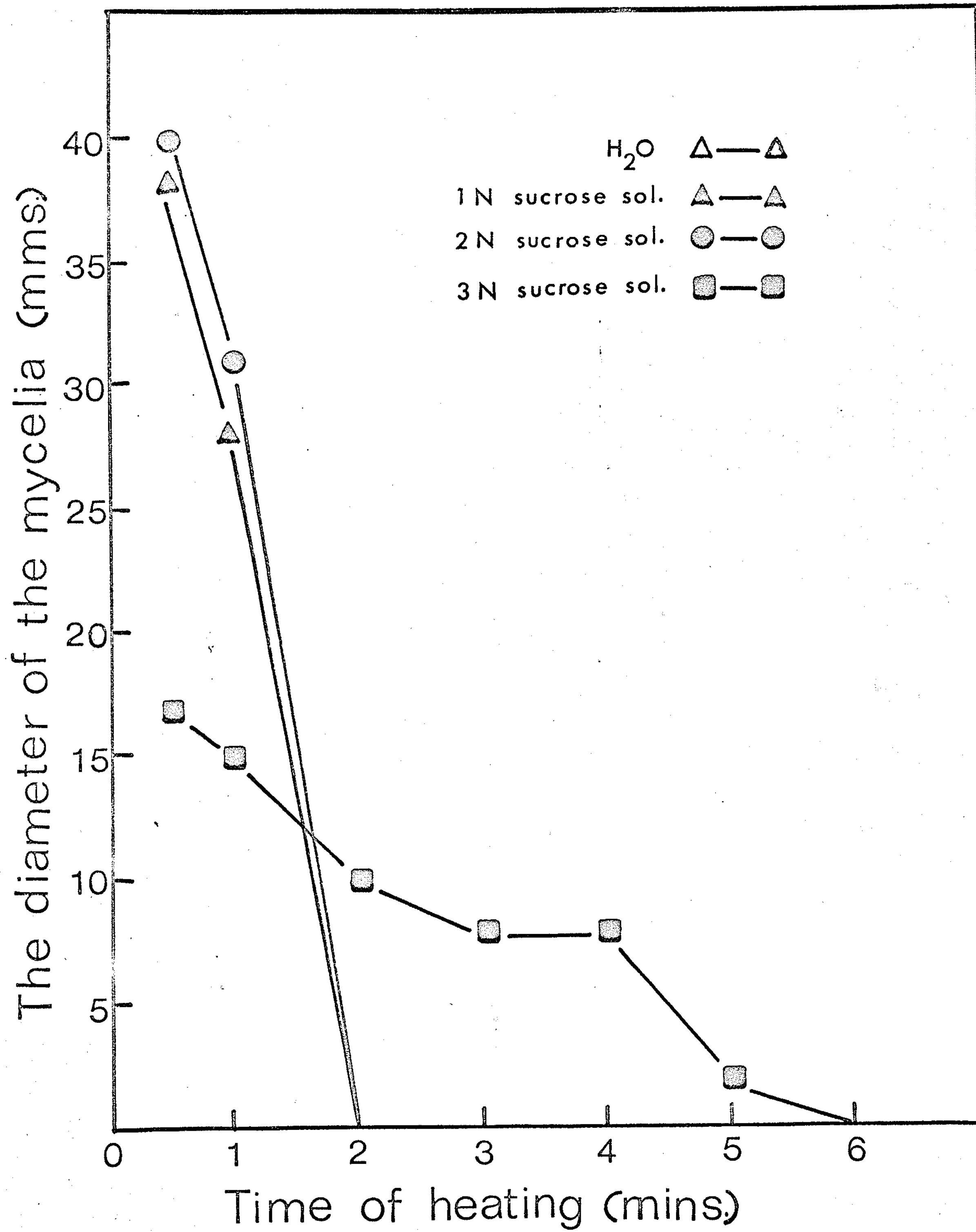



FIGURE 37. Similar to Figure 35 at 50°C.

Note: No growth for the culture treated at 50°C in distilled $H_2O$.

