Studies in the Storage Rots of Potatoes Caused by *Phoma* Species.

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INTRODUCTION

Considerable loss is incurred annually in stored potato stocks, due to storage rots caused by fungal organisms. Among such rots, Dry Rot, caused by *Fusarium caeruleum* (Lib.) Sacc. is particularly prevalent.

In the year 1936, attention was drawn to a form of potato storage rot which was suspected to differ from the usual Dry Rot. On investigation, the causal organism was found to be a member of the Sphaerioidaceae (Alcock and Foister, 1936) which on potato tissue gave rise to symptoms similar to those of Dry Rot. Owing to the similarity of symptoms, the disease was not regarded as being new, but was considered to be an old disease which had remained unrecognised. Probably much of the rot formerly attributed to *Fusarium caeruleum* was, in fact, due to this fungus.

Further study of the causal organism referred it to the genus *Phoma*. However, since no known *Phoma* species would satisfactorily accommodate the organism it was recommended that a new species be erected (Foister, Tervet and Alcock, 1936). In a subsequent diagnosis (Foister,1940) the fungus was named *Phoma foveata* Foister.

The disease caused by *Phoma foveata* is commonly known as Gangrene, and occurs in most of the potato growing districts of Scotland. Infection of the tuber is believed to take place in the soil since the fungus is capable of living as a soil saprophyte. However, development of the disease does not take place until the tubers are placed in the requisite storage conditions. In storage, the disease may be spread through the stocks from a source such as a single diseased tuber. Dissemination of the pycnospores from such a source, by air draughts, mites and other fauna, probably brings about further infection.

Gangrene does not usually appear early in the storage period, and usually incidence of the disease is greater among tubers stored in boxes and bags than those stored in pits. In Scotland, where a considerable trade in seed potatoes is conducted, Gangrene has proved troublesome, in that consignments of potatoes, dispatched in an apparently healthy condition, have been found to be infected on reaching their destination. In such cases infection may be as high as 90%. Most of the disease reported in England has been found on seed potatoes brought from Scotland. However, it has been recorded on potatoes grown in England. Naturally, there is some concern among the English growers lest the planting of diseased tubers would so infect the soil as to render it dangerous for potato growing in the future.

During the last twenty years, a tuber disease, known as Skin Necrosis, has frequently been reported on tubers grown on varying types of soil (Moore, 1947). 1943 No particular causal organism has been reported as responsible for this condition, but species of Phoma, Cephalosporium and Fusarium have been isolated from the diseased tubers.

As far as is known, attempts to reproduce Skin Necrosis have failed; therefore it is believed that infection with a fungus alone is not responsible for this disease.

A Skin Necrosis condition associated with Phoma eupyrena Sacc. was studied in 1938, but the results of the investigation were not published. At that time it was not found possible to reproduce the symptoms by inoculating tubers. Since the necrotic regions on naturally infected tubers did extend, though slowly, when kept in moist conditions, the investigators were of the opinion that the Phoma sp. was a primary parasite.

It seems that some factor operating with a fungus is responsible for the Skin Necrosis condition.

SYMPTOMS OF GANGRENE AND SKIN NECROSIS.

The symptoms of Gangrene are variable, depending on the age and variety of the tuber, the age of infection and storage conditions.

In most cases the first sign of infection is a minute circular depression on the tuber surface. Several such depressions may occur on one tuber, each arising from a separate infection. With further growth of the fungus, the depression enlarges, and at an early stage can be likened to a "thumb mark".(Pl.I, fig.1). Since this form also resembles an early stage of infection with Dry Rot, caused by Fusarium caeruleum it is not diagnostic. As the disease progresses the depression may enlarge to a considerable diameter, but its size is not always indicative of the extent of internal rot. Frequently the disease may progress considerably before external signs of a depression become visible while a shallow rot may subtend a large depression. The margin of the depression may become irregular in outline (Pl.I,fig.2), but it usually remains well defined, even in very old rots.

The skin covering the diseased area of the tuber may remain taut (Pl.I,fig.2), or it may show an irregular wrinkling (Pl. I,fig.3). These wrinkles are not so characteristic as in the case of Dry Rot where they usually form concentric rings over the diseased area.

Under very damp conditions a greyish white mycelium may be produced over the surface of the depression. However, this mycelial growth is quite distinct from the white pustules with blue bases which are the external fructifications which are characteristic in older stages of Dry Rot.

On cutting, tubers infected with Phoma foveata may show a rot of varying depth. In some cases the rot is confined to the surface cells of the tuber

although it may spread laterally to a considerable extent. This is the condition known as Skin Necrosis and differs from typical Gangrene in which deep rots are produced. In Gangrene the rot may progress, even to the opposite side of the tuber, and where the advancing rot touches the skin, further small depressions occur.

In the case of Gangrene, when first infected, the tissues become light brown in colour and somewhat mealy in appearance. This colour soon deepens and the rot becomes pink with grey areas. The tissue then commences to dry, and the resulting shrinkage causes small mycelium-lined cavities to form. When examined under the microscope, an abundant septate mycelium is seen, especially in the intercellular spaces. These hyphae are hyaline or dark coloured, and occasionally form in masses between the cells. The tissues soon become dry and friable with a pink, dark grey or black colour. At this stage the cell walls are destroyed (Pl.II, fig.1) and the rot then consists of starch grains held together by hyphae (Pl.II, fig.2). Large cavities, involving the greater part of the diseased tissue, are usually formed at this stage (Pl.III, fig.1). These cavities are lined with a white or pink mycelium within which the black pycnidial fructifications of the fungus may be found (Pl.III, fig.1). Pycnidia may also occur just under the skin and, when mature, they push through the cork layer and extrude a tendril of pycnospores. (Pl. II, fig.3).

The region of the rot is generally well defined, and can be separated easily from the intact tissue. It is often possible to prise out the entire rot (Pl.III, fig.2), but the cavity remaining is lined with intact but infected tissue which subsequently rots. However, the rot is often cut off by a wound cambium which prevents further infection (Pl.II, fig.1).

Externally, the symptoms of Skin Necrosis are similar to those of Gangrene (Pl.IV,fig.1), but the depth of the depression is only slight. The irregular necrotic areas which have an uneven surface, spread from small pockets of infection (Pl.IV,fig.1) until the greater part of the surface of the tuber becomes involved (Pl.IV,fig.2). On cutting,tubers infected in this way show a shallow rot extending only a few cells deep (Pl.III,fig.3). The infected tissues are light brown at first, but darken rapidly as the cell walls become destroyed. The diseased tissue then consists of starch grains and hyphae, and is found underlying the whole of the necrotic region.

The skin of the tuber is no longer functional in the necrotic areas,and as a result, the deeper tissues of the tuber are killed by dessiccation. The fungus is then capable of living as a saprophyte on this dead tissue. The result, therefore, is the same as in a Gangrene type of rot; the tuber dries, shrivels and hardens (Pl.IV,fig.3), and enclosed within the skin of the tuber is a mass of debris and starch grains, held together by the dark, very septate mycelium of the fungus.

In Skin Necrosis, pycnidia are formed just under the skin of the tuber. Where these are produced singly, they penetrate the cork layer (Pl. IV,fig.2) and set free their tendrils of pycnospores. When they occur in a mass, however, the cork layer may be lifted off in a sheet then the pycnospores are released (Pl. IV,fig.3).

A CONSIDERATION OF THE GENUS PHOMA AND THE SPECIES OCCURRING ON POTATOES.

General Consideration.

The generic name, Phoma, has given rise to much controversy since it was instituted by Fries in 1819. Fries (1823) based the genus on Phoma pustula (Pers.) Fr. (now Hypospila of the Pyrenomycetes) which bears no relation to Phoma in its modern sense. In subsequent years, various characters were ascribed to Phoma (including asci) until Desmazieres (1849) redefined the genus, with a new diagnosis. Desmazieres' conception of Phoma has been accepted by mycologists for the last hundred years; therefore, it has been recommended (Wakefield,1939) that Phoma Desm. (1849) be conserved against Phoma Fries (1823), with Phoma herbarum Westend. as the nomenclatural type. This recommendation has been accepted for the present work.

In considering the taxonomy of the Sphaeropsidales,the criteria mostly employed have mostly been morphological. The data used in allocating pycnidial fungi to the form genus, Phoma, have been the structure of the pycnidium, the shape, size, septation and colour in the mass of the spores, and the very unstable vegetative characters. Since these characters show great variability, they have generally been considered unsatisfactory. In consequence, other criteria, such as the nature of the host plant or substratum, have been stressed. This, too, has proved to be equally unsatisfactory since it is known that many species of Phoma have a wide host range and a correspondingly extensive synonymy, within the genus, and even in related genera. Similarly, the same name can be given to quite different fungi.

To indicate the taxonomic situation with the genus, Phoma, one need only quote Dennis who, as recently as 1946, wrote "what is urgently needed is a

reinvestigation of the small-spored Sphaeropsidales, based on isolations from material matched with the existing host-species and subsequently grown for comparison in artificial culture and on suitable differential hosts."

At least seven species of Phoma have been recorded on the potato, but only three of those have been credited with causing disease. The three are Phoma solanicola Prill. and Del., Phoma tuberosa Melhus, Rosenbaum and Schultz and Phoma foveata Foister.

Phoma solanicola was first described (Prillieux and Delacroix, 1890) as causing a stem rot of potatoes in France. However, its pathogenicity was not proved and Köhler (1928) who rediscovered the species in Germany, proved it to be non-pathogenic on healthy potato stems. Both P. foveata and P. tuberosa have been found to be parasitic on sound potato tubers. The original description of P. solanicola is too inadequate to be used for comparative purposes, but as identified and described later by Köhler, it is recognizable. From the descriptions of P. solanicola and P. tuberosa it seems that the only criterion separating these fungi is their substratum. But is their substratum so different? Such a criterion can scarcely be sufficient to separate species, especially when one considers that the tuber of the potato is nothing other than a modified stem. Moreover, before naming those fungi, no attempt seems to have been made to inoculate potato tubers with P. solanicola or haulms with P. tuberosa. Grove (1935) considers that P. tuberosa may be the same species as P. solanicola. There is no doubt that they are one and the same fungus. Considering this to be the case, according to the International Rules of Botanical Nomenclature, the correct name of the fungus would be Phoma solanicola Pril. and Del. sensu Kohler.

In addition to the above species, P. solani Cke. and Harkness, P. oleracea Sacc., P. nebulosa (Pers.) Mont. and P. eupyrena Sacc. have all been recorded on potato stems. Wollenweber (1920) regarded P. eupyrena to be the cause of spots on the skin of potato tubers. However, in 1923, Shapovalov indicated that the Phoma was probably a secondary invader, the real disease being Skin Spot (Oospora pustulans Owen and Wakef.).

Detailed Consideration.

In view of the difficulties involved in taxonomic work with the Sphaeropsidales, an endeavour has been made to obtain several differential criteria before attempting to classify the Phoma isolates used in the present investigation. Any conclusions regarding their identity, however can only be accepted as provisional, pending a more satisfactory classification of Phoma and related genera.

During the investigation, isolations were made from tubers showing typical Gangrene and Skin Necrosis symptoms. Frequently the rots showed contamination by other organisms, but generally, pure cultures of Phoma could be obtained by taking the inoculum from the advancing edge of the rot. This has shown that three distinct types of culture may be obtained, and probably they represent three different Phoma species. For convenience, the three types of culture have been named isolate 1, isolate 2 and isolate 3 respectively.

Isolate 1 was originally obtained from a tuber of the variety Majestic while isolates 2 and 3 were first isolated from Craigs Defiance tubers. Unless otherwise stated, these were the isolates used in the experimental work. In addition, the same fungi were obtained from several sources as is shown in Table I.

Description of the Isolates on Agar.

Observations on the three isolates were made when they were grown in plate cultures on malt and

potato dextrose agars. The isolates retained their identity on these media, and macroscopically, each isolate was similar on both media, but growth on malt agar was much slower.

Isolate	Tuber variety	Locality	Symptoms
1	Majestic	Echt	Gangrene
	Arran Viking	Corstorphine	Gangrene
	Majestic	"	"
	Various tubers	"	"
	Up-to-Date	Gamrie	"
	Arran Banner	Strathallan	Skin Necrosis
	" "	Montrose	" "
	Majestic	Laurencekirk	" "
	"	Various parts	Gangrene
	Red Skin	Stirling	"
2	Majestic	Echt	Gangrene
	Craigs Defiance	Corstorphine	"
	Arran Viking	"	"
	Arran Banner	Strathallan	Skin Necrosis
	Redskin	Stirling	Gangrene
	Various tubers	unknown	"
3	Majestic	Echt	Gangrene
	Arran Banner	Strathallan	Skin Necrosis
	" "	Montrose	" "
	Majestic	Aberdeen	Gangrene
	"	Laurencekirk	Gangrene

Table I.

In order to facilitate comparison with the results of other workers, the following descriptions of the isolates are based on Petri-dish cultures on malt agar. Colours are according to Ridgway (1912).

Isolate 1.

Basal plectenchyme.

The sparse growth of hyaline hyphae produced at first, later darkens through shades of reddish brown, until finally it becomes a dark, almost black-brown. Many of the hyphae become filled with a yellow crystalline substance, and usually yellow, needle-like crystals may be found in the medium associated with empty hyphae. These crystals (Pl.VI,fig.1) give the colony a greenish yellow appearance, and are probably the same as the crystals which form on the aerial hyphae.

Aerial Mycelium.

At first this is short and white, occurring over the whole colony. When older it becomes drab coloured and tends to form faintly concentric zones. Finally, it felts down, and usually the peripheral region assumes a greenish yellow colour, due to the formation of yellow needle-like crystals on the hyphae.

Pycnidia.

Mature pycnidia did not form on malt agar but scattered sclerotial bodies, formed from dark hyphae, were found. These bodies, which occur sunk in the agar medium, are probably immature pycnidia.

Pycnospores.

None observed on malt agar.

Chlamydospores.

Occasionally, dark chlamydospores formed on old submerged hyphae.

Remarks.

The original isolation from potato tubers formed sub-globose pycnidia in agar culture, but sub-cultures taken from it never produced mature pycnidia, and seldom formed immature fructifications. However, other isolations continued to form mature pycnidia, the spores measuring 6.2μx2.48μ(5.6-7.3x1.67-4.67)μ (Pl.9,a)in a three weeks old culture. In the mass the

spores were pink, and appeared biguttulate when viewed under oil immersion.

Monospore cultures gave rise to two types of colony, the one producing pycnidia,and the other, sterile mycelium with very few pycnidia. The difference between the two types remained constant over several series of transfers. Multispore isolations produced few pycnidia.

Most isolations of this fungus produced a red pigment in the agar, but occasional transfers from these isolations failed to produce any pigment.

In pure culture, intrahyphal growths were frequently observed connecting living portions of hyphae which had become separated by the death of the intervening cells. Such growths were quite distinct from the intrahyphal germination of chlamydospores.

Isolate 2.

Basal plectenchyme.

In young cultures the dense growth of hyaline to white hyphae first produced, gives rise to an irregular band of hair-brown just within the marginal zone. This colouring is due to the darkening of groups of hyphae, and pycnidia are particularly abundant in this region. The marginal area is formed of hyaline mycelium. Old cultures become mouse-grey to black in colour and show a vague zoning. Growth of the colony is irregular; consequently, from an early stage the outline is crenate.

Aerial mycelium.

At first this consists of long white hyphae which cover all but the marginal zone of the colony. With age, these hyphae turn a dark mouse-grey in colour, especially in the centre of the culture. In old cultures the hyphae felt down and the dark mouse-grey becomes dominant.

Pycnidia.

Spherical to sub-globose,and frequently compound, mature pycnidia form on the margin of the plectenchyme, even before this becomes covered with aerial hyphae. These are produced in abundance, singly or in irregular groups, immersed or almost superficial, and are black in colour.

Pycnospores.

Spores are colourless, cylindrical, and with rounded ends. Septate spores were not observed. When viewed under oil immersion, the spores were biguttulate but occasionally the guttules were visible under the magnification of the high power objective. The spore dimensions for a one month old culture are 6.5µx3.0µ (5.33-7.67x2.33-3.67)µ, and in the mass the spores are pink. (Pl. IX, a).

Chlamydospores.

In old submerged hyphae, individual cells commonly form black chlamydospores.

Remarks.

After several series of sub-cultures, this isolate no longer formed pycnidia. Monospore cultures separated into two groups of which one produced an abundant growth of mycelium, with few or no pycnidia, and the other having a scant mycelium and many pycnidia. The group forming pycnidia continued to do so over several transfers and showed no sign of losing this property. The pycnidial group had a much slower rate of growth than the other. Multispore isolations produced only a few pycnidia.

Isolate 3

Basal Plectenchyme.

The dense growth of white hyphae in young cultures darkens quickly, leaving a regular, white margin. The dark region is dark mouse-grey at first, and later becomes black. The older hyphae are generally black, but occasional hyaline, empty hyphae are present. Growth is uniform, therefore the colony is circular.

Aerial Mycelium.

At first this forms a short growth which, although white, appears dark when viewed from above, due to the dark underlying mycelium. With age, it darkens to a blackish mouse-grey in the centre, and olivaceous black towards the edge. The peripheral region has short, white hyphae, except in very old cultures.

Pycnidia.

Mature pycnidia form singly or in groups, but are not abundant. They are sub-globose, black, usually immersed, and distributed throughout the culture.

Pycnospores.

These are colourless, cylindrical, and with rounded ends. Septate spores were not observed. Under the high power objective they appeared biguttulate. The spore dimensions for a one month old culture are 4.67μx2.83μ(4.0-5.33x2.33-3.33)μ. In the mass the spores form a greyish-white slime. (Pl. IX, a).

Chlamydospores.

Abundant, characteristic chlamydospores are produced, mostly in short chains. These spores are black and contribute very much to the black appearance of the cultures. (Pl. IX,c).

Remarks.

Monospore cultures did not differ to any extent from the original isolate.

Temperature Studies.

Method.

The rate of growth of isolate 1, isolate 2 and isolate 3 was measured in this investigation. The three isolates were grown on malt and potato dextrose agars, 15 Petri dishes of each medium being inoculated centrally with isolate 1,15 with isolate 2 and 15 with isolate 3. Since isolate 1 did not form pycnospores readily, mycelium was used to inoculate the plates; thus conditions were made as uniform as possible for the three isolates. The inoculum in each case was from single spore stock cultures. In order to standardize the quantity of inoculum used, small discs were cut with the dummy objective of the microscope, and used as inoculum. The inoculated plates were allowed to stand at room temperature for four days until an appreciable growth of the fungus was made. The diameter of the colony of each isolate was then measured to ensure that growth in each series was approximately the same. Three cultures of each isolate on the two media were then kept:—

(a) at 26°C in an incubator,

(b) at 23°C in an incubator,

(c) at 18°C in an incubator,

(d) at 0°-4°C in a refrigerator,

(e) at room temperature (18°-20°C) in the light.

Measurements of growth were made each day during the experimental period, until the first instance of complete overgrowth of the agar was observed.

Following exposure to the various temperatures, sub-cultures were made on tubes of malt agar at room temperature. In this way it was possible to determine whether the temperature conditions of the experiment had any particular influence on the fungi.

Results.

The results of the temperature studies are

expressed on Graphs 1 to 7. Since three cultures of each isolate were made on each medium, the daily rates of growth, calculated from the daily increase in diameter of the fungal colony, were taken as an average of the three plates in each case. The results are given to the eighth day of the experimental period, the initial four days' growth, prior to this period, being taken as zero.

Growth in daylight at room temperature gave the same result as the series kept at 23°C, therefore the cultures were not considered further. From Graph 7, which shows the average daily growth rates of the isolates, it may be seen that the most favourable conditions for the growth of isolates 1 and 3 were on potato dextrose agar at 18°C. However, at 18°C which was the optimum temperature for the growth of isolate 2, growth was better on malt agar.

Considering Graphs 1 to 6 it is evident that all the isolates made an appreciable growth at 18°C and 23°C, while temperatures of 0°C and 26°C tended to have an adverse effect. In the case of isolate 1, growth on malt agar was better than on potato dextrose agar at 26°C, while at 0°C potato dextrose agar was the more favourable medium. Isolate 2 showed very little growth on either medium at 0°C but at 26°C on potato dextrose agar a measurable amount of growth occurred. At 26°C growth of isolate 2 on malt agar was more or less inhibited. Evidently, neither medium was suitable for the growth of isolate 3 at 26°C, but at 0°C growth was possible. This isolate showed better growth on potato dextrose agar at 0°C.

Excepting isolate 2, growth was generally better on potato dextrose agar at all the experimental temperatures. With isolate 2 malt agar was the more favourable medium for growth at lower temperatures, but at higher temperatures better growth occurred on potato dextrose agar.

It may be concluded that the three isolates were quite distinct from one another in their growth rates on malt and potato dextrose agars. Moreover since the growth rate of the same isolate can vary on different artificial media, then there is every possibility that it will vary on tubers of different potato varieties.

In all cases, sub-cultures taken from the experimental cultures were able to grow, but transfers from the cultures kept at 26°C were very much slower in producing cultures equivalent to those produced normally. Sub-cultures from the isolates kept at 0°C showed growth above the average.

Studies in the Change in pH of the Agar Surrounding the Fungal Colony.

An estimation was made of the change in pH of the agar in the region of advancing hyphae, in order to determine 1) if the three isolates differed in the amount of change they cause in the medium, and 2) if growth rates and pH change could be correlated.

Method.

Twenty nine plates of potato dextrose agar were prepared and nine inoculated with isolate 1, nine with isolate 2, and nine with isolate 3. Discs of inoculum, similar to those used for the temperature studies, were used as inoculum. Of the two plates of medium which remained, one was used to find the initial pH of the agar and the other to determine the pH at the end of the experimental period. All the plates were stored at a temperature of 18°C to 20°C, and four days allowed to elapse before the first estimation of pH was made.

The indicators used were B.D.H. Universal and Capillator Indicators.

An area of agar, about 5mm. wide, was removed

from the region immediately surrounding the colony. This agar was transferred to a watch glass, and warmed until it melted. A small quantity was then added to a drop of the Universal Indicator, and a rough measure of the pH obtained. Equal quantities of the melted medium and the appropriate Capillator Indicator for the pH indicated, were then mixed and drawn into a capillary pipette. By comparing the colour of the mixture in the pipette with the indicator's buffer tubes, a more accurate determination of the pH was obtained.

Estimation of the pH of the agar was made at intervals of two days for the first nine days of the experiment. Afterwards the estimations were recorded daily until the experiment was discontinued on the fourteenth day.

Results.

The pH estimations made during the experiment are shown on Table II.

Isolate	pH Agar	Estimated pH on day indicated								
		1	3	5	7	9	10	11	12	13
1	5.4	5.6	5.6	5.8	6.8	7.1	8.0	7.4	5.4	5.4
2	5.4	5.6	6.4	7.2	7.8	8.8	9.2	8.6	8.4	8.0
3	5.4	5.6	5.8	6.3	6.4	6.6	8.2	8.3	8.5	8.6

The daily difference in pH is represented in Graph 8, and this indicates that each of the isolates had a different effect on the change in pH of its agar substrate in the region of growing hyphae. The experimental data show that there was a change in the medium from an acid to an alkaline condition.

Although the pH estimations were made in the region of advancing hyphae, these hyphae cannot be held entirely responsible for the change, but the whole of the colony requires to be considered. The condition must be considered by taking into account

the relative quantity of agar uncovered by the colony, since this, at first, must have a stabilizing effect on any change caused by the fungus. Therefore, one would expect the increased change which occurred towards the end of the experimental period, because the influence of the fungus would then be dominant.

The changes in pH are considered along with the growth rates of the isolates in Graphs 9, 10 and 11. Isolates 1 and 3 showed similar curves for those two factors, but with isolate 2 there was a higher pH value and a much lower growth rate throughout. After certain pH values with isolates 1 and 3, further rises in the pH of the medium were accompanied by a depression of the growth rate. Evidently, an increase in the pH of the medium above certain values, tends to lower the growth rate of the isolates. However, pH cannot be the only factor influencing growth rate, but changes, due to the metabolism of the colony, also require to be taken into consideration.

Growth on Special Media.

An attempt was made to obtain further differential criteria for the three isolates from their reaction to growth on different media.

Growth on Gelatine.

The isolates were compared with regard to their capacity to liquefy 10% gelatine. The gelatine was prepared as recommended by Dowson (1949), substituting Yeastrel broth for meat-infusion broth. Two parallel tubes which were set as cylinders, were inoculated with each isolate , the level of the gelatine marked, and the cultures incubated at 18°C for thirty days.

In all cases the gelatine was liquefied, showing the presence of a proteolytic enzyme. The gelatine was liquefied to an extent of 19mm. with isolate 1, 21.5mm. with isolate 2 and 14mm. with isolate 3.

Growth on Potato Plugs.

All isolates showed good growth on sterilized potato plugs.

Isolate 1 formed a chocolate brown mycelial mat with a short,pinkish,aerial mycelium except at the upper end where the aerial mycelium was greyish. At the points where the plugs made contact with the tube there were dark brown bands, arising from which were dark hyphae. Among those hyphae,masses of yellow crystals formed. Those crystals were similar to those produced on malt agar. This isolate formed only a few scattered immature pycnidia.

On potato plugs, isolate 2 produced a black mycelial mat with a short,greyish white, aerial mycelium. Where the plug made contact with the tube, a black band formed. The mycelial strain of this fungus failed to produce pycnidia on this substrate whereas many pycnidia were produced by the pycnidial strain. The copious spore exudate was pink in colour. Somewhat less mycelium mat was produced by the pycnidial form, and it showed a tendency to be more brown than black in colour.

Isolate 3 showed a dense, olivaceous black growth of both basal and aerial mycelium which verged into a dark grey at the upper end of the plug. The pycnidia which were formed in masses produced a greyish white spore exudate.

Growth on Media Containing Carbohydrates.

Four series of test tube and Petri dish cultures were made on Czapek agar in which the carbohydrates incorporated were glucose,sucrose, maltose and starch respectively. A fifth series,containing no carbohydrate was used as the usual control. To begin with, 1% of each carbohydrate was used, with the exception of starch which was always incorporated as a 0.5% paste. However, later,the carbohydrates were increased to 3% without making any difference to the

results obtained.

In addition to the three isolates, the pycnidial strain of isolate 2 was also tested.

Medium Incorporating Sucrose. (Pl.X).

Isolate 1: The colony was stunted, pale yellow - orange in colour and had a very undulating surface. The cells of the hyphae showed abnormal swelling and rapid division in several directions; subsequently forming unicellular and multicellular chlamydospores. All the hyphae were finally replaced by these spores.

Isolate 2: Growth was good, forming a brown basal plectenchyme which became pinkish buff at the margin. The aerial hyphae were close, short and greyish white. Abundant, black chlamydospores formed in the basal mat.

Isolate 2: pycnidial strain. This was the same as isolate 1 but russet in colour. Many dark, unicellular and multicellular chlamydospores were formed, but a good proportion of normal hyphae was present. The colony showed a tendency to be stunted.

Isolate 3: This isolate formed a black, stunted, irregular colony with well developed olivaceous black, aerial hyphae. Many dark unicellular and multicellular chlamydospores were formed.

Medium Incorporating Glucose. (Pl.X).

Isolate 1: The colony was stunted and chocolate coloured with a pale yellow-orange margin. Normal hyphae were present, but the aerial hyphae divided into dark multicellular chlamydospores.

Isolate 2: On this medium the mycelium was almost identical with that grown on the sucrose medium. In addition to dark chlamydospores, a few small immature pycnidia were present.

Isolate 2: pycnidial strain. The colony was similar to that of isolate 1 but bone brown in colour. The colony was stunted.

Isolate 3. The mycelium was identical with that grown on the medium containing sucrose.

Medium Incorporating Maltose. (Pl.X).

Isolate 1. The colony was regular in outline, smooth and pale yellow-orange. Most of the cells of the hyphae showed abnormal swelling, and abundant multicellular chlamydospores were formed. The colony was stunted.

Isolate 2. The colony was identical with the colony produced on the glucose-containing medium.

Isolate 2: pycnidial strain. The colony was similar to that grown on the glucose-containing medium but it had a buff coloured margin.

Isolate 3. The colony was identical with that grown on the medium incorporating glucose.

Medium Incorporating Starch.

All isolates showed better growth on the starch-containing medium than with the other carbohydrates. The cells of the hyphae were normal, but development of aerial mycelium was suppressed, except with isolate 3. In all cases the mycelial mat was white with zones of buff. However, with isolate 3 the buff colour was dominant.

The pycnidial strain of isolate 2 was the only isolate which formed mature pycnidia on this medium. Indeed, mature pycnidia did not form with any of the isolates on any of the other media containing carbohydrates.

Utilization of Starch.

After the isolates had grown for six weeks on the starch-containing medium, a uniform blue-black colour was produced when the iodine test for starch was applied to the medium. This indicated that no amylase was produced by the isolates, and that they do not utilize starch.

Growth on the medium containing no carbohydrate was the same as that on the medium containing starch.

Inoculation of Different Host Plants.

In addition to growth on artificial media, inoculations were also made on potato tubers, potato haulms, tomato stems and Swede turnip roots.

All three isolates proved to be parasitic on potato tubers, forming a gangrene type of lesion. These inoculations will be considered in more detail in a later section.

Healthy potato haulms were inoculated by spraying with spore suspensions of each of the isolates. Since isolate 1 did not produce many pycnidia, another pycnidial form of the isolate was used to obtain the suspension. One dozen potato plants were inoculated with each isolate and kept under observation during the full growing season.

Isolates 2 and 3 were both recovered from the pycnidia which formed on the dead haulms of the plants inoculated with these isolates. Neither isolate proved to be parasitic on the living haulms. Isolate 1 was also recovered from dead haulms but in one instance it was found girdling the living stem of an inoculated Arran Banner plant. Since isolate 1 was the only organism ever to be recovered from this lesion, it seemed that this fungus was the parasite responsible forits formation. (Pl. V, fig.3).

An identical set of inoculations was made with the pycnidial strain of isolate 2. These did not cause any lesions on the living plants, but later the isolate was recovered from pycnidia on the dead haulms. It is interesting to note that only the pycnidial strain was reisolated from those dead haulms, but on two occasions it was recovered from plants inoculated with the mycelial strain.

Inoculations on the stems of tomato plants (variety, Melville Castle) were made with the three isolates. In each case a minute V-shaped cut was made on the lower part of the stem and a piece of

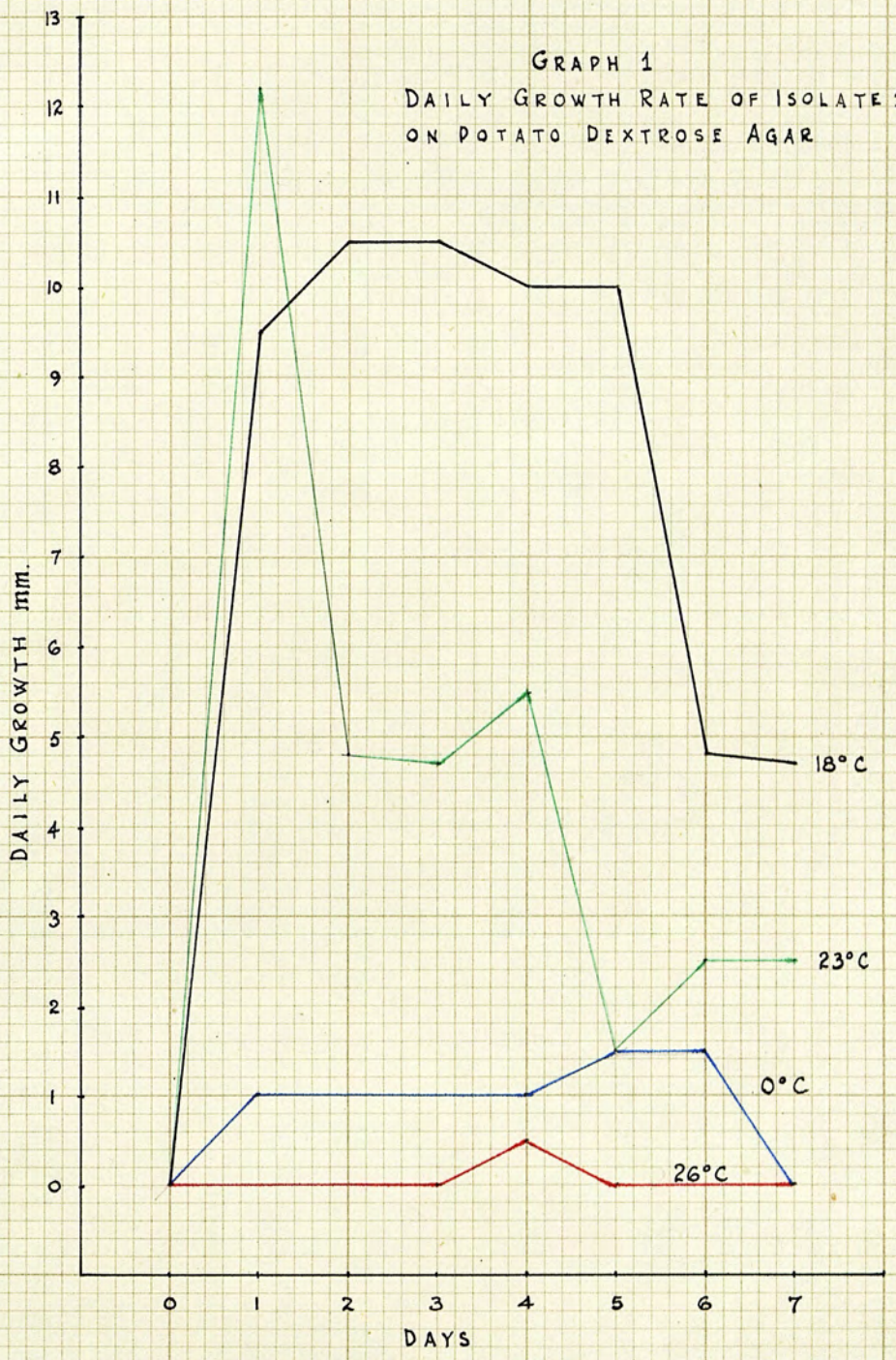
mycelial inoculum inserted under the flap of tissue. The area was then covered with moist, sterile cotton wool, and bound with adhesive cellophane. Two plants were inoculated with each isolate.

