1942

The effect of sucrose and dextrose on the thermal resistance of some acid food spoilage organisms.

Harvey Fram
University of Massachusetts Amherst

The Effect of Sucrose and Dextrose on the Thermal Resistance of Some Acid Food Spoilage Organisms

THE EFFECT OF SUCROSE AND DEXTROSE ON THE THERMAL RESISTANCE OF SOME ACID FOOD SPOILAGE ORGANISMS

Harvey Fram

Thesis Submitted for Degree of

Master of Science

Massachusetts State College

Amherst

May, 1942

TABLE OF CONTENTS

INTRODUCTION AND PURPOSE OF INVESTIGATION

The fact that high concentrations of sugar will prevent the growth of microorganisms, and consequently preserve foods, has long been known. With the great increase in the use of other sugars besides sucrose in foods, it is of both academic and practical interest to investigate more thoroughly the inhibitive effect which different sugars have on various microorganisms.

Since 1930, when the Department of Agriculture finally allowed the use of corn sugar (dextrose) without declaration on the label, the commercial use of this sugar has increased tremendously. With the present shortage of cane sugar (sucrose), an attempt will be made by the food industries to substitute more and more dextrose in their products. It is generally agreed that the partial substitution of dextrose for sucrose in various foods results in products that are equal or superior in quality to those made with all sucrose. However, information concerning the comparative inhibitory action of sucrose and dextrose on microorganisms is still lacking.

Because of the supposed difference in the inhibitive or preserving properties of dextrose and sucrose, this investigation was undertaken. It is the purpose of this study to compare the action of sucrose and dextrose on the heat resistance of certain microorganisms encountered in the spoilage of acid food products and to determine the practical value of these findings.

REVIEW OF LITERATURE

Comparative Inhibitory Action of Sucrose and Dextrose on Microorganisms.

The inhibitive action of sugars on the growth of microorganisms is generally agreed to be due to osmosis. This assumption is useful in explaining the differences observed in the inhibitive effect of various sugars. Jordan(1940) stated that the larger the molecule, the greater the viscosity and the lower the osmotic pressure. It follows, then, that a disaccharide such as sucrose, with a molecular weight of 342, will have a lower osmotic pressure than an equal weight of a monosaccharide such as dextrose with a molecular weight of 180. Thus, dextrose should inhibit microorganisms to a greater extent than sucrose. Tarkow(1940) found this to be true. He reported that weight for weight dextrose inhibited the growth of Saccharomyces cerevisiae and Aspergillus niger to a much greater extent than did sucrose. Nunheimer and Fabian(1940), working with food poisoning staphylococci, found that a concentration of 40-45 per cent dextrose was required to exert an antiseptic action on various strains of staphylococci, as compared with a 50-60 per cent concentration in the case of sucrose. They noted a great difference between the action of dextrose and sucrose. When sucrose was used, vigorous growth of the organisms took place, even at high concentrations of the sugar. This period of activity was followed by a phase during which the growth was materially checked, although no decided reduction in numbers was apparent. At the end of the fifth day, the organisms rapidly decreased, and at the end of the

seventh day, all were killed. When dextrose was used, however, the organisms reacted quite differently. At no time was there vigorous growth, and the number of organisms was found to decrease continually. These authors, too, explained this differential action on the difference in osmotic pressure between the two sugars.

Erickson and Fabian(1942), in studying the preserving and germicidal action of various sugars, found that the order of preserving and germicidal action was fructose⟩dextrose⟩sucrose⟩lactose. In order to bring about a preserving effect on Saccharomyces cerevisiae, only 42-47.5 per cent dextrose was needed as compared to 57.5 per cent sucrose. With Saccharomyces ellipsoideus, 45 per cent dextrose, or 60 per cent sucrose were needed to bring about this preserving effect. On the other hand, to bring about a germicidal effect, 50 per cent dextrose, or 60 per cent sucrose, were needed with S. cerevisiae. With S. ellipsoideus, 47.5 per cent dextrose exerted this same germicidal effect, while sucrose would not kill in any concentration.

Dozier(1934) concluded from his studies on the influence of sugars and salt on C. botulinus, that glucose inhibited toxin production to a greater extent than did sucrose. This superior inhibitory action of glucose was considered to be due to the increased acidity resulting from the sterilization of the glucose. Buchanan(1932) showed that with carbonated beverages, at higher temperatures, yeast spores were killed more easily in sucrose solutions, but at lower temperatures, dextrose had a greater inhibitory action on the growth of yeast spores.

The Effect of Heat on the Inhibitive Action of Sugars.

The widespread use of sugar syrups in the canning industry necessitates an investigation of what effect processing or heat treatment has on the inhibitive action of sugar. Rahn(1928) stated that sugar not only retards the growth of yeasts and other microorganisms, but that it also retards the action of heat upon microorganisms, i.e., it will take more heat to kill a bacterium or yeast in a sweetened fruit juice than in the same juice without sugar. His results showed that it takes four to six times as long to kill the same yeast in a 60 per cent syrup as it does in a 10 per cent syrup, and in an 80 per cent syrup it takes ten times as long. His explanation of this protective action of sugar was based on the fact that with higher concentrations of sugar, there is less moisture available for the microorganisms. It is generally agreed that dry organisms can withstand much more heat than moist ones. Fay(1934), in studying the effect of hypertonic sugar solutions on the thermal resistance of bacteria, also found that a number of microorganisms showed increased resistance to heat in the presence of high concentrations of sugars. He found that the protective action increased with the osmotic pressure when the same sugar was used, although equimolar solutions of different sugars gave different results. Lactose and maltose showed little or no protective action, while sucrose and dextrose showed a definite protective action. Wallace and Tanner(1931) found that it took longer to kill Aspergillus niger and Rhizopus nigricans in sugar solutions than in distilled water or salt solutions.

Baumgartner and Wallace(1934) showed that in concentrations up to 50 per cent, sucrose had no effect on the thermal resistance of the spores of aerobic or anaerobic organisms in acid or alkaline solutions. These authors obtained similar results when pseudo yeasts were used, but in the case of vegetative cells, tests with Escherichia coli showed that with a minimum concentration of about 10 per cent, sucrose afforded appreciable protection at pasteurizing temperatures in neutral solutions. This protective action varied with the sugar concentration. However, the investigators pointed out that in very acid fruit syrups, this protective action was masked by the toxicity of the hydrogen ion concentration. They showed that in concentrations up to 55 per cent, sucrose had no inhibitive action on either the vegetative cell growth or spore formation of organisms encountered on underprocessed canned fruit. The inhibitory factor seemed to be the hydrogen ion concentration.

Braun, Hays, and Benjamin(1941) stated that the thermal death time of organisms causing spoilage in canned food products varied directly with increasing concentrations of the sugar solutions in which the organisms were heated. This increase was especially marked in sugar solutions which approached saturation. They noted a marked increase in the thermal death time for all spores heated in 70 per cent sucrose solution when compared with lower concentrations. The factor of heat penetration might be assumed to be the reason for this increased resistance; however, these investigators presented data and calculations to show that heat penetration was not a factor. In

addition, the fact that a definite correlation between the thermal death time and the syrup density was not observed for all types of organisms studied would indicate that heat penetration was not involved in this instance. They found that putrefactive anaerobes were not affected in solutions of 50 per cent sugar or less. Irish, Joslyn, and Parcell(1928) noted that sugar exerted only a slight retarding effect on heat penetration at low concentrations, but an appreciable retarding effect at concentrations above 50 per cent.

Work done with ice cream seems to further substantiate the protective action of sugar in microorganisms. Beavans(1930) found a protective action when Escherichia coli was grown in nutrient broth containing from 4-20 per cent lactose. He concluded that the survival of members of the Escherichia-Aerobacter groups in pasteurized ice cream mixes may be caused by the protective action of the high sugar content. Fabian and Coulter(1930) found that the amount of sugar usually found in ice cream mixes exerts a slight protective action on E. coli at pasteurizing temperatures. Anzulovic(1932) stated that among the ingredients of ice cream mixes, sugar showed the greatest protective action against bacteria, followed by gelatin, serum solids, and fat.

Another factor influencing the effect of heat on the inhibitive action of sugars is the method of sterilizing the sugar solutions. Baumgartner(1938) reported that the method of sterilizing reducing sugars greatly influenced the results obtained. He found that sterilizing sugar solutions by autoclaving at 112° C. for 15 minutes or

steaming for 30 minutes on three successive days resulted in the formation of a material which was directly toxic for E. coli at 54° C. and capable of delaying the growth of this organism at 37° C. The toxic material did not occur when buffered or broth solutions of reducing sugars were sterilized by filtration. It was found that filter sterilized glucose, galactose, maltose, sucrose, mannitol, and glycerol solutions, in 0.5 M concentration in a buffer or a broth protected E. coli against thermal destruction. Fulmer, Williams, and Werkman(1931) noted a growth stimulation of yeast and certain bacterial species, including E. coli, in a medium containing 0.6 gram NH_4Cl, 0.2 gram K_2HPO_4, and five grams glucose per 100 ml., which had been sterilized at 15 pounds pressure for 15 minutes. Lewis(1930) showed that Phytomonas malvacearm failed to grow in culture media containing glucose, maltose, lactose, galactose, or levulose, and various nitrogenous compounds when sterilized at 122° C. for 15 minutes. He proved that inhibitory substances produced by sterilization at high temperatures were responsible for the failure of growth. Tarkow(1940) also found that pasteurization temperatures altered slightly the relative inhibitive action of dextrose and sucrose.

Factors Influencing the Thermal Death Time of Organisms.

By thermal death points in relation to time is meant the length of time at different temperatures necessary to completely destroy a definite concentration of organisms in a medium of a known hydrogen ion concentration and composition (Bigelow and Esty(1920)).

Esty(1928) reviewed the literature on this subject very thoroughly and stated that the thermal death time of organisms was variable, the factors effecting it being the strain, type, age, and concentration of the organism; the composition, acidity, pH and pH value of the suspending media; and the temperature and conditions of incubation.

Among the factors that have received considerable attention is the pH value of the suspending media. Weiss(1921), working with Clostridium botulimus spores, reported that when the size of the container was constant, there were at least two factors that determined the length of time of exposure and degree of heat required to accomplish sterility: (1) the hydrogen ion concentration; the more acid the medium, the shorter the time; and (2) the physical character of the food; the more fluid products requiring a shorter period of exposure at a given temperature. Esty and Meyer(1922) noted that the thermal resistance of Clostridium botulimum spores was markedly decreased in food juices with a pH value lower than 4.5. Murray and Headlee(1930) found that an acid reaction had a greater effect in lowering the thermal resistance of the spores of Clostridium tetani than did an alkaline reaction. Townsend(1938) studied the thermal resistance of a bacillus causing spoilage in tomatoes, figs, and pears, and reported that the heat resistance of the organism was reduced considerably as the pH value of the suspending media decreased. He suggested the possibility of acidification before processing with many products which are injured by excessive cooking. Bigelow and Esty(1920) also reported that the hydrogen ion concentration of a medium inoculated with a spore suspension was most important, and that the time necessary for

destruction of bacterial spores was largely dependent on this factor.

As regards the factor of strain and type of organism, Esty and Meyer (1922) compared the thermal resistance of many strains of the spores of the Clostridium group and found remarkable variations in all their results.

Weiss(1921a) showed that age of the culture was an important factor in heat resistance tests. He reported that young moist spores of Clostridium botulinus had a higher thermal resistance than did old moist spores. Spores one month old were found to be three times as resistant as spores five months old. The thermal resistance of emulsions of young spores increased as the concentration of the emulsions increased.

For further literature on thermal death times, the reader is referred to the bibliographies prepared by Magoon(1926) and Esty(1928).

EXPERIMENTAL

Scope of Work

The purpose of this investigation was to study the action of sucrose and dextrose on the thermal resistance of various microorganisms that have been found to cause spoilage in canned acid food products. An attempt was made to determine the practical value of the differential inhibitive action of sucrose and dextrose solutions on yeasts, molds, and bacteria.

The first part of the investigation included the determination of the thermal death times of the typical spoilage organisms, using various concentrations of sucrose and dextrose as the suspending medium. Because of the fact that sugar syrups are used to a great extent in canning acid fruits, it was also deemed important to determine the thermal death points of the organisms in sucrose and dextrose syrups that were first adjusted to pH 4.0. This work was designed to show the influence of the pH value of the suspending medium on the thermal resistance of the index organisms.

In order to compare the action of sucrose and dextrose in a more practical manner, efforts were made to determine the required process time and temperature needed to destroy typical spoilage organisms in experimentally inoculated packs. In these inoculated pack studies, apples were used as a typical fruit product and a sugar concentration of 40 per cent was employed. Uninoculated controls were observed to note the differences in quality between all sucrose and all dextrose packs.

Index Organisms

The organisms used in this investigation were the yeasts, Saccharomyces cerevisiae and Saccharomyces ellipsoideus; the molds, Aspergillus niger and a strain of Penicillium: and the bacteria, Lactobacillus lycopersici and Clostridium pastorianum. These organisms were chosen because of their association with the spoilage of acid canned food products.

The yeasts are the two most common species of the Saccharomyces group. S. cerevisiae is known as the common brewing and baking yeast, and S. ellipsoideus is known as the wine yeast. Both are widespread and commonly encountered in nature. They are active fermenting organisms, attacking sugars even at high concentrations, and growing over a wide range of pH values.

The molds used are the two most troublesome organisms to the fruit and vegetable manufacturer. They develop on a variety of fruit and vegetable products, such as jams, jellies, and insufficiently sterilized fruit juices; due to their ability to grow in high concentrations of sugar and in high acidity.

L. lycopersici has been described as the organism responsible for the spoilage of various tomato products. Mickle(1924) first described and isolated this organism from samples of spoiled tomato catsup. Pederson(1929) reported that the same organism was the causative agent in the spoilage of a variety of tomato products. The organism was also found to be an active sugar fermenter, somewhat acid resistant, but not heat resistant.

C. pastorianum has been described as an acid tolerant, butyric acid producing, spore forming anaerobe. Products that have been reported to be susceptible to this type of spoilage are tomatoes, pears, figs, ripe apricots, ripe nectarines, pineapple, and the juices from these fruits.

Methods

Sterilization and Media

The technique used throughout this investigation was of necessity aseptic. Glassware, such as test tubes and flasks, was sterilized in an autoclave at 250° F. (121° C.) for 30 minutes. Pipettes were sterilized in a hot air oven at 400° F. (204° C.) for two hours. Nutrient media and distilled water were sterilized in an autoclave at 250° F. (121° C.) for 20 minutes.

In order to obtain sterile sugar media, a Seitz filter was employed. Sterilization of sugar solutions by heat has been found to markedly effect the resultant growth of microorganisms therein. The sugar solutions were made by dissolving enough sugar by weight in distilled water to give the desired concentration. The solutions were then passed through a sterile Seitz filter into a sterile suction flask. The solutions were tubed aseptically in 10 cc. amounts into sterile culture tubes. The sugar concentration was checked before and after filtration by means of a refractometer.

The sub-culture nutrient media used in the thermal death time determinations varied with the organisms tested. With the

yeasts and molds, Difco dextrose broth (pH 7.2) was used. In the case of L. lycopersici, a tomato juice yeast extract broth (pH 6.6-6.8), as suggested by Pederson(1929), was used. Liver broth (pH 7.2) made up according to the method of Cameron, Williams, and Thompson (Tanner(1932)) was used with C. pastorianum.

The pH value of both sugar solutions and nutrient media was determined before use by means of a Beckman pH meter. Adjustment of the pH values of the nutrient media was made with 1 N HCl and 1 N NaOH. The sugar solutions were adjusted to a pH 4.0 with 1 N HCl.

Preparation of Inoculum

The yeasts used in this investigation were obtained from departmental cultures. The organisms were grown at room temperature on Difco dextrose agar slants, and when visible growth was obtained, the slants were stored at 40° F. (4.4° C.) as stock cultures. To obtain an actively growing culture of the yeasts for use in the experimental determinations, a broth transfer, made from the stock culture, was incubated for 24 hours. At the end of this time, another broth transfer was made from the first tube, and this procedure repeated two more times. The fourth broth tube was then used in each test. This actively growing culture was diluted so that the final concentration of the suspension was 500,000 yeast cells per ml. as determined by direct count. A haemocytometer was used in making the direct count. One-tenth ml. of this standardized suspension, containing 50,000 yeast cells, was used to inoculate each thermal death time tube.

The strain of A. niger was obtained from a departmental culture, while the strain of Penicillium was obtained from a vanilla bean. The organisms were grown at room temperature on Difco dextrose agar slants, and when the cultures had sporulated, they were stored at 40° F. (4.4° C.) as stock cultures. For use in all determinations, a freshly inoculated slant was allowed to sporulate at room temperature and the spores, washed from the slant with sterile distilled water, were used. The spore suspension was diluted with sterile water so that the suspension had a final concentration of 100,000 spores per ml. as determined by direct count using the haemocytometer. One-tenth ml. was used as an inoculum and 10,000 spores were inoculated into each tube.

The strain of L. lycopersici was obtained from the New York Agriculture Experiment Station. The culture was grown at 86° F. (30° C.) for three days on dextrose agar and stored at 40° F. (4.4° C.) as a stock culture. An actively growing broth suspension of the organisms, prepared in the same manner described above for the yeasts was used in all determinations. The medium employed was tomato juice yeast extract broth (Pederson(1929)). The final broth suspension was diluted with sterile broth to give a final concentration of 500,000 bacterial cells per ml. as determined by direct count. One-tenth ml. of this standardized suspension was used in each test giving an inoculum of 50,000 bacterial cells per tube.

The strain of C. pastorianum was obtained from J. R. Esty of the National Canner's Association Laboratory in San Francisco, California. The spores were obtained by growing the organism in a

flask of liver broth, stratified with mineral oil to provide anaerobic conditions, for 20 days at 86° F. (30° C.) The spores were then concentrated by centrifuging, washed with sterile distilled water, and stored in some of the original medium at 40° F. (4.4° C.). For use in each determination the spores were diluted with sterile distilled water so that the final concentration of the suspension was 500,000 spores per ml. As with the other organisms, one-tenth ml. of the spore suspension, which contained 50,000 spores was used as the inoculum per tube.

Thermal Death Time Technique

The effect of different concentrations of sucrose and dextrose on the thermal resistance of the organisms described above was studied. Concentrations of 10, 20, 30, 40 and 50 per cent of dextrose and of sucrose, and a distilled water control were used as the suspending media. The pH values of the sucrose solutions ranged from pH 6.0 to pH 6.5; and those of the dextrose solutions from pH 5.8 to pH 6.1. The pH value of the distilled water was 5.7. To note what effect the pH value of the suspending medium would have on the thermal resistance of the organisms, determinations were also made in sugar solutions that were first adjusted to pH 4.0. Three different temperatures were used with each organism; 140° F. (60° C.), 160° F. (71° C.), and 175° F. (80° C.) with the yeasts and molds; 150° F. (66° C.), 160° F. (71° C.), and 170° F. (77° C.) with L. lycopersici; and 190° F. (88° C.), 200° F. (93° C.), and 212° F. (100° C.) with C. pastorianum.

One-tenth ml. of the standardized suspension of the test organism was inoculated into test tubes containing 10 cc. of sterile sugar solution. With the yeasts and bacteria, this inoculation consisted of 50,000 organisms per tube. In the case of the molds, the inoculation consisted of 10,000 spores per tube. The inoculated sugar solution tubes were then placed in a water bath maintained at the given temperature. Individual tubes were removed and immediately cooled at such time intervals that both survival and destruction of the particular organism would be evident. One ml. was then removed from each tube aseptically by means of a sterile pipette, and inoculated into the sub-culture medium tubes. For the yeasts and molds, Difco dextrose broth was used. For L. lycopersici, a tomato juice yeast extract broth was used, and for the spores of C. pasterianum liver broth was employed. It was necessary to provide anaerobic conditions for the growth of the spores of C. pasterianum, and this was accomplished by stratifying each inoculated liver broth tube with sterile, three per cent agar before incubation.

The yeasts and molds were incubated at room temperature for five days; L. lycopersici at 86° F. (30° C.) for two days; and C. pasterianum at 86° F. (30° C.) for three days. At the end of the incubation period, the sub-culture tubes were observed for evidence of growth.