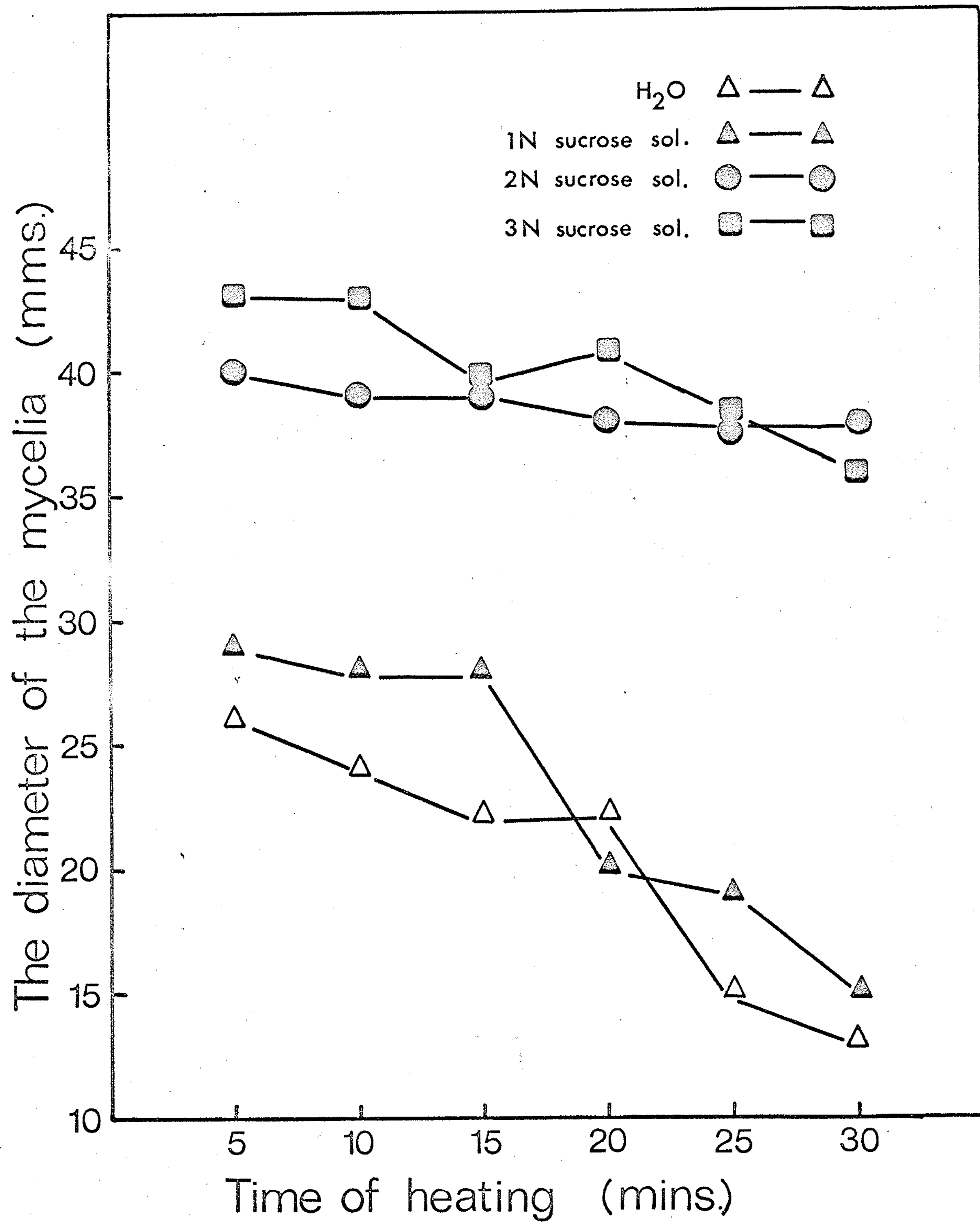



FIGURE 38. Graph comparing the thermal resistance of Sclerotinia sclerotiorum after heating in distilled water and in various concentrations of sucrose solution at 40°C.

