A STUDY OF

THE ALTERNARIA LEAFSPOT COMPLEX

ON POTATOES AND TOMATOES

IN THE MANAWATU.

This Thesis is presented as partial fulfillment of the requirements for the M.AGR.Sc. Degree of Massey University College of the Manawatu.

B.T. HAWTHORNE. September 1963.

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"YOU WILL FIND A FUNGUS AND DETERMINE ITS CHARACTERISTICS. YOU TURN TO THE BOOKS AND DECIDE ON ITS GENUS. THEN YOU LOOK FOR THE SPECIES. AND YOU LOOK, AND YOU LOOK, AND AFTER A WHILE YOU FIND IT!

IN ANOTHER GENUS!"

DR. J.J. DAVIS.

I N T R O D U C T I O N.

The fungus Alternaria (Macrosporium) solani is associated with a foliage disease of potatoes and tomatoes throughout the world. Although there are often several phases of attack on these hosts by the fungus e.g. tuber damage in the potato and seedling loss with tomatoes, the disease has been named on the basis of the foliage symptoms which are characteristic. Two names are commonly used (1) Target Spot

(2) Early Blight,

and of the two, 'Target Spot' is the more descriptive since foliage lesions are circular to irregular dark brown areas with a very characteristic zonation effect due to a series of more or less concentric rings within the lesion.

Early investigators studied the diseases of the respective hosts from a practical plant pathology standpoint, concerned, in the main, with tracing the Disease Cycle and formulating methods of control.

A number of independent studies of the disease(s) caused by A.solani on potatoes and tomatoes showed that the causal fungus was extremely variable in phenotype and genotype. This inherent variability has added to the confusion and complexity surrounding the taxonomy and nomenclature of the fungus through the years. An additional source of complication with respect to taxonomy and nomenclature has arisen from the slowly evolved conception of the genus Alternaria to its present day delimitations e.g. early workers placed the fungus in the genus Macrosporium on the basis of characters which, according to modern views, as put forward by Wiltshire in 1933, place the fungus in the genus Alternaria.

More particularly in the U.S.A. and Australia Alternaria solani seems capable of 'causing' widespread epiphytotics of 'Early Blight' of potatoes, which result in heavy loss. In addition, the several phases of attack, by the fungus, on tomatoes have proved very costly over the years due to seedling loss and reduction in fruit yield. In N.Z. in 1927 'Early Blight' was regarded as one of the most serious of potato diseases, latterly, however, the disease seems of no national importance although in localized areas it can cause heavy and often unsuspected loss e.g. the observation that 'Late Blight' resistant potato varieties Rua and Tahi in a recent season in the Manawatu were heavily affected with 'Early Blight' in later stages of growth - sufficient to cause local growers to doubt the claimed resistance to 'Late Blight' !

The consistent prevalence of leaf spotting and Early Blight on potatoes and tomatoes in the Manawatu area (of the N.Is.,N.Z.) over several seasons together with the apparent absence of records of a comprehensive study of this disease in N.Z. has led to this study.

At the outset a straightforward study of the disease in the field and in the glasshouse was envisaged. This theme had to be considerably modified however because regular inspection of a representative 8 - 10 crops in the Manawatu showed that the incidence of 'Early Blight' on potatoes and tomatoes was extremely low in the two seasons covered by the duration of the work.

As a result the emphasis was shifted and the aims of this study became:

(1) To make a study of the characteristics of the disease under glasshouse conditions.

(2) To compare isolates of the fungus from three solanaceous hosts namely, Potato, Tomato and Black nightshade with reference to morphology, physiology and pathogenicity and to gauge the extent of variability between 'strains' and its importance in taxonomy.

MATERIALS and METHODS.

Materials and methods specific to particular parts of this study are described in detail in their respective sections. Materials and methods applicable to several sections of the work are, however, outlined below.

MATERIALS and METHODS USED in the LABORATORY.

(a) PREPARATION OF MEDIA.

The detailed preparations of all media are described in Appendix I. Oxoid potato dextrose agar (P.D.A.) was used for all normal culturing work. This medium was prepared in 2 - 4 litre quantities as required, and stored after autoclaving in partly filled 250 ml. flasks stopped with cotton wool plugs.

(b) INOCULATION OF MEDIA.

Inoculum discs 5 mm in diameter, cut with a sterile cork borer from the edge of an actively growing colony on P.D.A. were used in all experimental work. The inoculum disc was always placed mycelium-side down on the media being inoculated. The diameter of the inoculum disc i.e. 5 mm, was subtracted from the measured diameter of all colonies recorded.

(c) MEASUREMENT OF GROWTH RATE ON ARTIFICIAL MEDIA.

Work by Brancato and Golding (1953) indicates that colony diameter is a valid measure of the effects of environmental factors such as medium constituents, pH, and temperature. Throughout this study where colony diameters were to be measured 3 - 4 colonies were used per treatment, 2 diameters at right angles were recorded for each colony and results expressed as an average. Since growth proved to be at a constant and linear rate records of colony diameters were made only once, normally 8 days after inoculation.

(d) INDUCTION OF SPORULATION AND PREPARATION OF INOCULUM.

Prolific sporulation of Alternaria solani on artificial media is notoriously difficult to obtain and a wide variety of different methods involving various media, treatments with ultra violet light and combinations of light and high humidity, appear in the literature.

The technique used in this study is an adaptation of a method described in 1962 by Ludwig, Richardson and Unwin. The surface of approximately 2 week old cultures of the fungus growing on laboratory P.D.A., in petri dishes, was scraped with the end of a clean glass slide so that all the aerial mycelium was removed. The plates were then washed with lids removed, in running tap water for 24 hours. To prevent the agar floating away the plates were covered with a thin muslin cloth. Following washing the plates were stacked in an inverted, slanted position. The method of stacking ensured that conditions of high humidity obtained at the surface of all colonies.

This method provided heavy and consistently reliable crops of conidia from all isolates within 48 hours of stacking.

Spore suspensions used in all inoculation work were obtained quickly and easily.

A fine jet of distilled water from a plastic 'wash' bottle was directed onto the surface of the sporulating colony until there were about 7-8 ml. of water in the petri dish. A trace of TEEPOL was added to improve wetting and a smooth glass rod rubbed over the colony surface to further improve the release of conidia into the water. The concentrated spore suspension was poured into a measuring cylinder to determine the volume and then the actual concentration was measured with the aid of a Neubauer haemocytometer. Desired concentrations were obtained by adding the required volumes of distilled water.

(e) EXAMINATION OF DISEASED MATERIAL AND ISOLATION OF FUNGI FROM LESIONS.

Lesions from diseased material were placed on 2 glass slides in a petri dish containing a moist filter paper. The petri dishes were left on the laboratory bench. If the fungus was viable within the lesion 24-36 hours was sufficient time for production of conidia.

Conidia were identified by microscopic examination and then single spore isolations were made to laboratory P.D.A. slopes preparatory to subsequent culturing.

Since the conidia of Alternaria solani are comparatively large and readily discernible with a binocular microscope the technique used for isolation and identification was both simple and rapid.

The main contaminates found on naturally infected material were species of Stemphylium and Alternaria tenuis. These were readily distinguishable and caused no concern in making single spore transfers of the conidia of Alternaria solani.

MATERIALS and METHODS USED in the GLASSHOUSE.

(a) SOIL MIXTURES.

Sand, loam and peat were used, in conjunction with chemical fertilizers and dried blood, to make up 2 different soil mixtures.

(i) Seedbox soil Mixture 2 Loam : 1 Peat : 1 Sand plus ¾ oz lime per bushel of soil mixture and 1½oz superphosphate per bushel.

(ii) Pricking out soil Mixture 7 Loam: 3 Peat: 2 Sand plus 4 oz per bushel of a basic fertilizer mix containing the following ingredients:- 1 part, by weight, Potassium sulphate; 2 parts dried blood; 2 parts superphosphate 1 oz per bushel of lime.

The peat and loam were partially sterilized before use. A thin layer of the peat and/or loam was spread on a concrete floor, dampened slightly, and covered with a polythene sheet which was securely held in position, around the edges, with heavy weights. Methyl bromide gas was then released under the sheeting which was left in place for 24 hours. Experience showed that a 1 lb. tin of Methyl bromide prevented subsequent growth of all weed seeds in approximately a cubic yard of peat or loam spread out in a thin layer. After treatment with Methyl bromide the peat and loam were stored in separate bins and used as required. The sand used was deep river sand and hence did not need methyl bromide treatment to kill weed seeds.

(b) PRODUCTION OF PLANTS.

Seeds of all plant species except potato were sown in small seed boxes (18"x12"x3"). When the seedlings were sufficently advanced they were pricked out into pots. In most cases there was 1 plant per pot but with plants such as Zinnia, Carrot, Ageratum and Godetia, used in host-range studies, 3 seedlings were pricked out into each pot.

Potato plants were raised by planting seed potatoes in pots containing pricking out mix.

Where it was warranted, especially with tomatoes and black nightshade plants, staking and tying of the plants was carried out. This produced a

better plant and greatly facilitated movement of the pots plus plants together with economizing on bench space.

Watering of plants was carried out night and morning during the summer months (November to mid March.) since the tomato and black nightshade plants especially were prone to wilting. During cooler weather however the plants needed watering only every 2 - 3 days.

Maximum temperatures recorded in the glasshouse during hot weather were about 95°F but only for short periods. During the winter months an average temperature of approximately 70°F was maintained in the glasshouse by means of thermostatically controlled heaters placed under the benches.

Since potato seed proved difficult to obtain, in quantity, when required, and even more difficult to store in good condition most of the "Disease Cycle" studies were carried out with tomato plants. Hence, a constant supply of tomato plants was maintained by sowing seed every 2 - 3 weeks and pricking out the seedlings into pots.

(c) CONTROL OF PESTS.

Some trouble was experienced with White Butterfly Caterpillar damage but good control was obtained by dusting the plants with 'Derris Dust'. Occasionally an aphid population started to build up and this was controlled by shutting the glasshouse down at night and burning a NEXA strip inside. Carried out at weekly intervals this was an efficient control measure.

(d) INOCULATION OF PLANTS.

Plants were inoculated with spore suspensions sprayed onto the foliage in a fine misty spray from a patent "WINDEX" sprayer.

After inoculation plants were placed in a high humidity chamber constructed out of light, clear "Armathene" sheeting.

SELECTION AND DESIGNATION OF ISOLATES USED IN THIS STUDY.

In April 1962 crops of potatoes and tomatoes and volunteer plants of black nightshade, showing symptoms typical of attack by Alternaria solani, were examined and single spore isolates obtained.

The isolates were designated according to:

(i) The host from which they were isolated. This was indicated by using 2 capital letters representing the botanical name. Thus an isolate from potato (Solanum tuberosum) was designated ST ---; from tomato (Lycopersicum esculentum), LE - - -; from Black nightshade (Solanum nigrum), SN - - -.

(ii) The date on which the isolation was made. This was indicated by using the day and the month. Hence an isolate made on the 8th. of April (4th month) becomes ST) LE) SN) 84 -

(iii) The locality where the diseased material was obtained. This was indicated by a letter of the alphabet. Usually 5 single spore isolations were made from each diseased specimen.

Eleven isolates were selected for study. These were:

ex Black nightshade	ex Potato	ex Tomato
SN84A	ST84B	LE84E
SN84D	ST84C	LE84G
SN264A	ST264B	LE264B.
SN264D	ST264C	

An additional isolate was obtained from the Auckland area through the services of Dr.F.J.Morton of Plant Diseases Division D.S.&I.R. This isolate was off potato and designated A46. This isolate was incorporated into the work part way through the study and hence it was not used in all studies.

THE DISEASED PLANT.

INTRODUCTION.

The first description of the leafspot on potato caused by Alternaria solani was given by Galloway in 1891. Jones (1891,1893) extended this work and distinguised 'Early Blight' symptoms and those of Late Blight and tipburn. A major contribution to the understanding of the etiology of the disease was made by Rands in 1917 in a detailed and prolonged study at Wisconsin.

Eason and Shapovator (1918) were the first to demonstrate that the fungus caused stem lesions on potato and tomato plants as well as the typical leaf symptoms. In 1925 Folsom and Bonde described a dry rot of potato tubers due to attack by the fungus and they demonstrated this pathogenic action by infection experiments.

Since the early work there have been many studies on various aspects of Early Blight as it affects potato and tomato crops and on the development of suitable control measures.

In N.Z. Early Blight on potatoes was first recorded by Kirk in 1895 and later by McAlpine (1903, 1911) in Australia and N.Z.

Early Blight occurs throughout the world where potatoes and tomatoes are grown but it only appears to have caused consistent major losses in the U.S.A., since the latter part of the 19th. century. In European countries attack by the fungus on potato leaves usually is not of any particular significance since the attack does not set in until relatively late - in contrast to the early attacks in the U.S.A. Indeed, Stapel (1943) in Denmark was able to show that even severe attacks on foliage resulted in only a small yield reduction probably because the attack did not become severe until after tuber development was nearly complete.

When an outbreak of Early Blight does occur however losses of up to 50% or more are common and to be expected.

e.g. For outbreaks on potatoes in Wisconsin Rands (1917) estimated losses between 10-25%

" " " " " Sth.Africa Wager (1931) " " " 8-50%

" " " " Germany Braun (1938) " " " 20-40%

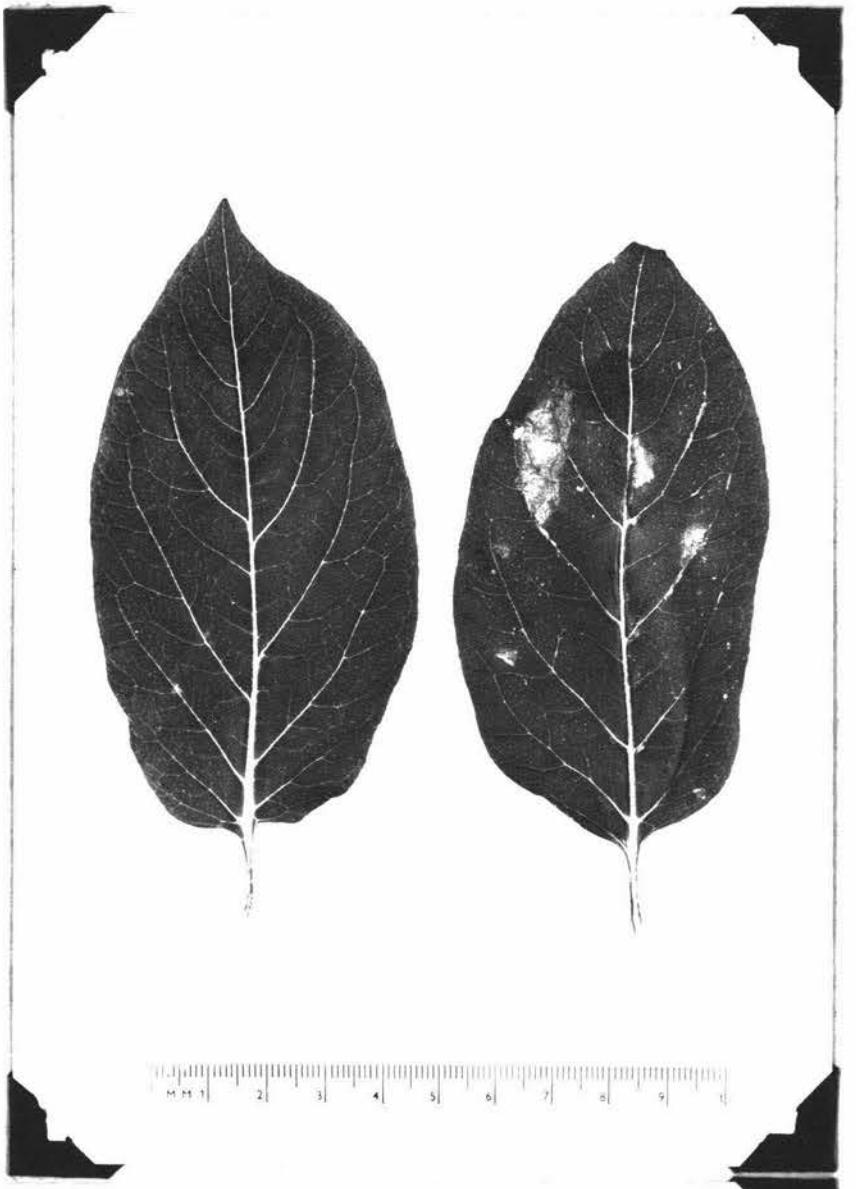
and on tomatoes in Virginia, McMurtrey (1927) estimated losses to be 35%

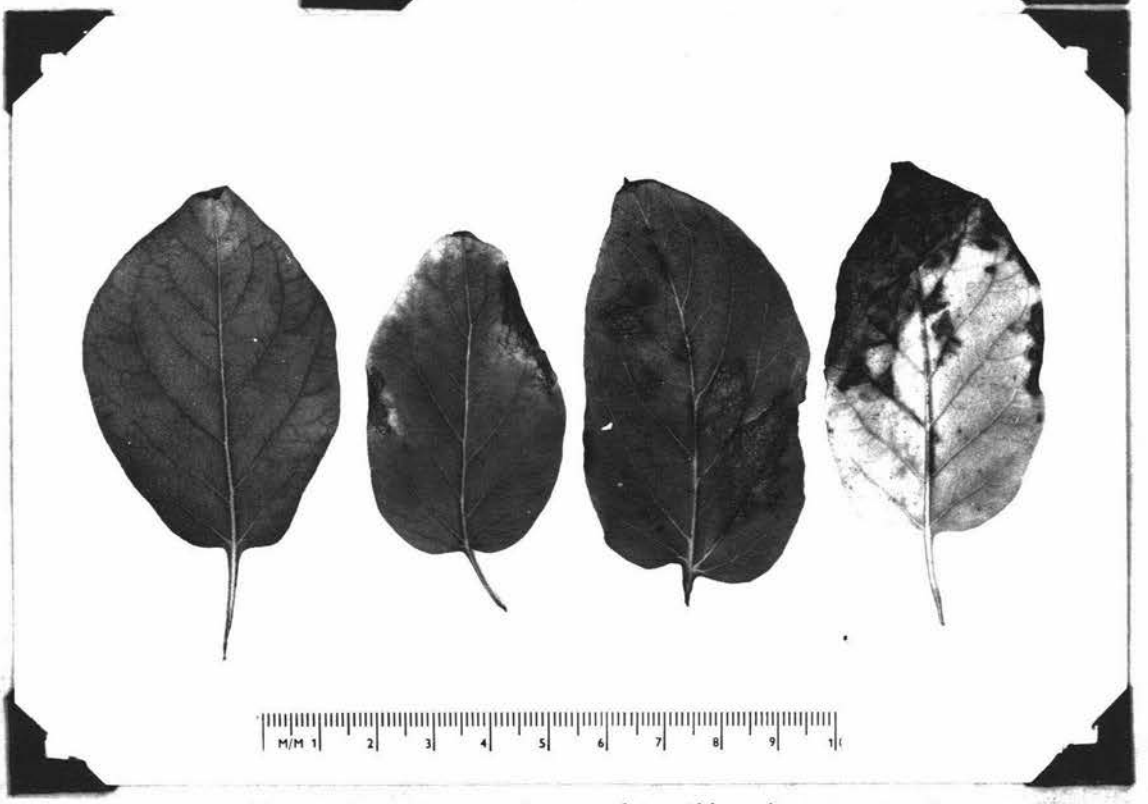
" " " Denmark, Neergaard (1945) " " " 75%

Although, in 1927, Early Blight was one of the most serious potato foliage diseases in N.Z. it is not regarded as important now. Similarly, on tomatoes the disease has never assumed large scale proportions in N.Z. One of the major reasons for this is that potatoes and tomatoes are rarely attacked until very late in their growing season. and the marketable crop is little affected. In addition, the regular sprays for Late Blight, where carried out e.g. in the Pukekohe and Manawatu areas, are effective against Alternaria solani. Feddersen (1962), in South Australia found that Maneb sprays (2lb./acre) applied to potatoes after flowering, and continued at fortnightly intervals until harvest, gave excellent control in areas where the disease was common.

Although Alternaria solani is a comparatively weak pathogen in European countries and in N.Z. it reacts differently in the U.S.A. and on tomatoes, especially, it initiates several phases of attack which follow the maturity of the plant starting with damping off of seedlings then collar rots, foliage damage and finally fruit rotting. Moore (1942) and Moore et al (1943) in the U.S.A. developed a seed treatment for tomatoes which controlled the initial phases of Alternaria solani attack from seed-borne inoculum. They found that prolonged storage of seedlings and wilting increased the incidence of attack. This problem seems peculiar to parts of the U.S.A. where there is a large scale shipping of seedlings from nursery areas to the commercial growing areas.

(A.) Healthy and diseased leaves.





(B.) Gradation of symptoms from healthy to severely diseased.

FIG.1 SYMPTOMS OF EARLY BLIGHT ON POTATO

SYMPTOMS OF EARLY BLIGHT ON POTATOES AND TOMATOES IN THE MANAWATU.

The disease is predominantly one of the foliage of plants of both crops from when they are nearly mature until senescence. The symptoms are very similar for both plants.

The fungus may attack the leaves, petioles or stems. It is most common on leaves causing circular to irregular dark brown lesions which are characterized by a series of concentric ridges which give the so called Target Spot effect. Infection usually occurs around the margins of the lower, older leaves. The lesions spread fairly rapidly on the older tissue and the leaves soon become chlorotic and then brown and brittle. Leaf lesions on younger leaves spread slowly or else may become isolated and die leaving a small brown area. Lesions on the stem and petioles may be up to an inch long and are dark brown and more or less superficial. Fig shows typical foliage symptoms on potato.

Infections of tomato seedlings in the glasshouse did not produce severe damage of the leaves and stems and the majority of plants grew out of the disease which was confined to the initial areas of infection.

Symptoms of Alternaria solani attack on Black nightshade are more or less similar to those on potato and tomato, however, the lesions are generally more irregular and have a less prominent ridged or zonate appearance. Also, the lesions are not restricted in development due to the absence of big veins in the leaf in contrast to the leaves of potato and tomato.

ASPECTS OF THE DISEASE CYCLE ON TOMATOES UNDER GLASSHOUSE CONDITIONS.

There are several stages of host-parasite interaction in plants which are collectively known as the "Disease Cycle" viz:

Stage 1: Penetration of host tissue and infection.

Stage 2: An incubation period during which the pathogen becomes established in the host tissue.

Stage 3: Appearance of visible symptoms on the plant indicating the 'disturbing' presence of the pathogen.

Stage 4: Production of new infective particles by the pathogen to provide for secondary spread to fresh host material.

Stage 5: The pathogen goes into an 'overwintering' or resistant stage, which may leave the host, in order to survive adverse conditions.

Observations were made, under glasshouse conditions, of some of the variables which could affect stages of the Disease Cycle and the development of Early Blight. Throughout these studies tomatoes were used as the test plant because:

(1) all the other inoculation work indicated that potatoes and tomatoes reacted identically to the same treatments,

(2) tomatoplants were far easier to raise and gave economies of glasshouse space, time, materials and labour.

Spore suspensions used during this work were made up with conidia from the following isolates:

SN84A, SN84D, SN264A, ST84C, ST264B, LE84E, LE84G and A46. It was hoped to cut down any variation due to strain effects by this method and thus get a more uniform idea of the possible behaviour of the fungus in the field from glasshouse observations.

PENETRATION and INFECTION:

Several tomato plants were inoculated with a concentrated spore suspension and put in a high humidity chamber for 24 hours after which inoculated leaves were removed from the plants. Small sections were cut out of the leaves and cleared by warming in 70 per cent ethyl alcohol for 1½ - 2 hours. When clear the sections were stained

in 0.2% Cotton Blue until the desired contrast was obtained. The sections were mounted in lactophenol on glass slides and examined under the microscope.

RESULTS:

The majority of conidia had produced germ tubes which grew out over the surface of the leaf. Many germ tubes appeared to be indeterminate in growth and showed no sign of penetrating the leaf. In quite a number of cases however the germ tubes had ceased growth and swollen at the ends to produce a flat pad or appressorium-like structure. In a few cases, by manipulating the light intensity and focus of the microscope it was possible to trace a blue stained fungal thread from the appressorium into the cleared host tissue.

Rands (1917) found that germ tubes of Alternaria solani penetrated either directly through the cuticle or else through the stomata and Angell (1929) observed that germ tubes of A.porri penetrated through the stomata. In this work however penetration was only seen to occur directly through the cuticle but this was after a period of only 24 hours and it is probable that later germinating spores or more extensively ramifying germ tubes may have penetrated via the stomata.

Fig 2. gives a semi diagrammatic picture of spores germinating on host tissue and in some cases producing the appressorium-like structures and infection threads.

DEVELOPMENT OF INFECTION:

It was noticeable during the initial cross inoculations on the 3 hosts, Potato, Tomato and Black nightshade with the freshly obtained isolates that symptoms of infection were evident within 48-72 hours if the temperatures were 24°C or slightly above and the relative humidity was kept high. Under these conditions leaf lesions extended rapidly and within a week most inoculated plants were severely diseased and dying. However if the plants were removed from the inoculation cabinet where conditions were kept optimum for infection and disease development, leaf lesions were slow to develop and in many cases the plants continued active growth.

When isolates were several months old however up to 5 days elapsed before small lesions appeared on inoculated plants even though the conditions for infection were optimum.

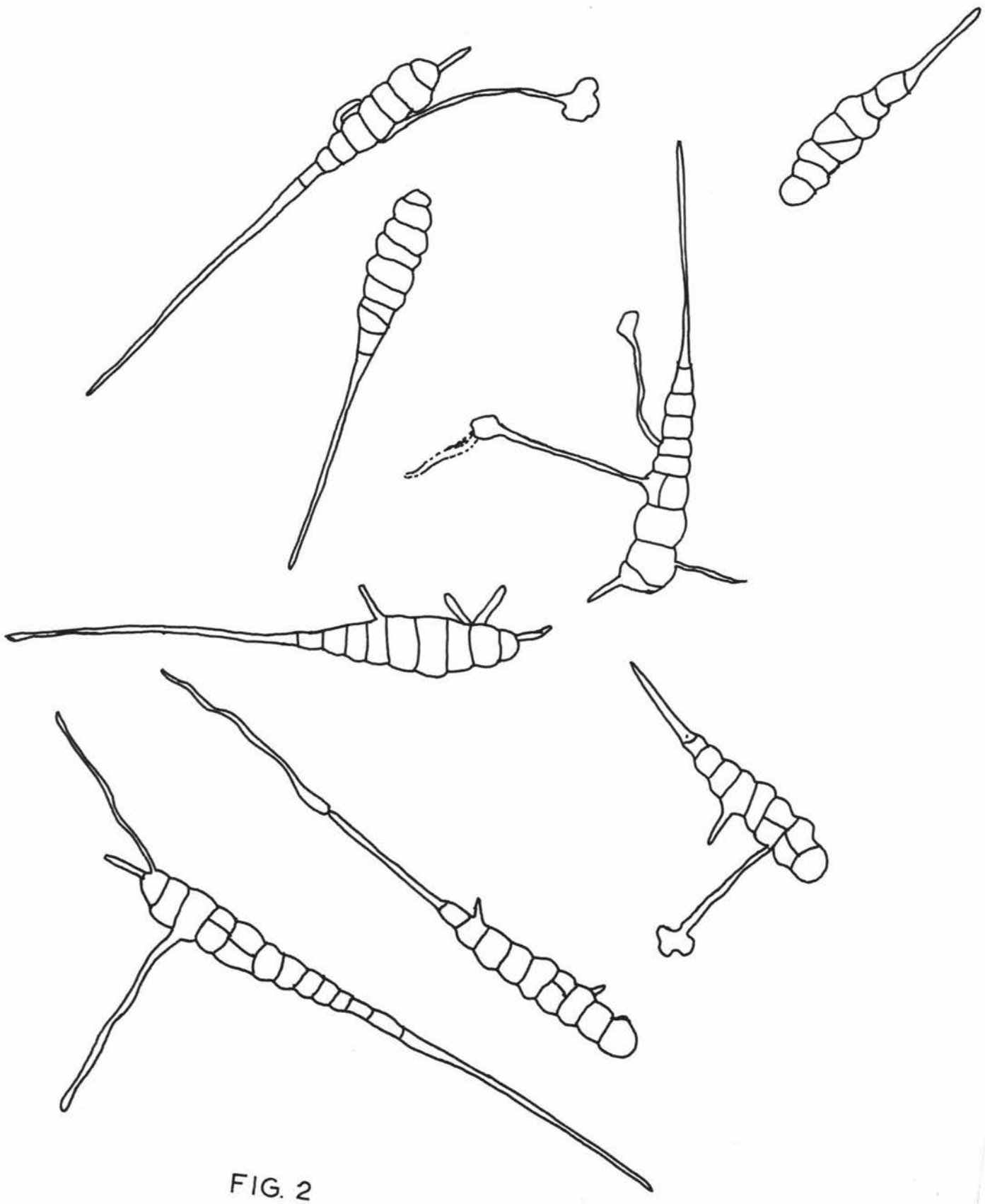


FIG. 2

This observation showed the importance of 'pathogen vigour' in the development of the disease. An inoculum potential experiment was carried out to test the effect of increasing doses of the pathogen on development of infection. Four levels of spore concentration i.e. 10.5×10^4, 5×10^4, 1×10^3, and 1×10^2 spores/ml were used and 6 plants were inoculated at each level. (All the plants were the same age - 14 weeks from seed sowing.) A count of numbers of lesions in each group after 5 and 10 days from inoculation showed that there was no difference between levels of spore concentration. This seemed an anomalous result until the results were obtained from an experiment which showed the profound effect of age of the plant on incidence and development of infection.