Inoculated Pack Technique

The minimum required process time and temperature needed to destroy the index organisms in a typical acid food product were

determined by inoculating experimental packs of apples. To observe the differences in the effect of sucrose and dextrose on the process time and temperature, the apples were packed in 40 per cent sucrose syrups and 40 per cent dextrose syrups. The apples used in this part of the investigation were MacIntosh (pH 4.0), and Rhode Island Greenings (pH 3.2).

In the inoculated packs, No. 2 plain tin cans were used for the yeasts and bacteria, while one-half pint glass jars were used for the molds. To insure comparable results, the containers, whether tin or glass, were exhausted to 140° F. (60° C.). They were then inoculated with 50,000 yeast cells, 10,000 mold spores, 50,000 L. lycopersici cells, or 50,000 C. pastorianum spores, respectively, sealed and processed. The containers were processed at time intervals ranging from 0-15 minutes at a given temperature. For each time interval, five inoculated containers were prepared along with five uninoculated controls. The containers were incubated at 95° F. (35° C.) for two weeks. At the end of this time, presence or absence of growth was determined by gas production with the yeasts and bacteria, and by observation in the case of the molds. Observations were also made on the uninoculated controls for color, texture, and flavor of the product.

EXPERIMENTAL RESULTS

Thermal death time studies

Part of the procedure in the determination of a safe process time and temperature for canned products, consists in the determination of the thermal death times of the particular organisms responsible for the spoilage of the product.

Tables 1-24 show the thermal death times of certain spoilage organisms in different concentrations of sucrose and dextrose at pH values of approximately 6.0 and 4.0. Thermal death time curves based on these data are presented in Figures 1-24.

Tables 1 and 2 show the thermal death times of S. cerevisiae in sucrose and dextrose solutions with a pH value of approximately 6.0. The similarity of the curves in figures 1 and 2 indicate that there is little difference in the effect of either sucrose or dextrose on the thermal death times of this organism. It is also evident from these results that in sugar concentrations of 30 per cent or more, the thermal resistance is increased, i.e., it takes five minutes to destroy the organism in 50 per cent sugar at 140° F. (60° C.) and only two minutes in distilled water at the same temperature.

When the pH value of the sugar solutions was adjusted to pH 4.0, (tables 3 and 4 and figures 3 and 4), the thermal resistance of the organism was still practically the same in both sucrose and dextrose solutions, but the protective action of increased sugar concentrations was somewhat reduced. That is, higher concentrations

of sugar showed less protective action at a pH value of 4.0 than at 6.0. In distilled water and in solutions of low sugar content, the thermal resistance was not influenced by the pH value.

With S. ellipsoideus (tables 5-8 and figures 5-8), the same general results and trends were observed. The results with A. niger (tables 9-12 and figures 9-12) seem to indicate that this organism was slightly more resistant to heat than were the yeasts. This mold was destroyed in six minutes at 140° F. (60° C.) in 30, 40, or 50 per cent sucrose solutions. This organism was the only one that showed a somewhat reduced thermal resistance in dextrose as compared to sucrose solutions, as may be seen from the difference in the slopes of the curves in figures 9 and 10. When the pH value of the sugar solutions was adjusted to 4.0, (tables 11 and 12 and figures 11 and 12), the same general results were obtained with this mold as were obtained with the yeasts. The thermal resistance of the organism was reduced only slightly in the higher sugar concentrations.

The results obtained with the strain of Penicillium (tables 13-17 and figures 13-17) simulate very closely those obtained with the yeasts.

In the case of L. lycopersici (tables 17-20 and figures 17-20), it was again observed that there was no difference in the effect of sucrose and dextrose on the thermal death times of the organism. When the pH value of the sugar solution was adjusted to 4.0, the thermal death time of the organism was very little affected. The similarity of the curves in figures 17-20 show these results more clearly. Further evidence is provided in these results to show that sugar in high

Table 1. Thermal death time of S. cerevisese in sucrose solutions. *

Temperature	Exposure period	Control distilled water (pH 5.7)	Sucrose concentration in per cent 10 (pH 6.1)	20 (pH 6.1)	30 (pH 6.2)	40 (pH 6.2)	50 (pH 6.4)
° F.	minutes						
140	1	+	+	+	+	+	+
	2	-	-	-	+	+	+
	3	-	-	-	+	+	+
	4	-	-	-	-	+	+
	5	-	-	-	-	-	-
160	1/2	+	+	+	+	+	+
	3/4	+	+	+	+	+	+
	1	-	-	-	+	+	+
	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
175	1/4	+	+	+	+	+	+
	1/2	+	+	+	+	+	+
	3/4	-	-	-	+	+	+
	1	-	-	-	-	-	-
	2	-	-	-	-	-	-

* Inoculation consisted of approximately 50,000 yeast cells per tube.

+ indicates survival

- indicates destruction

Figure 1. Thermal death time curves of S. cerevisiae in different concentrations of sucrose.

1. distilled water (pH 5.7)
2. 10 per cent sucrose (pH 6.1)
3. 20 per cent sucrose (pH 6.1)
4. 30 per cent sucrose (pH 6.2)
5. 40 per cent sucrose (pH 6.2)
6. 50 per cent sucrose (pH 6.4)

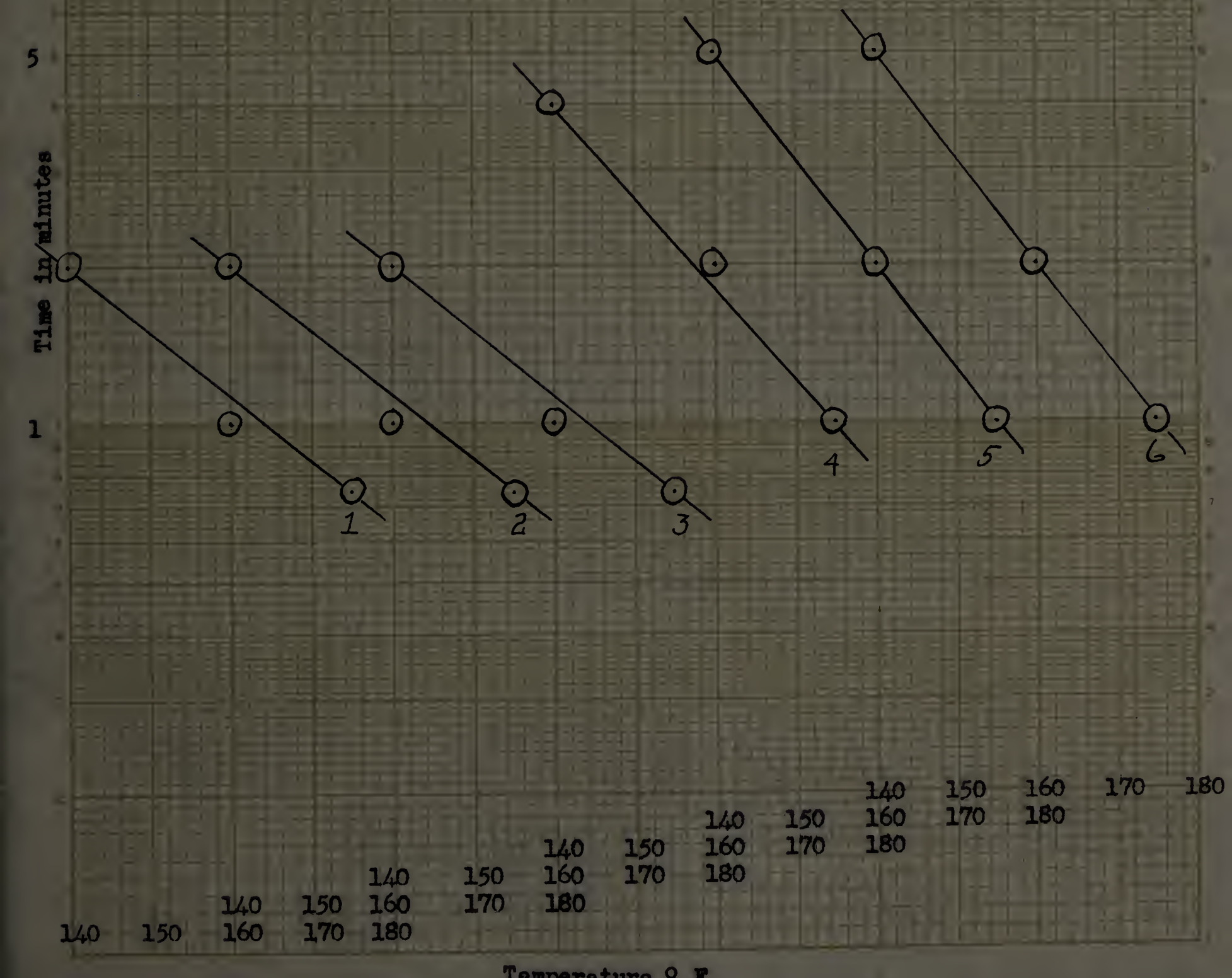


Table 2. Thermal death time of S. cerevisene in dextrose solutions. *

Temperature	Exposure period	Control distilled water (pH 5.7)	Dextrose concentration in per cent 10 (pH 5.9)	20 (pH 5.9)	30 (pH 6.0)	40 (pH 6.0)	50 (pH 6.1)
° F.	minutes						
	1	+	+	+	+	+	+
	2	-	-	-	+	+	+
140	3	-	-	-	-	+	+
	4	-	-	-	-	-	+
	5	-	-	-	-	-	-
	1/2	+	+	+	+	+	+
	3/4	+	+	+	+	+	+
160	1	-	-	-	+	+	+
	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
	1/4	+	+	+	+	+	+
	1/2	+	+	+	+	+	+
175	3/4	-	-	-	-	+	+
	1	-	-	-	-	-	-
	2	-	-	-	-	-	-

* Inoculation consisted of approximately 50,000 yeast cells per tube.

+ indicates survival

- indicates destruction

Figure 2. Thermal death time curves of S. cerevisiae

in different concentrations of dextrose.

1. distilled water control (pH 5.7)
2. 10 per cent dextrose (pH 5.9)
3. 20 per cent dextrose (pH 5.9)
4. 30 per cent dextrose (pH 6.0)
5. 40 per cent dextrose (pH 6.0)
6. 50 per cent dextrose (pH 6.1)

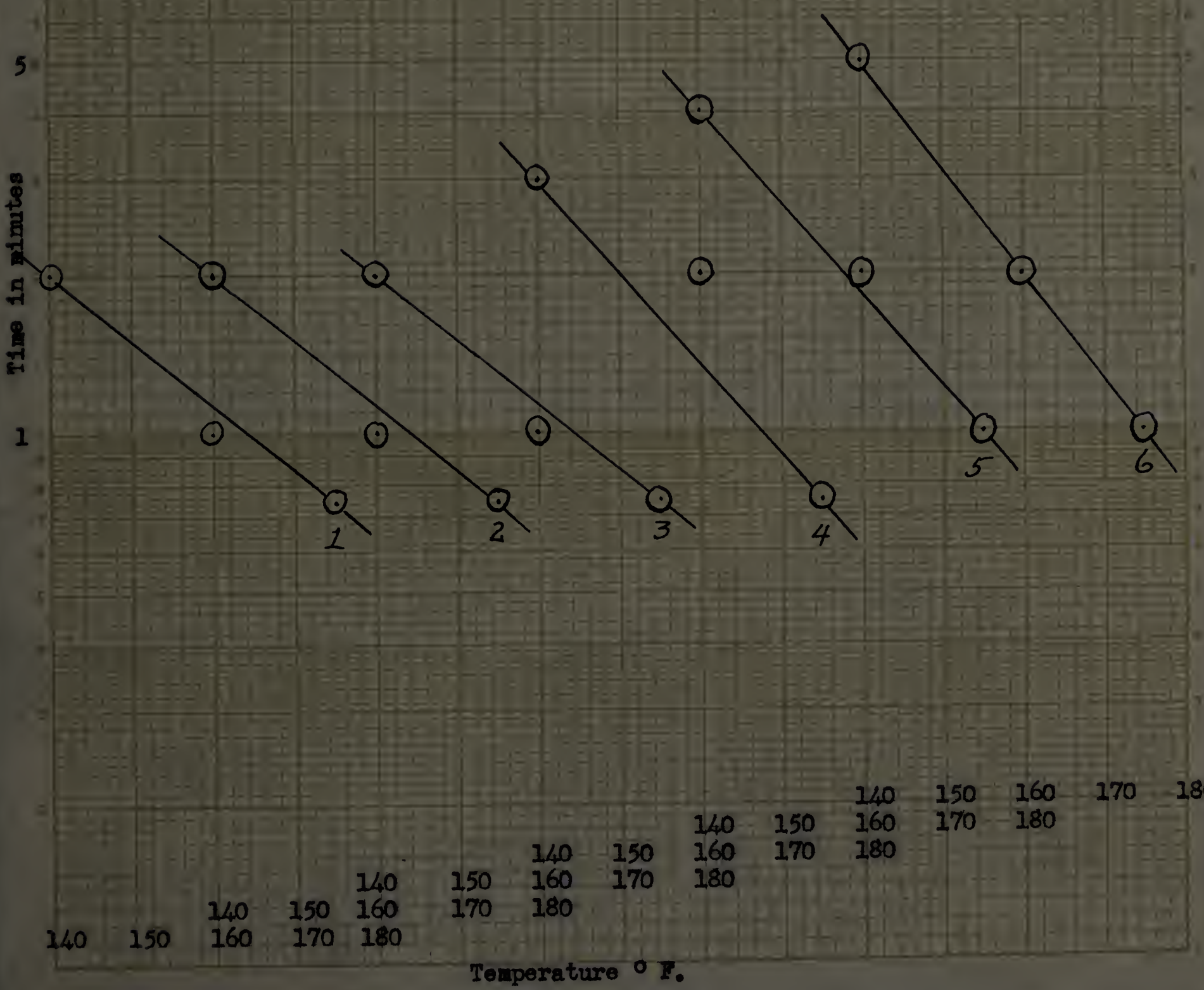


Table 3. Thermal death time of S. cerevisiae in sucrose solutions adjusted to pH 4.0 ± 0.1. *

Temperature	Exposure period	Control distilled water	Sucrose concentration in per cent 10	20	30	40	50
° F.	minutes						
	1	+	+	+	+	+	+
	2	-	-	-	+	+	+
140	3	-	-	-	-	-	+
	4	-	-	-	-	-	-
	5	-	-	-	-	-	-
	1/2	+	+	+	+	+	+
	3/4	+	+	+	+	+	+
160	1	-	-	-	-	+	+
	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
	1/4	+	+	+	+	+	+
	1/2	+	+	+	+	+	+
175	3/4	-	-	-	-	-	-
	1	-	-	-	-	-	-
	2	-	-	-	-	-	-

* Inoculation consisted of approximately 50,000 yeast cells per tube.

+ indicates survival

- indicates destruction

Figure 3. Thermal death time curves of S. cerevisiae in different concentrations of sucrose adjusted to pH 4.0 ± 0.1

1. distilled water control
2. 10 per cent sucrose
3. 20 per cent sucrose
4. 30 per cent sucrose
5. 40 per cent sucrose
6. 50 per cent sucrose

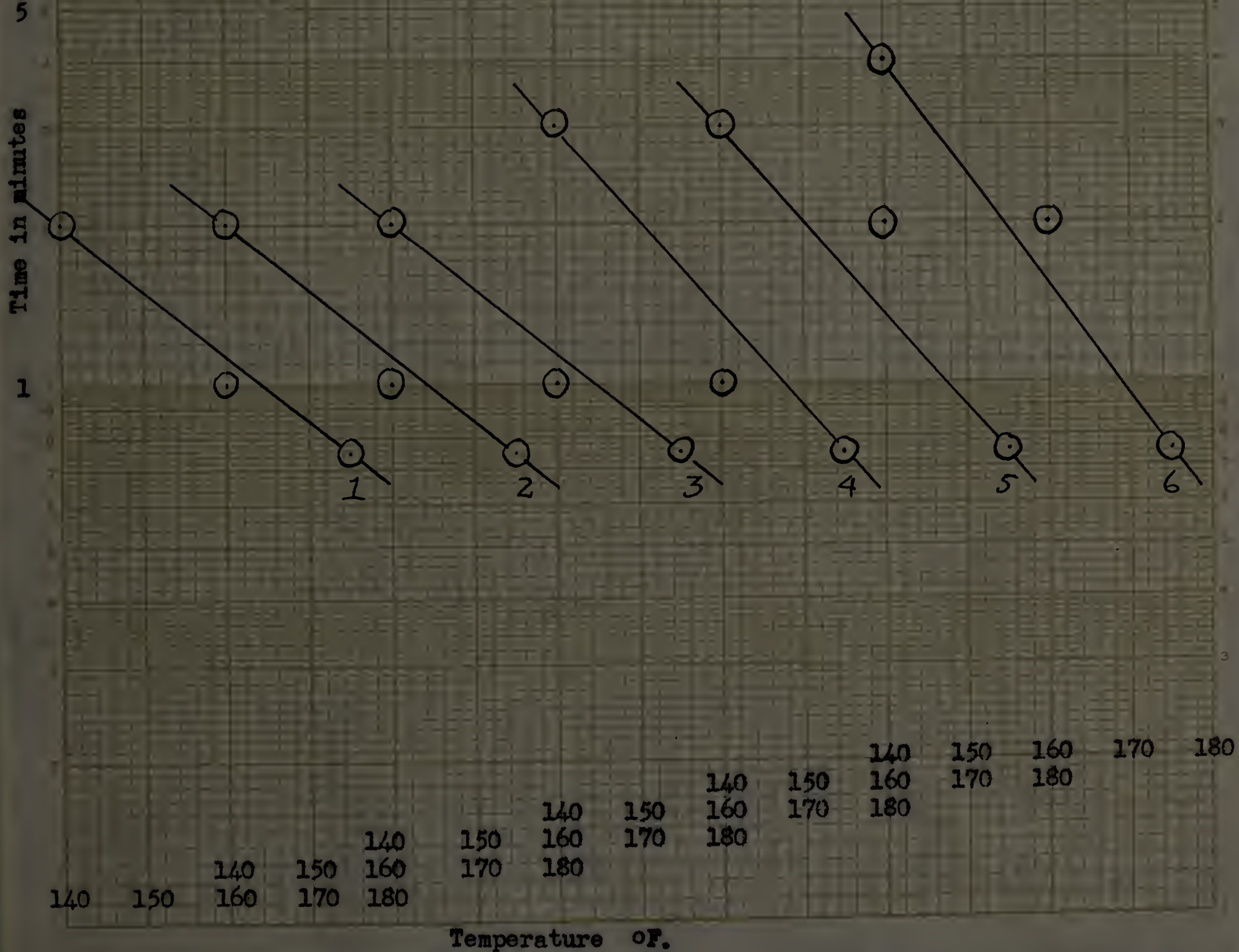


Table 4. Thermal death time of S. cerevisiae in dextrose solutions adjusted to pH 4.0 ± 0.1. *

Temperature	Exposure period	Control distilled water	Dextrose concentration in per cent				
			10	20	30	40	50
° F.	minutes						
140	1	+	+	+	+	+	+
	2	-	-	-	-	+	+
	3	-	-	-	-	-	-
	4	-	-	-	-	-	-
	5	-	-	-	-	-	-
160	1/2	+	+	+	+	+	+
	3/4	+	+	+	+	+	+
	1	-	-	-	-	-	+
	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
175	1/4	+	+	+	+	+	+
	1/2	+	+	+	+	+	+
	3/4	-	-	-	-	-	-
	1	-	-	-	-	-	-
	2	-	-	-	-	-	-

* Inoculation consisted of approximately 50,000 yeast cells per tube.