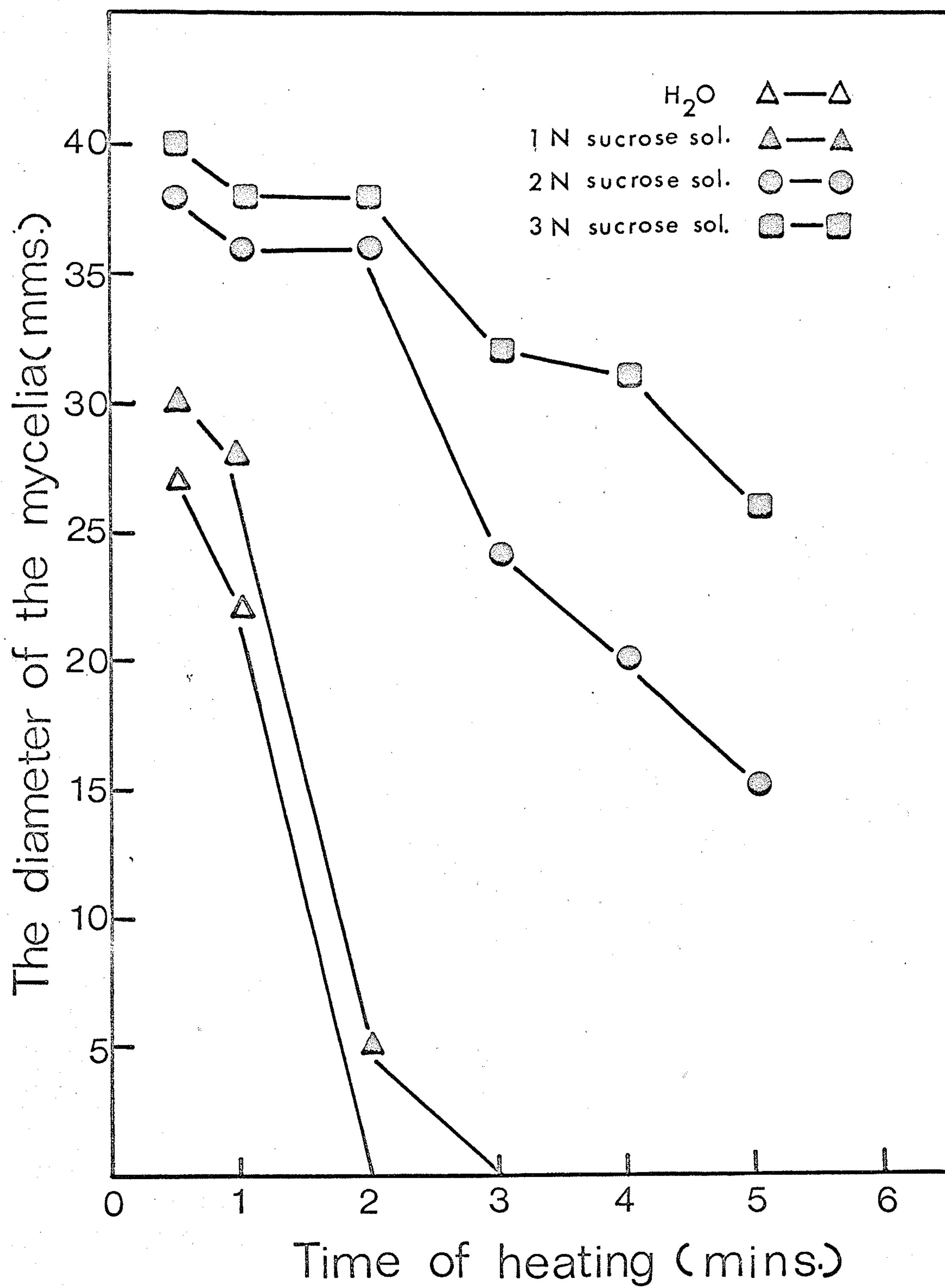



FIGURE 39. Similar to Figure 38 at 45°C.