Six plants from each of 5 different age groups were inoculated with a spore suspension containing 1×10^4 spores per ml. Results were recorded 5 and 10 days after inoculation and are presented in the table below.

TABLE I.

	AGE FROM SOWING (WEEKS)									
	27		24		17		14		4	
DAYS FROM INOCULATION	5	10	5	10	5	10	5	10	5	10
PLANT 1	S	D	L	S	L	M	M	S	S	M
PLANT 2	S	D	L	S	VL	M	L	M	M	M
PLANT 3	S	D	M	S	VL	L	VL	L	S	M
PLANT 4	M	S	M	S	L	L	L	L	S	M
PLANT 5	S	D	S	S	VL	M	VL	M	M	L
PLANT 6	S	D	M	S	NIL	L	NIL	VL	S	M

S = 90 - 100% of leaves heavily spotted. M = 50 - 75% of leaves lightly spotted.

D = leaves dead or dying. L = < 50% of leaves, very lightly, spotted.

It can be clearly seen that the older the plant, the more rapid and severe the infection and it seems that up until the plant is 17 - 20 weeks old it is fairly resistant to attack by Alternaria solani even if environmental conditions are suitable for disease outbreak. The seedlings, although initially severely infected

by the fungus, were able to grow out of the disease since the enviromental conditions were also optimum for rapid plant growth.

The results of the inoculum potential and age predisposition experiments make it obvious that host vigour is the major factor determining development of Early Blight in the glasshouse and very probably in the field. However, if the fungus gets firmly established on a plant it is capable of causing extensive damage to the leaves and stems so it must be regarded as severely pathogenic but only weakly or moderately parasitic.

SPORULATION ON THE HOST AND OVERWINTERING.

Rands (1917) appears to be the only worker to have investigated sporulation of the fungus on the host and he found that it produced only sparse crops of conidia under field conditions but even so these were enough to initiate epidemics.

Although a large number of lesioned leaves from diseased plants in the glasshouse were examined during the course of this study only rarely, were crops of conidia noted.

Rands also found that the conidia of Alternaria solani were able to remain viable in the soil throughout severe winters during which the ground was covered with snow for lengthy periods. He considered this to be the major method of 'overwintering' for the fungus.

Under conditions in the Manawatu it seems feasible to suppose that the fungus overwinters on Black nightshade plants since these abound in potato and tomato growing areas throughout the year. Spores must also remain viable in the ground and on seed potatoes although the potato tuber phase of "Early Blight" very seldom becomes noticeable.

DISCUSSION:

Early Blight is not a serious problem on potatoes and tomatoes in the Manawatu apart from infrequent isolated outbreaks. The weather is usually excellent for maintaining crop vigour and the intensive spray programme against Late Blight is also effective in controlling Early Blight.

Overseas work indicates that periods of high temperature (28°C -) combined with high humidity or dew deposition on the leaves are prerequisites for abundant infection in the field.

Due to the various probable modes of overwintering of the fungus in the field it is safe to assume that viable spore of Alternaria solani are always present about potato or tomato cropping areas. The low incidence of Early Blight in the Manawatu and other areas of N.Z. indicates that enviromental conditions, in the main, are such as to keep crops vigorous which is apparently enough to keep the weakly parasitic fungus from increasing. However, as soon as the crops begin to lose their vigour, for any reason, (generally with senescence.) the fungus can infect and spread relatively quickly.

THE FUNGUS.

INTRODUCTION.

Twelve single spore isolates of Alternaria solani were selected for a comprehensive study of their morphology and physiology on artificial media. Knowledge gained from this study was combined with that derived from cross-inoculation and host range studies in order to arrive at conclusions regarding the taxonomy and nomenclature of the fungus.

It was necessary to investigate the conditions required to induce abundant sporulation by the fungus on arti ficial media since without treatment all strains produced conidia very sparsely.

A STUDY OF ISOLATES ON ARTIFICIAL MEDIA.

MATERIALS AND METHODS:

This study was carried out in 2 Parts.

In Part I the isolates were grown on a range of 7 Media in order to compare cultural characteristics and variations in growth rate. In Part 2 the isolates SN84A, ST84B and LE264B (i.e. one isolate from each host) were grown on potato leaf extract agar, tomato leaf extract agar and for purposes of comparison, on water agar.

Preparations of the media used are described in Appendix I. 3 plates per isolate were inoculated with 5mm discs of mycelium taken from the edge of an actively growing colony on laboratory P.D.A. for each medium. After 8 days incubation at 26°C the colony diameters were recorded.

RESULTS:

Colony diameters from Parts I and 2 are recorded in Tables I and 2 respectively.

All isolates grew most rapidly and luxuriantly on laboratory P.D.A. although growth on Oxoid P.D.A. was nearly as good. Although individual isolates performed differently on different media there was usually uniformity in colony characteristics of all isolates on any given medium.

One exception to this uniformity of isolates occurred on Tomato juice agar and V8 juice agar since the isolates from Black nightshade always grew slowly and formed puckered, crinkled, very dense colonies in contrast to the regular colonies formed by the other isolates.

The small trial of the 3 isolates, SN84A, ST84B and LE264B showed that potato tissue extract substantially increased growth in comparison with tomato tissue extract. Somewhat surprising however was the greater radial growth made by all 3 isolates on water agar in comparison with tomato extract agar.

TABLE II

COLONY DIAMETER (mm.) OF 8 - DAY COLONIES EX DIFFERENT MEDIA.							
ISOLATE	Oxoid P.D.A	Lab P.D.A.	Prune Agar	Corn-meal Agar	Czapek Dox Agar	Tomato juice Agar	V8Juice Agar
SN84A	73.2	77.5	57.1	53.0	53.3	32.5	41.4
SN84D	74.5	78.5	51.0	65.5	56.0	32.0	54.2
SN264A	62.2	72.3	52.5	51.0	39.6	26.5	41.0
SN264D	71.0	73.2	59.6	58.7		36.0	
ST84B	72.5	82.5	53.5	69.0	68.5	55.0	57.5
ST84C	62.0	78.1	53.5	60.0	65.0	53.0	55.0
ST264B	65.6	71.3	48.5	59.0	51.5	48.0	49.0
ST264C	63.6	73.2	51.3	60.0	65.6	51.6	48.0
A46		80.5	54.5	62.0	67.3	63.0	
LE84E	69.8	78.0	24.6	62.0	41.0	60.0	56.2
LE84G	47.5	77.1	47.2	61.8	66.6	57.1	54.0
LE264B	70.8	75.7	49.0	58.1	62.5	56.0	53.0

TABLE III

COLONY DIAMETER (mm.) OF 8 - DAY COLONIES EX DIFFERENT MEDIA .			
ISOLATE	Water Agar	Tomato leaf Extract Agar	Potato leaf Extract Agar
SN84A	22.3	14.8	54.6
ST84B	52.8	40.5	65.8
LE264B	44.7	35.5	61.0

DESCRIPTION OF COLONY CHARACTERISTICS ON SEVERAL MEDIA.

Laboratory P.D.A.
Oxoid P.D.A.

Aerial mycelium loose and cottony, 1-3mm high with a tendency to flatten out on the medium in older colonies. The colonies were usually regular and frequently there were a number of dark concentric rings.

Colouration of mycelium was light olive grey to dark brownish grey. Pigment production by chromogenic isolates resulted in a range of medium colouration from yellow red to a deep crimson. This colouration deepened with the age of the colony.

Prune Agar.

Little or no aerial mycelium and a fairly sparse below surface growth of hyphae. Mycelial growth was even and regular but not dense. Hyphae were coloured a light to deep brown.

Czapek Dox Agar.

Aerial mycelium dense, 1 - 2mm high. Growth was irregular and hyphae tended to bunch together in strands below the surface. Colouration of hyphae was a dark brown.

PIGMENT PRODUCTION.

Isolates from Potato and Tomato when grown on P.D.A. produced an intense red colouration which became more pronounced with age of the colony, Isolates from Black nightshade however did not produce any pigmentation of the medium.

The production of pigment was not noticeable on the other media used apart from a yellowish colouration of the cornmeal agar.

Pigment production was greatest on P.D.A. when the conditions were optimum for growth.

SPORULATION.

None of the isolates produced conidia on any of the media under ordinary conditions. Laboratory P.D.A. was by far the best medium for supporting sporulation once the optimum conditions were applied.

DISCUSSION:-

Apart from the finding that isolates from Black nightshade were not chromogenic this study revealed that, with the exception of growth rate, there was a striking uniformity of cultural characteristics both within and between isolates on a variety of culture media.

The maximum growth attained by all isolates on P.D.A. together with the performance of the 3 isolates, SN84A, ST84B and LE264B on potato leaf extract points to the possibility of a growth factor from potato being important for optimum development of the fungus. Further work on this aspect could provide interesting information on inter-relationships of host and fungus.

Maximum pigment production occurred on P.D.A. when conditions were optimum for growth. This confirms the findings of Angell (1929) who made an intensive study of conditions affecting pigment production by Alternaria porri.

Neergaard (1945) reported that isolates tended to change their form after long periods in culture on artificial media and in some cases the 'degeneration' was such that after 2 months an isolate would produce atypical spores. A tendency towards loss of vigour of the stock isolates was noticed in this study but cultural characters remained stable.

Some loss of pathogenicity was, however, quite marked in all isolates after several months on artificial media.

It is tempting to attach special significance to the fact that isolates from Black nightshade were not chromogenic but without further isolations and more detailed work of a chemical nature it would be unwise to speculate, despite the apparent stability of this character through inoculation and reisolations from other hosts.

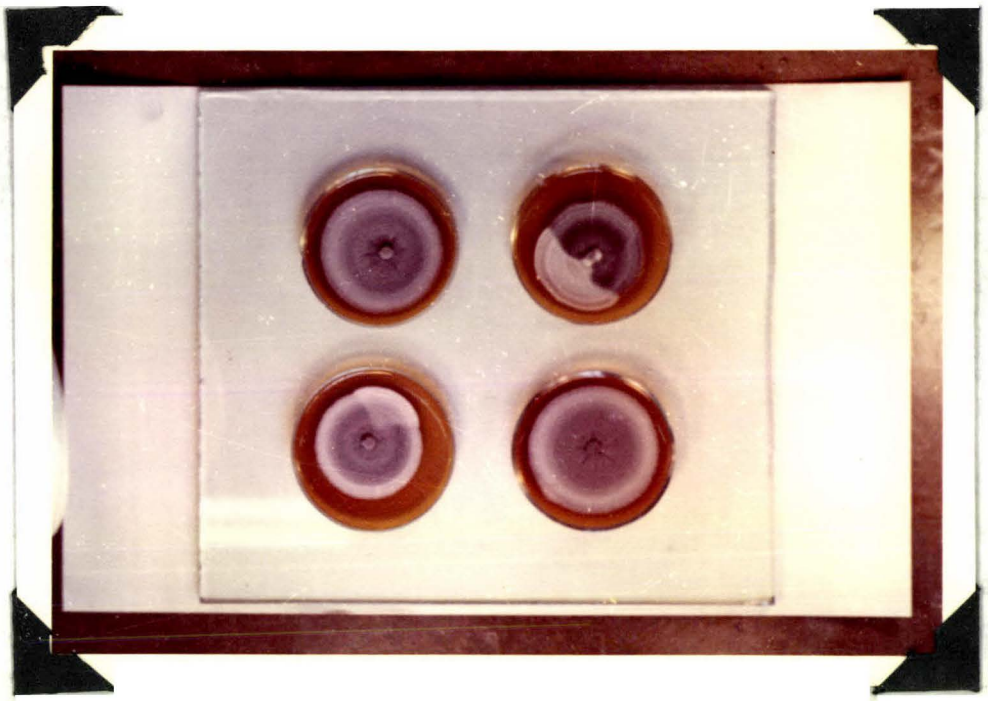


FIG. 3 SECTORING OF AN ISOLATE ON TOMATO JUICE AGAR

THE EFFECT OF TEMPERATURE ON COLONY GROWTH OF ISOLATES ON OXOID P.D.A.

MATERIALS AND METHODS:

Three plates per isolate of Oxoid P.D.A. were inoculated with 5 mm discs taken from the edge of an actively growing colony on Laboratory P.D.A. This procedure was carried out for 11 isolates over the following range of temperatures: 5°C, 10°C, 17°C, 20°C, 24°C, 26°C, 28°C, 30°C, 33°C, 37°C.

Colony diameters were recorded for each isolate at each temperature after 8 days incubation.

RESULTS:

The average colony diameters are recorded for all isolates at all temperatures in Appendix II. In addition, the performance of each isolate over the temperature range is shown in histogram form.

Prolonged exposure to 37°C was apparently lethal to 8 of the 11 isolates studied since they failed to commence growth when returned to the bench top after 8 days incubation at 37°C. The 3 exceptions were: ST26.B, LE8.E, and LE26.B which made very slight growth during their incubation at 37°C.

Cultural characteristics remained stable for all isolates throughout the temperature range.

The optimum temperature for growth varied, for different isolates, from 24°C to 30°C although in several cases e.g. LE8.G, ST8.B the optimum was not clearly differentiated.

TABLE IV.

TABLE SHOWING ISOLATES AND THEIR OPTIMUM TEMPERATURE

OPTIMUM TEMPERATURE.	ISOLATES
24°C	SN8.A, SN8.D, LE8.E
28°C	SN26.A, ST8.B, ST8.C, ST26.B, ST26.C, LE26.B
28 - 30°C	LE8.G

The minimum temperature for growth of the 11 isolates would probably be

between 3 - 5°C. Prolonged storage of cultures at 0°C did not affect their viability.

DISCUSSION:

The following table gives the optimum temperature for growth of A.porri f.sp.solani strains as determined by different workers.

TABLE V.

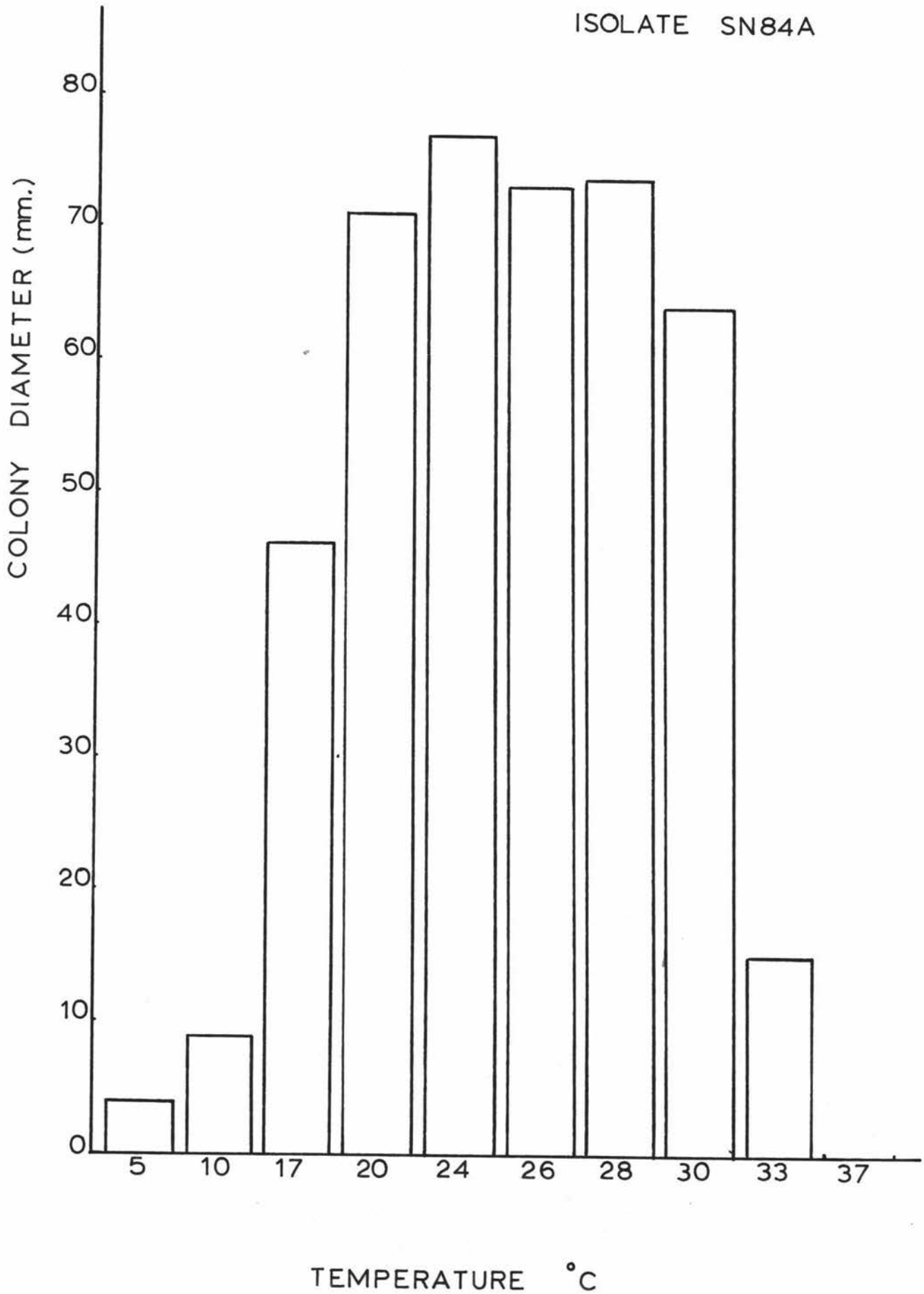
AUTHOR	YEAR	OPTIMUM TEMPERATURE	CULTURE MEDIUM
LANDS	1917	26 - 28°C	Unspecified agar medium
KLAUS	1940	26.1°C	potato agar
NEERGAARD	1945	27°C	standard nutrient agar.

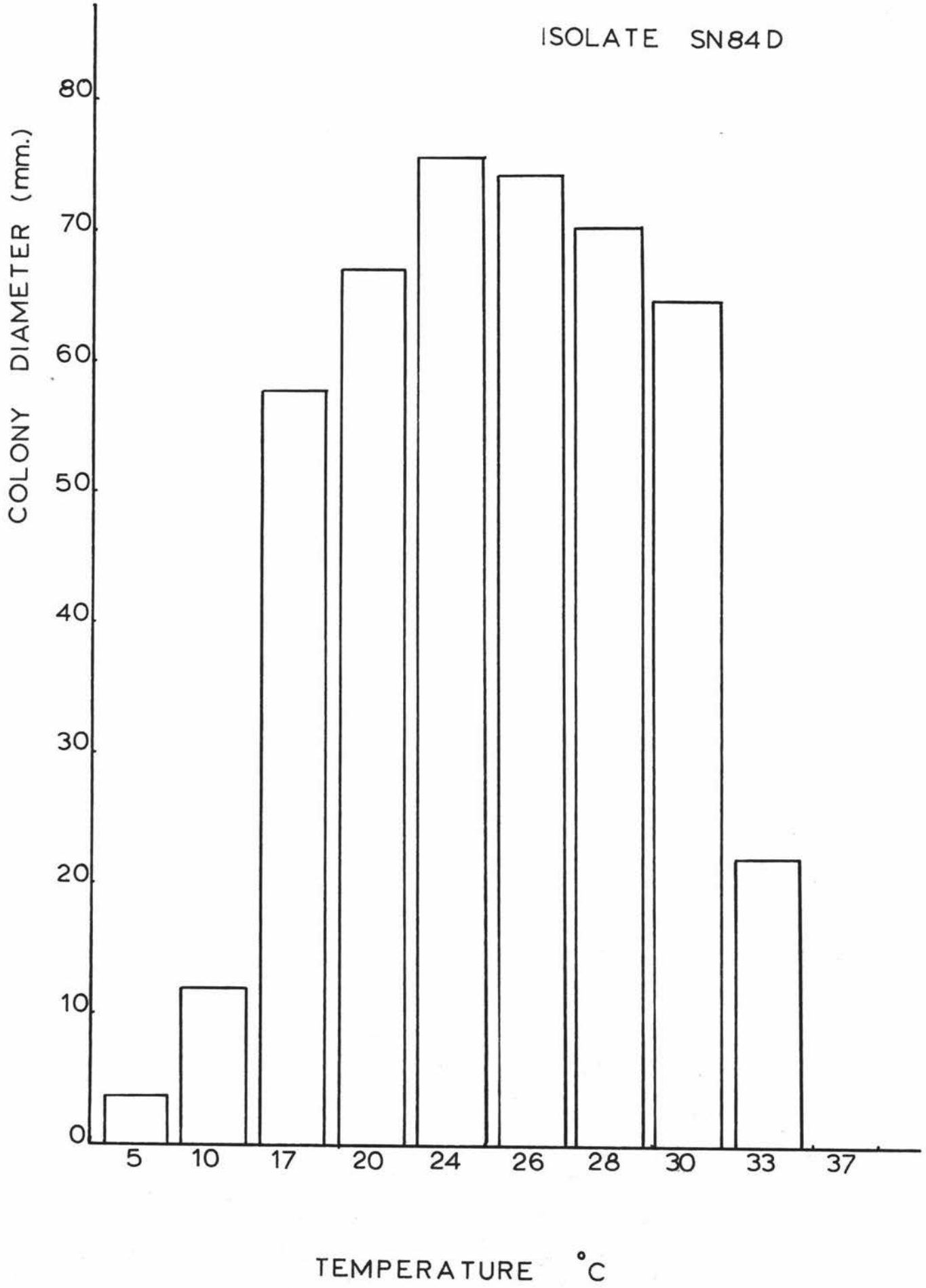
The range in optimum temperatures, for the isolates studied, from 24°C to 30°C seems to be quite different from the close agreement obtained by the 3 overseas workers quoted in the above table. There are however several contributing factors which make the variability seem large.

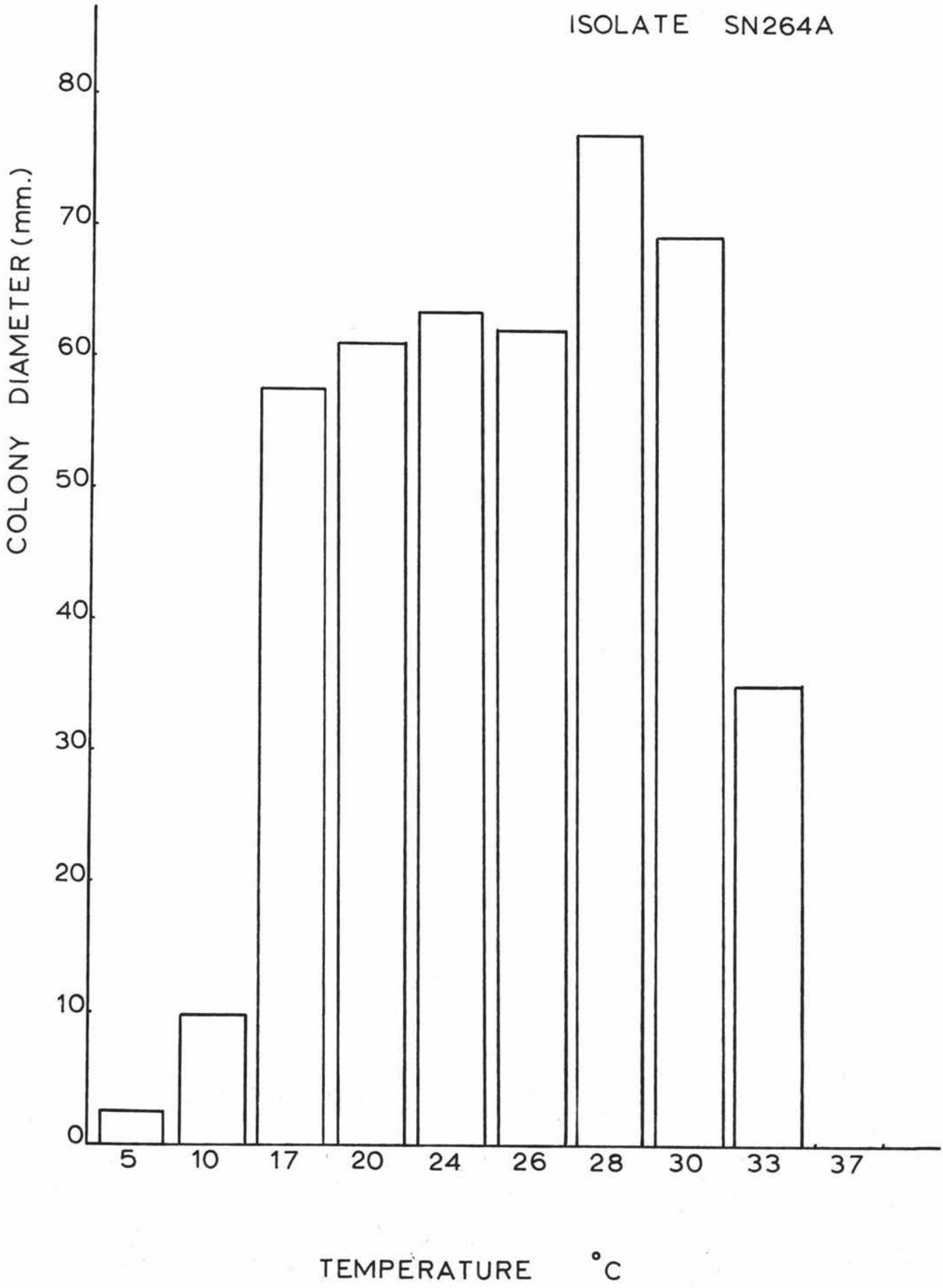
The small temperature intervals for 2°C between 24°C and 30°C tend to heighten any small differences between isolates whereas larger intervals of, say 4°C or 5°C, would tend to 'lump' the isolates into a seemingly more homogeneous group. Further, it should be noted that the temperatures were maintained to within only - 1°C and this could also serve to nullify the usefulness of small temperature intervals.

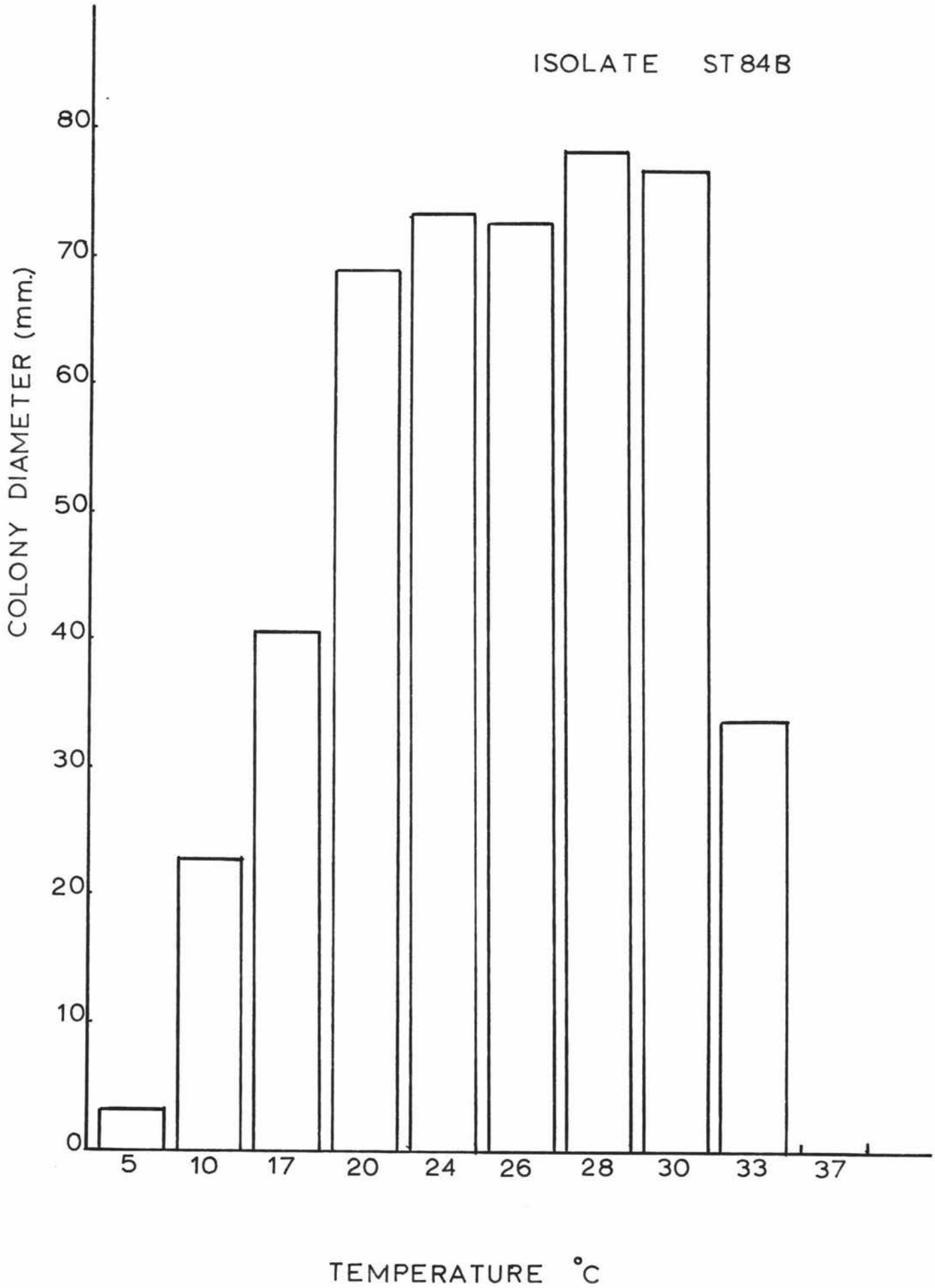
With the exception of SN264A and LE264B all isolates show a definite plateau of response to temperatures between 24° and 30°C so the merit of using small temperature intervals with the intention of differentiating between isolates would appear debatable.