+ indicates survival

- indicates destruction

Figure 4. Thermal death time curves of S. cerevisiae in different concentrations of dextrose adjusted to pH 4.0 ± 0.1

1. distilled water control
2. 10 per cent dextrose
3. 20 per cent dextrose
4. 30 per cent dextrose
5. 40 per cent dextrose
6. 50 per cent dextrose

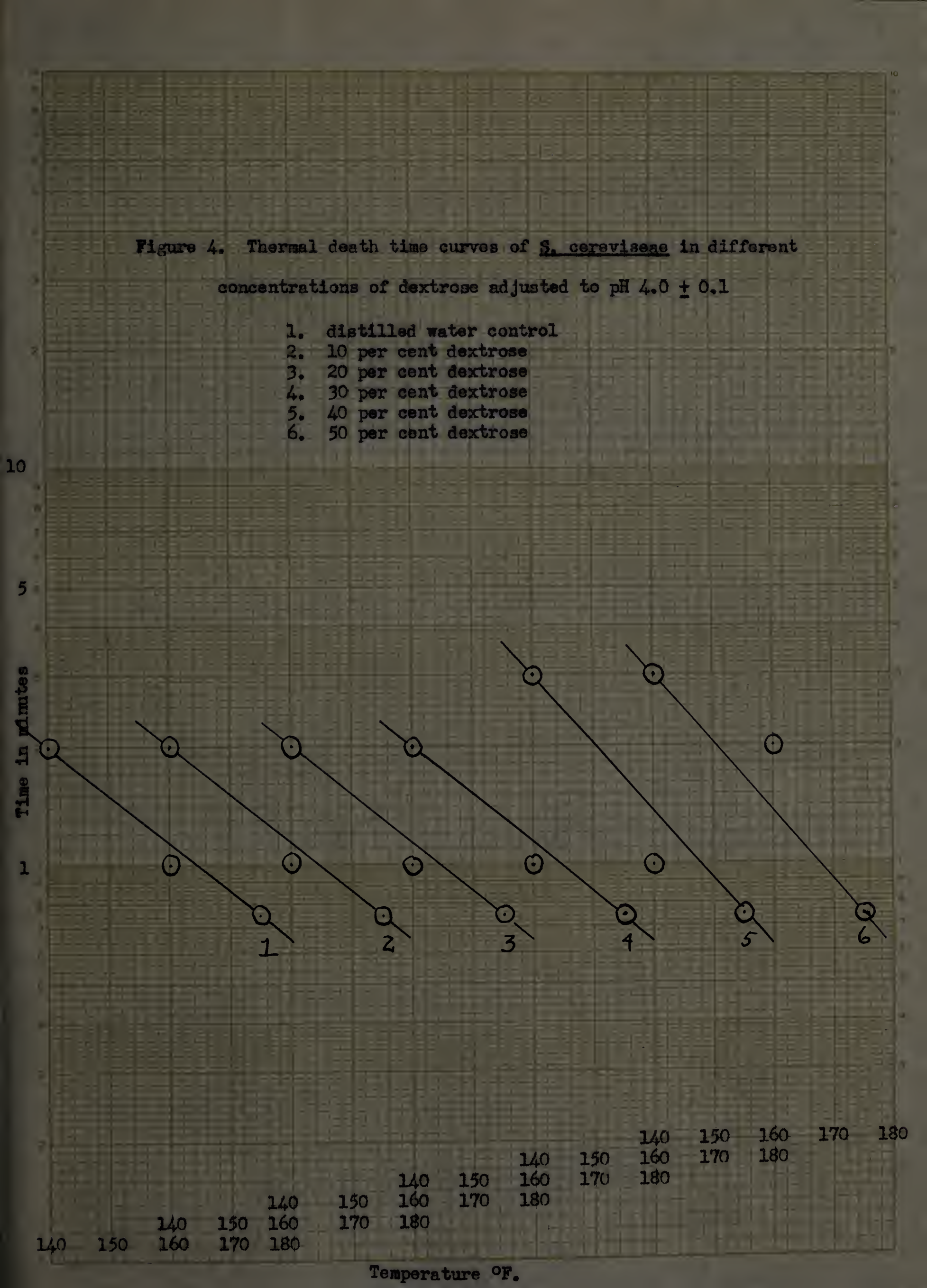


Table 5. Thermal death time of *S. ellipsoideus* in sucrose solutions. *

Temperature	Exposure period	Control distilled water (pH 5.7)	Sucrose concentration in per cent 10 (pH 6.1)	20 (pH 6.1)	30 (pH 6.2)	40 (pH 6.3)	50 (pH 6.5)
° F.	minutes						
140	1	+	+	+	+	+	+
	2	+	+	+	+	+	+
	3	-	-	-	+	+	+
	4	-	-	-	-	+	+
	5	-	-	-	-	-	-
160	1/2	+	+	+	+	+	+
	3/4	+	+	+	+	+	+
	1	-	-	-	+	+	+
	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
175	1/4	+	+	+	+	+	+
	1/2	+	+	+	+	+	+
	3/4	-	-	-	+	+	+
	1	-	-	-	-	-	-
	2	-	-	-	-	-	-

* Inoculation consisted of approximately 50,000 yeast cells per tube.

+ indicates survival

- indicates destruction

Figure 5. Thermal death time curves of S. ellipsoideus

in different concentrations of sucrose.

1. distilled water control (pH 5.7)
2. 10 per cent sucrose (pH 6.1)
3. 20 per cent sucrose (pH 6.1)
4. 30 per cent sucrose (pH 6.2)
5. 40 per cent sucrose (pH 6.2)
6. 50 per cent sucrose (pH 6.4)

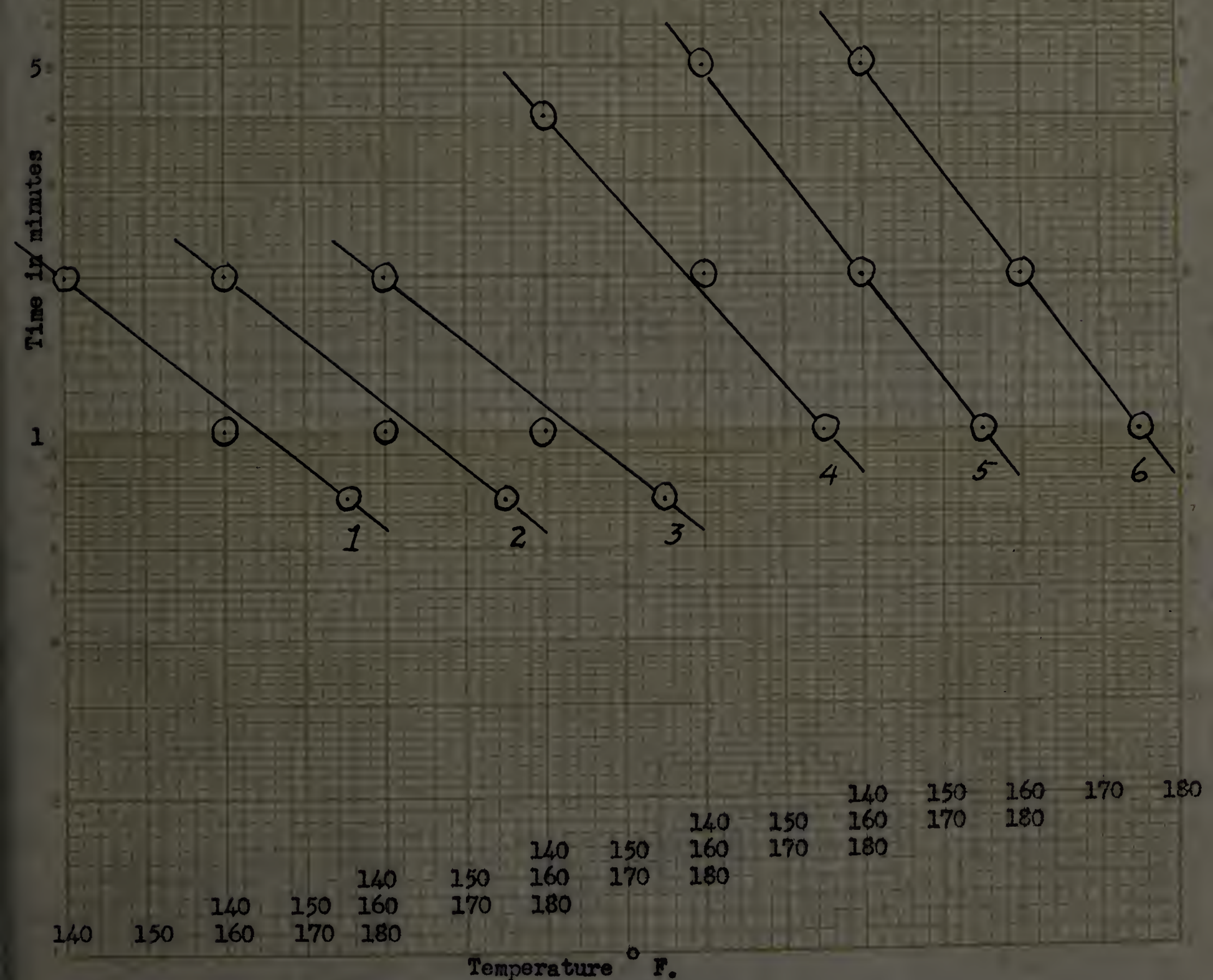


Table 6. Thermal death time of S. ellipsoideus in dextrose solutions. *

Temperature	Exposure period	Control distilled water (pH 5.7)	Dextrose concentration in per cent 10 (pH 5.8)	20 (pH 5.9)	30 (pH 5.9)	40 (pH 6.0)	50 (pH 6.1)
° F.	minutes						
140	1	+	+	+	+	+	+
	2	-	-	-	+	+	+
	3	-	-	-	+	+	+
	4	-	-	-	-	+	+
	5	-	-	-	-	-	-
160	1/2	+	+	+	+	+	+
	3/4	+	+	+	+	+	+
	1	-	-	-	+	+	+
	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
175	1/4	+	+	+	+	+	+
	1/2	+	+	+	+	+	+
	3/4	-	-	-	+	+	+
	1	-	-	-	-	-	-
	2	-	-	-	-	-	-

* Inoculation consisted of approximately 50,000 yeast cells per tube

+ indicates survival

- indicates destruction

Figure 6. Thermal death time curves of S. ellipsoideus

in different concentrations of dextrose

1. distilled water control (pH 5.7)
2. 10 per cent dextrose (pH 5.8)
3. 20 per cent dextrose (pH 5.9)
4. 30 per cent dextrose (pH 5.9)
5. 40 per cent dextrose (pH 6.0)
6. 50 per cent dextrose (pH 6.1)

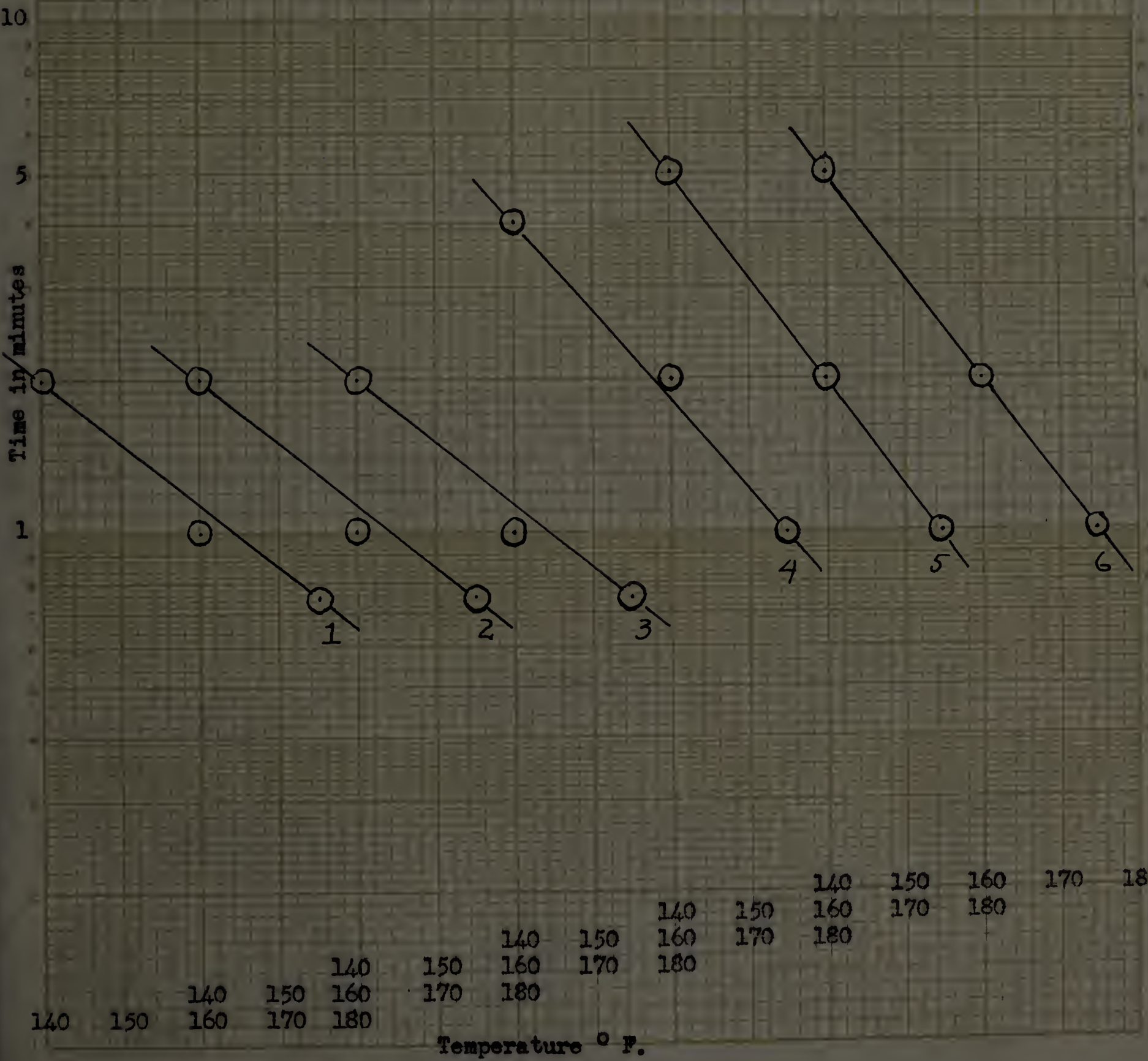


Table 7. Thermal death time of S. ellipsoideus in sucrose solutions adjusted to pH 4.0 ± 0.1. *

Temperature	Exposure period	Control distilled water	Sucrose concentration in per cent				
			10	20	30	40	50
° F.	minutes						
140	1	+	+	+	+	+	+
	2	-	-	-	+	+	+
	3	-	-	-	-	-	+
	4	-	-	-	-	-	-
	5	-	-	-	-	-	-
160	1/2	+	+	+	+	+	+
	3/4	+	+	+	+	+	+
	1	-	-	-	-	+	+
	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
175	1/4	+	+	+	+	+	+
	1/2	-	+	+	+	+	+
	3/4	-	-	-	-	-	-
	1	-	-	-	-	-	-
	2	-	-	-	-	-	-

* Inoculation consisted of approximately 50,000 yeast cells per tube.

+ indicates survival

- indicates destruction

Figure 7. Thermal death time curves of S. ellipsoideus in different concentrations of sucrose adjusted to pH 4.0 ± 0.1

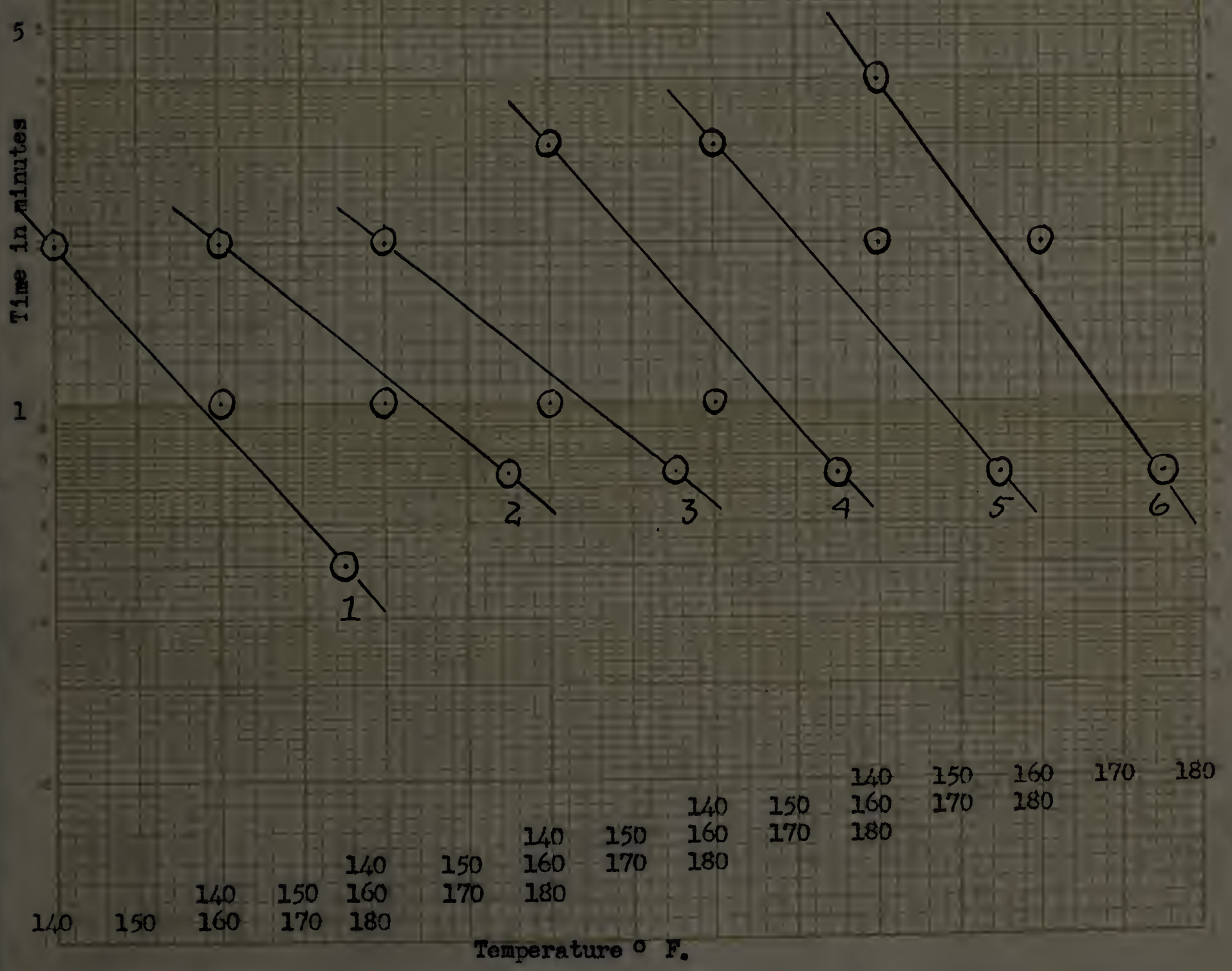


Table 8. Thermal death time of S. ellipsoideus in dextrose solutions adjusted to pH 4.0 ± 0.1. *

Temperature	Exposure period	Control distilled water	Dextrose concentration in per cent				
			10	20	30	40	50
° F.	minutes						
140	1	+	+	+	+	+	+
	2	-	-	-	+	+	+
	3	-	-	-	-	-	+
	4	-	-	-	-	-	-
	5	-	-	-	-	-	-
160	1/2	+	+	+	+	+	+
	3/4	+	+	+	+	+	+
	1	-	-	-	-	+	+
	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
175	1/4	+	+	+	+	+	+
	1/2	-	+	+	+	+	+
	3/4	-	-	-	-	-	-
	1	-	-	-	-	-	-
	2	-	-	-	-	-	-

* Inoculation consisted of approximately 50,000 yeast cells per tube.

