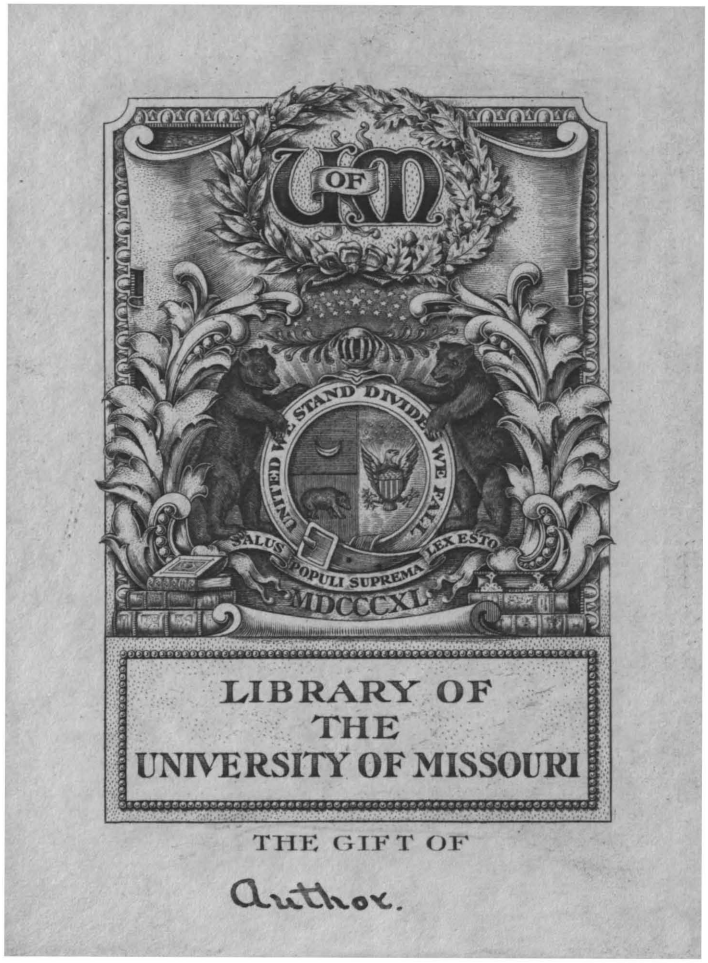

TEMPERATURE as a FACTOR Modifying the TOXIC ACTION of Certain Chemicals on the GERMINATION and DEVELOPMENT of a few FUNGI.

- CHARLES BROOKS -

Submitted to the Faculty of the GRADUATE DEPARTMENT in partial fulfillment of the Requirements for the Degree of MASTER of ARTS.

UNIVERSITY of MISSOURI,
1905.

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The purpose of the experiments, the results of which are presented in this paper, was to determine what might be the modifying effect of temperature on the toxic properties of certain chemicals as shown by the effect of these substances on germination and growth in certain Fungi. Since chemical processes as well as plant activities are influenced by temperature, it was thought that additional knowledge in regard to the nature of the physiological action of poisons might be obtained by comparing their effects at the optimum temperature for germination and growth of the plant with results secured under otherwise similar conditions, but at temperatures below and above that which is most favorable for the development of the particular plant.

So far as the writer has been able to determine the problem of toxic action has never been investigated from this stand-point. It is, however, a well-known fact,

that temperature is an important factor in plant activities and that changes in temperature may often serve as a stimulus to germination and development. HEALD has shown that a high temperature may serve as a stimulus in the germination of fern spores,but that this factor does not have a similar effect upon the spores of the moss. ERIKSSON and HENNING found that that exposure for a short time to low temperature served to increase the germination of the aecidiospores and uredospores of Puccinia graminis. DUGGAR found that temperature had an influence upon the germination of certain fungous spores. Botrytis and Phycomyces were both injured at a temperature of 32° C.,while Aspergillus flavus and Coprinus fimetarius were slightly benefitted by the same temperature,but in general,he found very little difference between the germination in water of fungi at 25° C.,and at temperatures nearer the maximum. MATTHEWS reported that a small rise in room temperature increased the toxic action of certain salts upon the eggs of the fish Fundulus heteroclitus,but no data in regard to the extent of the injury were given.

Considerable work has been done in recent years on the effect of toxic agents upon the germination and development of fungi. CLARK determined the concentration

of various chemical solutions necessary to produce injury, inhibition, and death in certain fungi. He found that 1/4 of a normal solution of HNO_3 killed the spores of Sterigmatocystis nigra within 48 hours, that n/8 and n/16 solutions of the same acid produced total inhibition of the spores and that n/32 gave great injury in the development of the fungus. Botrytis vulgaris spores were killed by n/16 HNO_3, and n/32 gave great injury to the plant. The spores of Penicillium glaucum were killed by n/4 solution, were totally inhibited by n/8 and n/16, and n/32 gave decided injury. H_2SO_4 gave similar results, but n/2 concentration was required to kill the spores of Sterigmatocystis and Penicillium. With $CuSO_4$, n/4 killed the spores of Sterigmatocystis, n/8 to n/16 gave total inhibition, and n/32 to n/64 caused decided injury. Botrytis spores were killed by n/16 $CuSO_4$, inhibited by n/32, and the plant greatly injured by n/64. The spores of Penicillium were killed by 2n/1 and inhibited by n/1 to n/64 while decided injury resulted from n/128. DUGGAR[3] has reported upon special factors that influence the germination of fungous spores, and Miss Ferguson[6] has given some of the conditions for germination in various basidiomycetous fungi. These recent papers have only an indirect bearing upon the

work that follows but have been very suggestive in the obtaining of methods for the solution of the problem.

- METHODS -

The effect of the various toxic solutions at the different temperatures was observed by means of the ordinary Van Tieghem Cells. The manner of constructing and the method of using these have been fully described by CLARK and DUGGAR. These cells were never used a second time without being taken apart and thoroughly cleaned as described below.

All cells used in these experiments were boiled for twenty or thirty minutes,first in an alkali,then in an acid,and finally in distilled water. They were dried from alcohol and made up in the usual manner. The covers were treated as the cells except that in each instance they were heated for a a longer time and that they were given one or two final boilings in distilled water. All flasks,vials, etc.,used in these experiments were cleaned with alkali, acid and distilled water by boiling,as described for the cells.