Within three weeks isolate 1 formed extensive lesions which more or less encircled the stems. Isolate 2 also produced large lesions but in places the fungus was restricted from further growth by the formation of a wound cambium. Isolate 3 formed very small lesions which were encircled by a wound cambium. Pycnidia were not produced on any of the lesions but cultures, identical with the original inoculum, were obtained from the advancing edges of the rots.

Isolate 1 produced small lesions of about half an inch in depth and diameter when inoculated on Swede turnip roots. The pycnidial strain of isolate 2 penetrated for a quarter of an inch into the Swede turnip roots but the mycelial type of this fungus was not found to be pathogenic. Isolate 3 did not form any lesion. Cultures, identical with the original inoculum were recovered in each case.

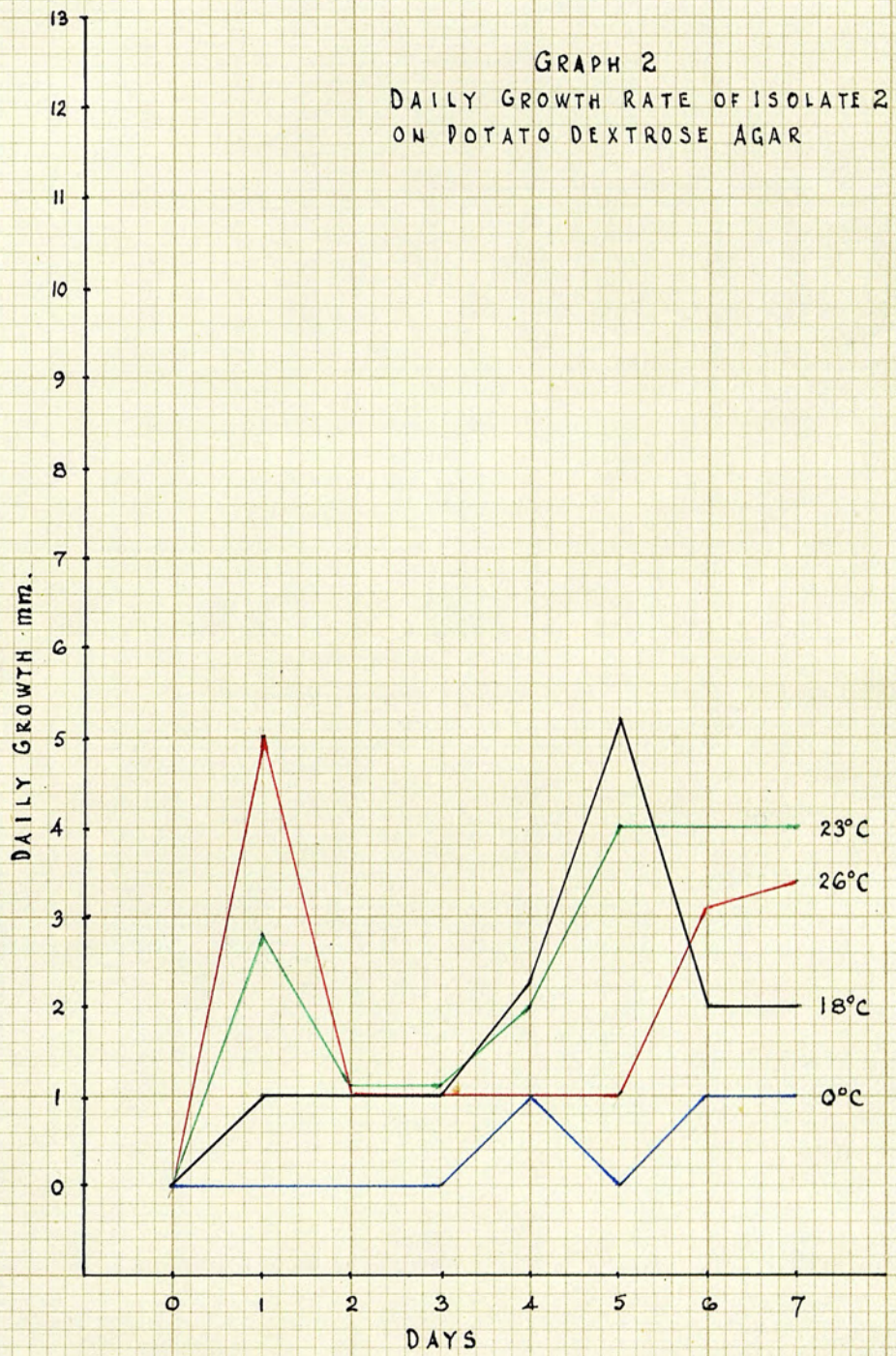
GRAPH 1

DAILY GROWTH RATE OF ISOLATE 1 ON POTATO DEXTROSE AGAR



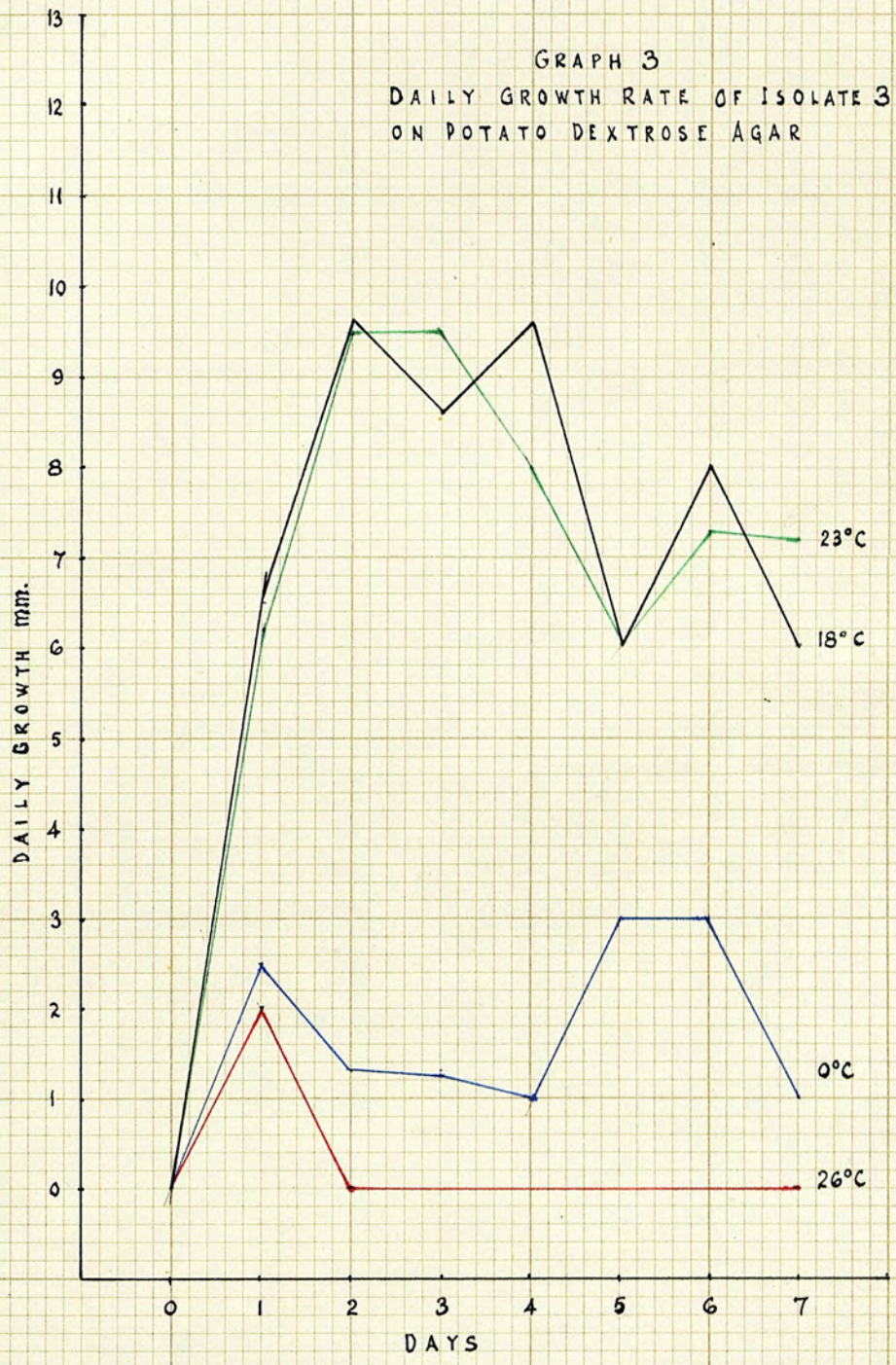
GRAPH 2

DAILY GROWTH RATE OF ISOLATE 2 ON POTATO DEXTROSE AGAR



GRAPH 3

DAILY GROWTH RATE OF ISOLATE 3
ON POTATO DEXTROSE AGAR



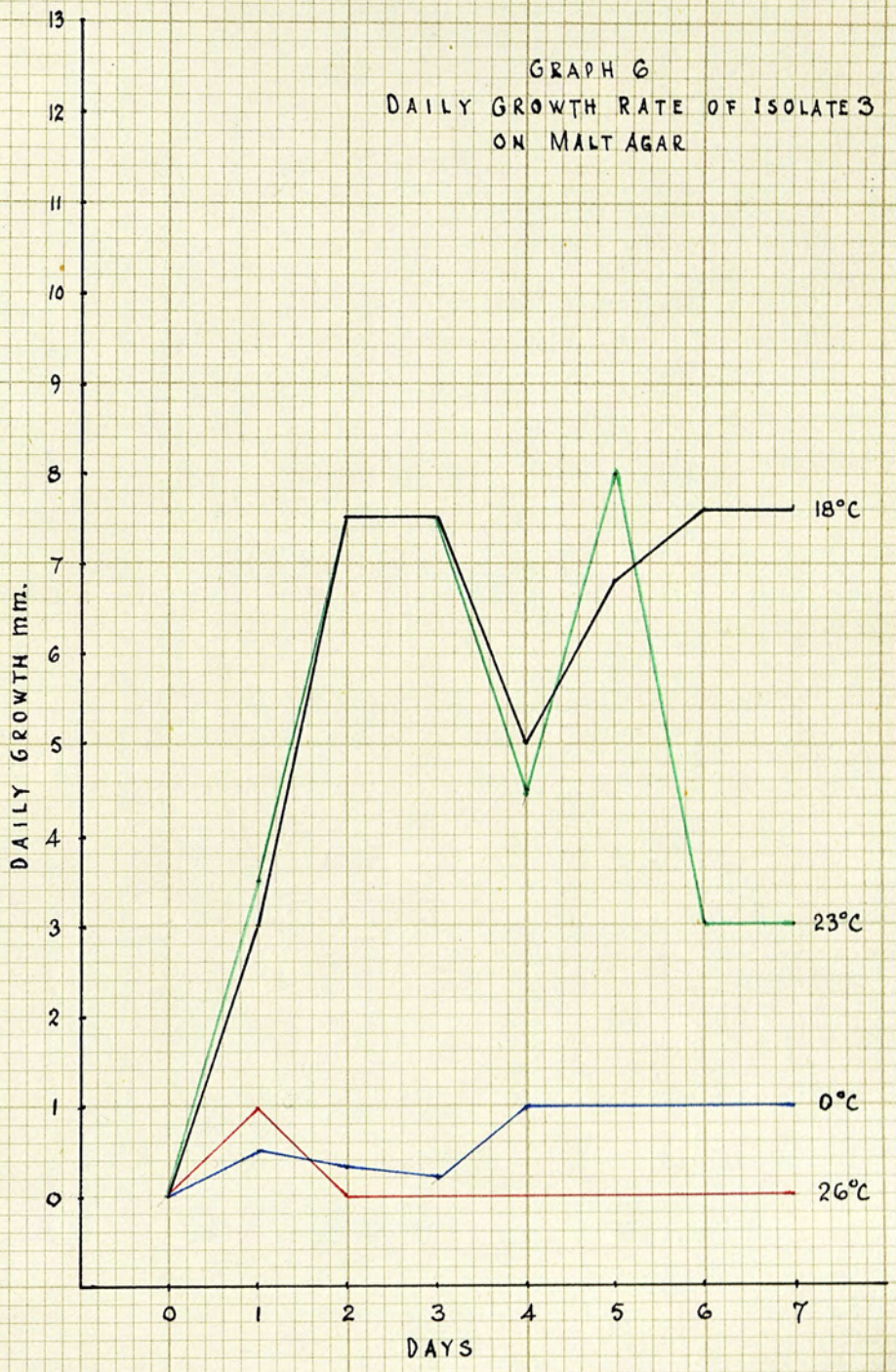
GRAPH 4

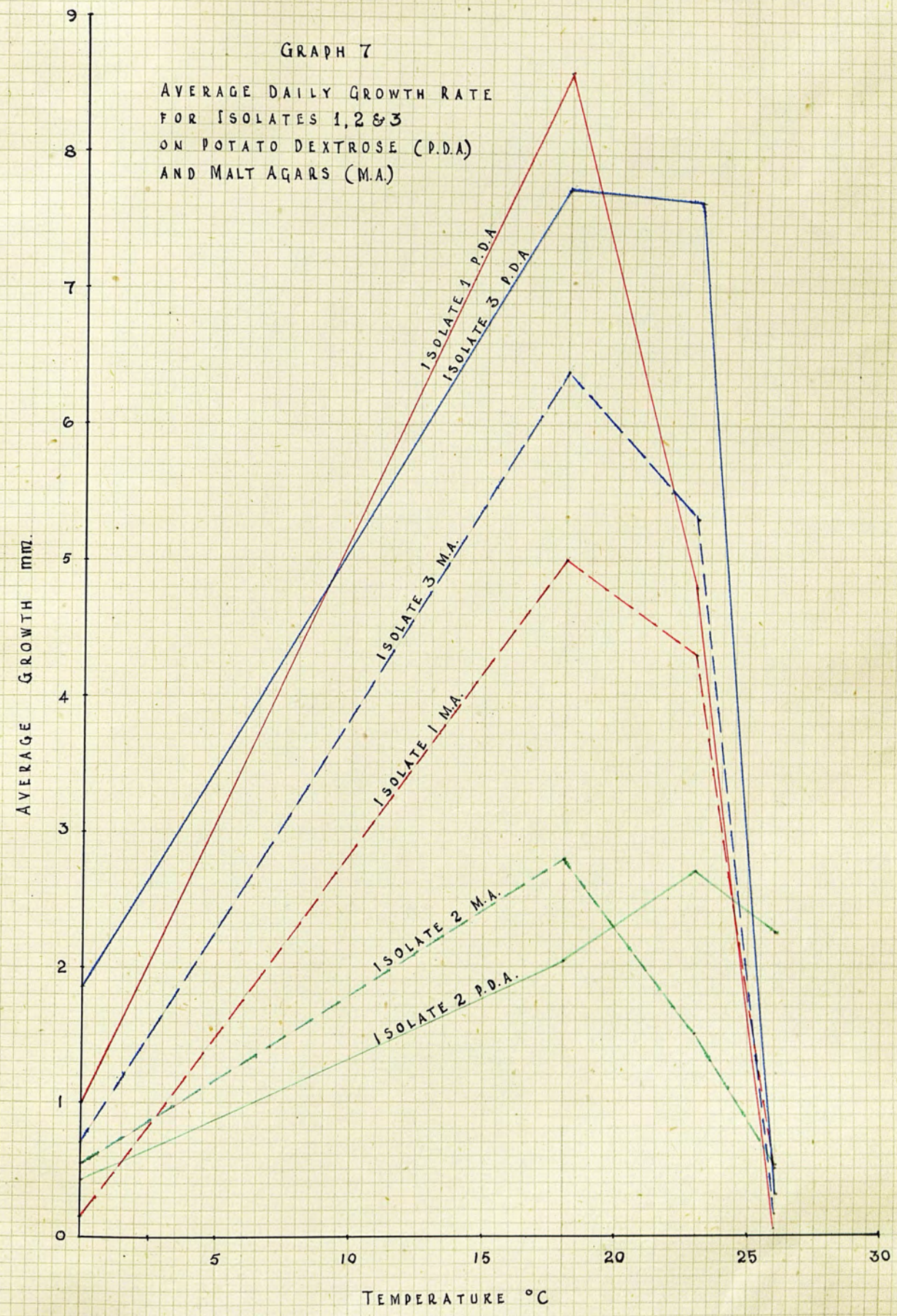
DAILY GROWTH RATE OF ISOLATE 1 ON MALT AGAR

GRAPH 5
DAILY GROWTH RATE OF ISOLATE 2
ON MALT AGAR

GRAPH 6

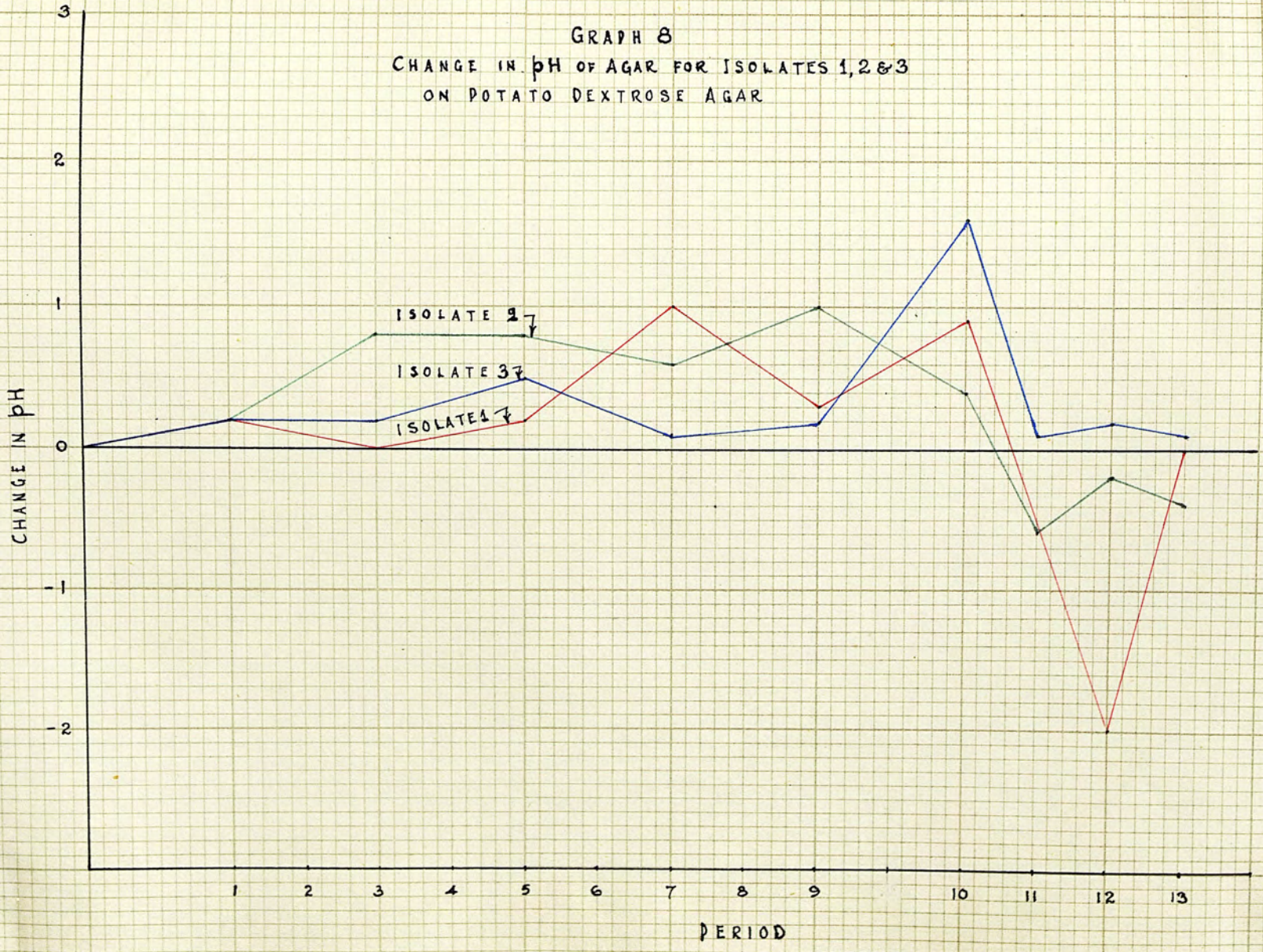
DAILY GROWTH RATE OF ISOLATE 3 ON MALT AGAR




GRAPH 7
AVERAGE DAILY GROWTH RATE FOR ISOLATES 1, 2 & 3 ON POTATO DEXTROSE (P.D.A.) AND MALT AGARS (M.A.)
ISOLATE 1 P.D.A.
ISOLATE 3 P.D.A.
ISOLATE 3 M.A.
ISOLATE 1 M.A.
ISOLATE 2 M.A.
ISOLATE 2 P.D.A.
AVERAGE GROWTH mm.
0
1
2
3
4
5
6
7
8
9
5
10
15
20
25
30
TEMPERATURE °C

GRAPH 8

CHANGE IN pH OF AGAR FOR ISOLATES 1, 2 & 3
ON POTATO DEXTROSE AGAR

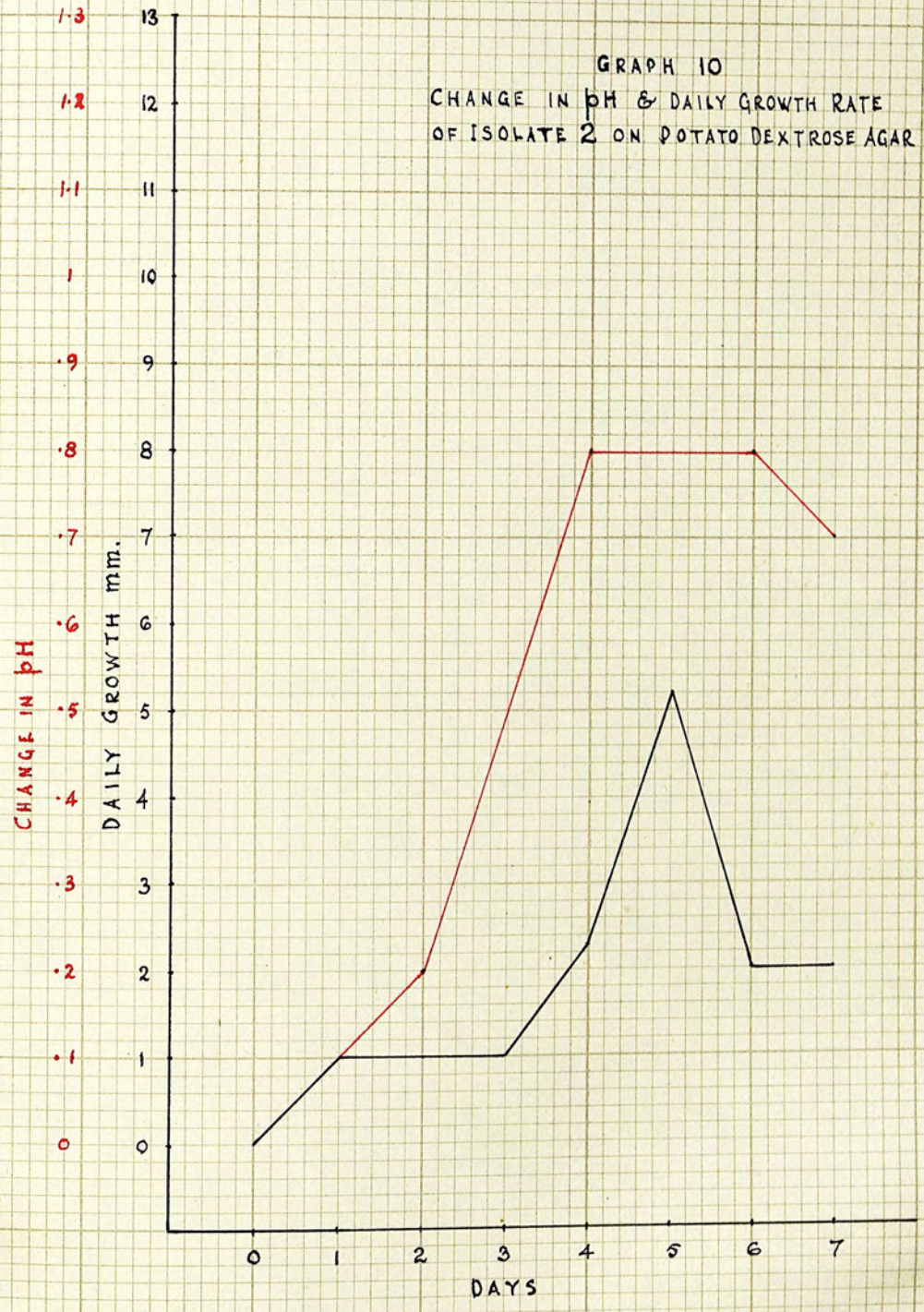


GRAPH 9

CHANGE IN pH & DAILY GROWTH RATE OF ISOLATE 1 ON POTATO DEXTROSE AGAR

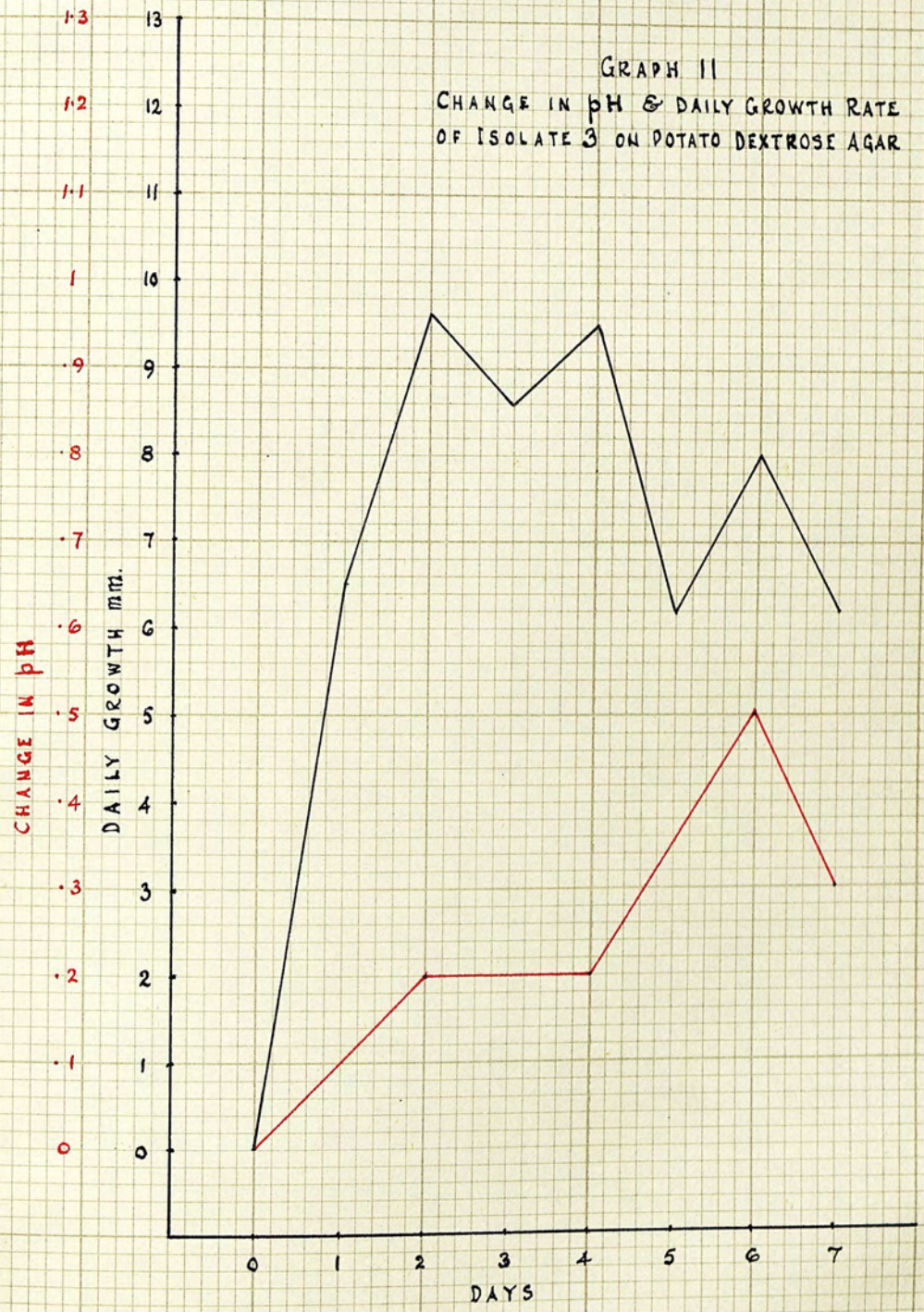
GRAPH 10

CHANGE IN pH & DAILY GROWTH RATE OF ISOLATE 2 ON POTATO DEXTROSE AGAR



GRAPH II

CHANGE IN pH & DAILY GROWTH RATE OF ISOLATE 3 ON POTATO DEXTROSE AGAR



CONCLUSIONS AND COMMENTS.

Experimental evidence shows that the Phoma isolates used in the investigation were distinct in growth rate, action on the pH of their substrate, reaction to different artificial media and in pathogenicity to different host plants. However, the extent to which this evidence would support their separation into species requires further investigation

Isolate 1 compared favourably with the description of Phoma foveata Foister, and comparison with a culture accepted as P. foveata by Foister, confirmed its identity.

Isolate 2 is probably Phoma solanicola Prill. and Del. sensu Kohler which, as stated previously, is considered to be the same as Phoma tuberosa Melhus, Rosenbaum and Schultz. Authentic material of neither fungus was available for comparison, but the descriptions and material used by other workers compared favourably with this isolate.

Isolate 3 is undoubtedly Phoma eupyrena Sacc.. Authentic material of this fungus was not available, but comparison with herbarium material of P. eupyrena at the Commonwealth Mycological Institute, established its identity.

Monospore cultures of isolates 1 and 2 segregated into two types, the one mostly producing mycelium and the other forming abundant pycnidia with less mycelium. This is an instance of the 'Dual Phenomenon' as observed by Hansen (1938), the former type representing Hansen's "M" type and the latter his "C" type. Both types remained constant over several series of transfers, and the M type showed more vigorous growth. Multispore isolations which contain a mixture of M and C types, showed an increasing tendency to form fewer pycnidia when kept in artificial culture. Owing to the greater growth rate of the M type it is possible that it tends to be dominant in multispore

isolations; consequently the M type has an increased chance of being transferred during sub-culturing. In this way, the more often transfers are made, the sooner can the capacity to produce pycnidia be lost. Inoculation experiments show that both the M and C types are capable of retaining their identity when grown on living plants and that they differ in pathogenicity. Since they behave as separate entities under experimental conditions, there is every reason to believe that they do likewise in nature. This variability in types found within the species must necessarily be taken into account in their classification.

As previously discussed the taxonomic position of the Sphaeropsidales is entirely unsatisfactory. The system of nomenclature in most general use is that proposed by Grove (1935) where the species are identified according to their morphological characters and host species. However this method does not account for such species as Phoma hibernica Grimes et al, which is only known as a saprophyte, divorced from any host plant. Grove's system is convenient for the majority of species.

On the basis of Grove's classification it is difficult to find justification for separating the species, Phoma foveata and Phoma solanicola. Both fungi are parasitic on potato tubers and cause the same type of rot. In its original description, P. solanicola was described as a parasite on living potato stems, but in the present investigation this was found to be true of P. foveata also. (Pl.V, fig.3).