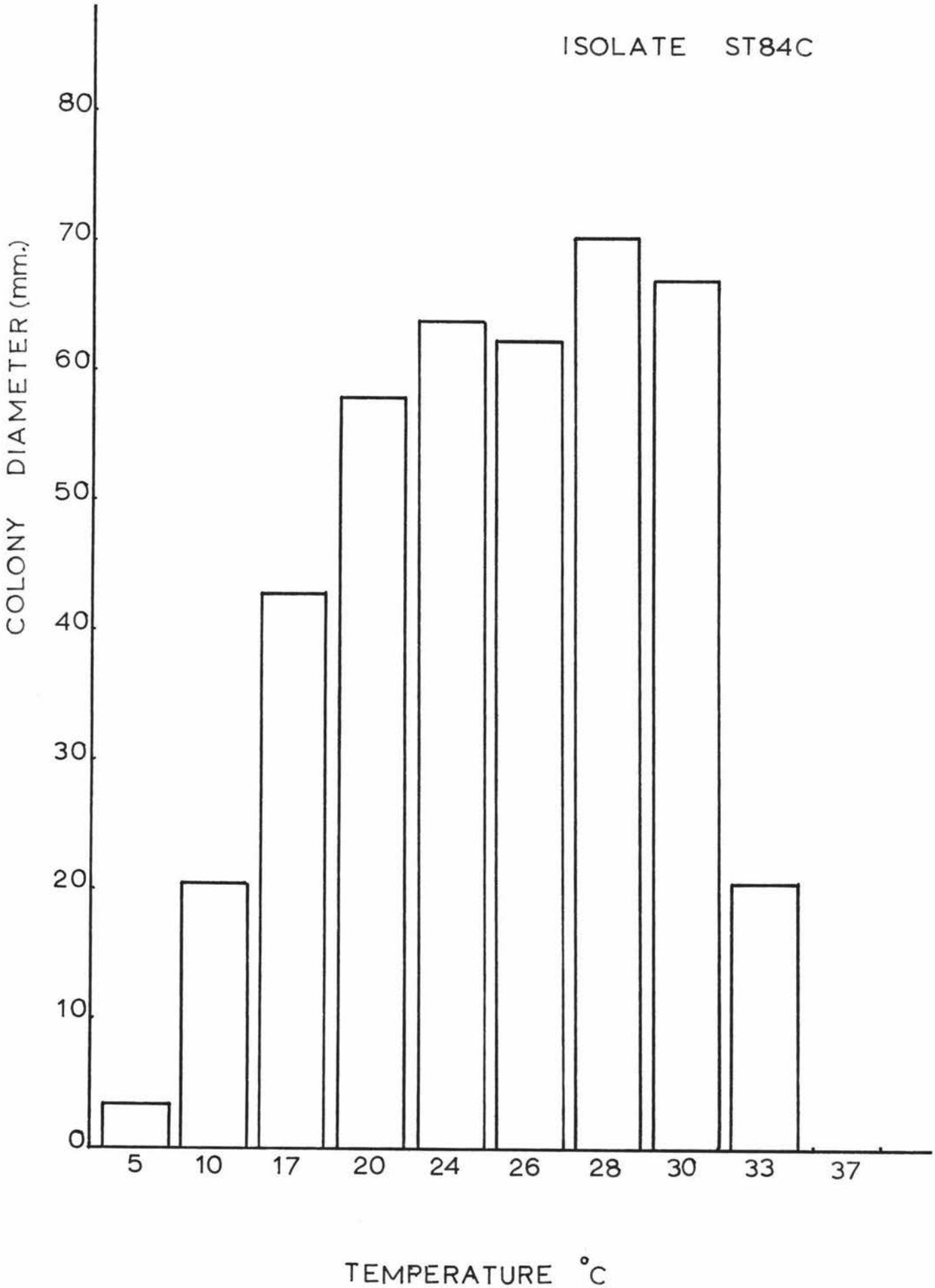
The variations in response to the temperature range by the 11 isolates are nevertheless good evidence of the variability of the fungus and an indication that the preciseness of the figures for optimum temperature given by Klaus and Neergaard are misleading.

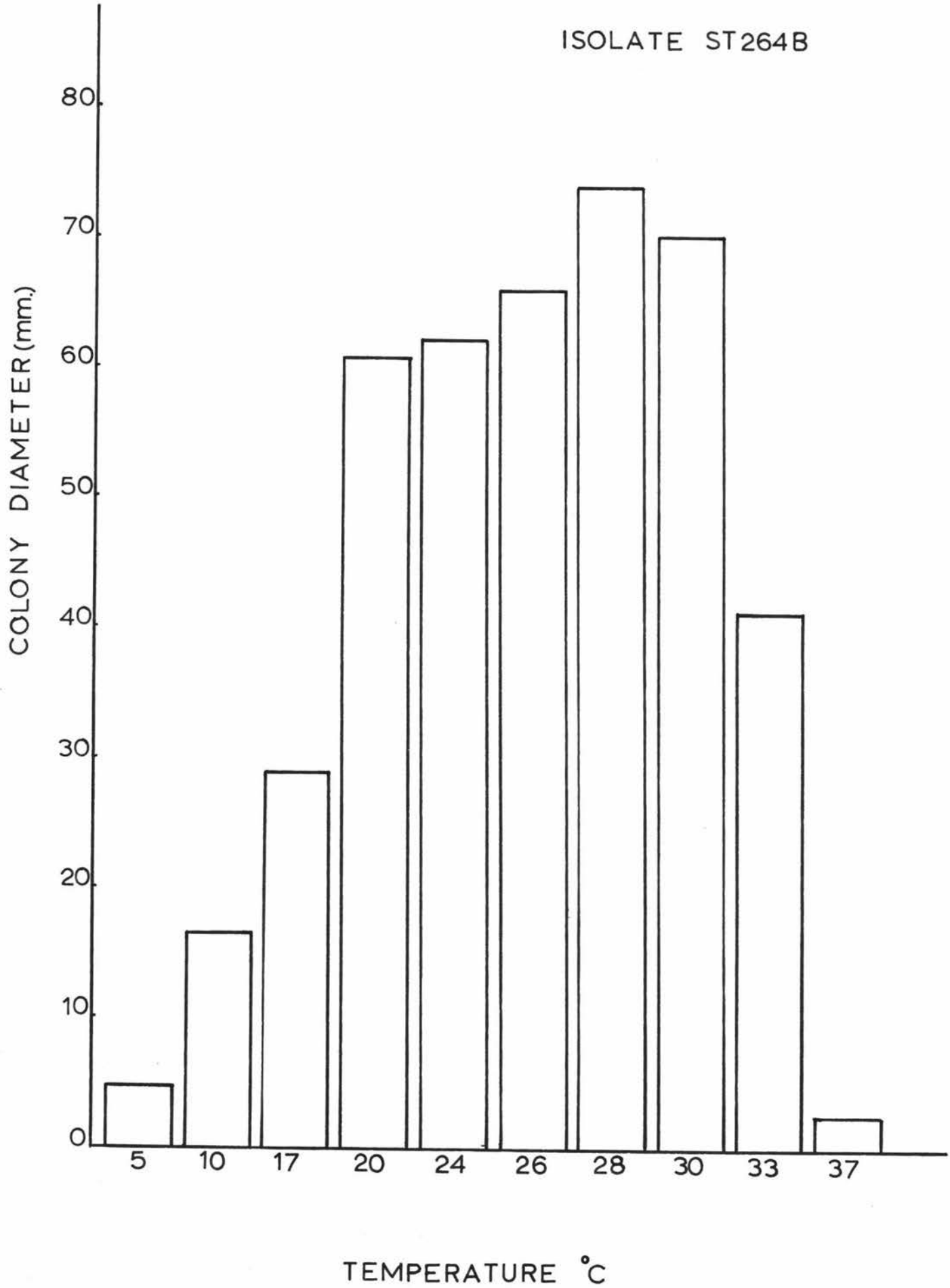

ISOLATE SN84A
COLONY DIAMETER (mm.)
0
10
20
30
40
50
60
70
80
5
10
17
20
24
26
28
30
33
37
TEMPERATURE °C

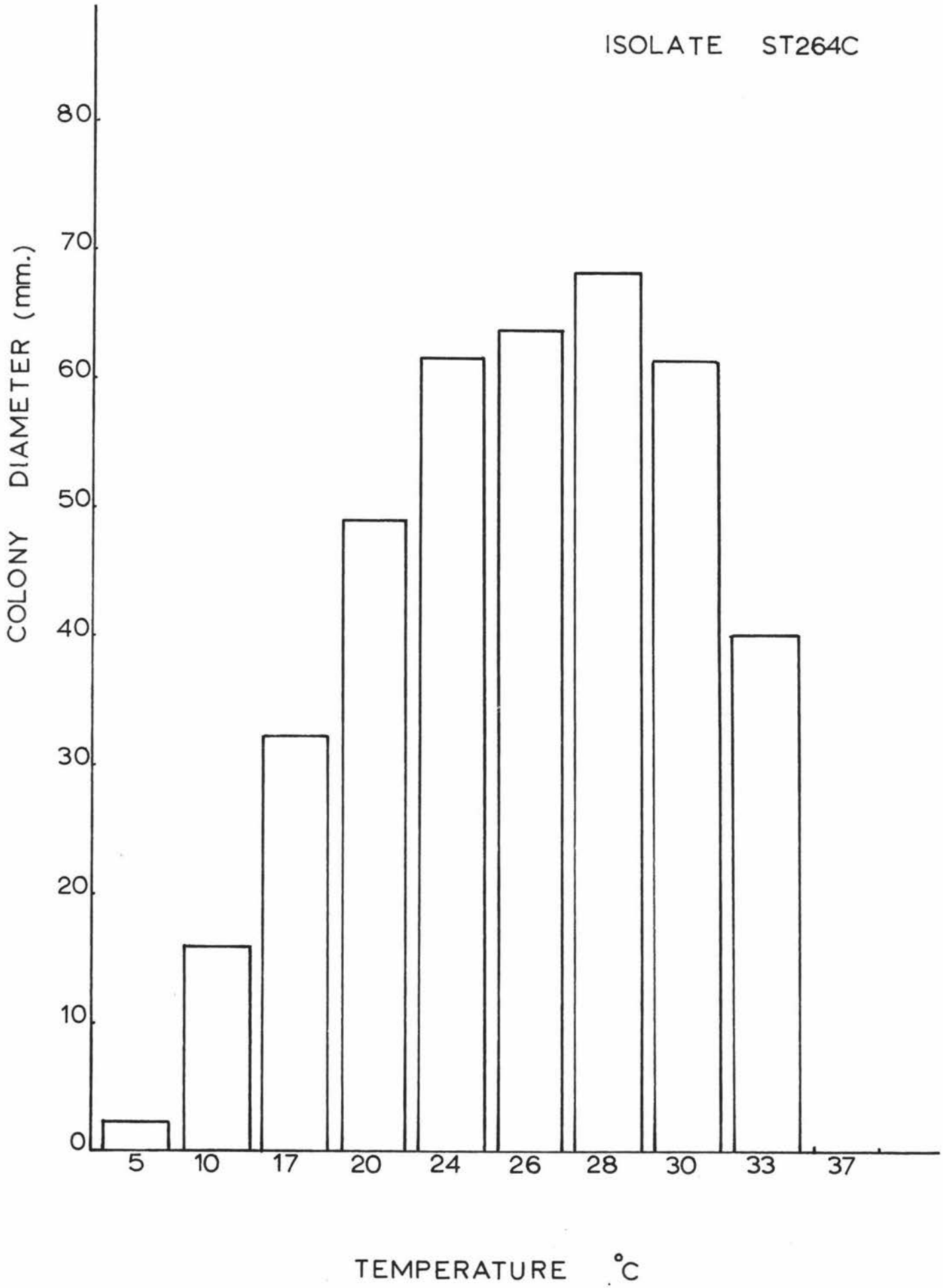

ISOLATE SN84D
COLONY DIAMETER (mm.)
0
10
20
30
40
50
60
70
80
5
10
17
20
24
26
28
30
33
37
TEMPERATURE °C

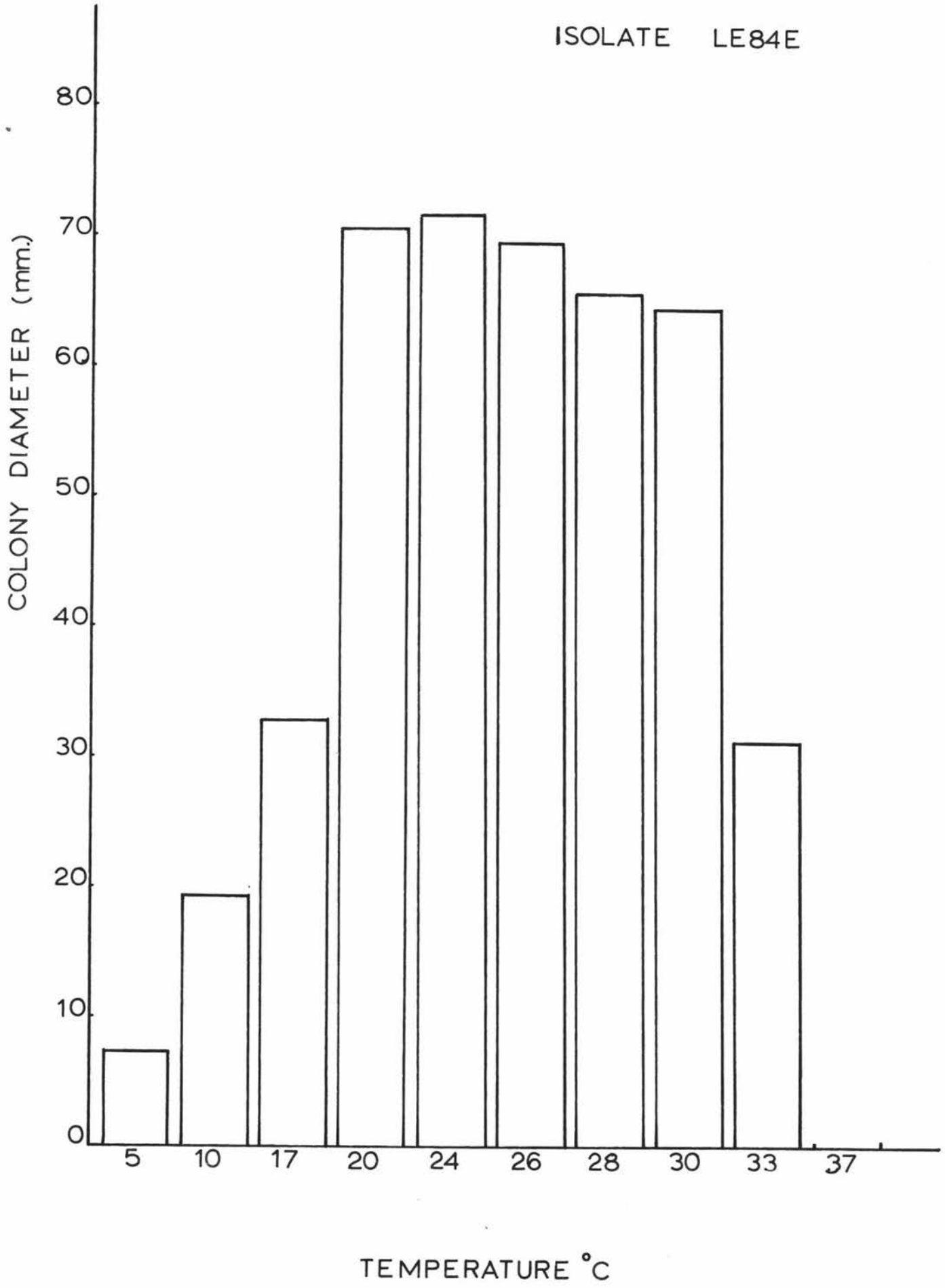

ISOLATE SN264A
COLONY DIAMETER (mm.)
0
10
20
30
40
50
60
70
80
5
10
17
20
24
26
28
30
33
37
TEMPERATURE °C

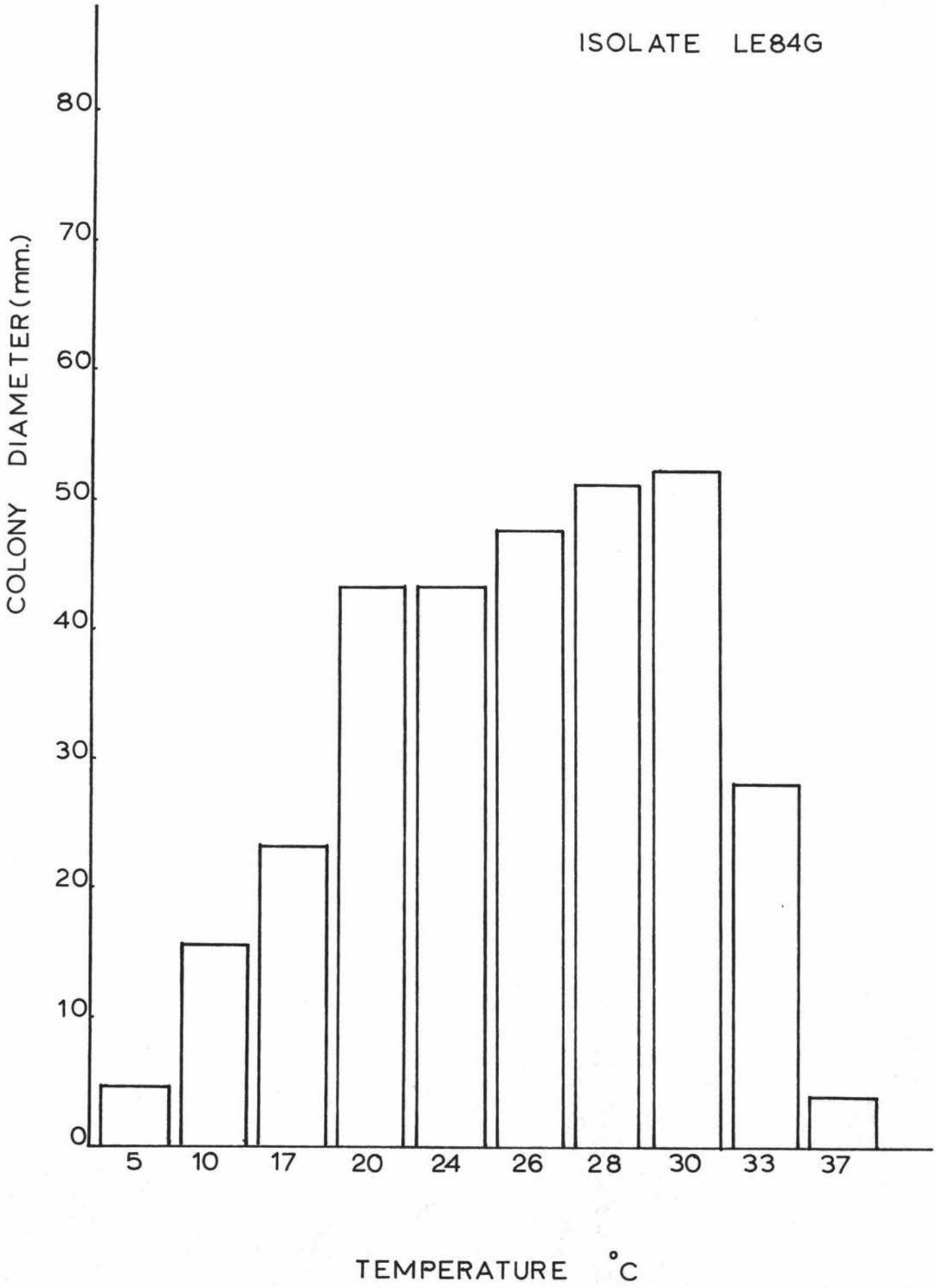

ISOLATE ST84B
COLONY DIAMETER (mm.)
0
10
20
30
40
50
60
70
80
5
10
17
20
24
26
28
30
33
37
TEMPERATURE °C

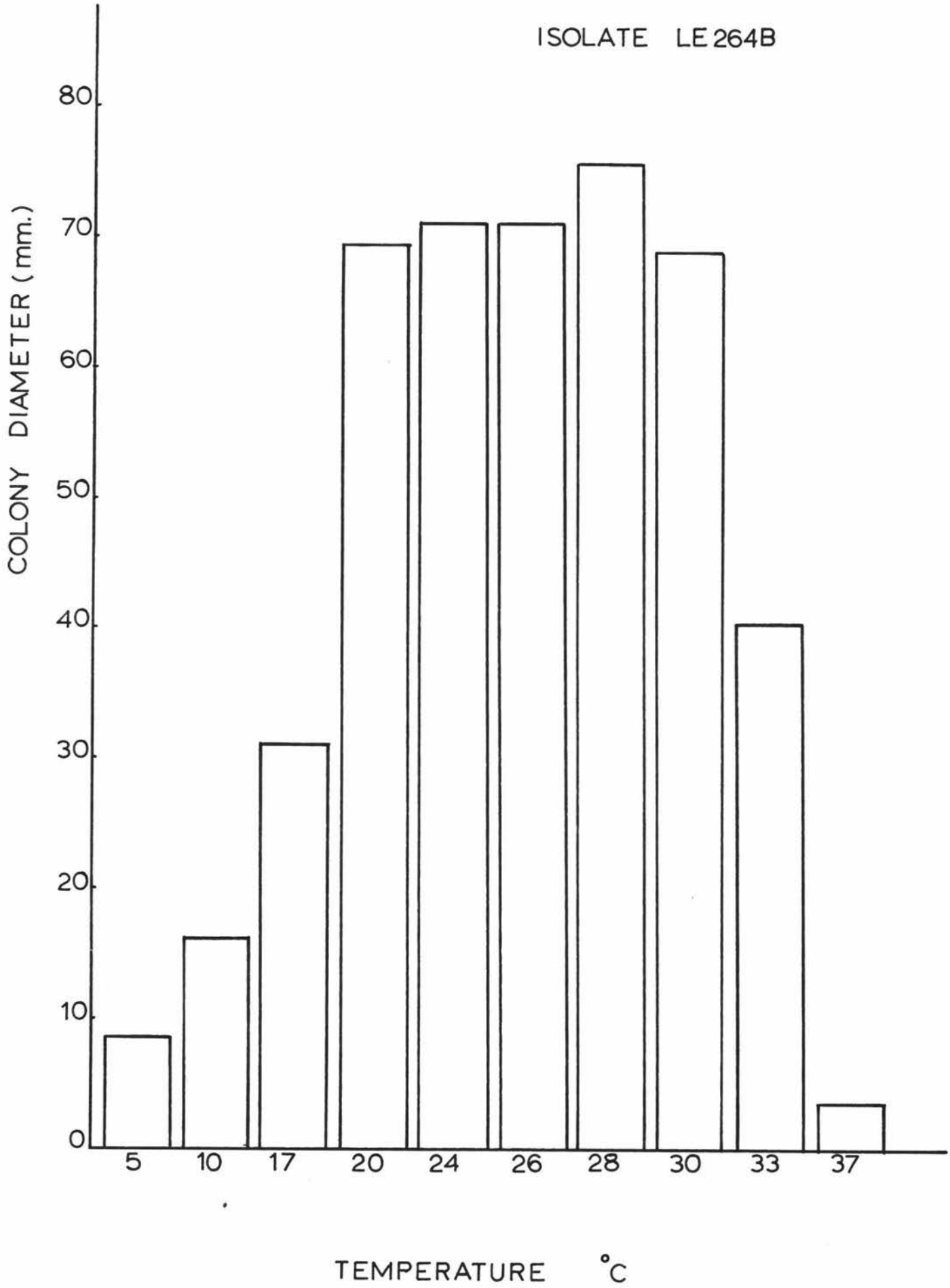

ISOLATE ST84C
COLONY DIAMETER (mm.)
0
10
20
30
40
50
60
70
80
5
10
17
20
24
26
28
30
33
37
TEMPERATURE °C


ISOLATE ST264B
COLONY DIAMETER (mm.)
0
10
20
30
40
50
60
70
80
5
10
17
20
24
26
28
30
33
37
TEMPERATURE °C


ISOLATE ST264C
COLONY DIAMETER (mm.)
0
10
20
30
40
50
60
70
80
5
10
17
20
24
26
28
30
33
37
TEMPERATURE °C


ISOLATE LE84E
COLONY DIAMETER (mm.)
0
10
20
30
40
50
60
70
80
5
10
17
20
24
26
28
30
33
37
TEMPERATURE °C


ISOLATE LE84G
COLONY DIAMETER (mm.)
0
10
20
30
40
50
60
70
80
5
10
17
20
24
26
28
30
33
37
TEMPERATURE °C


ISOLATE LE 264B
COLONY DIAMETER (mm.)
0
10
20
30
40
50
60
70
80
5
10
17
20
24
26
28
30
33
37
TEMPERATURE °C

THE EFFECT OF MEDIUM pH ON COLONY GROWTH.

MATERIALS AND METHODS:

A pH range from pH 3.5 to pH 9.0 was obtained by adding 2N hydrochloric acid or sodium hydroxide to buffered Oxoid P.D.A. The buffer was made up as follows:-

GLYCINE	3.8 gm
POTASSIUM DIHYDROGEN PHOSPHATE	3.8 gm
CITRIC ACID	2.6 gm
DISTILLED WATER	100 ml

For each pH, 7 flasks each containing 100 ml Oxoid P.D.A. were autoclaved and then placed in a 50°C water bath to keep the medium liquid.

5 ml of buffer were added aseptically to each flask. Drops of sodium hydroxide or hydrochloric acid were then added with a standard dropper in accordance with the amounts stated in Table

TABLE VI

QUANTITIES OF ACID AND ALKALI ADDED TO BUFFERED OXOID P.D.A. TO OBTAIN A RANGE OF pH.

NUMBER OF DROPS OF 2N HCl	APPROX pH
16 - 17	3.5
BUFFER ONLY	4.1
NUMBER OF DROPS OF 2N NaOH	
12	4.6
18	5.1
23	5.6
25	6.1
29 - 30	6.6
43	7.0
51	7.5
57 - 58	8.0
62	8.6
73	9.0

To determine the pH of each flask 1 ml of medium was withdrawn with a sterile pipette and added to 9 ml of distilled water and the pH of the resulting mixture measured with a Beckman glass electrode pH meter. Generally, the required pH was obtained in all flasks. Occasionally however it was necessary to add a few more drops of the alkali (acid) to bring the pH to that required. (Particular care was taken to use only thoroughly washed and rinsed glassware and this prevented any serious discrepancies in pH.)

Each flask was used to pour 6 petri plates. The twelve isolates were studied at each pH level - three plates per isolate were inoculated with 5mm discs from the

edge of an actively growing colony on Laboratory P.D.A.

Colony diameters were recorded after 8 days incubation at 26°C.

RESULTS:

The average colony diameters for each isolate over the range of pH are recorded in Appendix III. In addition the performance of each isolate is shown in histogram form.