As a culture medium several vegetable decoctions were tried. It was found that the five fungi used in these

experiments grew well upon decoctions made from onions, beets, tomatoes, grapes, parsnips, beans, mushrooms, and sugar beets. Several series of experiments were made with tomato decoction as a medium, but it was found that a sugar-beet solution gave less precipitate in the presence of $CuSO_4$ and was in general more satisfactory for the work. In all the experiments reported in this paper beet decoction was used as the nutrient medium. In making the infusion, 600 grams of beets were used for every liter of water. At the time of using, the decoction was diluted, by the addition of the toxic solution and water, to one half of its former nutrient value.

The toxic agents used were HNO_3, H_2SO_4, and $CuSO_4$. The chemicals were of the highest quality that could be obtained and the acid solutions were standardized before using. It is a well-known fact that strong concentrations of $CuSO_4$ precipitate proteids. In solutions at ordinary temperatures in which both are present, this precipitation continues for a long time, thus continually changing the nature of the liquid. Therefore, as it was necessary to make experiments at times some distance apart, the toxic agent was not added to the beet decoction until the time for using. Stock solutions of chemicals were made in water that had been

carefully re-distilled from glass in the presence of an oxidizing agent. Normal or 1/2 normal solutions were made and these stored in flasks provided with closely fitting rubber stoppers. These stock solutions were diluted and mixed with the beet decoction at the time of using by means of a series of graduated vials.

The fungi used were Botrytis vulgaris, Monilia fructigena, Sterigmatocystis nigra, Mucor mucedo, and Penicillium glaucum. The first two may be and usually are parasitic, and have an optimum temperature that is comparatively low ; the last three are saprophytic and grow well at temperatures considerably above the optimum for the first two. It was thought by this selection to obtain more interesting results than with forms more closely related physiologically. Only pure cultures were used. In the test tube cultures from which the spores were obtained for use, the fungi were grown upon cylinders of potato or sugar-beet. In either case the liquid in the tubes was a decoction of sugar-beets. Other nutrient substances were tried for the test-tube cultures but these usually produced modifications in the growth of the fungi and it was not found advisable to use spores produced on different media in the course of a series of experiments the results of which were to be

compared. The spores used were always taken from cultures that were from twelve to sixteen days old.

Fairly constant temperatures were secured by means of incubators and a refrigator. Comparative results could have been obtained more readily if all the temperatures desired had been available at once,but at the time of beginning the experiments no way was seen of obtaining more than two or at most three different temperatures at one time. It was thus impossible to use spores from the same culture for all the temperatures. The cultures of the five fungi at a particular temperature were made on the same day, thus giving a good opportunity to compare the effects produced on the various fungi when exposed at exactly the same temperature.

CLARK[6] has pointed out certain sources of error for Van Tieghem cell-cultures exposed to ordinary temperatures, but the placing of cells made up under laboratory conditions at temperatures ranging from 5° to 30°C.,gave opportunity for additional error. The cells were not entirely closed until they had been left for several minutes in the temperature at which they were to remain. Opportunity was thus given for an adjustment of air pressure in the cell,but it

did not in all cases prevent the condensation of water vapor upon the cover glass. The small drops of water thus formed not only increased the evaporating surface but also modified the vapor pressure in the cell. The small water drops adjacent to the hanging ones of the nutrient solution seemed to sometimes unite with them, thus changing both their size and concentration. When the cultures were made in the dry air of a furnace heated room no difficulty was experienced, but cells made up on sultry days, or when the air of the culture room was humid from any cause, gave a visible condensation when placed at the lower temperatures. Even with the greatest precaution in closing the cells this difficulty was not entirely overcome.

It was also found difficult to examine the cultures placed at various temperatures without interfering with the structure and condition of the cells. Examinations were made at temperatures as near those at which the fungi were growing as possible, and results obtained from damaged cells were rejected. All cultures were observed every twenty-four hours and notes taken of per cent of germination length of germ tube, fruiting, and any peculiarities in germination or development. More frequent observations would

have been of interest,but they were not made on account of the increased source of error that would have been thus introduced. Any sources of error that were not otherwise provided for were guarded against by always making duplicate cultures. The experiments with the three chemicals were always made at different times and as control cultures were made in every case,the growth of a fungus in a nutrient medium at a particular temperature was tested three times.

The vitality of spores that had been subjected to the action of an inhibiting toxic agent was tested by transfer to a nutrient,non-toxic medium. An attempt was made to accomplish this transfer by removing the drop of the toxic solution with sterilized filter paper and replacing it by a drop of beet decoction. This method left some part of the former solution as well as any precipitate that had been formed,adhering to the cover glass,and was therefore abandoned. All transfers that are concerned in the following data were made by means of a sterilized platinum needle. The spores were in every case transferred to a drop of beet decoction on a clean cover glass. The medium used in the bottom of the cells was in this,as well as in all other cases,the same as that of the hanging drop. It is quite

evident that the above method of transferring did not prevent a small amount of the toxic solution being carried into the new drop by the spores and the needle,but the results obtained indicated that this small per cent.of the toxic agent either served as a very slight stimulus to germination and growth or exerted no appreciable influence upon the spores.

Early in the work it was seen that results obtained from the exposure of the fungous spores to the toxic agent must be considered entirely apart from data secured in cases where the mycelium was acted upon by the toxic solution. Therefore,when a particular toxic solution gave no germination at one temperature but did at others,the ungerminated spores were in no case transferred, i.e.,transfers were made only with those solutions that gave no germination even at the optimum temperature at the end of the given time. By a series of preliminary experiments strengths of toxic solution and time of exposure were determined,such as would give the greatest contrast in the results obtained at the various temperatures.

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- DATA and DISCUSSION -

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In order to put the results obtained in a form as concise as possible, charts have been arranged, and the greater part of the data secured is expressed in these by means of curves.

In the first ten charts the abscissae, marked off at the top of each plate, indicate the temperatures at which the fungus was kept in culture, and the ordinates, marked off in the middle of the page show the per cent. of germination at those temperatures.

All the points indicating per cent of germination at the various temperatures for a particular toxic solution are joined by solid or broken lines ; the fractions at the ends of these curves show the strengths of the toxic agent used. The name of the fungus is given at the top of the chart and the sets of curves below show the results obtained by using various solutions of the three chemicals with this fungus. As a further illustration of the meaning of these charts ;- in No.I. the curves showing data obtained by using $CuSO_4$ with Botrytis are given in the upper portion of the chart. With 1/16 of a normal solution no germination

was obtained at 30°, but at 10°,31 per cent of the spores germinated and at 5°,40 per cent.

