STUDIES ON THE ASSOCIATION OF CERTAIN PHYTOPATHOGENS

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A Thesis

Presented to the Faculty of Graduate Studies and Research, McGill University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Plant Pathology.

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Introduction:

Association of plants is by no means a new subject for study, though so far reports dealing with association of phytopathogens have been comapartively rare, as a survey of the literature on the subject indicates. Most attention has evidently been turned to other branches of research.

Recently, during an extensive survey of storage rots at Macdonald College and vicinity, it has been observed by the writer that even though the rots were relatively abundant and many vegetables and fruits were attacked, the number of species of pathogenic organisms on a single specimen was very limited. It was only rarely that more than two species of these organisms were found at the same time on the same host. In advanced stages of the rots, the number of species increased, but these additional species were usually of a saprophytic nature. Sometimes two or more wound parasites were found on different specimens in the same lot of the host plants, but only in a few cases were they associated intimately. Association of parasites with saprophytes was much more common.

Large numbers of fungus spores are continually dispersed over exposed objects. It seems strange, therefore, that the association of phytopathogens is not more general. It is by no means a rare occurrence to have several diseases present on the same plant, but they are seldom closely associated.

The study of the association of phytopathogens involves the knowledge of a large number of scientific data. A detailed knowledge of taxonomy of plants is essential, so that the many

species of plants and and their fungous and bacterial parasites can be distinguished. A good knowledge of plant physiology and biochemistry is needed to differentiate the many processes that are involved in the activities of the parasites on the host. Then, finally, a knowledge of the effects of ecological factors on the above relationship is necessary, for these factors are of major importance.

The importance of phytopathogenic association cannot be overestimated, for it is found to occur frequently in nature. It is a well known fact that cereal plants attacked by smut fungi are rendered more susceptible to the attack of rust. Apples bearing lesions caused by the Scab fungus, Venturia inaequalis, may become rotted in storage by the Pink Rot fungus, Cephalothecium roseum. Many other such examples may be named. In most cases the primary invasion does not do very much harm, but the penetration of the wound caused by the primary parasite is invaded by secondary organisms which are able to cause a very destructive rot. Such associations include the following: potato tubers attacked by Synchytrium endobioticum, later rotted by bacteria; tomato fruit attacked by Cladosporium fulvum, but the destructive rot being initiated later by Botrytis sp.; the rot of onion bulbs, caused by Botrytis allii and Erwinia caratovora; and Sclerotinia fructicola on apples, later followed by Penicillium expansum. These associations can be often found.

Review of Literature:

Faulwetter (30), when studying a leaf spot of cotton caused by an organism which he considered identical with Alternaria tenuis, noticed that on the margins of the lesions caused by

by the fungus, spots caused by Phytomonas malvacearum were found. He also found the organism present in spots surrounding red-spider injuries, and also on leaves where there had been no previous injury whatsoever.

Heald and Pool (37) observed that perithecia of Melanospora pampeana were produced in large numbers when the fungus was grown in association with Fusarium moniliforme, Basisporium gallarum and also Fusarium culmorum. In single spore cultures perithecial production was rare.

McCormick (57) had observed that perithecial development in Thielavia basicola was greatly stimulated when cultured together with Thielaviopsis basicola. It likewise would be stimulated when grown together with Cladosporium fulvum, Aspergillus umbrosum, A. glaucus, Eurotium amsteoldami, and Fusicladium pirinum.

Schmitz (74) grew mixed cultures of four isolations of Fomes pinicola. He observed that intermixing took place between inoculations of the same strain, but when two different strains were grown together they did not intermingle at all, and in these cases a clear line of demarcation was observed between the two colonies.

Cook (19) made a study of the succession of fungi on culture media. He finds that either the character of the food, or the possible formation of toxins checking growth of the first organism were the most logical explanations. The failure of the used medium to produce further growth of the primary organism may be due to lack of food, presence of toxic substances, or to both.

Porter (64) observed that in the association of fungi several degrees of inhibition may prevail. Any one of the following types may be found: mutual intermingling, growth of one organism over the other, slight inhibition, growth around the contending

organism,and inhibition at a considerable distance.In most cases the inhibition or influence of one organism over another was often marked by a change in physiological and morphological characteristics.

Rege (65) showed that Acremoniella sp.did not show any activity on cellulose material and Aspergillus sp. took a fortnight to start,while Coprinus sp.gave slow decomposition.Combination of these organisms in pairs gave better or more rapid cellulose decomposition,while where all three were associated best cellulose decomposition occurred.

Millard and Taylor (60) showed that the addition of green manure caused a great increase in the number of Actinomycetes in the soil,but the fast-growing saprophytic forms managed to increase more rapidly and so the parasitic forms may be temporarily or permanently starved out.

Adams (1) observed that Darluca filum parasitized the aecia of Peridermium Peckii.When immature fruiting structures were attacked,especially the aecia and pycnidia,they became destroyed.

Weston (87) observed that "bunted" Little Joss wheat was also more susceptible to the attacks of the orange leaf rust, Puccinia glumarum.More detailed investigation showed that other varieties of wheat also showed the same realtionship between rust and smut.

Camp (16) found that Penicillium stoloniferum readily attacked lemons and oranges which had been partially rotted by other fungi.

Millard and Beeley (57) found that there are several types of Actinomyces scabies on potato and mangel.Some of these were pathogens,others were not.When grown together in certain combin-

ation, sometimes the typical scab developed, while in other combinations no scab formed, even when the pure cultures of the organisms produced a definite lesion. He considers it to be a real case of suppression of pathogenicity due to association of a parasite and saprophyte.

Other reports dealing with fungus association are available, but these being of a physiological nature, will be mentioned in the accounts of the experiments which follow.

Object of Work

The purpose of this investigation is to study the association of certain phytopathogens on the host, and also to determine the physiological relationships of the associated organisms by cultural experiments.

Preliminary Considerations

The survey of storage rots brought out several problems dealing with the physiology of association. Among the most prominent of these were the following: cause of limitation of the number of pathogens in a rot; the reasons for the inhibition of growth of some of these organism when associated with others; the reasons for the change in a rot type due to association of the causal organisms; the effect of the H-ion concentration on the association; the effect of spore number or each organism; and the effect of temperature

Answering and clarifying the questions involved in the above problems requires a great expenditure of time and labor, especially if the number of organisms associated in a culture is more than two at a time. On account of the rapid increase in the complexity of the whole problem of association when many organisms were studied, the number of these studied in detail was limited to six.

Experimental Procedure

Materials:

As phytopathogenic association was studied on the host as well as in artificial culture media, large supplies of both healthy and diseased specimens of fruit and vegetables were required. Three main groups of rots were studied, those of the apple, the onion, and the tomato; for in each of these groups the organisms concerned were able to cause a rot under similar conditions, and so could be compared readily in their effect on the host, whether separate or in association.

When diseased specimens were needed for examination, only those which were partially rotted were selected. This was necessary in order to prevent needless isolation of saprophytes which grow on decaying plant tissues. The presence of the saprophytes probably had considerable influence on the development and physiological activities of the pathogens, but if the study of many pathogens and non-pathogens in a certain type of rot was undertaken, the resultant mass of data would be too complicated for clear comprehension. Therefore, specimens which were rotted slightly, and which yielded only one or two pathogens were selected.

When healthy specimens were selected, they had to be of the same variety, the same degree of maturity, and the same size. As nearly all the experimental work dealt with "wound parasites", it was necessary that all the specimens were as sound as possible. Small injuries to the epidermis often served as a means of entrance to contaminating organisms, and so during preliminary trials some specimens had to be discarded, for this necessarily interfered with preparation of data. Therefore apples which were used in the study of apple rots were all hand-picked, graded,

and belonged to the varieties MacKintosh Red,Fameuse,and McMahon White.Similarly,three varieties of onions,Red Weathersfield, Yellow Globe,and Silverskin,were selected.All the onions were examined for traces of rots,or the production of sclerotia.This examination was facilitated by the removal of the outer dry scales from the bulbs.Neck-rots were comparatively easily distinguished by the production of external sclerotia,but when the rots were due to bacterial action they were very difficult to distinguish, unless very advanced.In the study of tomato rots,only one variety, Livingstone Globe,was used for experimental purposes.

For the culture of fungi or bacteria,Richard's nutrient solution,or the same solution modified by the addition of two grams of "Difco" peptone per litre of the solution,were used . In future references in this paper,this Richard's-peptone nutrient solution will be known as Richard's modified solution.In this modified medium better growth of fungi and bacteria was obtained than in the unmodified Richard's nutrient solution,though the latter proved to be useful in the study of plant extracts on fungus and bacterial growth,for it contained only substances of known composition.

The solid media used included "Difco" potato-dextrose agar, made up ready for use by the addition of 40 grams of the prepared material to one liter of water,and autoclaving;and also nutrient gelatine.The gelatine was not used for extensive cultural experiments,but served as a means of identifying certain Penicillia found during the survey of rot-producing phytopathogens.

Methods:

The Survey.

During the survey mentioned above, the store-rooms of several concerns were examined fortnightly for the development of storage rots, and the rotted specimens that were selected were carried to the laboratory for isolation of the rot-producing organisms. During the course of the survey the following fruits or vegetables were studied: cabbage, carrot, celery, potato, parsnip, onion, beet, tomato, and apple. As mentioned above, in selecting the specimens, care was taken to obtain those which were moderately rotted, so as to reduce to the minimum the number of saprophytic organisms present in the rot. In the rotted tissues of the onion, yeasts were found to be relatively abundant, particularly members of the genus Schizosaccharomyces. On some specimens, the rot, in some measure, was assisted in its spread by nematodes.

Isolation:

Isolations were made from rotted tissue by the following methods. The surface of the decayed specimens was first washed thoroughly with distilled water, and then the superficial portions of the rotted tissue were removed with a sterile scalpel. Afterwards small portions of the decayed material were transferred to solidified potato-dextrose agar in Petri dishes. After incubation for several days, the mycelium which grew out from the rotted tissue was used as a basis for making pure cultures of pathogenic organisms. It was found that usually only one species of an organism occurred in the margin of the lesions that occur

on the host,and this was usually responsible for the rot.If the inoculum was obtained from the margins of the lesion,after the superficial layers had been removed,the number of saprophytic organisms present in the inoculum was reduced to the minimum,and there was little difficulty in obtaining pure cultures of the desired organisms.