+ indicates survival

- indicates destruction

Figure 8. Thermal death time curves of S. ellipsoideus in different concentrations of dextrose adjusted to pH 4.0 ± 0.1

1. distilled water control
2. 10 per cent dextrose
3. 20 per cent dextrose
4. 30 per cent dextrose
5. 40 per cent dextrose
6. 50 per cent dextrose

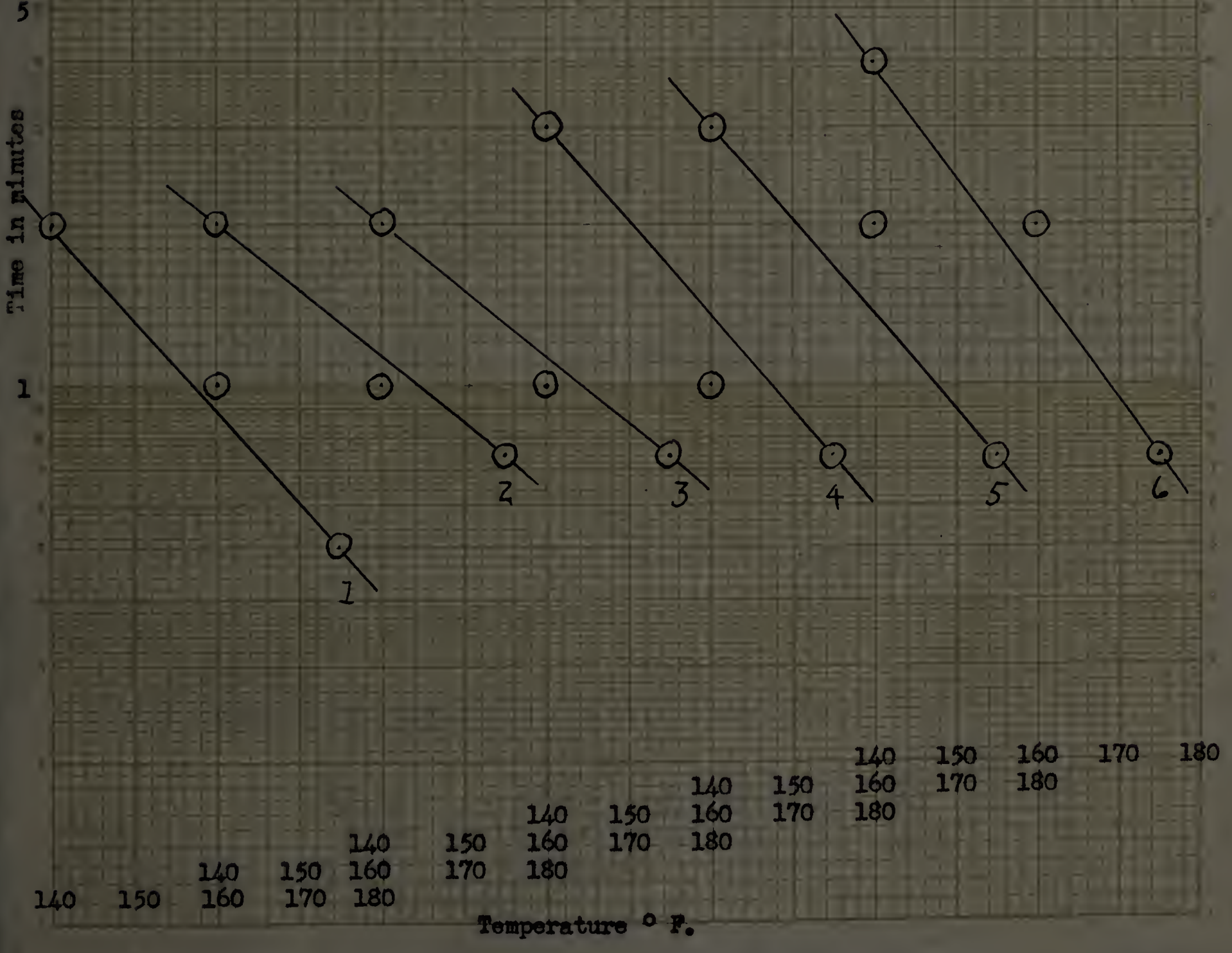


Table 9. Thermal death time of A. niger in sucrose solutions. *

Temperature	Exposure period	Control distilled water (pH 5.7)	Sucrose concentration in per cent 10 (pH 6.1)	20 (pH 6.2)	30 (pH 6.2)	40 (pH 6.3)	50 (pH 6.5)
° F.	minutes						
140	1	+	+	+	+	+	+
	2	-	+	+	+	+	+
	3	-	-	+	+	+	+
	4	-	-	-	+	+	+
	5	-	-	-	+	+	+
	6	-	-	-	-	-	-
160	1/2	+	+	+	+	+	+
	3/4	+	+	+	+	+	+
	1	-	+	+	+	+	+
	2	-	-	-	-	-	+
	3	-	-	-	-	-	-
175	1/4	+	+	+	+	+	+
	1/2	+	+	+	+	+	+
	3/4	-	-	-	+	+	+
	1	-	-	-	-	-	-
	2	-	-	-	-	-	-

* Inoculation consisted of approximately 10,000 spores per tube.

+ indicates survival

- indicates destruction

Figure 9. Thermal death time curves of A. niger

in different concentrations of sucrose

1. distilled water control (pH 5.7)
2. 10 per cent sucrose (pH 6.1)
3. 20 per cent sucrose (pH 6.2)
4. 30 per cent sucrose (pH 6.2)
5. 40 per cent sucrose (pH 6.3)
6. 50 per cent sucrose (pH 6.5)

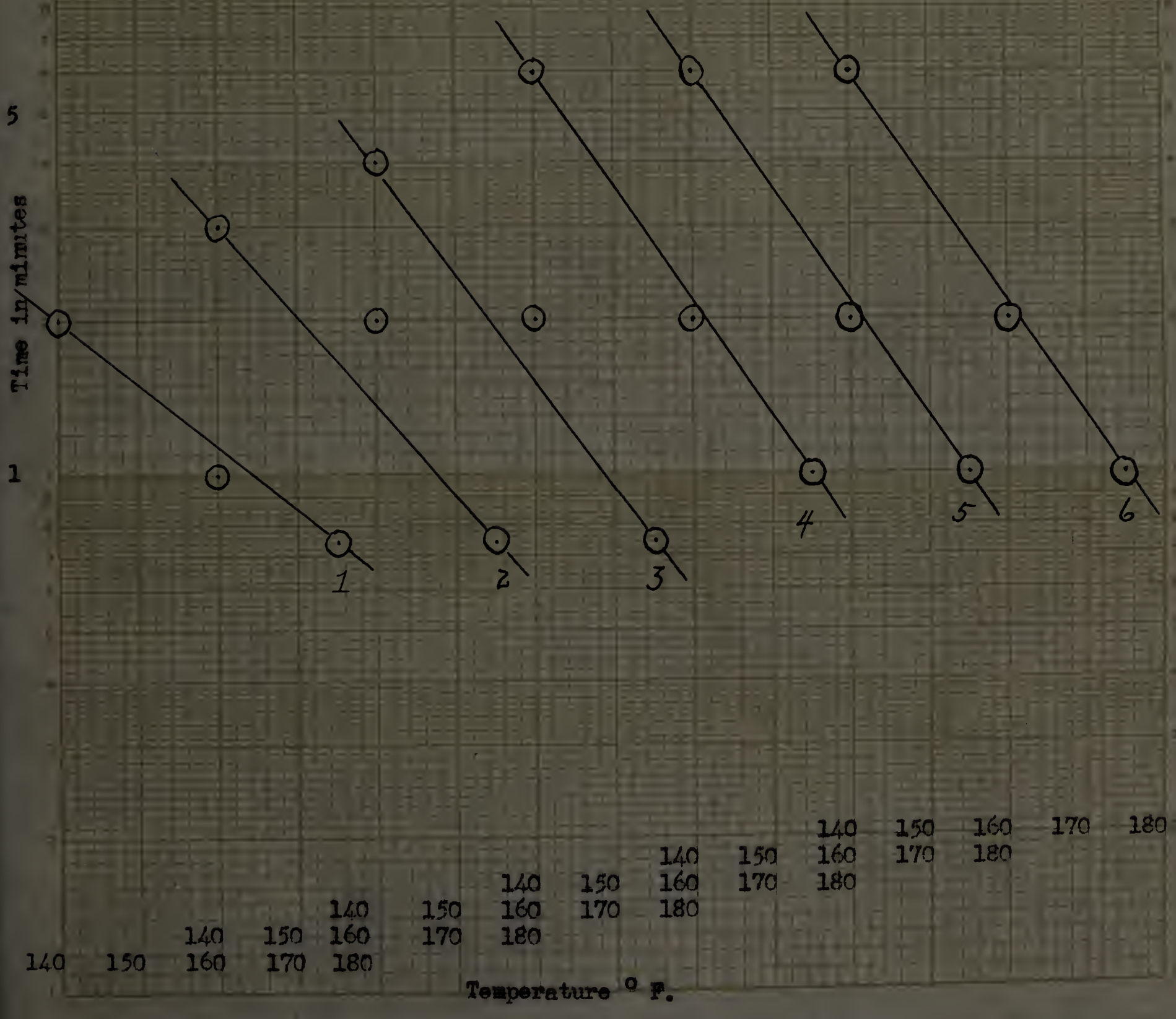


Table 10. Thermal death time of A. niger in dextrose solutions. *

Temperature	Exposure period	Control distilled water (pH 5.7)	Dextrose concentration in per cent 10 (pH 5.8)	20 (pH 5.9)	30 (pH 6.1)	40 (pH 6.1)	50 (pH 6.1)
° F.	minutes						
140	1	+	+	+	+	+	+
	2	-	-	-	+	+	+
	3	-	-	-	-	+	+
	4	-	-	-	-	+	+
	5	-	-	-	-	-	+
	6	-	-	-	-	-	-
160	1/2	+	+	+	+	+	+
	3/4	+	+	+	+	+	+
	1	-	-	-	+	+	+
	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
175	1/4	+	+	+	+	+	+
	1/2	+	+	+	+	+	+
	3/4	-	-	-	+	+	+
	1	-	-	-	-	-	-
	2	-	-	-	-	-	-

*Inoculation consisted of approximately 10,000 spores per tube

+ indicates survival

- indicates destruction

Figure 10. Thermal death time curves of A. niger

in different concentrations of dextrose

1. distilled water control (pH 5.7)
2. 10 per cent dextrose (pH 5.8)
3. 20 per cent dextrose (pH 5.9)
4. 30 per cent dextrose (pH 6.1)
5. 40 per cent dextrose (pH 6.1)
6. 50 per cent dextrose (pH 6.1)

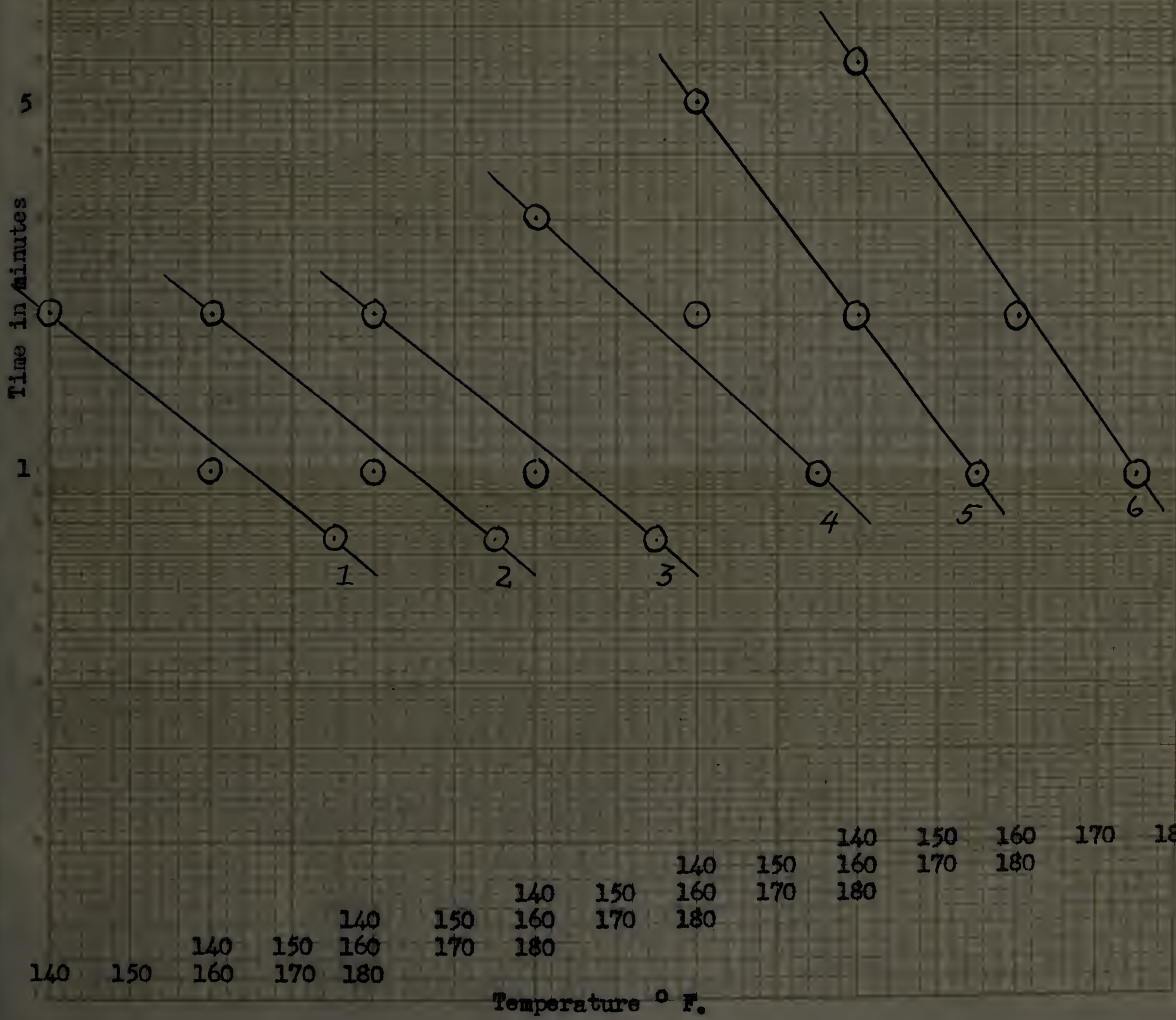


Table 11. Thermal death time of A. niger in sucrose solutions adjusted to pH 4.0 ± 0.1. *

Temperature	Exposure period	Control distilled water	Sucrose concentration in per cent				
			10	20	30	40	50
° F.	minutes						
140	1	+	+	+	+	+	+
	2	-	-	-	+	+	+
	3	-	-	-	-	+	+
	4	-	-	-	-	-	-
	5	-	-	-	-	-	-
160	1/2	+	+	+	+	+	+
	3/4	+	+	+	+	+	+
	1	-	-	-	+	+	+
	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
175	1/4	+	+	+	+	+	+
	1/2	+	+	+	+	+	+
	3/4	-	-	-	-	-	-
	1	-	-	-	-	-	-
	2	-	-	-	-	-	-

* Inoculation consisted of approximately 10,000 mold spores per tube.

+ indicates survival
- indicates destruction

Figure 11. Thermal death time curves of A. niger in different concentrations of sucrose adjusted to pH 4.0 ± 0.1

1. distilled water control
2. 10 per cent sucrose
3. 20 per cent sucrose
4. 30 per cent sucrose
5. 40 per cent sucrose
6. 50 per cent sucrose

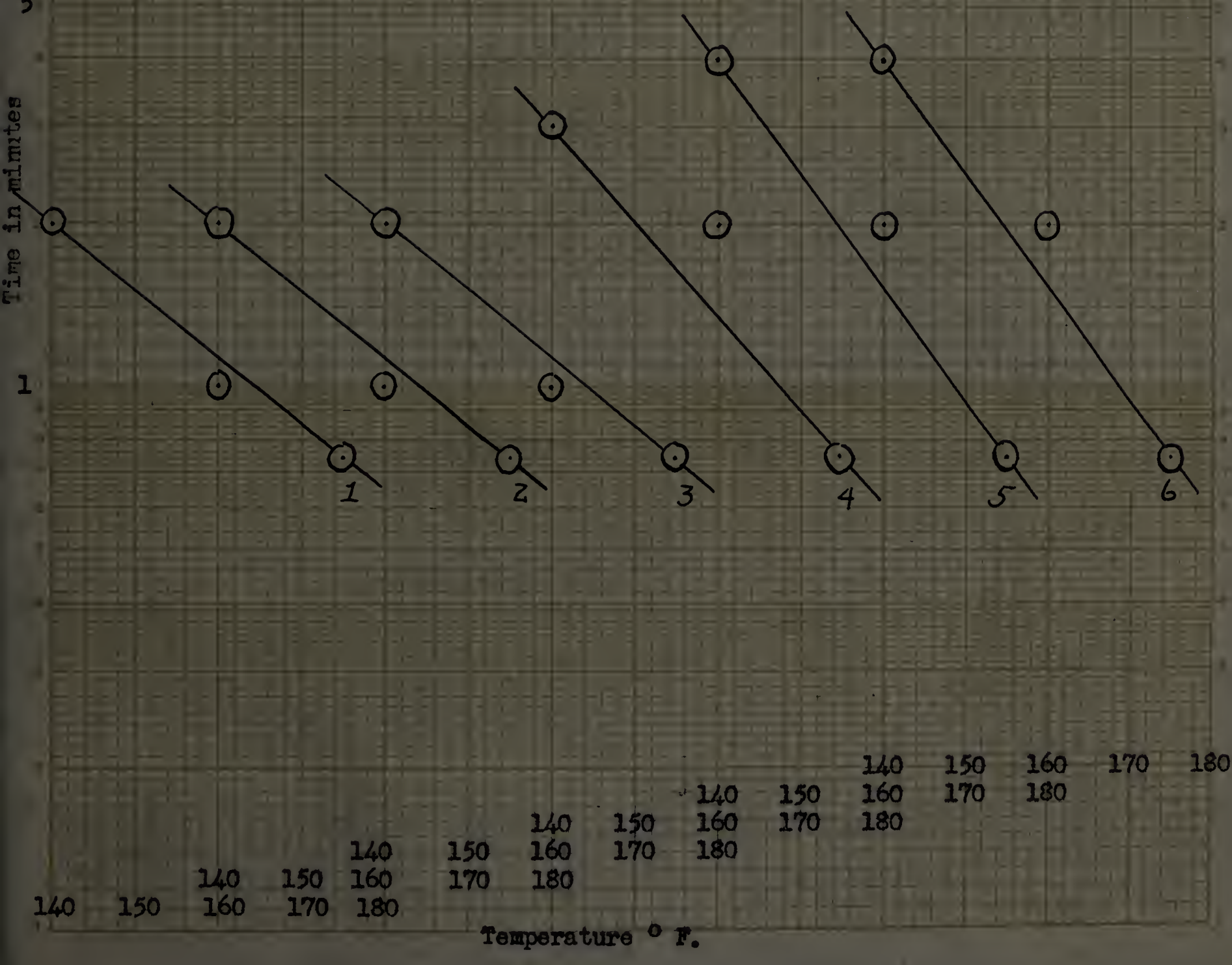


Table 12. Thermal death time of A. niger in dextrose solutions adjusted to pH 4.0 ± 0.1. *

Temperature	Exposure period	Control distilled water	Dextrose concentration in per cent				
			10	20	30	40	50
° F.	minutes						
140	1	+	+	+	+	+	+
	2	-	-	-	+	+	+
	3	-	-	-	-	+	+
	4	-	-	-	-	-	-
	5	-	-	-	-	-	-
160	1/2	+	+	+	+	+	+
	3/4	+	+	+	+	+	+
	1	-	-	-	-	+	+
	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
175	1/4	+	+	+	+	+	+
	1/2	+	+	+	+	+	+
	3/4	-	-	-	-	-	-
	1	-	-	-	-	-	-
	2	-	-	-	-	-	-

* Inoculation consisted of approximately 10,000 mold spores per tube.