In charts I.to V.inclusive the results were obtained by exposing the spores for twenty-four hours at the various temperatures in the toxic solution indicated and then transferring them as previously described. The charts are based entirely upon the data secured on the first and second days after transferring. The solid lines indicate the total germination at the end of the second day. The broken lines show the per cent of germination twenty-four hours after transferring. Where the record of germination was the same for the two days only the solid line is used. It will be noticed that only in a very few instances did spores germinate on the second day.

It is readily seen that the deleterious action of the toxic agents increased very rapidly with the rise in temperature. A comparison of the charts for the various fungi indicates that the nature of the fungous spore may have a modifying influence on these germination curves. Thus, there is a marked drop between 5° and 10° in the germination curve for Botrytis and Monilia,but for no other fungus. With Penicillium the fall comes either between 10° and 15° or between 15° and 20°, while with Mucor and Sterigmatocystis

the downward curves begin at 15° or 20°. This variation may be the result of using solutions that were, without regard to temperature, more injurious to some of the fungi than to others; but strong concentrations, such as n/4 and n/8 HNO_3, when used with Mucor and Sterigmatocystis, have not given the rise in the curves from 10° to 5° that has been repeatedly obtained with Botrytis and Monilia. There is some indication that the minimum temperatures for germination have had a modifying influence that may account for some of these variations. Thus, Botrytis and Monilia are not only the fungi that gave a marked decrease in germination on passing from 5° to 10°, but are also the only ones of the five which germinated in the control cultures at 10° by the end of one day.

With Penicillium the curves in the $CuSO_4$ and HNO_3 charts show a tendency to drop between 10° and 15°, in the H_2SO_4 chart the fall comes beyond 15°. Along with these data should be noted the fact that the control with the H_2SO_4 cultures gave no germination in one day while those with the HNO_3 and $CuSO_4$ cultures had germinated in this time. This variation in the controls was probably due to a slight change in the temperature of the refrigerator,

together with the fact that 15° approaches the lower limit of temperature for obtaining the germination of Penicillium in one day. Mucor gave no germination the first day in the controls at 15° with the exception of about 14 per cent with the $CuSO_4$ series, and Sterigmatocystis in no instance germinated in one day at this temperature. As has been already mentioned the curves for these fungi descend between 15° and 20° or at a higher temperature. It will be noticed that the spores exposed to a harmful agent at a temperature so low that it inhibits germination have not been greatly injured. It should be mentioned here that the two forms that have been most greatly injured are the ones that require the least stimulus for germination. Thus DUGGAR[3] found that both Botrytis and Monilia germinated readily in distilled water, while Aspergillus and Penicillium did not germinate.

In the cultures from which the data in charts VI. to X. inclusive were obtained the spores were in no instance transferred. The cells in the $CuSO_4$ series remained at the temperature indicated for four days, those with the acids were removed at the end of two days. In the former six daily observations were made, in the latter only four. Control cultures of spores in beet decoction were kept with

the toxic cultures at all times. These controls were subjected to the various temperatures for the same length of time as the other cultures. The per cents indicated in these charts do not in every instance represent the actual germination but were in all cases obtained by dividing the per cent of germination in the particular culture by that in the control at the same temperature. It was found more difficult to represent in graphical manner the results obtained from these experiments since the per cent. of germination did not always seem to agree with the extent of the injury. The solid lines show the germination at the time of the final removal from the given temperature. The per cent of germination at the end of 24 hours is indicated by the broken lines. These unite with the solid lines as they approach the optimum temperatures. Where no broken line is given the germination was the same at the end of the first day as at the time of removal from the given temperature. The results obtained at temperatures at which the controls had not germinated were omitted from the curves. This accounts for the fact that a number of the curves are not extended to the lower temperatures.

In all instances the injurious effects were least at the optimum for the fungus. This optimum was determined

by the germination and development in the controls. The harmful effects were shown by decreased germination as indicated in the charts and by abnormal development. The toxic solutions that gave but partial germination at the optimum for the fungus usually gave only abnormal development above and below that optimum. Thus, Botrytis in n/32 HNO_3 gave mycelial development approaching the normal only at 15° and 20°; Penicillium in n/128 $CuSO_4$ gave medium growth at 20° but at no other temperature ; at 30° the germ tubes seldom became more than a few spore diameters in length even after removal to a more favorable temperature and many spores swelled without germinating. Sterigmatocystis has its optimum above 35° and it is the only fungus in which the injurious effects decreased above that temperature.

Both Mucor and Sterigmatocystis germinated and grew well at 35°, the other three fungi gave little or no germination at that temperature.

The charts do not show the results obtained at low temperatures but in every instance cultures were placed at 5° and 10°. Spores kept for two days in a particular toxic solution at a temperature so low that it inhibited their germination, upon removal to the room temperature gave a germina-

tion and development that was but little inferior to that obtained from the fresh spores under like conditions of medium and temperature. Spores inhibited at a temperature that did not prevent germination were more greatly injured. Spores of Mucor gave fair growth in n/32 H_2SO_4 and n/32 HNO_3 after removal from 5°, but after removal from 10° did not germinate. Sterigmatocystis spores in n/16 HNO_3 grew almost as well after removal from 10° as from 5°, but in the cultures removed from 15°, (a temperature not inhibiting germination in the control) ,no germination was obtained.

A comparison of the curves obtained with the different chemicals shows that those for weak concentrations of HNO_3 do not drop so rapidly at high temperatures as the curves for weak solutions of the other toxic agents.

In order to obtain additional information in regard to the significance of the results secured with the cells,a series of flask cultures was made. In every instance 25 c. c. of the given solution was placed in a 100 c. c. flask. The effect of the toxic agent was determined by taking the dry weight of the fungus growth at the end of the given time. With the exception of n/64 $CuSO_4$ duplicate cultures were made and the average weight used in

estimating the effect. Flask cultures were made of Mucor, Penicillium, and Sterigmatocystis. The results obtained from cell cultures were used as a basis in determining what strengths of the toxic agents should be used in the flasks. So much greater concentration is required to give injury in flask cultures than in cells that no definite results were obtained with Mucor and Penicillium. It was found that Penicillium would not grow in flask cultures placed at 30° C., In the first series with Sterigmatocystis the air in the incubator was dry while that in the refrigerator was kept damp by the melting ice. It was feared that the evaporation might have been enough greater at 35° and 30° than at lower temperatures to cause an appreciable change in the cultures, and the series was repeated.