In order to obtain pure cultures of various Schizomycetes, it was necessary to insert a sterile inoculating needle into the lesion,after due care had been taken to eliminate outside contamination,and then to transfer the inoculum to a tube of melted agar,which was well shaken and poured into a sterile Petri dish.When the numbers oforganisms in the agar suspension are too great,the suspension should be diluted,so that single colonies can be isolated without difficulty after they have grown.The cultures were incubated at 20-24°C.for two or three days,after which period the bacterial,or yeast colonies were large enough for isolation purposes.

The Preparation of Culture Media

Both Richard's nutrient solution and the modified form were prepared as follows.The required quantities of the components were weighed out and placed in a clean,distilled-water washed, Erlenmeyer flasks,and distilled water added according to the formula.The media were first heated for a short time to dissolve the solid materials,and then the solution was filtered through an absorbent-cotton filter.This process of filtering removed the coarse precipitate which formed during the process of heating. After filtering,the medium was autoclaved for 15 minutes at 15 pounds pressure.A rapid cooling of the autoclaved medium in

is advocated,for a slow cooling or a prolonged autoclaving tended to produce in the medium an undesirable,reddish-brown color, which interfered with H-ion determinations made by the colorimetric method.

When it was necessary to distribute the medium in small quantities into culture flasks,50 c.c. portions were removed from the filtered medium and transferred to small,150 c.c., Erlenmeyer flasks.These flasks were all previously cleaned by the use of a cleaning solution composed of potassium dichromate ($K_2Cr_2O_7$) and concentrated sulphuric acid (H_2SO_4),and by washing with distilled water afterwards.A 50 c.c. pipette was admirably suited for rapid distribution of the medium into the small flasks.The flasks were thenplugged with cotton and autoclaved as above.

Hydrogen-ion Determinations.

In the case of media already mentioned,little difficulty was found in making H-ion determinations.After repeated tests, it was foundthat there was very little change in acidity during the autoclaving process if the autoclaving was performed for the stated time and at the pressure recommended.An increase in length of time of autoclaving raised the acidity considerably. It was found that by following this procedure,Richard's nutrient medium had a pH value of about 4.7,while that of the modified medium usually was nearly 6.5 .Therefore,in the preparation of the media,in order to maintain a uniformity,the acidity in each case was corrected by adding N/10 potassium hydroxide,or N/10 hydrochloric acid solutions.The H-ion concentration standard used in all experiments requiring a uniformly acid medium was pH 6.5 .

All the H-ion determinations were made with the aid of a complete set of the LaMotte colorimetric standards,with a working range of H-ion concentrations from pH 1.2 to pH 10.0 .The readings were all made induplicate,according to the method advised by the manufacturers of the standards.A rapid determination of the H-ion content of the culture media was possible by this method which proved very valuable when many culture flasks were examined.

The above media used in the experiments were comparatively well buffered,especially the modified Richard's solution.Here the peptone proved a very efficient buffer.

All H-ion determinations were made before or after the full culture period.In most cultural experiments this period extended over ten days.It was found that,in general, there was a slight change in the H-ion concentration of the prepared media,though in cultures the change in pH value of the medium was considerable. The general tendency in all flasks,except those containing cultures growing on a very acid medium,was to have an increase in acidity,whether the medium was inoculated or not.This increase in acidity of the medium was usually constant for all experiments.

Preparation of Plant Extracts:

Only fresh,unheated plant juices were used in all experiments where the influence of such plant extracts on growth was studied. The following procedure for the preparation of these extracts was employed.

In the case of the preparation of extracts from apples or tomatoes,the fruit in each case was first washed in warm tap-water,then in distilled water.After washing,the fruit was sliced and then ground to a pulp in a clean mortar.It was necessary to

add some clean,burnt,river sand,so that in grinding the plant cells were broken.The juice was then separated from the pulp by means of a fruit press,and prepared for use by filtering through a clean,sterile Berkefeld filter (size N) so that the bacteria,etc,were removed.The H-ion concentration of the juice was determined after some of the liquid had been removed by means of a sterile pipette.The juice so prepared was used undiluted in the experiments.

When onion extract was prepared,the outer scales of the bulbs were removed,the dry neck and basal portions cut off,and the bulbs washed.During the grinding of the pulp it was found necessary to add water to the pulp,in order to facilitate grinding,and also to increase the amount of the juice.

The plant extracts must be used at once for a rapid,enzymatic decomposition takes place.The life of the plant juice may be prolonged if it is kept in cold storage,at about 4-8°Centigrade.

In all experiments where these plant extracts were employed, Richard's nutrient solution was used,for being of a known composition,and not bringing in sources of error,any stimulus found in the growth of the organisms would be known to be due to the effects of the extracts alone,not to the medium itself.

Preparation of the Inoculum

When the effect of spore number in the inoculum on the association of phytopathogens was studied,it was necessary to know the approximate number of spores in a certain amount of inoculum.In a few cases sporulation was not abundant,so here the mycelium of the fungus had to be used for inoculation purposes. When the spores were produced in sufficient quantities,the

following method was employed.

Freely sporulating,pure cultures of the selected fungi were used.The cultures were grown on potato-dextrose agar in test-tubes,so that the purity of the cultures was easily maintained. In the preparation of the spore suspension,about 10 c.c. of sterile tap-water was poured into each tube,which was then shaken. In this manner a moderate suspension was obtained in each tube. To get a uniform suspension the mixture from each tube was poured into a large,sterile,cotton-plugged flask.This was then shaken well,so that the spores were set free from one another as much as possible.

After the spore suspension had been prepared,the number of spores in a given quantity of the suspension was determined.From preliminary trials it was found that this number could be determined by microscopic examination.If a small droplet of the spore suspension was placed on a glass slide and covered with a coverslip,and examined under the high power lens of a microscope,the number of spores in a field could be counted.The average of the spore count from several fields could then be correlated with the number in a given quantity of the same suspension,as determined by the plating-out method.It is necessary to use the same quantity of the suspension,and the same lens,when making several examinations.To obtain a high degree of accuracy in the count, clean glass slides,and coverslips of the same weight and size should be used.A small,wire loop is adequate for obtaining equal amounts of the suspension,if the loop is protected from change. A table was prepared,so that it was possible to read from it the number of spores found in one cubic centimeter of any suspension if the number derived from the average of several fields of

microscope was first determined. If another microscope or another loop were used, then the correlation table had to be made up anew. In order to protect the loop, it can be kept safely in a clean test-tube. To make the determinations more accurate, cover-slips of the samesize and weight should be used. This method described above was found to be rapid and accurate enough for all practical purposes.

In making the spore suspensions, the microscopic method was used to maintain uniformity in the number of spores used in the inoculum. Usually the most concentrated spore suspension used contained about 10,000 spores per cubic centimeter. Further dilutions could be made from this standard.

Inoculation of the Host

All inoculations were made in the same manner. The surface of the specimens being prepared for inoculation was first washed with distilled water, and then wiped with a soft cloth saturated with a solution of corrosive sublimate ($HgCl_2$), strength 1-500. The epidermis was then punctured with a sterile scalpel and the inoculum inserted into the wound. The inoculum was either in the form of spores or mycelium, and was obtained from a pure culture of the desired organism. Only one organism was used to inoculate a single wound, but where association was studied, two organisms were placed in the same wound. The puncture was then sealed with melted paraffin. It was observed that this method of sealing did not interfere with the growth of the rot-producing fungi, and yet allowed the wound to remain free from outside contamination. Punctured and sealed checks showed that this method was very efficient. After sealing the wounds, special precautions were

unnecessary,for the wound did not open readily afterwards.The layer of paraffin also checked moisture evaporation,so that infection was more easily secured.

Determinations of Amounts of Rot:

In apple fruit it was possible to obtain nearly exact data concerning the amount of rot produced by a pathogen in a definite length of time.The lesions here were distinct,for the decayed tissue differed very much in character from healthy tissue.The The volume of the rotted tissue found on diseased specimens could be determined by scraping out carefully each decayed portion,and then finding the difference in volume of the sound apple and the same apple after rotting had taken place and the rotted tissue had been removed.

In determining volumes,the specimens were submerged in water,and the amount of water displaced was calculated.If a graduated vessel is used,the readings can be made directly,though for more accurate work it is best to fill a cylinder of known volume,with water,and then measure the overflow after the measured specimen had been immersed,by means of a finely graduated glass cylinder.

A certain amount of shrinkage occurred in the apples during the experiment,especially when the apples were placed in a high temperature.To determine the amount of shrinkage,apples were punctured and sealed,but not inoculated,and placed at the same temperature as the inoculated apples.The loss in volume was thendetermined as before.

To prevent the specimens from becoming mixed,the data,which

showed the volume of the specimen,kind of inoculum,date of inoculation,etc;;were entered on a small tag attacked to the stem of each specimen.This tag remained on the specimen during the entire experiment and served as a ready means of identification of each type of rot.

Determination of the amount of rot was practically impossible with onions and tomatoes.In the former the amount of rot could not be exactly calculated as each scale of the bulb was rotted to a different extent,most rot appearing in the outer scales,and the line of demarcation between the healthy and diseased tissue was indistinct.In the tomato the soft pulpy interior rendered it impossible to separate the healthy and diseased portions with any accuracy.Therefore the extent of the rot had to be judged solely by the spread of the lesion on the surface,even though in the tomato the major part of the rot occurred inside the inner pulp.

The Study of Anaerobiosis:

The purpose of this investigation was to obtain data concerning the growth of rot-producing fungi in the absence of free-oxygen;and therefore the cultures had to be kept as free from atmospheric oxygen as possible.

Richard's modified solution was used for the culture of the fungi.To some tubes of the medium,after they have been autoclaved and cooled,small portions of raw,sterile,potato-tuber tissue were added,this being done in order to determine if the peroxidase present in the fresh tissue would enable fungi to grow in the absence of oxygen,as was found with aerobic bacteria in the work of Thoytta and Avery (81).

After the media were prepared,and the inoculum added,the

culture tubes were inserted into tubes larger than the first, which served as a means of maintaining anaerobic conditions. These were prepared by inserting into the tubes quantities of crystalline pyrogallic acid which were held in the bottom of the tubes by plugs of absorbent cotton. Just before the culture tubes were inserted into the containers, about 5 c.c. of concentrated solution of caustic potash (KOH) were poured into the latter tubes. After the culture tubes were in, the containers were rendered air-tight by sealing with corks impregnated with melted paraffin. The tubes were then placed in an upright position on a rack, and incubated at room temperature, 20-24°C., for about fourteen days or more. Observations on spore germination and growth were made daily, but final observations were not made until after the period of incubation had been completed. A more detailed account of the experiment will be given below.

Experiments and Results:

Results of the Survey of Some Vegetable and Fruit Rots.