Most of the isolates showed a fairly sharp optimum between pH 6.1 and pH 6.6 with the range from pH 4.6 to pH 8.0 being satisfactory for growth.

The appearance of colonies of all isolates was similar at each pH level. In addition, the chromogenicity, or otherwise, of the isolates was maintained throughout the pH range.

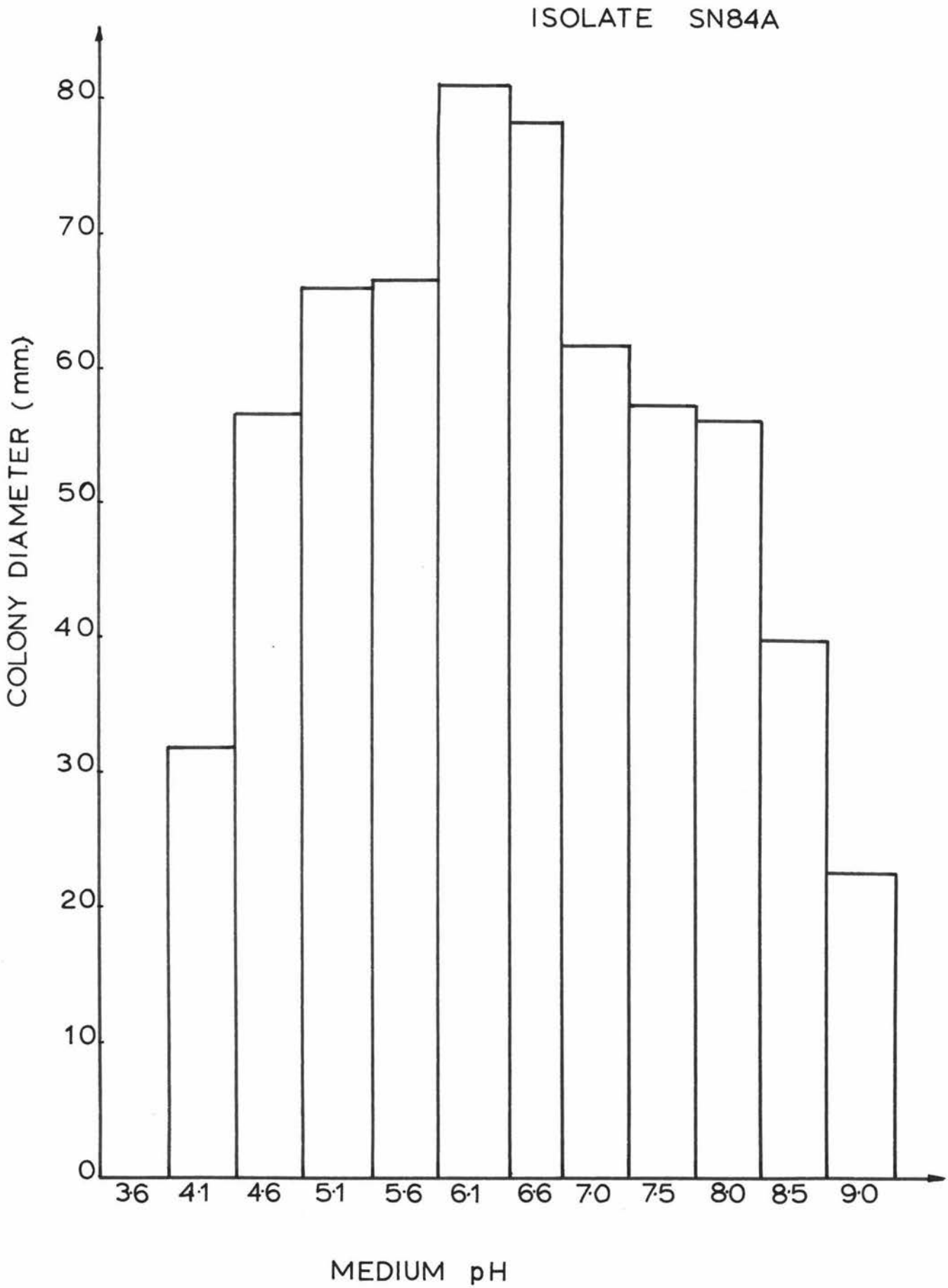
DISCUSSION:

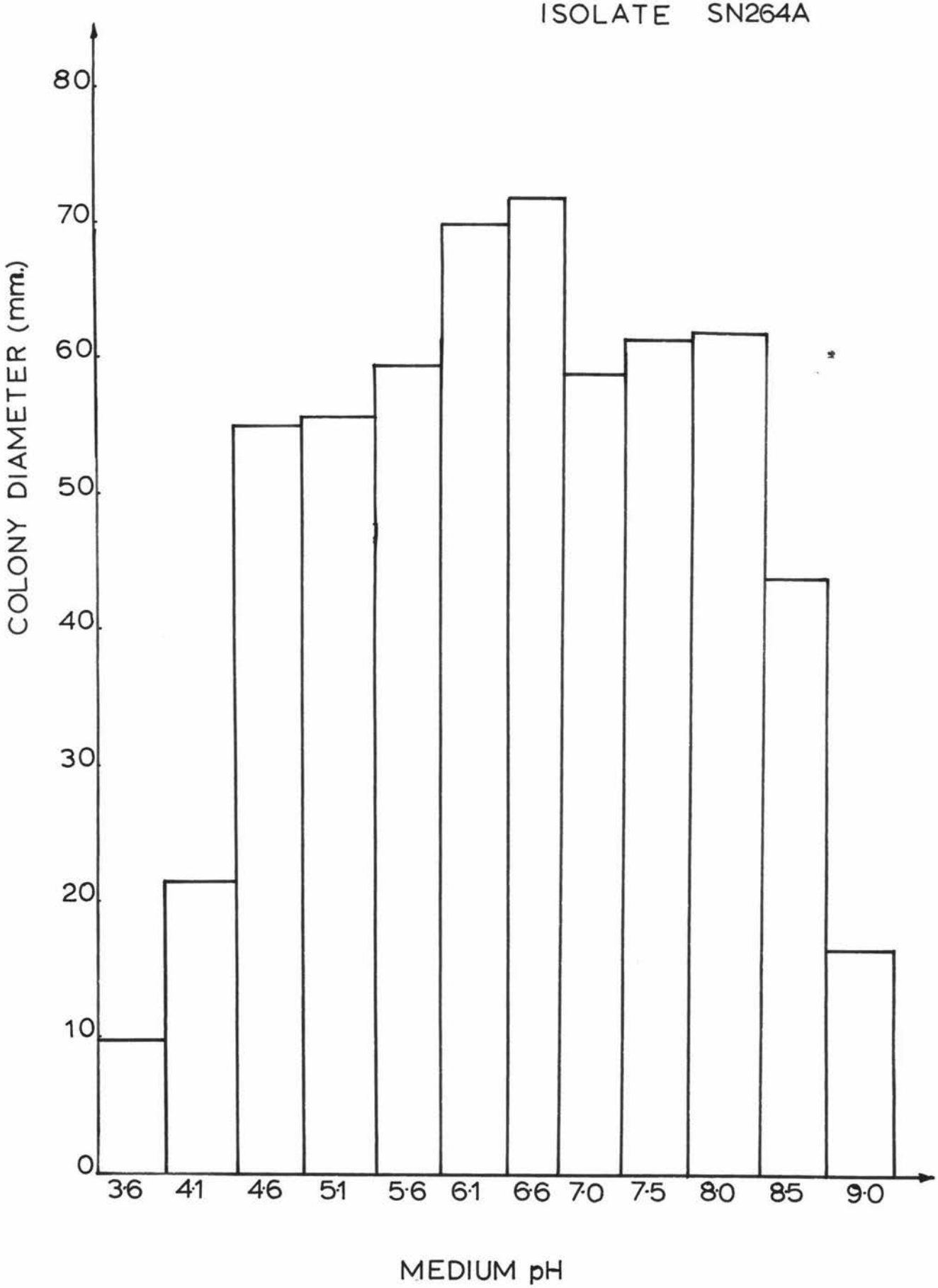
No figures for the pH range and optimum pH of strains of A.solani have been noted in the extensive literature on the fungus so it is not possible to make a comparison.

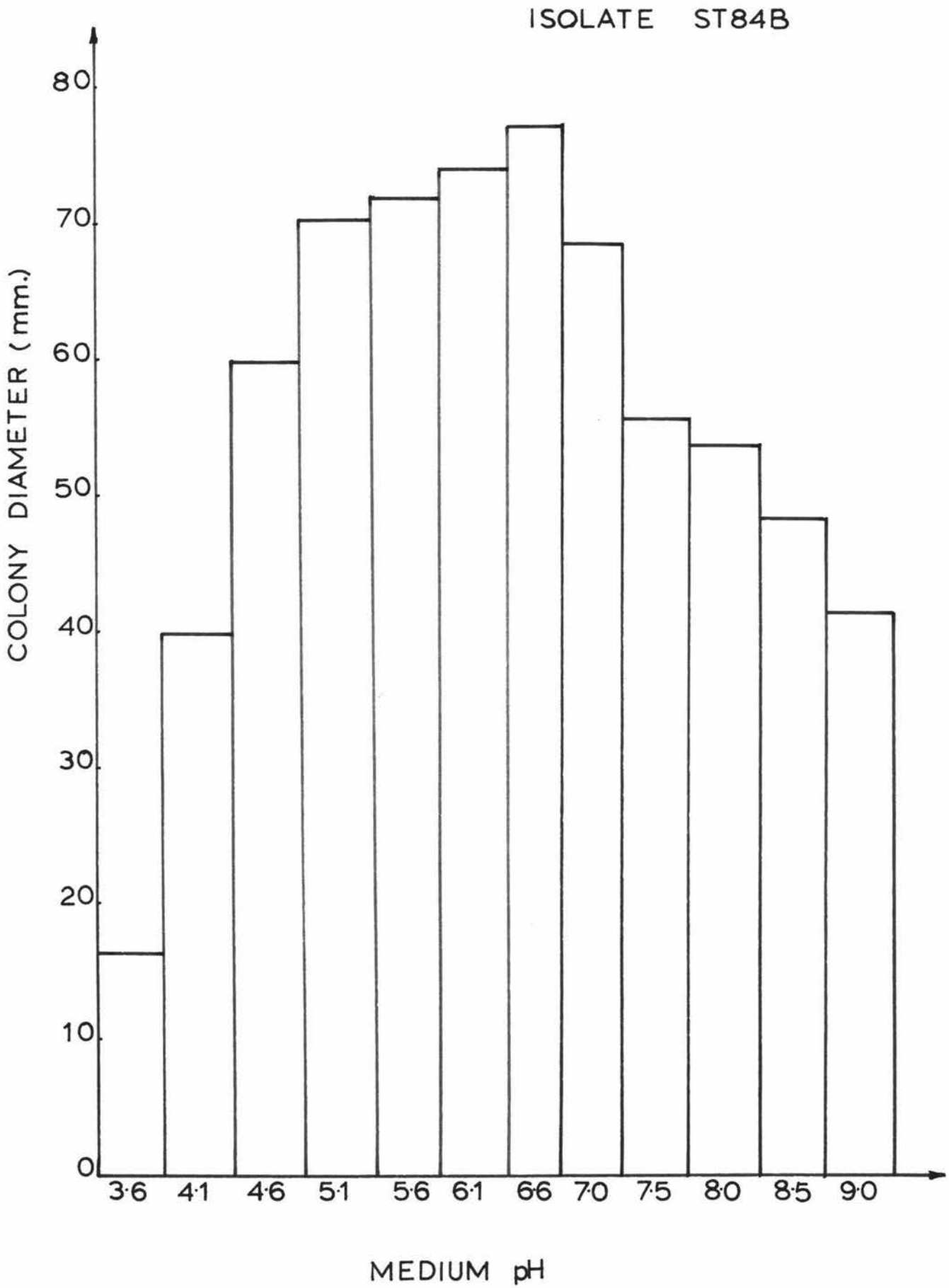
Unfortunately only 10 of the original 12 isolates were recorded over the entire pH range since the isolates SN84D and SN264D had both mutated before this part of the study was completed.

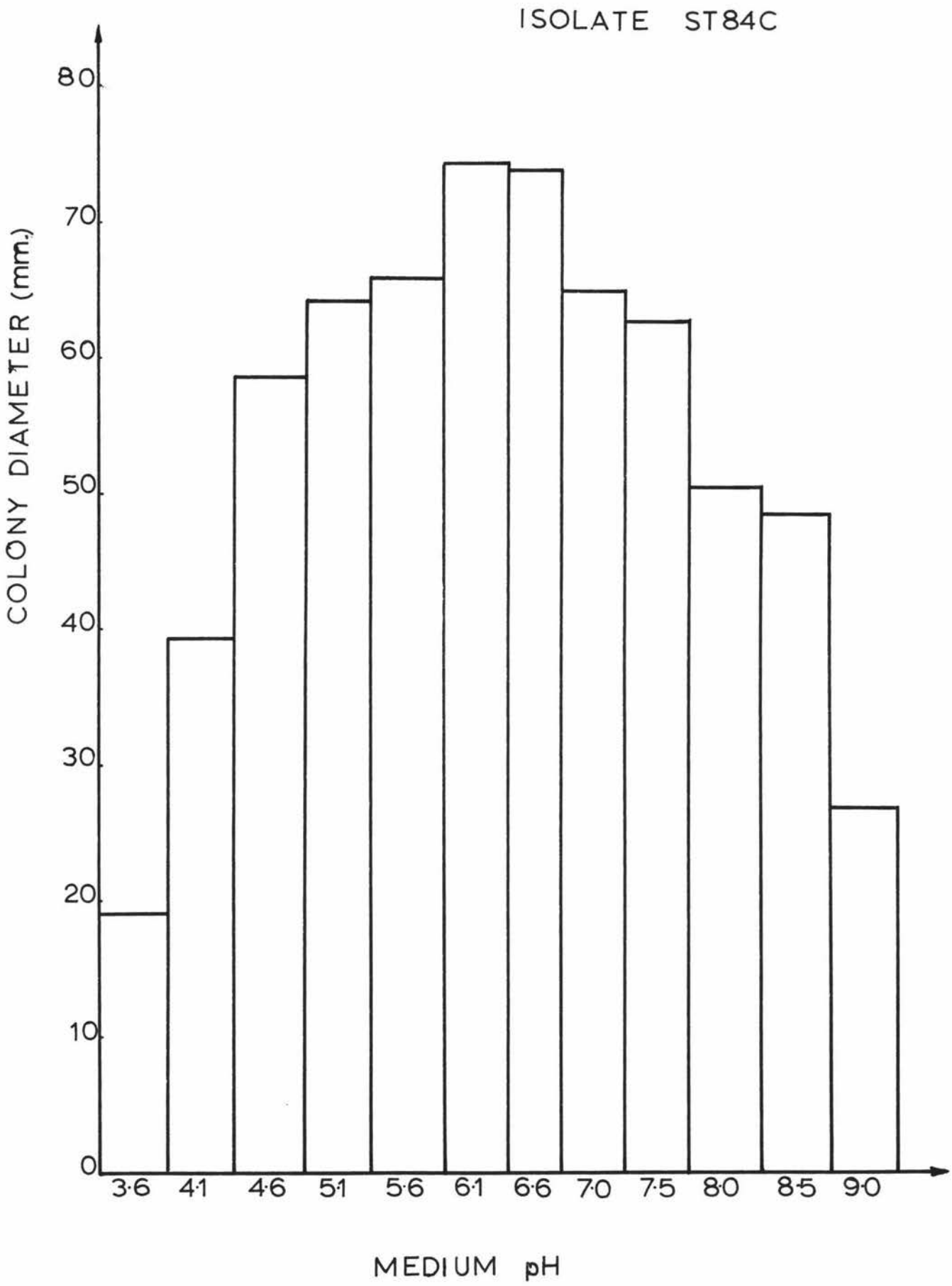
Isolates varied in their response over the pH range and isolates SN84A, SN84D, SN264A and ST264B were noticeably more sensitive to the lower pH's than other isolates.

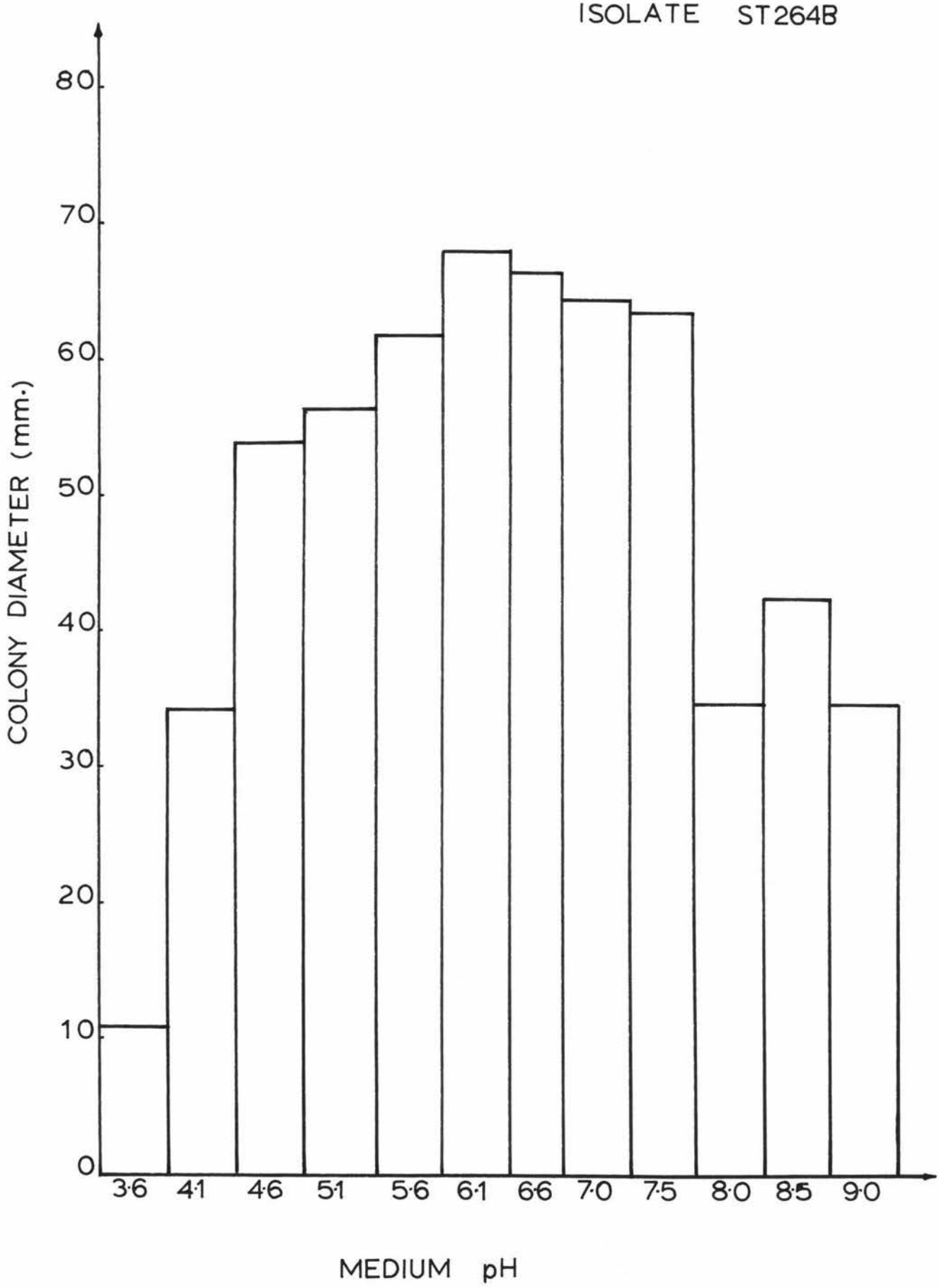
For the 10 isolates studied, however, all had an optimum pH between pH 6.1 and pH 6.6. This uniformity is a marked contrast to the variation obtained in the temperature range study and gives a fair degree of certainty to the optimum pH figures obtained.