Chart XI. gives the results obtained from the first series, while XII. shows the results of the last series. In the first set of experiments the cultures were allowed to grow two weeks , in the last but one week ; in the former the cultures for the various temperatures were started at different times , in the latter all were started on the same day and the spores used were from one stock culture. The curve at the bottom of each chart was obtained from the controls by taking the greatest weight as 100 and determi-

ning what per cent the other weights were of this. In the sets of curves in the upper portion of the charts the ordinate marked 100 represents the controls. The fungous weight obtained in a toxic solution at a particular temperature was compared with the weight in the control at that temperature considered as 100.

It will be seen that injury, as shown by weights obtained, decreased with rise of temperature. The action of the different chemicals varied greatly. Nitric acid served as a check to growth at low temperatures but was a decided stimulus at 25° and 30°. It should be remembered that a similar rise at high temperatures was obtained with HNO_3 in the cell cultures. Sulphuric acid has checked the growth at low temperatures but has served as only a very slight stimulus in other cases. It is also worthy of note that the harmful effects of $CuSO_4$ were not as great, comparatively, at 15° as at 20°. This was true of neither of the acids.

Further experimentation would probably give additional information in regard to the meaning of the difference in action of the chemicals and the varying effects upon the different fungi.

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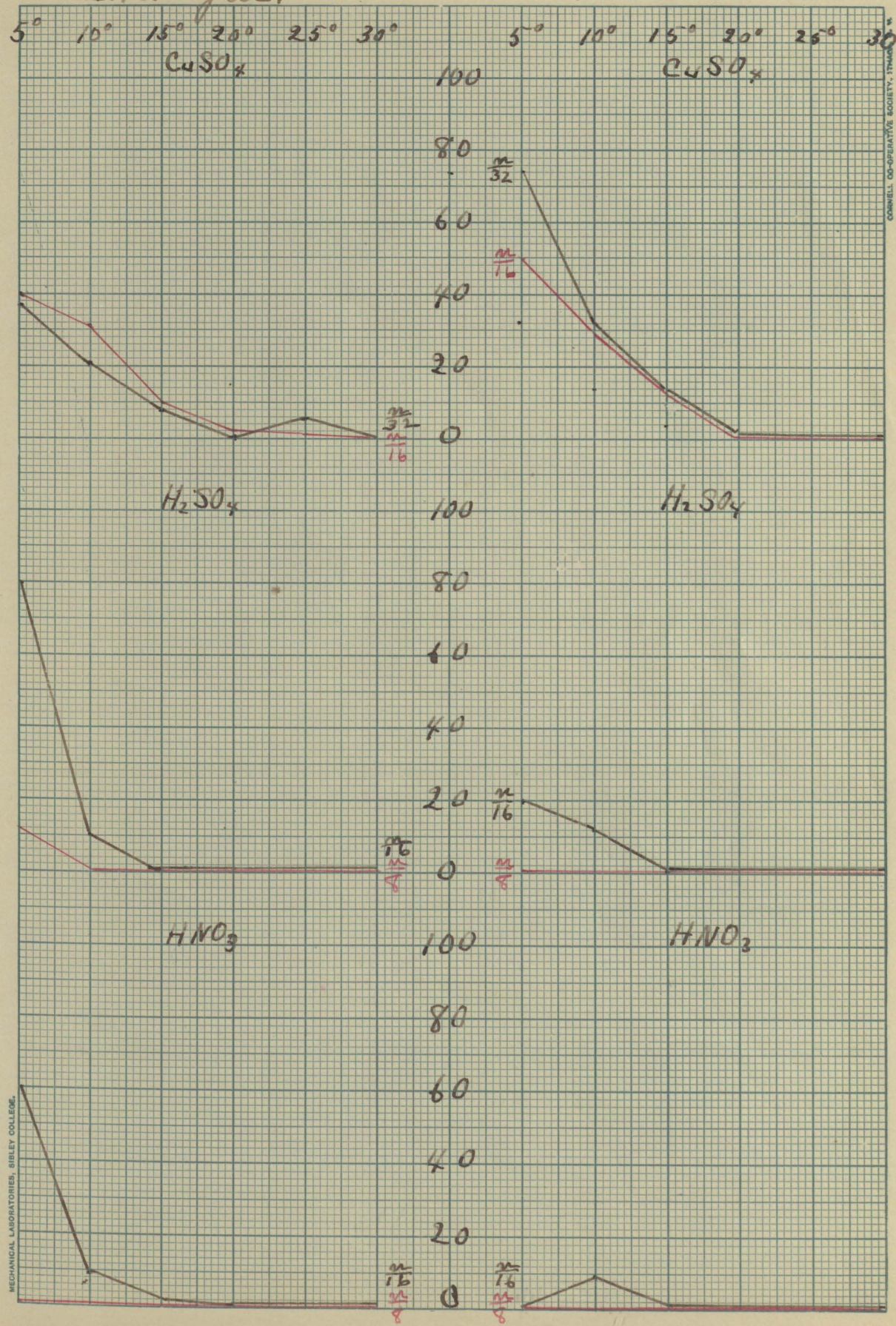
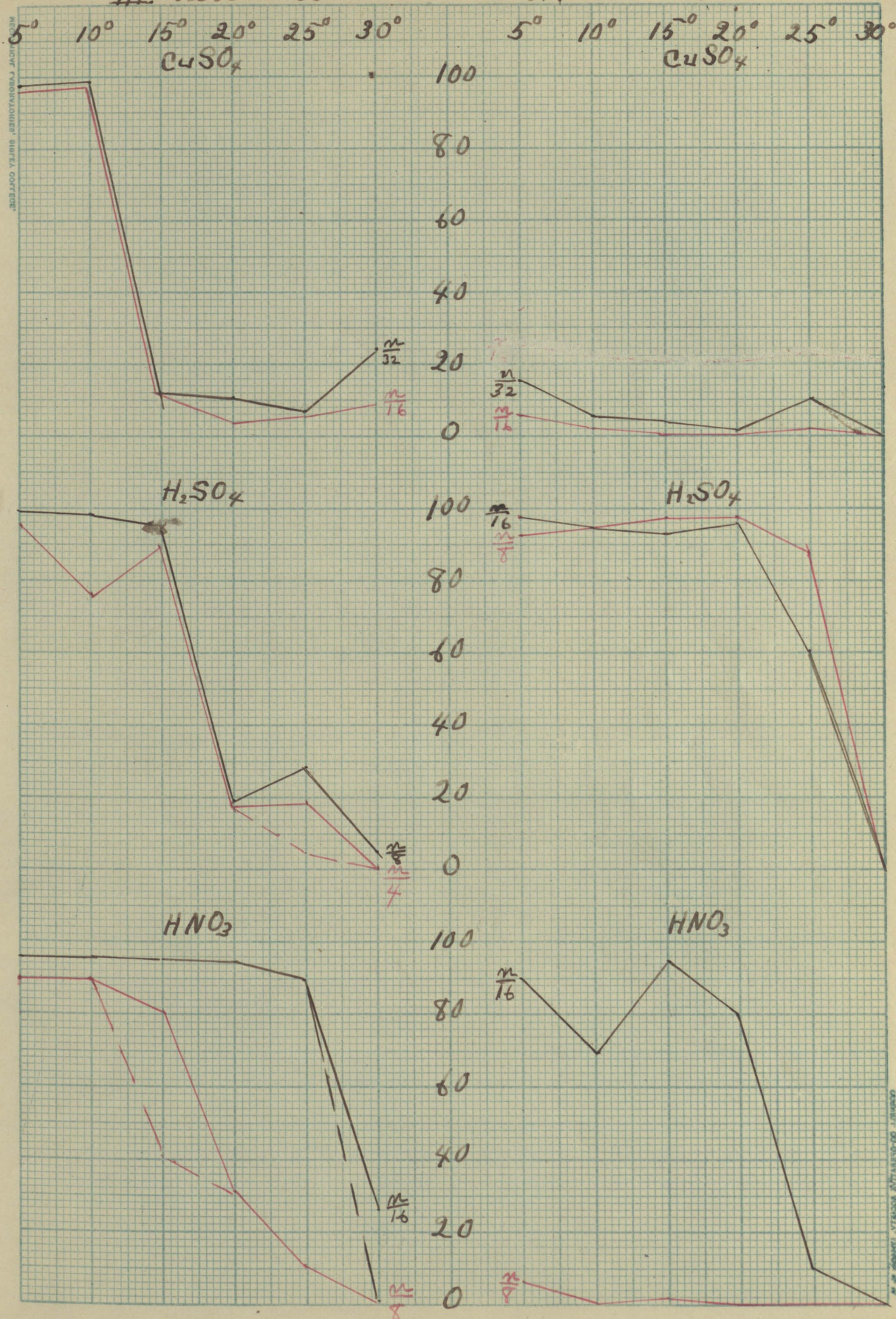
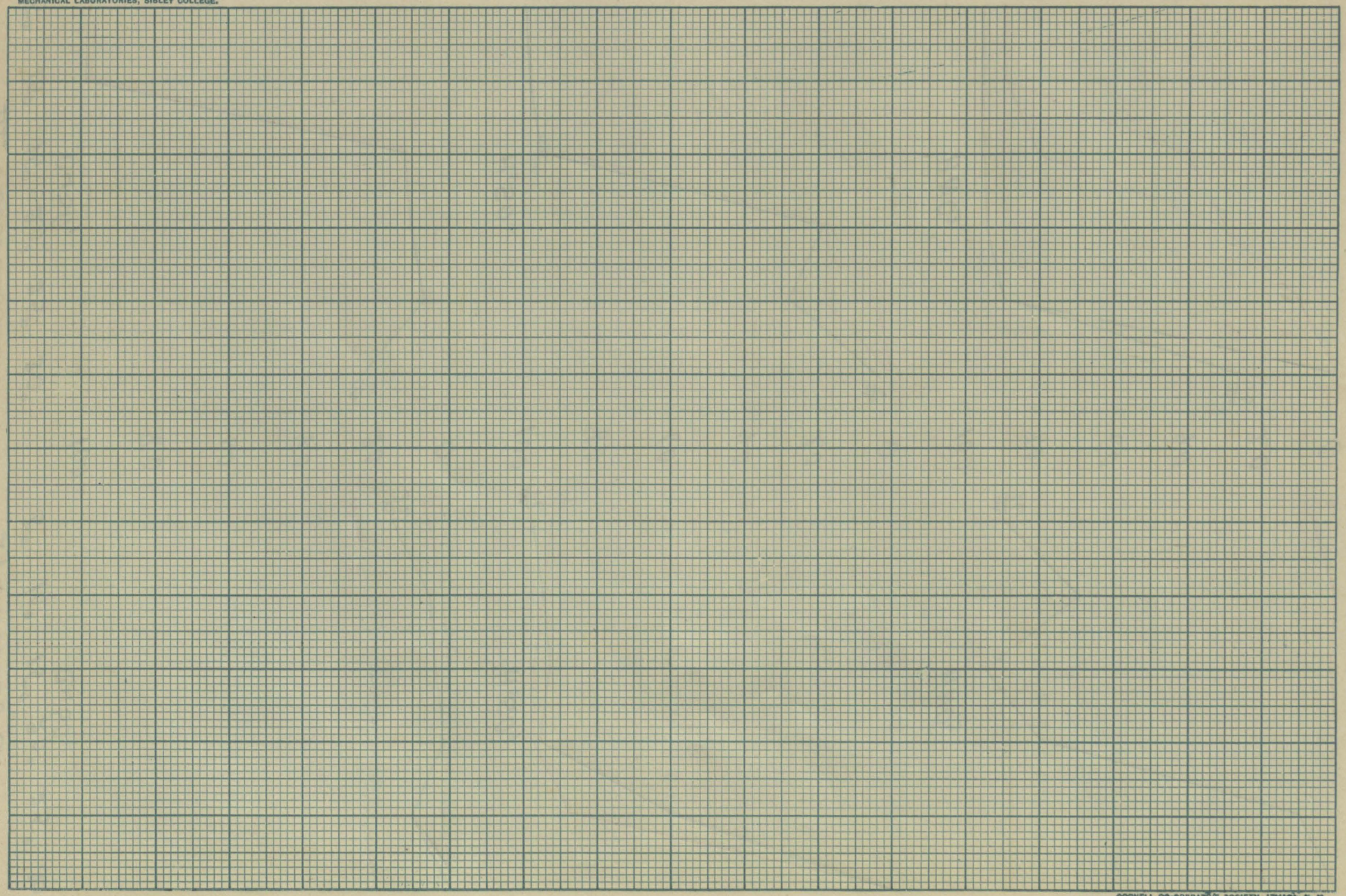
I. Botrytis.