The spore dimensions given by different authors for P. foveata, P. tuberosa and P. solanicola are summarised in Table III. It can be seen that the spore dimensions cited for each species show great variation within similar ranges; therefore they cannot be of much value in determining the species.

In shape and colour in the mass the spores are similar.

Identification and Authority	Spore measurements	Substr-atum.
P. foveata		
Foister	5.7x1.7µ(3.2-7.7x1.1-2.1)µ	tuber
Dennis	6.6x2.9µ(4.8-8.2x2.8-3.4)µ	"
"	7.5x2.9µ(5.6-9.3x3.6-4.8)µ	malt agar
Isolate 1	7.1x3.1µ(4.3-8.6x2.0-4.0)µ	tuber
" (type)	6.2x2.5µ(5.0-7.3x1.7-4.7)µ	malt agar
P. solanicola		
Prillieux et al	7.5x3.0µ	haulm
Kohler	5.9x2.8µ(unextruded).	"
"	8.6x3.5µ(extruded).	"
Grove	6.0x2.5µ(4-7 x 2-3)µ	"
Dennis	8.0x3.4µ(6.7-9.0x2.8-4.0)µ	"
"	7.9x3.2µ(6.4-10.1x2.8-3.9)µ	malt agar
Isolate 2	6.5x3.0µ(5.3-7.7x2.3-3.7)µ	" "
P. tuberosa		
Melhus et al	(3.7-6.1x1.8-3.7)µ	tuber
Dennis		
C type	7.0x3.5µ(4.8-8.7x2.8-4.5)µ	malt agar
M type	7.0x3.6µ(5.0-10.1x3.1-5.0)µ	"" "

Table III.

The ostiolate pycnidia produced by both fungi on inoculated potato stems are identical (Pl.V,fig.1). On potato tubers the pycnidia of both species are similar and ostioles infrequent. The sub-epidermal pycnidia on potatoes are similar and sometimes situated on small cushion-like stromata but on haulms ther is no stroma.

With regard to the taxonomic value of stromata, it is interesting to note that in the original description of P. tuberosa (Melhus, Rosenbaum and Schultz, 1916) it was stated that a distinct stroma was absent. On the other hand, the fungus described by Alcock and Foister (1936) and later described as P.foveata (Foister, 1940), had pycnidia set in a stroma. However, the pycnidia of P. foveata as figured by Dennis (1946) show no sign of a stroma, but

his strain 2 (representing P. tuberosa) has its pycnidia situated on a stroma. It seems that the presence or absence of a stroma is a variable factor in those fungi, and consequently, is of little diagnostic value.

In using the above mentioned criteria it is evident that no satisfactory distinction can be made between P. foveata and P. solanicola. The use of additional criteria, based on cultural and physiological properties, may now be considered to estimate their value in the identification of these species.

To judge from the observations made on isolates 1 and2, the growth rates and effect on pH of the medium is quite distinct for both isolates. From general observations, however, it seems that different isolations of the same fungus may vary in growth rate and, as observed in isolate 2, the M and C strains can differ in rate of growth. Dennis (1946) found that even the same isolate at different transfers varied considerably in growth rate. Therefore, comparison of growth rates is of little assistance in the identification of the species.

Information from the growth of the fungi when grown on media containing different carbohydrates is as unsatisfactory as other methods in providing differential criteria, because the isolates tend to give similar reactions. Indeed, on these media alone, there would seem to be more justification for separating the M and C types of isolate 2 than separating the species.

As recommended by Grimes et al. (1932) the capacity of the isolates for liquefying 10% gelatine was tested. Grimes et al. used this as a criterion for the initial grouping of their Phoma species and Dennis (1946) considered this to be a character which could be used with some confidence as a

differential criterion. In this respect it is interesting to note that the isolate of P. foveata used by Dennis liquefied 21mm. of gelatine in 30days while his strains 2 and 3, interpreted as P. tuberosa and P. solanicola respectively, liquefied gelatine to an extent of 21mm. and 19mm. respectively. These figures compare favourably with the results obtained for isolates 1 and 2 but they do not differ sufficiently from each other to be used as specific characters.

The results obtained from inoculations on different host plants are the same for isolates 1 and 2, and do not differ from Dennis' results for the corresponding strains. Certainly, isolate 2 is somewhat less virulent in each case, but the M and C types of this isolate differ in pathogenicity, so that one cannot reasonably expect isolates 1 and 2 to give exactly the same result.

As a rule, the macroscopic appearance of the isolates in culture is quite distinct. The main difference is that isolate 1 produces a red pigment and yellow crystals which colour the colony. Occasionally, transfers of isolate 1 fail to produce pigment and crystals and such colonies are similar to those of isolate 2. Isolate 2 also varies, but it has never been observed to form pigment or crystals like isolate 1. Although the two isolates are usually distinct in appearance, in view of their tendency to vary, the macroscopic appearance of the colony cannot be used with certainty to separate the species. The appearance of most Phoma species in culture is variable, and generally, this is regarded as too unstable a factor to be of value as a criterion in taxonomy.

Taking into account the evidence available, and comparing it with the results of other workers, it is difficult to find justification for separating

the species P. foveata and P. solanicola. In all probability they are no more than strains of one fungus.

The extensive production of chlamydospores is a character which readily distinguishes isolate 3 from the other two isolates. On media containing glucose, maltose and sucrose all the isolates formed abundant muriform chlamydospores (PLX), which are quite distinct from the type produced on malt agar. The production of those chlamydospores is generally associated with stunted colonies. Stunting of the colonies is apparently correlated with the presence of soluble carbohydrates since it was not observed in the colonies on the medium incorporating starch, or on the control cultures.

In the hyphae which give rise to chlamydospores, certain cells enlarge and by the formation of septa, both lengthways and crossways, muriform spores result. In old cultures, the chlamydospores, which separate easily on contact, are so abundant that normal hyphae are almost excluded. When placed in water the spores germinate by one or several germ tubes.

A similar type of agamic multiplication has been recorded by Goidanich et al (1948) in the closely alied genus, Deuterophoma. In the species D. trachei-phila Petri, irregularly shaped mono- and bi-cellular conidia have been observed both in culture and in the host. In culture, the conidia have been observed especially in stunted colonies. Moreover, in the host, the conidia were observed particularly in the xylem, which according to Adams (1923), frequently contains considerable quantities of sugars. Can it be that the appearance of similar characters in Phoma and Deuterophoma is due to the presence of sugars? Since in both cases the chlamydospores were associated with stunted colonies it seems that they are the response to an unfavourable environment.

In a consideration of different spore forms, Mason (1933) includes the little understood genus, Coniothecium. The example cited, Coniothecium scabrum McAlp. produces chains of unseparated dictyothallospores which, as illustrated, are formed in exactly the same way as the spores observed in the stunted Phoma colonies. The figures accompanying the original description of C. scabrum (McAlpine, 1899) show its spores to be exactly the same as those of the Phoma isolates. In addition, Mason states that any one of the C. scabrum spores can become a Phoma pycnidium by further enlargement and septation.

The case of Coniothecium is similar to that of Phoma alternariacearum Brooks and Searle which produces characteristic multicellular chlamydospores. Grove (1935) considers these spores to be an immature form of Ascochyta Lycopersici Brun. and Wollenweber and Hochapfel (1936) list the Phoma as synonymous with Phoma glomerata (Corda) Wr. & Hochl.. Bisby (1939) in mentioning those two Phoma species as synonymous gives C. scabrum as a synonym also. Observations made on P. alternariacearum during the present investigation certainly supports the view that the chlamydospores can, by further division and septation, form pycnidia (Pl. X).

From the point of view of taxonomy it seems that by growing isolates 1, 2 and 3 on certain media it is possible to place them in two different genera of families belonging to different orders. Similarly, at different stages of growth, C. scabrum can be placed in the same two genera. If such a situation can arise so readily in artificial culture then it is possible that it may occur in nature also, especially if the fungi meet with unfavourable circumstances. Such a situation can only add further complication to the taxonomy of an already confused genus.

THE ANTAGONISM OF TRICHODERMA VIRIDE AND ACROSTALAGMUS CINNABARINUS.

On one occasion it was observed that a plate culture of isolate 1 was contaminated with a species of Trichoderma. The Phoma hyphae had ceased growth, but the Trichoderma continued to grow and fructify. Examination under the microscope showed that the Phoma had degenerated, even before the Trichoderma hyphae were in contact with the colony. These observations resembled the parasitic action of Trichoderma lignorum (Tode) Harz recorded by Weindling (1932) on soil fungi, and further investigation proved this to be a similar case.

By means of monospore isolations, the Trichoderma contaminant was brought into pure culture and identified as Trichoderma viride Pers. ex Fries.

On potato dextrose agar a sparse, whitish felt of septate vegetative hyphae was formed. Conidiophores and sterile hyphae arose in tufts which were generally white at first but later became green. Spore-bearing phialides arose from the conidiophores at various positions, and frequently in whorls of three. Heads of about 10 to 15 spores, held together by slime, surmounted the phialides. The spores which measured 2.3 to 3.7u in diameter were globoid, green and smooth.

Plates of potato dextrose agar were inoculated simultaneously at different points with isolate 1 and Trichoderma viride. The Trichoderma formed normal cultures but the Phoma colonies extended to an average of two centimetres in diameter. After four days, examination under the microscope showed that the Phoma hyphae were very distorted and contained a dark yellow, granular substance. Surrounding the hyphae were yellow needle-like crystals, similar to those formed in pure cultures. Sheaves of yellow crystals were also formed, especially at points where

the hyphae appeared to have burst open. (Pl. V, fig.4).

To acertain whether the abnormal appearance of the Phoma was due to an unsuccessful competition with the Trichoderma for food, or to an anti-fungal effect of Trichoderma, a series of cultures was set up in which the Phoma was allowed to grow for three days before inoculation with Trichoderma. The Phoma showed normal growth until 24 to 48 hours after the Trichoderma was introduced. After this period, the Phoma made little further growth, the hyphae became distorted and crystals formed. Since this occurred rapidly in all the hyphae long before the Trichoderma hyphae were in contact with the colony, it seemed to indicate the presence of a toxic substance diffusing from the Trichoderma.

To estimate the effect of the toxic substance, plate cultures of Trichoderma viride were inoculated with isolate 1 at daily intervals for two weeks. The inoculations made up to the fifth day made no growth; yet in those prepared in the second week of the experiment, the Phoma made considerable progress. This shows that the active principle of the Trichoderma is not constantly produced and does not have an accumulative effect in the medium.

In order to study the reaction of the Phoma hyphae when in contact with those of the Trichoderma, slide cultures were prepared (Noble 1937), the two fungi being inoculated simultaneously at opposite ends of the slide. Under the microscope it was possible to observe that when the hyphae of opposed colonies made contact, growth of the Phoma hyphae stopped almost abruptly while the Trichoderma hyphae advanced into the Phoma colony. Any Trichoderma hypha which made contact with that of the Phoma was observed to react in either of two ways. When the hyphae in contact were submerged, or aerial, the Trichoderma hyphae coiled tightly round the Phoma as it advanced.

(Pl.VII,fig. 1). However, when the hyphae made contact on the surface of the agar the Trichoderma was observed to grow in close contact with the Phoma and proliferate over its surface.

The cells of the Phoma became filled with dark yellow, granular contents, similar to those described above. Later this granular substance disappeared concurrently with the appearance of yellow needle-like crystals in the medium immediately surrounding the hyphae.

Direct attack of the Phoma by Trichoderma was also observed to be independent of any culture medium through which diffusion of toxic substances could take place. This was observed by placing two drops of potato dextrose agar at opposite end of the cover slip which formed part of a van Tieghem cell. The drops were inoculated with Phoma and Trichoderma respectively, and incubated at 18°C until the hyphae had grown out on to the glass. When opposing hyphae made contact, the Trichoderma hyphae were observed to coil round those of the Phoma which then stopped growth and reacted similarly to those described above (Pl. VII,fig.2).

It was observed that growth of the Trichoderma was much enhanced in the region of the Phoma, suggesting strongly that nutrient materials were obtained from the host hyphae.

Antagonism between fungi has been recorded on several occasions and the antagonistic action of Trichoderma lignorum (Tode) Harz (= T. viride Pers. ex Fries) has been studied considerably by Weindling (1932, 1934, 1937 and 1941). Undoubtedly the above mentioned antagonism between T. viride and isolate 1 is similar to that observed by Weindling on other fungi.

The antagonism of T. viride is known to be induced by toxic substances. A crystalline toxic substance was first isolated from T. lignorum by

Weindling and Emerson (1936) and this was subsequently named gliotoxin (Weindling, 1941). This substance was studied further by Brian (1944) and Brian and Hemming (1945). Another antibiotic substance, viridin, has also been obtained from certain strains of T. viride (Brian and McGowan, 1945) and some of its biological activity and chemical and physical properties investigated (Brian et al, 1946; Vischer et al, 1950).

Weindling (1934) classified the strains of Trichoderma lignorum (= T. viride) according to the following scheme:

O series, having a coconut-like odour; producing no pigment.

P series, having no odour, producing a bright yellow pigment on certain media.

Q series, having no odour and no pigment.

In the light of Bisby's observations on Trichoderma (1939) it is doubtful whether the presence or absence of odour is significant in distinguishing the O and Q series. However, as indicated by Brian et al (1946) the viridin producing strains seem to fall into Weindling's P series while those producing gliotoxin appear to belong to the O or Q series.

Since the Trichoderma strains used in the present investigation produced pigment when grown on Czapek-Dox agar it belongs to Weindling's P series. Therefore, in all probability the antibiotic substance involved in the attack on isolate 1 was viridin.

The antagonism observed during the present investigation closely resembled the parasitism of T. lignorum on Rhizoctonia solani Kuhn, described by Weindling (1932). In both cases inhibition and death of the host hyphae is brought about in the same two ways: a) by close contact and coiling of the parasite round the host hyphae; b) by a long range attack without hyphal contact.

Weindling associated the formation of yellow

needle-like crystals and sheaves of crystals with the parasitism of Trichoderma. Similar crystals were observed when isolate 1 was attacked, but the same type of crystals were also formed in old, pure cultures of isolate 1. Therefore, in this case, crystal formation is not merely the result of parasitism. The crystals were formed almost as soon as parasitism commenced, but in pure cultures they were formed in association with old hyphae. This seems to suggest that they appear soon in parasitised cultures due to the earlier degeneration of the host hyphae caused by the parasite.

The effect of Trichoderma on isolates 2 and 3 was also tested. The killing of the host hyphae in advance of the parasite was not observed on either isolate. However, direct hyphal attack on both isolates did occur. With these isolates, growth did not cease until the Trichoderma colony made contact with the Phoma.

Owing to diffusion processes, the toxic substance produced by the Trichoderma cannot be so concentrated at a distance from the colony. It is possible that to be effective with isolates 2 and 3 the toxic substance must be at a higher concentration than is necessary in the case of isolate 1. Thus dilution by the medium renders the substance ineffective with those isolates. On the other hand, the high concentration produced around the coiling hyphae is sufficient to induce parasitism.

The effect of the active principle may also be considered from another angle, assuming viridin to be the toxin involved. Decomposition of viridin in aqueous solutions at or about neutrality is known to occur (Brian et al, 1946). In the pH changes in the medium described in an earlier section, it was found that in the medium in which isolates 2 and 3 were grown, the change towards neutrality was brought

about more rapidly than with isolate 1. Therefore, it is possible that the viridin is decomposed by the higher pH in the medium growing isolates 1 and 2 and is not available to influence their hyphae. Aerial attack is possible since it is active without the intervention of any medium which might affect the toxin. Therefore in the case of isolates 2 and 3 the effect of Trichoderma on the host hyphae is more localized than with isolate 1.

External parasitic attack was most prevalent on the Phoma isolates but internal parasitism was observed in the case of isolate 2 (Pl. VII, fig.3). Similar internal parasitism was observed by Weindling (1932) on a strain of Rhizoctonia solani parasitised by T. lignorum. As is seen in the illustration, penetration of the isolate 2 hypha was by mechanical pressure and the inpushing of the host cell wall, prior to actual penetration, can be observed.

Occasionally some cells of the Phoma isolates survived the attack by the Trichoderma, and once the effect of the parasite diminished, those cells grew and re-established the colony. Usually growth was resumed intrahyphally and where the new hypha penetrated the wall of the dead hypha, some of the yellow, granular contents of the old hypha were released into the medium (Pl.VI,figs.2 and 3). Intrahyphal growths were never observed deep in the agar but always occurred on the surface of the medium.

That growth should invariably be resumed intrahyphally was somewhat striking. A possible explanation is that within the parasitised hypha metabolic changes rapidly deactivate the very unstable Trichoderma toxins while within the surrounding medium, the toxins may still be active. Thus the dead hypha forms a favourable environment for growth within an unfavourable medium.

Trichoderma viride was frequently found within the lesions of Gangrene infected potato tubers.

An attempt was made to investigate any association between Phoma and Trichoderma on this substrate.

The effect of T. viride on potato tubers was tested by spraying half tubers with a concentrated spore suspension of the fungus and storing them in boxes limed with moist peat. Of the twenty tubers inoculated in this way all were quite healthy when examined one month later; therefore, under the conditions of the experiment T. viride was not parasitic on potato tubers.

Similar inoculations were made on tubers of the variety, Catriona, using isolates 1 and 2 as inocula. In each case, after 1 month, the whole of the cut surfaces of the tubers were found to be invaded by the isolates to an extent of 6mm.

A further series of similar inoculations was made in which mixed spore suspensions of the isolates and Trichoderma were used. With both isolates in mixed culture, the depth of rot produced was, on an average, three times more than with either fungus alone.

The results of the above inoculations show that on healthy potato tubers, T. viride does not have the same inhibitory effect on isolates 1 and 2 as in artificial culture media. Thus, the possibility of using T. viride as a means of controlling Gangrene is not likely; indeed, the results suggest the reverse to be the case.

Weindling (1932) suggested the control of certain pathogenic soil organisms by inoculation of the soil with T. lignorum. The fungicidal control of plant infections by gliotoxin has also been considered (Brian and Hemming, 1945) and has shown the substance to be inferior to other fungicides due particularly to its instability in aqueous solutions at different pH values. Since viridin is known to be equally, if not more, unstable its fungicidal value cannot be high. Therefore it seems

that T. viride or its fungistatic products can provide a suitable control for Gangrene on potatoes. Apart from considering the effect of the tuber, in treating potatoes with such a fungicide, one factor which would require particular consideration would be the soil on which the potatoes were grown. Substances coming into solution from adhering soil might alter the pH or other factors in the solution and so render the fungicide inactive. Thus the fungicide may be successful in treating samples from acid soils but ineffective with those from alkaline soils.

Examination of naturally and artificially infected tubers on which the Phoma and Trichoderma isolates were present together, showed that the starch grains in the tubers were corroded, indicating hydrolysis. Tubers in which the Phoma was growing alone showed only intact starch grains. Therefore, it seems that in the case of the former, Trichoderma was the agent responsible for starch hydrolysis. This was confirmed by growing Trichoderma on an inorganic medium to which a 0.5% starch paste was added. After growth on this medium for three weeks the iodine test for starch gave a negative result. With the Phoma isolates the same test gave a positive result.

With the Phoma isolates and Trichoderma the ability to produce a pectic enzyme was tested in the following manner: the fungi were allowed to grow for three weeks in tubes of Yeastrel broth, then the mycelial growth was separated from the medium. Microtomed discs of potato tissue of uniform dimensions were then completely immersed in the medium and a few drops of toluene added as an antiseptic. Control tubes of Yeastrel broth in which no fungus was grown, were treated in the same way. With the Phoma isolates, complete disintegration of the potato tissue occurred after four days incubation

at 23°C. In the case of Trichoderma the coherence of the potato tissue was not lost, even after 10 days, and the tissue was as intact as that in the control tubes. It was concluded that the Phoma isolates produced a pectinase but the Trichoderma did not.

Based on the above observations, the following is presented as a possible explanation for deeper rots being induced by mixed inocula. Disorganization of host tissue is induced by the solution of certain cell wall constituents, mainly pectic in nature. This is facilitated usually by pectic enzymes, but with a few fungi other substances are involved (Brown, Brooks and Bawden, 1948). since T. viride does not produce a pectic enzyme, it cannot produce a rot on potato tissue by this means. However, the Phoma isolates were found to produce a pectic enzyme which must enable them to rot the tubers, providing a path of entry for the Trichoderma at the same time. Within the rot, starch is hydrolised by Trichoderma and not Phoma. Sugars released from the starch are available to both fungi and this supernormal nutrient supply results in a more vigorous growth of the parasitic Phoma.

Among tubers naturally infected with Gangrene the fungus, Acrostalagmus cinnabarinus, was frequently observed as a contaminant on the rot. As was found to be the case in contamination with T. viride, the rots infected with this fungus were always much deeper than in those in which the Phoma isolates were present alone. These observations seemed to suggest that Acrostalagmus might associate with the Phoma isolates in the same way as T. viride. To determine this an investigation was carried out.

A. cinnabarinus, the imperfect state of Nectria inventa Pethyb. is a common soil fungus and in in culture formed an orange-red, orbicular colony. The erect conidiophores, like the vegetative hyphae, were septate. The conidiophores bore two to three

series of whorled branchlets which were terminated by three tenpin-like phialides. The oval conidia, borne on the phialides, measured (5.3-8.0 x 2.9-5.0)u and were produced in a head enveloped by slime.

Plates of potato dextrose agar and malt agar were inoculated simultaneously with the Acrostalagmus and the Phoma isolates 1, 2 and 3. All fungi made good growth and examination under the microscope showed the Phoma hyphae to be normal in appearance. When opposed colonies first made contact with each other, growth of the Phoma was arrested for a short time but was later resumed and continued normally. Within the Phoma colony, the Acrostalagmus grew more rapidly and fructified more luxuriently than when in culture by itself.

From the above observations ther is no conclusive evidence of the production of a fungistatic substance by Acrostalagmus cinnabarinus similar to that formed by T. viride. It is possible that the growth of the Phoma isolates was temporarily arrested by a fungistatic substance, but if such a substance was responsible, then it must have been in too low a concentration or too quickly inactivated to bring about the death of the Phoma hyphae.

Direct hyphal attack by the Acrostalagmus, similar to that occurring with T. viride, was observed on all the Phoma isolates in plate and slide cultures. On reaching the Phoma colony, the A. cinnabarinus hyphae which made contact with those of the Phoma coiled round them and grew in great profusion in that region. Aerial hyphae were also attacked in the same way. In the case of isolate 1, the attacked hyphae became filled with a yellow granular substance and crystals, like those formed in association with Trichoderma, made their appearance. On attack, the hyphae of isolates 2 and 3 lost their vacuolate structure and became devoid of contents.

External parasitism formed the usual mode of attack but internal parasitism also occurred.(Pl.VIII, figs.1 and 2). In such instances the host hypha became swollen in the region of penetration and its vacuolate structure was lost. Soon after penetration the hyphal contents of the host disappeared. Since this loss is progressive along the host hypha, it seemed to indicate that those contents were used in the nutrition of the parasite.

In the absence of conclusive evidence regarding the presence of a fungistatic substance being produced by the Acrostalagmus it is conceivable that its hyphae may act only as food competitors while incidentally killing the other fungus in culture. However, more than mere competition for food is suggested by the aerial and intrahyphal attack. The fact that the Acrostalagmus hyphae are more abundant in the region of the Phoma and that the host hyphae become devoid of contents when attacked, suggests that nutrients are obtained from the host. Moreover, parasitism occurred in the absence of any intervening medium. This was demonstrated by growing the Phoma isolates with the Acrostalagmus in van Tieghem cells in the manner described previously for the study of T. viride. When opposing hyphae met on the glass, those of A. cinnabarinus coiled round the Phoma hyphae and grew rapidly in that region. Intrahyphal parasitism was also observed under those conditions.

In Gangrene rots which were contaminated with A. cinnabarinus, the starch grains showed corrosion in the same manner as in the rots contaminated with T. viride. When cultured on a medium containing 0.5% starch, the Acrostalagmus completely hydrolysed the starch within two weeks; therefore the Acrostalagmus must have been responsible for the starch hydrolysis in the contaminated Gangrene rots.

A. cinnabarinus did not cause any rot on halved potato tubers which were inoculated by a spore

suspension of the fungus and stored in boxes lined with moist peat. However, similar tubers inoculated with a mixed spore suspension of Acrostalagmus and each of the Phoma isolates in turn, produced rots which averaged two to three times the depth of those caused by the Phoma isolates alone.

In comparing the rots produced by mixed inocula it was noticed that destruction of the cell walls in the rot was more rapid and complete than with any of the Phoma isolates. In addition, the intermediate zone which separates the completely rotted tissue from the tissue in the region of advancing hyphae (Pl.II,fig.2) was almost absent. This seemed to suggest that A. cinnabarinus had a role in destroying the host tissue. When the test for the presence of a pectic enzyme was applied to A. cinnabarinus it proved positive and by taking that into account there is no doubt that the Acrostalagmus was responsible for the more rapid destruction of the potato tissue.

It seems that the deeper rots resulting from contamination of Gangrene rots with A. cinnabarinus can be explained in the same way as the rots contaminated with T. viride. However, unlike the latter, A. cinnabarinus has the additional power of destruction in its production of a pectic enzyme.

With both T. viride and A. cinnabarinus it was not possible to determine to what (if any) extent they antagonised the Phoma isolates in potato tubers. There was the possibility that antagonism was a phenomenon only occurring in artificial culture. Alternatively, since neither fungus was able to induce rot on potato tubers, they must have followed the Phoma into the rot. In doing this they may have parasitised the older Phoma hyphae. All isolations made from the advancing edges of contaminated rots produced only pure cultures of Phoma, showing that

the contaminants must have been excluded from this region. Consequently, even if the old Phoma hyphae were parasitised, the young advancing hyphae were not attacked and remained available to continue the rot.

THE PATHOGENICITY OF THE PHOMA ISOLATES TOWARDS POTATO TUBERS.

During the investigation experiments were conducted to compare the virulence of the isolates with each other on different potato varieties at different times during storage.

Methods.

a) Sterilization of tubers.

The tubers were washed free of dirt and allowed to dry. They were then surface disinfected by dipping in a 2% formalin solution for 30 seconds. On removal from the disinfectant, the tubers were spread in a single layer over a sterilized bench until all trace of the smell of formalin had disappeared. The tubers were then placed in sterilized sacks and stored in an unheated room until required.

b) Storage of inoculated tubers.

Small cardboard boxes, large enough to hold eight seedling tubers, were sterilized by spraying with methylated spirit then, with their lids closed, were set aside to dry.

The boxes were next lined with a layer of moist, sterile peat and the inoculated tubers placed in them.

The boxes had the advantage of being easily handled when placed in the requisite storage conditions and maintained a sufficiently humid atmosphere for growth of the fungus without causing damage to the tubers.

c) Inoculation method.

The following three treatments were used in tuber inoculations;

(i) A portion of inoculum was placed on the tuber without any tuber treatment.

(ii) The inoculum was placed on the surface of the tuber where it had been bruised by a good blow without breaking the skin.

(iii) The inoculum was introduced into the tuber:

a minute "V" shaped cut was made on the skin of the tuber, a portion of the underlying flesh removed, the culture placed in the space thus formed and the "V" shaped hinge replaced.

These treatments are subsequently refered to as intact, bruised and cut respectively.

d) Inoculum.

The most convenient method for inoculating tubers would have been to inject spore suspensions of the isolates into the tubers with a hypodermic syringe. Since the isolates did not spore readily it was found necessary to use mycelium as inoculum. In order to standardize the quantity of inoculum used, single spore, plate cultures of the isolates were divided into equal portions with the dummy objective of the microscope. The small discs of culture thus obtained were removed and transferred to the tubers.

Tuber Inoculations.

Series I.

This series of inoculations was of a preliminary nature in order to determine the relative pathogenicity of the Phoma isolates. The potato varieties used were Arran Pilot, Doon Star and Catriona, and the three types of treatment described previously, were applied to the tubers.

The results of these inoculations are given in Table IV.

It was evident from the results that the three isolates differed in pathogenicity, isolate 1 being the most virulent and isolate 3 the least virulent. Moreover, the pathogenicity of each isolate varied with the host variety concerned. No infection occurred on intact tubers and incidence of the disease among bruised tubers was low. Therefore the isolates were most important as wound parasites.

Variety	Treatment	% Infection		
		Isolate 1	Isolate 2	Isolate 3
Arran Pilot	Intact	0	0	0
	Bruised	8.4	16.7	0
	Cut	44.5	60.4	0
Doon Star	Intact	0	0	0
	Bruised	6.3	4.2	0
	Cut	62.5	39.6	6.3
Catriona	Intact	0	0	0
	Bruised	0	0	0
	Cut	77.2	6.3	25.0

Table IV.

In each case the fungus used as inoculum was reisolated from the tubers.

Series II.

This series of inoculations was made to determine the influence of the maturity of the tubers on the development of Gangrene. Since the maximum number of infections was desired for this experiment, only cut tuber treatment was used. In addition, to keep temperature conditions as natural as possible, the tubers were kept in an unheated, insulated store, the temperature of which varied between 8° and 10°C, i.e. the temperature considered to be ideal for potato storage.

The three Phoma isolates were used for the inoculations which were made on seven potato varieties. However, only three of these varieties were available in sufficient quantity to conduct the experiment through the full storage period.

The first set of inoculations was made in late August immediately after the tubers were lifted. Further sets were inoculated during the first week in November, the first week in January and the last week in March. The tubers were kept under the experimental conditions for eight weeks before being examined.

During March a set of inoculations was also made on tubers which were stored in a cool greenhouse (temperature approximately 15°C). Owing to the higher temperature in the greenhouse those tubers sprouted earlier than those kept in the store room. Therefore, it was possible to compare the rots with those on tubers at an earlier stage of maturity.

The tubers were stored at a relative humidity which fluctuated between 95% and 98%.

Six uninoculated control tubers were included in each set and those were never observed to develop Gangrene symptoms.

Approximately 40 tubers of each variety were inoculated with each isolate and at the end of the experimental period, the diameter and depth of each rot was measured. The two sets of measurements were treated separately throughout and for convenience in analysis, they were divided into groups. Group 0 included all the inoculations where no infection resulted.

Group 1 contained the measurements from 1mm. to 5mm.
" 2 " " " " 6mm. to 10mm.
" 3 " " " " 11mm. to 15mm.
" 4 " " " " 16mm. to 20mm.
" 5 " " " " 21mm. to 25mm.
" 6 " " " " 26mm. to 30mm.
" 7 " " " " 31mm. to 35mm.

Since the number of tubers inoculated varied by two or three the numbers included in each Group were standardized by expressing them as percentages.

The results for isolates 1 and 2 are expressed on histograms 1 to 8. The percentage of the measurements obtained in each set of inoculations are placed together in their corresponding Groups; thus, e.g. all the rots for January, March, August and November occurring in Group 1 can easily be compared.

Isolate 3 did not cause any rot on the tubers except on those which were stored in the greenhouse and inoculated in March. However, those rots were too small to be significant and were not considered further.

Reisolations from the inoculated tubers yielded pure cultures of the isolates used for inoculation.

Series III.

In this series an endeavour was made to compare the pathogenicity of isolates 1 and 2 on different potato varieties according to pretreatment and subsequent storage conditions. The varieties used were Arran Banner, Arran Pilot, Catriona, Doon Star, Golden Wonder, Kerr's Pink and Home Guard. Only cut tuber treatment was applied and the tubers were stored in boxes in the usual way.

Before inoculations commenced isolate 1 was lost and another isolation of the same fungus was used to replace it. This fungus which was also designated isolate 1 was a pycnidial strain.

Two sets of inoculations were made, one during the last week of December and the other during the last week in March. In the case of the former, one half of the tubers inoculated with isolate 1 was kept at room temperature for one month before inoculation. After inoculation, half of these were retained at room temperature while the remainder were stored in an insulated store room at a temperature of 5°to 8°C. Similarly, all other tubers in this set were kept in the store room before inoculation and subsequent to inoculation, one half of these were stored at room temperature while the remainder were placed in the store room.

In the first set of inoculations the tubers which were inoculated with isolate 2 were stored in the store room before inoculation and at room temperature afterwards.

In the second set of inoculations the tubers inoculated with isolates 1 and 2 were treated in the same way. They were kept in the store room before inoculation and afterwards, one half of the inoculated tubers were kept at room temperature while the remaining portion was placed in the store room.

The tubers remained under the experimental conditions for 6 weeks before being examined.

The results of those experiments were treated in the same way as those of Series II and are expressed on histograms 9 to 22 for isolate 1 and 23 to 29 for isolate 2.

With each set of inoculations the fungus used for inoculum was recovered from the advancing edges of the rots.

Series IV.