As mentioned above, special attention was given during the survey to rots of cabbage, carrot, celery, potato, parsnip, onion, apple and tomato. The rots of the last three plants were selected for detailed study. The external appearance of each diseased specimen was noted, the object being to discover the nature of the rot. This was defined by the texture, odour, formation of sclerotia, and discoloration of the tissues.

After the external examination was complete, the organisms found in the rot were isolated by removing small portions of the decaying tissue and growing it on potato-dextrose agar, or where

it was found that schizomycetes were present,by the dilution method.

In obtaining the inoculum from the diseased bulbs of onion, it was sometimes difficult to cleanse the exterior of the bulbs on account of the formation of superficial sclerotia which caused the fusing together of some of the outer scales.Therefore,in this case, a number of the outer scales had to be removed to obtain inoculum from those parts of the lesion which were free from any external contamination.

In many soft-rotted vegetables,especially those emitting an offensive odour,numerous nematodes could be found.These were believed to be responsible for a rapid spread of saprophytic organisms through the rotted tissues.Yeasts were present in abundance wherever these nematodes were found.

When the survey was complete,it was noted that comparatively few of the organisms were responsible for the rotting of their host.In most cases the association of parasite with saprophyte was the rule.The pathogenicity of each was tested by experiment.

In Table I the fungi or other organisms are listed in groups according to the host on which they were found.In most cases a single parasite was associated with many saprophytes,while in others two or more parasites were found in the same lesion.

In apples rotted by Penicillium expansum,no other genus of micro-organisms was found present.In specimens rotted by the "Pink Rot" fungus,Cephalothecium roseum,the rot was caused by a primary infection by the Scab fungus,Venturia inaequalis,which was then followed by the Cephalothecium.This organism grew fast

and caused the specimen to become completely destroyed. The Brown rot fungus, Sclerotina fructicola, was found on a few specimens, this being partially due to the unfavourable temperature of the store-room. In such cases, a secondary infection by various Penicillia accompanied the Brown rot.

In the cabbage the dominant rot was caused by Sclerotinia perplexa, the Botrytis stage being most in evidence. A moderate production of spores was observed, as well as a few small sclerotia. In a few specimens the rot was rendered watery due to invasion of saprophytic bacteria and yeasts. An abundant growth of Phycomyces sp. was observed sometimes, but it was strictly saprophytic in nature.

In the carrot the predominating rot was caused by Sclerotinia sclerotiorum. Some of the rotted tissues were also invaded by Erwinia caratovora, but the low temperature of the store-rooms inhibited the growth of this pathogen considerably. Saprophytic bacteria and yeasts were also present in the lesions caused by the Sclerotinia, but these were not identified.

In the potato the typical rot was caused by Fusarium sp. probably species oxysporum . In one or two cases Verticillium albo-atrum was found in the lesions. A considerable amount of rot caused by the Late Blight fungus, Phytophthora infestans, was also observed. Some saprophytic fungi, such as Rhizopus nigricans and various Mucors, were observed.

In the onion, Botrytis allii was the predominating pathogen. Botrytis byssoidea and B. squamosa were also found on some of the decayed specimens, the latter fungus chiefly on the white skinned onions. Botrytis allii was responsible for at least seventy-five percent of the decayed bulbs. Yeasts and bacteria

were usually found to be present also,and sometimes Pythium debaryanum,was found as a saprophyte.It is not absolutely certain that Pythium of the species debaryanum was always present,probably other species of Pythium were sometimes present,and were confused with Pythium debaryanum.Spore production was not always observed and so it was sometimes difficult to determine the species of the fungi found.

In the parsnip Sclerotinia sclerotiorum caused a soft rot of the cortex of the root.Some saprophytic yeasts were also found in the lesions.

In the tomato the dominant rot seemed to be caused by Botrytis sp.The rot was found usually in the fruit which had become fully grown but had not yet changed color.The progress of the rot before or after this stage was relatively slow.The "leaf mould"fungus,Cladosporium fulvum,was sometimes found on the green fruit,but it was mostly superficial and did not cause a deep rot to develope.However,in some cases,the surface of the lesions was cracked due to the drying out of the tissues beneath,and then these cracks became infected by Botrytis sp.at once,so that the typical rot produced by the latter organism was soon developed.

From the above survey,the writer decided that association of phytopathogens was of three types,as follows:

(1) Where associated organisms are seldom found,as in rots produced by Penicillium expansum.

(2) Where pathogens can mix freely,as in the case of rots of the carrot,where Sclerotinia sclerotiorum and Erwinia caratovora can be found in the same lesion.

(3) Where the parasite is usually followed by a saprophyte, as in the onion rots.

TABLE I. ORGANISMS FOUND IN SOME VEGETABLE AND FRUIT ROTS.

host.	parasites.	saprophytes.
Apple	Sclerotinia fructicola Penicillium expansum Cephalothecium roseum	None.
Beet	Phoma betae	Schizosaccharomyces sp.
Cabbage	Sclerotinia perplexa	Phycomyces sp. Schizosaccharomyces sp. Bacteria.
Carrot	Sclerotinia sclerotiorum Erwinia caratovora	Schizosaccharomyces sp.
Celery	Sclerotinia sclerotiorum Erwinia caratovora	None.
Onion.	Botrytis allii Botrytis byssoidea Botrytis squamosa	Pythium debaryanum (?) Schizosaccharomyces sp. Bacteria.
Parsnip	Sclerotinia sclerotiorum	Schizosaccharomyces sp.
Potato	Fusarium oxysporum (?) Verticillium albo-atrum	Rhizopus nigricans.
Tomato	Botrytis sp. Cladosporium fulvum	None.

Association of Phytopathogens in Culture:

Zeller and Schmitz (98)grew many fungi in mixed culture and found that there was an inhibition of growth of one fungus due to the effect of the presence of another fungus,but in a few cases one colony grew over the other.Sometimes the overgrowing fungus was greatly stimulated.Two colonies of the same fungus generally intermixed.However,the findings in general show that the fungi in their growth show a marked tendency to grow away from the medium influenced by their own growth metabolism.There apparently was no connection between active acidity and growth.

Schmitz (74)grew mixed cultures of four isolations of Fomes pinicola.In all cases the fungus mats from two inoculations of the same fungus mixed without inhibition.On the other hand,when plates were inoculated with two strains of the same fungus,the colonies did not intermingle,and a clear line of demarcation occurred between the two colonies.

Cook(19)found that fungi succeed one another in a more or less regular order.

Porter(64) found that the growth of fungi is checked when two or more species are contiguous.Such inhibition may be mutual or the growth of one individual may be hindered more than that of the other.He recognizes five types of inhibition,from mutual intermingling of both colonies to complete inhibition of growth at a considerable distance.

Brown (15) discusses intermingling of colonies.According to his results two colonies will never meet if they produce staling substances toward one another.If only one of these produces staling substances toward the second colony,this colony will become inhibited,while the first colony will continue its growth.

Where staling substances are produced there is no mutual intermingling of colonies.

Davis(20) observed that colonies of Ophiobolus graminis did not intermingle to any extent.Where two colonies came together a line of dark-colored mycelium was formed and perithecia were formed along this line.According to the results of Kirby, the production of the ascogenous stage may be due to the intermingling of plus and minus strains,or heterothallism.

Practically all the experiments of the workers mentioned above were carried out on solid culture media.The writer,therefore, purposely experimented with mixed cultures on solid as well as liquid nutrient media."Difco" potato-dextrose agar was used as the solid medium,and Richard's or modified Richard's liquid media as the nutrient solutions.

The following fungi were chosen for the study of association of phytopathogens in culture;Penicillium expansum,Sclerotinia fructicola,Botrytis sp.(tomato),Cladosporium fulvum,Sclerotinia sclerotiorum,Botrytis allii,and a slow growing,undetermined fungus isolated from decaying carrots.The latter fungus produced no spores whatsoever,but its mycelium was septate,The inoculations were so made that each fungus was grown in pure culture,and also was paired with each of the other fungi.All the cultures were made in duplicate.The main object of this preliminary experiment was to determine the effect of the association on the growth, sporulation,mycelial and sclerotial development,acid production, and to determine which organism was dominant in the mixed culture where any inhibition occurred.

The fungi used in the above experiment were grown on modified Richard's nutrient solution in small Erlenmeyer flasks of 150 c.c.

capacity.The flasks were made chemically clean,and into each was placed fifty cubic centimeters of the medium which was adjusted to pH 6.5.The cultures were then incubated at room temperature for ten days,observations being made daily.At the end of the experiment the Hydrogen-ion concentration was determined for the medium in each flask,in order to determine the change due to the growth of the fungi in culture.The data obtained from the above experiment are given in Table II.

The above results show that when fungi are grown in association,there is a tendency for some species to inhibit other species,the amount of dominance varying.The final Hydrogen-ion concentration of the medium after the ten-day growth of the culture is a fair indication of the amount of dominance,for when almost complete dominance occurs,the Hydrogen-ion concentration is about the same as that of the pure culture of the dominant organism.Where the dominance of one organism is not complete,then the final acidity is at some point between the acidities of the two organisms grown in culture.In a few cases the fungi grew equally well in mixed culture as in pure culture.Sometimes a certain amount of stimulation to the growth of the overgrowing organism was given by the association.

Because there seemed to be such a difference in growth of fungi found in rots of different plants due to the association the writer performed a similar experiment to that given above,but this time using only fungi found on the same host.The fungi were all isolated from decaying carrot roots and included Erwinia caratovora,Schizosaccharomyces sp.,three strains of Sclerotinia sclerotiorum,and the unidentified fungus,number seven in Table II.Observations were made daily as before,but it was

noticed that in the case of the yeast and the bacterium the maximum growth was reached on the fourth day.The fungi kept on growing throughout the whole ten-day period.

The results of this experiment show that there is some dominance of fungi over bacteria in mixed culture,but in such cultures the growth of the fungi is materially lessened.However, the fungi were still able to grow very well in cultures after the growth of the bacterium and yeast had ceased.No data were obtained concerning the growth of the bacterium or yeast on used medium following the growth of the fungi.However,it appears that the acidity of the used media would be too high to allow bacterial growth to take place,for the limit of Hydrogen-ion concentration allowing the growth of bacteria is pH 4.0 .

The results of the above experiment are given in tabular form in Table III.

Effect of Plant Extracts on Association.

It has been known for a number of years that vitamines,or "essential food factors",are necessary for the proper physiological functioning of living matter.The chemical composition of these substances is not fully understood,but they appear to be classified according to their physiological functions.They are present in all living matter,and for a time in dried remains of such living matter,but they will not live indefinitely.