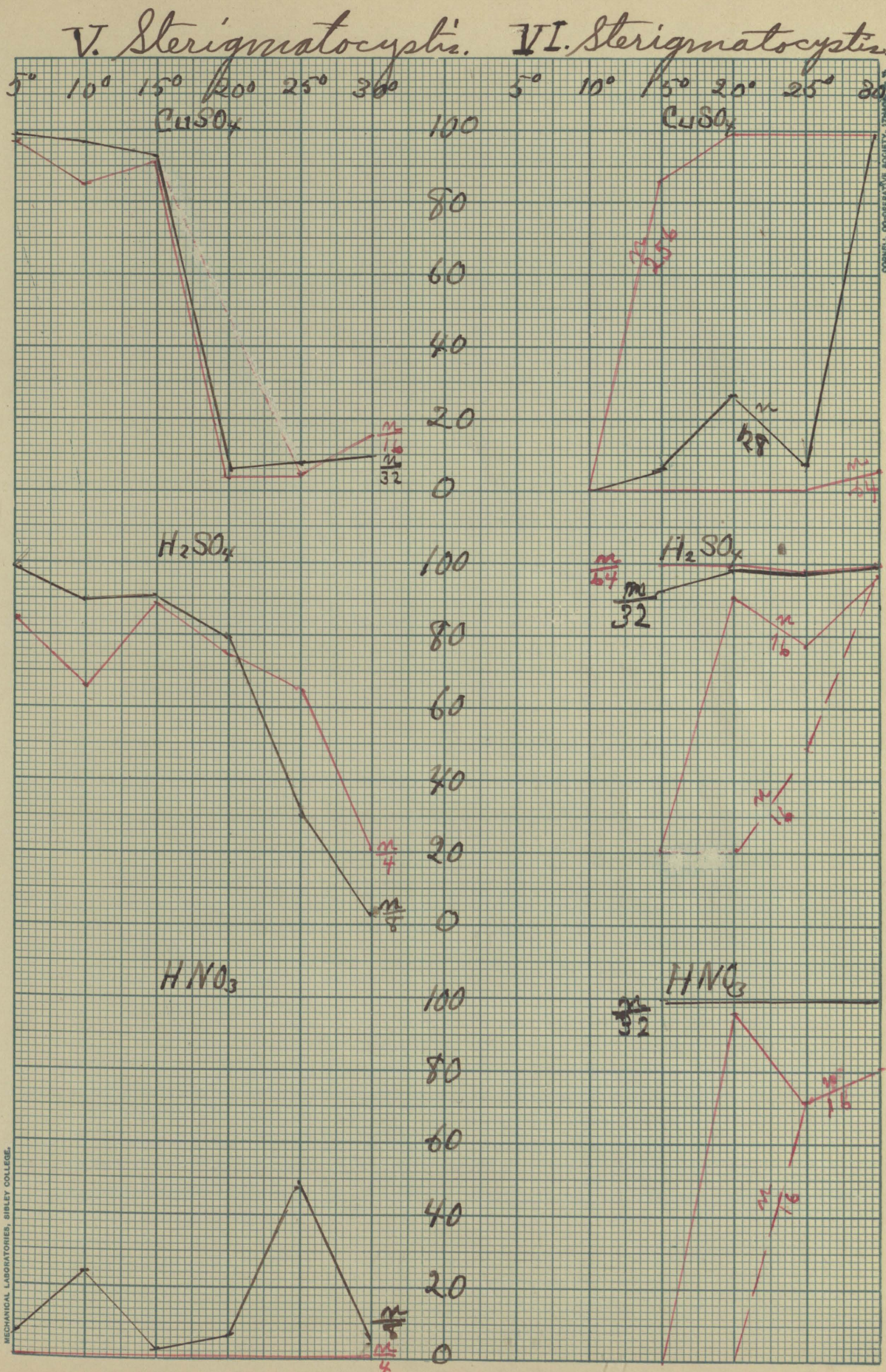
II. Monilia.