+ indicates survival

- indicates destruction

Figure 12. Thermal death time curves of A. niger in different concentrations of dextrose adjusted to pH 4.0 ± 0.1

1. distilled water control
2. 10 per cent dextrose
3. 20 per cent dextrose
4. 30 per cent dextrose
5. 40 per cent dextrose
6. 50 per cent dextrose

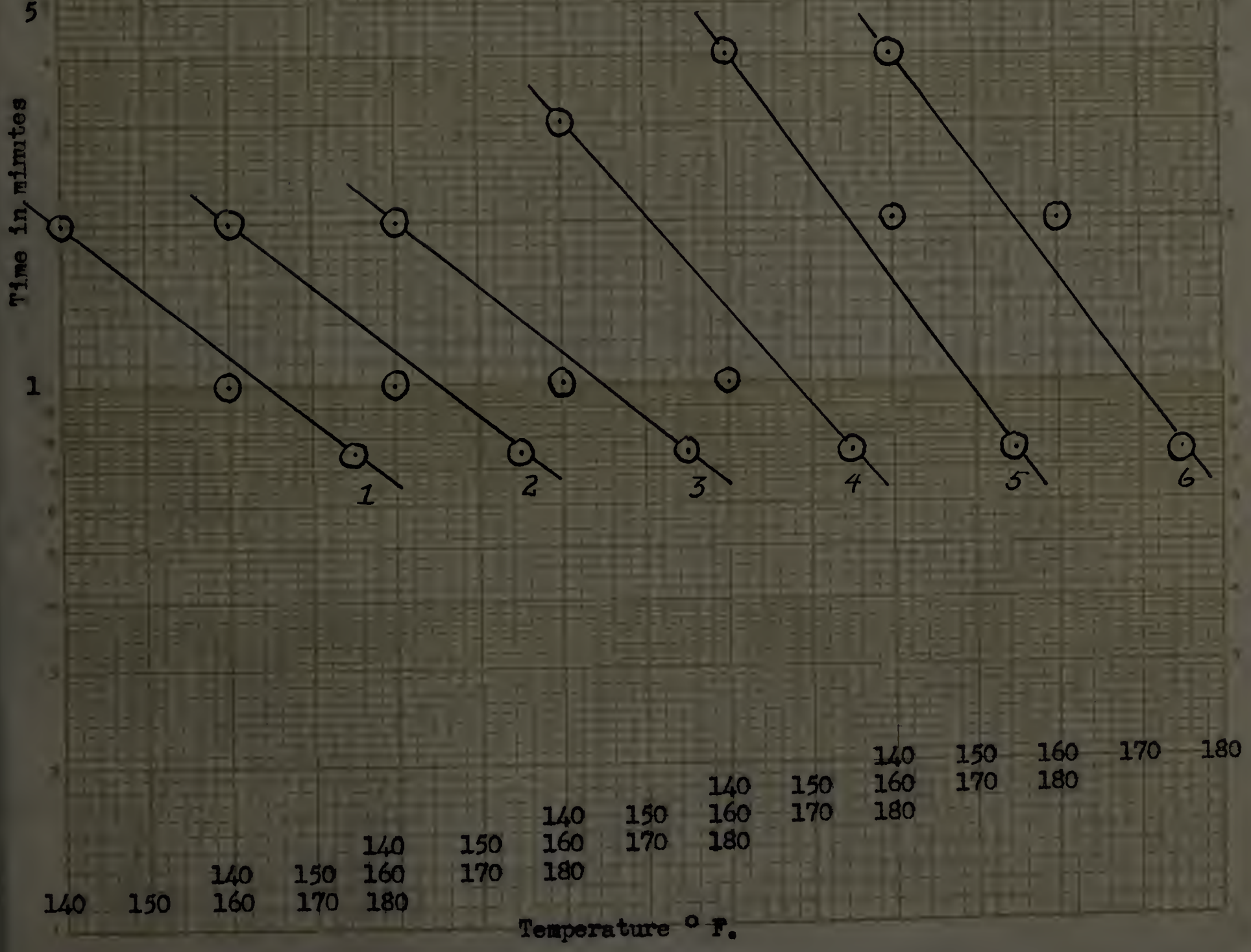


Table 13. Thermal death time of a Penicillium in sucrose solutions. *

Temperature	Exposure period	Control distilled water (pH 5.7)	Sucrose concentration in per cent 10 (pH 6.0)	20 (pH 6.1)	30 (pH 6.2)	40 (pH 6.4)	50 (pH 6.4)
o F.	minutes						
	1	+	+	+	+	+	+
	2	-	-	-	+	+	+
140	3	-	-	-	-	+	+
	4	-	-	-	-	-	+
	5	-	-	-	-	-	-
	1/2	+	+	+	+	+	+
	3/4	+	+	+	+	+	+
160	1	-	-	-	+	+	+
	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
	1/4	+	+	+	+	+	+
	1/2	+	+	+	+	+	+
175	3/4	-	-	-	-	+	+
	1	-	-	-	-	-	-
	2	-	-	-	-	-	-

* Inoculation consisted of approximately 10,000 spores per tube.

+ indicates survival

- indicates destruction

Figure 13. Thermal death time curves of a Penicillium in different concentrations of sucrose

1. distilled water control (pH 5.7)
2. 10 per cent sucrose (pH 6.0)
3. 20 per cent sucrose (pH 6.1)
4. 30 per cent sucrose (pH 6.2)
5. 40 per cent sucrose (pH 6.4)
6. 50 per cent sucrose (pH 6.4)

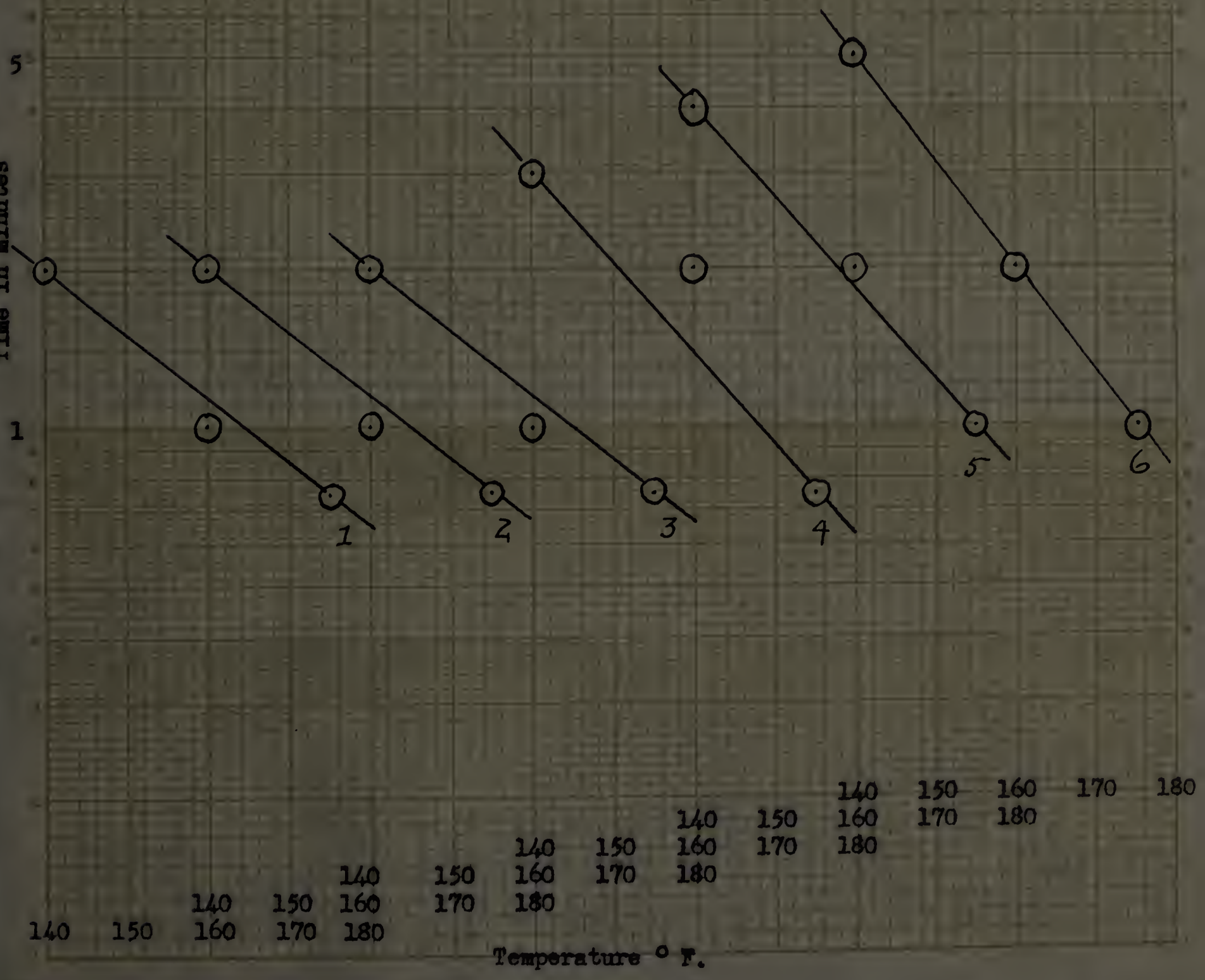


Table 14. Thermal death time of a Penicillium in dextrose solutions. *

Temperature	Exposure period	Control distilled water (pH 5.7)	Dextrose concentration in per cent 10 (pH 5.8)	20 (pH 5.8)	30 (pH 6.0)	40 (pH 6.0)	50 (pH 6.1)
° F.	minutes						
140	1	+	+	+	+	+	+
	2	-	-	-	+	+	+
	3	-	-	-	+	+	+
	4	-	-	-	-	-	+
	5	-	-	-	-	-	-
160	1/2	+	+	+	+	+	+
	3/4	+	+	+	+	+	+
	1	-	-	-	+	+	+
	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
175	1/4	+	+	+	+	+	+
	1/2	+	+	+	+	+	+
	3/4	-	-	-	+	+	+
	1	-	-	-	-	-	-
	2	-	-	-	-	-	-

* Inoculation consisted of approximately 10,000 spores per tube.

+ indicates survival

- indicates destruction

Figure 14. Thermal death time curves of a Penicillium

in different concentrations of dextrose

1. distilled water control (pH 5.7)
2. 10 per cent dextrose (pH 5.8)
3. 20 per cent dextrose (pH 5.8)
4. 30 per cent dextrose (pH 6.0)
5. 40 per cent dextrose (pH 6.1)
6. 50 per cent dextrose (pH 6.1)

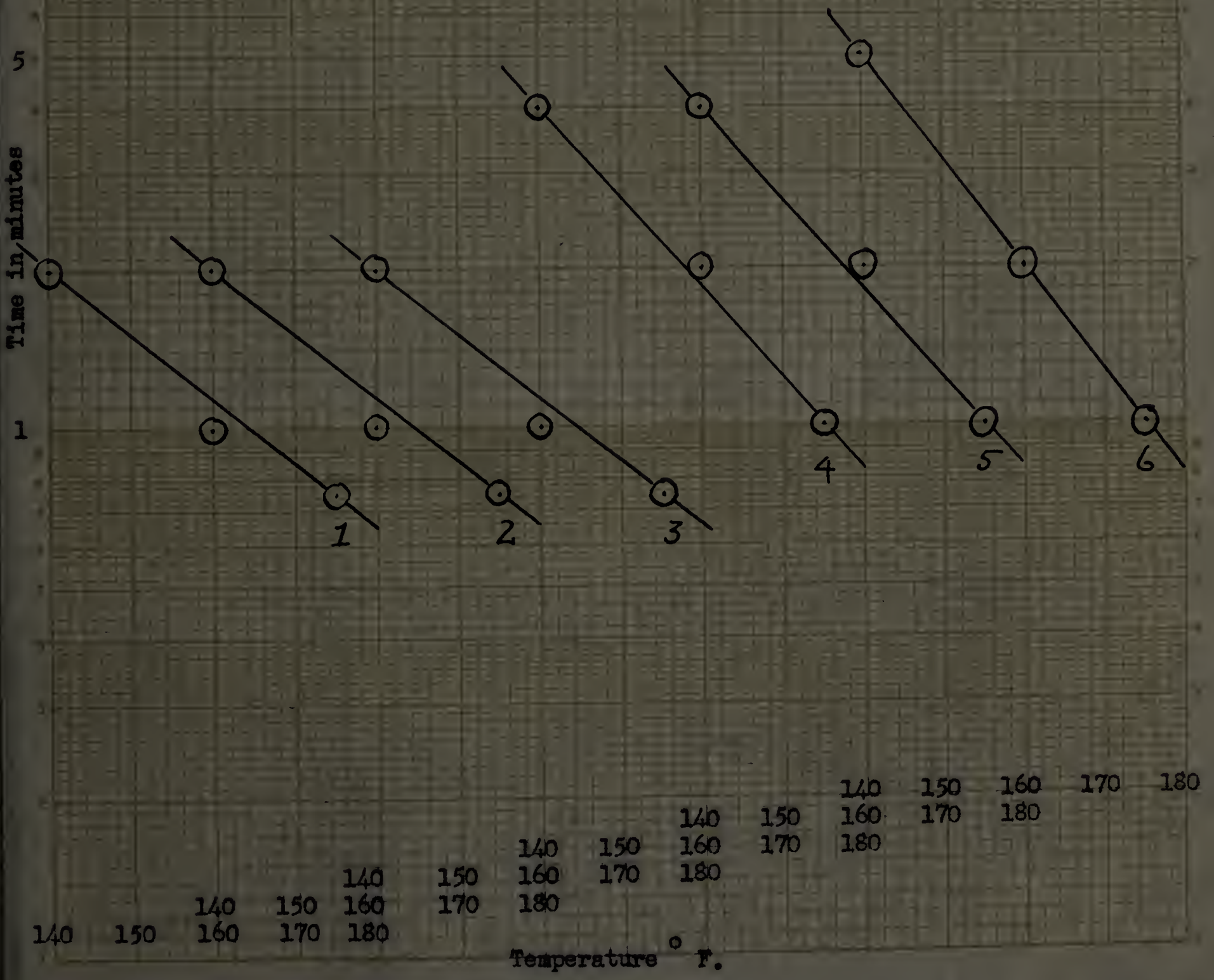


Table 15. Thermal death time of a Penicillium in sucrose solutions adjusted to pH 4.0 ± 0.1. *

Temperature	Exposure period	Control distilled water	Sucrose concentration in per cent				
			10	20	30	40	50
° F.	minutes						
140	1	+	+	+	+	+	+
	2	-	-	-	+	+	+
	3	-	-	-	-	-	-
	4	-	-	-	-	-	-
	5	-	-	-	-	-	-
160	1/2	+	+	+	+	+	+
	3/4	+	+	+	+	+	+
	1	-	-	-	+	+	+
	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
175	1/4	+	+	+	+	+	+
	1/2	+	+	+	+	+	+
	3/4	-	-	-	-	-	-
	1	-	-	-	-	-	-
	2	-	-	-	-	-	-

* Inoculation consisted of approximately 10,000 mold spores per tube.

+ indicates survival

- indicates destruction

Figure 15. Thermal death time curves of a Penicillium in different concentrations of sucrose adjusted to pH 4.0 ± 0.1

1. distilled water control
2. 10 per cent sucrose
3. 20 per cent sucrose
4. 30 per cent sucrose
5. 40 per cent sucrose
6. 50 per cent sucrose

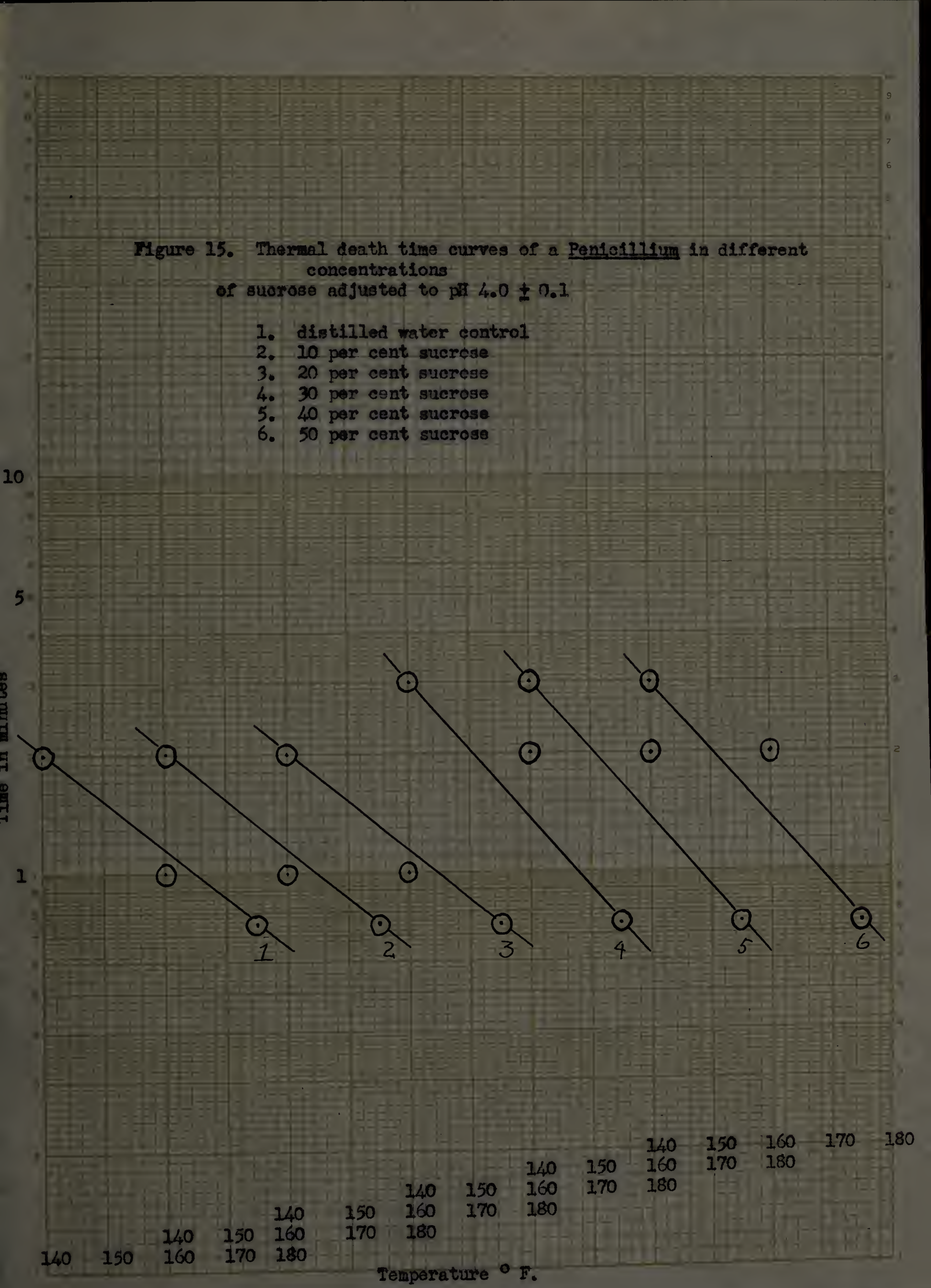


Table 16. Thermal death time of a Penicillium in dextrose solutions adjusted to pH 4.0 ± 0.1. *

Temperature	Exposure period	Control distilled water	Dextrose concentration in per cent				
			10	20	30	40	50
° F.	minutes						
140	1	+	+	+	+	+	+
	2	-	-	-	+	+	+
	3	-	-	-	-	-	-
	4	-	-	-	-	-	-
	5	-	-	-	-	-	-
160	1/2	+	+	+	+	+	+
	3/4	+	+	+	+	+	+
	1	-	-	-	-	+	+
	2	-	-	-	-	-	-
175	1/4	+	+	+	+	+	+
	1/2	+	+	+	+	+	+
	3/4	-	-	-	-	-	-
	1	-	-	-	-	-	-
	2	-	-	-	-	-	-

* Inoculation consisted of approximately 10,000 mold spores per tube.