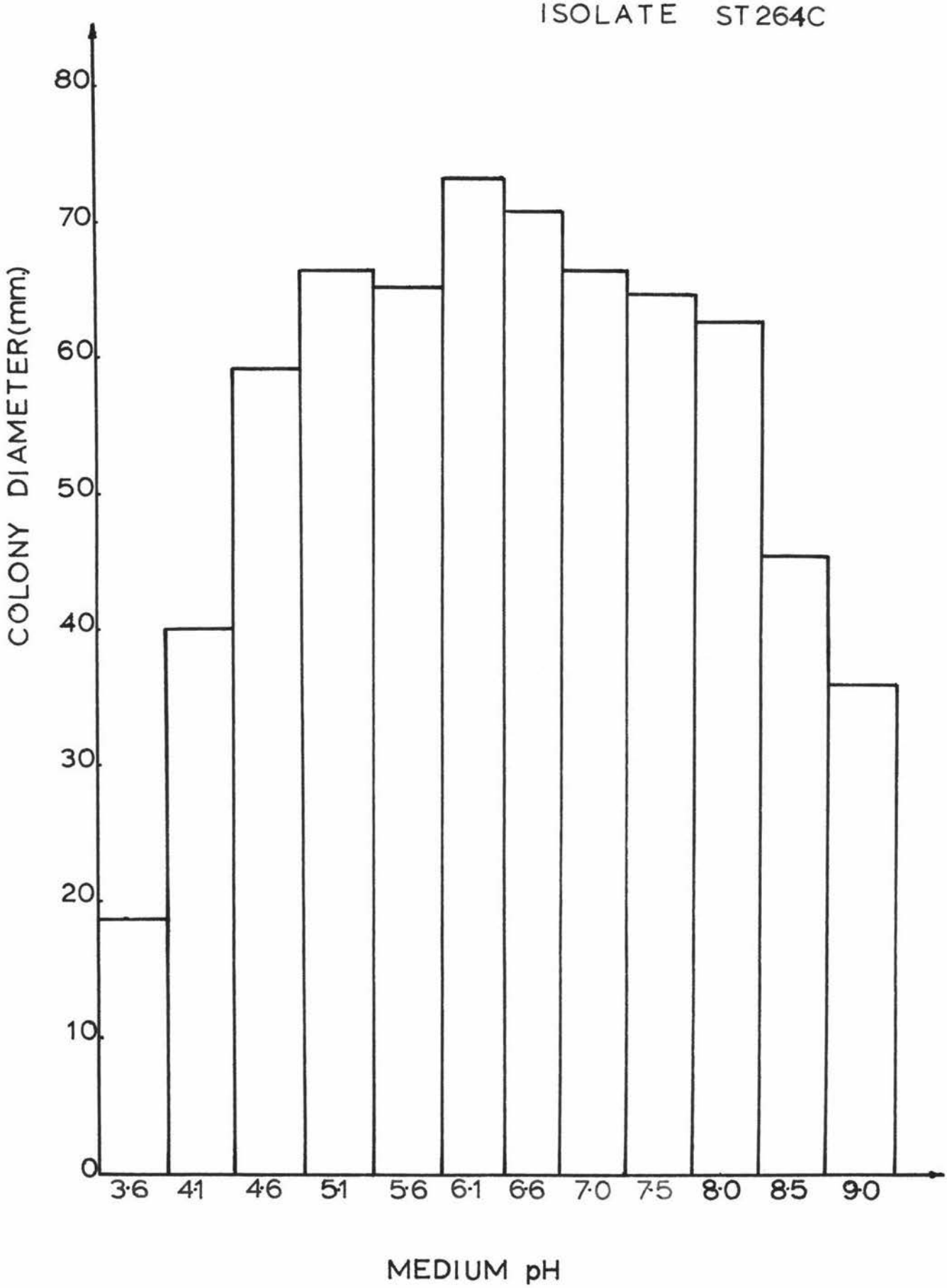

ISOLATE SN84A
COLONY DIAMETER (mm.)
0
10
20
30
40
50
60
70
80
3·6
4·1
4·6
5·1
5·6
6·1
6·6
7·0
7·5
8·0
8·5
9·0
MEDIUM pH

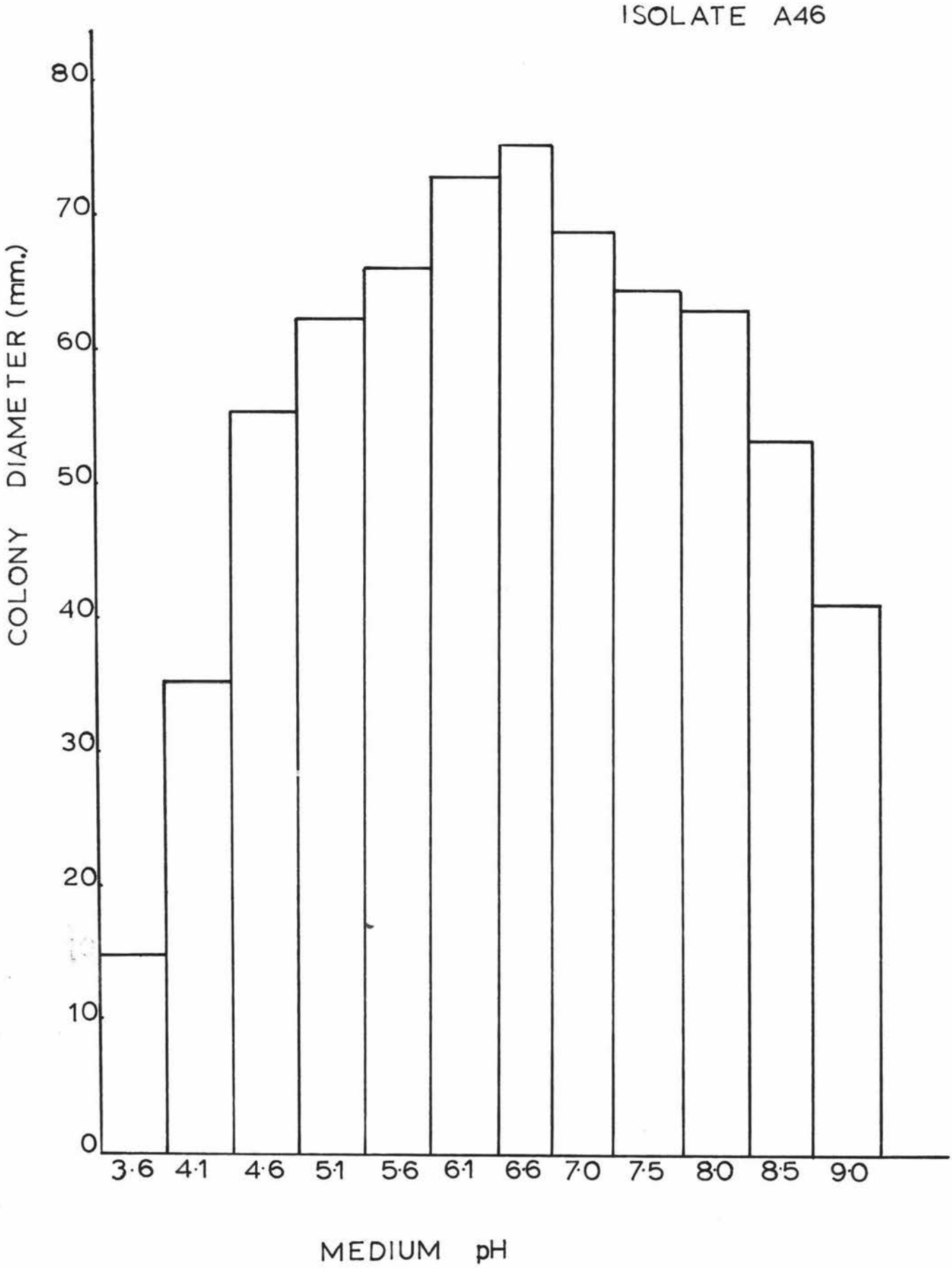

ISOLATE SN264A
COLONY DIAMETER (mm.)
0
10
20
30
40
50
60
70
80
3·6
4·1
4·6
5·1
5·6
6·1
6·6
7·0
7·5
8·0
8·5
9·0
MEDIUM pH

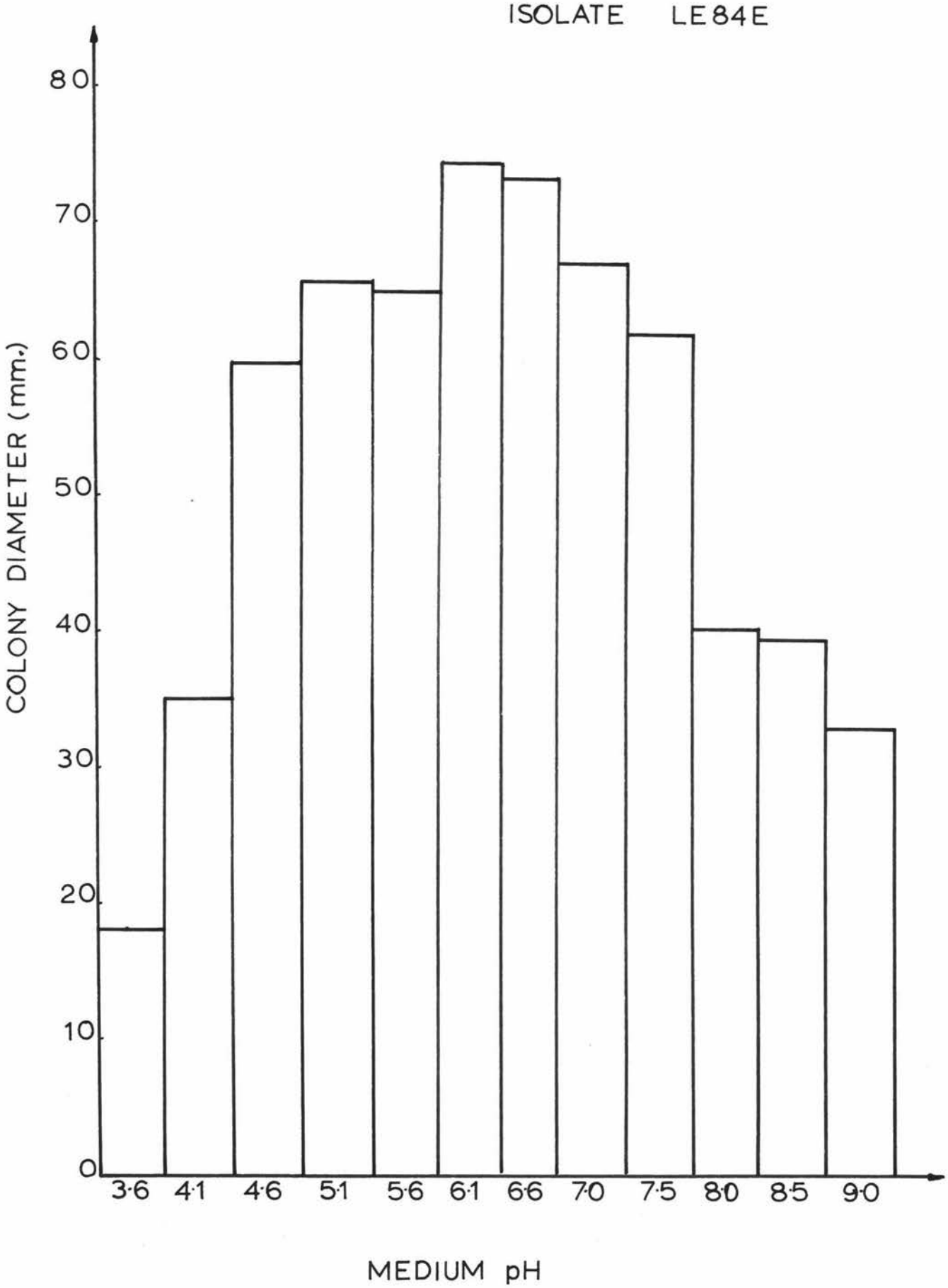

ISOLATE ST84B
COLONY DIAMETER (mm.)
0
10
20
30
40
50
60
70
80
3·6
4·1
4·6
5·1
5·6
6·1
6·6
7·0
7·5
8·0
8·5
9·0
MEDIUM pH

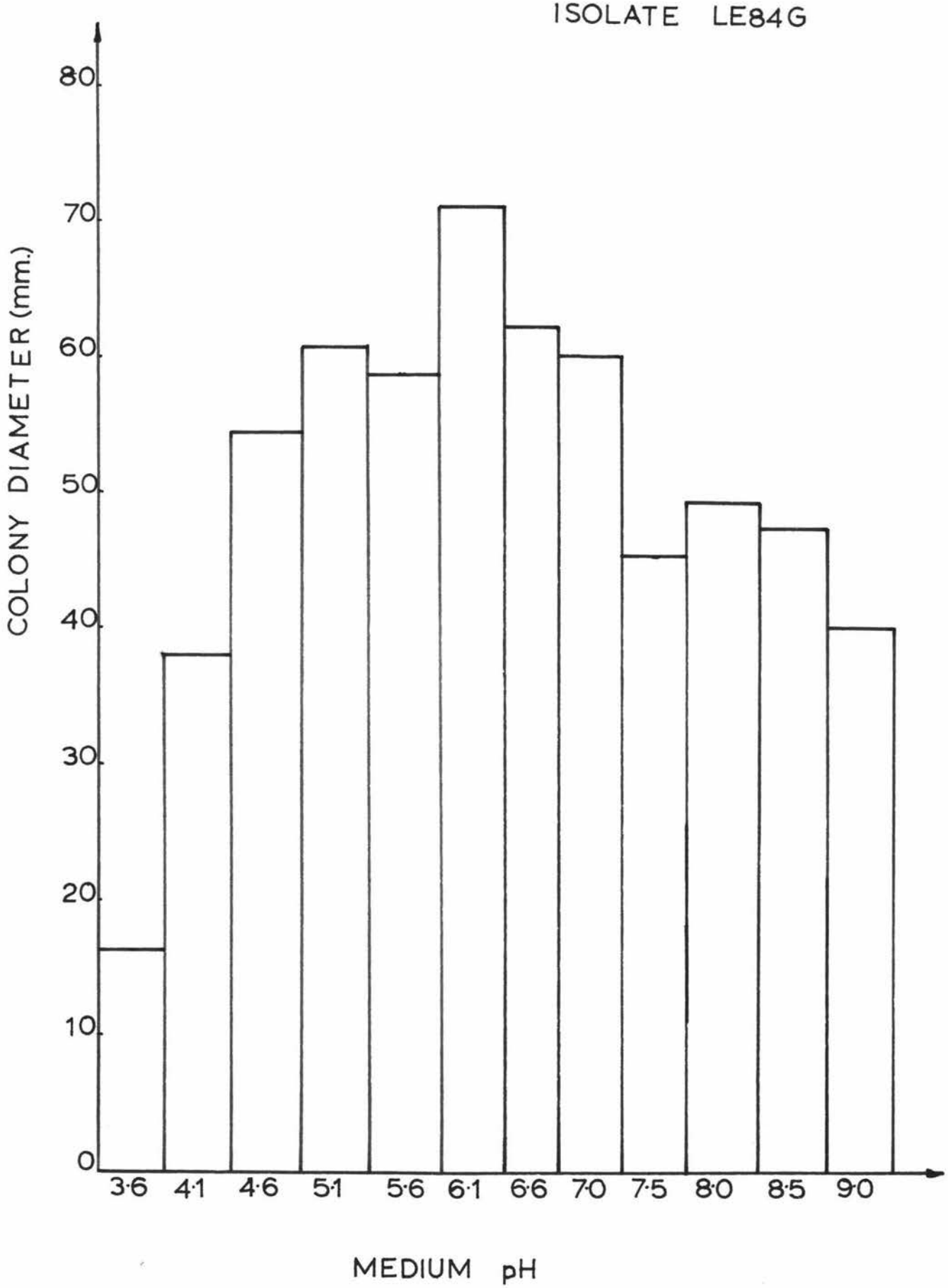

ISOLATE ST84C
COLONY DIAMETER (mm.)
80
70
60
50
40
30
20
10
0
3·6
4·1
4·6
5·1
5·6
6·1
6·6
7·0
7·5
8·0
8·5
9·0
MEDIUM pH

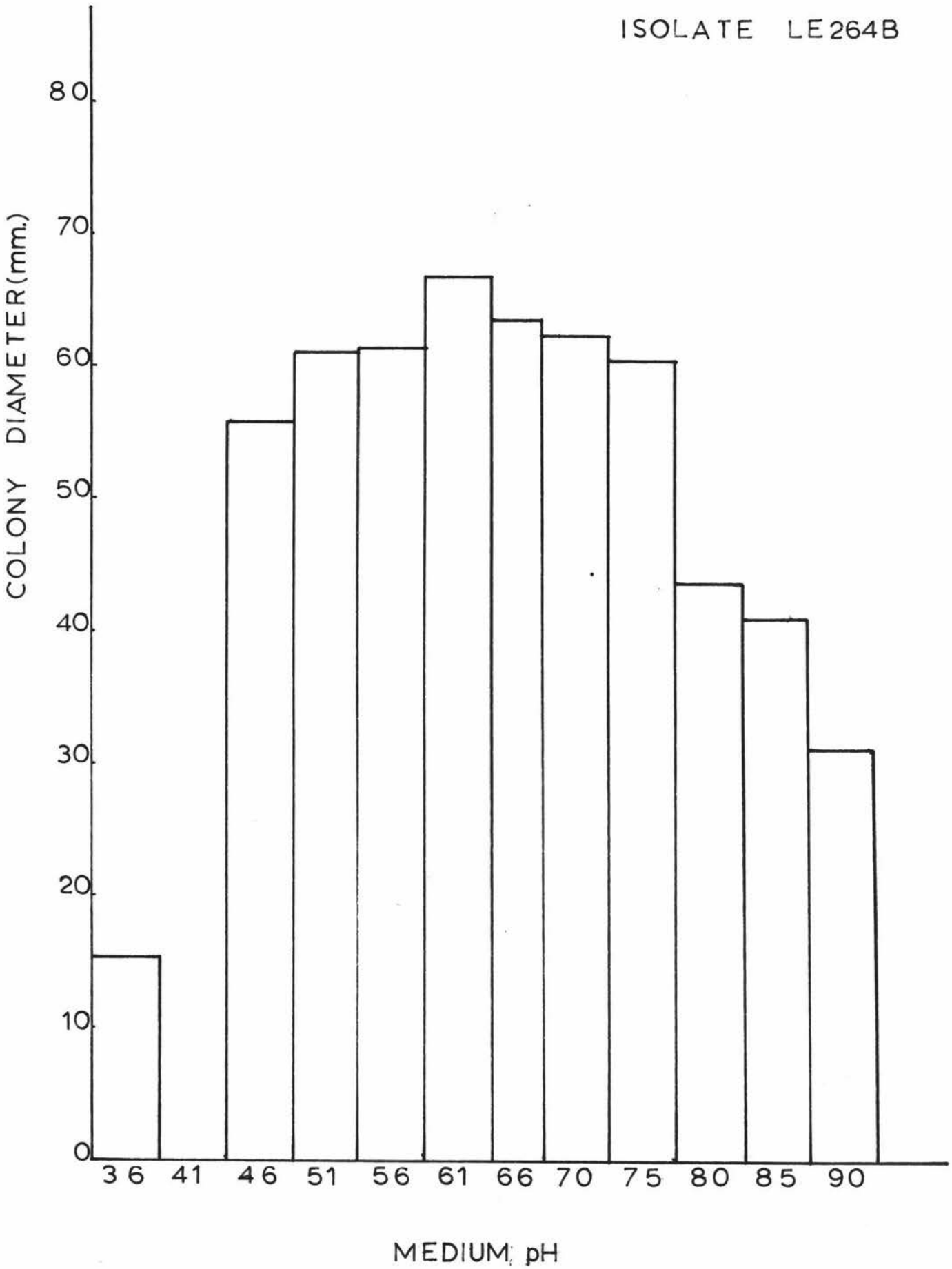

ISOLATE ST264B
COLONY DIAMETER (mm.)
0
10
20
30
40
50
60
70
80
3·6
4·1
4·6
5·1
5·6
6·1
6·6
7·0
7·5
8·0
8·5
9·0
MEDIUM pH


ISOLATE ST 264C
COLONY DIAMETER(mm.)
0
10
20
30
40
50
60
70
80
3·6
4·1
4·6
5·1
5·6
6·1
6·6
7·0
7·5
8·0
8·5
9·0
MEDIUM pH


ISOLATE A46
COLONY DIAMETER (mm.)
0
10
20
30
40
50
60
70
80
3·6
4·1
4·6
5·1
5·6
6·1
6·6
7·0
7·5
8·0
8·5
9·0
MEDIUM pH


ISOLATE LE84E
COLONY DIAMETER (mm.)
0
10
20
30
40
50
60
70
80
3·6
4·1
4·6
5·1
5·6
6·1
6·6
7·0
7·5
8·0
8·5
9·0
MEDIUM pH


ISOLATE LE84G
COLONY DIAMETER (mm.)
0
10
20
30
40
50
60
70
80
3·6
4·1
4·6
5·1
5·6
6·1
6·6
7·0
7·5
8·0
8·5
9·0
MEDIUM pH


ISOLATE LE264B
COLONY DIAMETER(mm.)
0
10
20
30
40
50
60
70
80
36
41
46
51
56
61
66
70
75
80
85
90
MEDIUM pH

SPORULATION ON ARTIFICIAL MEDIA.

Very sparse production of conidia by strains of the fungus Alternaria solani on artificial media and often on natural host material is a fact established by people working with the fungus in countries throughout the world. A simple method of inducing abundant sporulation greatly facilitates "Disease Cycle" studies and is prerequisite to any study of conidial germination "in vivo"or "in vitro".

REVIEW OF LITERATURE.

Rands (1917) reported successful induction of sporulation in Alternaria solani following wounding the mycelium of a 10-12 day old culture on hard P.D.A. by shredding the agar and then exposing to sunlight. Moisture relations were controlled so that complete drying out of the agar was avoided. Sporulation was obtained within 24-48 hours. Rands stated that initial stimulus for sporulation,

> "seems to be the result of wounding the mycelium in connection with changes in the relative humidity of the air in which it is exposed."

Unfortunately Rand's technique proved erratic and unreliable in results when applied by other workers to strains of the same fungus although Angell (1929) and Neergaard (1945) both used the method successfully.

Ramsey and Bailey (1930) stimulated spore production in an Alternaria species by irradiating with ultra violet light (U.V.L.) a culture, grown on P.D.A. They found wavelengths between 2535 and 2800 Å produced greatest stimulation.

Dillon Weston (1936) found that high intensity white light and not U.V.L. increased sporulation. Because the white light produced a stimulus to sporulation while acting through the glass cover of a petri dish, Dillon Weston concluded that U.V.L. radiation was not the vital component.

Horst Klaus (1940), a German worker, investigated very thoroughly the conditions for inducing sporulation of Alternaria solani and concluded that optimum humidity (100%) and optimum temperature (26°C) were the main contributing factors, with strong light also playing a part since he found that given optimum humidity and temperature cultures failed to sporulate in the dark.

McCallan and Chan (1945) investigated factors influencing sporulation of Alternaria solani and their results showed that exposure of a scraped culture, of the fungus on P.D.A., to U.V.L. of 250 m.u wavelength for 20 seconds at a distance of 10 cm from the lamp gave excellent sporulation. Factors of secondary importance were optimum temperature (20°c for their strain) and optimum humidity.

Beecher (1945) found that light by itself was often limited in its stimulating effect by the medium used for culturing the fungus. Accordingly he varied the culture medium and found that irradiation for 30 minutes at a distance of 30 - 32 inches with an S - I lamp of cultures on BRAN EXTRACT* medium was very good for producing sporulation.

Johnson and Halpin (1952) in experiments investigating the effect of light on morphology and spore production of several species in the Dematiaceae found that light of intensity greater than 200 foot candles inhibited sporulation by their strain of Alternaria solani.

A different approach to inducing sporulation was incorporated in a technique developed by Ludwig, Richardson and Unwin (1962). These workers obtained abundant sporulation after washing scraped V-8 juice agar cultures of the fungus for 24 hours and then stacking the plates in an inverted slanted position under laboratory conditions of light and temperature. Conidia were produced in 1 - 2 days and several crops could be obtained before the agar became too dessicated. The washing was thought to eliminate an 'antisporulating factor' which may be produced by the fungus in culture.

*1oz.bran/litre, boiled 10-15 minutes, strained and decanted; 10ml/litre of a 5% $FeCl_3$ solution; 2% agar.

SPORULATION TECHNIQUES USED IN THIS STUDY.

A technique which was virtually that of Klaus was used with only moderate success.

A large number of discs were cut, with a 5mm. diameter cork borer, from 7 - 8 day old cultures of the fungus and transferred with a sterile needle to the inside surface of a petri dish lid. The discs were placed with the mycelium end uppermost. A piece of filter paper was placed in the bottom half of the petri dish and moistened with distilled water. The lid was placed in position and the dishes left on the bench at room temperature.

Results from this method were variable between strains on the same medium and often there was no sporulation. A number of different media were tried and only on P.D.A. did all isolates produce at least a few conidia within 1 or 2 days.

The method was used, in the main, for obtaining conidia for measurement of isolates from 2 or 3 different media.

The scraping and washing technique of Ludwig, Richardson and Unwin described on page 4 was employed with great success for all isolates. Large crops of conidia were consistently obtained.

DISCUSSION:

The factor common to all successful methods of inducing sporulation in Alternaria solani is an initial moderate to severe wounding of the mycelium. Rands accomplished this by shredding the P.D.A. culture whereas others such as McCallan, Chan and Ludwig et al scraped the aerial mycelium from cultures before applying other treatments. The success of Ramsay and Bailey's method involving U.V. irradiation may well be attributed to a lethal action on the aerial mycelium. Klaus inadvertently wounded the mycelium by cutting the P.D.A. cultures into pieces in order to subject them to high humidity hence the initial stimulus or "physiological shock" provided by wounding was also included in his method.

Lilly and Barnett in their text on Physiology of Fungi note that a number of different fungal species can be induced to form sexual fruiting bodies if the mycelium is wounded. The explanation offered is that the products of autolysis may be stimulating or even vital to sexual reproduction. Such a hypothesis could well be advanced to explain the wounding stimulus to asexual reproduction which is evident with Alternaria solani.

Secondary factors of light, culture medium, temperature and humidity play an important part in obtaining prolific sporulation in strains of Alternaria solani.

SPORE GERMINATION.

Five isolates were used throughout this work viz: SN84A, SN84D, ST84B, LE84E and LE264B.

A preliminary trial was carried out with these isolates to obtain a rough idea of the temperature requirements for germination. Three substrates were compared for suitability for germination tests, (i) Leaf tissue from tomato and black nightshade
(ii) A thin film of P.D.A. placed on a slide
(iii) A drop of distilled water.

A spore suspension was made up in distilled water for each isolate, a drop put on the glass slide, on the P.D.A. film and on the host tissue pieces. These were then incubated at 26°C and inspected after 3 hours. Germination of all isolates was nearly 100% on P.D.A. and on the host tissue but very slight or nil on the drops of spore suspension.

The very low germination in distilled water was investigated in a second trial. Conidia from the 5 isolates were placed on coverslips (3 coverslips per isolate) and on each cover slip was placed a drop of distilled water. The cover slips were placed on glass slides and were incubated in petri dishes containing a moist filter paper at 25°C and 30°C. The slides were examined at intervals and it was found that after 24 hours at 25°C there was no germination of conidia from 4 of the isolates, the exception being LE84E which germinated well. At 30°C SN84A, LE84E and ST84B gave very slight germination and the other 2 isolates failed to germinate. It was concluded that distilled water was not a favourable substrate in which to stimulate germination of the isolates.

Since the germination percentage was almost identical on host tissue and P.D.A. it was decided to use P.D.A. films for the rest of the work.

EFFECT OF TEMPERATURE ON GERMINATION:

Concentrated spore suspensions were made up for each isolate and a drop of suspension was placed on a P.D.A. film on a glass slide. The slide was placed on a second slide which was resting on a piece of moist filter paper in the bottom of a petri dish. Six slides were put up for each isolate at each temperature. Nine temperatures were used: 5°, 10°, 16°, 20°, 25°, 30°, 34°, 37°, 40°. Trial runs were

carried out at each temperature to get a rough idea of the rapidity and extent of germination. As a result it was necessary to take counts of germinating spores every half hour at temperatures from 25°C upwards and every 4 hours for 5°, 10° and 16°C.

At the correct time intervals the slides were removed from the incubators and set up on the microscope stage. Two 'Veeder' tally counters were used to record the numbers of germinated spores and the number of ungerminated spores seen in 3 or 4 low power fields of the microscope. Approximately 100 spores were counted each time and the number of germinated spores was expressed as a percentage of the total number of spores counted. Counts were stopped when germination reached 95 - 100 per cent.

Criterion of Germination:

A spore was considered to have germinated when the germ tube(s) had parallel sides. This generally occured when the germ tube was approximately the same length as the width of the spore. It was necessary to adopt this criterion because at higher temperatures, more especially 37 and 40°C, germ tubes would push out from the spore for a slight distance and then just remain as 'bumps' on the spore for quite some time before continuing elongation.

RESULTS:

These are presented in Table 7 which shows the time taken for germination percentage to reach several levels viz: 10, 25, 50, 75, and 95 - 100%. The figures in the table were obtained from a graph of germination percentage against time for each isolate at each temperature.

The optimum temperature for germination for all 5 isolates was between 30 and 34°C. At 34°C the time taken for 95-100% of the spores to germinate was between 1.5 hours and 2.2 hours. Following an initial lag phase which varied with each isolate and was greater at the lower temperatures, germination proceeded almost linearly with respect to time.

At 40°C spores of all isolates were either killed immediately or else they started to germinate before being killed by producing very short 'knobbly' swollen germ tubes which quickly ceased to grow. Observations were carried on for 24 hours after which the slides were placed in a 30°C incubator to test viability. In each case the spores

TABLE VII

ISOLATE	% GERM.	5 ± 1	10 ± 1	16 ± 1	20 ± 1	25 ± 1	30 ± 1	34 ± 1	37 ± 1	40 ± 1
		FIGURES REPRESENT HOURS TO REACH THE PERCENTAGE GERMINATION TEMPERATURE °C.								
SN84A	10	5.5	3.5	1.2	1.1	0.8	0.7	0.7	0.75	
	25	8.5	5.5	1.5	1.4	1.1.	1.0	0.9	1.2	N
	50	13.0	8.0	2.75	1.8	1.25	1.25	1.3	2.1	I
	75	19.0	10.5	4.25	2.4	1.75	1.6	1.75	2.5	L
	95-100	24.0	13.0	8.0	2.7	2.5	2.0	2.0	3.0	
SN84D	10	3.2	1.0	1.0	1.1	0.6	0.6	0.6	0.8	N
	25	4.6	1.6	1.8	1.4	1.2	1.2	1.1	1.4	
	50	5.4	2.0	2.4	2.0	1.8	1.6	1.5		I
	75	6.3	2.8	3.0	2.5	2.0	2.0	1.8		
	95-100	7.8	4.0	3.7	2.9	2.6	2.5	2.1		L
ST84B	10	4.3	1.5	1.6	1.1					N
	25	5.0	1.8	1.8	1.4					
	50	6.0	2.8	2.1	1.7					I
	75	7.2	5.2	2.4	2.3					
	95-100	8.2	7.6	2.6	2.7	1.5	1.5	1.5	1.5	L
LE84E	10	4.2	2.0	1.1	1.0	0.6	0.6		0.8	N
	25	5.5	3.0	1.3	1.2	0.8	D.8	1.0	1.0	
	50	6.2	4.0	1.8	1.6	1.2	1.1	1.2	1.25	I
	75	7.5	6.0	2.6	2.2	1.8	1.5	1.3	1.7	
	95-100	8.5	8.0	4.0	2.8	2.4	2.0	1.5	2.4	L
LE264B	10	4.2	2.0		1.2	1.0	0.8	0.6	0.9	
	25	5.0	2.3		1.5	1.4	1.2	0.9	1.4	N
	50	6.0	2.8	N.R.	1.8	1.7	1.6	1.5	2.1	
	75	7.0	3.4		2.3	2.0	1.8	1.7	2.6	I
	95-100	8.0	4.2		2.9	2.7	2.4	2.2	3.1	L

N.R. = Not recorded.

were killed by exposure to 40°C. Thus the maximum temperature for germination of isolates lies between 37° and 40°C. The minimum temperature would be between 3° and 5°C.

There was the usual variation of response between isolates although the 3 cardinal temperatures were similar. Worth noting, perhaps, is the wide range of temperature (25 - 37°C) over which isolate ST84B germinates equally rapidly and also the fact that the optimum temperature for germination (30 - 34°C) is somewhat higher than the optimum temperature for growth.

DISCUSSION:

The cardinal temperatures of germination for the fungus are 3 - 5°C, 30 - 34°C, 37 - 40°C. These figures are very broad and this is an indication of the variability of the fungus and also of a lack of precision in the temperature intervals. A smaller temperature interval however would have been difficult to maintain and the results would, probably, not have been more meaningful since the fungus is capable of wide variation between isolates. The isolate SN84D did not germinate beyond 45% at 37°C and after 4 hours the level remained at 45% although the spores remained viable since they continued growth when removed to a lower temperature.

The very slight germination, in distilled water, of the isolates tested bears out Angell's(1929) report that germination of Alternaria porri was very low in distilled water. Reasons for this type of behaviour are not fully understood but it does seem that any study of conidial germination should be carried out on a nutrient medium to ensure repeatable results (Tompkins, 1932).

It was noticeable that spores produced nearly twice as many germ tubes at the optimum temperatures (30 - 34°C) than at the extremes of the range. Also, germ tube length was about 1½ times greater at the optimum temperatures.

All isolates germinated in aqueous suspension on a P.D.A. film and in one small experiment it was found that dry spores placed on a P.D.A. film germinated in an atmosphere of high humidity (90 - 100% R.H.) whereas dry spores placed on a glass slide and incubated at optimum temperatures with high humidity did not germinate. This result indicates that moisture by itself is insufficient to promote germination in Alternaria solani and that some factor in the nutrient medium is required in addition to moisture.

The optimum temperature for germination (30 - 34°C) is higher than the optimum temperature for growth (24 - 30°C). This contrasts with the finding of both Rands (1917) and Angell (1929) who found that the optimum temperatures for growth and germination were practically the same e.g. Rands' figures for the cardinal temperatures of germination are 1-3, 26-28, 37-45°C. It seems that figures for the cardinal temperature of germination vary according to the method of recording results. Angell found an optimum temperature for germination of 24-26°C for Alternaria porri and this was based on rate of germ tube elongation. This criterion however is more suited to a comparison of conidia which have been subjected to a variety of treatments and are then germinated under the same conditions. In a temperature study of germination, rate of germ tube elongation is very similar to a growth rate study of the fungus hence the close correlation between the cardinal temperatures of growth and germination.

The method of recording germination used in this study is more suitable than germ tube elongation with time since it shows the trends of germination percentage with time at the different temperatures.

HOST SPECIALIZATION.

To test whether or not there was any difference between isolates in degree of host specialization a host range study was carried out.

The study was carried out in 2 parts viz:

Part I:

Isolates from Black nightshade were inoculated onto potato and tomato.

" " Potato " " " Black nightshade and tomato.

" " Tomato " " " Black nightshade and potato.

Part II:

With the exception of SN264D and LE84G the isolates were inoculated onto a range of host plants given in the table below. Plants used in the study were all mature and disease free.

TABLE VIII

FAMILY	HOST SPECIES	COMMON NAME
Compositae	Ageratum haustonianum Lactuca sativum Zinnia elegans	Ageratum Lettuce Zinnia
Solanaceaen	Solanum nigrum Solanum tuberosum Lycopersicum esculentum Nicotiana tabacum	Black Nightshade Potato Tomato Tobacco
Oenotheraceae	Godetia spp.	Godetia
Allium		Wild Onion
Umbelliferae	Daucus carota	Carrot

METHODS:

Part I was carried out fairly soon after the isolations were made from the field with the exception of isolates SN84A and LE84G. To make results comparable between isolates a standard inoculum dose of 5×10^4 spores/ml was used for each isolate on each host. Three plants per isolate of each host were inoculated with the spore suspension and one plant was sprayed with distilled water to serve as a control. Inoculated plants and controls were placed in a high humidity chamber constructed of clear plastic sheeting for 24 - 48 hours and then removed to a bench in the glasshouse. Once lesions had developed single spore reisolations were made in the laboratory onto laboratory P.D.A. as a final check.

Part 2 was carried out along similar lines to Part 1 with a standard inoculum dose of 5×10^4 spores / ml and three host plants plus a control for each isolate on each host. The results, however, proved to be very variable since the isolates had been kept in culture for about 9 months and were possibly losing pathogenicity. Because of this a second trial was carried out using high concentration spore suspensions inoculated onto unwounded leaves of the host and also onto leaves wounded by pricking with a needle. Reisolation of the fungus was attempted from any lesions occuring on unwounded and/or wounded leaves. As a check on pathogenicity of isolates in this 're-run' of the trial Black nightshade and tomato plants were included and inoculated in the same manner as the host range plants.

RESULTS:

The results from Parts I and 2 are combined in Table 9 which also shows details of symptom type.

Results of Part I reveal that all isolates successfully cross-inoculated between the three hosts, Potato, Tomato and Black nightshade. As a rule, however, isolates from Black nightshade took 2-3 days longer to produce symptoms on potato and tomato than isolates from potato and tomato inoculated onto Black nightshade and tomato or potato respectively. In addition, although there was a standard inoculum dose used, Black nightshade isolates produced fewer lesions on the alternate hosts than did isolates from potato and tomato on their alternate hosts.