III. Penicillium. IV. Mucor.

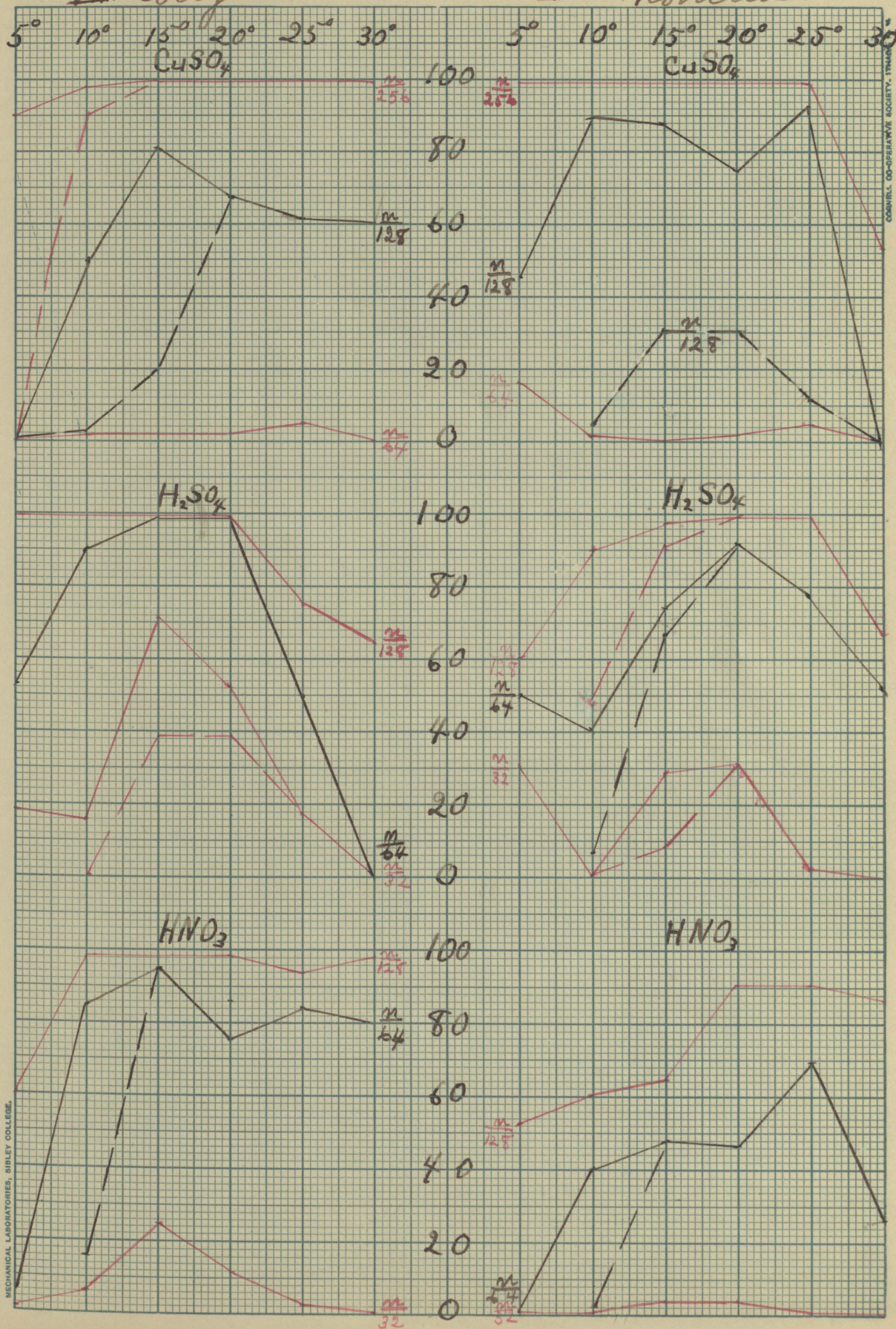





V. Sterigmatocystis.
VI. Sterigmatocystis.
5° 10° 15° 20° 25° 30°
5° 10° 15° 20° 25° 30°
CuSO4
CuSO4
H2SO4
H2SO4
HNO3
HNO3
100
80
60
40
20
0
MECHANICAL LABORATORIES, SIBLEY COLLEGE.
CORNELL CO-OPERATIVE SOCIETY, ITHACA, N.Y.

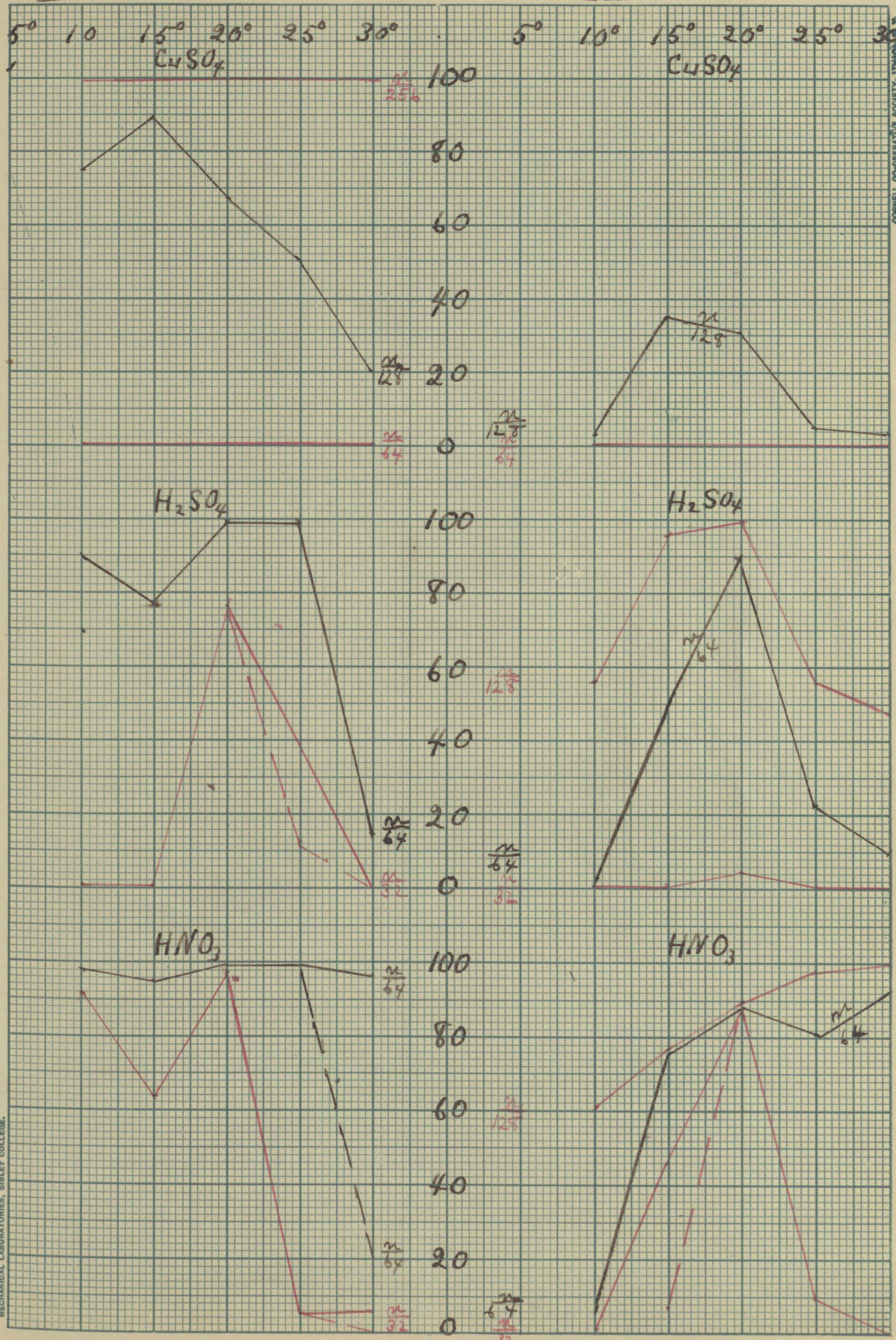
VII. Botrytis.