+ indicates survival

- indicates destruction

Figure 16. Thermal death time curves of a Penicillium in different concentrations of dextrose adjusted to pH 4.0 ± 0.1

1. distilled water control
2. 10 per cent dextrose
3. 20 per cent dextrose
4. 30 per cent dextrose
5. 40 per cent dextrose
6. 50 per cent dextrose

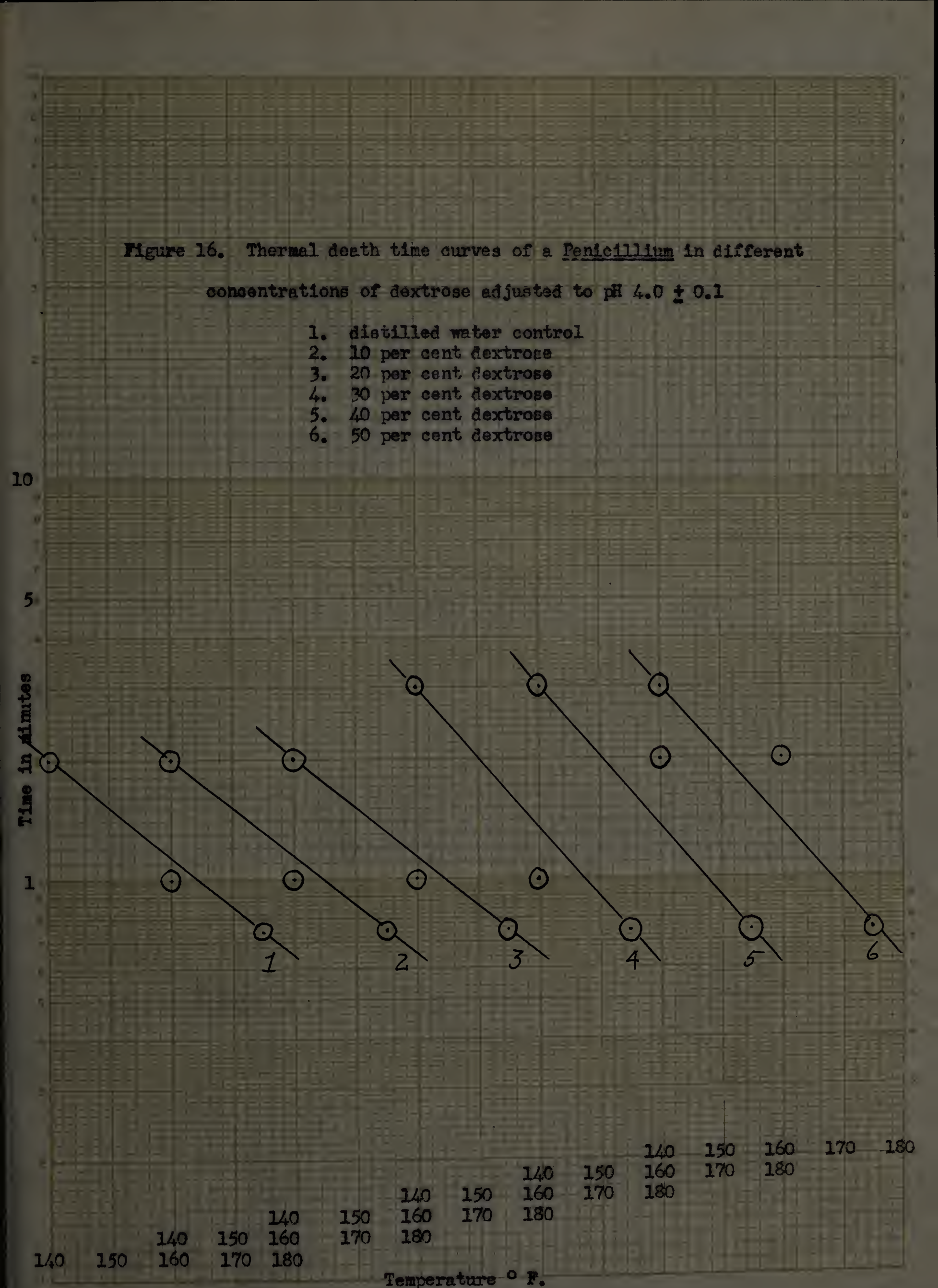


Table 17. Thermal death time of L. lycopersici in sucrose solutions. *

Temperature	Exposure period	Control distilled water (pH 5.7)	Sucrose concentration in per cent 10 (pH 6.1)	20 (pH 6.1)	30 (pH 6.2)	40 (pH 6.4)	50 (pH 6.4)
° F.	minutes						
150	1	+	+	+	+	+	+
	2	+	+	+	+	+	+
	3	-	-	-	+	+	+
	4	-	-	-	-	+	+
	5	-	-	-	-	-	+
	6	-	-	-	-	-	-
160	1	+	+	+	+	+	+
	2	-	-	-	+	+	+
	3	-	-	-	-	-	+
	4	-	-	-	-	-	-
170	1/2	+	+	+	+	+	+
	3/4	+	+	+	+	+	+
	1	-	-	-	+	+	+
	2	-	-	-	-	-	-
	3	-	-	-	-	-	-

* Inoculation consisted of approximately 50,000 bacterial cells per tube

+ indicates survival

- indicates destruction

Figure 17. Thermal death time curves of L. lycopersici

in different concentrations of sucrose

1. distilled water control (pH 5.7)
2. 10 per cent sucrose (pH 6.1)
3. 20 per cent sucrose (pH 6.1)
4. 30 per cent sucrose (pH 6.2)
5. 40 per cent sucrose (pH 6.4)
6. 50 per cent sucrose (pH 6.4)

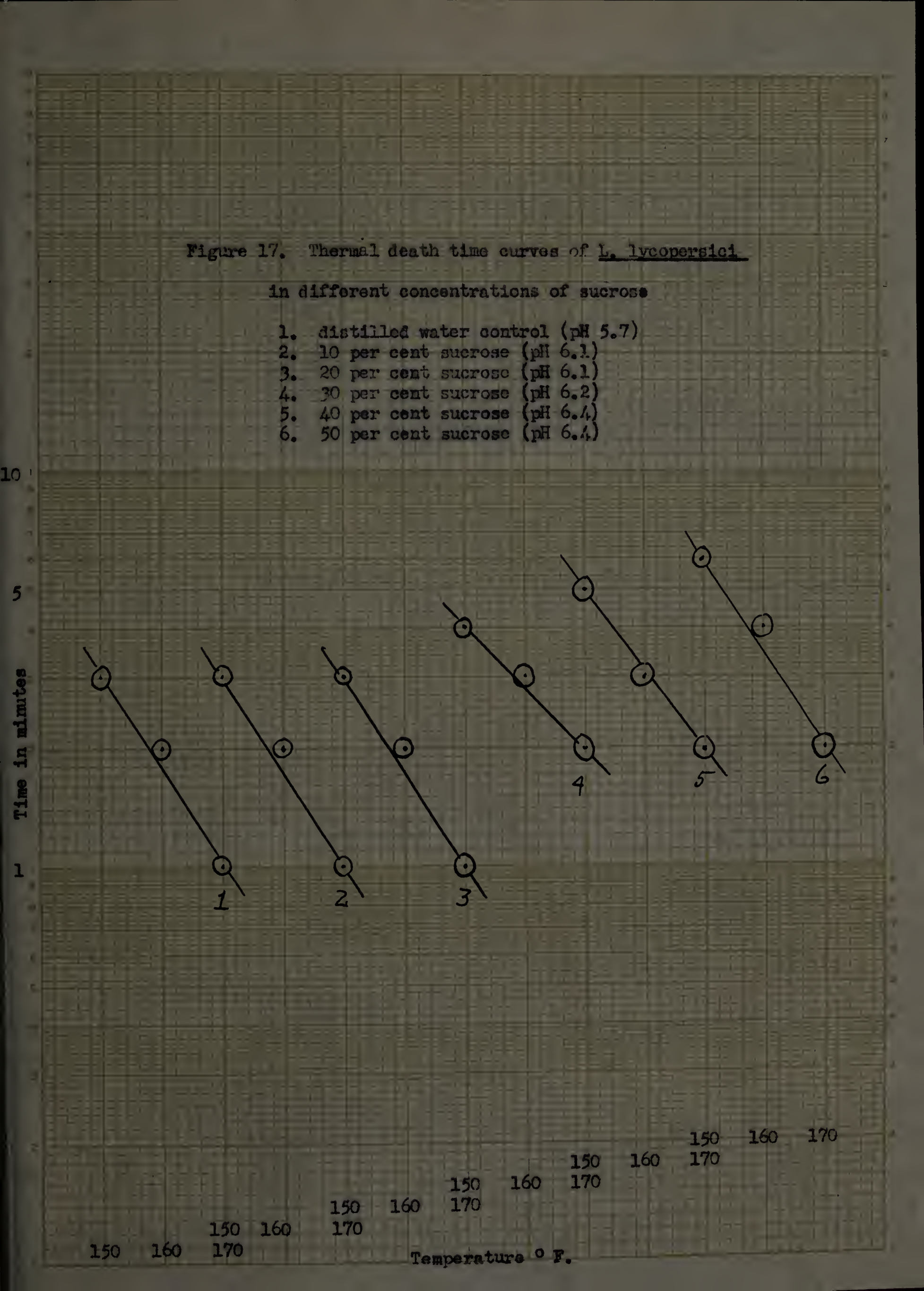


Table 18. Thermal death time of L. lycopersici in dextrose solutions. *

Temperature	Exposure period	Control distilled water (pH 5.8)	Dextrose concentration in per cent 10 (pH 5.9)	20 (pH 5.9)	30 (pH 6.0)	40 (pH 6.0)	50 (pH 6.1)
° F.	minutes						
150	1	+	+	+	+	+	+
	2	+	+	+	+	+	+
	3	-	-	-	+	+	+
	4	-	-	-	-	+	+
	5	-	-	-	-	-	+
	6	-	-	-	-	-	-
160	1	+	+	+	+	+	+
	2	-	-	-	+	+	+
	3	-	-	-	-	-	+
	4	-	-	-	-	-	-
170	1/2	+	+	+	+	+	+
	3/4	+	+	+	+	+	+
	1	-	-	-	+	+	+
	2	-	-	-	-	-	-
	3	-	-	-	-	-	-

* Inoculation consisted of approximately 50,000 bacterial cells per tube.

+ indicates survival

- indicates destruction

Figure 18. Thermal death time curves of L. lycopersici

in different concentrations of dextrose

1. distilled water control (pH 5.8)
2. 10 per cent dextrose (pH 5.9)
3. 20 per cent dextrose (pH 5.9)
4. 30 per cent dextrose (pH 6.0)
5. 40 per cent dextrose (pH 6.0)
6. 50 per cent dextrose (pH 6.1)

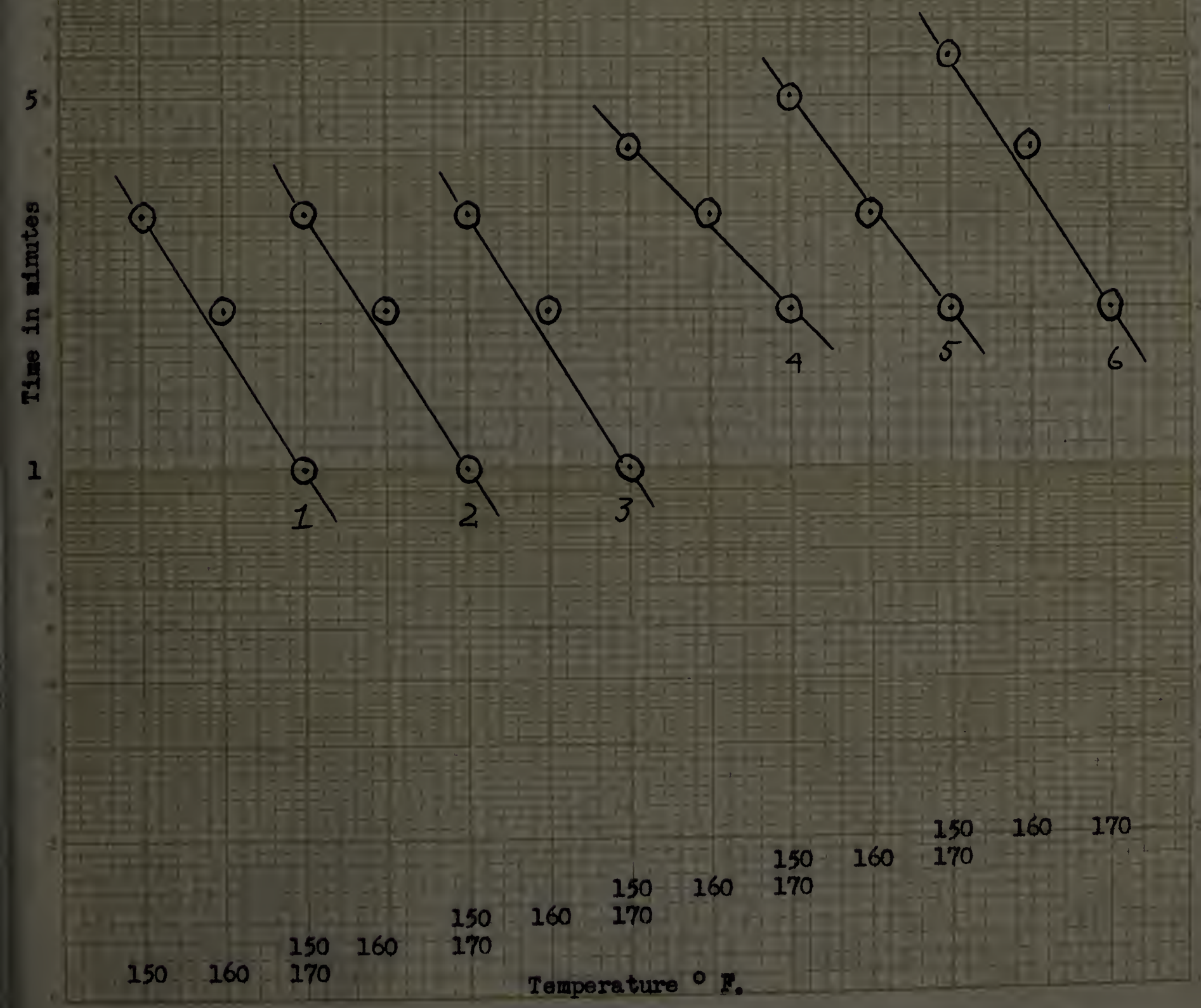


Table 19. Thermal death time of L. lycopersici in sucrose solutions adjusted to pH 4.0 ± 0.1. *

Temperature	Exposure period	Control distilled water	Sucrose concentration in per cent				
			10	20	30	40	50
° F.	minutes						
150	1	+	+	+	+	+	+
	2	+	+	+	+	+	+
	3	-	-	-	+	+	+
	4	-	-	-	-	+	+
	5	-	-	-	-	-	+
	6	-	-	-	-	-	-
160	1	+	+	+	+	+	+
	2	-	-	-	+	+	+
	3	-	-	-	-	-	+
	4	-	-	-	-	-	-
170	1/2	+	+	+	+	+	+
	3/4	+	+	+	+	+	+
	1	-	-	-	-	+	+
	2	-	-	-	-	-	-

* Inoculation consisted of approximately 50,000 bacterial cells per tube.

+ indicates survival

- indicates destruction

Figure 19. Thermal death time curves of L. lycopersici in different concentrations of sucrose adjusted to pH 4.0 ± 0.1

1. distilled water control
2. 10 per cent sucrose
3. 20 per cent sucrose
4. 30 per cent sucrose
5. 40 per cent sucrose
6. 50 per cent sucrose

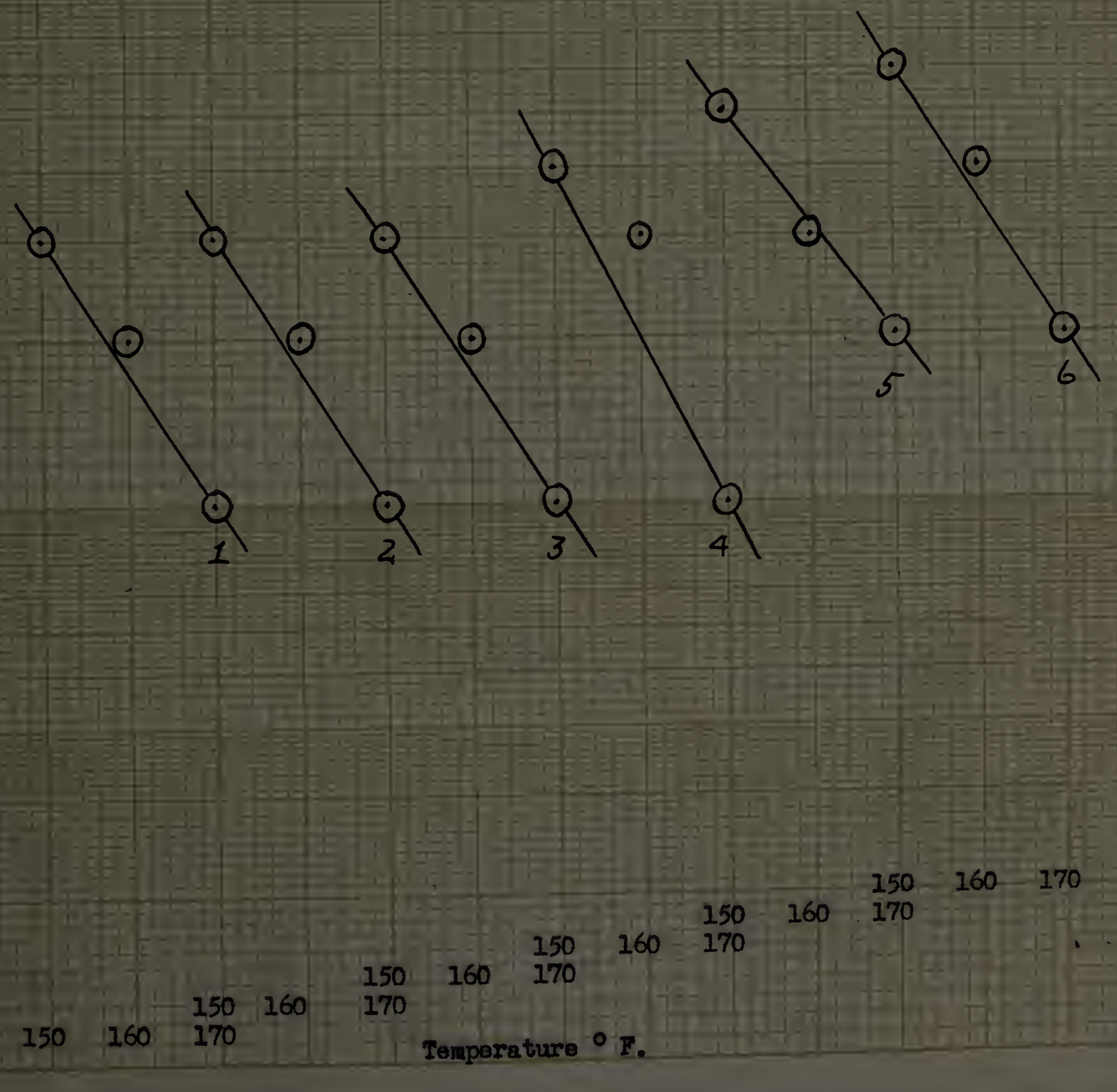


Table 20. Thermal death time of L. lycopersici in dextrose solutions adjusted to pH 4.0 ± 0.1. *

Temperature	Exposure period	Control distilled water	Dextrose concentration in per cent				
			10	20	30	40	50
° F.	minutes						
150	1	+	+	+	+	+	+
	2	+	+	+	+	+	+
	3	-	-	+	+	+	+
	4	-	-	-	-	+	+
	5	-	-	-	-	-	+
	6	-	-	-	-	-	-
160	1	+	+	+	+	+	+
	2	-	-	-	+	+	+
	3	-	-	-	-	-	+
	4	-	-	-	-	-	-
170	1/2	+	+	+	+	+	+
	3/4	+	+	+	+	+	+
	1	-	-	-	-	+	+
	2	-	-	-	-	-	-

* Inoculation consisted of approximately 50,000 bacterial cells per tube.