The writer recently carried out a joint experiment in connection with soil bacteriology with the object of investigating the effect of some plant extracts on the growth of some Nitrogen-fixing bacteria.Extracts from various plant materials were prepared as follows.Twenty-gram samples of several plant tissues

TABLE II. THE ASSOCIATION OF PHYTOPATHOGENS IN MIXED CULTURES.

Culture	Final pH	Sporulation	Mycelium	Sclerotia	Dominance
(1) P. expansum	3.3	++++	+++	0	
do.	3.3	++++	+++	0	
(2) S. fructicola	4.8	+++	++++	0	
do.	4.7	+++	++++	0	
(3) Botrytis sp.	3.6	++++	+++++	++	
do.	3.6	++++	++++	++	
(4) C. fulvum	5.7	+++++	+++	0	
do.	5.7	+++++	+++	0	
(5) S. sclerotiorum	4.8	++	++++	++++	
do.	4.9	++	++++	++++	
(6) B. allii	4.9	++++	++++	++	
do.	4.9	++++	++++	++	
(7) Unknown fungus.	5.9	0	++	0	
do.	5.7	0	++	0	
(1) and (2)	3.1	++++	+++	0	(1) dominant
do.	3.3	++++	+++	0	" "
(1) and (3)	3.3	++++	++++	++	equal
do.	3.5	++++	+++	++	"
(1) and (4)	3.3	++++	+++	0	(1) dominant
do.	3.3	++++	+++	0	" "
(1) and (5)	3.2	++++	+++	0	equal
do.	3.1	++++	+++	0	"
(1) and (6)	3.3	+++	+++	0	(1) dominant
do.	3.3	+++	++-	0	" "
(1) and (7)	3.2	++++	+++	0	(1) dominant
do.	3.2	++++	+++	0	" "

TABLE II.(continued)

(2) and (3)	4.8	+++	++++	++	(3)dominant
do.	4.7	+++	++++	++	" "
(2) and (4)	4.9	++++	+++	0	equal
do.	5.0	++++	+++	0	"
(2) and (5)	3.9	+++	++++	0	(5)dominant
do.	3.6	+++	++++	0	" "
(2) and (6)	3.7	+	++++	0	(6)dominant
do.	3.6	+	++++	0	" "
(2) and (7)	3.5	+++	++++	0	(2)dominant
do.	3.3	+++	+++	0	" "
(3) and (4)	3.6	++++	+++++	0	(3)dominant
do.	3.7	++++	+++++	0	" "
(3) and (5)	3.8	+++	++++	++	(3)dominant
do.	3.8	+++	++++	++	" "
(3) and (6)	3.8	+++	++++	++	(3)dominant
do.	3.8	+++	++++	++	" "
(3) and (7)	3.9	+++	++++	++	(3)dominant
do.	3.8	+++	++++	++	" "
(4) and (5)	3.9	+	++++	++	(5)dominant
do.	3.9	+	++++	++	" "
(4) and (6)	4.2	+	++	0	(6)dominant
do.	3.9	++	+++	0	" "
(4) and (7)	5.4	++++	++++	0	(4)dominant
do.	5.5	++++	++++	0	" "
(5) and (6)	3.9	+++	++++	++	equal
do.	3.9	+++	++++	++	"
(5) and (7)	3.9	+++	+++++	+++	(5)dominant
do.	3.9	+++	+++++	+++	" "
(6) and (7)	5.1	+	++++	0	(6)dominant
do.	5.0	+	++++	0	" "

TABLE III. ASSOCIATION OF CARROT-ROTTING MICRO-ORGANISMS.

Culture	Final pH	Sporulation	Mycelium or cells	Sclerotia	Dominance
1) Schizosaccharomyces sp.	4.9	0	++++	0	
do.	4.95	0	++++	0	
2) E.caratovora	4.9	0	++++	0	
do.	4.9	0	++++	0	
3) S.sclerotiorum strain I.	3.8	++	++++	+++	
do.	3.8	++	++++	+++	
4) S.sclerotiorum strain II.	3.1	+	++++	+++	
do.	3.2	+	++++	+++	
5) S.sclerotiorum strain III.	3.5	++	++++	++++	
do.	3.6	++	++++	++++	
(6) Unknown fungus	4.9	0	++	0	
do.	5.0	0	++	0	
(1) and (2)	4.9	0	++++	0	(2) dominant
do.	4.9	0	++++	0	" "
(1) and (3)	3.3	++	++++	+++	(3) dominant
do.	3.6	++	++++	+++	" "
(1) and (4)	3.1	++	++++	+++	(4) dominant
do.	3.15	++	++++	+++	" "
(1) and (5)	3.6	+	++++	+	(5) dominant
do.	3.4	+	++++	+	" "
(1) and (6)	4.9	0	++	0	equal
do.	4.9	0	++	0	"
(2) and (3)	4.2	++++	+++	++	(3) dominant
do	3.8	+++	+++	++	" "

TABLE III.(continued)

(2) and (4)	3.7	++	+++	0	(4)dominant
do.	3.2	++	++++	+	" "
(2) and (5)	3.8	+++	+++	0	(5)dominant
do.	3.8	+++	+++	0	" "
(2) and (6)	5.0	0	++	0	equal
do.	5.0	0	++	0	"
(3) and (4)	3.3	++	+++	0	nearly equal
do.	3.2	++	+++	++	" "
(3) and (5)	3.8	++	+++	++	equal
do.	3.8	++	+++	++	"
(3) and (6)	3.9	+++	++++	++	(3)dominant
do.	4.0	+++	++++	++	" "
(4) and (5)	3.2	++	++++	++	(4)dominant
do.	3.1	++	++++	++	" "
(4) and (6)	3.7	+++	++++	++	(4)dominant
do.	3.9	+++	++++	++	" "
(5) and (6)	3.9	++	++++	++	(5)dominant
do.	4.0	++	++++	++	" "

were ground up separately in hundred cubic centimeter lots of water.The mixture was then heated to about seventy degrees Centigrade for about one half hour,being stirred at intervals.Then the decoction was filtered through a sterile Berkfeld filter in each case and kept in a sterile,cotton-plugged flask ready for use.

Sterile Ashby's nitrogen-free nutrient medium was used for the culture of the organisms used.This medium was distributed in ten cubic centimeter lots in clean test-tubes of equal size, and sterilized by autoclaving.When cool,a drop of a homogeneous suspension of either Azotobacter chroococcum or Rhizobium radicicolum was placed in each culture tube,and varying amounts of the extracts added.The cultures were incubated at room temperature(20-24°C.) and observations made daily.After one day a distinct effect of the plant extract was noticed in the cultures and the final data were obtained after the fourth day.It was noticed that generally the turbidity of the culture increased with the increase in the amountof extract,showing that there had been a stimulus given to the growth of the organisms through its use.

The different extracts did not stimulate growth in the same way,nor was the amount of growth in each case proportional to the amount of extract added.The results of this experiment are shown in Table IV.

Itano (45) has shown that the addition of plant extracts may increase the power of Azotobacter to fix atmospheric nitrogen so that sixteen milligrams of this element were fixed per gram of sugar consumed instead of two milligrams when no extract was added to the culture.

TABLE IV. EFFECT OF PLANT EXTRACTS ON GROWTH OF N-FIXING BACTERIA

Extract	Amount of extract in drops	Amount of growth of Azotobacter	Rhizobium
Decomposed wheat straw containing also Azotobacter	1	+	++
	1	+	++
	5	++	+++
	5	+	+++
	10	++	+++
	10	+++	++++
Green alfalfa	1	+	++
	1	+	++
	5	+++	++++
	5	+	++++
	10	+	+++
	10	+++	++++
Barley meal	1	+	+++
	1	+	+++
	5	++	++++
	5	++	++++
	10	+++	++++
	10	+++	++++
Ground Yeast cells	1	++	++
	1	++	++
	5	+++	++++
	5	+++	++++
	10	++	+++
	10	++++	++++
Decomposed wheat straw without Azotobacter	1	+	+
	1	++	+
	5	+	+++
	5	+	+++
	10	+	+++
	10	+	+++
No extract	Tube 1	++	+
	" 2	+	+
	" 3	+	+
	" 4	+	+

Duggar, Severy, and Schmitz (25) grew many fungi on plant decoctions, but their results show that while the decoctions were able to produce considerable fungus growth, the addition of sugar and nitrogen gave a tremendous increase in growth as compared to that which occurred on the plant decoctions alone. They, however, autoclaved their decoctions for one hour at fifteen pounds pressure, so that it is probable that they precipitated out from the solution a large number of soluble proteins which may have been very useful in promoting growth.

Wildiers (88) found that he could not get normal growth of Saccharomyces cerevisiae in a synthetic medium if only small inoculations were made. Heavy inoculations resulted in an abundant, normal growth of the organism. Biologists took no notice of his paper for a while, but at present most workers believe that the Yeasts require vitamines for their growth metabolism.

Linossier (49) showed that without vitamines in the solution fungi were able to germinate slowly and with difficulty, but after about six days the controls caught up with the stimulated cultures.

Lumiere (52) denied any influence on the growth of fungi by this stimulus giving substance. The stimulus apparently was not due to any growth accessory substance, but to the addition of salts to poor, synthetic media. His experiments do not appear to be very conclusive, however.

Willaman (91) showed that Sclerotinia cinerea did not develope unless vitamines from some source were added to the medium. Willaman found that the amount of vegetative growth was not in proportion to the juice added, as large amounts of the latter caused an inhibition in growth in the majority of cases.

A large number of vitamine extracts were tried,but only a few showed any tendency to stimulate reproduction.The amount of sporulation was not necessarily proportional to the vegetative growth.

Schelling (73) found that Aspergillus niger could develope without any food accessory substance,but stimulation to the growth and reproduction was given by the addition of small amounts of Vitamine B to the solution on which the fungus grew. The main stimulus occurred during the first five hours after inoculation.She believes that it is probable that the stimulus is due to increased acidity of the medium,or that Vitamine B is catalytic in its action upon the growth of A.niger.The most probable reason for the stimulus is that there is an increased organic food supply through the addition of the vitamine,or that it acts as a stimulant in the sense that small amounts of toxic substances act as stimulants.

Sanborn (70) observed that food accessory substances had a definite stimulative effect upon the physiological efficiency of Cellulomonas folia,a cellulose decomposing organism.These substances were probably equivalent to Vitamine B.In a later paper(71) he describes the effect of the association of Azotobacter chroococcum with C.folia and notes that there is a remarkable increase in the growth and physiological efficiency of the latter organism due to the association.He finds the same effect in the association of C.folia with several other micro-organisms. In a third paper (72),the same author reports that the decomposing residues of many plants produce food accessory substances which stimulate the activities of soil organisms that are able to utilize these plant residues.