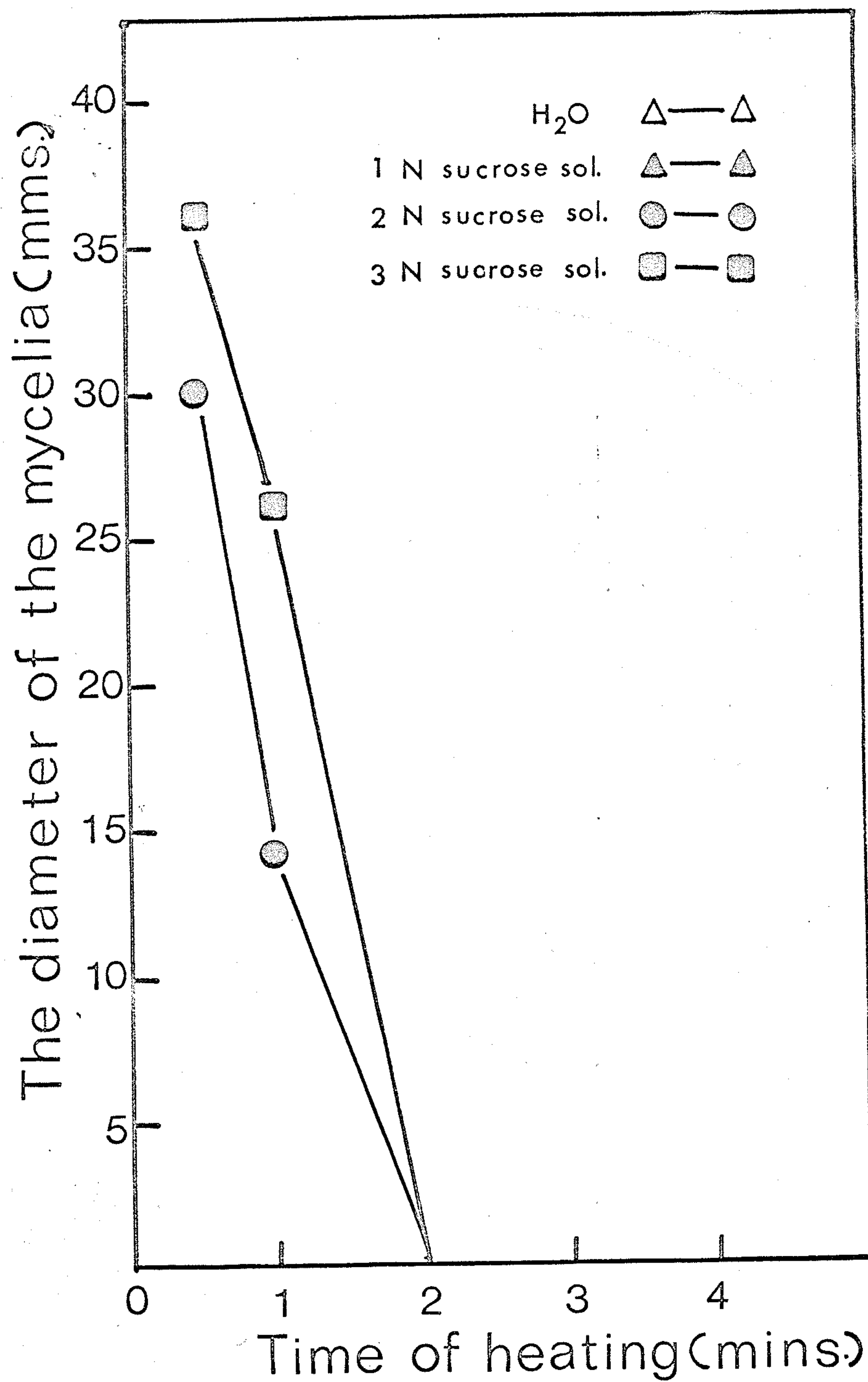



FIGURE 40. Similar to Figure 38 at 50°C.

Note: No growth for the cultures treated at 50°C in distilled $H_2O$ and in 1 N sucrose solution.