+ indicates survival

- indicates destruction

Figure 20. Thermal death time curves of L. lycopersici in different concentrations of dextrose adjusted to pH 4.0 ± 0.1

1. distilled water control
2. 10 per cent dextrose
3. 20 per cent dextrose
4. 30 per cent dextrose
5. 40 per cent dextrose
6. 50 per cent dextrose

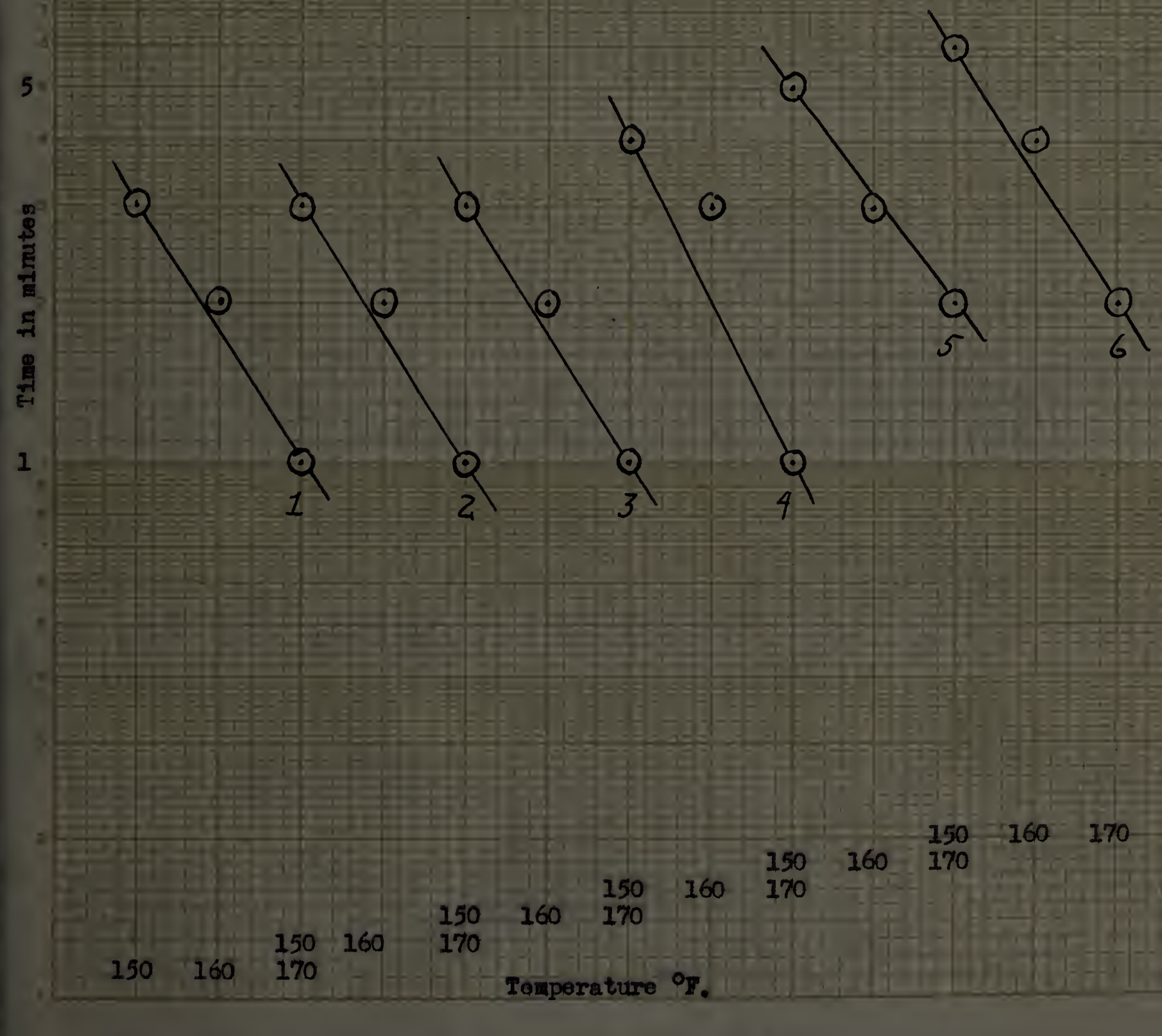


Table 21. Thermal death time of C. pastorianum in sucrose solutions. *

Temperature	Exposure period	Control distilled water (pH 5.7)	Sucrose concentration in per cent 10 (pH 6.1)	20 (pH 6.1)	30 (pH 6.2)	40 (pH 6.3)	50 (pH 6.4)
° F.	minutes						
190	7	+	+	+	+	+	+
	8	+	+	+	+	+	+
	9	+	+	+	+	+	+
	10	-	-	-	-	-	+
	11	-	-	-	-	-	-
200	2	+	+	+	+	+	+
	3	+	+	+	+	+	+
	4	+	+	+	+	+	+
	5	-	-	-	-	-	+
	6	-	-	-	-	-	-
212	1	+	+	+	+	+	+
	2	+	+	+	+	+	+
	3	-	-	-	-	-	+
	4	-	-	-	-	-	-

* Inoculation consisted of approximately 50,000 bacterial spores per tube.

+ indicates survival

- indicates destruction

Figure 21. Thermal death time curves of C. pastorianum

in different concentrations of sucrose

1. distilled water control (pH 5.7)
2. 10 per cent sucrose (pH 6.1)
3. 20 per cent sucrose (pH 6.1)
4. 30 per cent sucrose (pH 6.2)
5. 40 per cent sucrose (pH 6.2)
6. 50 per cent sucrose (pH 6.4)

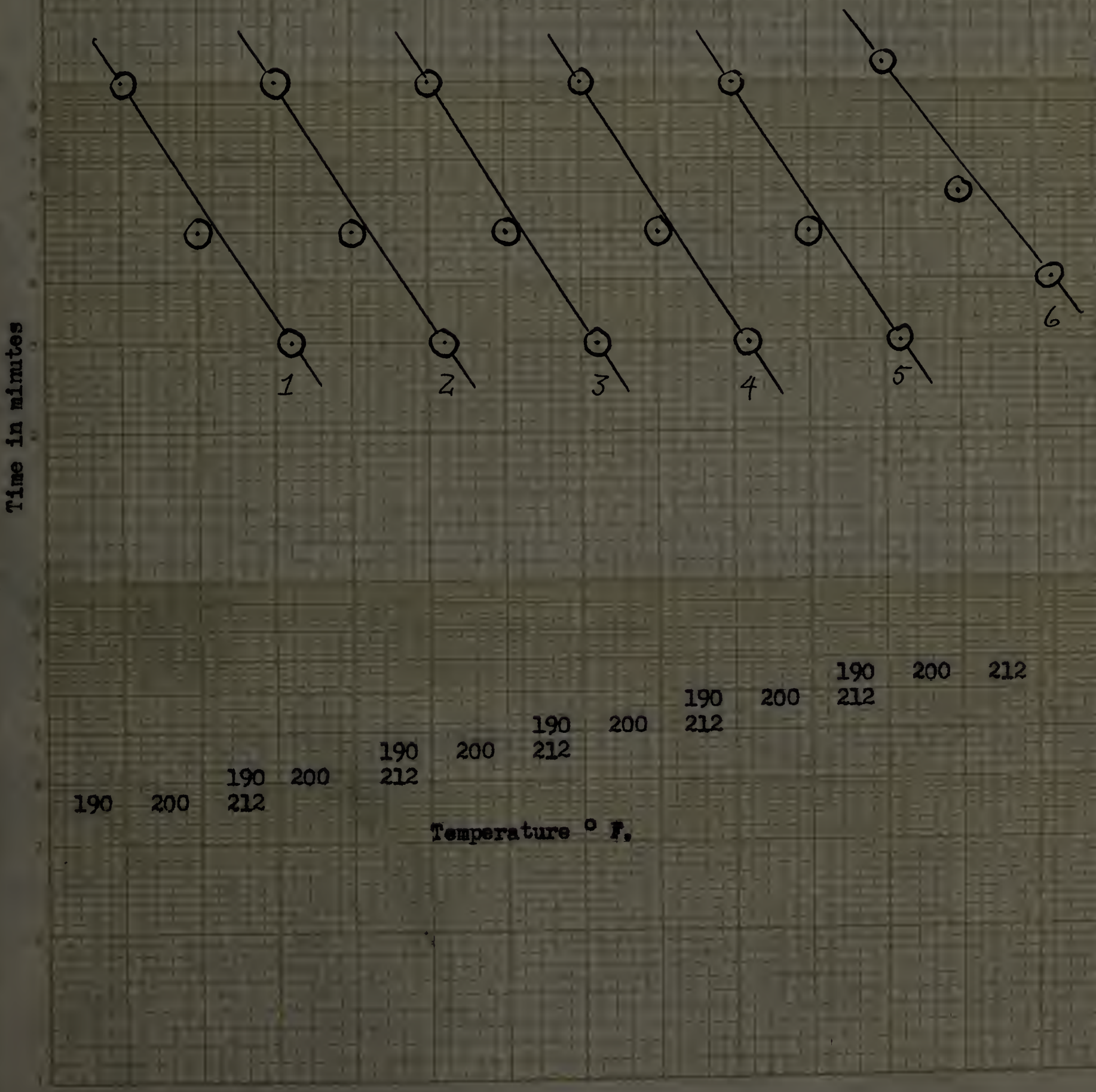


Table 22. Thermal death time of C. pastorianum in dextrose solutions. *

Temperature	Exposure period	Control distilled water (pH 5.7)	Dextrose concentration in per cent 10 (pH 5.9)	20 (pH 5.9)	30 (pH 6.0)	40 (pH 6.0)	50 (pH 6.1)
° F.	minutes						
190	7	+	+	+	+	+	+
	8	+	+	+	+	+	+
	9	+	+	+	+	+	+
	10	-	-	-	-	-	+
	11	-	-	-	-	-	-
200	2	+	+	+	+	+	+
	3	+	+	+	+	+	+
	4	+	+	+	+	+	+
	5	-	-	-	-	-	+
	6	-	-	-	-	-	-
212	1	+	+	+	+	+	+
	2	+	+	+	+	+	+
	3	-	-	-	-	-	+
	4	-	-	-	-	-	-

* Inoculation consisted of approximately 50,000 bacterial spores per tube.

+ indicates survival

- indicates destruction

Figure 22. Thermal death time curves of C. pastorianum

in different concentrations of dextrose

1. distilled water control (pH 5.7)
2. 10 per cent dextrose (pH 5.9)
3. 20 per cent dextrose (pH 5.9)
4. 30 per cent dextrose (pH 6.0)
5. 40 per cent dextrose (pH 6.0)
6. 50 per cent dextrose (pH 6.1)

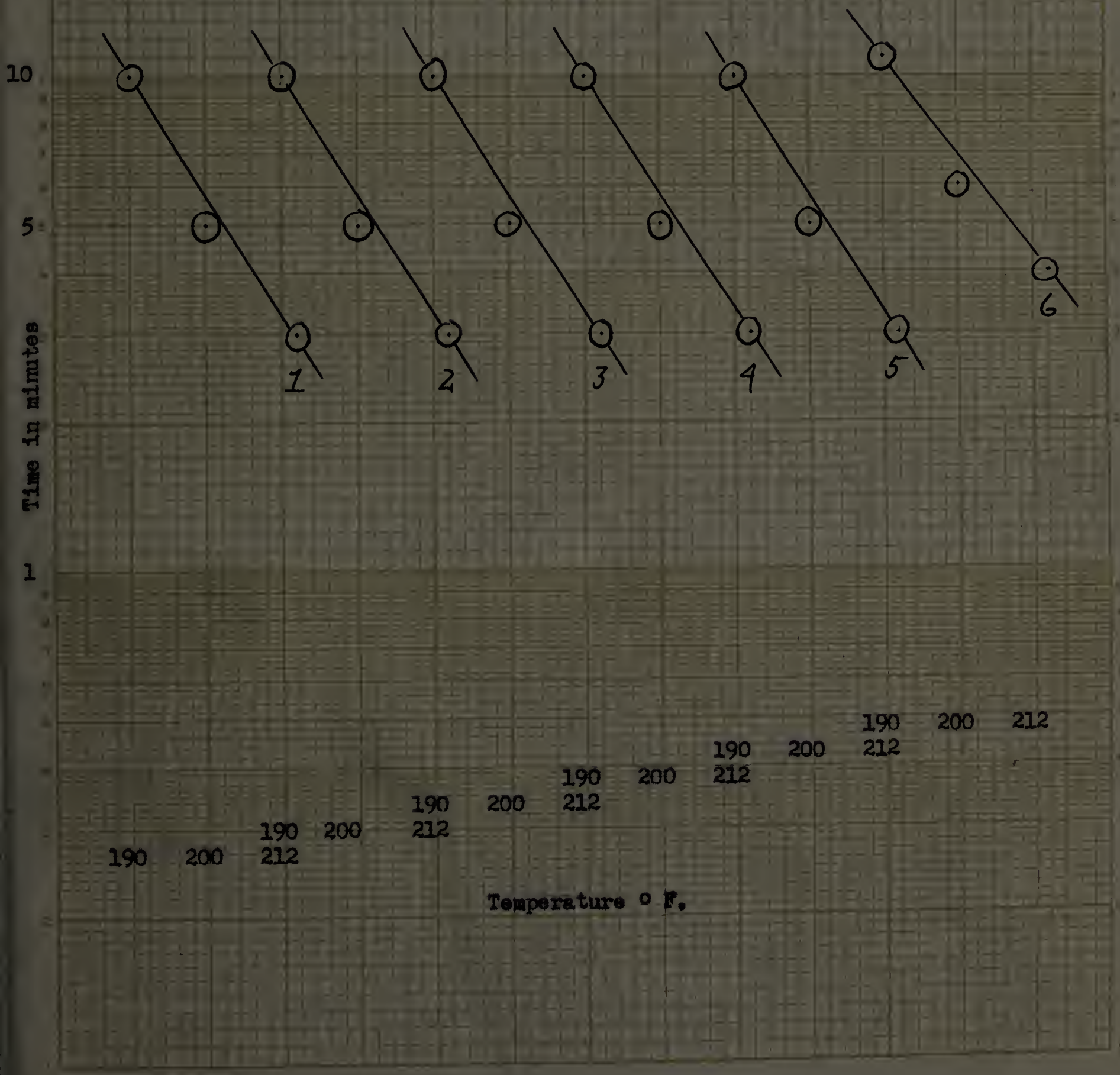


Table 23. Thermal death time of C. pastorianum in sucrose solutions adjusted to pH 4.0 ± 0.1. *

Temperature	Exposure period	Control distilled water	Sucrose concentration in per cent				
			10	20	30	40	50
° F.	minutes						
190	5	+	+	+	+	+	+
	6	+	+	+	+	+	+
	7	+	+	+	+	+	+
	8	+	+	+	+	+	+
	9	-	-	-	-	-	+
	10	-	-	-	-	-	-
200	1	+	+	+	+	+	+
	2	+	+	+	+	+	+
	3	+	+	+	+	+	+
	4	-	-	-	-	-	-
	5	-	-	-	-	-	-
212	1	+	+	+	+	+	+
	2	-	-	-	-	-	+
	3	-	-	-	-	-	-
	4	-	-	-	-	-	-

* Inoculation consisted of approximately 50,000 spores per tube.

+ indicates survival

- indicates destruction

Figure 23. Thermal death time curves of C. pastorianum in different concentrations of sucrose adjusted to pH 4.0 ± 0.1

1. distilled water control
2. 10 per cent sucrose
3. 20 per cent sucrose
4. 30 per cent sucrose
5. 40 per cent sucrose
6. 50 per cent sucrose

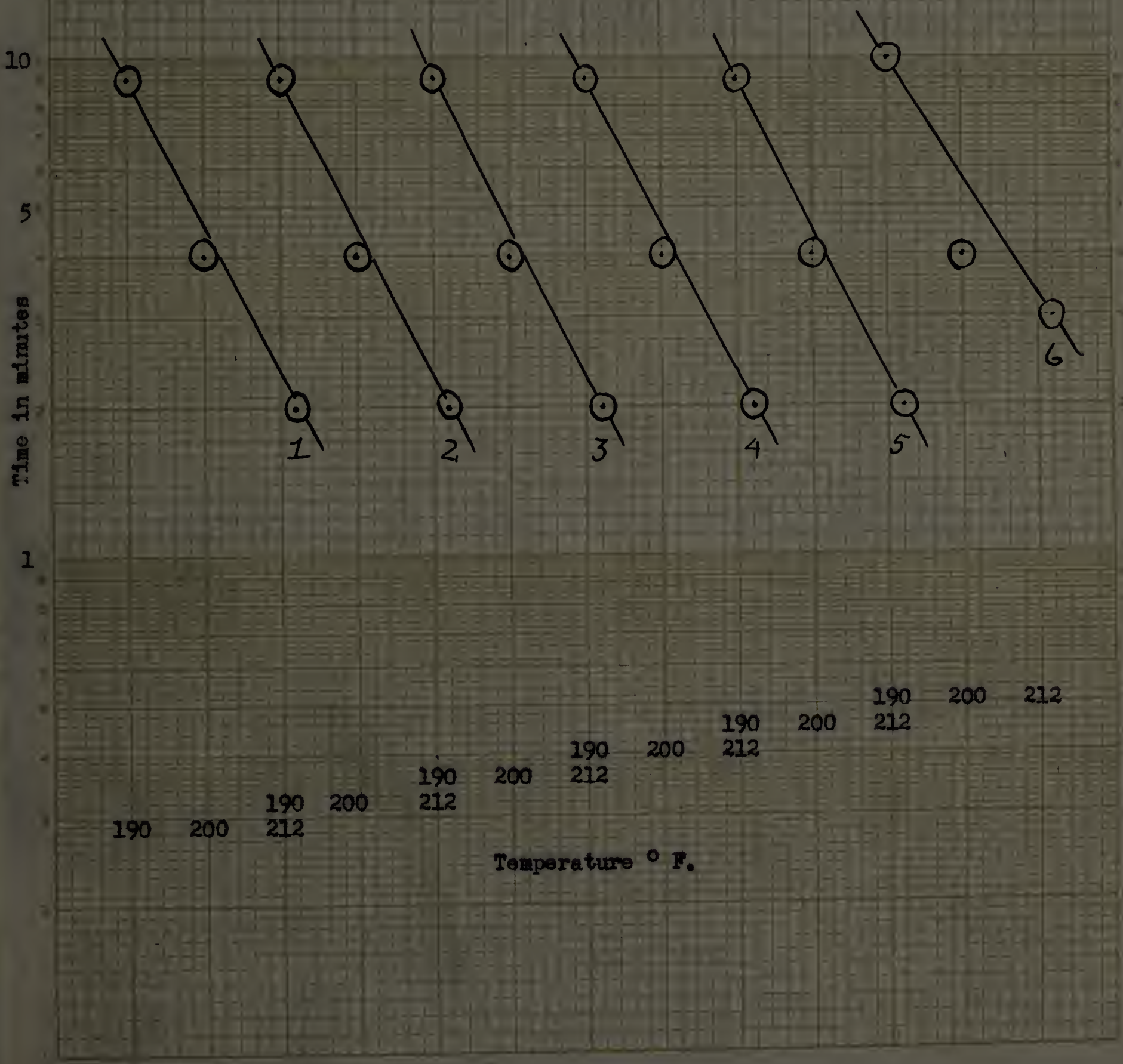


Table 24. Thermal death time of C. pastorianum in dextrose solutions adjusted to pH 4.0 ± 0.1. *

Temperature	Exposure period	Control distilled water	Dextrose concentration in per cent				
			10	20	30	40	50
° F.	minutes						
190	5	+	+	+	+	+	+
	6	+	+	+	+	+	+
	7	+	+	+	+	+	+
	8	+	+	+	+	+	+
	9	-	-	-	-	-	-
	10	-	-	-	-	-	-
200	1	+	+	+	+	+	+
	2	+	+	+	+	+	+
	3	+	+	+	+	+	+
	4	-	-	-	-	-	-
	5	-	-	-	-	-	-
212	1	+	+	+	+	+	+
	2	-	-	-	-	-	+
	3	-	-	-	-	-	-
	4	-	-	-	-	-	-