The writer performed an experiment to see if food accessory substances,as described above,would also stimulate the growth of rot-producing fungi and bacteria.These substances were in the form of fresh plant extracts prepared by grinding washed and sliced apples or onions,and filtering the juice through a clean, sterile Berkefeld filter(size N) into a sterile flask.Autoclaving of the extracts was avoided in order to prevent the precipitation of the soluble proteins in the extract.

The extracts were added to the medium (Richard's) at the rate of one drop to one cubic centimeter per flask containing fifty cubic centimeters of the medium.Spore suspensions of the organisms used was prepared,so that each cubic centimeter of the suspension contained approximately ten thousand spores,the spore suspensions being prepared according to the method described above.One cubic centimeter of the suspension was added to each flask.

The effect of apple extract on the growth of Sclerotinia fructicola and Penicillium expansum was studied.These two fungi were grown either in pure cultures or in association,and the effect of the extract was observed in each case.The cultures were incubated at room temperature (20-24°C.) for ten days,and at the end of the period the growth of the mycelium in the flasks was charted in diagram form.These are shown in Figure I.

Though daily observations were made,there appeared to be no stimulus given to the growth of the fungi by the extract.Where Penicillium expansum was grown in culture with Sclerotina fructicola the latter organism was almost completely suppressed,though it grew very well in the pure cultures,but in neither case was any increased growth observed.

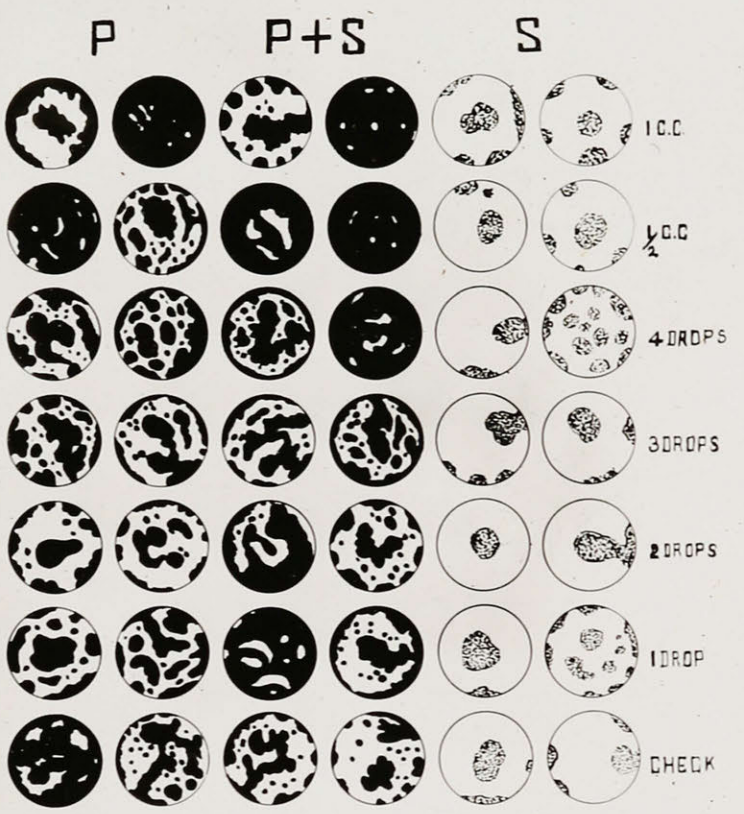


Figure I. Showing the effect of the addition of apple extract on the growth of Penicillium expansum (P), and Sclerotinia fructicola (S) in pure cultures or in combination (P-S). No increased growth of either organism was observed. Incubations made at 20-24°C.

A similar experiment was carried out with two organisms found associated in onion rots. One of these was a fungus, Botrytis allii, and the other a bacterium, Erwinia caratovora. The purpose of this experiment was to determine the effect of onion extract on the growth of the above organism. Plant extracts were found to be able to stimulate the growth of Nitrogen-fixing bacteria, so the writer decided to see if a similar result could be obtained in this case. The fungus, B. allii, could be used as a check on the results obtained from the experiment dealing with the effect of apple extract on the growth of apple-rot fungi, such as mentioned above.

The suspension of spores of the fungus was prepared as above, but the preparation of a definite bacterial suspension was a more difficult matter. It was found by preliminary experiment that the number of organisms per given volume of bacterial suspension was from ten to fifteen times the number of spores in an equal volume of the suspension of the fungus which showed a turbidity equal, or nearly equal, to that of the bacterial suspension. Though the numbers of bacteria could be judged only approximately by this method, it was the most rapid one, for the microscopic method used for the determination of the numbers of fungus spores was useless in this case. Therefore the suspension of bacteria had to be diluted about ten times in order to equalize the number of spores present in the inocula used in the experiment.

Inoculations were made as in the experiment with the apple-rotting fungi, and the same cultural conditions were used. However, it was found to be impractical to leave the bacterial cultures for the full ten days as was done with the fungus cultures. The effect

of the extract was most noticeable on the third or fourth day after inoculation in these bacterial cultures;and it was found that after this period the amount of growth in all the flasks became more nearly equal.

In making an estimate of the amount of growth in the flasks, it was found that there did not appear to be enough difference in the fungus cultures to warrant the conclusion that onion extract as prepared above had any influence on the growth of Botrytis allii.However,the obvious increase in growth in the bacterial cultures could be measured,and the procedure employed was as follows.

The medium in the flasks containing the pure and mixed cultures were well shaken for a short time in order to make the suspension of cells in the medium as homogeneous as possible. The media containing the bacterial cultures were then mixed so that the duplicate cultures were combined;and well shaken after. One drop of this suspension was then removed with a sterile pipette and transferred to a flask containing one hundred cubic centimeters of sterile tap-water.This mixture was well shaken,and then one cubic centimeter of this dilute suspension was plated out withapproximately ten cubic centimeters of potato-dextrose agar in sterile Petri dishes.After incubation at room temperature for three days the colonies on the surface of the agar were counted and the number used as an index of the effect of the onion extract. The results are shown in Figure II and Table V.

It was noted that while the onion extract stimulated all the cultures of Erwinia caratovora,whether in pure culture or in combination withBotrytis allii,but the growth was greater in the pure cultures than in the mixed.Apparently only small amounts of

the extract were necessary to bring about a definite stimulus.

From the above experiments,although they are of a preliminary nature,it is found,in agreement with other workers,that there is little or no visible effect of the plant extract on the growth of fungi,although such effect has been observed in bacterial cultures.It is possible,however,that a proper technique has not been devised,so that any stimulative effect of plant extracts on fungus growth cannot be demonstrated.

The Influence of Temperature on Growth and Association of Some Phytopathogens in Artificial Media.

An attempt has been made to determine the influence of temperature on the development and association of some rot-producing organisms.It has been observed during the survey of rots in storage that apples kept at low temperatures were more liable to be rotted by Penicillium expansum than by any other fungus,while Sclerotinia fructicola rotted apples more readily when they were kept at a higher temperature than fifteen degrees Centigrade.Apples kept at room temperature were about equally susceptible to the attacks of both organisms,but the rot caused by S.fructicola progressed much faster than the other.Therefore, it was thought that temperature could have a considerable influence on the association of these fungi,and so the study of the effect of temperature on the growth of the apple, onion, and tomato rotting fungi was undertaken.

Ames (2) has noted that in refrigerating experiments,temperatures near the freezing point of water must be maintained if the development of rot-producing fungi is to be avoided.Germination of spores occurred even at this temperature,but growth was

TABLE V.

EFFECT OF ONION EXTRACT ON THE GROWTH OF ERWINIA CARATOVORA

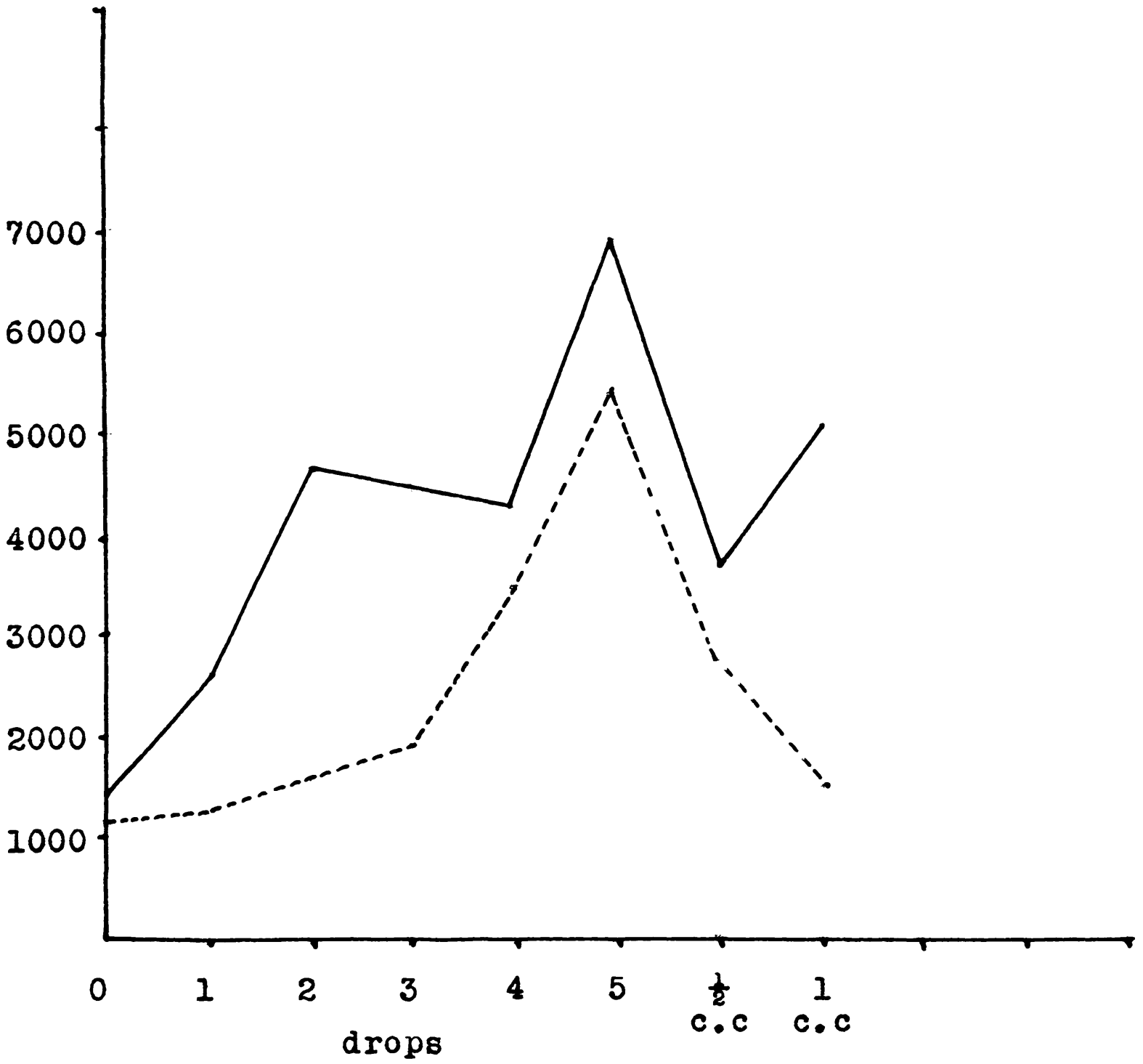
amount of extract per 50 cc. of medium	schizomycete in pure culture @@			schizomycete plus fungus @		
	plate 1	plate 2	Average	plate 1	plate 2	Average
no extract	1376	1520	1448	1520	840	1180
one drop	2800	2480	2660	1574	912	1243
two drops	4560	4738	4649	1728	1480	1604
three "	4640	4380	4510	2080	1840	1960
four "	4416	4190	4303	3520	3040	3280
five "	7280	6450	6855	5600	5428	5514
½ cc.	3360	3940	3650	2848	2752	2800
one cc.	4885	5286	5085	1448	1680	1564

@ Erwinia caratovora growing together with Botrytis sp.