During the investigation, Skin Necrosis was reported on three occasions. In every case the Phoma isolates were obtained from the diseased tubers and the land on which the tubers were grown was fairly acid (pH below 5) and classified as "marginal". At other times the same tuber varieties grown on different soil, showed typical Gangrene symptoms. This seemed to suggest that the acid soil so influenced the potato tubers as to alter their reaction to the Phoma species.

With a view to examining the relationship between Gangrene and Skin Necrosis, Majestic tubers were obtained from a peat soil and an average light soil and the following inoculations made. The two lots of tubers were sterilized and stored in the usual way. One half of each lot was stored at room temperature and the remainder were placed in the store room.

This series of inoculations was made during the last week in March and the results were taken six weeks later. Forty inoculations were made with each fungus for each set of inoculations and the results which were calculated in the same way as those of

Series II are expressed on histograms 30 and 31 for isolate 1 and 32 for isolate 2.

Isolates 1 and 2 were both reisolated from the tubers inoculated with those fungi.

CONCLUSIONS AND COMMENTS.

Examination of the results of Series II inoculations shows that in each vatiety tested, susceptibility to Gangrene, as caused by either isolate, increases with the length of the storage period. The varieties are immune at lifting and during most of the dormant period. However, with sprouting, susceptibility is increased.

Isolates 1 and 2 differed in their pathogenicity depending on the tuber variety concerned. Thus, Doon Star, Catriona and Kerr's Pink were more susceptible to isolate 1, and Golden Wonder and Home Guard were more susceptible to isolate 2.

As regards susceptibility to isolate 1, the varieties may be placed in the following order:- a) Catriona and Home Guard. b) Doon Star, c) Golden Wonder and Kerr's Pink. Similarly, with isolate 2 the varieties are placed in the order, a) Home Guard b) Golden Wonder, c) Doon Star and Kerr's Pink and c) Catriona.

No conclusive evidence was produced regarding the pathogenicity of isolate 3 on any of the varieties tested. It seemed that this isolate was not a virulent parasite.

The results of Series III inoculations show that storage at a higher temperature before inoculation renders the tubers more susceptible to Gangrene. However, storage at a low temperature subsequent to inoculation increases susceptibility to Gangrene, the rots formed being more extensive in the tubers kept in the insulated store room than in those kept at room temperature.

Series III inoculations also indicated that different potato varieties can be graded according to their susceptibility. The first set of inoculations showed that the tubers tested could be placed in the following order of increasing susceptibility with regard to isolate 1: a) Kerr's Pink, b) Doon Star and Arran Banner, c) Golden Wonder, d) Arran Pilot, e) Catriona and Home Guard. Similarly, with regard to isolate 2, the order of the varieties is a) Kerr's Pink, b) Golden Wonder, c) Arran Banner and Doon Star, d) Arran Pilot, e) Home Guard, f) Catriona.

In the second set of inoculations the order of increased susceptibility to isolate 1 is a) Home Guard, Doon Star and Kerr's Pink, b) Arran Pilot, c) Arran Banner, d) Golden Wonder and e) Catriona. With isolate 2 the sequence is a) Doon Star, Kerr's Pink and Golden Wonder, b) Arran Pilot, c) Arran Banner, d) Home Guard and e) Catriona.

It is also evident from the results that some varieties, e.g. Kerr's Pink, Arran Banner and Catriona were more susceptible to isolate 2 in both sets of inoculations. In addition, Doon Star tended to be more susceptible to this isolate in the later set of inoculations.

Series IV inoculations showed conclusively, that soil and storage conditions can be responsible for the development of a Skin Necrosis type of rot on infected tubers. The Skin Necrosis symptoms developed on only 51.3% of those tubers which were grown on peat soil and kept under low temperature storage conditions. The remaining tubers all developed deeper rots which , although shallow, were quite distinct from those showing Skin Necrosis symptoms.

DISCUSSION.

The results of the investigation showed that three Phoma isolates which probably represent two Phoma species could cause disease in stored potato stocks. No distinction could be made among the rots caused by the isolates, all of which were responsible for two manifestations of disease, viz. Gangrene and Skin Necrosis. In the field, Gangrene was the more common of the two types of disease and occurred on many varieties from different soils and from different storage conditions.

During most seasons Gangrene causes a greater amount of rot in tubers stored in boxes and bags than among those stored in pits. A storage disease which is generally of greater importance than Gangrene is Dry Rot, caused by Fusarium caeruleum. However, in some seasons it has been noted that Gangrene can be more prevalent than Dry Rot, and this was found to be the case in many districts in the spring of 1951. Examination of stored stocks, both in Scotland and England, in 1951 indicated that incidence of Gangrene was high under all types of storage conditions.

Experimental evidence indicated that the development of Gangrene was favoured by storage at lower temperatures. Tubers inoculated with Fusarium caeruleum and stored under the same conditions failed to rot whereas at higher temperatures, Dry Rot set in. Therefore it seemed that temperatures low enough to inhibit Dry Rot were more favourable for the development of Gangrene. Inhibition of Dry Rot was due to the growth of the Fusarium having stopped with the low temperature. With the Phoma isolates, growth was possible but the reaction of the tubers was slow due to the temperature, consequently, Gangrene developed.

From the above observations it is suggested that the very cold conditions prevailing in 1951 were

responsible for the higher incidence of Gangrene in that season.

Alcock and Foister (1936) believed Gangrene, as caused by Phoma foveata, to be most prevalent late in the storage period, particularly on early varieties. The results obtained from the inoculations with isolate 1 confirmed this view.

Gangrene, as caused by isolate 2 did not give the same result as the isolate 1 inoculations. In the first series of inoculations with isolate 2 Home Guard, a first early, was almost equal in susceptibility to the maincrop varieties, Doon Star and Kerr's Pink; Golden Wonder, a late maincrop variety was next in susceptibility to the second early variety, Catriona. Certainly, the second set of inoculations showed the early varieties to be more susceptible at that time of inoculation. However, in determining the relative susceptibility of the varieties it is necessary to bring into consideration the stage of maturity of the tubers. Thus, it is not correct to draw the conclusion that Home Guard is more susceptible than Golden Wonder because deeper rots formed. Home Guard, by nature of its being an early variety, was at a more advanced stage of maturity at the time of inoculation. Consequently, the inoculations made during December, when the maturity of the varieties is more closely related, must give a better indication of their relative susceptibility. Therefore, susceptibility to isolate 2 was not correlated with early or late varieties. Among the early varieties, Arran Pilot was more susceptible than Home Guard in December, but Home Guard was more susceptible in March. Similarly, Golden Wonder was more susceptible than Kerr's Pink in December but later, both varieties were equally susceptible.

Under natural conditions the occurrence of Skin

Necrosis is less frequent than that of Gangrene . Moore (1947) mentioned several fungi which were associated with Skin Necrosis conditions but could not find any satisfactory evidence that they were the causal organisms. Probably the condition was due to several causes each giving the same end result. These causes may have included that studied during the present investigation.

The symptoms of Skin Necrosis were found to be associated with the Phoma isolates in three cases observed during the investigation. The varieties concerned were Arran Banner and Majestic, the tubers being grown on marginal land in which the soil had a low pH value. Reproduction of Skin Necrosis symptoms was possible by inoculating Majestic tubers with isolate 1 and subsequently storing them at a low temperature. (Pl. XI).

It is interesting to note that both Arran Banner and Majestic can suffer from the deep Gangrene type of rot also, but Skin Necrosis is the only type of Phoma infection ever to have been recorded on the variety Alness. Therefore, Skin Necrosis as caused by Phoma species, seems to be a condition associated with tuber quality whether this be determined by the genotype (var. Alness) or through other factors (soil and storage).

The obvious question remaining to be answered is "where and when does the fungus gain entry?"

True Gangrene is never reported on potatoes until they are in storage. Observations on naturally infected tubers usually indicated that the fungus had gained entry through a wound and the experimental evidence showed that the Phoma isolates were most important as wound parasites. From this it seems that injury at lifting and during dressing provides an opening for the fungus, the growth of which depends on the maturity and storage conditions of the tuber.

In one case of Skin Necrosis on Arran Banner, the tubers were known to be infected to a small extent at lifting. Since there was no question of mechanical injury on these tubers it seemed that some factor, possibly influence of the soil, allowed the fungus to gain entry.

Doubtless, the original source of inoculum under natural conditions is the fungus contained among the soil particles adhering to the tubers. Further infection by the fungus can be caused by contact with diseased tubers. In addition, these tubers can spread infection further by producing pycnospores in pycnidia on the surface of the rots. These spores can be carried by various means through the stocks.

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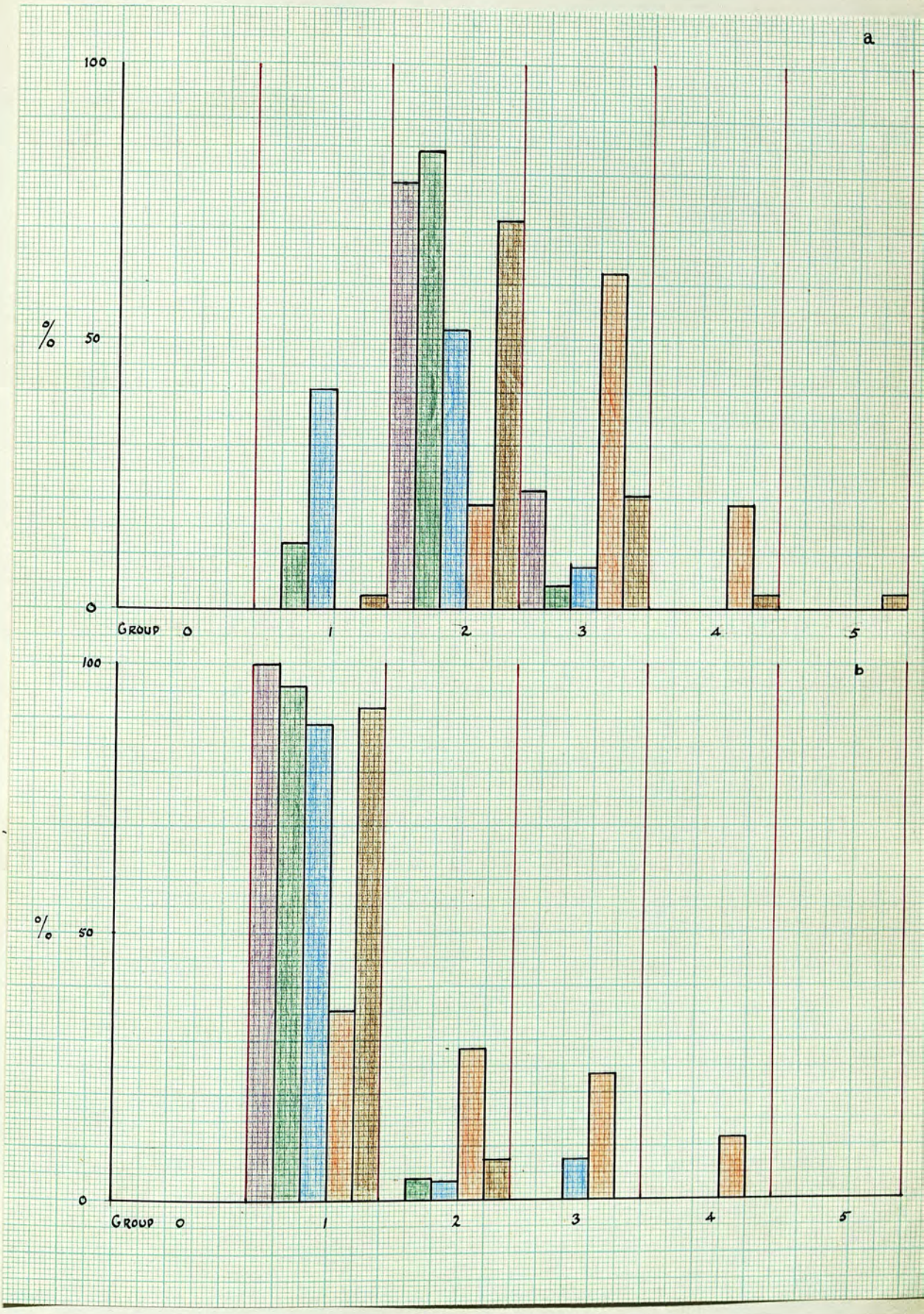
HISTOGRAMS 1 to 32.

Histogram 1.

This shows the diameter (a) and the depth (b) of the rots produced on Catriona by isolate 1.

Colours are according to the key attached to histogram 6.

Histogram 1.

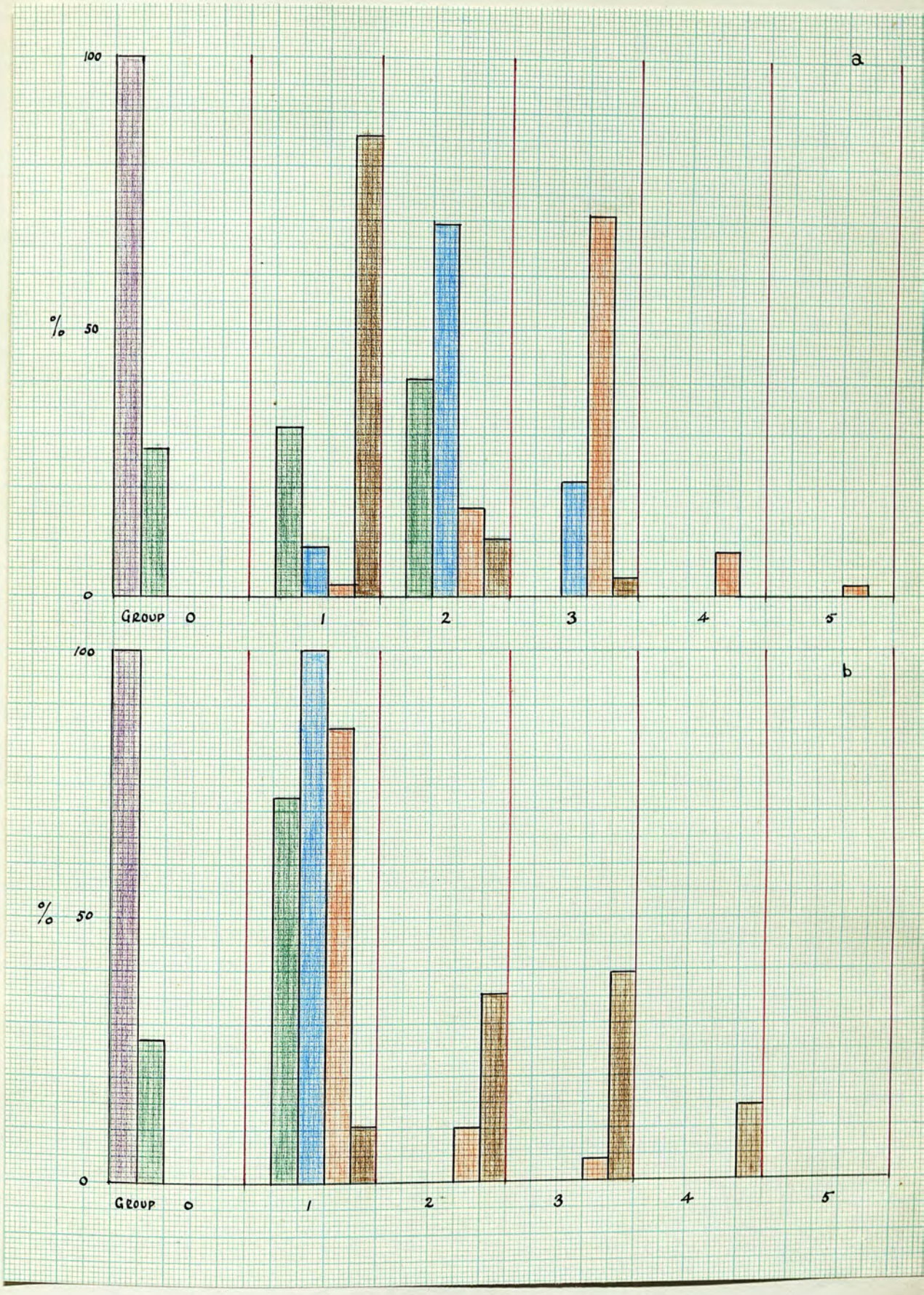


Histogram 2.

This shows the diameter (a) and the depth (b) of the rots produced on Doon Star by isolate 1.

Colours are according to the key attached to histogram 6.

Histogram 2.

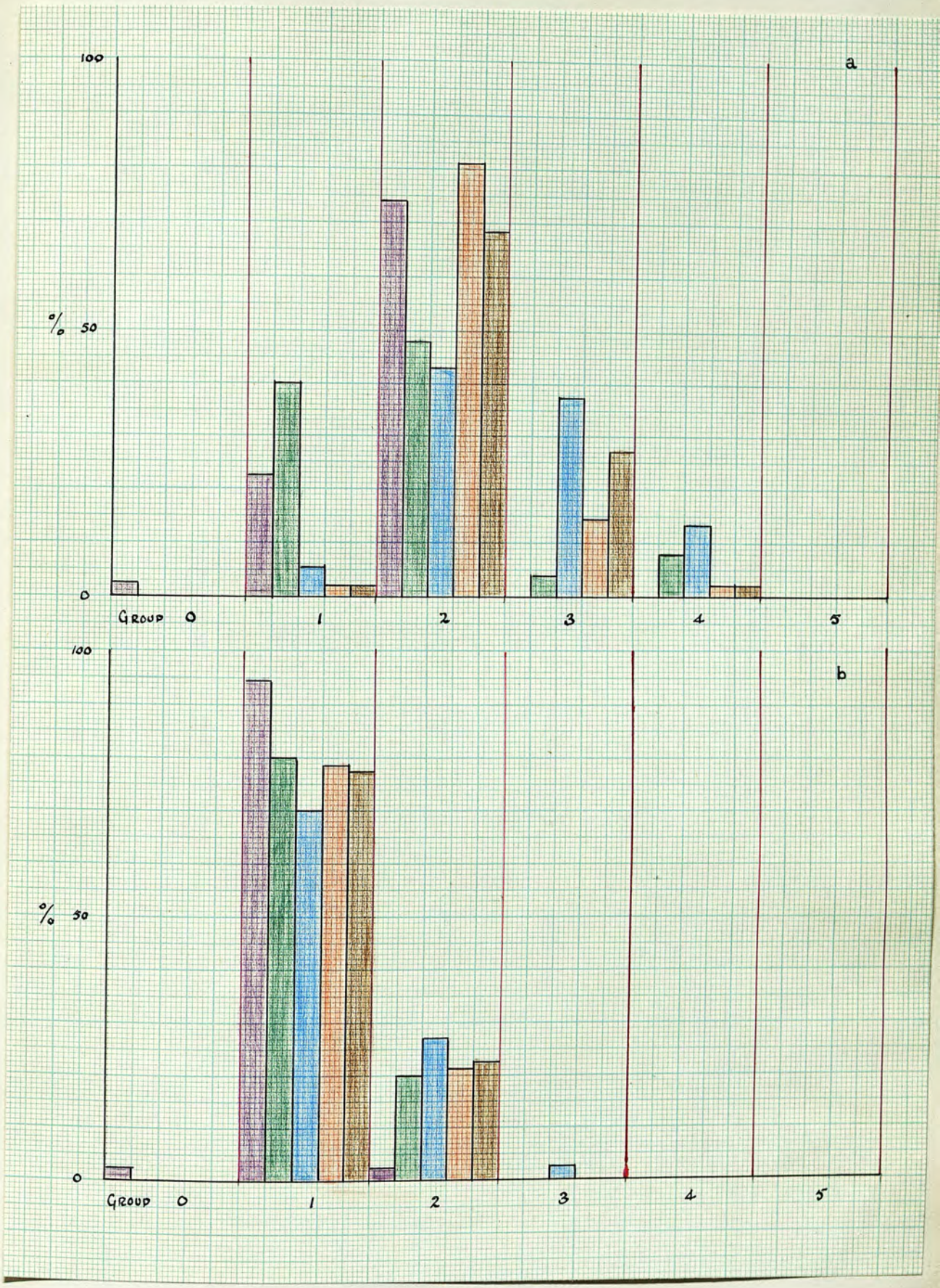


Histogram 3.

This shows the diameter (a) and depth (b) of the rots produced by isolate 1 on Golden Wonder.

Colours are according to the key attached to histogram 6.

Histogram 3.

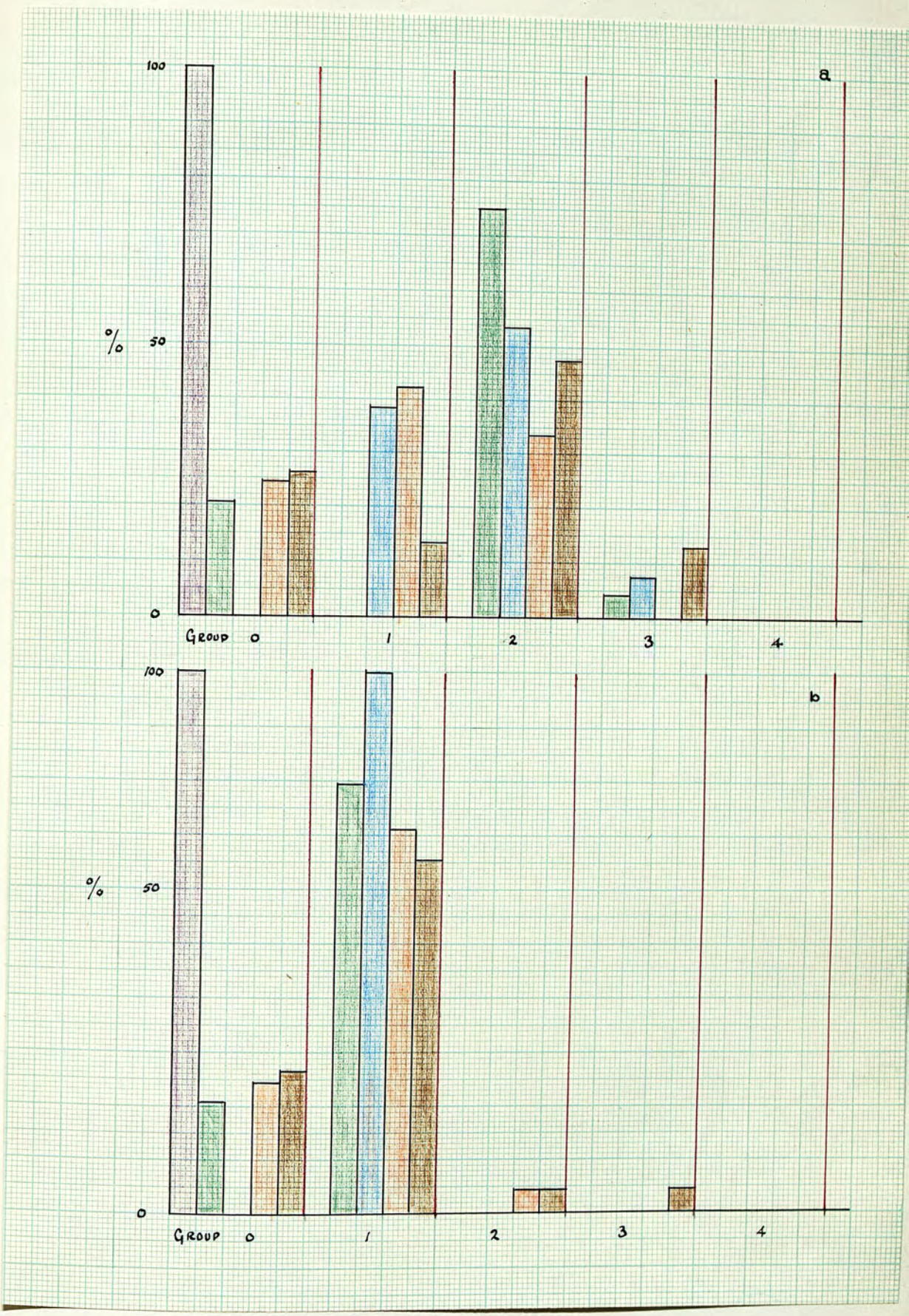


Histogram 4.

This shows the diameter (a) and the depth (b) of the rots produced on Catriona by isolate 2

Colours are according to the key attached to histogram 6.

Histogram 4

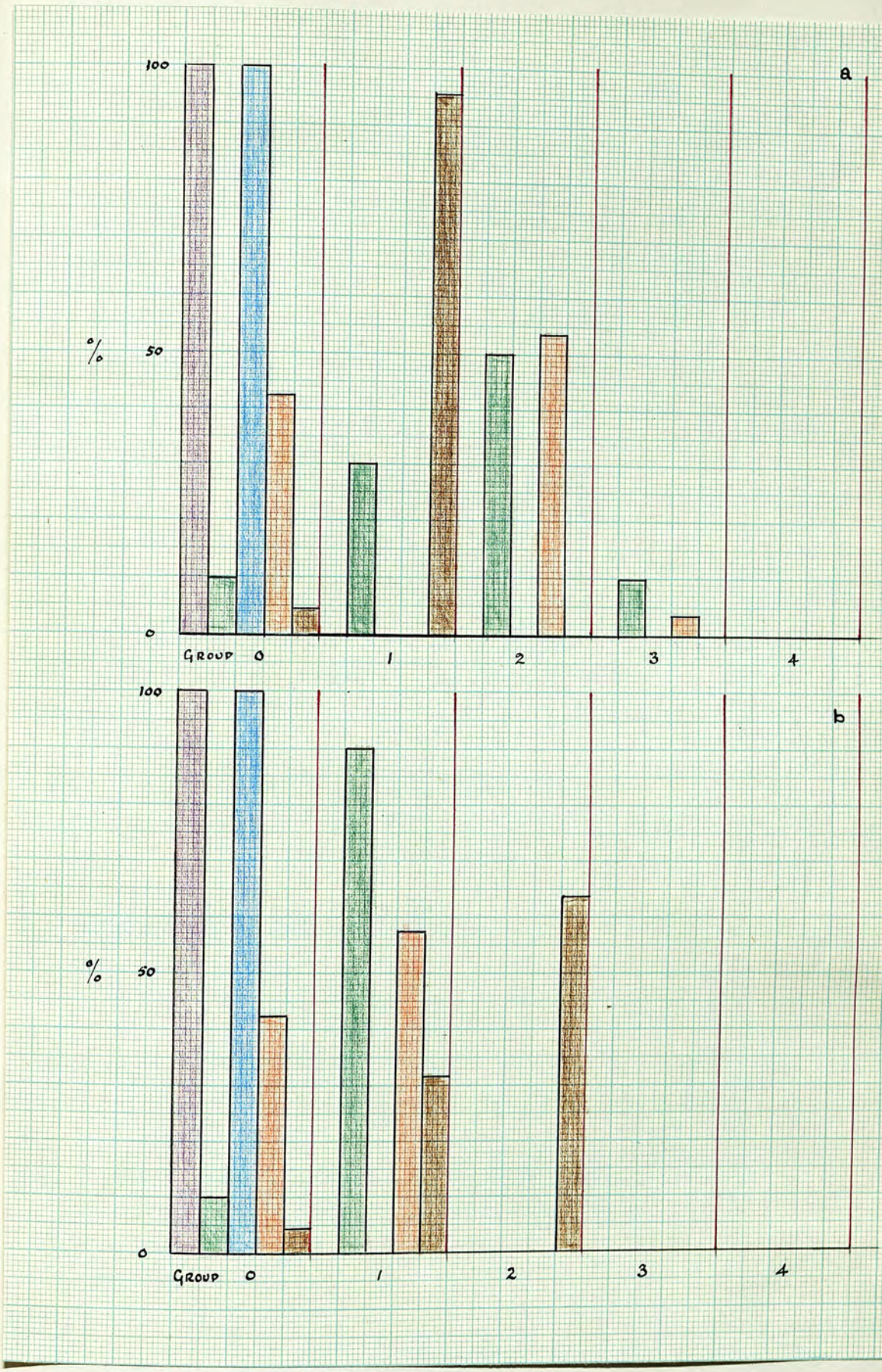


Histogram 5.

This shows the diameter (a) and depth (b) of the rots produced on Doon Star by isolate 2.

Colours are according to the key attached to histogram 6.

Histogram 5.

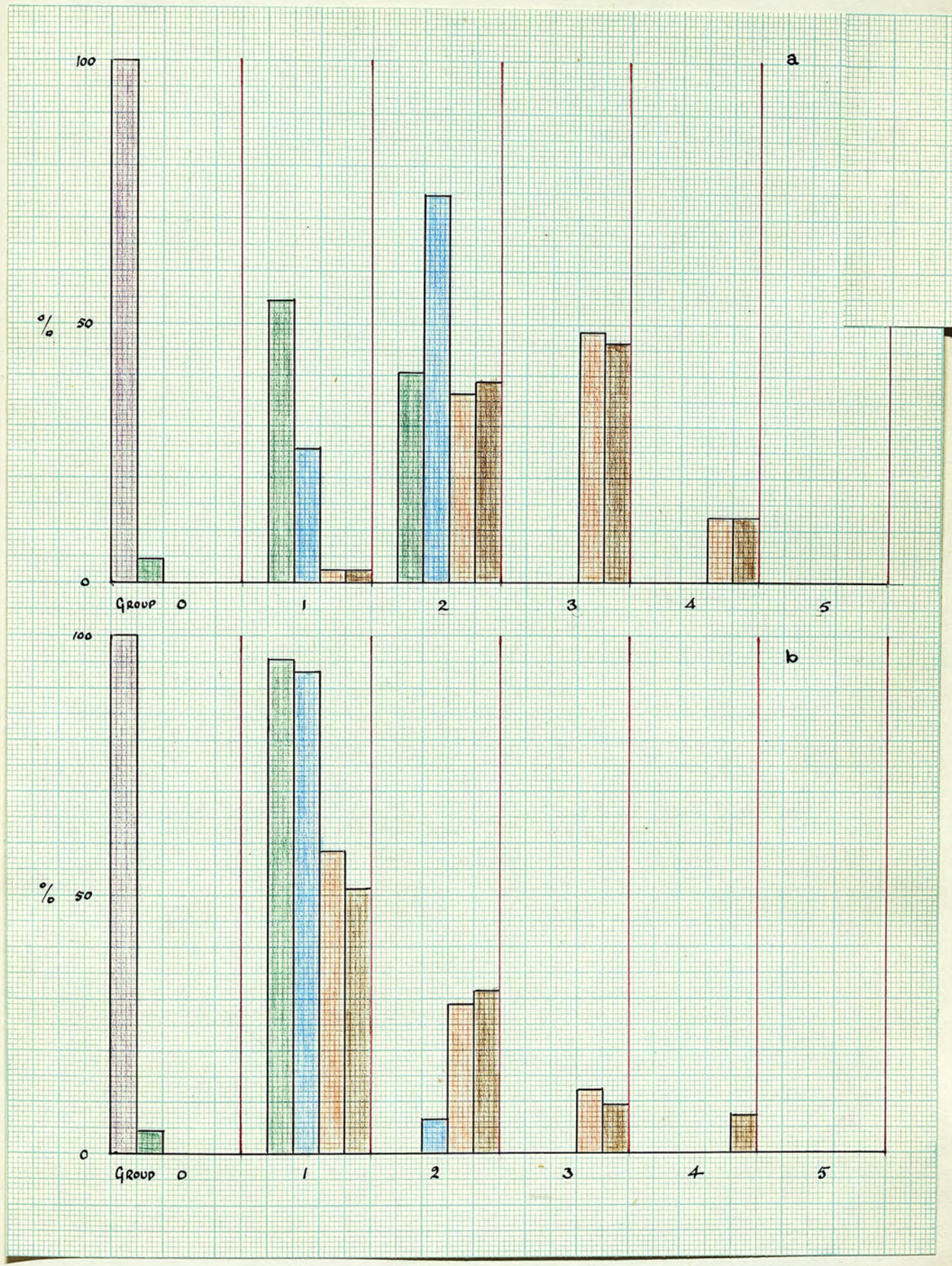


Histogram 6.

This shows the diameter (a) and the depth (b) of the rots produced on Golden Wonder by isolate 2.

Colours are according to the key attached to the histogram.

Histogram 6.