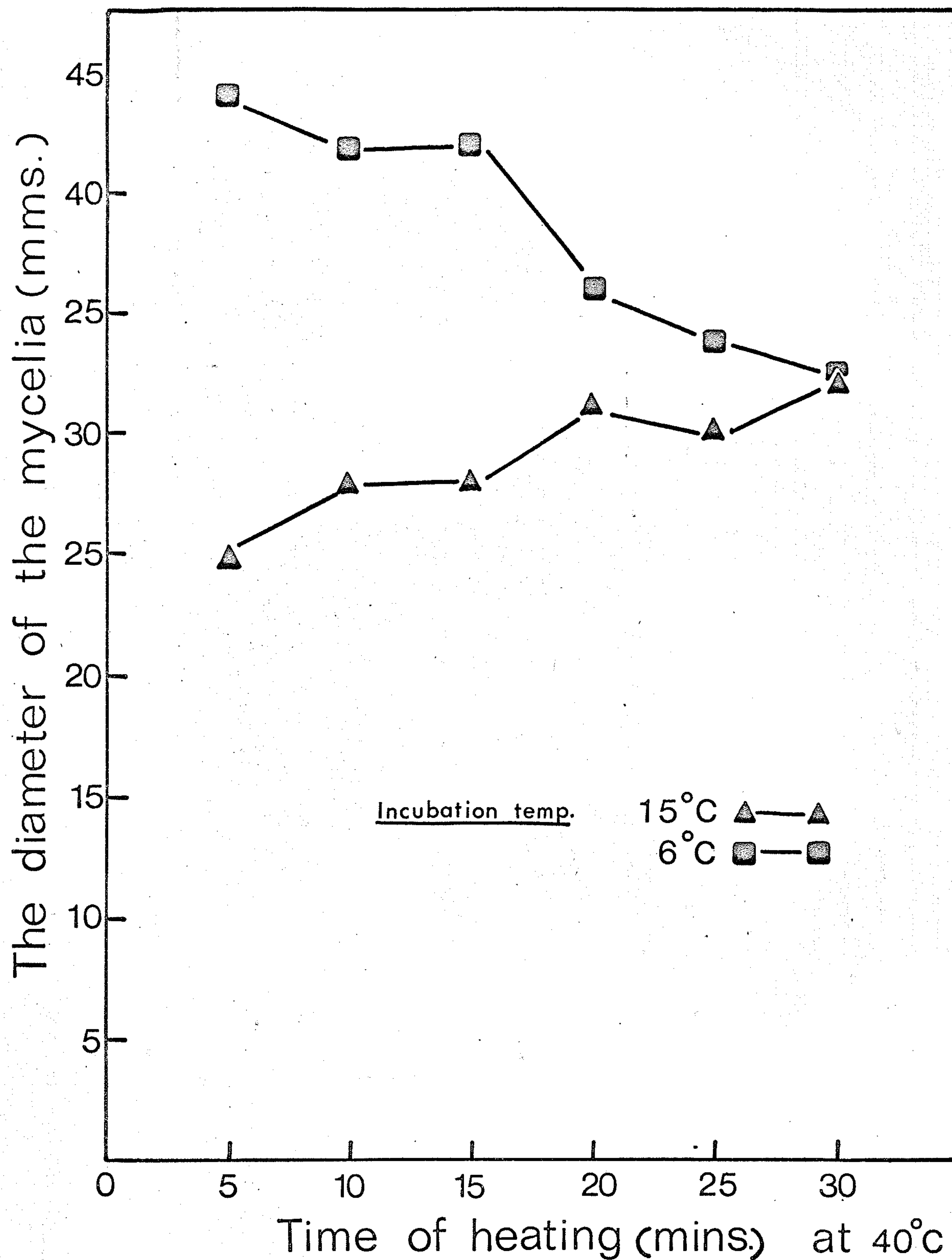



FIGURE 41. The effect of the saturated sucrose solution (concentration = < 3 M) on the heat resistance of Diplocladium sp. incubated at 6°C and 15°C before treatments.