@@ Erwinia caratovora alone in pure culture.

Note:- The lesser numbers of bacteria in the cultures which include the fungus may be due to increased acidity of the medium beyond the point of tolerance of the bacterium to the acid.

FIGURE II. THE EFFECT OF ONION EXTRACT ON GROWTH OF ERWINIA CARATOVORA.



Pure cultures of Erwinia caratovora (_____).

Mixed cultures of E.caratovora and Botrytis allii.(---------)

very slow.Growth is possible at a lower temperature than that which is the minimum for spore germination,so if germination was prevented no rotting took place.

Brooks and Cooley (11) found that peaches inoculated with two stone-fruit rotting fungi,Monilia fructigena and Rhizopus nigricans, more growth occurred than on potato-dextrose agar at the same temperature.Likewise the fungi were able to grow at a lower temperature on ripe fruit than on green fruit.Storing at ten degrees Centigrade immediately after inoculation prevented the rots of these fungi from developing,for at least five days after the rot had developed on fruit which had been kept in storage for one day at twenty-five degrees Centigrade before placing in storage at the former temperature.

The writer studied the effect of temperature on the development and association of fungi in the following manner.Three distinct and widely separated temperatures were used;one,a relatively high temperature,30-34°C.;the second,room temperature, 20-24°C.;and a third,store-room temperature,4-8°C.Six different fungi were used,and grown together in pairs as follows,Botrytis allii and Botrytis byssoidea,Botrytis sp.and Cladosporium fulvum, Penicillium expansum and Sclerotinia fructicola.Each pair of organisms caused rots of a different plant,the onion,tomato, and apple respectively.In each case the fungi were grown on potato-dextrose agar in Petri plates,or in small Erlenmeyer flasks of 150 cc.capacity,each containing 50 c.c. of Richard's modified nutrient solution.The inoculum was added in the form of spores,where they were available in sufficiently large numbers, or small squares of mycelium on agar.Approximately 10,000 spores

were used in the inoculation of each flask.Where the fungi were grown on potato-dextrose agar in Petri plates,the inoculum was placed in the center of the dish,in the case of pure cultures, or near the edge on opposite sides where the fungi were grown in pairs on the same plate.The cultures were incubated at the three temperatures for ten days,after which final observations were made.

In the apple-rotting fungi,Sclerotinia fructicola was found to grow best at 30-34°C.and 20-24°C.,slightly better in the latter,but very slowly at the cold-storage temperature,4-8°C. At the highest temperature the mycelium was closely appressed to the surface of the medium.Sporulation was moderate at this temperature,but the spores were found to be unable to survive for more than four days after formation,for in most cases,after this period they were found to be collapsed.No zonation was visible in the colonies growing on potato-dextrose agar.At 20-24°C. sporulation was abundant,spores were viable for a long time,and zonation was distinct.At 4-8°C.growth was very slow, for germination is retarded.Practically no spores are produced at this temperature.

Penicillium expansum grew best at 20-24°C.Sporulation and zonation is abundant and distinct.At 30-34°C.growth was very slow, sporulation less abundant,no zonation;and a reddish-brown pigment diffused into the medium around the colony.No pigment was formed at the other temperatures used.At 4-8°C,germination of spores was noticeably retarded,but the growth afterwards was slightly more rapid than at 30-34°C.

In mixed cultures there was a distinct retardation in growth

of each fungus as the colonies approached each other on the solid medium in Petri plates,no matter at what temperature they were growing.Otherwise,sporulation,growth,and zonation were exactly the same in mixed cultures as in pure cultures.The relative amounts of growth varied considerably with the differences in temperature,for at 30-34°C.Sclerotinia fructicola occuppied most of the surface of the medium.At 20-24°C. growth of both fungi was rapid,but nearly equal.At 4-8°C. growth was retarded,but very nearly equal,even though Penicillium expansum was able to germinate at least a day before the other.The relative growths of these fungi are shown in graphic form in Figures III,IV,and V.

Somewhat similar results were obtained in the cultural studies of the onion-rotting organisms Botrytis allii,and B.byssoidea.

Botrytis allii grew well at 30-34°C. or 20-24°C.,but at 4-8°C.the growth was considerably retarded.Best growth was obtained at 20-24°C.Sporulation was abundant at both higher temperatures, but very much reduced at the lower temperature.In all cases the mycelial growth was appressed to the medium,not flocculent and raised as with Botrytis byssoidea.

This latter organism grew best at 20-24°C.and considerably less at the two extremes of temperature.Growth was about equal at 30-34°C.as at 4-8°C.and at both these temperatures the growth exceeded that of Botrytis allii.Spore production was slight at all temperatures,but best at 30-34°C.At this latter temperature the mycelium was comparatively compact,not loose and flocculent as at the other temperatures.Germination in cold storage occurred at least one day before that of Botrytis allii.

In mixed cultures the growth of both fungi was approximately

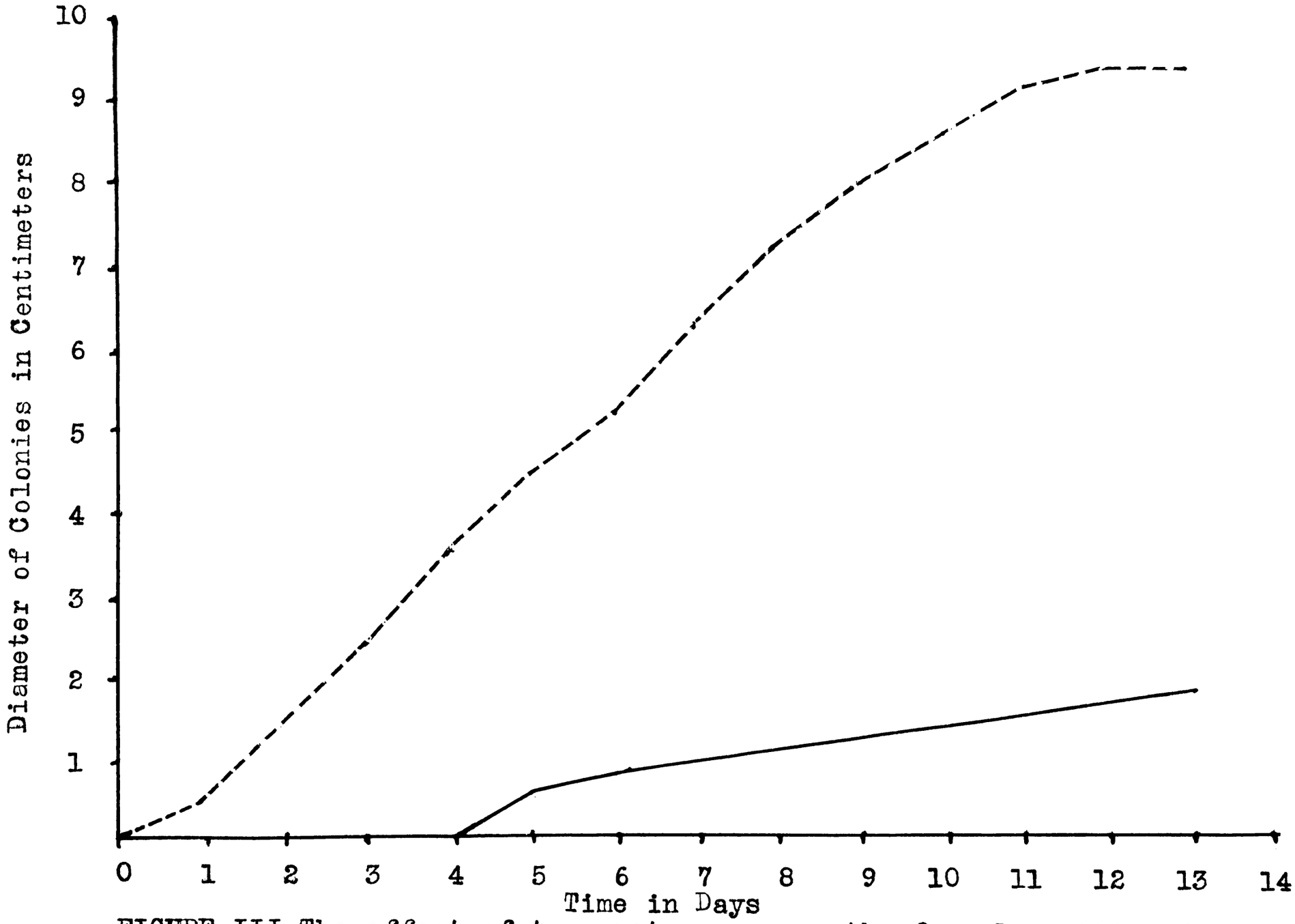
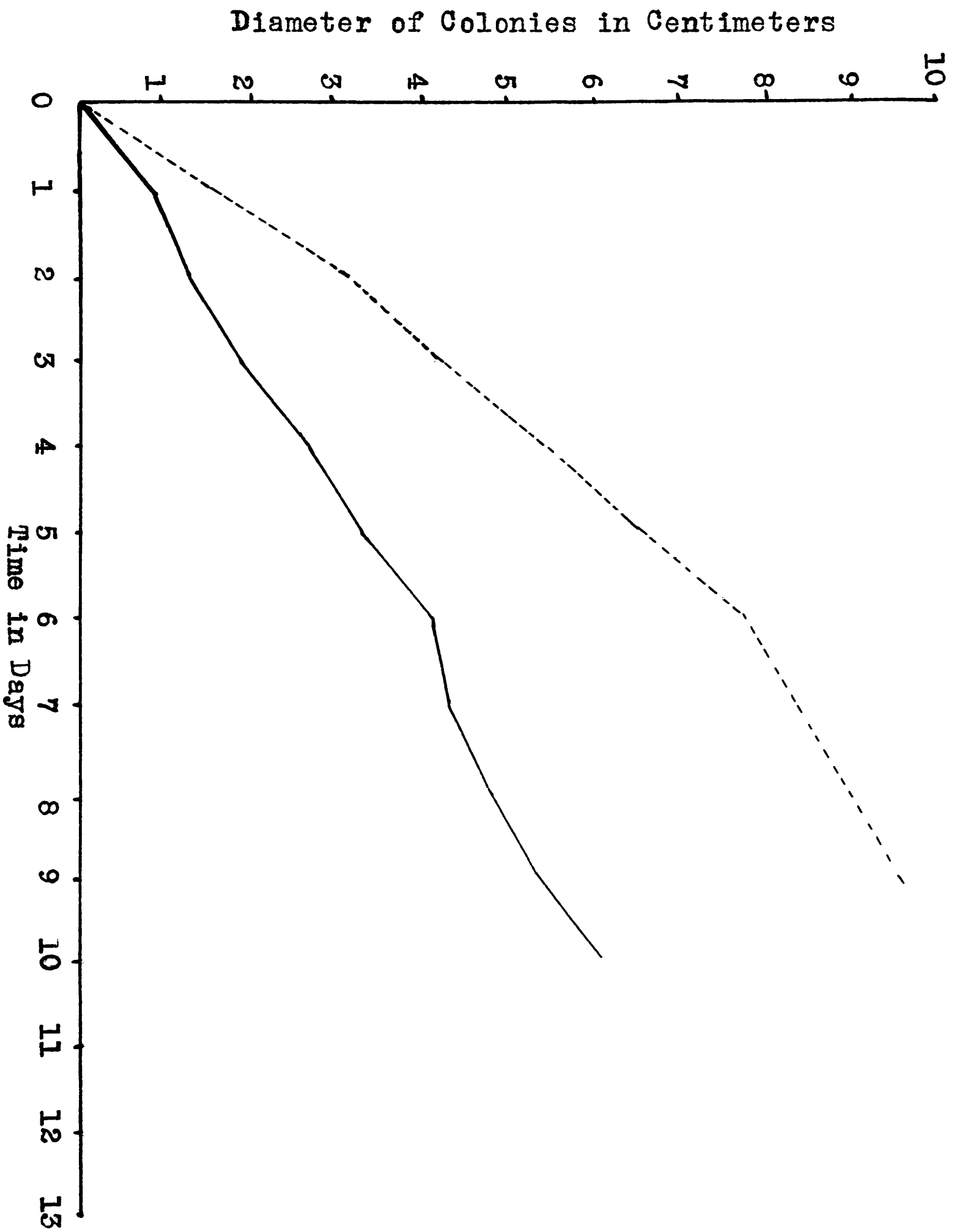