Symptoms of all isolates on all 3 hosts were similar i.e. brown irregular lesions up to 20 mm in diameter with the concentric ridging giving the characteristic 'target-spot' effect.

The concurrent check on pathogenicity in Part 2 using Black nightshade and tomato plants showed that all isolates were still capable of attacking these 2 hosts so it is fair to assume that the results of the host range trial are valid. The results show a wide variation in host range between isolates and also a wide range of symptom types. In most cases the isolates proved to be weakly pathogenic and only produced infection on wounded leaves of the hosts. Symptoms appeared on most plants after 8-10 days but in a few cases 14 - 15 days elapsed before any lesions formed.

DISCUSSION:

Spontaneous attacks of Alternaria solani have been recorded largely on members of the Solanaceae although Neergaard (1945) reported finding a spontaneous attack on Ageratum haustonianum of the Umbelliferae. Numerous investigators have extended the host range of the fungus by experimental inoculations onto wounded and unwounded hosts belonging to the Solanaceae e.g. successful inoculations have been recorded on S. ariculare, S.melongea, S.giganteum, S. carolinensis, Hyacyamus niger, Capsicum annum and C. globatum. Neergaard (1945) obtained infection on seedlings of a number of non-solanaceous hosts including Allium cepa, Zinnia elegans, Godetia hybrida, Brassica oleracea and Lactuca sativum.

The usefulness of experimentally induced infections is definitely limited since conditions are heavily biassed in favour of the fungus. The main justification for this type of work lies in the hope, often unfulfilled, of distinguishing clearcut races of a fungus by virtue of host specialization. Neergaard (1945) was able by this method to distinguish to his satisfaction, 3 groups or 'formae speciales' of the fungus A.porri of which strains of A.solani made up one of the groups.

No clearcut distinctions occured between isolates in this study although the Wild onion and carrot were not attacked by any isolate which tends to give weight to Neergaard's differentiation of 3 groups namely, A.porri mainly attacking species of Allium, A.porri f.sp.dauci mainly attacking species of Umbelliferae and A.porri f.sp.solani mainly attacking species of Solanaceae.

Whether or not a wider range of hosts would have shown up differences in host specialization is debatable. Probably, a greater variation between isolates is all that would be revealed.

TABLE IX

HOSTS

	PART ONE			PART TWO						
ISOLATES	POTATO	TOMATO	BLACK NIGHTSHADE	TOBACCO	LETTUCE	ZINNIA	AGERATUM	GODETIA	WILD ONION	CARROT
SN84A	x T	x T	x T	x X S	-	-	-	x M	-	-
SN84D	x T	x T	x T	N.R.	X S	x S	x S	x S	-	-
SN264A	x T	x T	x T	x X S	X L	x S	x S	x M	-	-
ST84B	x T	x T	x T	x X S	x X S	-	-	x M	-	N.R.
ST84C	x T	x T	x T	-	x S	x X S	x S	x S	-	-
ST264B	x T	x T	x T	x S	x M	x X L	-	-	-	-
ST264C	x T	x T	x T	-	x S	x X M	x M	-	-	N.R.
A46	x T	x T	x T	N.R.	x M	x S	x L	x M	-	N.R.
LE84E	x T	x T	x T	N.R.	x L	-	x X S	-	-	-
LE264B	x T	x T	x T	x X S	x X L	x	X S	-	-	-

SYMPTOM TYPES:

T = Typical target spot lesions, brown with concentric rings up to 15mm in diameter. Coalescencing of lesions.

S = Small dark brown spots up to 5mm in diameter.

L = Large, brown coalesced spots.

M = Brown irregular spots up to 10 mm in diameter.

x = Reisolation from lesion on wounded leaf.

X = Reisolation from lesion on unwounded leaf.

\- = No infection.

MORPHOLOGY OF THE FUNGUS.

The morphology of the fungus is discussed under several headings which are similar to the diagnostic criteria listed by Neergaard (1945) as an aid to differentiating between genera and / or species.

CONIDIA:

Conidial measurements were taken for all isolates from their natural host i.e. Potato or Tomato or Black nightshade, and from laboratory P.D.A. In addition, conidial measurements were made for some isolates on different culture media and for the following isolates SN84A, SN84D, ST84B, ST84C, LE84G and LE264B, on all three hosts.

The procedure for obtaining and measuring the conidia was the same in all cases. The agar cultures, or infected host material, were wounded and given high humidity for 24 - 48 hours in a petri dish. The resulting crop of conidia was transferred to a drop of lactophenol on a microscope slide and measured as soon as was convenient. Some slides were made into permanent mounts to enable the measuring to be spread over a 1 - 2 week period. Fifty spores were measured for all isolates off the 3 hosts and laboratory P.D.A. and 25 spores were measured for several isolates on several different media.
The following measurements were made on each spore:

(a) Spore body length.

(b) Spore body width (maximum)

(c) Beak length.

Defining where the beak began was not always easy but several features were taken into consideration e.g.

(i) the transition from coloured spore body to hyaline beak.

(ii) the position where the tapering sides of the spore body were nearly parallel.

(iii) the position where the distance between transverse septa increased by 2 or 3 times.

Drawings of conidia of all isolates, off their natural host and of laboratory PD.A. were made with the aid of a projection microscope giving a magnification

of approximately 500 times. A representative set of drawings is included for several isolates on different media. (Figs 4 - 7.)

The detailed results of the measurements i.e. the means and ranges, are presented in Appendix IV. For purposes of easy reference the measurements of all isolates off their natural host and off P.D.A. are given in Table 10 and, in addition, are presented diagrammatically in Figs 7 - 10.

The results of these investigations show the wide range of variation in conidial morphology both within and between isolates under the same and differing conditions. Perhaps the extreme example of this variation (within an isolate) is given by the difference between conidia of isolate SM2,D off P.D.A. and off Tomato juice agar. As a general rule however there were no distinctive differences for isolates between hosts or between culture media. Beak length was more variable than spore body length but it should be noted that conidia from isolates off Black nightshade generally had longer beaks than conidia of other isolates under the same conditions.

Normally, conidia were formed singly, and terminally, but occasionally branched conidiophores were seen, in culture, bearing 2 conidia as shown in Fig. 17. Small secondary conidia attached to the primary conidium were very rarely seen and tendencies toward catenulation were practically non-existent in all isolates. Another rarely seen phenomenon was the production of a secondary conidium on a branch put out by the parent spore. (Fig. 11.) This was observed a few times in conidia off culture media, and it may represent a special form of germination, or proliferation.

TABLE X.

CONIDIAL MEASUREMENTS EX DIFFERENT MEDIA (MEANS IN μ).

NATURAL HOST.

ISOLATE	SPORE	RANGE	BEAK	RANGE	WIDTH	RANGE
SN84A	95.9±33.0	53.3 - 203	144.9±47.2	71.8 - 244	20.9 ±3.38	14.4 - 28.7
SN84D	86.3±17.2	51 - 125	169.5±27.1	135 - 234	19.4 ±2.35	14.4 - 24.6
SN264A	75.8±12.3	43 - 96.4	129.4±24.5	43 - 184.5	14.9 ±2.15	10.25 - 20.5
SN264D	76.0±10.8	41 - 98.4	164.0±20.7	125 - 205	17.0 ±2.25	12.3 - 22.6
ST84B	85.3±14.1	55 - 115	86.3 ±17.7	55 - 133	20.7 ±2.05	14.4 - 22.5
ST84C	76.3±11.1	39 - 100	73.2 ±22.8	27 - 117	18.9 ±1.43	16.4 - 22.5
ST264B	67.7± 6.2	47.2 - 82	84.0 ±16.4	45.1-112.8	17.4 ±2.05	12.3 - 23.6
ST264C	74.2±10.8	49.2-102.5	85.1 ±18.5	47.2 -125	16.4 ± 0.20	12.3 - 20.5
A46	84.9±14.4	47 - 115	75.5 ±18.6	51 - 121	19.1 ±2.05	14.4 - 22.5
LE84E	85.3±19.9	55 - 131	75.0 ±23.5	28 - 139	17.2 ±2.76	10.3 - 20.5
LE84G	88.4±16.0	57.4 - 123	82.4 ±25.4	33 - 135	17.2 ±3.7	12.3 - 28.7
LE264B	87.3±14.4	61.5-131.2	89.1 ±18.2	47.2-149.7	18.5 ±2.87	12.3 - 24.6

P. D. A.

ISOLATE	SPORE	RANGE	BEAK	RANGE	WIDTH	RANGE
SN84A	54.3±12.4	26.6 - 86.1	103.7±30.4	43 - 199	14.6 ±2.15	10.3 - 18.5
SN84D	65.8±11.7	43 - 94.3	142.5±77.6	71.8 - 205	17.3 ±1.64	14.4 - 20.5
SN264A	51.9±10.9	35 - 90	123.0±26.7	59.5-188.6	15.6 ±2.45	12.3 - 22.5
SN264D	81.4± 9.2	39 - 80	146.0±33 9	53.3 - 209	15.8 ±1.85	12.3 - 20.5
ST84B	54.3± 8.7	32.8 - 71.8	73.8±17.1	36.9-112.8	17.2 ±2.05	12.3 - 22.5
ST84C	57.4± 8.1	43 - 77.9	74.4±15.7	43 - 112.8	15.0 ±1.23	12.3 - 18.5
ST264B	59.9± 7.6	37 - 82	87.1±17.3	33 - 119	16.4 ±1.95	12.3 - 22.5
ST264C	61.1± 8.5	39 - 84	98.8±24.2	45 - 147.6	14.8 ±1.43	12.3 - 18.5
A46	52.3± 7.4	39 - 63.6	65.8±10.4	39 - 92.3	15.4 ±1.90	12.3 - 20.5
LE84E	65.8±12.1	47.2 - 94.3	72.2±25.4	30.75-119	18.0 ± 2.05	14.4 - 22.5
LE84G	55.4± 6.8	39 - 71.8	91.0±20.7	57.4-141.5	15.6 ±1.95	12.3 - 20.5
LE264B	64.2± 9.8	32.8 - 84	100.3±24.2	59.5-157.9	16.0 ±1.64	12.3 - 18.5

DESCRIPTION OF CONIDIA:

The spore body is obclavate to elongated oval, constricted by 3 - 16 transverse septa and 0 - 6 longitudinal septa; colour varies from a light buff-brown to a dark olive-brown. The beak is long, filiform and hyaline with several transverse septa; occasionally the beak may be forked.

As a basis for comparison Table 11 records spore dimensions of the fungus given by several authors together with the dimensions of 3 isolates used in this study.

TABLE XI

AUTHOR	YEAR	MEDIUM	LENGTH including beak(μ)	WIDTH (μ)
ELLIS & MARTIN (from Neergaard)	1882	Potato leaves	100 - 140	15 - 18
RANDS	1917	Potato leaves	120 - 296	12 - 20
RANDS	1917	Potato Agar	104 - 184	14 - 18
ANGELL	1929	Onion Agar	140 - 370	14.5 - 33
RAABE (from Neergaard)	1939	Natural Medium	85 - 285	13 - 30
ISOLATE SN84A		Black Nightshade Leaves	125 - 447	14 - 29
ISOLATE ST84B		Potato leaves	110 - 248	14 - 23
ISOLATE LE264B		Tomato leaves	109 - 281	12 - 25

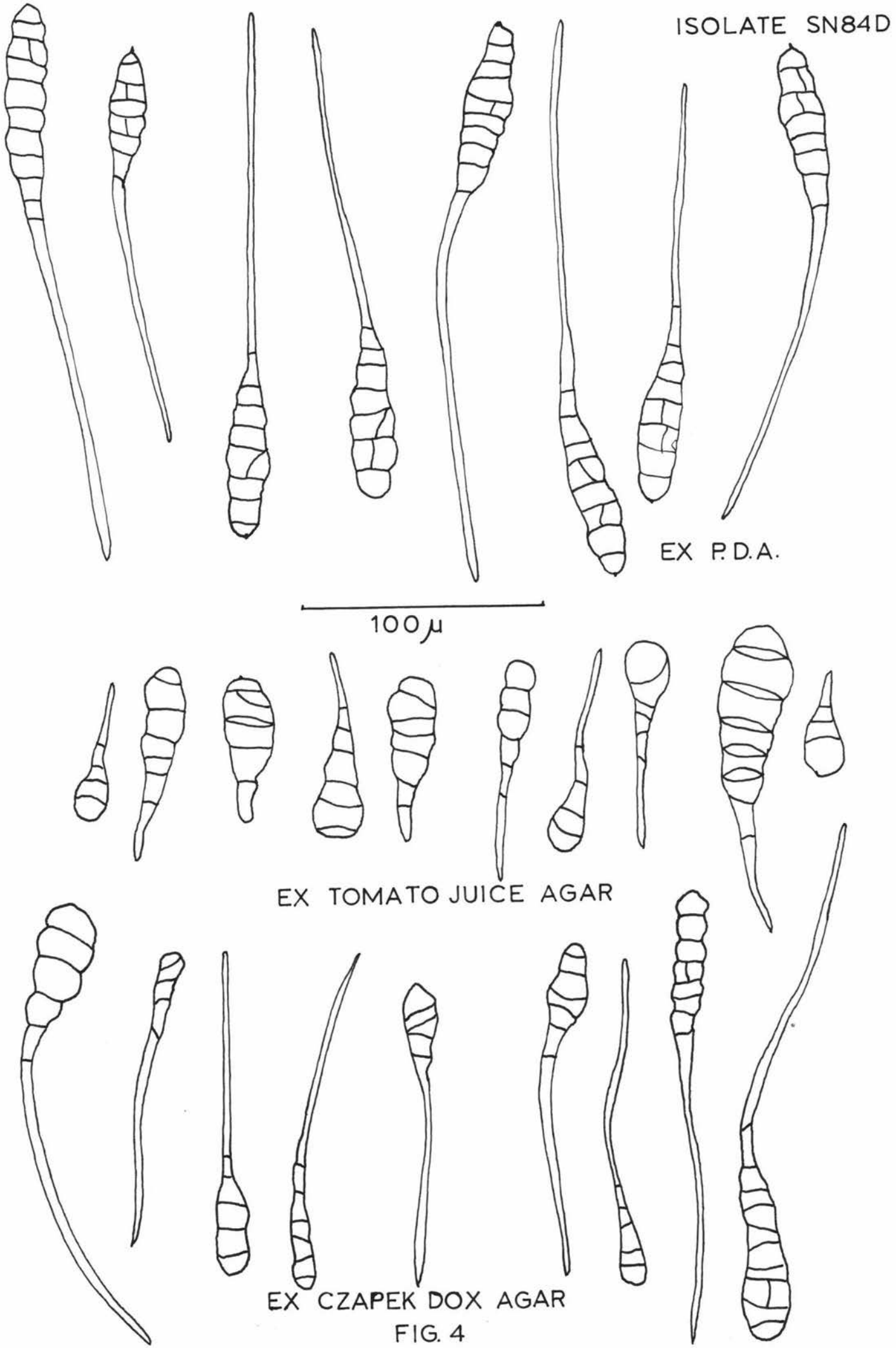
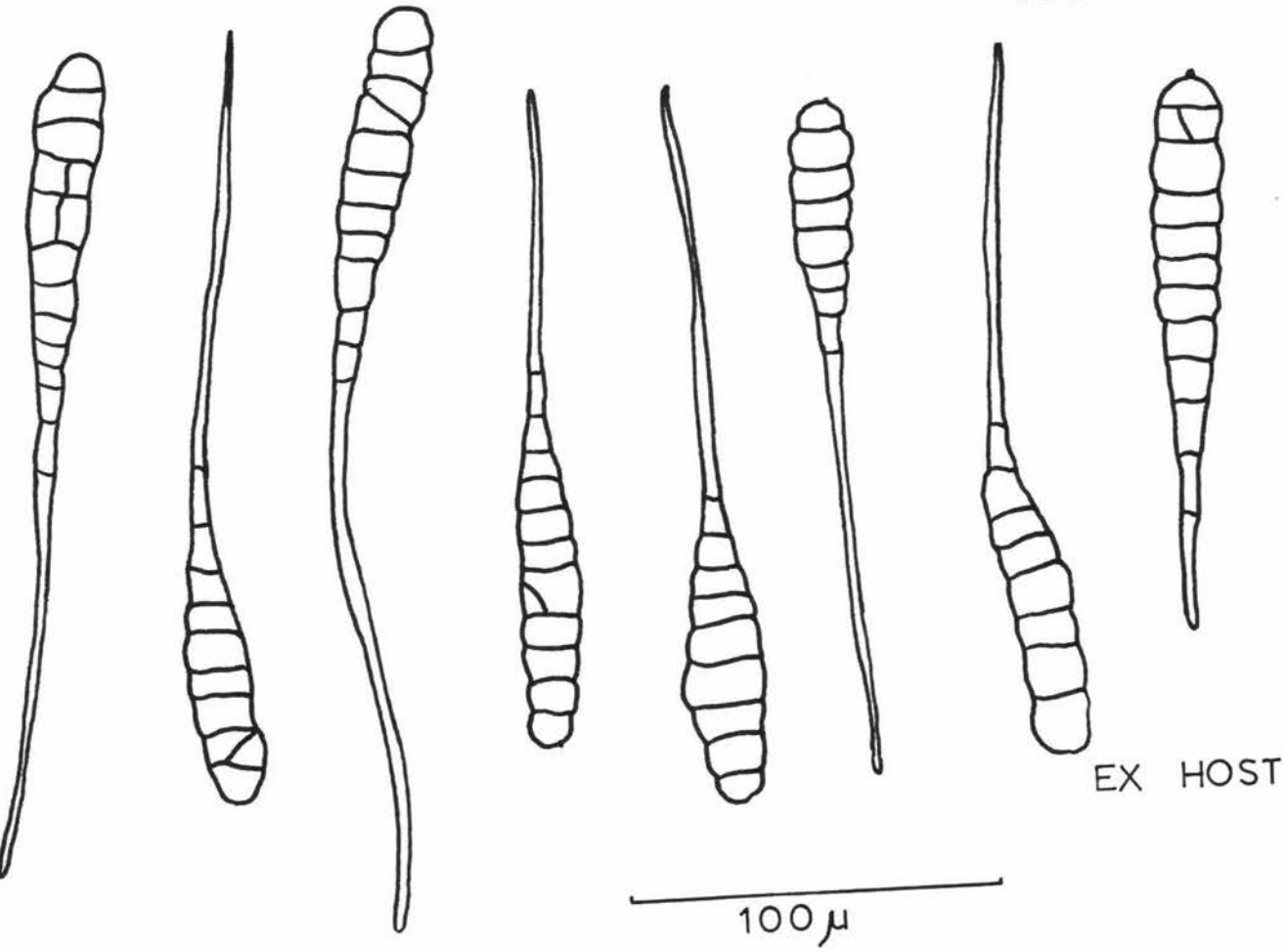

ISOLATE SN84D
EX P.D.A.
100 μ
EX TOMATO JUICE AGAR
EX CZAPEK DOX AGAR

FIG. 4

ISOLATE SN264A



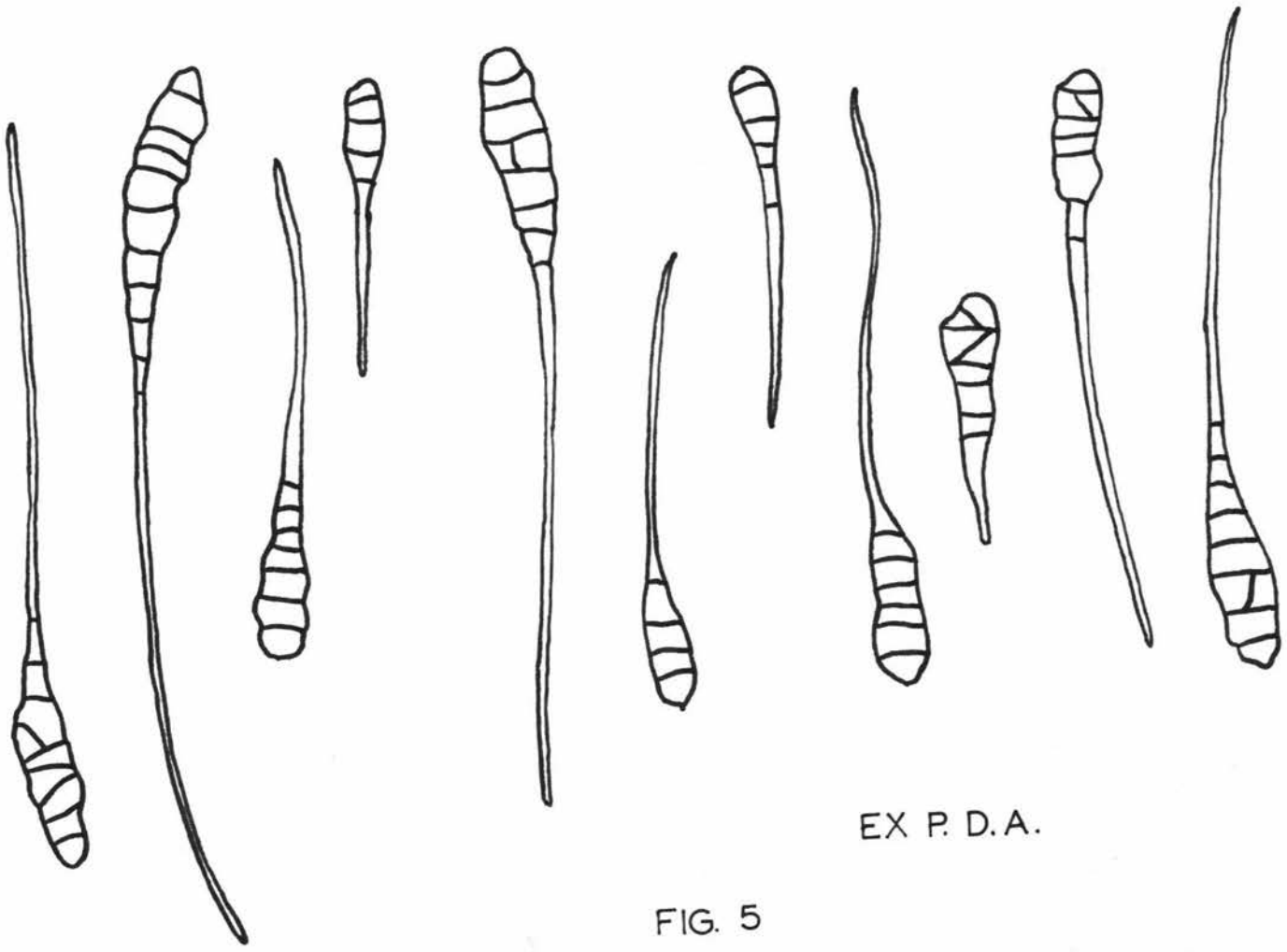
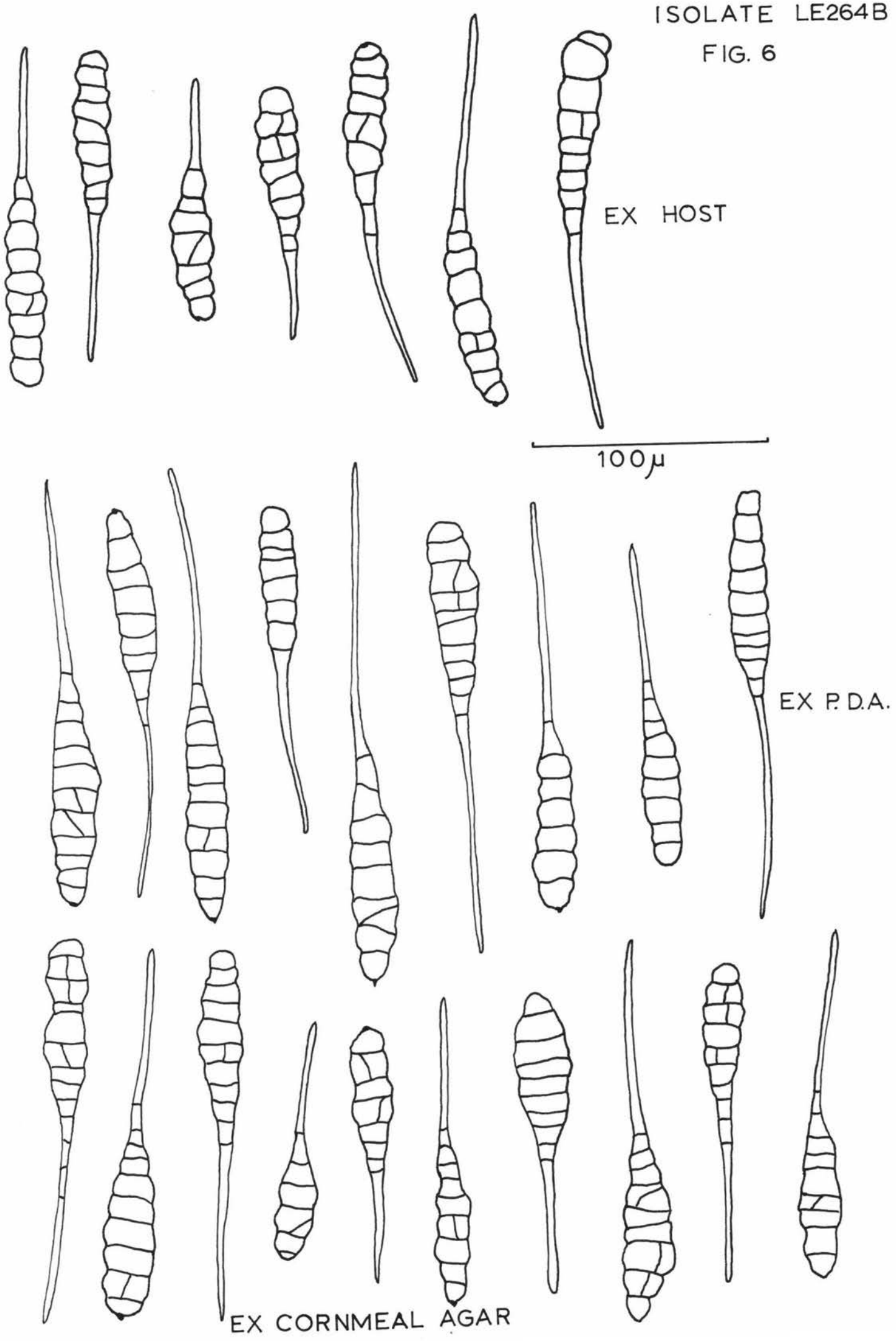


FIG. 5

ISOLATE LE264B

FIG. 6



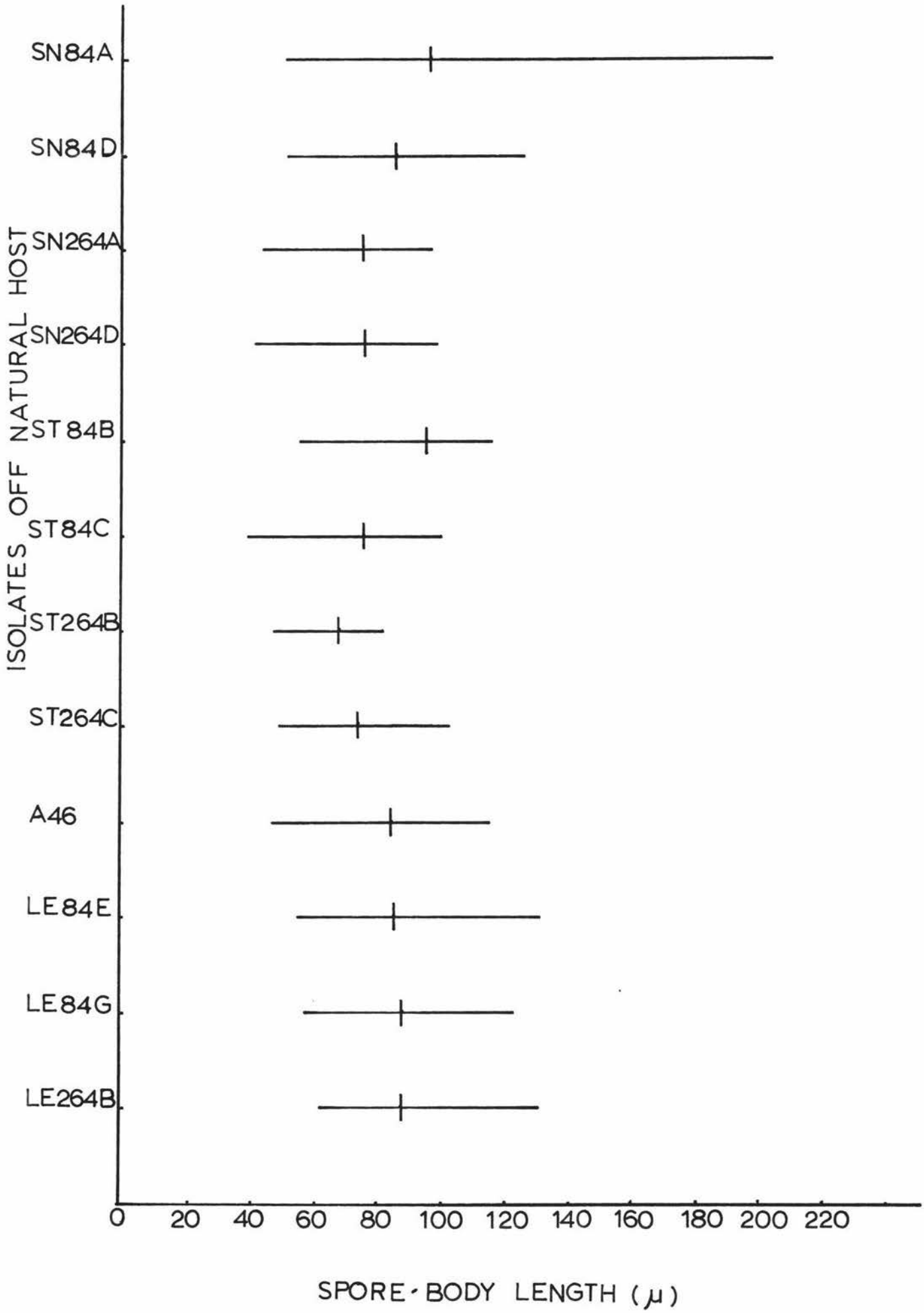

SN84A
SN84D
SN264A
SN264D
ST84B
ST84C
ST264B
ST264C
A46
LE84E
LE84G
LE264B
ISOLATES OFF NATURAL HOST
0
20
40
60
80
100
120
140
160
180
200
220
SPORE-BODY LENGTH (μ)