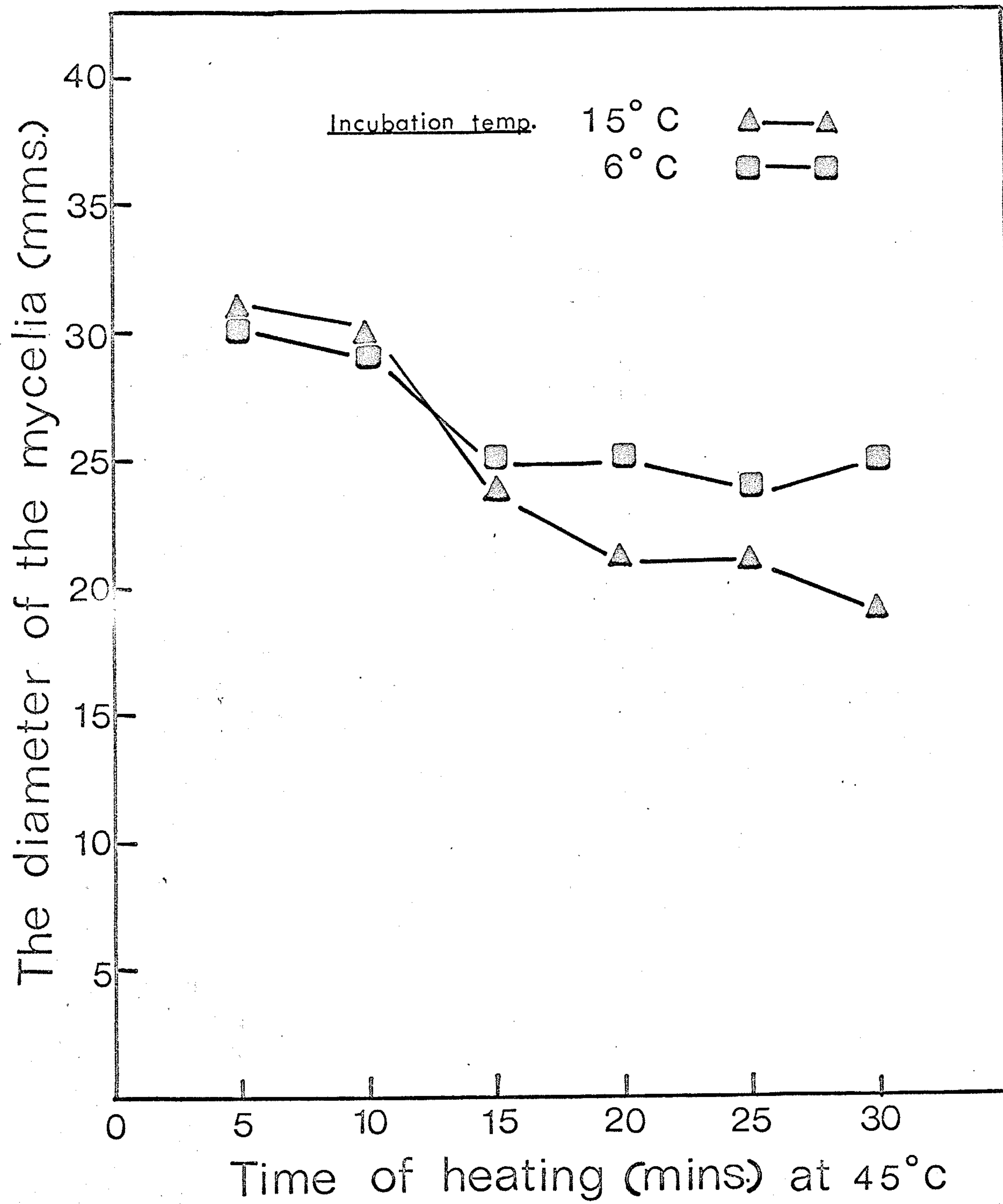



FIGURE 42. Similar to Figure 41 at 45°C.