FIGURE III. The effect of temperature on growth of apple-rotting fungi. Grown at 30-34°C. Sclerotinia fructicola (-------); Penicillium expansum (————).


Diameter of Colonies in Centimeters
1
2
3
4
5
6
7
8
9
10
0
1
2
3
4
5
6
7
8
9
10
11
12
13
Time in Days

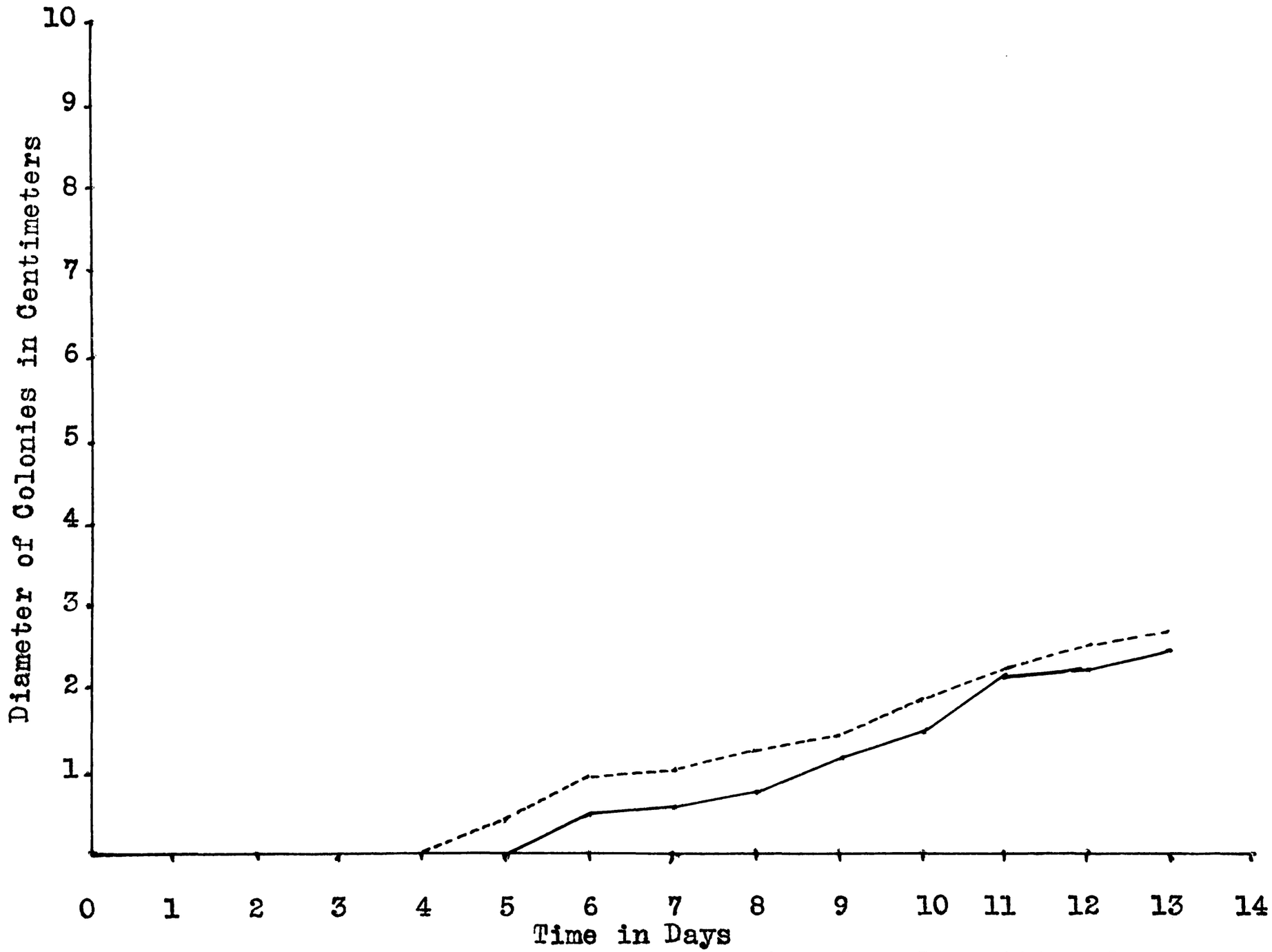


FIGURE V. The effect of temperature on growth of apple-rotting fungi. Grown at 4-8°C. Sclerotinia fructicola (------); Penicillium expansum (————).

similar to the growth obtained in the pure cultures of each fungus.There was no overgrowing of one fungus by the other,and the total area occuppied by each fungus depended directly on the temperature.At 30-34°C.Botrytis allii occupied at least nine-tenths of the available surface of agar in a Petri plate, while B.byssoidea covered the remainder.At 20-24°C.B.byssoidea covered about two-thirds of the medium,and B.allii one-third. At 4-8°C. complete overgrowth of the agar was not secured after ten days of growth,but Botrytis byssoidea grew approximately four times as fast as Botrytis allii.The results are illustrated in graphic form in Figures VI,VII,and VIII.

In the tomato-rotting organisms,Botrytis sp.and Cladosporium fulvum,they differed considerably in the amount of growth,for in all cases the former grew faster than the latter.There was a difference,however,in the rate of growth and sporulation of both fungi when grown at varying temperatures,whether in pure culture or in association.

At 30-34°C.growth was abundant in both cases,but the Botrytis grew much faster than the Cladosporium,and so covered practically all of the surface ofthe medium.Abundant spore production was obtained with both fungi.The latter fungus grew almost the same at this temperature as at 20-24°C.In the case of Botrytis the growth was slightly less than at room temperature,but sporulation was greater.The mycelium showed a tendency to produce small sclerotia much more rapidly than at the room temperature.A slight tendency towards the production of saltants was observed,the variation consisting of the formation of sectors of dark mycelium.