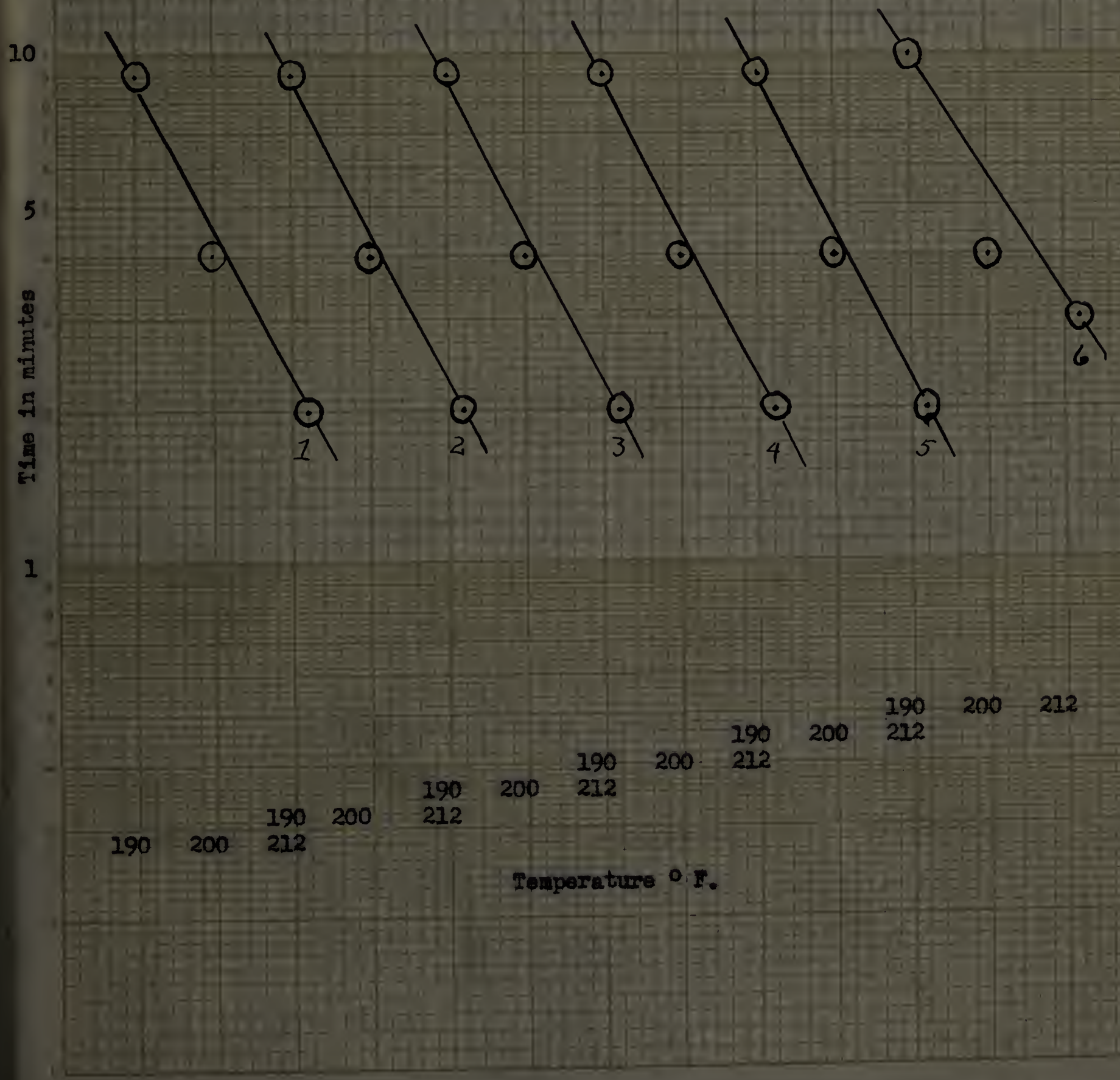
* Inoculation consisted of approximately 50,000 spores per tube.

+ indicates survival

- indicates destruction

Figure 24. Thermal death time curves of C. pastorianum in different concentrations of dextrose adjusted to pH 4.0 ± 0.1

1. distilled water control
2. 10 per cent dextrose
3. 20 per cent dextrose
4. 30 per cent dextrose
5. 40 per cent dextrose
6. 50 per cent dextrose



concentrations exerts a protective action on thermal resistance. It was also noted that L. lycopersici was found to be more heat resistant than were the yeasts or molds.

Tables 21-24 and figures 21-24 give the results obtained with the spores of C. pastorianum. As was expected, this organism was found to be the most heat resistant of all the index organisms. And here again, it was evident that the thermal resistance of the spores was the same when either sucrose or dextrose was used as the suspending medium. At a pH value of 4.0, the thermal resistance of the spores was somewhat reduced, but the results obtained in sucrose or dextrose solutions were still in agreement. Only a slight protective action was afforded these spores, and this action was observed only in 50 per cent sugar.

Experimental Pack Studies

Another part of the procedure in the determination of a safe process time and temperature for a canned product, consists in inoculating experimental packs of the product with the particular organism responsible for spoilage. The inoculated containers are then processed for varying lengths of time at a given temperature to determine the minimum process time and temperature needed to destroy the particular organisms. Tables 25 and 26 give the results obtained when the index organisms were inoculated into canned apples packed with 40 per cent sucrose or 40 per cent dextrose. It is evident from these tables that the minimum required process time

Table 25. Survival of the index organisms in canned apples packed in 40 per cent sucrose syrup.

Organism [1]	Process temperature	Process time in minutes			
		0	5	10	15
	° F.				
S. cerevisiae *	180	+	-	-	-
S. cerevisiae *	212	+	-	-	-
S. ellipsoideus *	180	+	-	-	-
S. ellipsoideus *	212	+	-	-	-
A. niger **	180	+	+	-	-
A. niger **	212	+	-	-	-
Penicillium **	160	+	+	+	-
Penicillium **	180	+	+	-	-
Penicillium **	212	+	-	-	-
L. lycopersici **	180	+	+	-	-
L. lycopersici **	212	+	-	-	-
C. pastorianum **	212	+	-	-	-

* Rhode Island Greenings (pH 3.2) used.
** MacIntosh (pH 4.0) used

[1] Inoculation consisted of 50,000 yeast cells, 50,000 bacterial cells or spores, and 10,000 mold spores per container.

+ indicates survival of organism and spoilage.

- indicates destruction of organism and no spoilage.

Table 26. Survival of the index organisms in canned apples packed in 40 per cent dextrose syrup.

Organism [1]	Process temperature	Process time in minutes			
		0	5	10	15
	° F.				
S. cerevisiae *	180	+	-	-	-
S. cerevisiae *	212	+	-	-	-
S. ellipsoideus *	180	+	-	-	-
S. ellipsoideus *	212	+	-	-	-
A. niger **	180	+	+	-	-
A. niger **	212	+	-	-	-
Penicillium **	180	+	+	+	-
Penicillium **	212	+	-	-	-
L. lycopersici **	180	+	+	-	-
L. lycopersici **	212	+	-	-	-
C. pastorianum **	212	+	-	-	-

* Rhode Island Greenings (pH 3.2) used.
** MacIntosh (pH 4.0) used.

[1] Inoculation consisted of 50,000 yeast cells, 50,000 bacterial cells or spores, and 10,000 mold spores per container.

\+ indicates survival of organism and spoilage.

\- indicates destruction of organism and no spoilage.

and temperature needed to destroy the organisms was the same when either sucrose or dextrose was used as the syrup..

The yeasts were the least resistant of the organisms, not withstanding five minutes at 180° F. (82.2° C.). The molds and L. lycopersici were somewhat more resistant, surviving five minutes at 180° F. (82.2° C.) but not for 10 minutes. All the organisms including the spores of C. pastorianum were destroyed in five minutes at 212° F. (100° C.).

The uninoculated controls prepared along with the inoculated packs were observed for difference in texture, flavor, and color. It was found that the all dextrose packed apples had an off flavor, a softer texture, and a darkened color when compared to the all sucrose packed product.

DISCUSSION

The inhibition of microbial growth by sugar is usually attributed to the osmotic pressure of the sugar. The differences observed by Tarkow(1940) in the inhibitive action of sucrose and dextrose on microorganisms were attributed to the difference in osmotic pressure of the two sugars. The osmotic pressure of a sugar solution is dependent on the number of particles suspended in it. Since the molecular weight of sucrose is 342, and that of dextrose 180, a given percentage of dextrose will result in solution of greater osmotic pressure than a solution of equal percentage of sucrose, due to the greater number of dextrose particles in solution. Thus, when a microorganism is suspended in solutions of equal concentrations of sucrose or dextrose, the greater number of particles in the dextrose solution will result in the organisms being subjected to a greater osmotic pressure, and consequently greater inhibition should result in the dextrose solution.

Jordan(1940) supported this hypothesis, and Nunheimer and Fabian(1940) also explained their differential inhibitive results on this basis.

The results presented in this paper, however, indicate that there was little or no difference in the effect of sucrose or dextrose on the thermal resistance of the organisms studied. A partial explanation of these results is offered on the following basis. In the determination of the thermal resistance of a microorganism in sugar solutions, the particular organism is only in contact with

the sugar solution during the heating period. In many cases, this was less than five minutes, and even with the spores of C. pastorianum, it never amounted to more than 11 minutes. It is likely that this short period of contact with the sugar solution did not allow for the full effect of the osmotic pressure to take place, and thus the similarity of results with both sugars. Previous investigators who have reported superior inhibitive action of dextrose over sucrose (Tarkow(1940); Nunheimer and Fabian(1940); and Erickson and Fabian(1942)) actually grew the organism in the sugar solutions and the particular organism was allowed to remain in contact with the sugar solution for as long as 7 to 10 days.

Generally speaking, a microorganism will be destroyed more effectively in an acid medium than in an alkaline medium. Yet, when the pH values of the sucrose and dextrose solutions were adjusted to 4.0, a slight decrease in the thermal resistance of the organisms was observed only in the higher sugar concentrations. There still was little or no difference between the action of sucrose and dextrose. Here again an explanation can be made on the basis of the short period of contact with the acid medium. Also, the index organisms have all been associated with the spoilage of acid products and have been described as more or less acid resistant. Aref and Cruess(1934) found that the pH value of the medium affected the death temperature and death rate of S. ellipsoideus relatively little over the range of pH 1.45 to 7.0. Wallace and Tanner(1931) reported that the thermal death time of six molds in sugar solutions, juice from pitted cherries,

and distilled water was the same. Cherry juice has a low pH value, thus their results are confirmed by those presented herein.

The question may arise as to the amount of inversion taking place in a tube of sucrose of pH 4.0 during thermal treatment. The author feels that the length of the heat treatment in each case was not sufficient to cause a significant amount of inversion; and if some inversion did take place, it would merely serve to stimulate more closely the conditions of practical canning. Since fruits usually have an acid pH value, and are packed with sucrose syrups, the amount of inversion taking place in the container would certainly be no less than that taking place in the test tube.

The protective action of sugars on the thermal resistance of microorganisms has been reported by many authors. Baumgartner and Wallace(1934), in explaining this protective action, reported that bacterial protein was analogous to albumin in that moist heat was more effective than dry heat for sterilization. It follows that if bacteria are heated in sugar syrups and the syrup exerts a non-toxic dehydrating influence on them, an increase in the thermal resistance of such cells should be observed. Rahn(1928) and Robertson(1927) also explained the protective action of sugars on the dehydrating influence of high sugar concentrations. The results obtained in this paper show that sugar exerts a greater protective action on the yeast and bacterial cells than on the C. pastorianum spores. These results are in agreement with those of Baumgartner and Wallace(1934) who also found that sugar exerted a greater protective action on the vegetative cells of E. coli, than on the spores of an aerobe or anaerobe.

The thermal death times of the various index organisms as reported by previous investigators are in general agreement with those presented here. Tracy(1932) found that one minute at 143.6° F. (62° C.) was necessary to destroy S. ellipsoideus. Pederson(1929) reported that L. lycopersici was destroyed in tomato juice at pH 4.4 in three to five minutes at 149° F. (65° C.) and in one minute at 168.8° F. (76° C.). Spiegelberg(1940) studied the thermal resistance of C. pastorianum spores and found that both the S and R spores were not highly resistant, since all failed to resist 212° F. (100° C.) for five minutes.

As was expected from the results obtained in the thermal death time studies, the yeasts were found to be the easiest of the index organisms to destroy in experimentally inoculated packs, followed by the molds and L. lycopersici. It is of interest to note that in spite of the fact that the thermal death time of the C. pastorianum spores was much greater than that of the other organisms, it did not survive five minutes process at 212° F. (100° C.) Spiegelberg(1940a) also reported that these spores were not very resistant in an acid fruit. He concluded from his studies on the factors in the spoilage of acid canned fruits, that a pH value of 4.4 was a critical value below which a process temperature of 190° F. (87.8° C.) would destroy these spores.

The factors that may have caused the molds to show increased heat resistance over the yeasts in the experimental packs may have been the pH values of the apples and the type of container used in each case. With the yeasts, Rhode Island Greenings (pH 3.2) packed

in No. 2 plain tin cans were used; while with the molds, MacIntosh (pH 4.0) packed in 1/2 pint glass jars were used.

The similar results obtained in the survival of the index organisms in either sucrose or dextrose syruped apples further supports the contention that there is no difference in the effect of sucrose or dextrose on the thermal resistance of the index organisms.

The author feels that the results presented in this paper are of practical value. A knowledge that the process time and temperature cannot be lowered if a dextrose syrup is used in place of sucrose syrup in the canning of fruits should be of value to the canner. Further work on the quantitative survival of various microorganisms in sucrose and dextrose syrups would be advisable in order to throw more light on the comparative inhibitive action of these two sugars.

SUMMARY

1. An investigation was made on the effect of different concentrations of sucrose and dextrose on the thermal resistance of certain microorganisms associated with the spoilage of acid food products. To compare in a more practical manner the effects of sucrose and dextrose on the thermal resistance of the index organisms, the minimum required process time and temperature needed to destroy these organisms was determined by inoculating experimental packs of apples.

2. The organisms studied were the yeasts, S. cerevisiae and S. ellipsoideus; the molds, A. niger, and a strain of Penicillium; and the bacteria, L. lycopersici, and C. pastorianum.

3. There was little or no difference in the effect of sucrose or dextrose solutions at a pH value of approximately 6.0 on the thermal resistance of the index organisms.

4. Sugar concentrations of 30 per cent or more increased the thermal resistance of the yeasts, molds, and L. lycopersici. A 50 per cent sugar solution afforded the spores of C. pastorianum only a slight protection.

5. When the pH value of the sugar solutions was adjusted to 4.0, there was still little or no difference observed in the effect of sucrose or dextrose on the thermal resistance of the organisms.

6. The thermal death times of the organisms in sugar solutions of pH 4.0, as compared to those in sugar solutions of pH 6.0, were reduced slightly at the higher sugar concentrations, except in the case of the spores of C. pastorianum. In this case, the thermal death time was reduced somewhat in all concentrations of sugar at the lower pH value.

7. In inoculated packs of apples with pH values of 3.2 and 4.0, packed with 40 per cent sucrose or dextrose syrup, all the organisms were destroyed in five minutes at 212° F. (100° C.)

8. When all dextrose was used as the syrup for canned apples, an off flavor, a dark color, and a soft texture resulted.

CONCLUSION

Sucrose and dextrose have similar effects on the thermal resistance of some microorganisms associated with the spoilage of acid food products.

LITERATURE CITED

Anzulovic, J. Venceslar, 1932. Study of E. coli in ice cream. J. Bact. 23, 56-58.

Aref, H., and Cruess, W. V., 1934. An investigation of the thermal death point of Saccharomyces ellipsoideus. J. Bact. 27, 443-452.

Beavens, E. A., 1930. The effect of lactose on the survival of E. coli when heated to 145° F. for 30 minutes. J. Dairy Sci. 13, 91-93.

Baumgartner, J. G., 1938. Heat sterilized reducing sugars and their effects on the thermal resistance of bacteria. J. Bact. 36, 369-382.

Baumgartner, J. G., and Wallace, M. D., 1934. Destruction of micro-organisms in the presence of sugars. I. Role of sucrose in the commercial processing of canned fruits. J. Soc. Chem. Ind. 53, 294-297 T.

Bigelow, W. D., and Esty, J. R., 1920. The thermal death point in relation to time of typical thermophilic organisms. J. Infectious Diseases 27, 602-617.

Braun, O. G., Hays, C. L., and Benjamin, H. A., 1941. Use of dry sugar in sweetening foods canned in syrups. Food Industries 13, No. 3, 64-65.

Buchanan, J. H., 1932. Corn sugar for beverages. Glass Packer 11, 357.

Dozier, C. C., 1924. Inhibitive influence of sugars and salt on viability, growth, and toxin production of B. botulinus. J. Infectious Diseases 35, 134-155.

Erickson, F. J., and Fabian, F. W., 1942. Preserving and germicidal action of various sugars and organic acids on yeasts and bacteria. Food Research 7, 68-79.

Esty, J. R., 1928. Determination of thermal death time. The Newer Knowledge of Bacteriology and Immunology by E. O. Jordan and I. S. Falk. University of Chicago Press, Chicago, Illinois. 1196 pp.

Esty, J. R., and Meyer, K. F., 1922. The heat resistance of the spores of B. botulinus and allied anaerobes. XI. J. Infectious Diseases 31, 650-663.

Fabian, F. W., and Coulter, E. W., 1930. Significance of Colon-Aerogenes groups in ice cream. 1. Survival of member of Escherichia-Aerobacter group to pasteurizing temperatures in ice cream. J. Dairy Sci. 13, 273-287.

Fay, A. C., 1934. The effect of hypertonic sugar solutions on the thermal resistance of bacteria. J. Agr. Research 48, 453-468.

Fulmer, E. I., Williams, A. L., and Werkman, C. H., 1931. The effect of sterilization of media upon their growth promoting properties toward bacteria. J. Bact. 21, 299-303.

Irish, J. H., Joslyn, M. A., and Parcell, J. W, 1928. Heat penetration in the pasteurizing of syrups and concentrates in glass containers. Hilgardia 3, 183-206. C.A. 22, 3710.

Jordan, S., 1940. How one sugar compares with another. Food Industries 12, No. 3, 45-47.

Lewis, I. M., 1930. Inhibition of Phytomonas malcaceara in culture media containing sugar. J. Bact. 19, 423-433.

Magoon, C. A., 1926. Studies on bacterial spores. I. Thermal resistance as affected by age and environment. J. Bact. 11, 253-283.

Mickle, F. L., 1924. Lactobacillus lycopersici n sp., the causative organism of the gaseous fermentation of tomato pulp and related products. Abs. Bact. 8, 403-404.

Murray, J. R., and Headlee, M. R., 1930. Thermal death point. I. Spores of Clostridium tetani. J. Infectious Diseases 48, 457-467.

Nunheimer, T. D., and Fabian, F. W., 1940. Influence of organic acids, sugars, and sodium chloride upon strains of food poisoning Staphylococci. Am. J. Pub. Health 30, 1040-1049.

Pederson, C. S., 1929. The types of organisms found in spoiled tomato Products. N. Y. State Agr. Exp. Sta., Tech. Bull. 150, 45 pp.

Rahn, O., 1928. Incomplete sterilization of food products due to heavy syrups. Canning Age 9, 705-706.

Robertson, M. I., 1927. Thermophilic and thermoduric microorganisms, with special reference to species isolated from milk. II. The thermal resistance of microorganisms. Vt. Ag. Ex. Sta. Bull. 274. 27 pp.

Spiegelberg, C. H., 1940. Cl. pastorianum associated with spoilage of an acid canned fruit. Food Research 5, 115-129.

Spiegelberg, C. H., 1940. Factors in spoilage of an acid canned fruit. Food Research 5, 439-455.

Tanner, F. W., 1932. The Microbiology of Foods. First edition. Twin City Printing Co., Champaign, Ill. 768 pp.

Tarkow, L., 1940. Master's Thesis. Mass. State College. 45 pp.

Townsend, C. T., 1938. Heat resistance and heat penetration studies in acid products. Canner 86, No. 13, 13.

Wallace, G. I., and Tanner, F. W., 1931. The effect of concentrated sugar and salt solutions on the thermal death time of molds. J. Bact. 21, 32. (abs.)

Weiss, H., 1921. The thermal death point of the spores of Bacillus botulinus in canned foods. J. Infectious Diseases 29, 352-368.

Weiss, H., 1921a. The heat resistance of spores with special reference to spore of Bacillus botulinus. J. Infectious Diseases 28, 70-92.

ACKNOWLEDGEMENTS

The author is indebted to Dr. William B. Esselen, Jr. for his invaluable aid and advice during the investigation.

Thanks are also due the graduate committee for their constructive criticism offered in the writing of the manuscript.

Approved by:

William B. Easley Jr.
Chairman

C. A. Peters

Mary E. Garvey
Graduate Committee

Date: May 13 1942