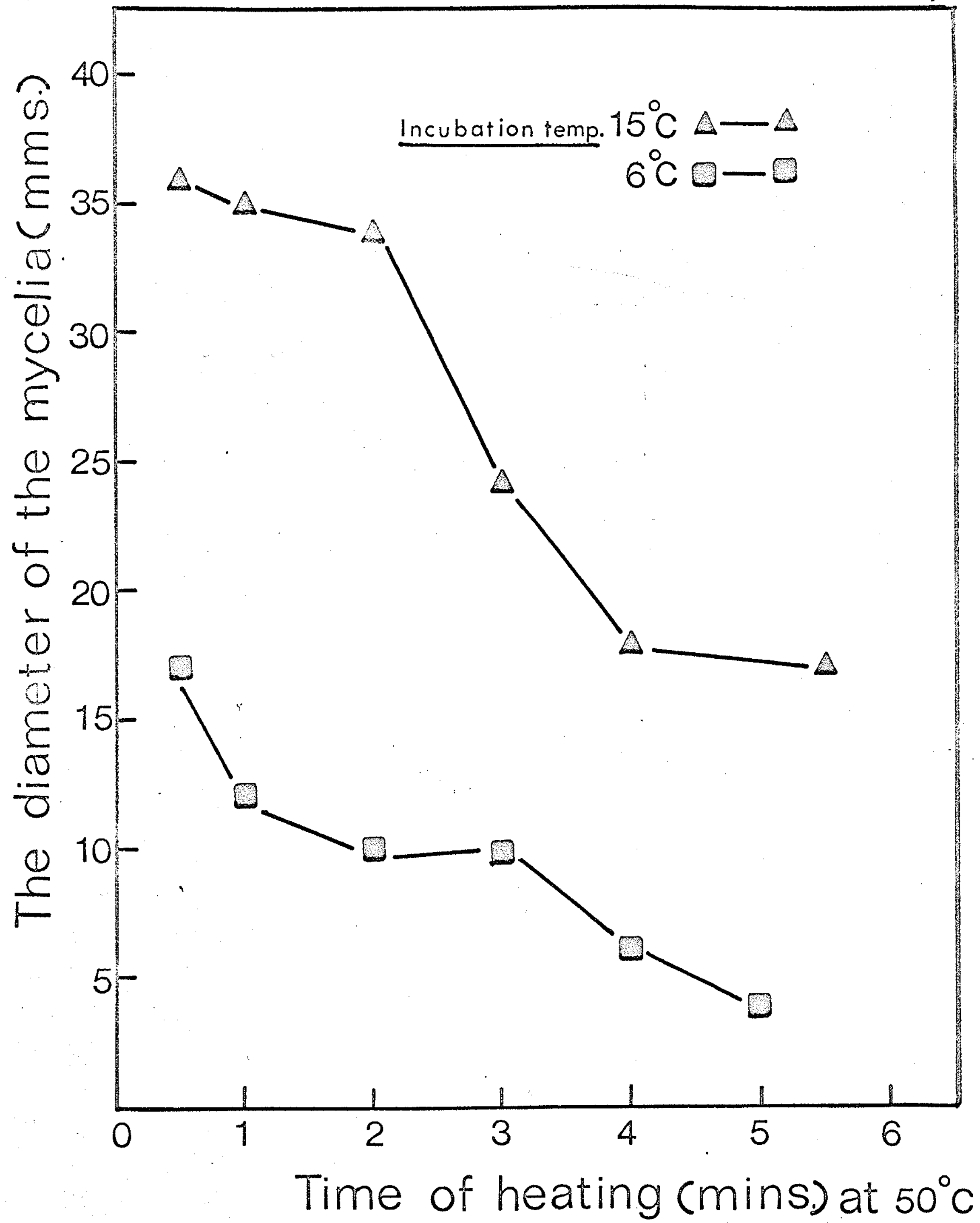



FIGURE 43. Similar to Figure 41 at 50°C.

# DISCUSSION

# DISCUSSION

## I. THERMAL ADAPTATION

Although the thermal adaptation has been clearly shown in animals, plants and some micro-organisms in the biological literature by Edney (1964), Parker (1963) and Yarwood (1961, 1963), however, results in the Part I of this thesis indicate only a very limited degree of thermal adaptation in the five fungi tested on the basis of the linear growth. The linear growth of each fungus tested varies with the incubation conditions as indicated in Tables I-V. It seems that temperatures, concentrations of the culture medium, time and the species of fungus are the four important factors which affect their growth rate in this study. It was found in general that the fungi maintained on 5 N sucrose peptone can survive longer or showed slight adaptation to the temperatures of 6°C and 30°C as compared to the corresponding cultures maintained on 1/5 or 1/125 N sucrose peptone media. From the data it can be seen that _S. sclerotiorum_ maintained at 6° and 30°C, and _P. cocos_ maintained at 30°C gave clear evidence in this aspect. In addition, it was noted that the older regions of the mycelium of _S. sclerotiorum_ maintained on 5 N sucrose peptone medium at 6°C turned greenish-yellow after 2-3 months. The explanation is not clear. As most fungi tested grew better on concentrated sucrose peptone medium at unfavorable conditions, this suggested that lowering the water content of the mycelia rendered the fungi more resistance to heat or cold. This is similar to the result obtained with plants by Osborne (1966) and Levitt (1951). However, there were some non-

consistent cases found, such as R. solani and Diplocladium sp. which showed fairly good growths on 1/125 N medium at 30°C. Diplocladium sp. maintained on 1/5 N sucrose peptone medium and S. purpureum kept on 1/125 N sucrose peptone medium at 6°C had the similar effect. Since many factors are involved in the growth of the organisms, it is, however, a far from adequate interpretation of this effect on the basis of the results secured so far.

It is apparent that the cultures of each fungus maintained on various concentrations of sucrose peptone medium at 15°C as control samples, showed either a steady response to this temperature or decreased the growth rate as the time of incubation increased. The investigation of the phenomenon of deterioration in R. solani is very interesting. The cultures maintained on 5 N sucrose peptone medium at the temperature of 6°C, 15°C and 30°C respectively showed signs of the change by turning a brownish colour as the incubation time increased. Since these deteriorated cultures ceased to grow after a few sub-cultures, it could be seen that the loss of vitality was a gradual process. The linear growth rates of most of the fungi tested showed a slight initial increase followed by a slowing down during the course of the experiment. The phenomenon of deterioration in R. solani and other fungi tested may be due to a similar, but more marked metabolic change with age under the influence of an unfavorable environment, such as insufficient nutrient or extreme high or low temperatures.

## II. THE EFFECT OF INCUBATION TEMPERATURE ON HIGH TEMPERATURE TOLERANCE

### A. THE RELATION BETWEEN HEAT RESISTANCE OF THE FUNGI AND INCUBATION TEMPERATURES AND TIME

From the data shown in Part II of this thesis, it is seen that the heat resistance of mycelia increases with the increase of the incubation temperatures in some fungi tested. In other words, the lethal temperature of the organisms rises with the incubation temperature. This characteristic was also demonstrated on spores of *Bacillus anthracis* by Weil (1899) and on woodlice by Edney (1964).