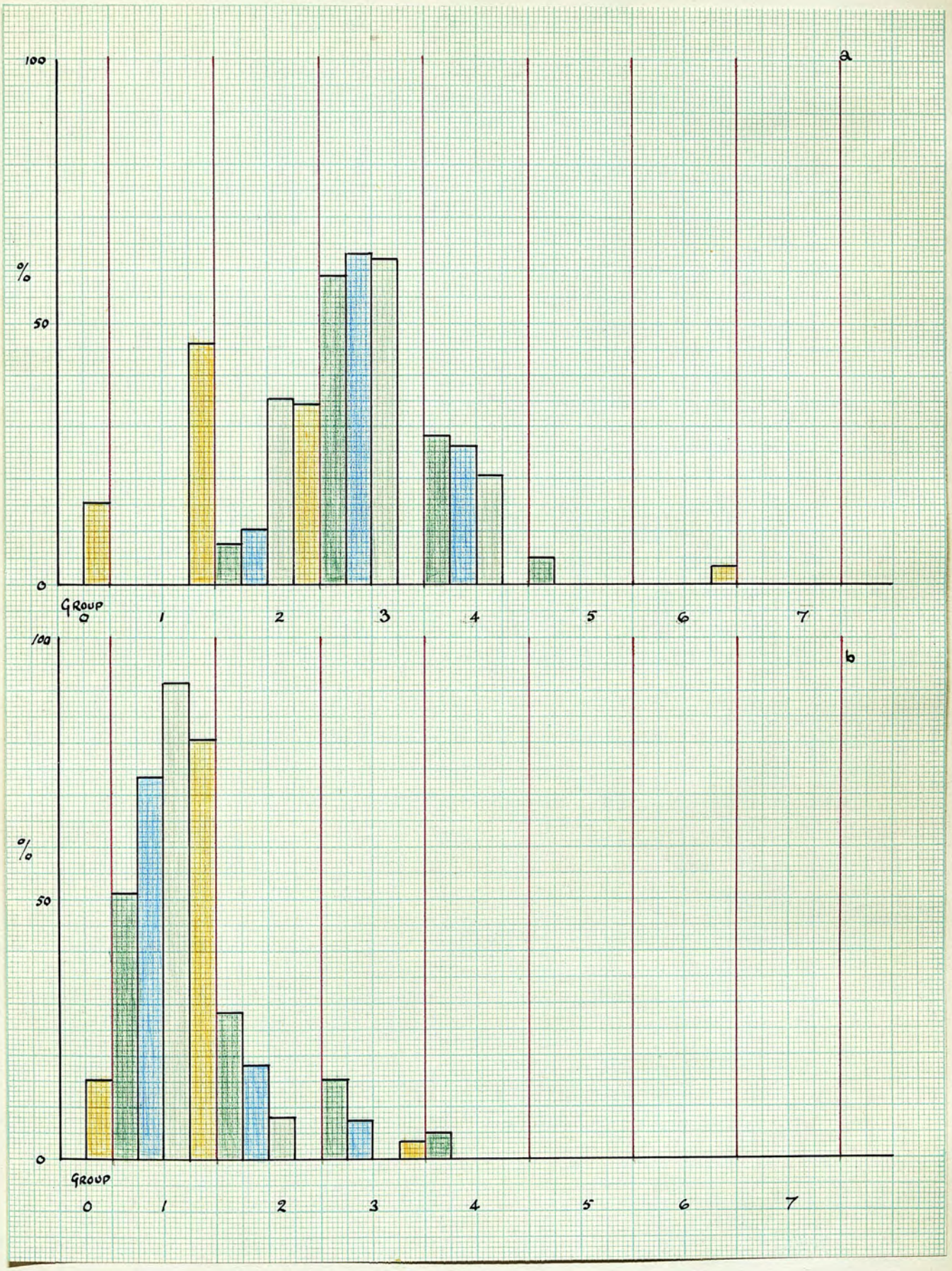
Inoculated in August.
Inoculated in November.
Inoculated in January.
Inoculated in March; stored in greenhouse
Inoculated in March; stored in store room.

Histogram 7.

This shows the diameter (a) and the depth (b) of the rots produced on Home Guard and Kerr's Pink by isolate 1.

Colours are according to the key attached to histogram 8.

Histogram 7.

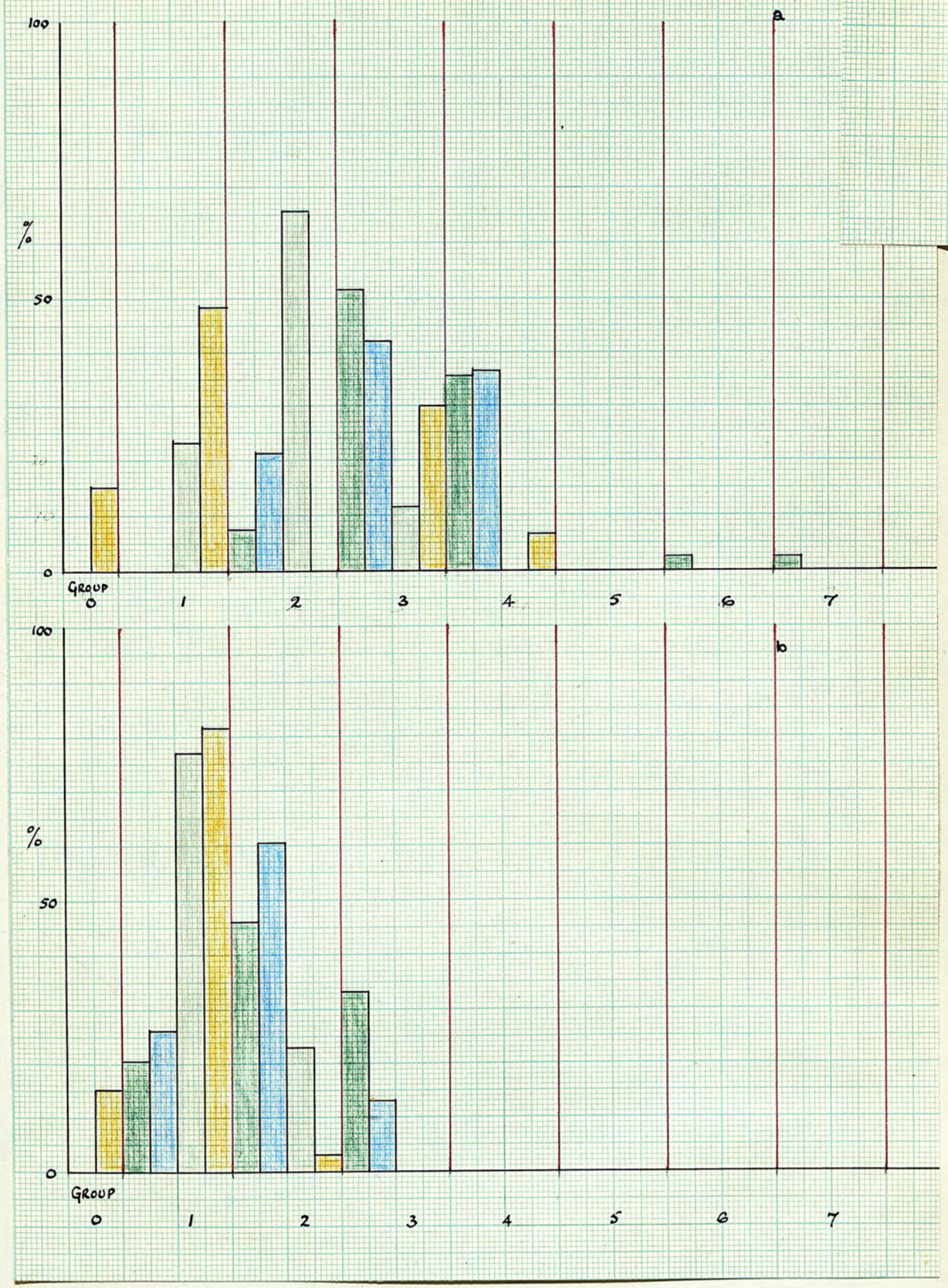


Histogram 8.

This shows the diameter (a) and the depth (b) of the rots produced on Home Guard and Kerr's Pink by isolate 2.

Colours are according to the key attached.

Histogram 8.



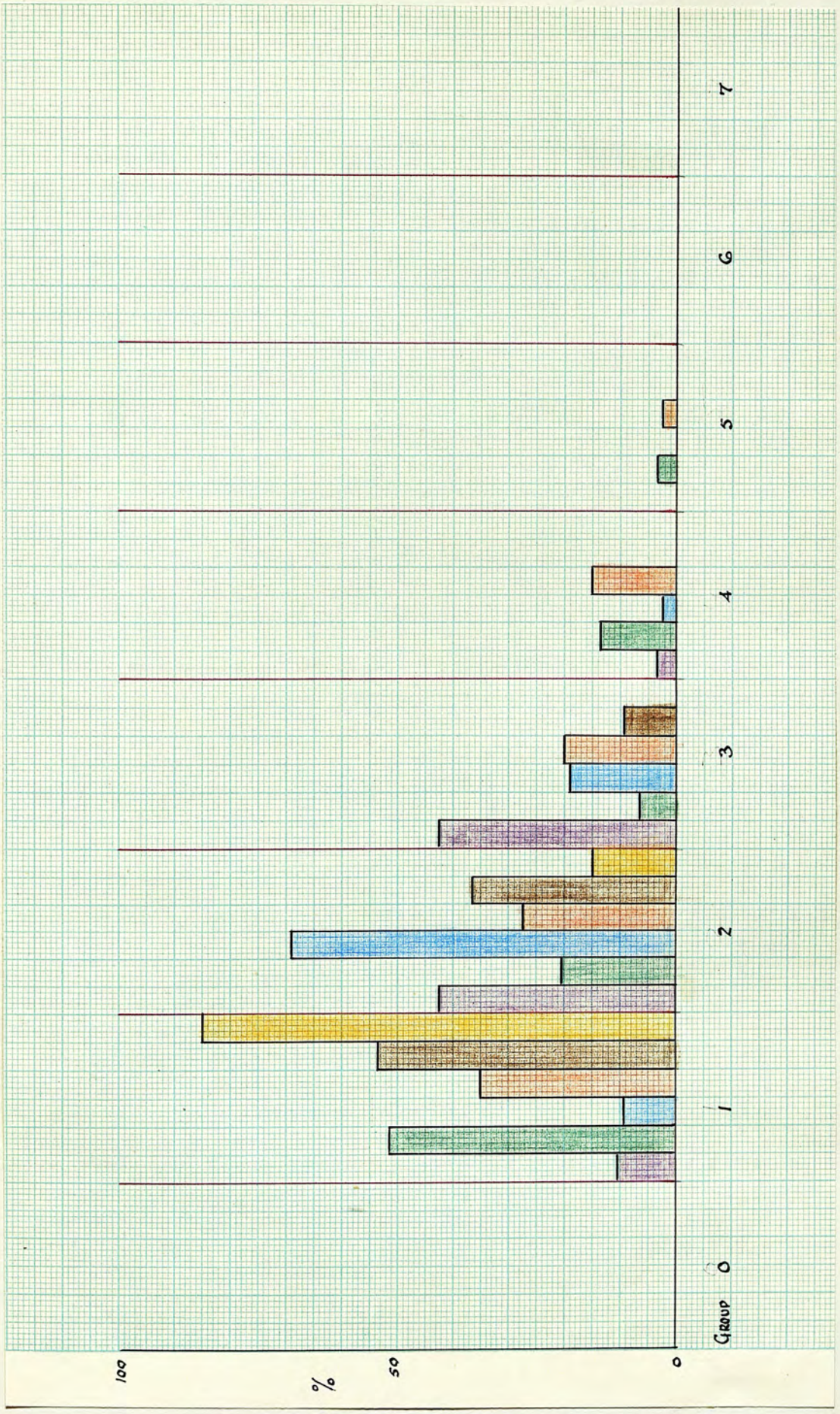
Home Guard; stored in greenhouse.
Home Guard; stored in store room.
Kerr's Pink; stored in greenhouse.
Kerr's Pink; stored in store room.

Histogram 9.

This shows the depth of the rots produced on Arran pilot by isolate 1.

Colours are according to the key attached to histogram 29.

Histogram 9.

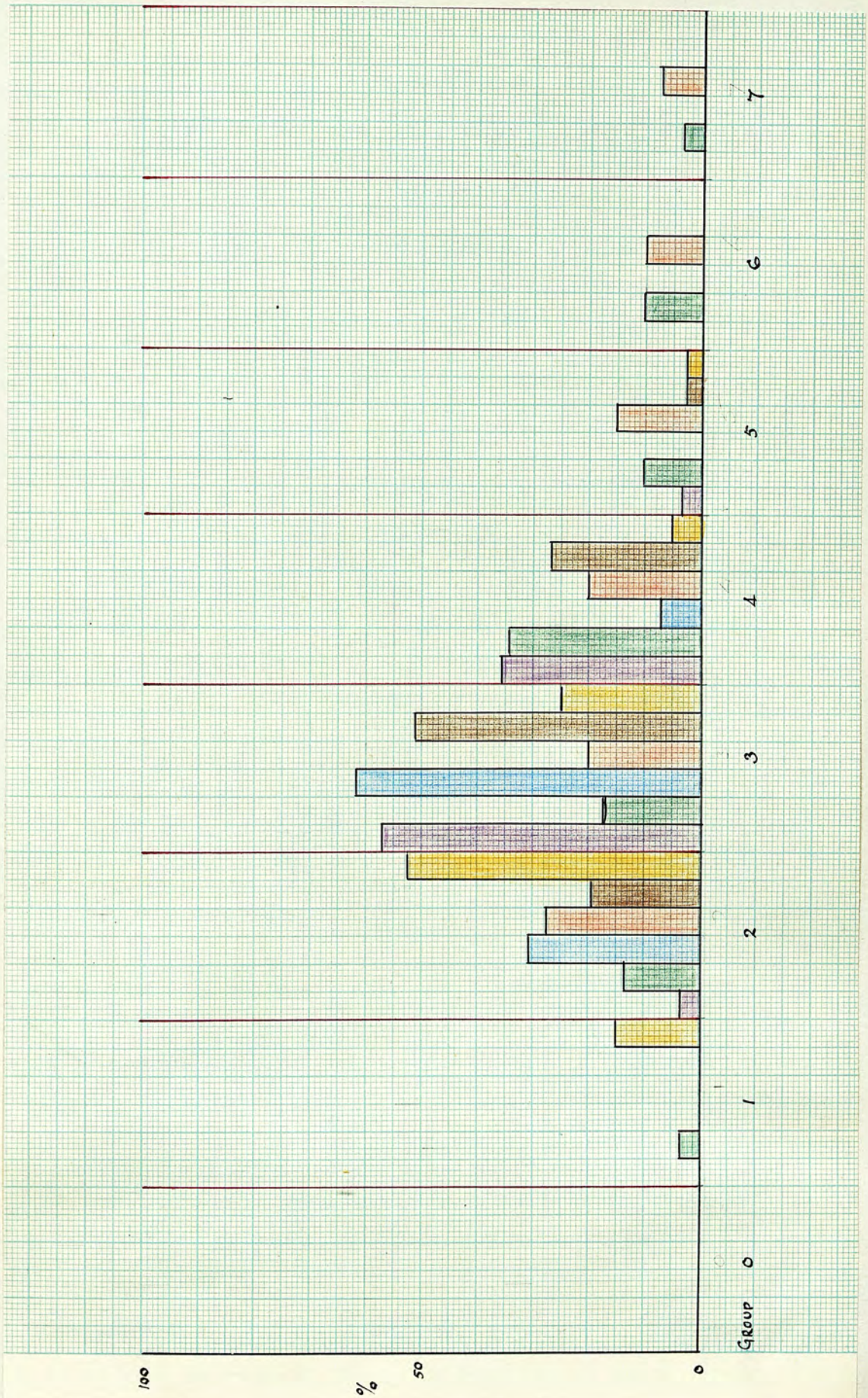


Histogram IQ

This shows the diameter of the rots produced on Arran Pilot by isolate 1.

Colours are according to the key attached to histogram 29.

Histogram 10

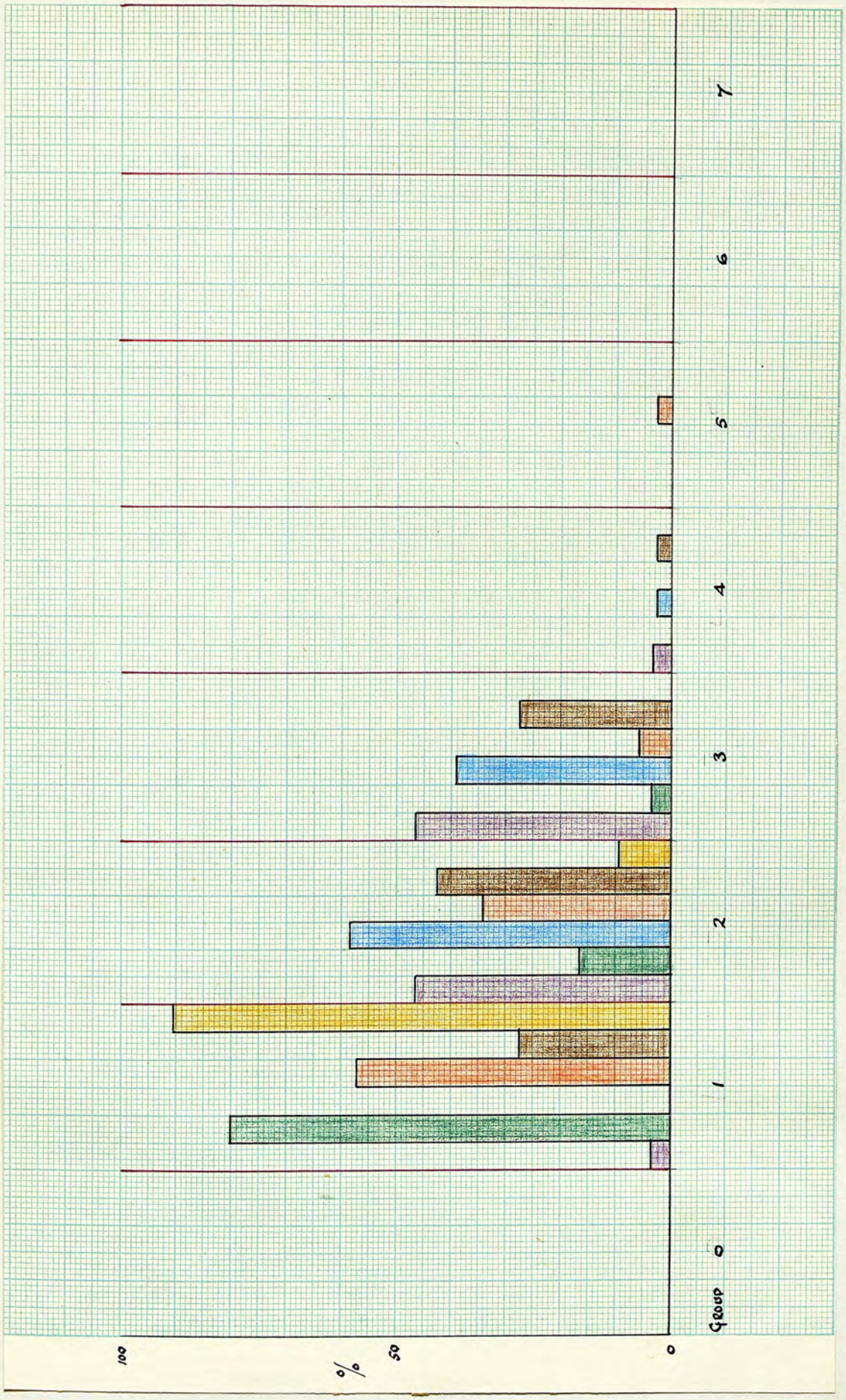


Histogram 11.

This shows the depth of the rots produced on Home Guard by isolate 1.

Colours are according to the key attached to histogram 29.

Histogram 11.

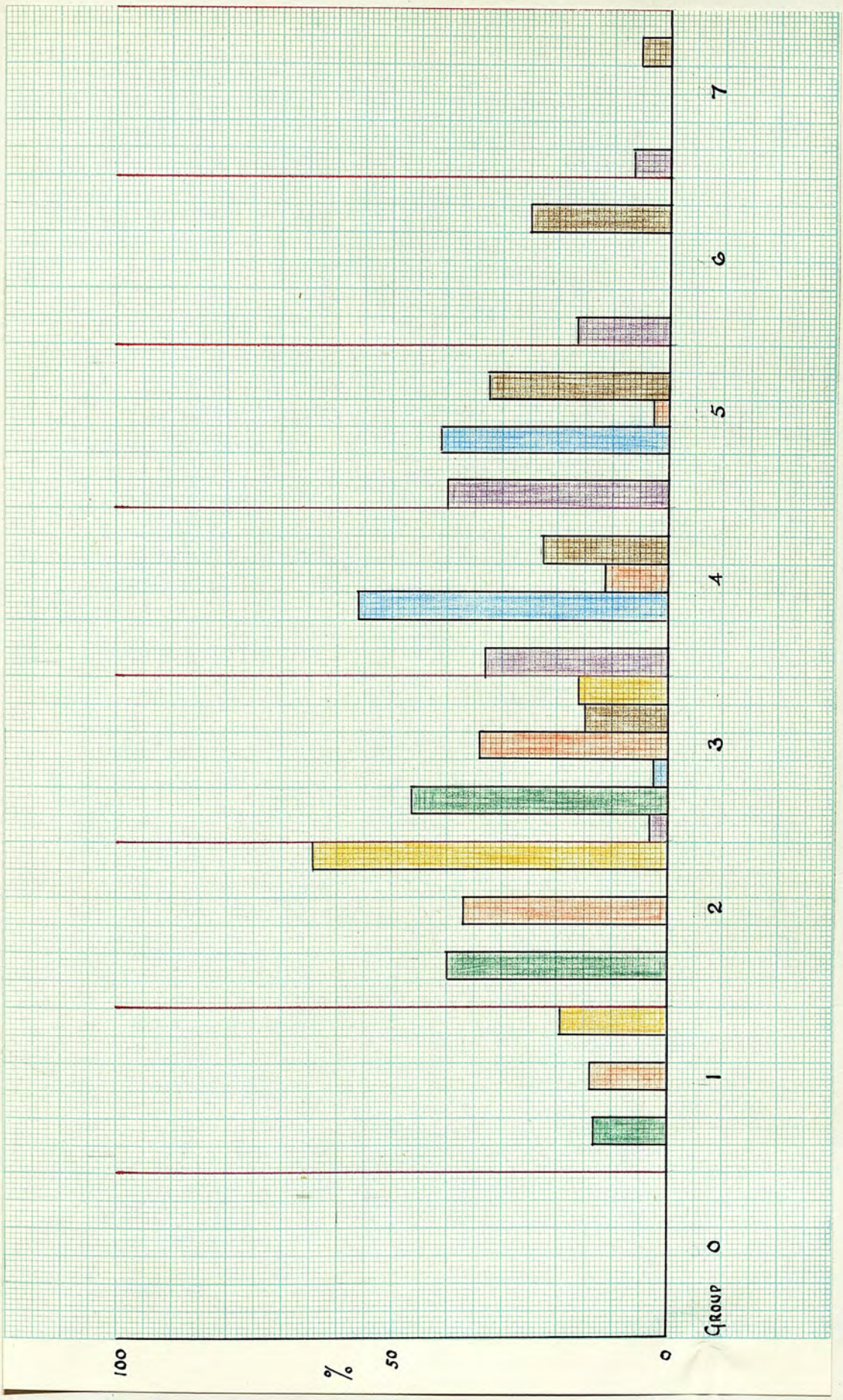


Histogram 12.

This shows the diameter of the rots produced on Home Guard by isolate 1.

Colours are according to the key attached to histogram 29.

Histogram 12.

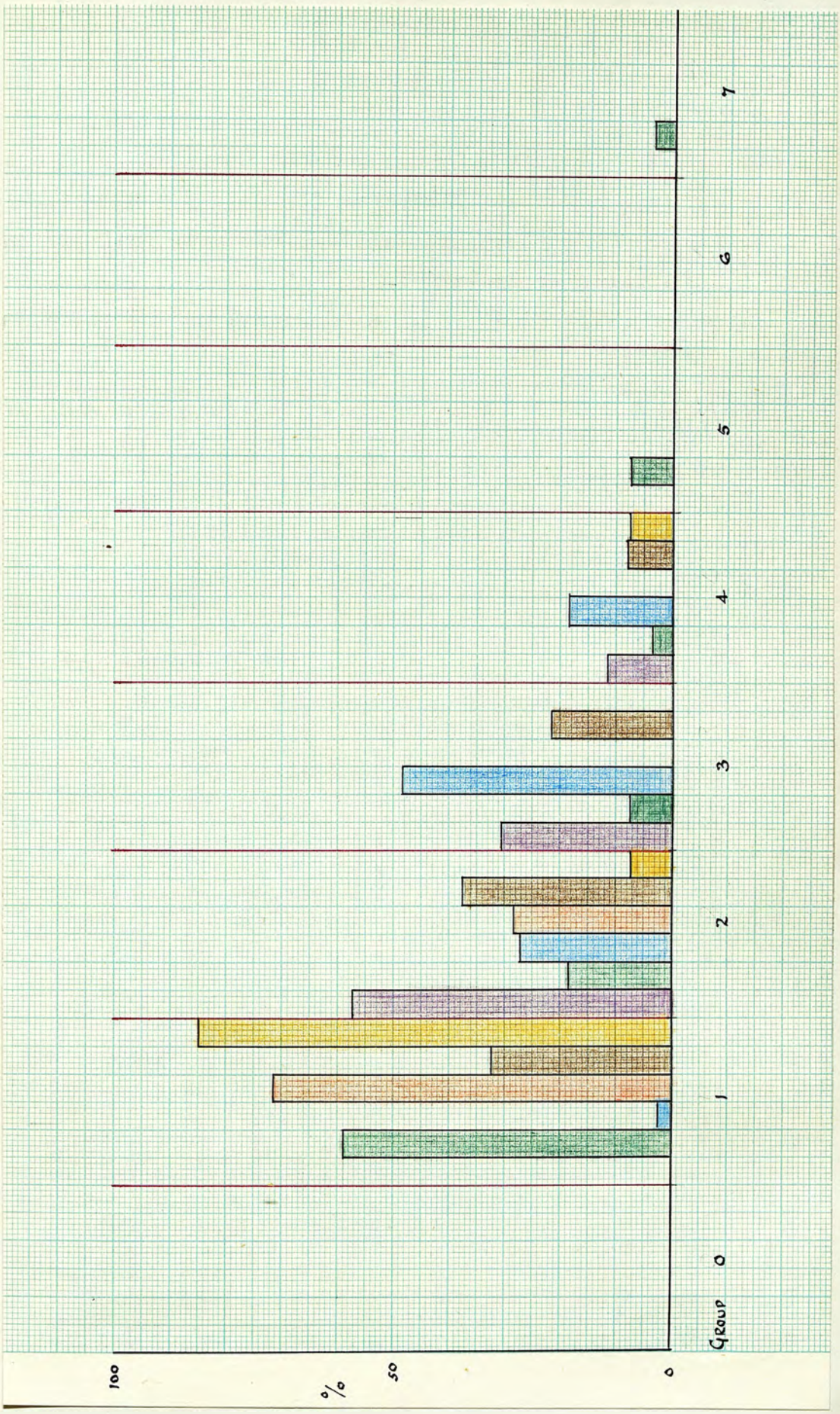


Histogram 13.

This shows the depth of the rots produced on Catriona by isolate 1.

Colours are according to the key attached to histogram 29.

Histogram 13.

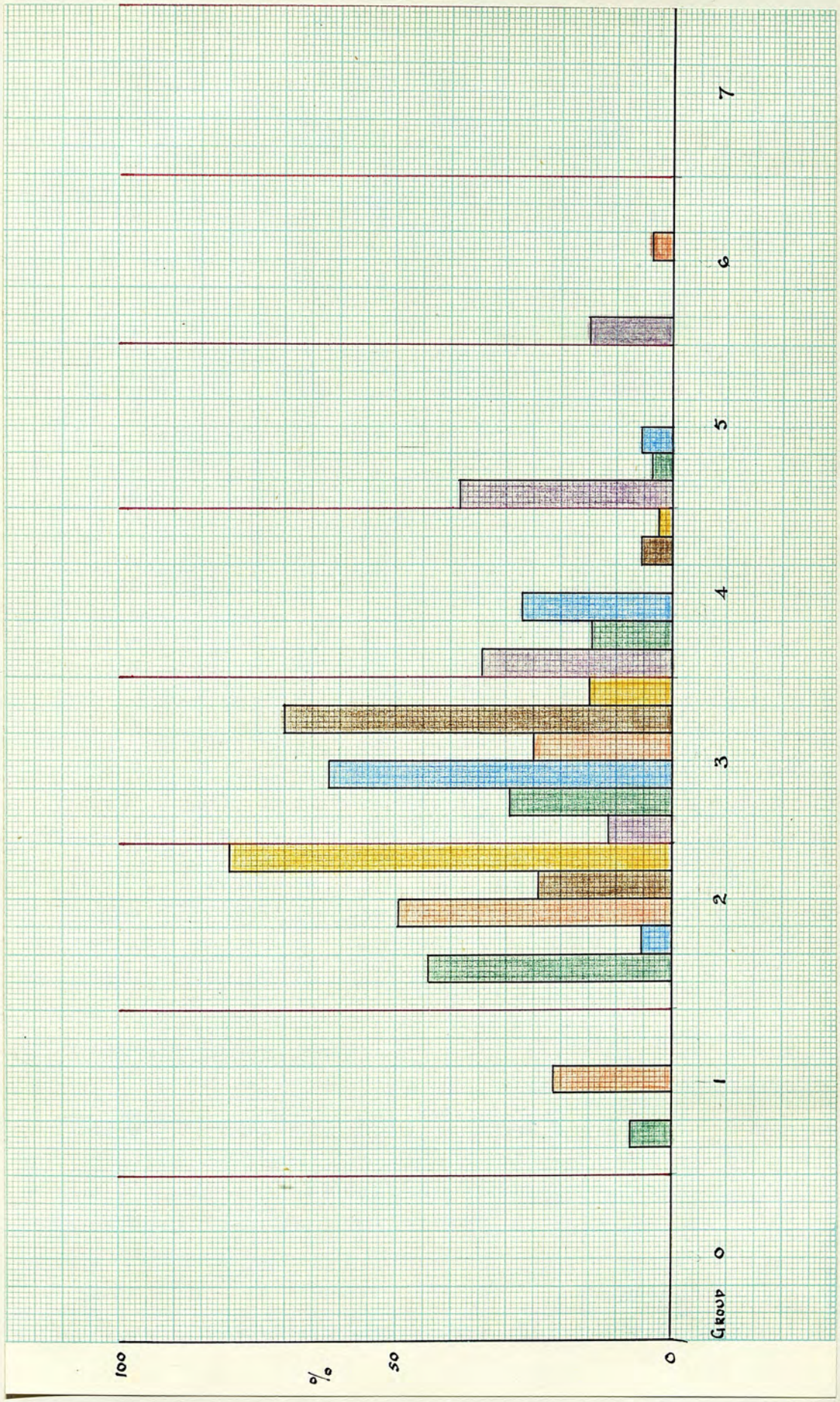


Histogram 14.

This shows the diameter of the rots produced on Catriona by isolate 1.

Colours are according to the key attached to histogram 29.

Histogram 14.

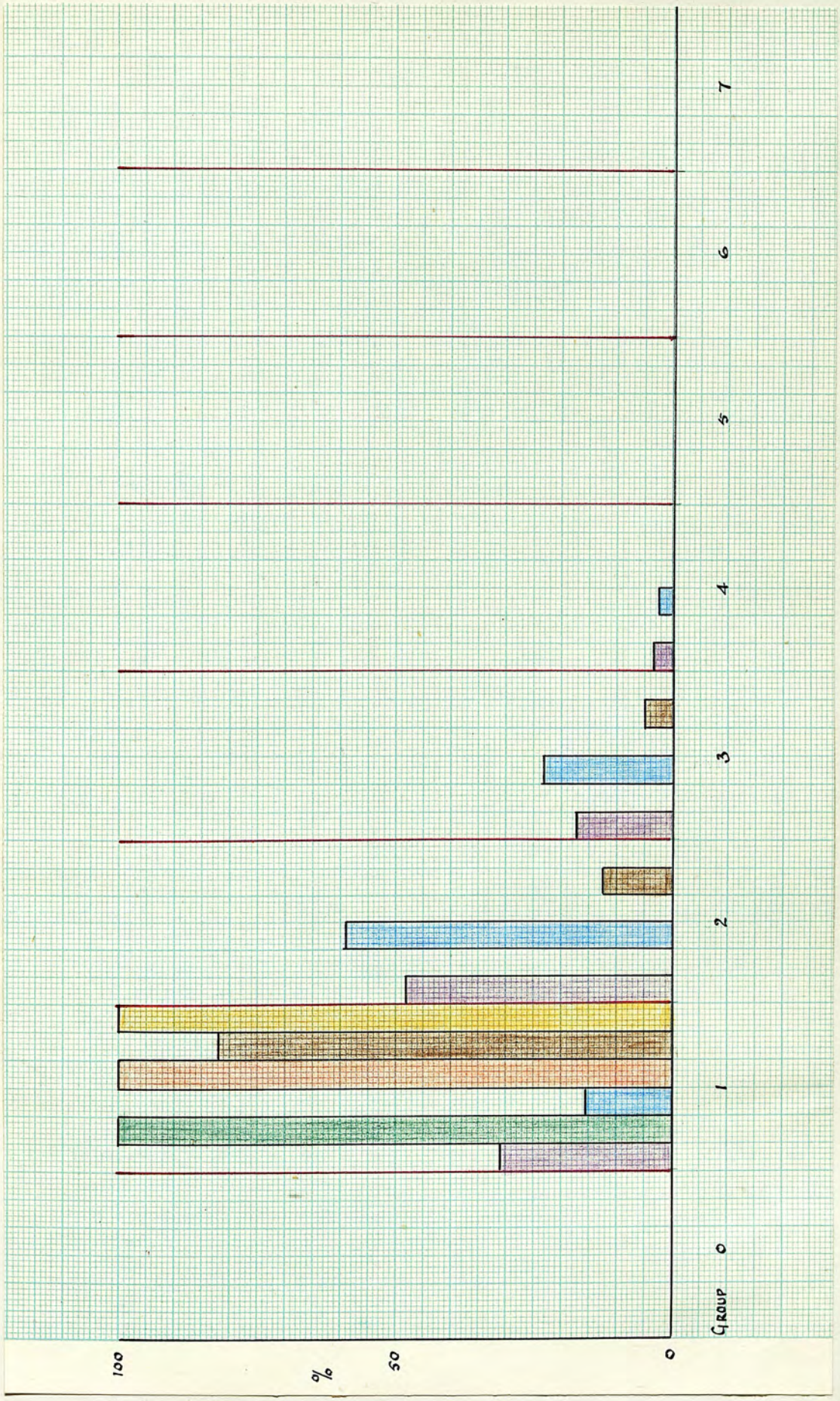


Histogram 15.