FIG. 7

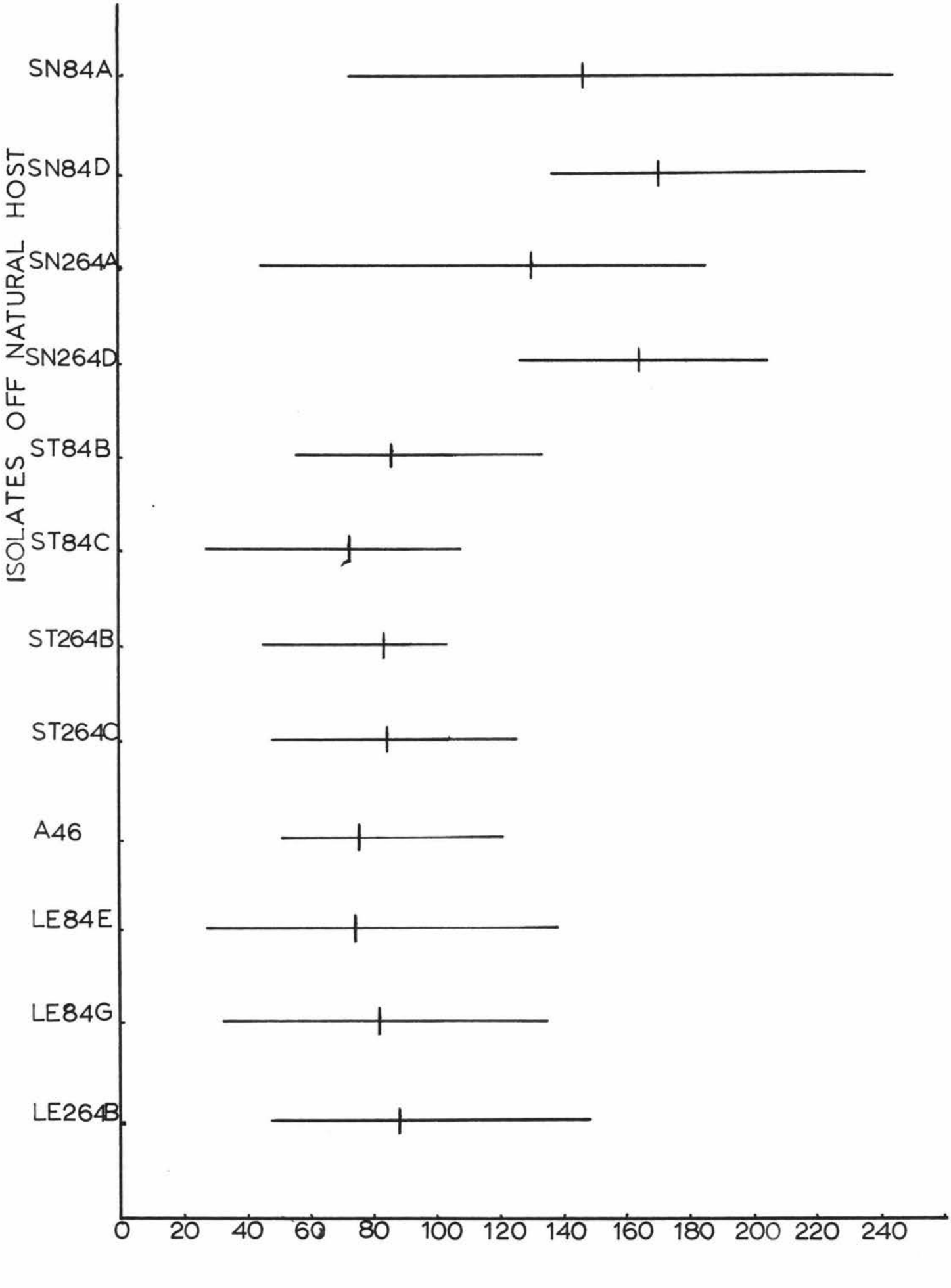

ISOLATES OFF NATURAL HOST
SN84A
SN84D
SN264A
SN264D
ST84B
ST84C
ST264B
ST264C
A46
LE84E
LE84G
LE264B
0
20
40
60
80
100
120
140
160
180
200
220
240
BEAK LENGTH (μ)

FIG. 8

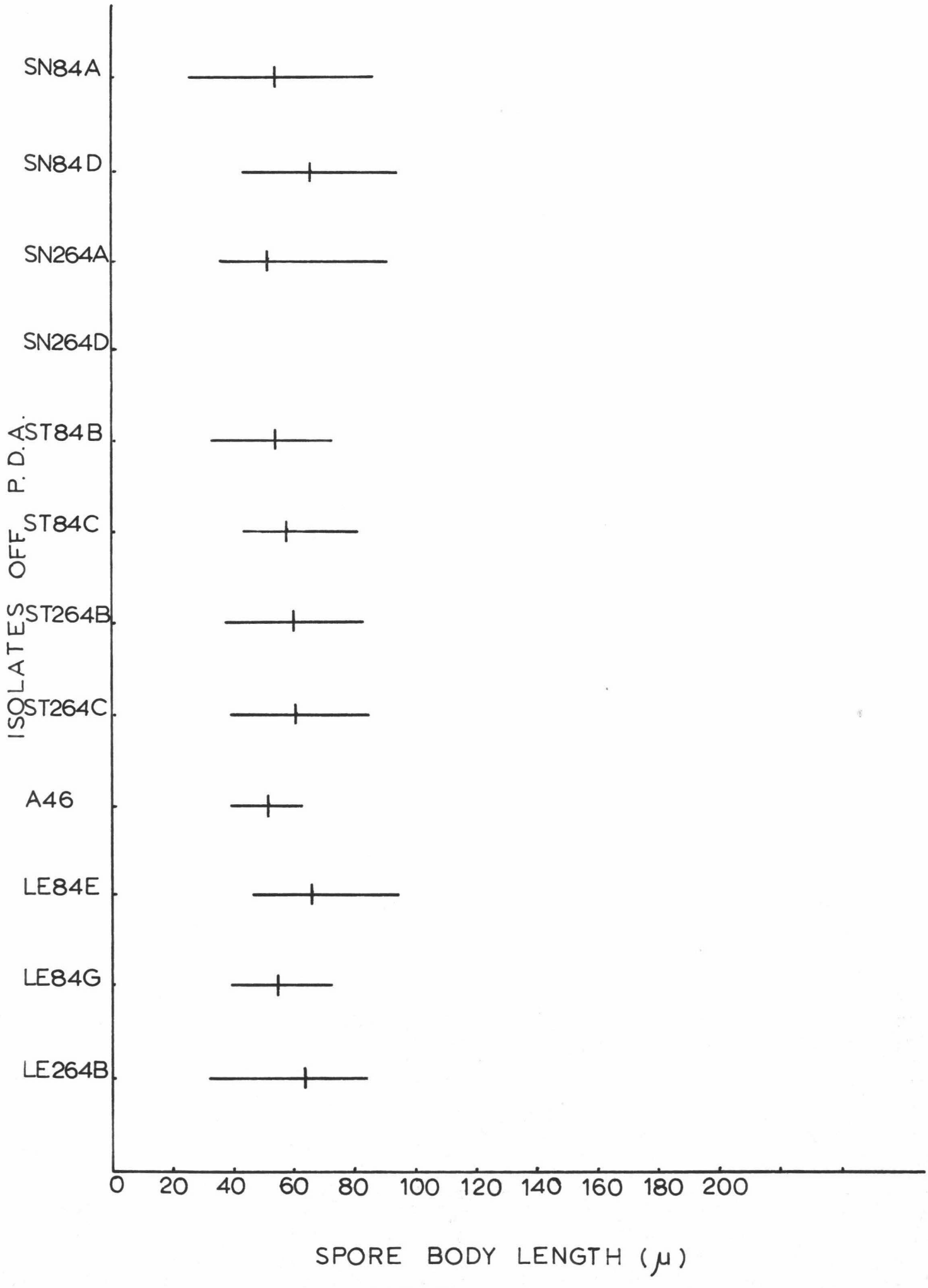

SN84A
SN84D
SN264A
SN264D
ST84B
ST84C
ST264B
ST264C
A46
LE84E
LE84G
LE264B
ISOLATES OFF P.D.A.
0 20 40 60 80 100 120 140 160 180 200
SPORE BODY LENGTH (μ)

FIG. 9

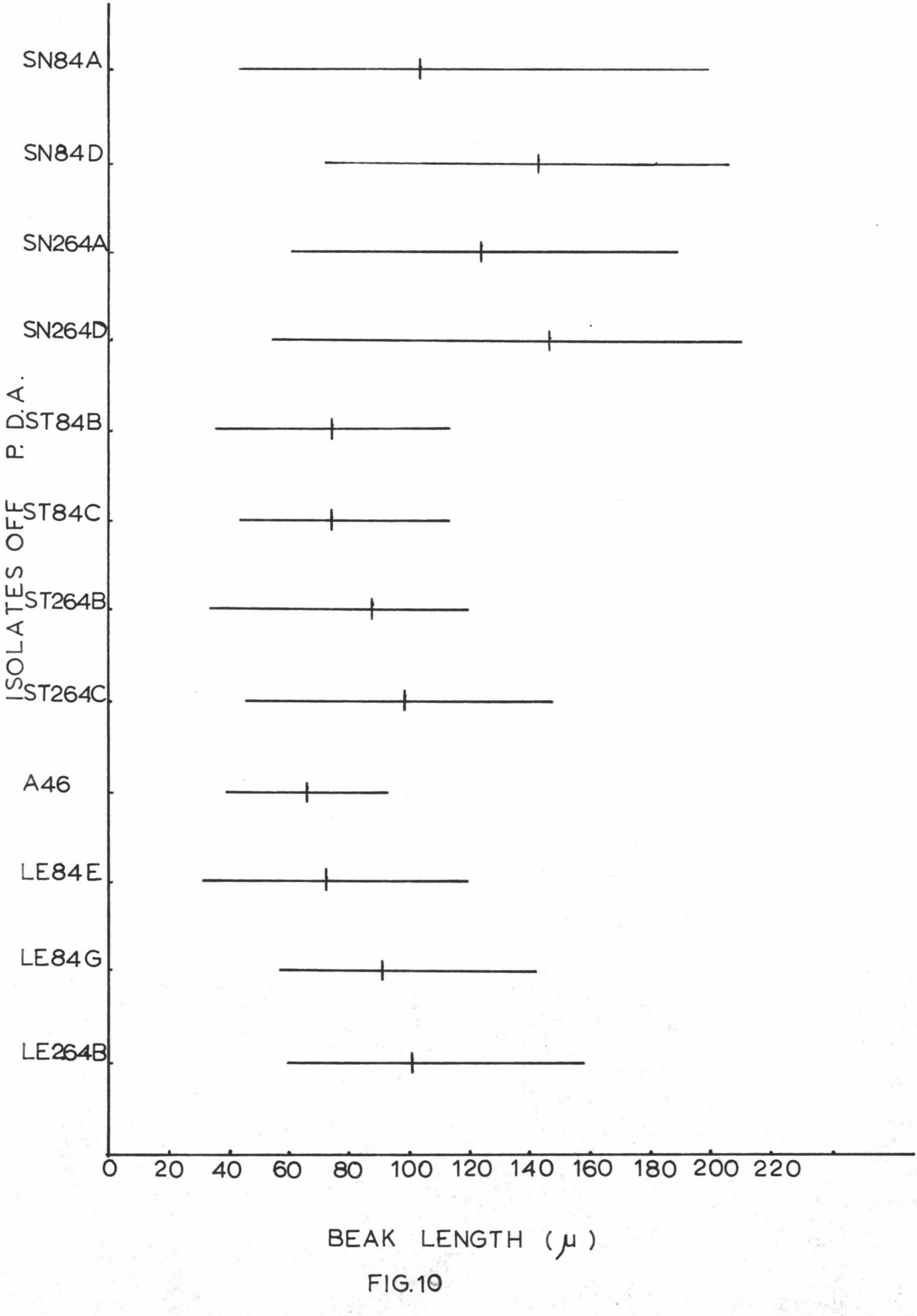

SN84A
SN84D
SN264A
SN264D
ST84B
ST84C
ST264B
ST264C
A46
LE84E
LE84G
LE264B
ISOLATES OFF P. D. A.
0 20 40 60 80 100 120 140 160 180 200 220
BEAK LENGTH (μ)

FIG. 10

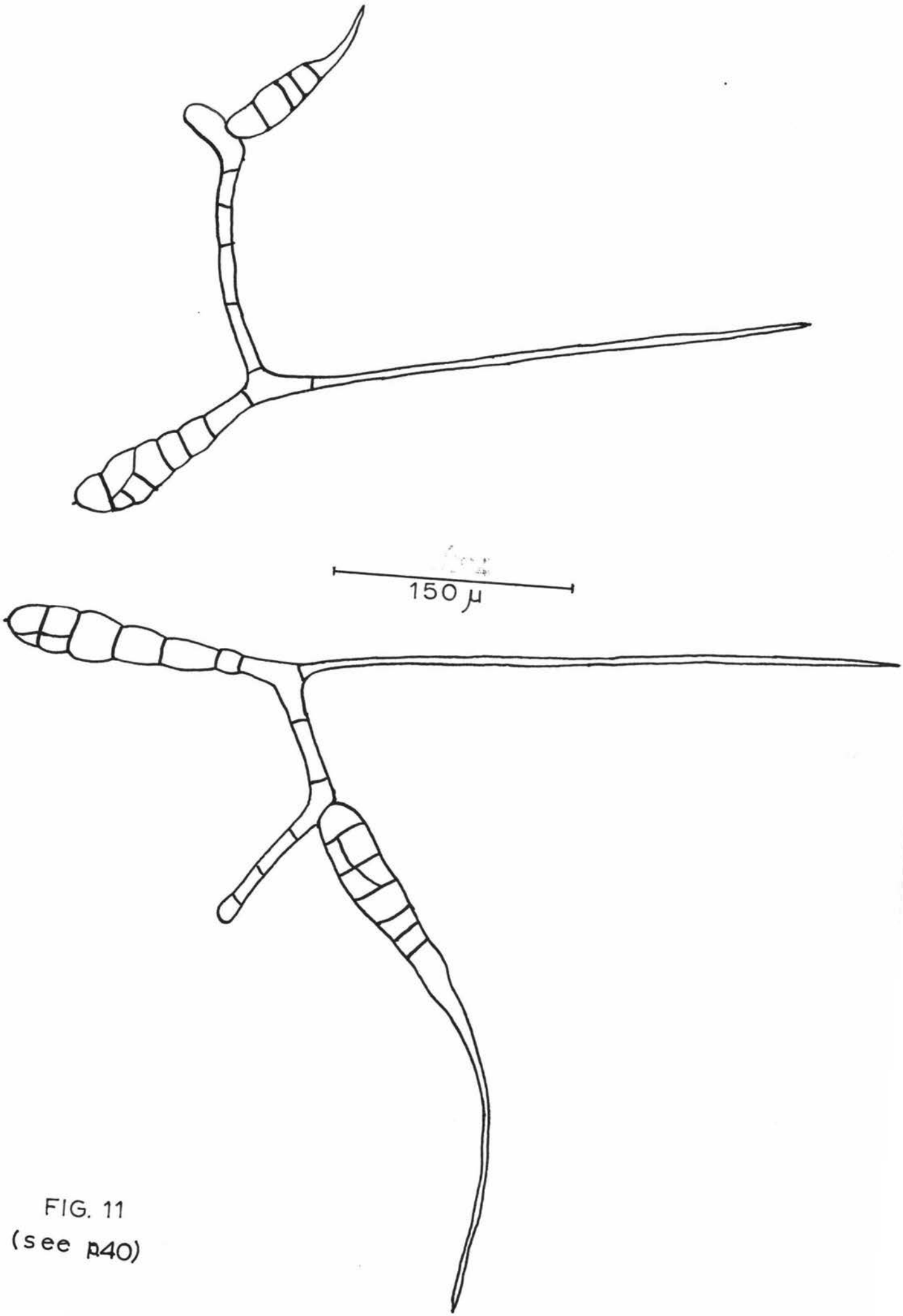


FIG. 11
(see p40)

DEVELOPMENT OF CONIDIA.

Growth and development of a conidium were followed under a microscope and microphotos were used to record the principal stages. The method used in these observations was to mount a small piece of agar, cut from a laboratory P.D.A. culture of the fungus, on the bottom of a petri dish which was placed on the microscope stage. The microscope was focussed onto the edge of the agar in order to examine conidium development against a clear background. Conidial production was ensured through giving the agar piece high humidity 'in situ' by putting a moist filter paper in the lid of the petri dish and placing this over the bottom section on the microscope stage.

The sequence of photographs on the following pages illustrates the development of a conidium quite clearly but a few words of description will also be useful.

Conidiophores grew out quite rapidly from the surface of the agar and varied from 50μ to 150μ in length before they produced a conidium. The conidium first became apparent, on the tip of the conidiophore, as a bud which rapidly swelled into the basal portion of the spore. Growth slowed down thereafter while the spore body slowly enlarged, became septate, and the beak developed. Under conditions of high humidity and optimum temperature (about 24^{o}C.) the development of a conidiophore plus conidium took about 18 - 20 hours on laboratory P.D.A.

CONIDIOPHORES:

Erect, nonbranching arising singly either as side branches or terminally, from hyphae. Dark brown in colour, septate and varying in length on laboratory P.D.A. from 50μ to 200μ . Neergaard (1945) reported that, on natural material, conidiophores sometime arose in bundles from sclerotioid cell clusters, but throughout the study this phenomenon was not once observed.

Frequently, isolates which sporulated relatively weakly even under optimum conditions produced numbers of conidiophores which grew to a length of up to 400μ and then began to curl up without producing a spore.

HYPHAE:

The hyphae range in colour from hyaline through to an 'olive-brown'. They are septate and up to 10μ wide.