In the case of *Diplocladium* sp. and *S. purpureum*, the thermal resistance is shown to be very markedly changed by the influence of the incubation temperatures and time, and they show a decrease in heat resistance as a result of adaptation to low temperatures. Whereas in *S. sclerotiorum* and *P. cocos*, there was very little effect. Time seems to be a very important factor in this study, because an appreciable change in thermal resistance occurred only after certain periods of incubation at particular temperatures. It is apparent that the time required to alter the heat resistance by the influence of the incubation temperatures varied with the organisms tested. In the case of *Diplocladium* sp., heat resistance was diminished after successive transfer at the temperature of 6°C for 4 months (Table VId). Similar results were also obtained for *S. sclerotiorum* after about 5 months (Table VIId). However, there was no such effect shown by *S. purpureum*. It was found that in *P. cocos* the thermal resistance was slightly increased after transfer at 30°C for 3 months (Table VIIIc),

but heat tolerance decreased again after 5 months as shown by the phenomenon of retarded growth after sublethal treatments. The decrease in heat tolerance after keeping at 30°C for long time may be explained by the phenomenon of lowering the vitality of the fungus under the unfavorable environment. It has been suggested by Loginova and Verhovtseva (1963) that the supplimentory amino acids are required to maintain the normal growth of thermotolerant yeast, because the amino acid synthesizing system is disrupted by the high temperature. Unfortunately, none of the fungi grew well at 30°C, so no material was available for the test. A similar phenomenon was seen in *Diplocladium* sp. maintained at 6°C for 5 months, showing a lowered resistance to heat, and retarded growth after sublethal treatments.

The phenomenon of retarded growth was shown by some cultures after the sublethal temperatures treatments. It might be due to one of two reasons (a) many of the hyphae may be killed in the heating process, giving rise to a long lag period in growth, or (b) it is possible that organisms were injured during the heat treatment. It would be very interesting to carry out further study on the effect of prolonged maintenance of the retarded organism on fresh media in order to investigate whether the organisms would eventually resume their normal growth rate.

In general, it was found that the higher the treatment temperatures, the longer the lag phase, and the extension of the lag period is

considered to be the time required for the organism to recover from heat injury.

The increase in the heat resistance of organisms under the influence of high incubation temperatures is considered as evidence of acquired thermal adaptation. Furthermore, it is a physiological adaptation, but not a genetical adaptation. Since time is required to cause the change, this may suggest that some progressive metabolic change may be involved in acquired heat resistance. It was reported by Stoner (1963) that the oxidation and turnover of glucose are increased in both fed and fasted rats as a result of acclimation to cold. At the same time, some adaptive changes are believed to occur in the enzyme system of the carbohydrate metabolism. A rise in protein -SH in plant was reported by Levitt et. al (1961) during exposing to hardening conditions. It is possible that the mechanism of thermal adaptation in animals, plants and micro-organisms is similar at the cellular level.

## B. THE EFFECT OF THE VARIOUS CONCENTRATIONS OF SUCROSE SOLUTIONS ON THE THERMAL RESISTANCE OF THE FUNGI

From the data given in Tables X a-c and Tables XI a-c, it is seen that the thermal resistance of the mycelia was greatly increased when heat treated in sucrose solution rather than when treated in distilled water, and that the heat tolerance increased with the rise of the concentration of sucrose solution. These results are similar to the results obtained with the spores of

*Micromonospora vulgaris* by Erikson (1952) and in *Rhizopus nigricans* and *Aspergillus niger* by Wallace and Tanner (1931). It was demonstrated by Fay (1934) that the heat resistance of several bacteria in solutions of dextrose and sucrose increased with the increase of the osmotic pressure of the solutions treated. Whereas the osmotic pressure of the solution was not considered as the only agency in the protective action to heat because equimolal concentrations of sucrose solution showed greater protective action against heat than the dextrose solution. The results secured so far in studies would appear to be related to the findings of Fay (1934) that hypotonic sugar solutions delayed the action of heat on egg albumin. In addition the fact that no retarded growth was seen after sublethal treatment in sucrose solutions further supports the evidence for a protective role for sucrose.

# SUMMARY

## SUMMARY

1. The fungi tested showed a slight adaptation to either $6^{\circ}C$ or $30^{\circ}C$ on the basis of linear growth.

2. It was found that the fungi maintained on 5 N sucrose peptone medium at $6^{\circ}C$ or $30^{\circ}C$ grew better and could survive longer comparing to those cultures maintained on 1/5 or 1/125 N media at the same temperature.

3. The phenomenon of progressive deterioration of fungi was investigated in this study. It is especially marked in the case of *Rhizotonia solani*.

4. The thermal resistance of the fungi tested was related to the incubation temperatures and showed the effect of adaptation to either low or high temperatures.

5. The protective action of sucrose solution rendered the fungi more resistant to thermal shock.

6. The resistance of the fungi to thermal shock increased with the concentration of the sucrose in the temperature bath.

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