This shows the depth of the rots produced on Doon Star by isolate 1.

Colours are according to the key attached to histogram 29.

Histogram 15.

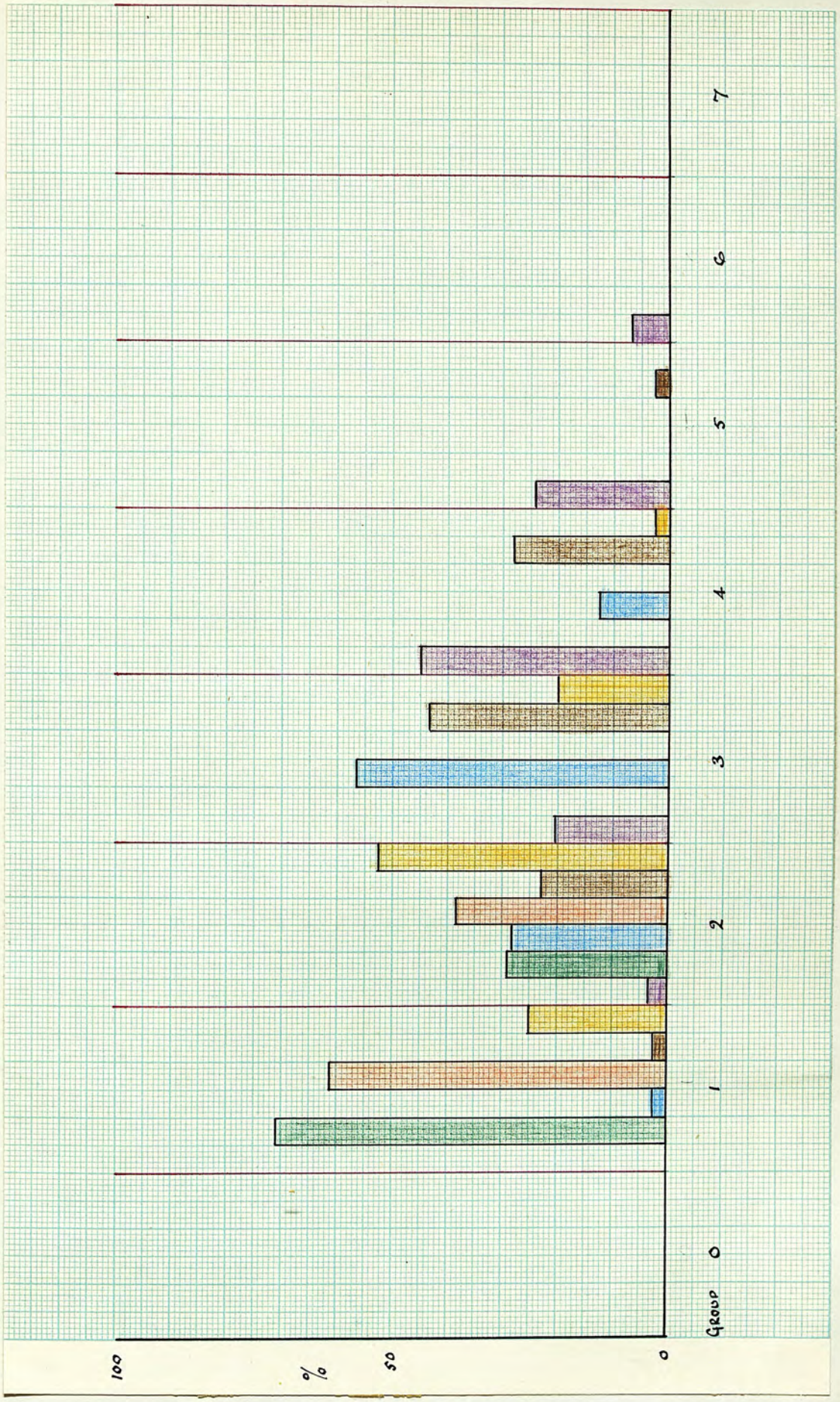


Histogram 16.

This shows the diameter of the rots produced on Doon Star by isolate 1.

Colours are according to the key attached to histogram 29.

Histogram 16.

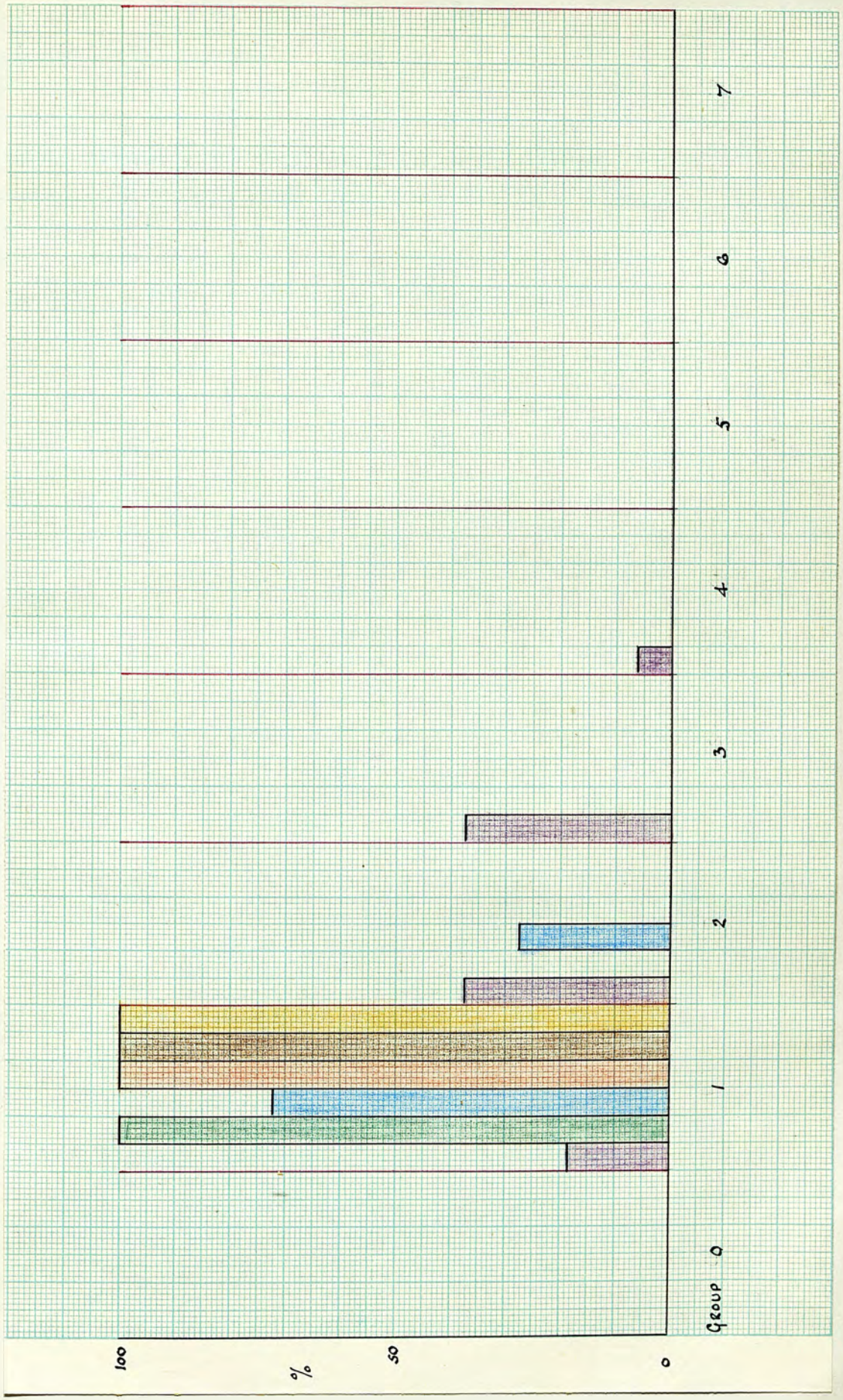


Histogram 17.

This shows the depth of the rots produced on Arran Banner by isolate 1.

Colours are according to the key attached to histogram 29.

Histogram 17.

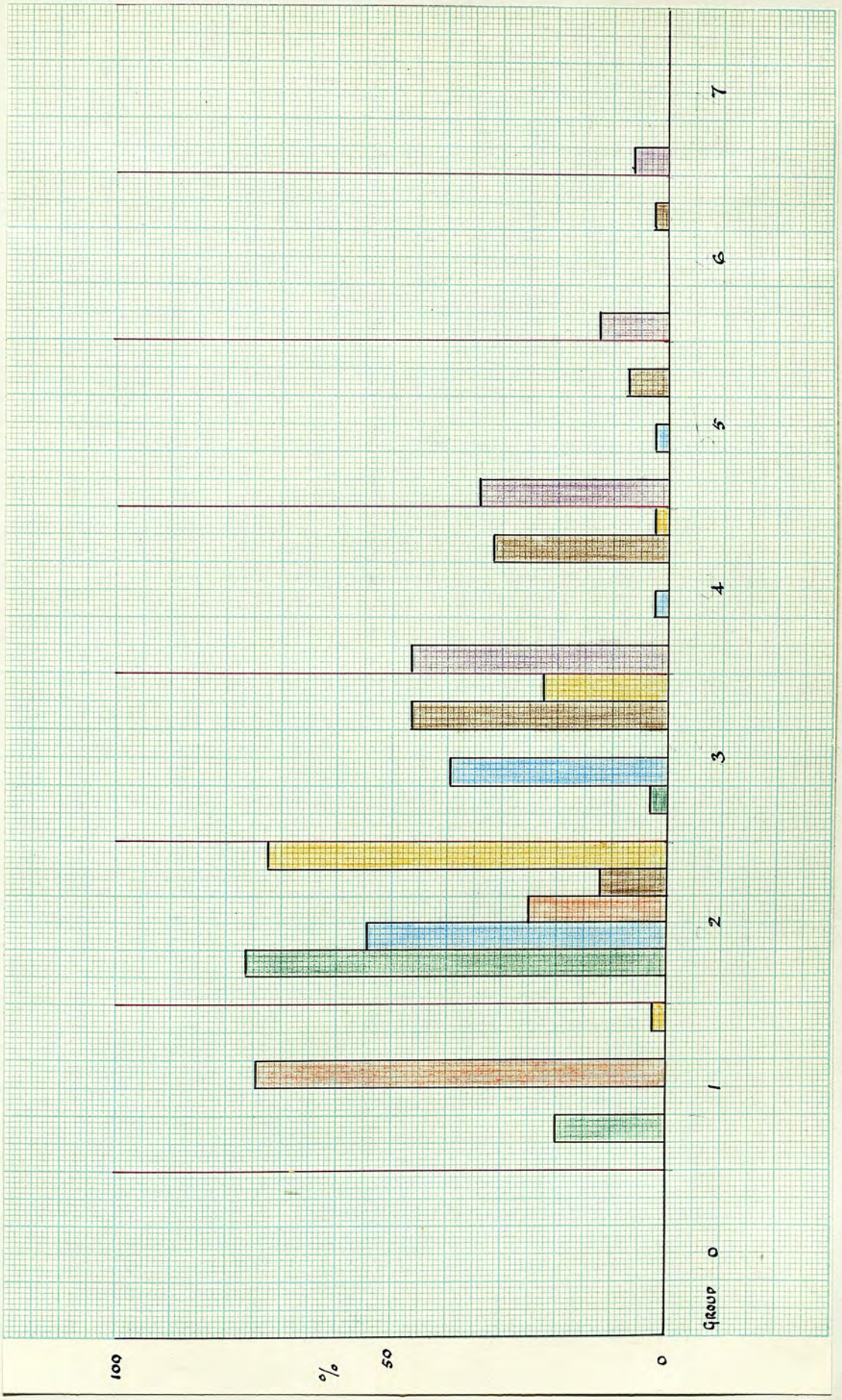


Histogram 18.

This shows the diameter of the rots produced on Arran Banner by isolate 1.

Colours are according to the key attached to histogram 29.

Histogram 18.

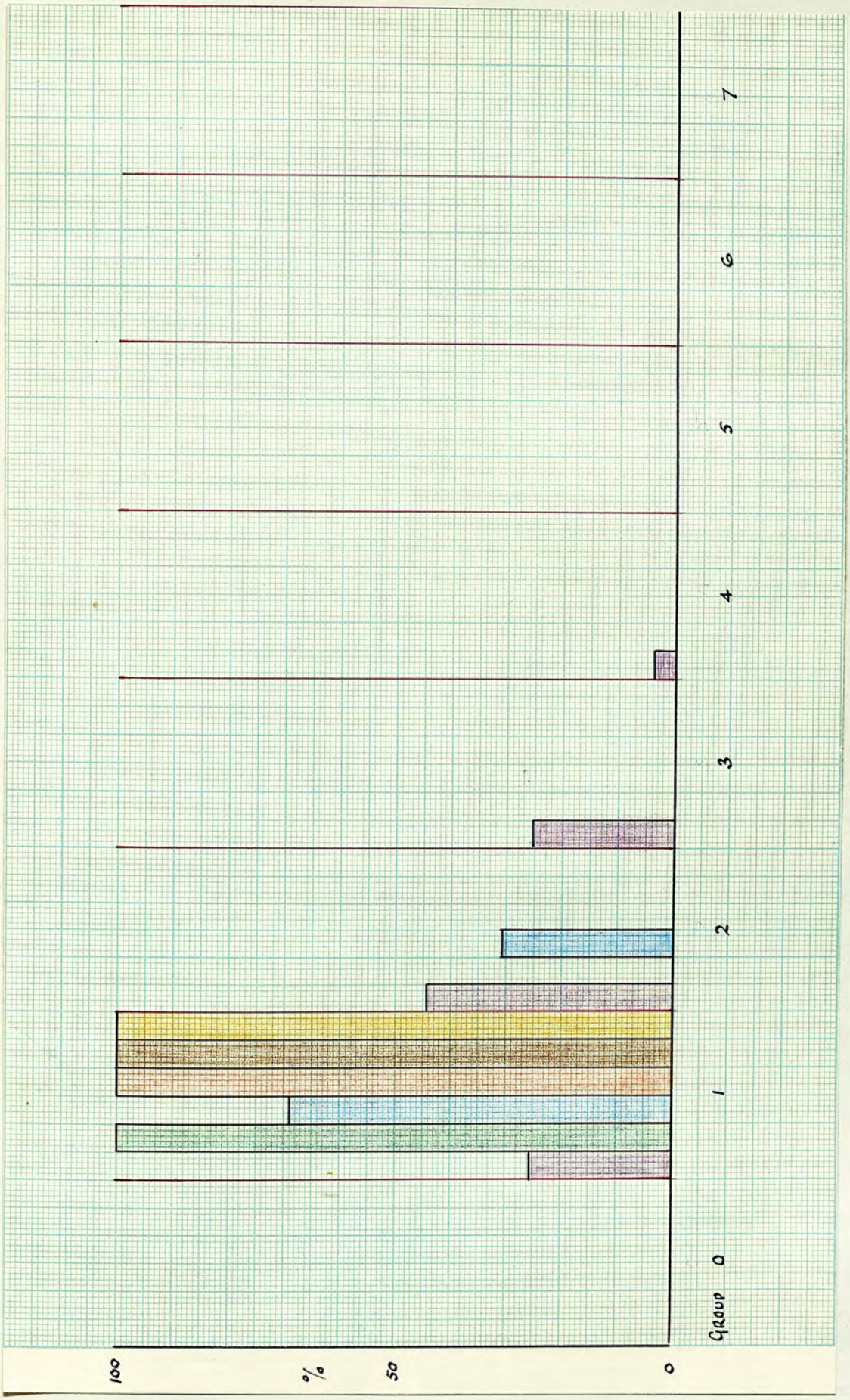


Histogram 19.

This shows the depth of the rots produced on Kerr's Pink by isolate 1.

Colours are according to the key attached to histogram 29.

Histogram 19.

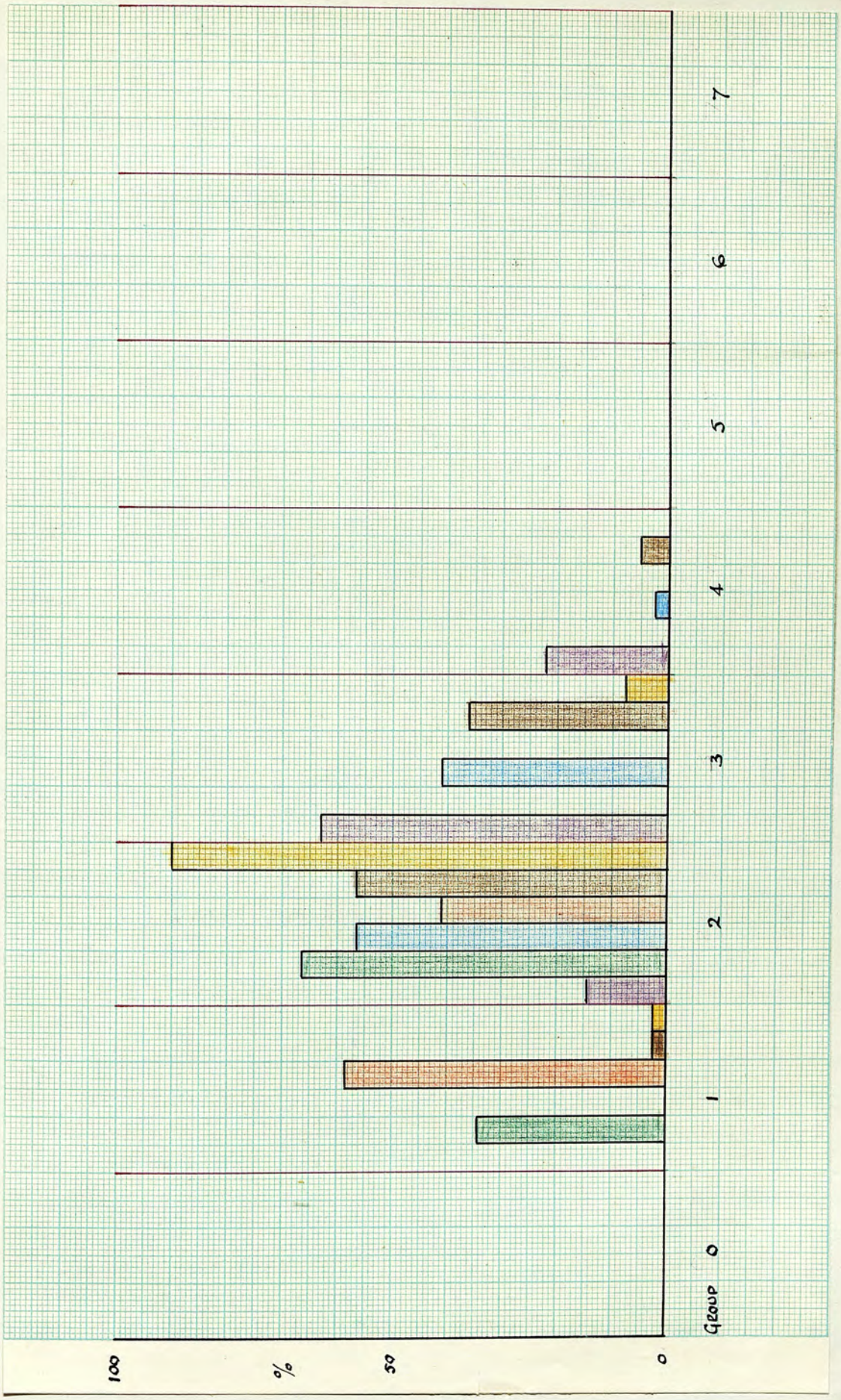


Histogram 20.

This shows the diameter of the rots produced on Kerr's Pink by isolate 1.

Colours are according to the key attached to histogram 29.

Histogram 20.

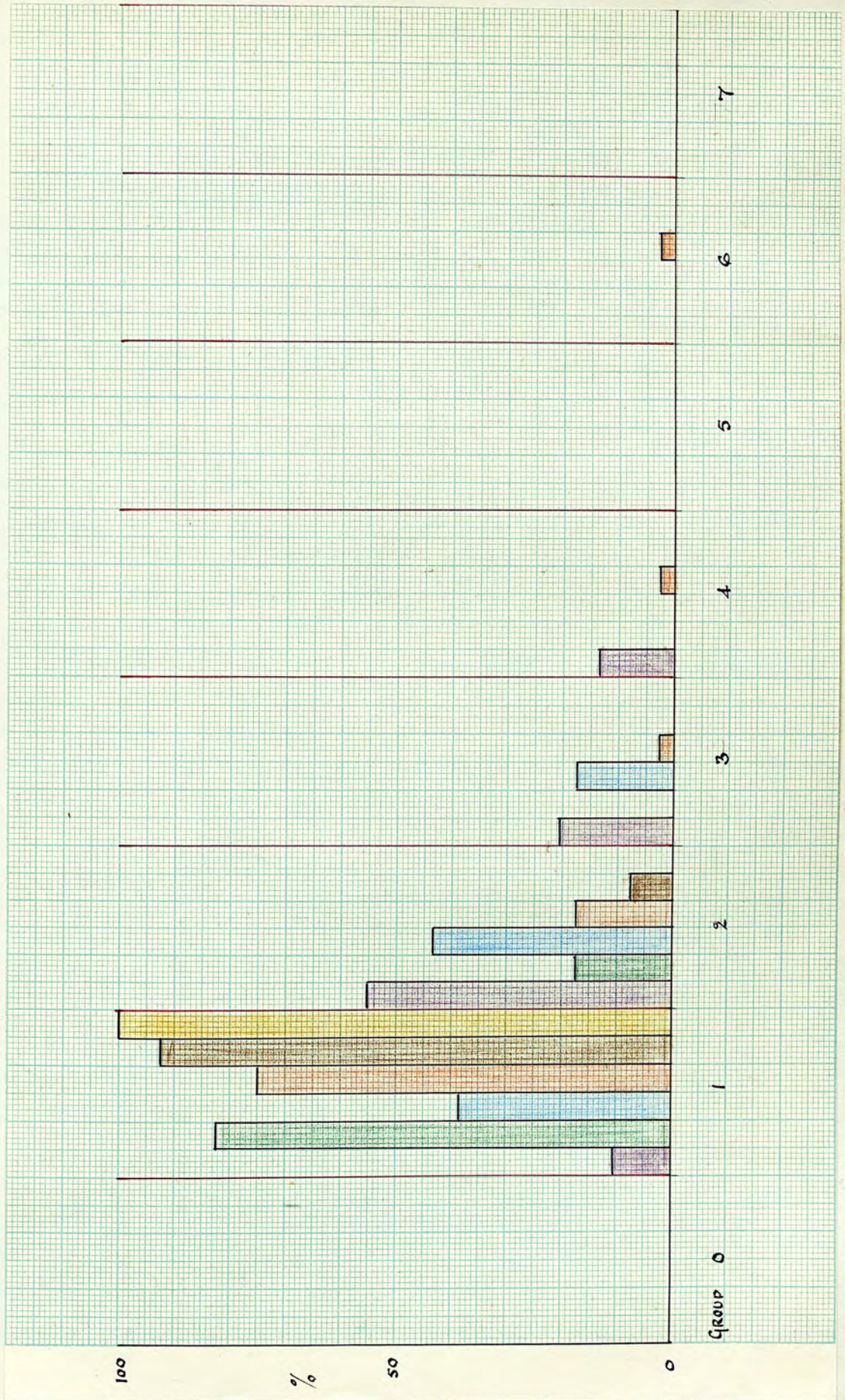


Histogram 21.

This shows the depth of the rots produced on Golden Wonder by isolate 1.

Colours are according to the key attached to histogram 29.

Histogram 21.

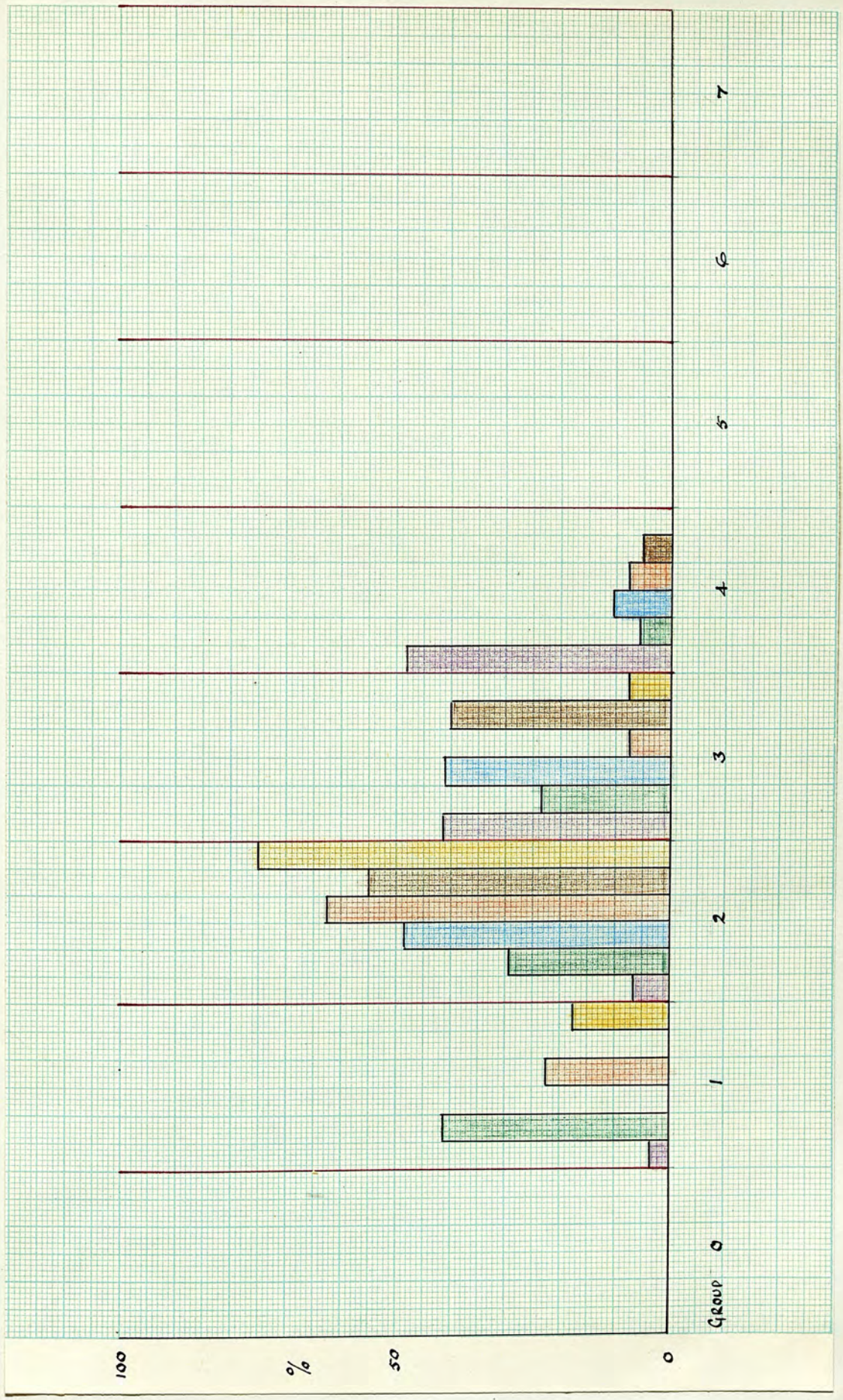


Histogram 22.

This shows the diameter of the rots produced on Golden Wonder by isolate 1.

Colours are according to the key attached to histogram 29.

Histogram 22.

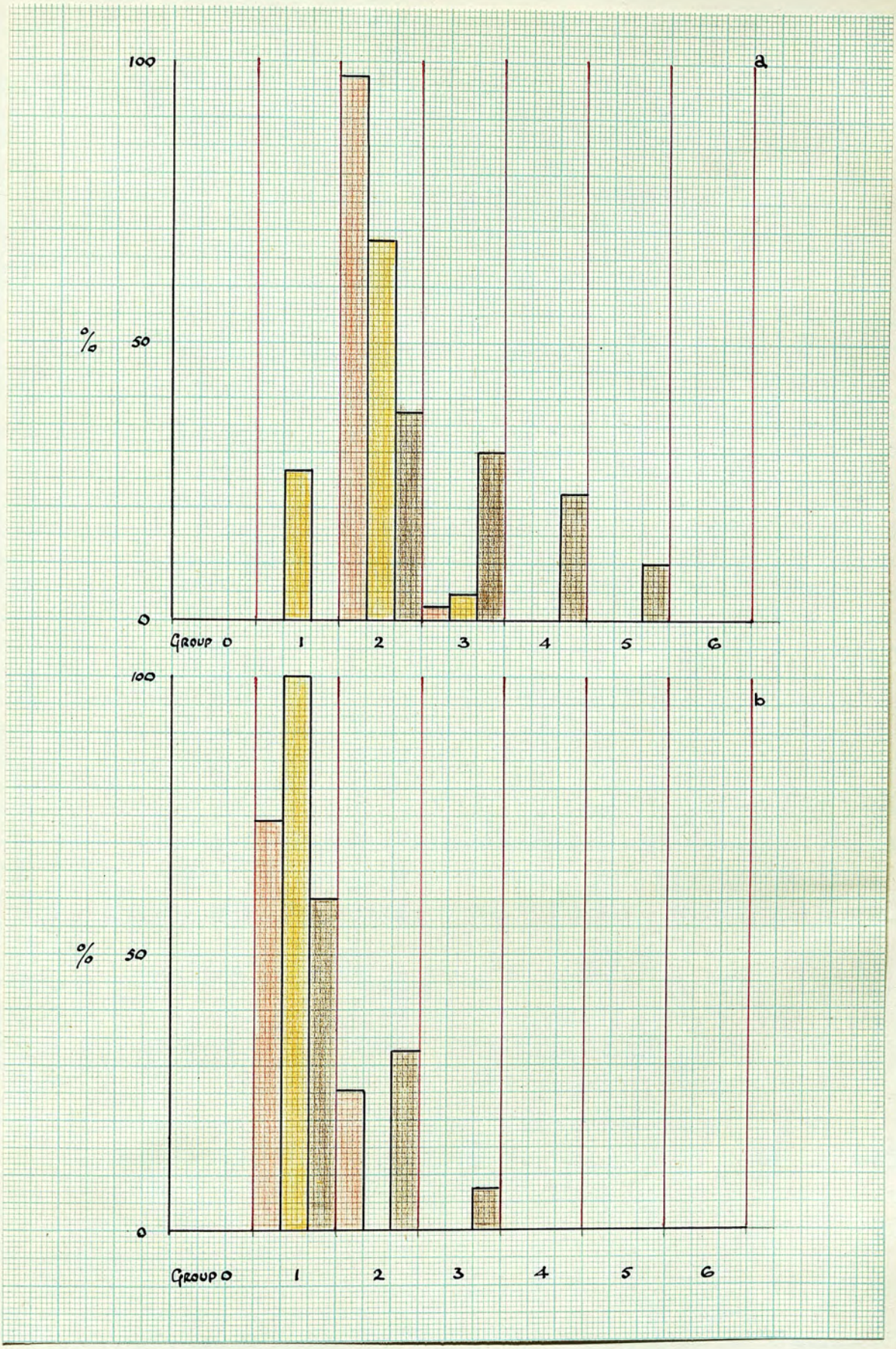


Histogram 23.