VIII. Monilia.

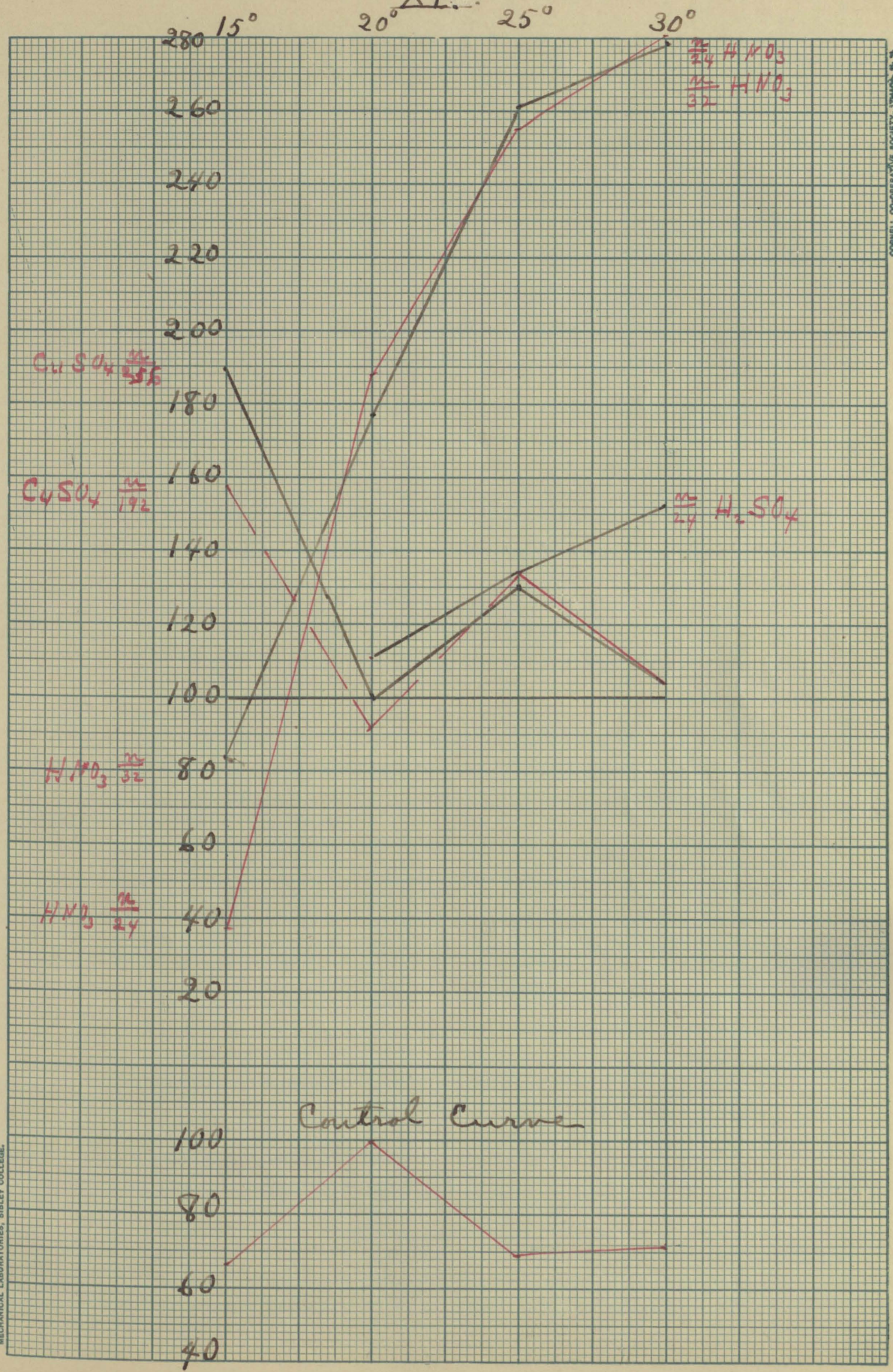


IX. Penicillium.

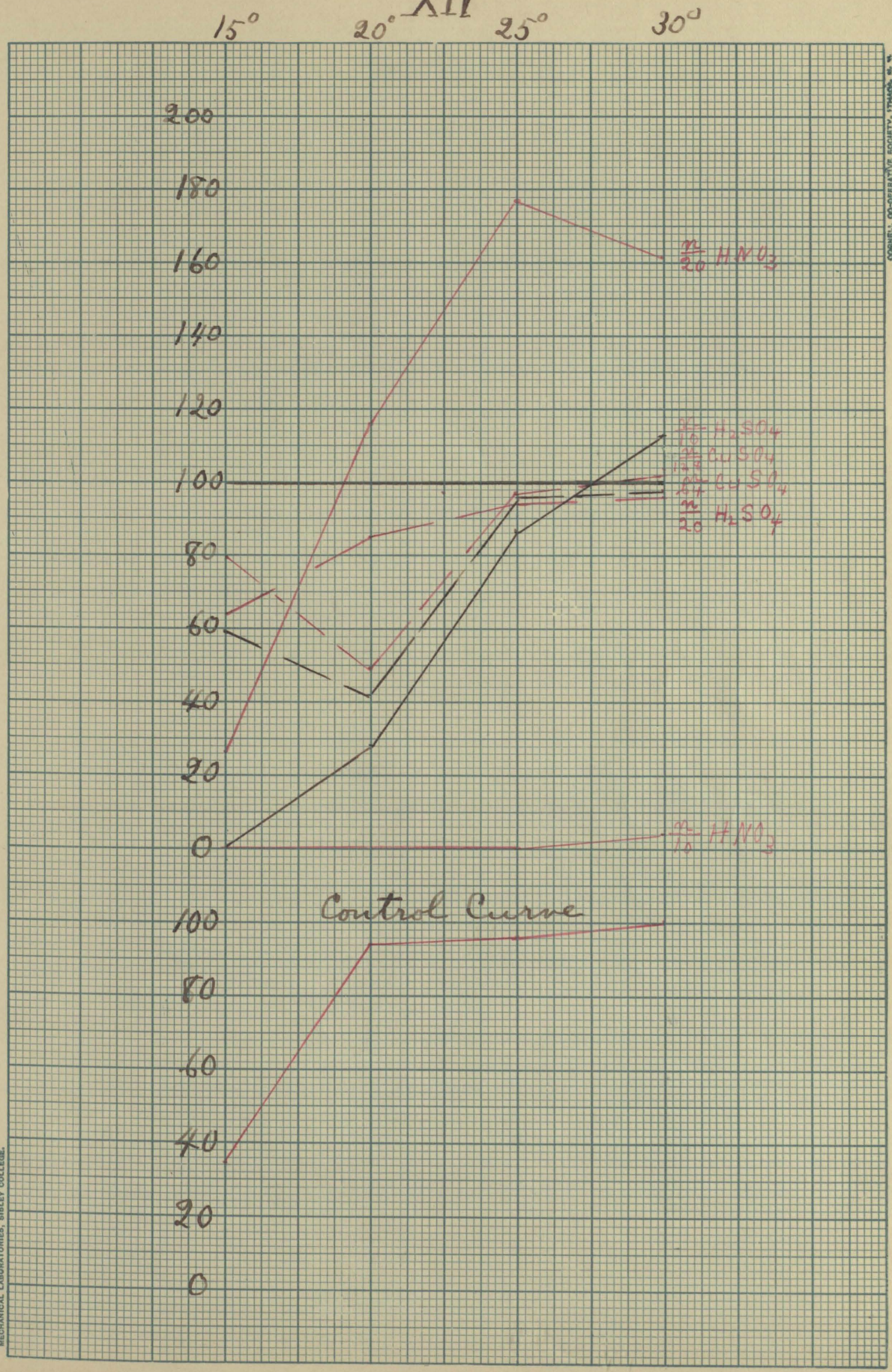
X. Mucor



XI.



XII



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