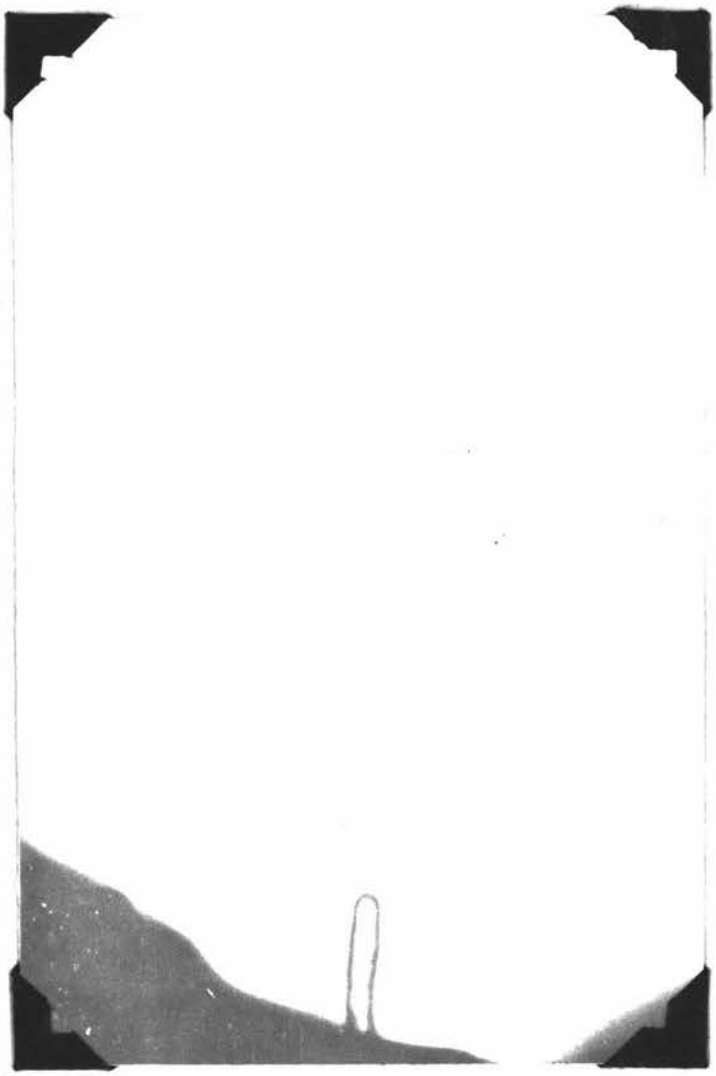


FIG. 12
A YOUNG CONIDIOPHORE

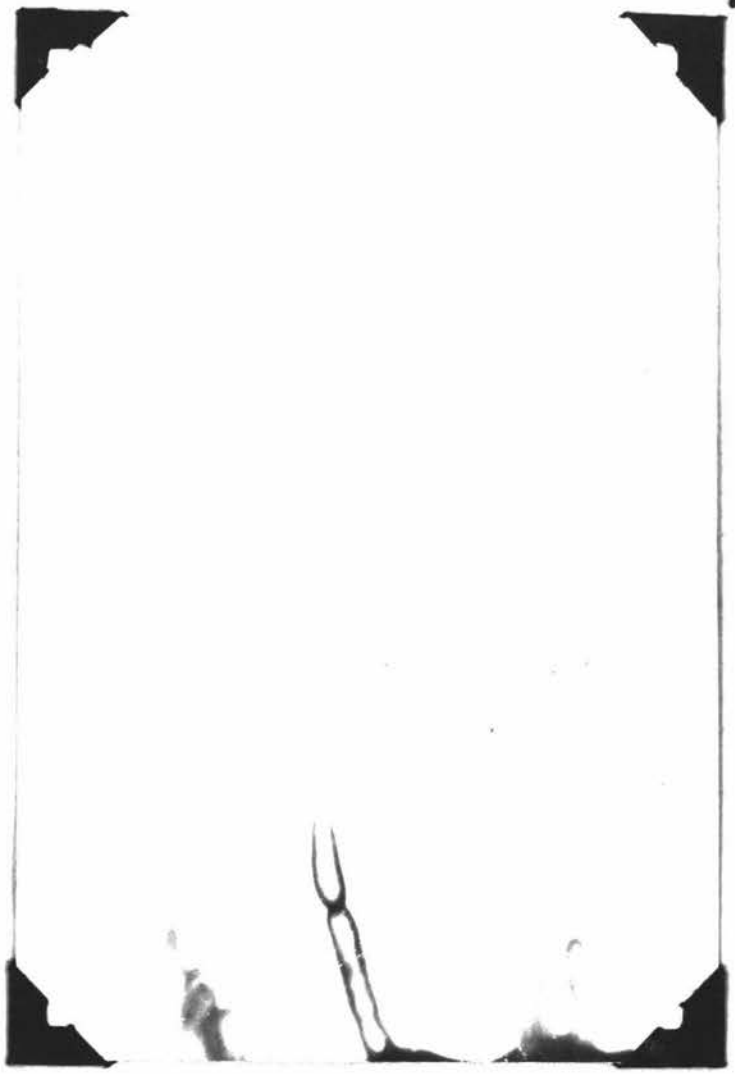


FIG. 13
FIRST STAGES OF
CONIDIUM DEVELOPMENT

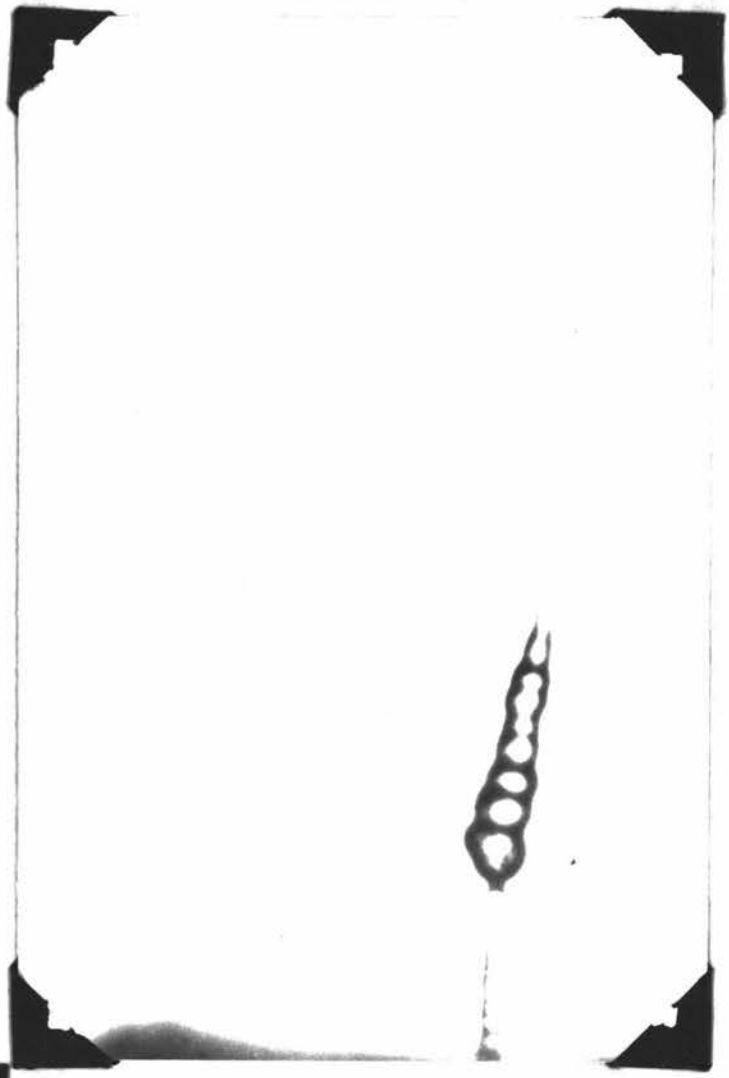


FIG. 14
A MATURE CONIDIUM

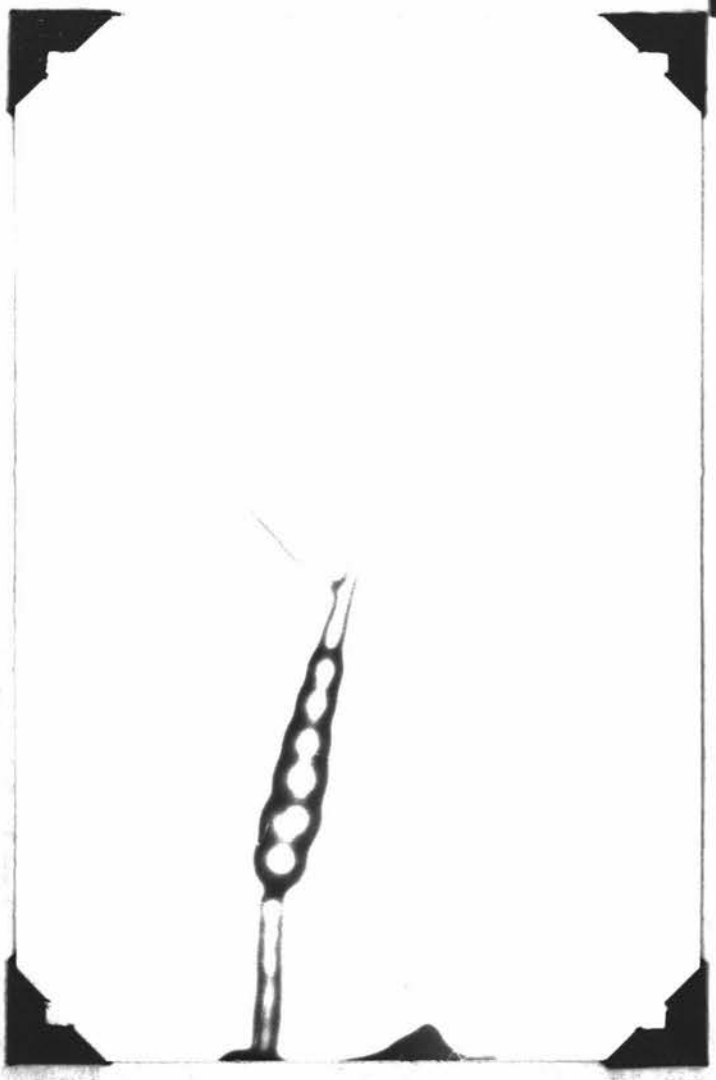


FIG. 15
A MATURE CONIDIUM
WITH A FORKED BEAK

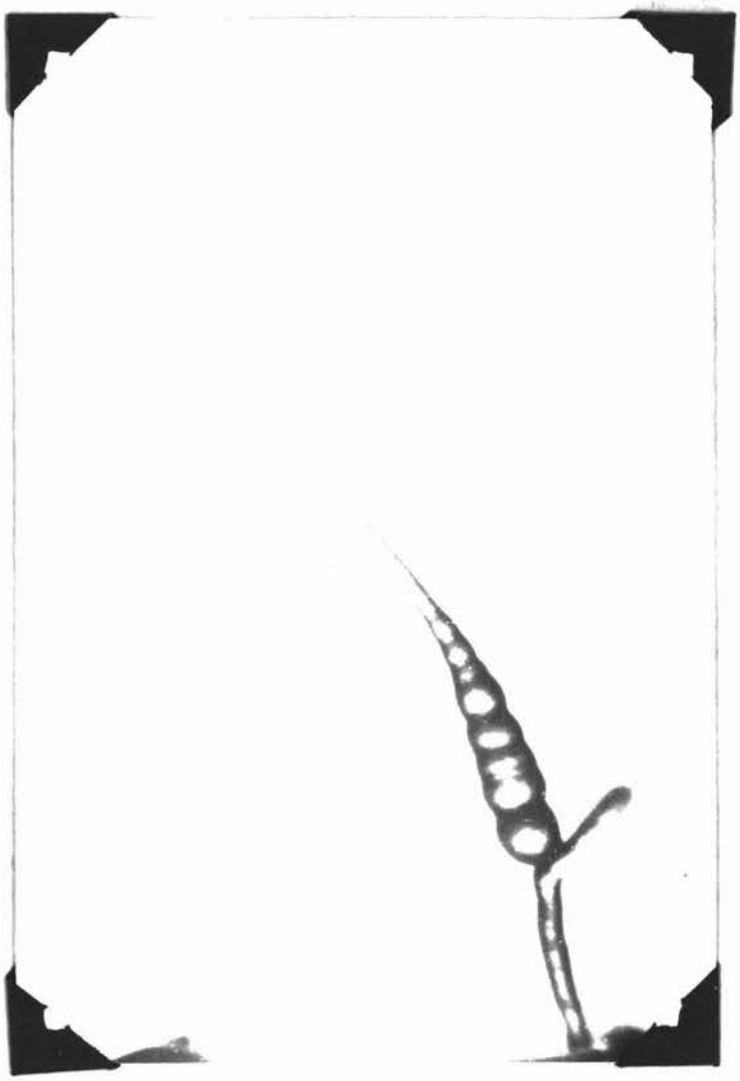


FIG. 16
BRANCHING CONIDIOPHORE

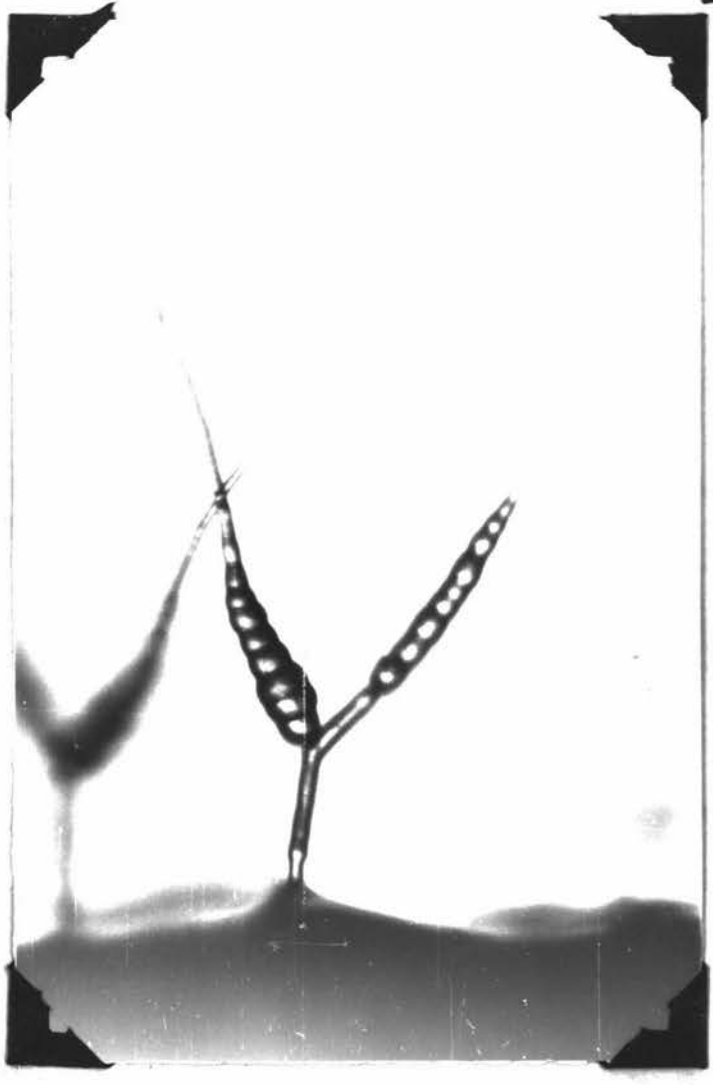


FIG. 17
CONIDIOPHORE BEARING
TWO CONIDIA

TAXONOMY AND NOMENCLATURE.

The genus Alternaria is classified in the Fungi Imperfecti in the order Moniliales and the family Dematiaceae.

HISTORY OF THE GENUS ALTERNARIA.

In the course of time a number of species have been described under Alternaria and Macrosporium.

In 1817 Nees set up the genus Alternaria with one species Alternaria tenuis in which the conidia were linked by "filiform connections". Fries in 1832 set up the genus Macrosporium with four species M.convallariae, M. tenuissimum, M. cheiranthi and M.caricinum, the first 3 of which are clearly shown by later workers to be congeneric with Alternaria. Fries erroneously regarded the conidial beak as a basal stalk and this misconception was continued by some later workers such as Berkeley who set up the species M. sarcinula in 1838.

In 1840 Corda published an illustration of A. tenuis which deviated from that of Nees since the conidia were formed into chains of up to 13 and the beaks were not filiform but tapered gradually from the conidial body. In addition, the beaks of most spores were hyaline in contrast to the dark brown of the spore body. Although the fungus described by Corda was quite different to that of Nees it has nevertheless formed the basis of the modern conception of the genus. Corda regarded Macrosporium Fr. as synonymous with Alternaria.

Elliott in 1917 emphasized that obclavate conidia often with a long beak should be a generic criterion in Alternaria. It was noted that chain formation may be suppressed under unfavourable conditions. Elliott asserted that species under the current conception of Macrosporium ought to be transferred to Stemphylium as he (?) considered there was nothing in the morphology of species of Macrosporium which would exclude them from the genus Stemphylium.

Bolle 1924 subscribed to Elliott's conception of Alternaria and suggested retaining the genus Macrosporium for forms with sarcinaeform spores thus ruling out Fries and basing the genus on Berkeley's description of M.sarcinula.

Tehon and Daniels 1925 set up the genus Thyrospora to which they suggested forms with sarcinaeform conidia be transferred.

In 1929, Angell, noted that forms with obclavate conidia borne singly, or in chains and forms with sarcinaeform conidia could be placed with a less equal justification into the genus Macrosporium or the genus Alternaria. Because fungi having obclavate conidia borne singly could, under certain circumstances bear them in chains Angell considered catenulation to be too unreliable to rate as a generic criterion. Hence he said that fungi in the 2 groups should be included in the same genus - Macrosporium Fr. in accordance with the earliest description of the characters of the genus by Fries in 1832.

In 1933 Wiltshire reviewed the evidence regarding the identity of the foundation species of Alternaria and Macrosporium. This meticulous and lucid account corrected previous misconceptions and resolved the several different interpretations regarding the importance of catenulation as a generic character.

Wiltshire found that the foundation species could be grouped into 3 different types:

(a) those forming long chains of shortly or comparatively shortly beaked spores.

(b) those forming chains only rarely, the spores possessing long filiform beaks.

(c) those with spores normally borne singly (though occasionally bearing secondary ones on short conidiophores), sarcinaeform, without any beak, usually with a major cross wall accompanied by a constriction.

Group (a) is, by common consent, placed in the genus Alternaria. It includes A. citri, A.Longpipes, A tenuis auct., A.fasciculata and is clearly congeneric with Alternaria tenuis Nees.

Members of group (b) are sometimes placed in Alternaria and sometimes in Macrosporium e.g. A. macrospora, A. solani, A.crassa, A. tomato (for long has been Macrosporium tomato), M.porri, M. sesami.

Group (c) is commonly placed in Macrosporium. The typical member of this group is the conidial stage of Pleospora herbarum which is usually regarded as M.sarcinula. Other species include M.parasiticum, M. sarcinaeform , Stemphylium solani etc.

There are intergrading forms between groups (a) and (b) but group (c) is sharply defined from the rest and in fact is identical with the original concept of Stemphylium: S.botryosum (Wallroth).

Popularly, the distinction between Alternaria and Macrosporium is that the former has conidia borne in chains and the latter, conidia borne singly. Bolle and Elliott however give evidence showing the variable nature of chain formation which can be suppressed almost entirely on culture media, or conversely occur on culture media but not in the natural host. Some weight however must be attached to the fact that group (a) has conidia usually in long chains.

The filiform beak of group (b) is also very variable e.g. in length and thickness. Furthermore, the beak on natural host material may be quite different from that developed in culture e.g. Alternaria gossypina (Thum) Hopkins on the cotton plant has only a short beak but in culture the beak is long. A.longpipes has a short beak on host material and practically no beak at all in culture.

The conidiophore in groups (a) and (b) consists of a branch of mycelium, which may be thickened and of a dark colour and may bear from one to several conidia in chains. The conidiophore may bear a single scar where the terminal spore or spore chain was attached but sometimes there are several scars.

The distinguishing character of groups (a) and (b) is the beaked spore, separating them from all other genera in the Phaedictyae. Associated with this characteristic is either a marked development of chain formation or of the length and narrowness of the beaks; as the beaks get longer the spores per chain get fewer.

In his study Wiltshire established that Alternaria Nees and Macrosporium Fries are based on congeneric species since of the four species described by Fries, M.cheiranthi and M.tenuissimum are congeneric and are clearly species of Alternaria in the current modern sense of the term. Although Wiltshire was unable to study any type material of M. convallariae Fries' description of the

species made it reasonable to assume that it was congeneric with M.cheiranthi. The fourth species, M.caricinum had no longitudinal walls and hence was distinctly different and does not belong to the Phaeddictyae.

Since Alternaria and Macrosporium are based on congeneric species one or other of the names must be discarded. Wiltshire concluded that although Macrosporium is the strictly legal name (being described by Fries in the "Systema" whereas Alternaria is not recognised) it has consistently but erroneously been applied to species forming no chains of conidia - group (c) and, in part, group (b). Consequently, owing to the confusion associated with this name he suggested relegating it to the nonima ambigua and employing Alternaria for groups (a) and (b).

Neergaard 1945 subscribes to this view since he suggests that employment of the name Macrosporium in strict accordance with the rules of nomenclature could only lead to further confusion.

A BRIEF REVIEW OF THE SPECIES ALTERNARIA.

First described by Ellis and Martin in 1882 and named: Macrosporium solani. In 1896 Jones and Grout transferred the species to Alternaria naming it A.solani (E.&.M.) Jones and Grout. From then on there were two schools, one classifying the species in Macrosporium and the other classifying it in Alternaria.

In 1929, Angell made an extensive comparative examination of M.solani (E.&.M.) and M.porri Ell. He concluded that these species were physiological races of the same species since (i) morphological variation was as great between species as between isolates within either species.

(ii) sporulation of both species in culture was very difficult to,obtain.

(iii) cultural characters of the 2 species grown on various media were practically identical.

One difference was noticeable however, M.porri forms attacked species of the Liliaceae whereas M.solani forms attacked species of Solanaceae.

Neergaard's (1945) investigations led him to support Angell and, in addition, he found that there was another form which should be considered a physiological race of the same species, namely: Alternaria brassicae v dauci (kühn) Lindau found on carrot.

Accordingly, Neergaard suggested that the 3 forms be classified as one species in which they constitute physiological races. In accordance with the rules of nomenclature the species name must be that of the first described species i.e. M.porri Ellis 1879. Thus the name given the species was:

Alternaria porri (Ell) Neergaard with 2 formae speciales.

A.porri f.sp.dauci (kühn) and A.porri f.sp.solani (E.&.M).

Neergaard gives the following description of the morphological characters of A.porri.

HYPHAE: hyaline to 'olive-buff' to dark 'olive-buff'; septate, 1 -10 μ wide.

CONIOPHORES:
deep 'olive buff' to'buffy brown'; septate (6-30μ between septa)

On natural media they are 30 - 100 μ in length and 4 - 9 μ in width.

On agar media they are 30 - 200 μ in length and 6 - 9 μ in width. As a rule they are non-branching, erect with one or seldom 2 scars.

On natural medium they form singly or in bundles, sometimes from sclerotoid cell clusters. In agar cultures the conidiophores form singly either as side branches on hyphae or terminally on hyphae, the narrower and lighter hypha gradually turning into the broader and darker conidiophore.

CONIDIA: Normally formed singly but very occasionally a secondary conidium is formed in cultures.

Smooth, obclavate to elongated oval, constricted by septa gradually tapering into a long filiform often bi or tri-forked beak,

Dark 'olive-buff', 'buffy brown', 'olive brown' to natal brown'.

Length of spore body: 25.5 - 137μ Width: 10.5 - 37.5 μ

Length of beak : 4.5 - 432μ " : 2 - 4 μ

Total spore length : 40.5 - 501μ

The spore may have from 3 - 14 transverse septa and 0 - 8 longitudinal septa.

The beak is hyaline to sub hyaline and has 0 - 7 tranverse septa.

DISCUSSION AND CONCLUSIONS.

There are many examples in fungal taxonomy where species, which were soundly based on morphological criteria, have been fragmented into a number of 'new' species on the basis of host specialization characteristics. One of the most extreme examples of this approach to taxonomy is given by Gaumann's (1918 ex Yerkes & Shaw) creation of 49 new species of Peronospora, from an original 4 or 5 species of P.parasitica, following some host range studies. The absurdity of creating species based largely on physiological characteristics is constantly being exposed by workers who study several closely related, so called species of many genera, under uniform conditions and find that they form a group of strains, of one morphologic species, which intergrade in any or all characteristics to such an extent that differentiation at species level is impossible and invalid e.g. the work of Yerkes and Shaw on the Peronospora species of Cruciferae and Chenopodiaceae.

Each genus has its own peculiar problems which accentuate different features as being diagnostic criteria at the species level. The importance of reliable morphologic criteria and to a lesser extent physiologic criteria is paramount. One of the more interesting taxonomic debates in very recent times, concerning the identity of the fungus causing the Spring Blackstem disease of alfalfa and redclover, has arisen because of two schools of thought about the importance attached to the production of uni-septate conidia by the fungus to various degrees under varying conditions. The disparity in thinking in this example is caused by the great similarity in the descriptions of the 2 species suggested by the 2 schools such that classification into one species or the other is based very much on an interpretation of English rather than any clearcut constant character.

The taxonomy of the genus Alternaria is difficult since constant morphological criteria are hard to find and it is only the very unwary who use conidial measurements by themselves as a means of differentiation. Several papers have been published showing the effect of changes in environment on conidial morphology in the genus Alternaria e.g. the paper by Rangaswami and Sambandam (1960) dealing with the influence of substrate on spore size of Alternaria melongenae. The results obtained, during this work, for conidial (Appendix IV and Table X) size of isolates on several substrates indicate the degree of

variability that can be expected within isolates and between isolates of Alternaria solani e.g. in Table X it is noticeable that spore body size is smaller on P.D.A. than on host material. The standard deviation is high for measurements of spore body length and beak length, again emphasizing the variation which occurs.

It is relatively simple however for any worker familiar with the genus Alternaria to differentiate a number of species on the basis of collective conidial morphology rather than on one isolated characteristic such as size or dimensions. There was a 'uniformity of variability' which became quite striking for the isolates of Alternaria solani used throughout this work and provided evidence that they all belonged to the one morphological species.

Neergaard regards Alternaria solani to be a physiological race of Alternaria porri since the two are morphologically similar and react similarly in culture but the 'solani' forms are parasitic mainly on Solanaceous hosts and the 'porri' forms are parasitic mainly on Allium species.

Even though it is possible to get isolates of one or other of these physiological forms to infect hosts from other families under experimental conditions this is artificial since everything is heavily biassed in favour of the fungus. Neergaard carried out extensive experimental inoculations on seedlings of host plants from several families but the infections were usually very slight with little affect on the plant.

There is extensive evidence however, for the occurrence of physiological strains within the 'solani' race. Bonde (1929), Klaus (1940) and Rex-Thomas (1943) are 3 workers who noted differences between isolates of Alternaria solani they studied. One constant difference between isolates studied in this work was in chromogenicity. Isolates from Black nightshade were all non-chromogenic in contrast to the isolates from Potato and Tomato which were strongly chromogenic.

There was no evidence in this study of any major physiological difference such as extreme virulence or avirulence of an isolate and taken as a group the isolates were sufficiently uniform to render any further classification on physiological or morphological grounds, into races or sub-forms, redundant.

Neergaard's system of classification, therefore, seems eminently suitable since

it is workable and while it recognizes that variation may often occur between isolates this is not allowed to assume false importance. Such a system, utilizing 'formae speciales' of a morphological species could well be adopted with other genera of Imperfect Fungi.

The name Alternaria solani has been used throughout this work in preference to Neergaard's A.porri f.sp.solani since the latter name has apparently not found acceptance with all workers in recent publications. However, provided the name Alternaria solani is used together with that of the host plant from which the fungus was isolated little confusion should result since the specialization of Alternaria solani forms for Solanaceous hosts is a very uniform characteristic.

In conclusion, it may be stated that the variation which was found between the 12 isolates of Alternaria solani studied, provided insufficient grounds for suggesting a further subdivision in classification and instead, was in substantial agreement with overseas results, notably those of Rands and Neergaard.

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APPENDIX I.

COMPOSITION AND PREPARATION OF MEDIA.

STERILIZATION :

Unless otherwise stated media were autoclaved at 15 pounds per square inch for 20 minutes immediately after preparation.

ARTICICIAL MEDIA:

(a) Potato Dextrose Agar (Oxoid)

Potato infusion	200 gm
Bacto Dextrose	20 gm
Bacto agar	15 gm

Preparation: 39 gms of the above mixture were suspended in 1000 ml of distilled water then heated to dissolve the medium. The medium was poured into 7 - 8 250 ml flasks which were stopped with cotton wool and autoclaved.

(b) Potato Dextrose Agar (Laboratory)

Potatoes (peeled and sliced)	200 gm
Dextrose	10 gm
Agar	12 gm

Preparation: The potatoes were cooked gently for an hour in 500 ml of distilled water. The liquid was strained through a cheesecloth and the filtrate added to the agar which had been melted in a further 500 ml of distilled water. The volume was adjusted to 1000 ml and the medium poured into 7 - 8 250 ml flasks which were stopped with cotton wool and autoclaved.

(c) Cornmeal Agar

Cornmeal	20 gm
Agar	17 gm

Preparation: The cornmeal was suspended in 500 ml of distilled water and simmered gently for 1½ hours after which, the liquid was strained through a cheesecloth. The agar was melted in 500 ml of distilled water added to the filtrate and the volume adjusted to 1000 ml.

(d) Czapek-Dox Agar - Modified (Oxoid)

Agar	12.00 gm
$NaNO_3$	2.00 gm
Magnesium glycero-phosphate	0.50 gm
KCL.	0.50 gm
$FeSO_4 . 7 H_2O$	0.01 gm
Sucrose.	30.00 gm

Preparation: 51.4 gm of the above medium were added to 1000ml of hot distilled water and allowed to dissolve for 15 - 20 minutes before pouring into flasks and autoclaving.

(e) Prune Agar (Difco)

Prune infusion	36 gm
Bacto agar	15 gm

Preparation: 24 gm of the medium were added to 1000 ml of distilled water the whole being boiled to aid solution of the medium. The medium was poured into 7 - 8 250ml flasks which were stopped with cotton wool and autoclaved.

(f) Water Agar

Preparation: 12 gm of Davis Agar were boiled in 1000 ml of distilled water until the agar dissolved. The medium was poured into separate flasks and autoclaved.

(g) Tomato Juice Agar

Oxoid Tomato Juice Agar	52 gm
Davis agar	7 gm

Preparation: The above mixture was boiled in 1000 ml of distilled water to dissolve the medium which was then poured into a number of flasks and autoclaved.

(h) V8 - Juice Agar

V8 juice	500 ml
Distilled water	250 ml
Davis agar	10 gm

Preparation: The ingredients were placed in a 1 litre flask and heated until the agar dissolved. The medium was then poured into 250 ml flasks and autoclaved.

NATURAL MEDIA:

Tomato)
Potato) Leaf Extracts

200 gm of mature leaves and petioles were macerated with 1000 ml of distilled water in a "Waring Blender". The resulting suspension was filtered through a pad of blotting paper. The filtrate was passed through a "Seitz Filter" under aseptic conditions to obtain a sterile extract.

A solution of 4% Davis agar was made up and held, together with the extract, in a 50°C water bath.

Plates were poured by pipetting 7.5 ml of 4% agar and 7.5 ml of extract into each plate and then agitating to ensure complete mixing.

APPENDIX II

ISOLATE	TEMPERATURE - COLONY GROWTH ON OXOID P.D.A. (COLONY DIAMETER (mm.) FOR 8-DAY COLONIES. TEMPERATURE °C.									
	5 ± 1	10 ± 1	17 ± 1	20 ± 1	24 ± 1	26 ± 1	28 ± 1	30 ± 1	33 ± 1	37 ± 1
SN84A	4.0	9.2	46.2	71.3	76.8	73.2	73.5	64.2	15.0	Nil
SN84D	3.6	11.8	57.7	67.0	75.6	74.5	70.4	64.8	22.0	Nil
SN264A	2.5	9.6	57.7	61.0	63.2	62.2	77.0	69.0	35.0	Nil
ST84B	3.1	22.8	40.3	68.7	73.0	72.5	78.3	76.6	33.5	Nil
ST84C	3.1	20.2	42.7	57.5	63.6	62.0	69.8	66.8	20.3	Nil
ST264B	4.3	16.5	28.8	60.3	62.0	65.6	73.8	70.0	41.0	2.3
ST264C	2.3	15.8	32.0	49.0	61.5	63.6	68.0	61.2	40.3	Nil
LE84E	7.1	19.2	32.7	70.3	71.0	69.3	65.5	64.5	31.0	Nil
LE84G	4.3	15.5	23.2	43.0	43.0	47.5	51.2	51.7	27.7	4.0
LE264B	8.5	16.2	30.8	69.4	71.0	70.8	76.3	68.8	40.5	3.5

APPENDIX III

COLONY DIAMETER (mm.) EX 8 - DAY COLONIES AT DIFFERENT pH LEVELS.												
ISOLATE	3.6	4.1	4.6	5.1	5.6	6.1	6.6	7.0	7.5	8.0	8.6	9.0
SN84A	10.2	32.0	57.0	66.1	66.7	81.3	78.6	61.8	57.5	55.7	40.0	22.4
SN84D	8.5	32.0	58.0	63.0	65.5	75.0	75.7					18.5
SN264A	10.2	21.5	55.0	55.3	59.5	70.1	72.0	59.0	61.6	62.0	44.0	16.4
ST84B	16.5	40.0	60.0	70.6	72.0	74.3	76.5	68.5	55.7	53.0	47.5	41.0
ST84C	19.2	39.3	58.1	64.0	66.1	74.6	74.0	65.0	63.1	50.0	48.0	27.0
ST264B	10.5	34.6	54.0	56.5	62.5	68.0	66.5	64.5	64.0	35.2	42.5	35.2
ST264C	18.6	40.0	59.0	66.5	65.0	73.0	70.5	66.0	64.0	62.2	45.0	35.5
A46	15.0	35.3	55.5	62.5	66.1	72.5	75.0	68.3	64.0	62.4	53.0	40.3
LE84E	18.2	35.0	60.0	66.2	65.1	74.0	72.8	67.1	61.5	40.0	39.5	32.5
LE84G	16.6	38.0	54.6	60.5	58.5	71.0	62.0	60.3	45.1	49.0	47.5	39.6
LE264B	15.2	N.R.	56.0	61.0	61.5	66.6	63.5	62.3	60.3	43.7	41.0	31.0

APPENDIX IV.

CONIDIAL MEASUREMENTS (MEANS IN μ)							
MEDIUM	ISOLATE	BODY	RANGE	BEAK	RANGE	WIDTH	RANGE
POTATO	SN84A	101.1 ± 19.5	73.8 - 139.4	135.0 ± 59.9	36.9 - 266.5	16.6 ± 2.05	12.3 - 20.5
TOMATO	SN84A	97.8 ± 18.5	65.6 - 125.0	171.0 ± 55.5	65.6 - 268.6	17.6 ± 3.39	12.3 - 24.6
POTATO	SN84D	94.1 ± 18.0	61.5 - 129.2	116.4 ± 54.9	49.2 - 219.4	18.5 ± 4.5	14.35 - 28.7
TOMATO	SN84D	79.5 ± 21.5	30.75- 133.3	144.0 ± 53.7	61.5 - 248.0	16.4 ± 2.46	12.3 - 22.55
BLACK NIGHTSHADE	ST84B	95.7 ± 16.9	73.8 - 133.3	88.6 ± 19.7	45.1 - 116.9	22.6 ± 3.10	14.35 - 26.7
TOMATO	ST84B	108.3 ± 16.6	73.8 - 131.2	87.3 ± 22.3	34.9 - 137.3	20.5 ± 2.87	14.35 - 28.7
BLACK NIGHTSHADE	ST84C	124.6 ± 22.7	84.0 - 164.0	92.7 ± 17.7	59.5 - 129.2	23.4 ± 2.87	20.5 - 30.75
TOMATO	ST84C	81.0 ± 16.8	49.2 - 121.0	76.7 ± 25.2	26.7 - 118.9	17.0 ± 2.05	12.3 - 20.5
POTATO	LE84G	64.4 ± 14.9	43.0 - 121.0	88.7 ± 22.9	49.2 - 137.4	15.6 ± 2.66	10.25 - 20.5
BLACK NIGHTSHADE	LE84G	98.0 ± 12.3	51.25- 131.2	98.6 ± 23.8	45.1 - 145.6	19.3 ± 4.5	12.3 - 26.7
POTATO	LE264B	69.7 ± 19.1	51.25- 102.5	61.7 ± 17.6	34.9 - 102.5	20.1 ± 1.64	18.45 - 22.6
BLACK NIGHTSHADE	LE264B	90.2 ± 22.8	71.8 -,121.0	100.6 ± 28.7	63.55- 143.5	24.0 ± 3.5	18.45 - 36.9
CORNMEAL AGAR	LE264B	63.6 ± 9.0	45.1 - 75.9	75.2 ± 18.65	36.9 - 110.7	19.3 ± 2.9	14.35 - 22.6
TOMATO JUICE AGAR	SN84D	42.9 ± 14.2	30.75- 92.3	46.5 ± 15.8	26.65- 75.9	19.7 ± 2.66	12.3 - 24.6
CZAPEK-DOX AGAR	SN84D	47.2 ± 12.9	28.7 - 77.9	109.9 ± 28.9	61.5 - 174.3	15.2 ± 4.5	10.3 - 22.6