This shows the diameter (a) and the depth (b) of the rots produced on Arran Pilot by isolate 2.

Colours are according to the key attached to histogram 29.

Histogram 23.

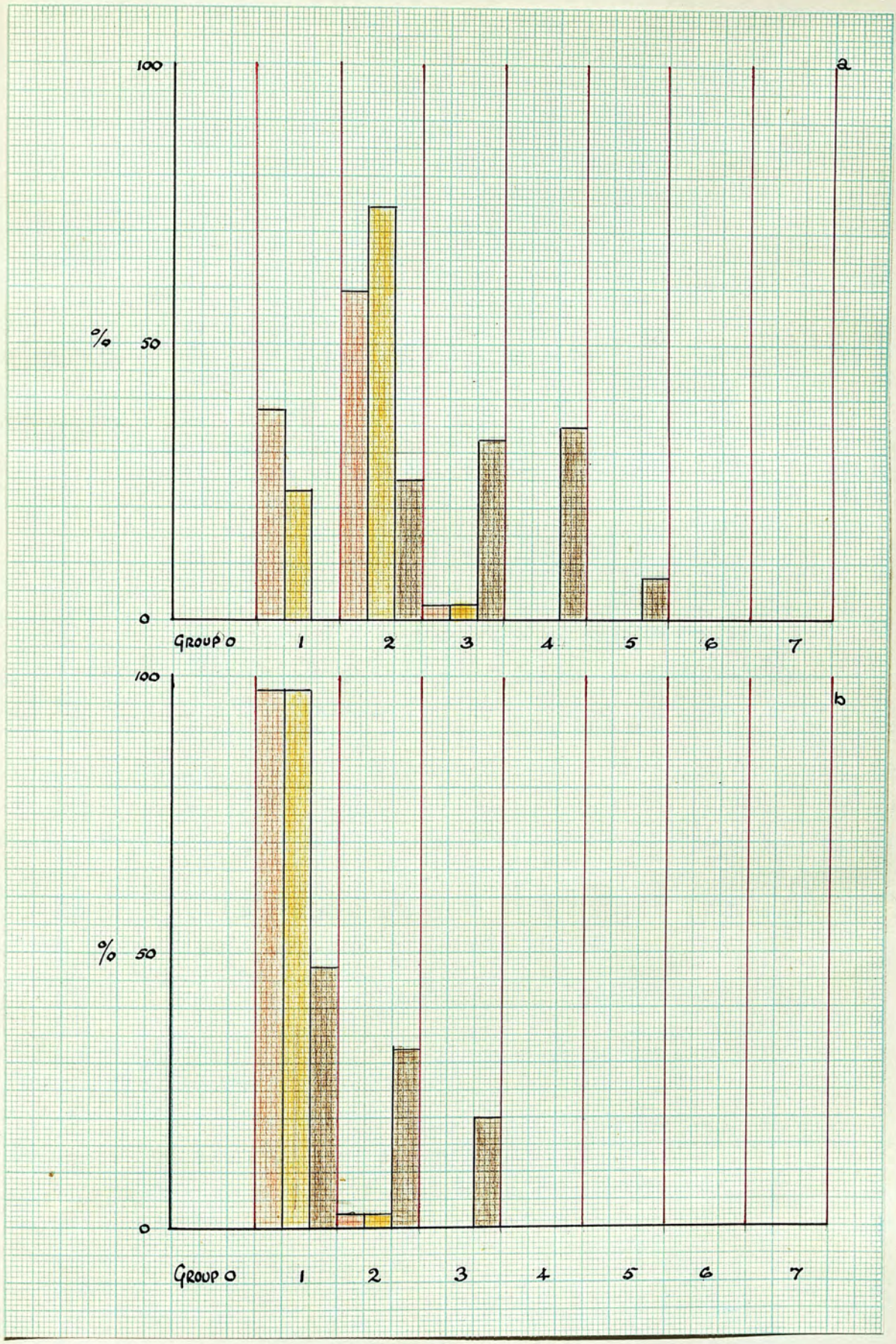


Histogram 24.

This shows the diameter (a) and the depth (b) of the rots produced on Home Guard by isolate 2.

Colours are according to the key attached to histogram 29.

Histogram 24.

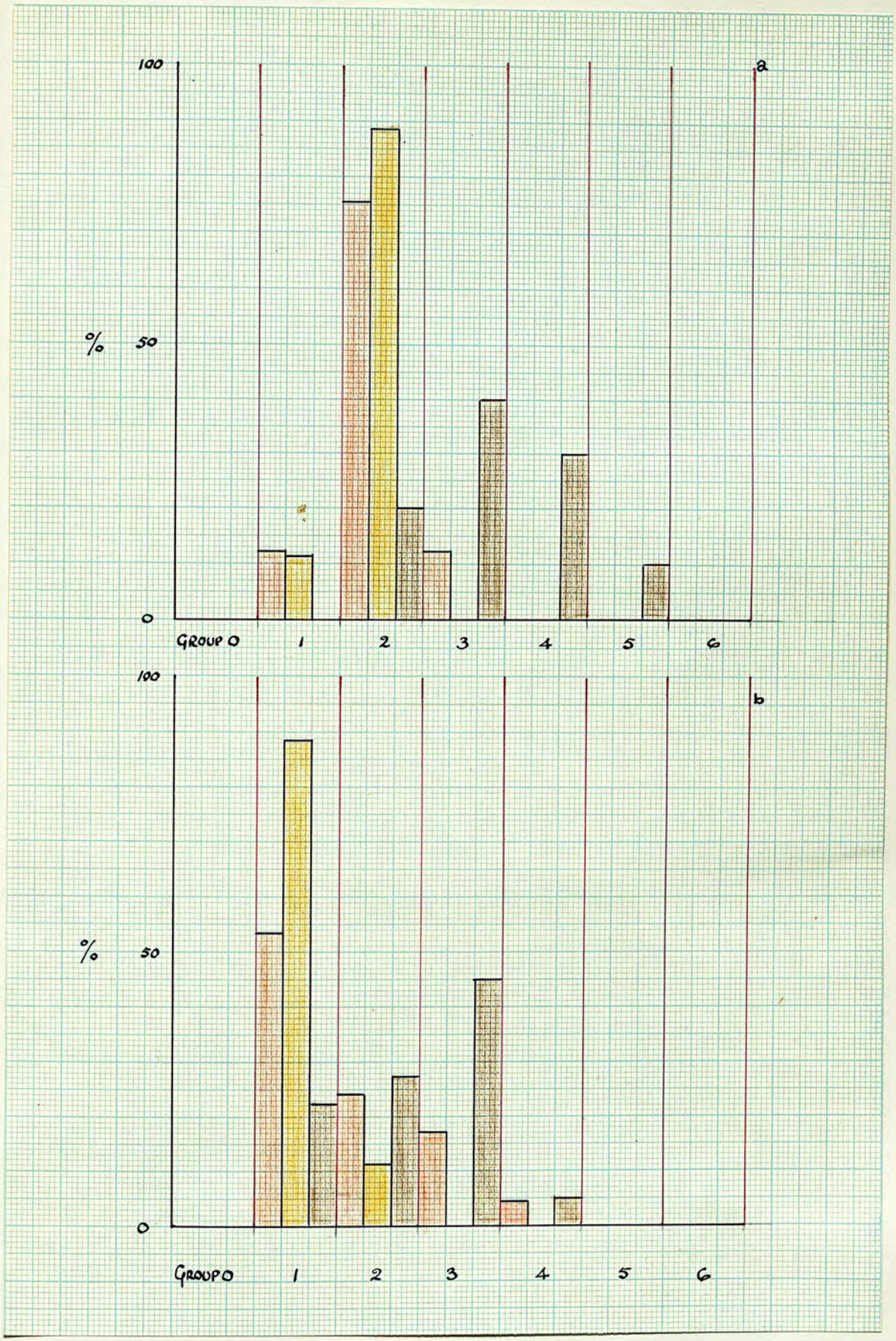


Histogram 25.

This shows the diameter (a) and the depth (b) of the rots produced on Catriona by isolate 2.

Colours are according to the key attached to histogram 29.

Histogram 25.

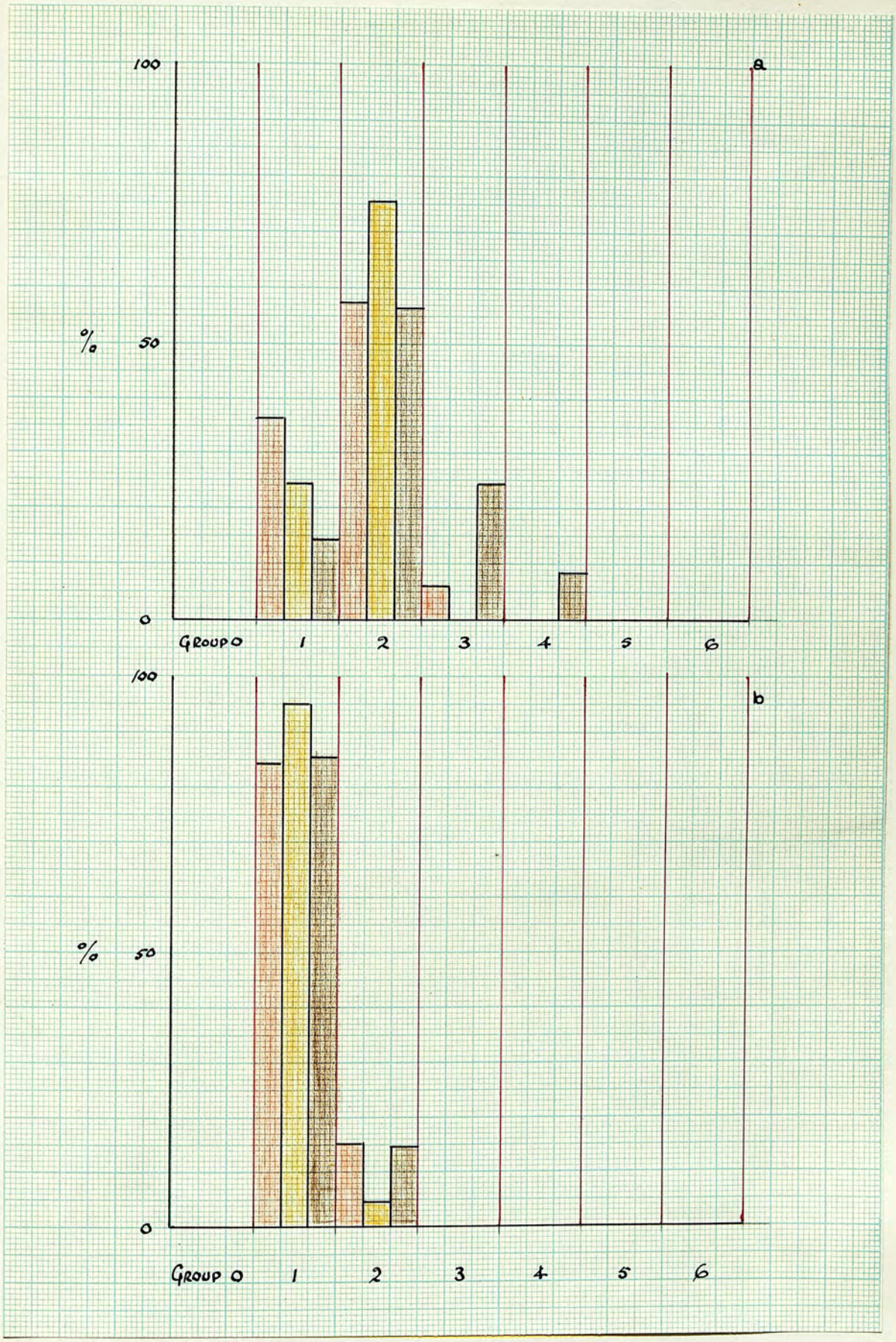


Histogram 26.

This shows the diameter (a) and the depth (b) of the rots produced on Doon Star by isolate 2.

Colours are according to the key attached to histogram 29.

Histogram 26.

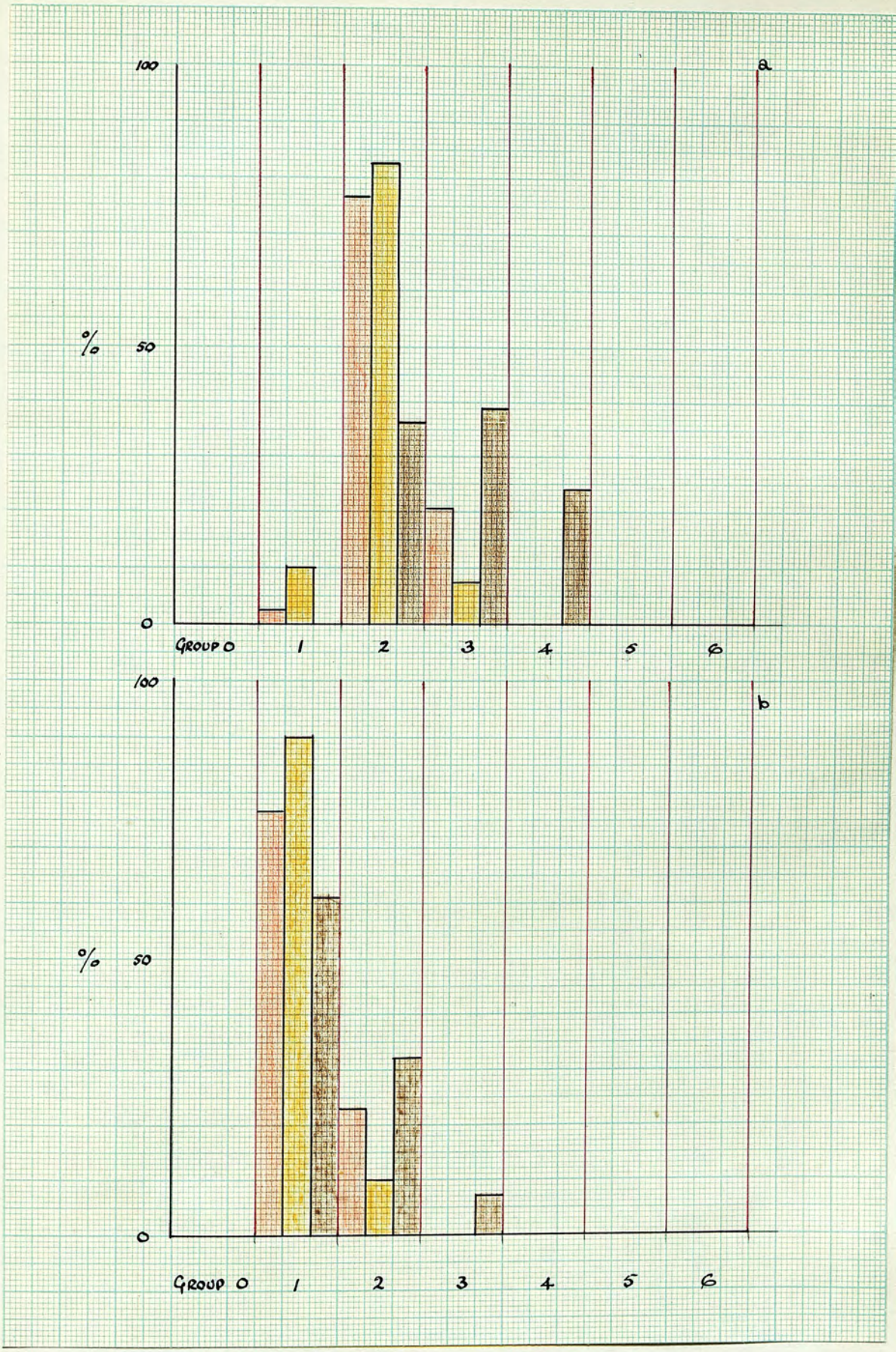


Histogram 27.

This shows the diameter (a) and the depth (b) of the rots produced on Arran Banner by isolate 2.

Colours are according to the key attached to histogram 29.

Histogram 27.

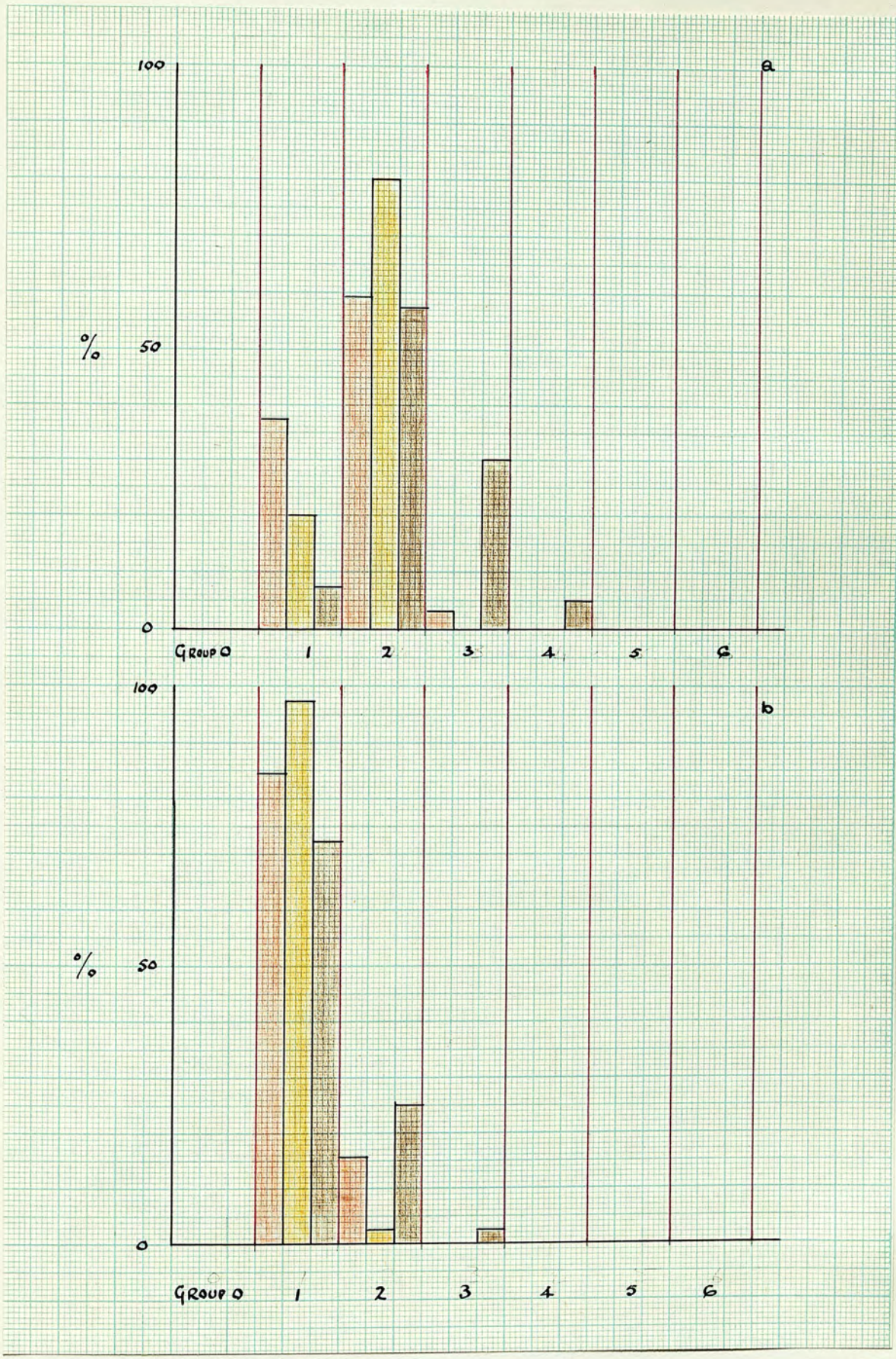


Histogram 28.

This shows the diameter (a) and the depth (b) of the rots produced on Kerr's Pink by isolate 2.

Colours are according to the key attached to histogram.29.

Histogram 28.

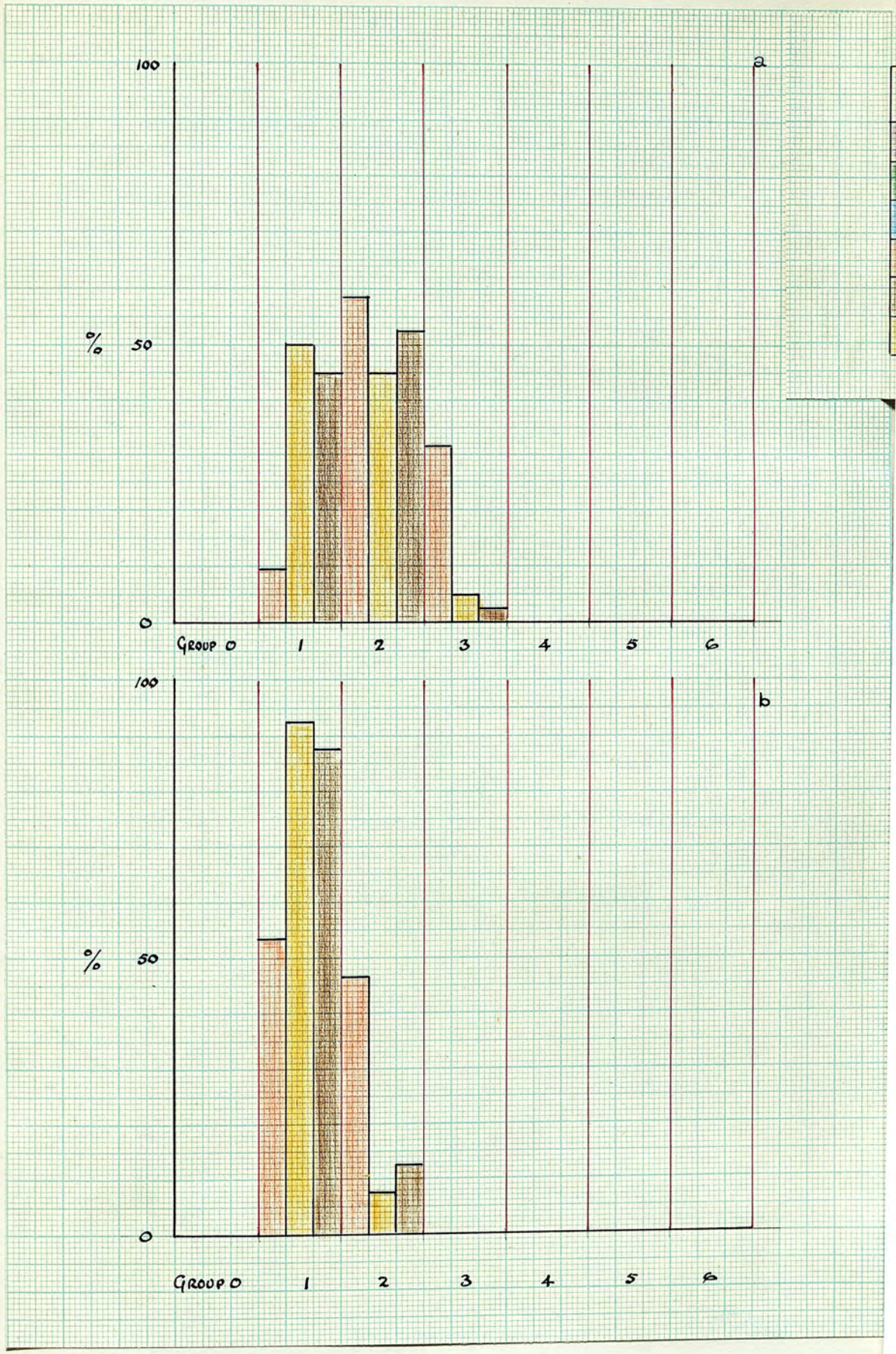


Histogram 29.

This shows the diameter (a) and the depth (b) of the rots produced on Golden Wonder by isolate 2.

Colours are according to the key attached.

Histogram 29.



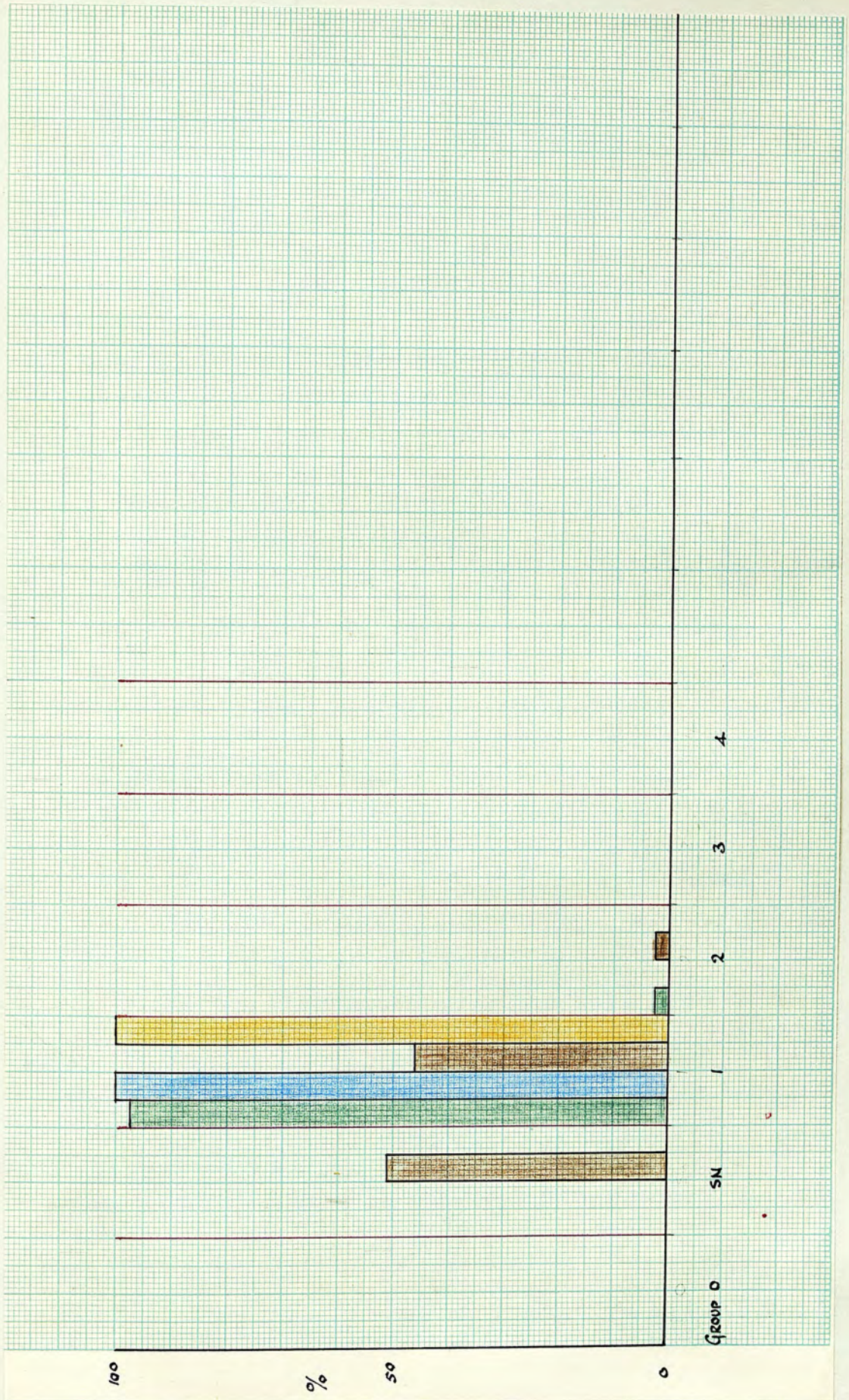
	Pre-inoculation.	Post inoculation.	Inoculated.
	Room temperature	Store room	December
	Room temperature	Room temperature	"
	Store room	Store room	"
	Store room	Room temperature	"
	Store room	Store room	March
	Store room	Room temperature	"

Histogram 30.

This shows the depth of the rots produced on Majestic by isolate 1.

Colours are according to the key attached to histogram 32.

Histogram 30.

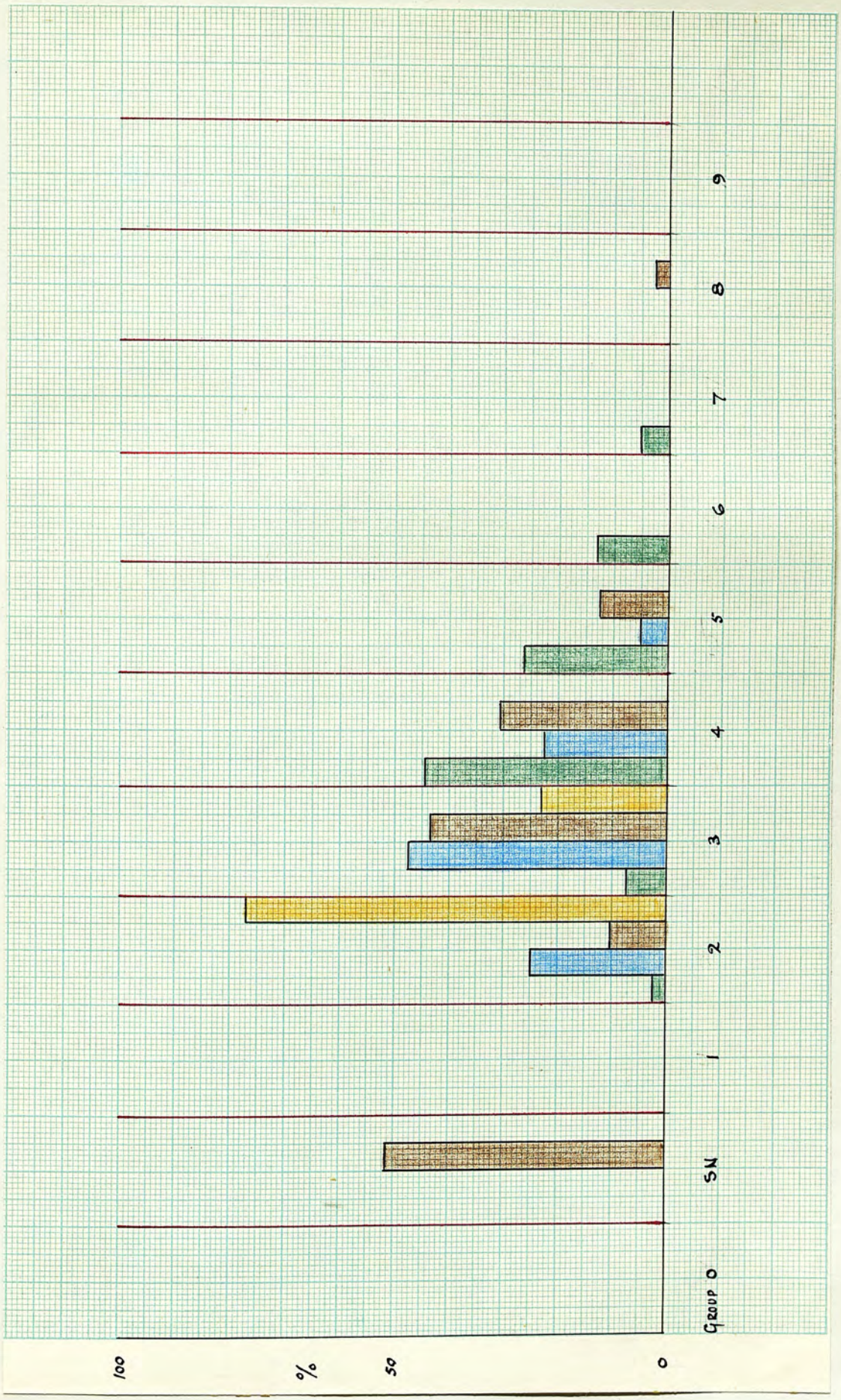


Histogram 31.

This shows the diameter of the rots produced on Majestic by isolate 1.

Colours are according to the key attached to histogram 32.

Histogram 31.

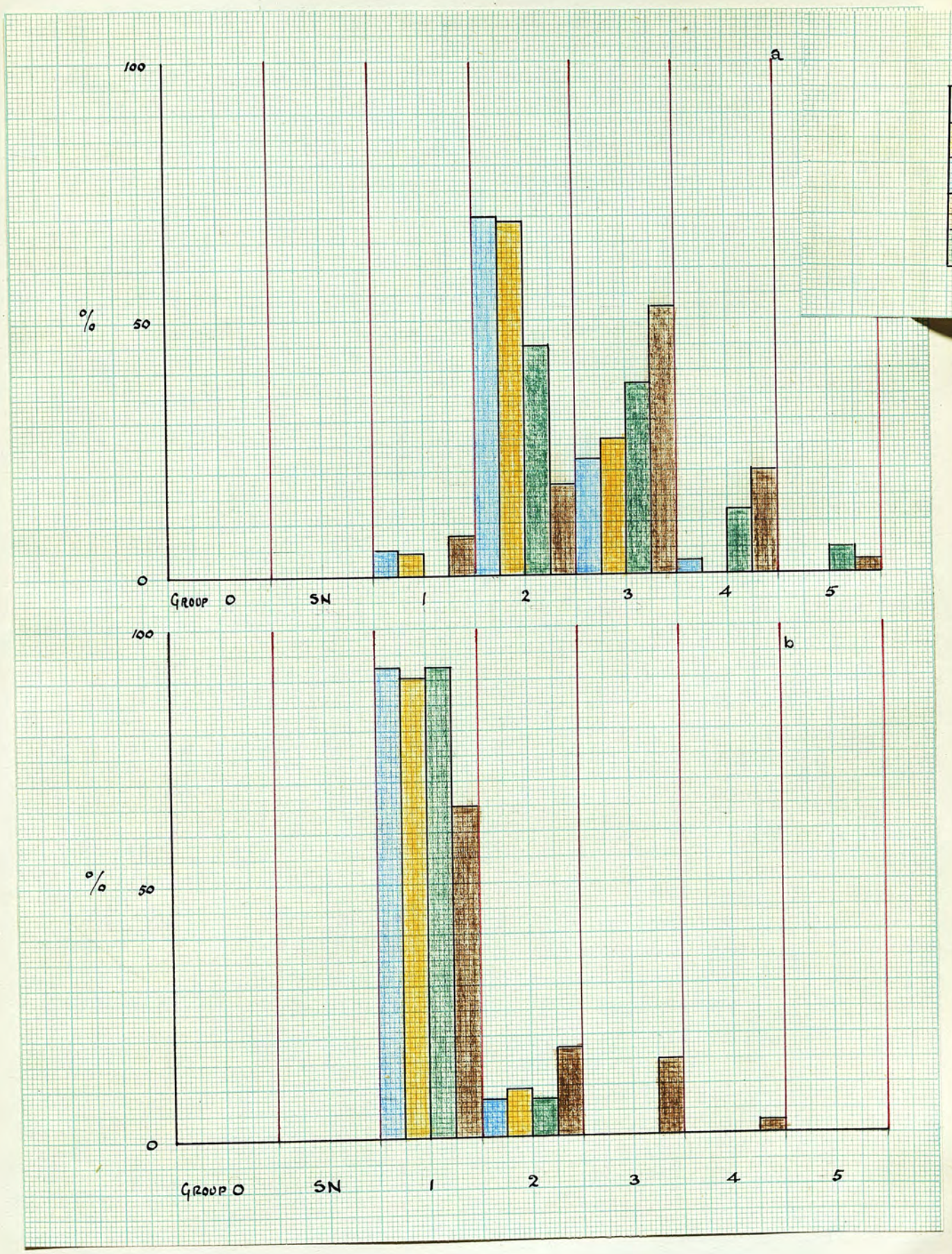


Histogram 32.

This shows the diameter and depth (a and b respectively) of the rots produced on Majestic by isolate 2.

Colours are according to the key attached.

Histogram 32.



	Soil	Post inoculation storage
	Peat	Room temperature
	Light	Room temperature
	Peat	Store room
	Light	Store room

PLATE I.

figure 1.

This shows a Majestic tuber with the typical "thumb mark" type of depression found in Gangrene.

figure 2.

This shows a naturally infected Great Scot tuber with a large, surface depression due to Gangrene. Note the irregular margin and taut skin of the depression.

figure 3.

This is a Gangrene infected Majestic tuber on which the skin covering the rot has become wrinkled. Pycnidia may be seen at A.

PLATE I.

figure 1.

figure 2.

figure 3.

PLATE II.

figures 1 and 2.

This shows a magnified view of the rotted tissue in a tuber suffering from Gangrene.

A— skin of the tuber.

B— Rotted tissue where the cell walls have been destroyed. This consists of starch grains held together by hyphae.

C— Margin of the rot where the tissue is in the process of decay. The cell walls have not yet been destroyed.

D— Unaffected tissue.

E— Pycnidium.

figure 3.

This is a magnified view of a section of a rot showing a pycnidium within the rotted tissue and in the sub-epidermal layers.

PLATE II.

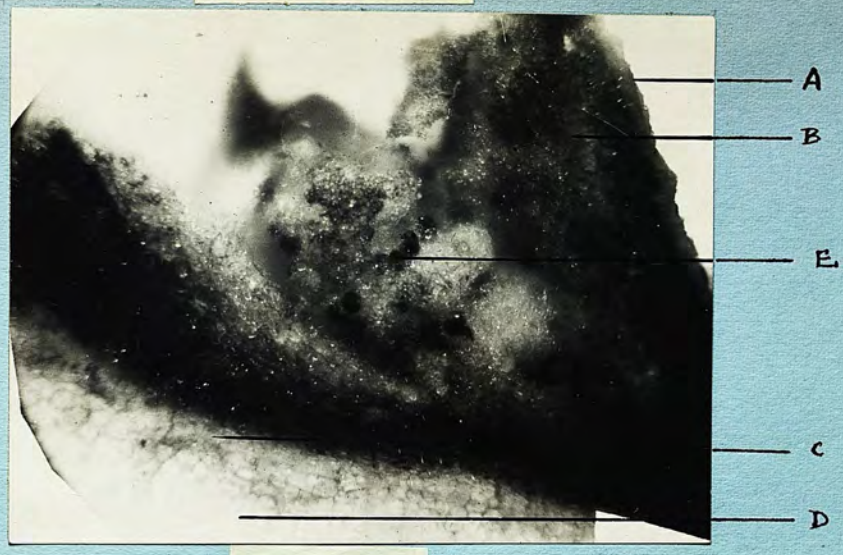


figure 1.

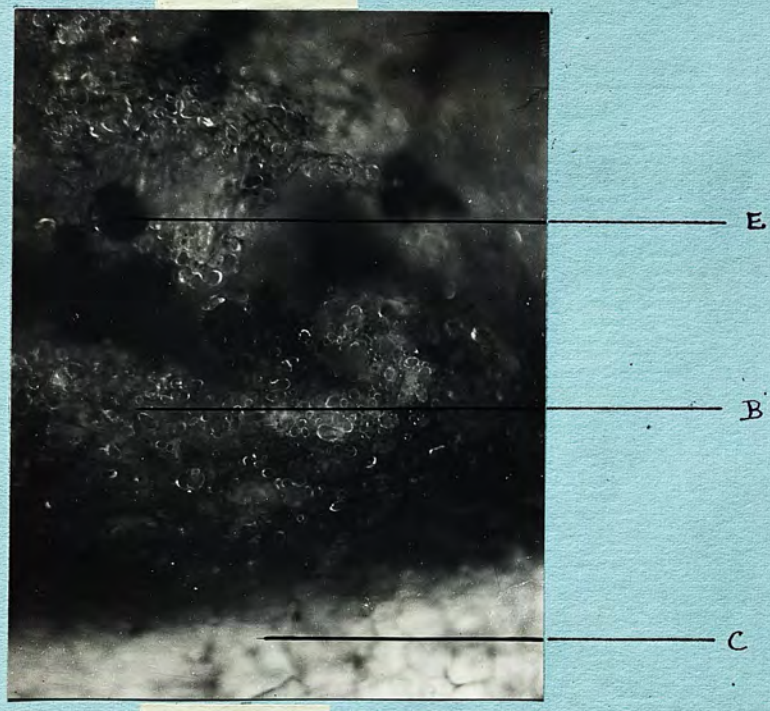


figure 2.

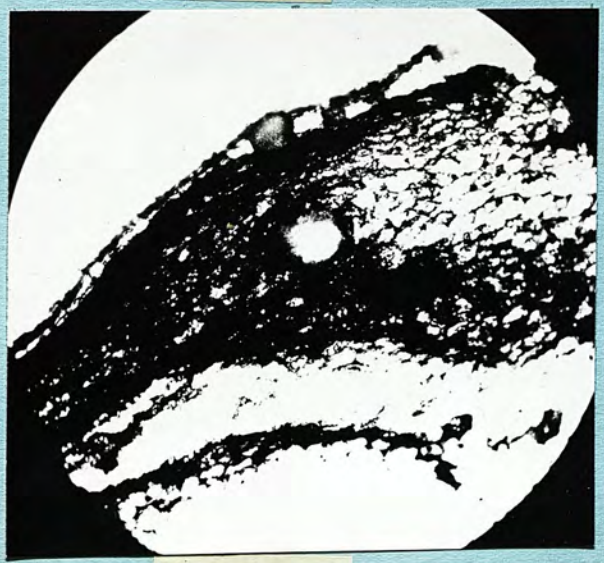


figure 3.

PLATE III.

figure 1.

This shows a Gangrene infected Great Scot tuber cut to show the internal symptoms of the disease. The rot contains large cavities and a group of pycnidia may be seen at A.

figure 2.

This shows a Gangrene infected tuber cut to show, on the left, a compact rot with small cavities starting to form. On the right it may be seen that the rot is so compact that it is possible to lift it out in one piece.

figure 3.

This shows an Arran Banner tuber cut to show the internal symptoms in a late stage of Skin Necrosis. The rot has progressed over the surface but has not penetrated to any depth.

PLATE III.

figure 1.

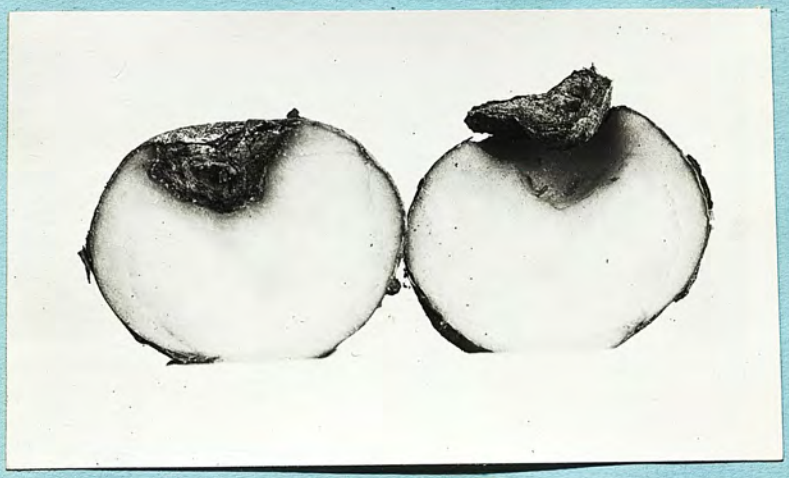


figure 2.

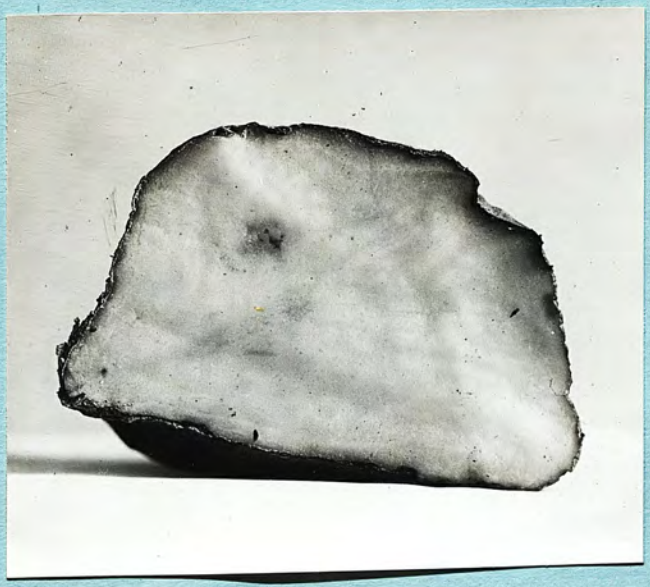


figure 3.

PLATE IV.

figure 1.

This is an Arran Banner tuber showing an early stage in Skin Necrosis. The disease is seen on small, irregular necrotic regions on the skin.

figure 2.

This is an Arran Banner tuber showing a late stage in Skin Necrosis.

A— diseased tissue.

B— healthy tissue.

C— pycnidia.

figure 3.

Arran Banner tuber showing the final stage in Skin Necrosis. Note how the tuber has become shrivelled. At A may be seen pycnidia which were produced under the skin which is now detached. At B the skin is in the process of being lifted off by the underlying pycnidia.

PLATE IV.

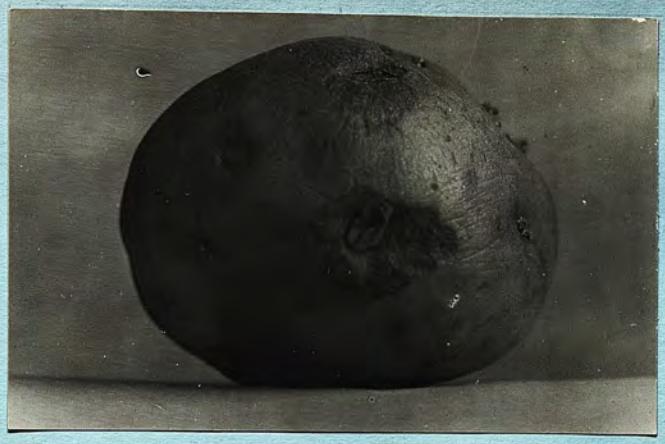


figure 1.

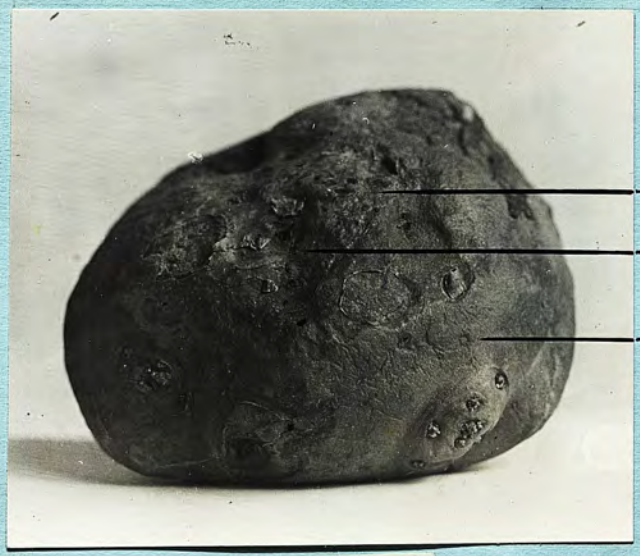


figure 2.

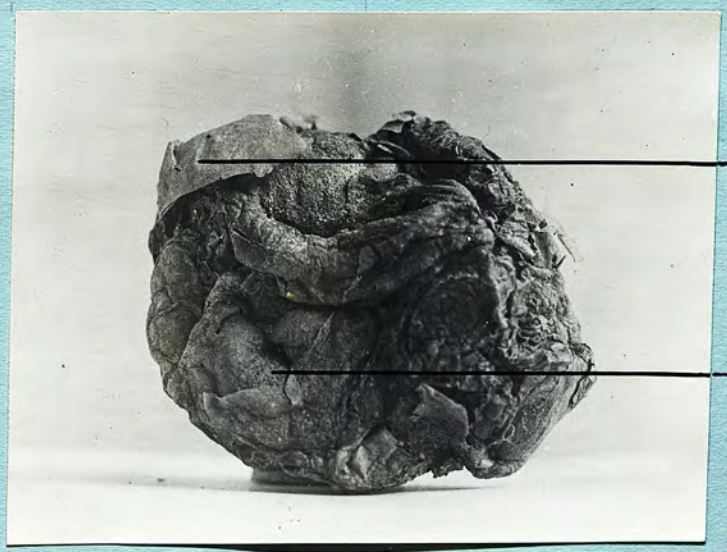


figure 3.

PLATE V.

figure 1.

Ostiolate pycnidia produced by isolate 1 on the lesion of the Arran Banner stem seen in figure 3

Figure 2.

Depression on the surface of a Gangrene infected tuber showing erumpent pycnidia.

figure 3.

Lesion caused by isolate 1 on a living Arran Banner stem.

figure 4.

Stellate crystals (A) produced in cultures of isolate 1.

PLATE V.

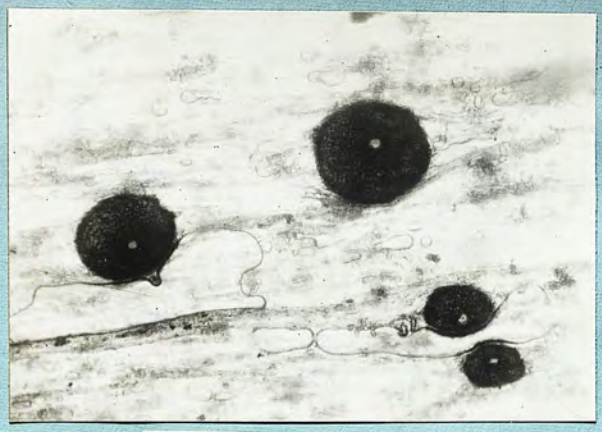


figure 1.

figure 4.

figure 2.

figure 3.

PLATE VI.

figure 1.

Crystals produced by isolate 1. Those needle like crystals occur in both pure and parasitised cultures.

figure 2.

This shows hyphal regeneration of isolate 1. Within the old hypha may be seen the new hypha which has disturbed the granular contents of the old hypha.

figure 3.

In hypha A may be seen the origin of the intrahyphal growth seen in figure 2.

PLATE VI.

figure 1.

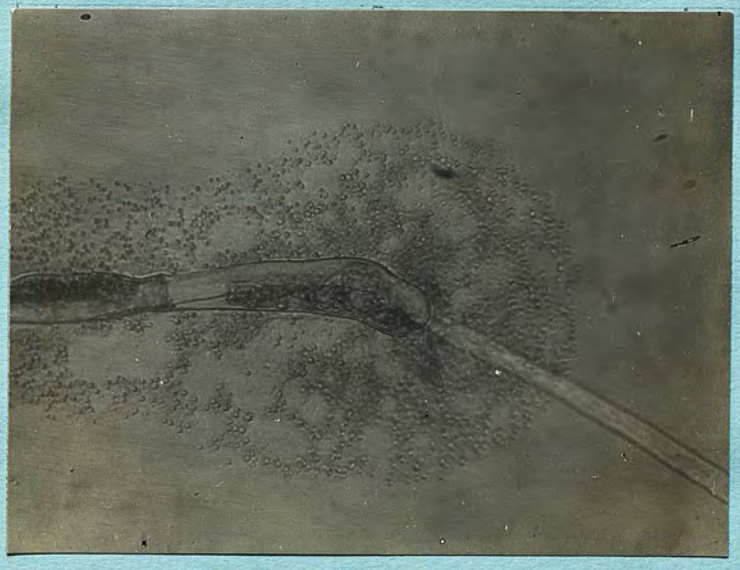


figure 2.

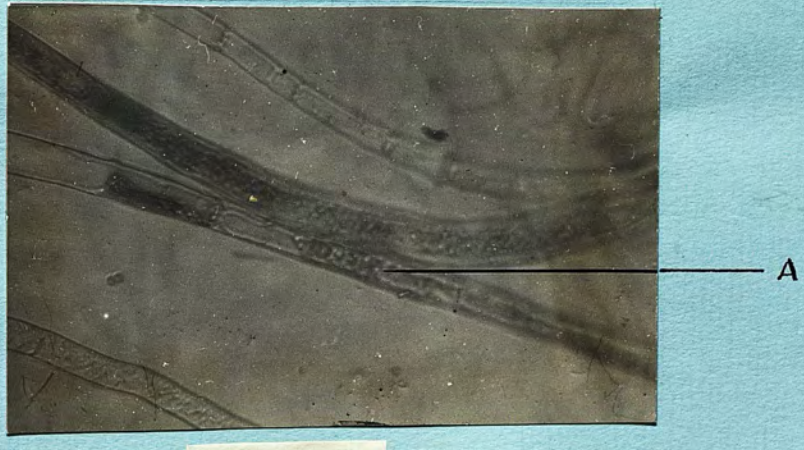


figure 3.

PLATE VII.

figure 1.

This shows a hypha of isolate 1 being attacked by *Trichoderma viride*. The *Phoma* hypha in the centre has the *Trichoderma* winding round it.

figure 2.

This shows a hypha of isolate 1 being attacked by *Trichoderma viride*. Note the profusion of *Trichoderma* in the region of the *Phoma*

A— isolate 1 hypha.

figure 3.

isolate 2 hypha (A) being attacked by *Trichoderma viride*. Observe the start of penetration by *Trichoderma* (B) which is pushing in the wall of the *Phoma*.

PLATE VII.

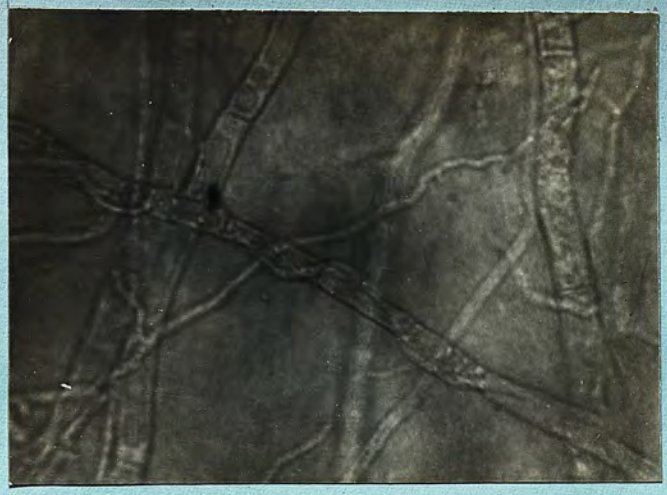


figure 1.

figure 2.

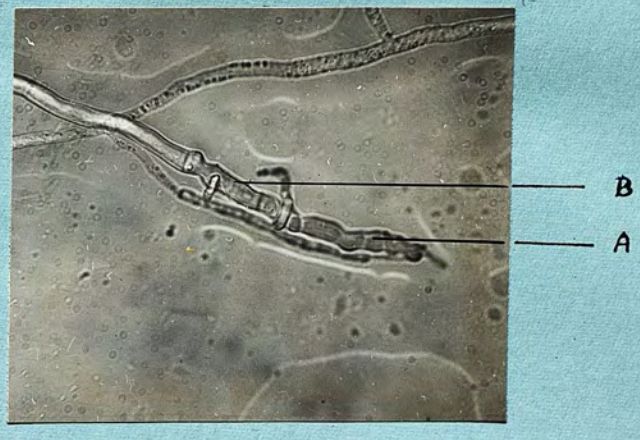


figure 3.

PLATE VIII.

figure 1.

Isolate 3 hypha being attacked by Acrostalagmus cinnabarinus. At A the Acrostalagmus hypha has penetrated that of the Phoma (B) which has become swollen. The Acrostalagmus hypha at C is producing two branches which are growing along the Phoma.

figure 2.

The hyphae seen in figure 1 three hours later. Note the rapid growth of the Acrostalagmus along the Phoma which at the points of contact has become devoid of contents.

PLATE VIII.

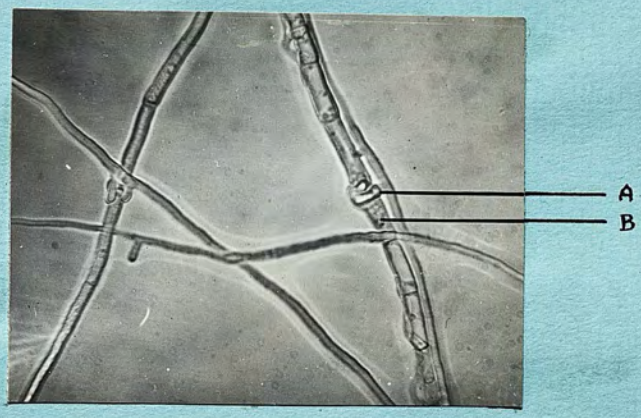


figure 1.

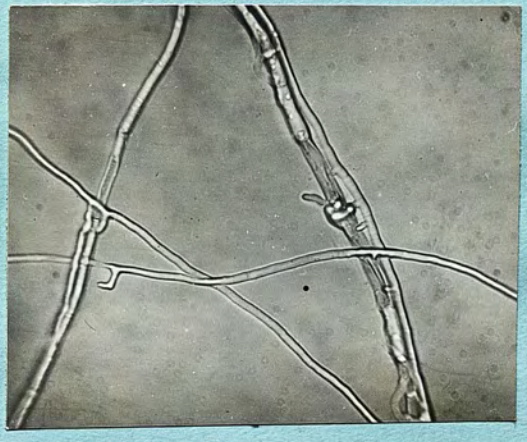


figure 2.

PLATE IX.

a) Pycnospores of the isolates indicated by number.
m= malt agar.
p= potato dextrose agar

b) chlamydospores of isolate 3 on malt agar

PLATE IX.

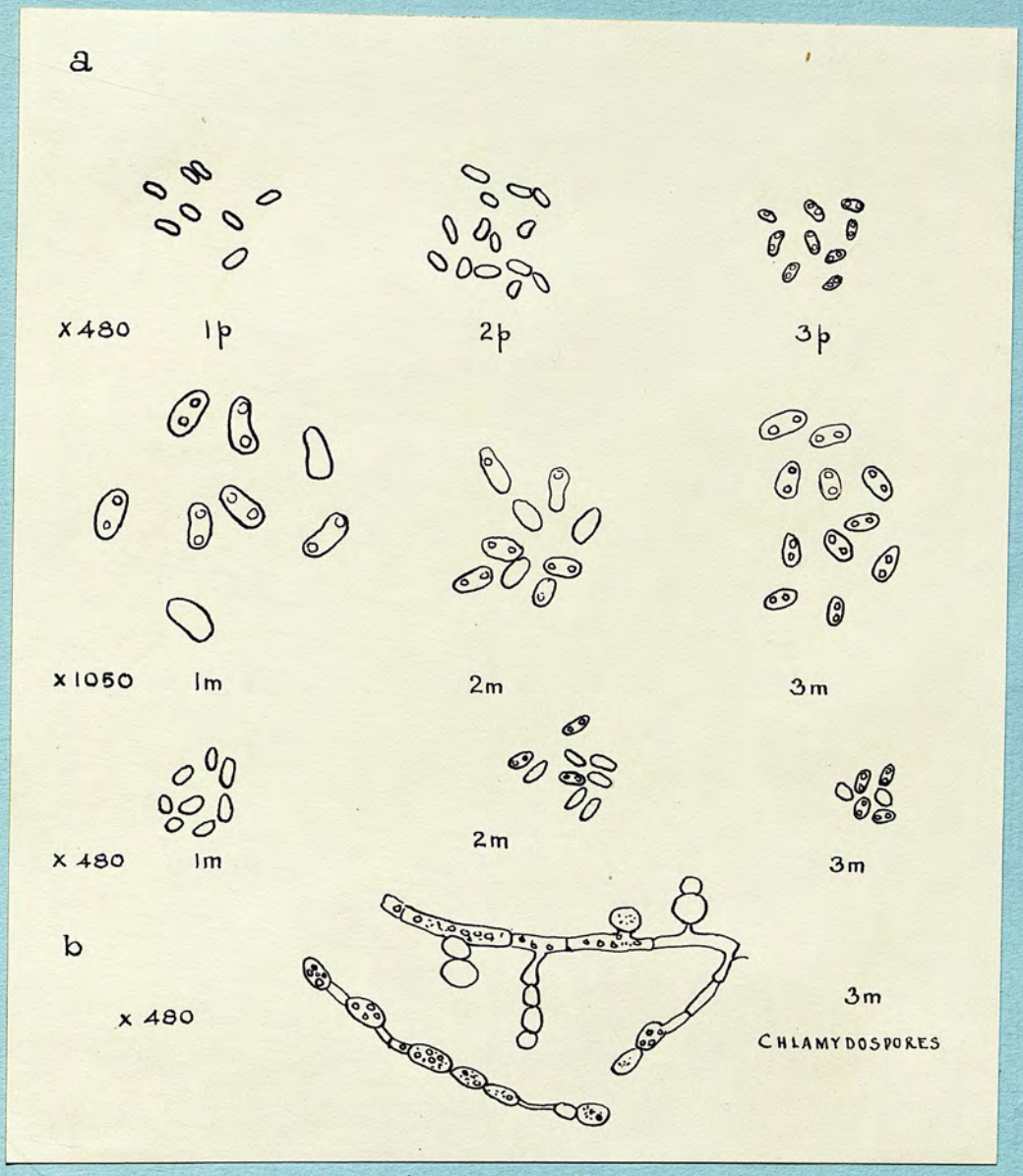

a
x480
1p
2p
3p
x1050
1m
2m
3m
x 480
1m
2m
3m
b
x 480
3m
CHLAMYDOSPORES

PLATE X.

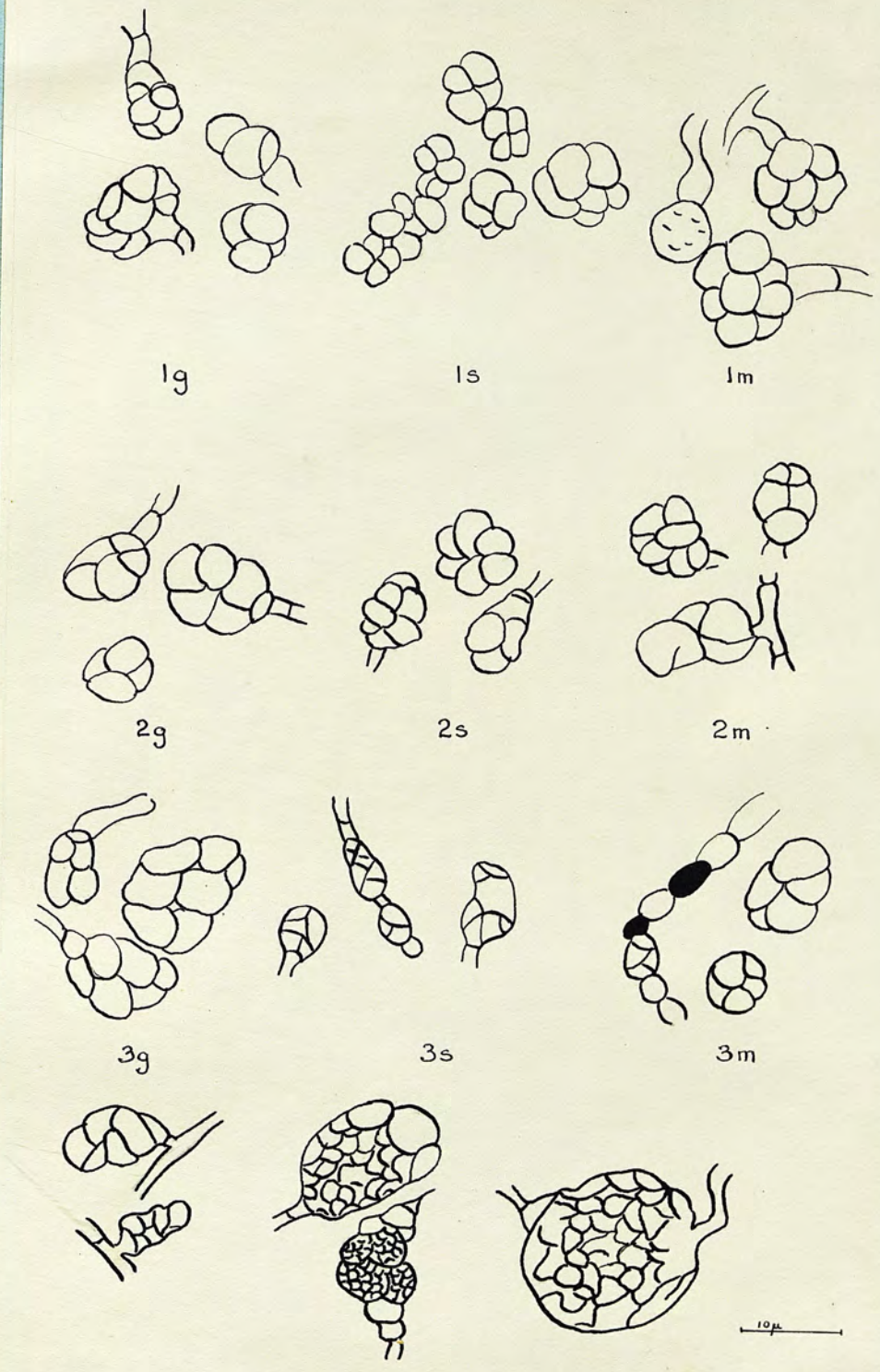
Chlamydospores formed on media containing different carbohydrates for the isolates indicated by number.

g...glucose medium

m...maltose containing medium

s...sucrose containing medium.

PLATE X.



PHOMA ALTERNARIACEARUM

PLATE XI.

figure 1.

Majestic tuber showing symptoms of Skin Necrosis.

figure 2.

Section of the tuber seen in figure 1 showing the shallow necrotic region underlying the skin.

PLATE XI.

figure 1.

figure 2.