At 20-24°C.growth of Botrytis sp. was greater than at the higher temperature.More aerial mycelium was formed,but comparatively

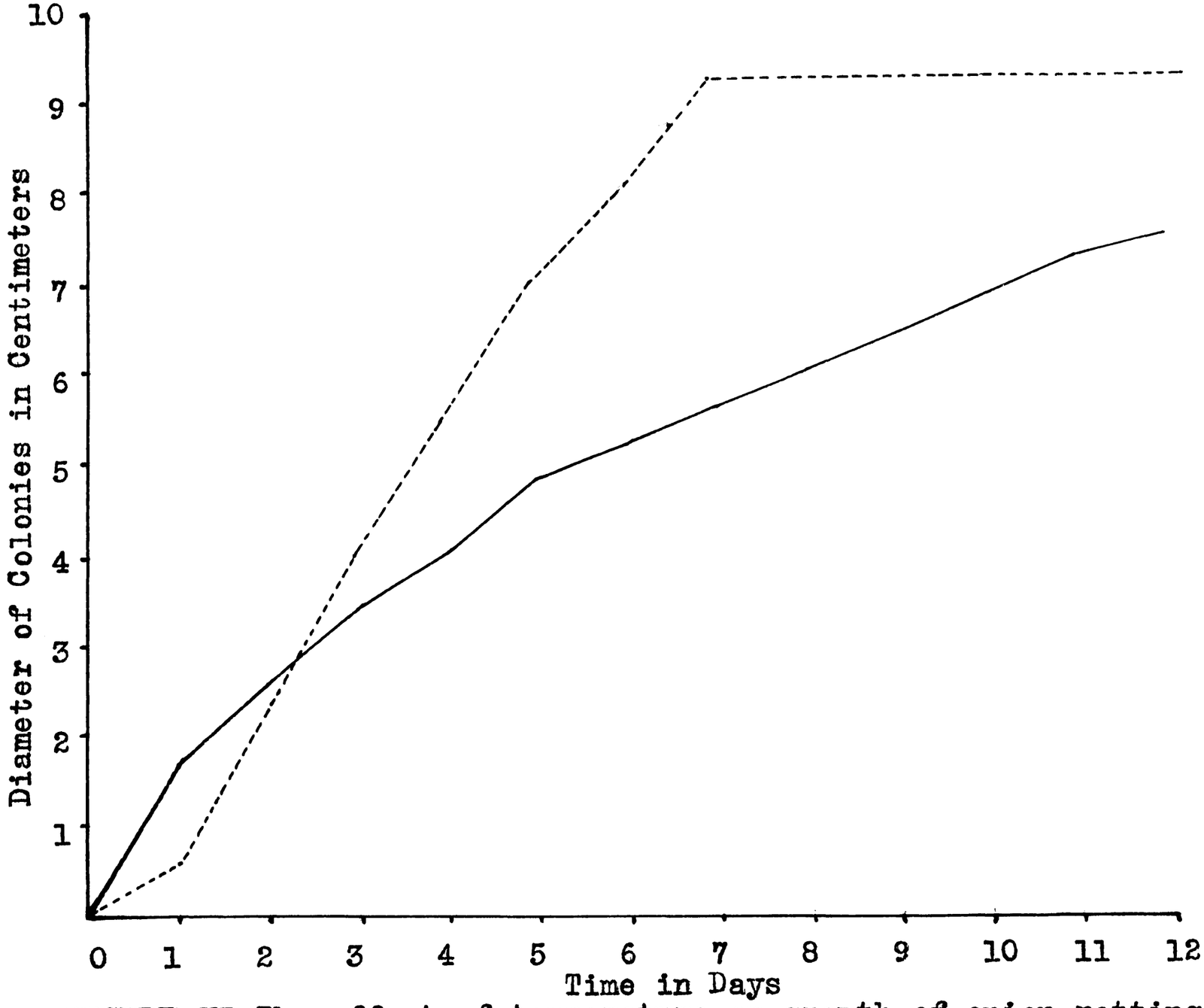


FIGURE VI. The effect of temperature on growth of onion-rotting fungi. Grown at 30-34°C. *Botrytis allii* (-------); *Botrytis byssoidea* (————).

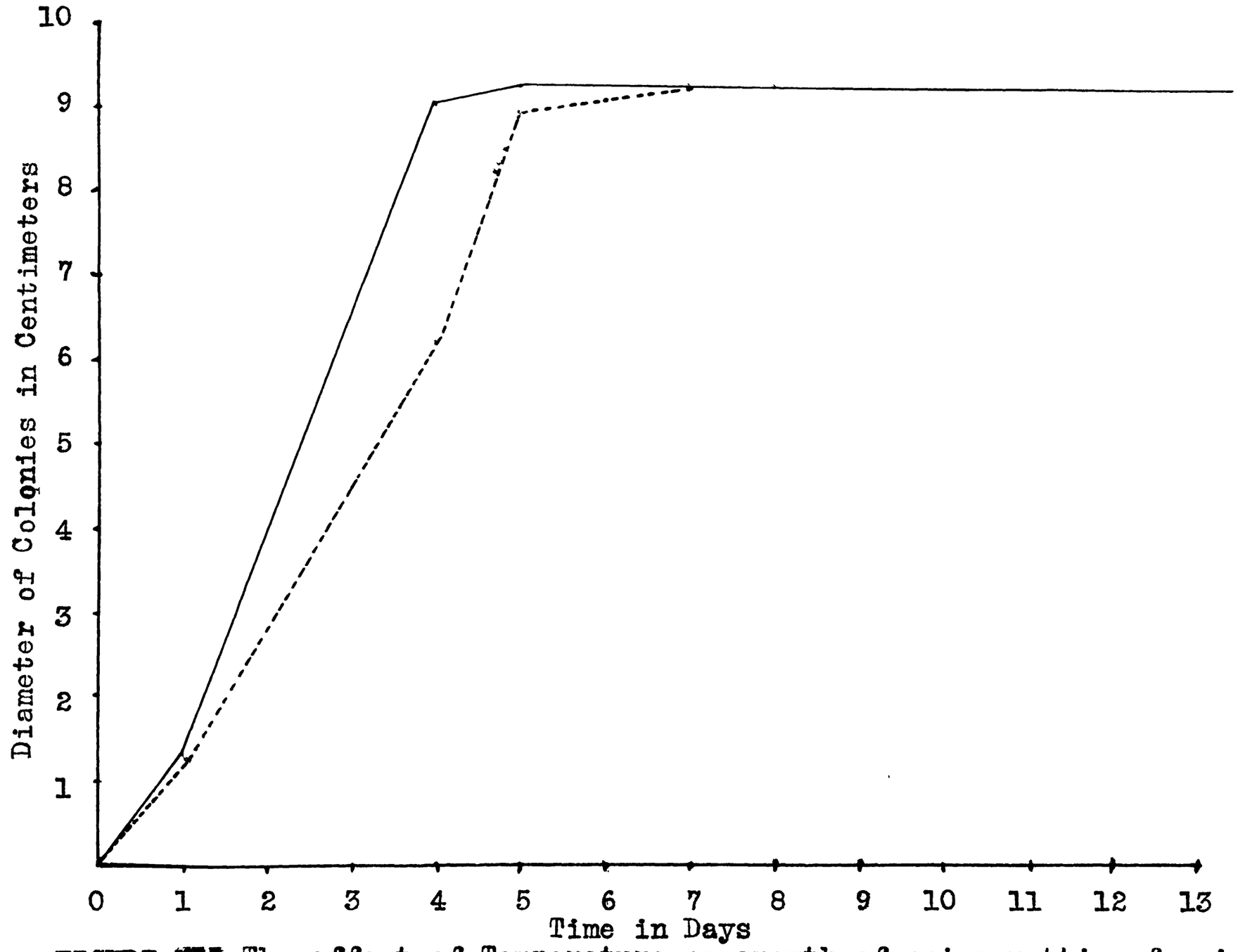


FIGURE VII. The effect of Temperature on growth of onion-rotting fungi. Grown at 20-24°C. Botrytis allii (-----); Botrytis byssoidea (———).

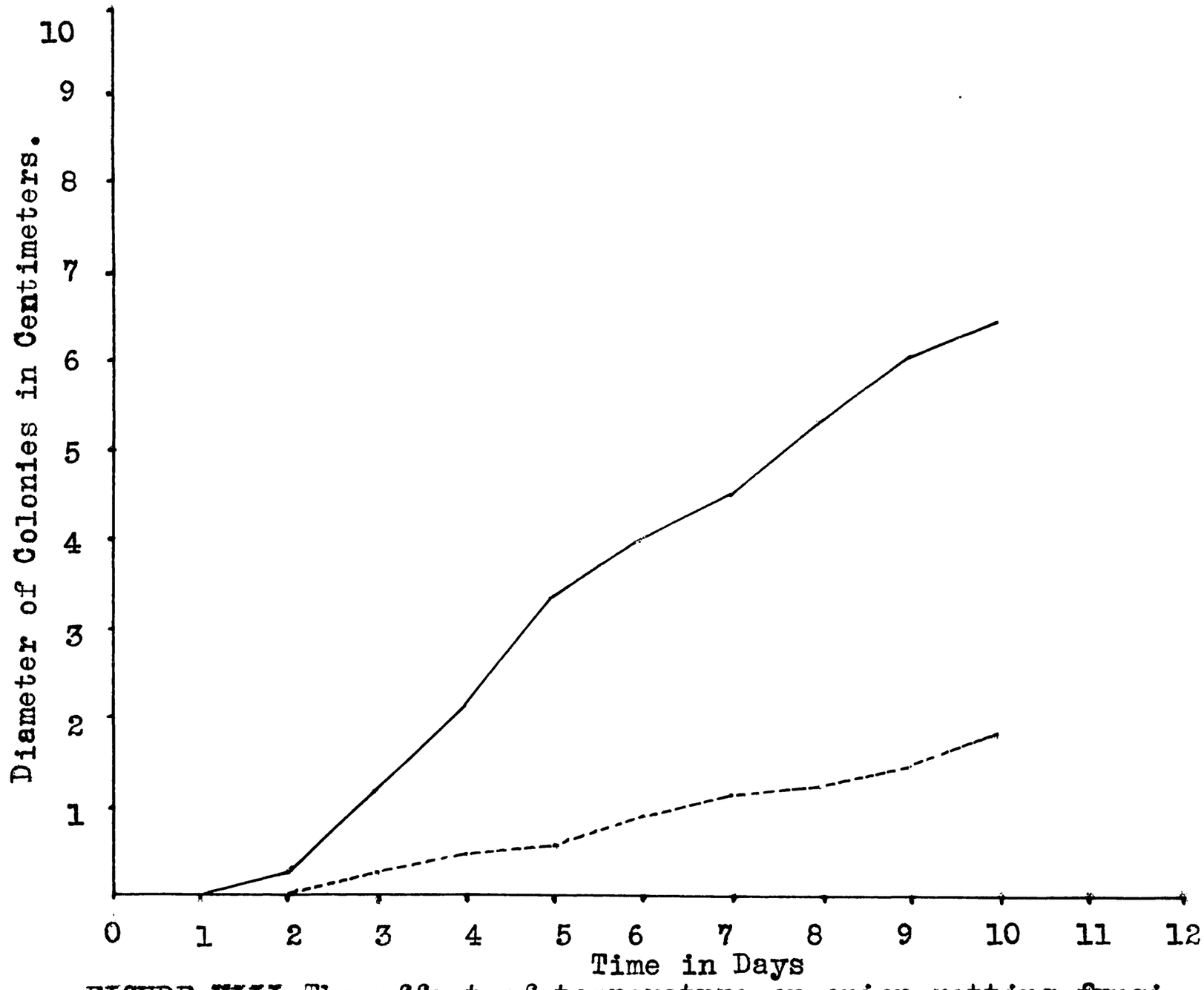


FIGURE VIII. The effect of temperature on onion-rotting fungi. Grown at 4-8°C. Botrytis allii (-------); Botrytis byssoidea (———).

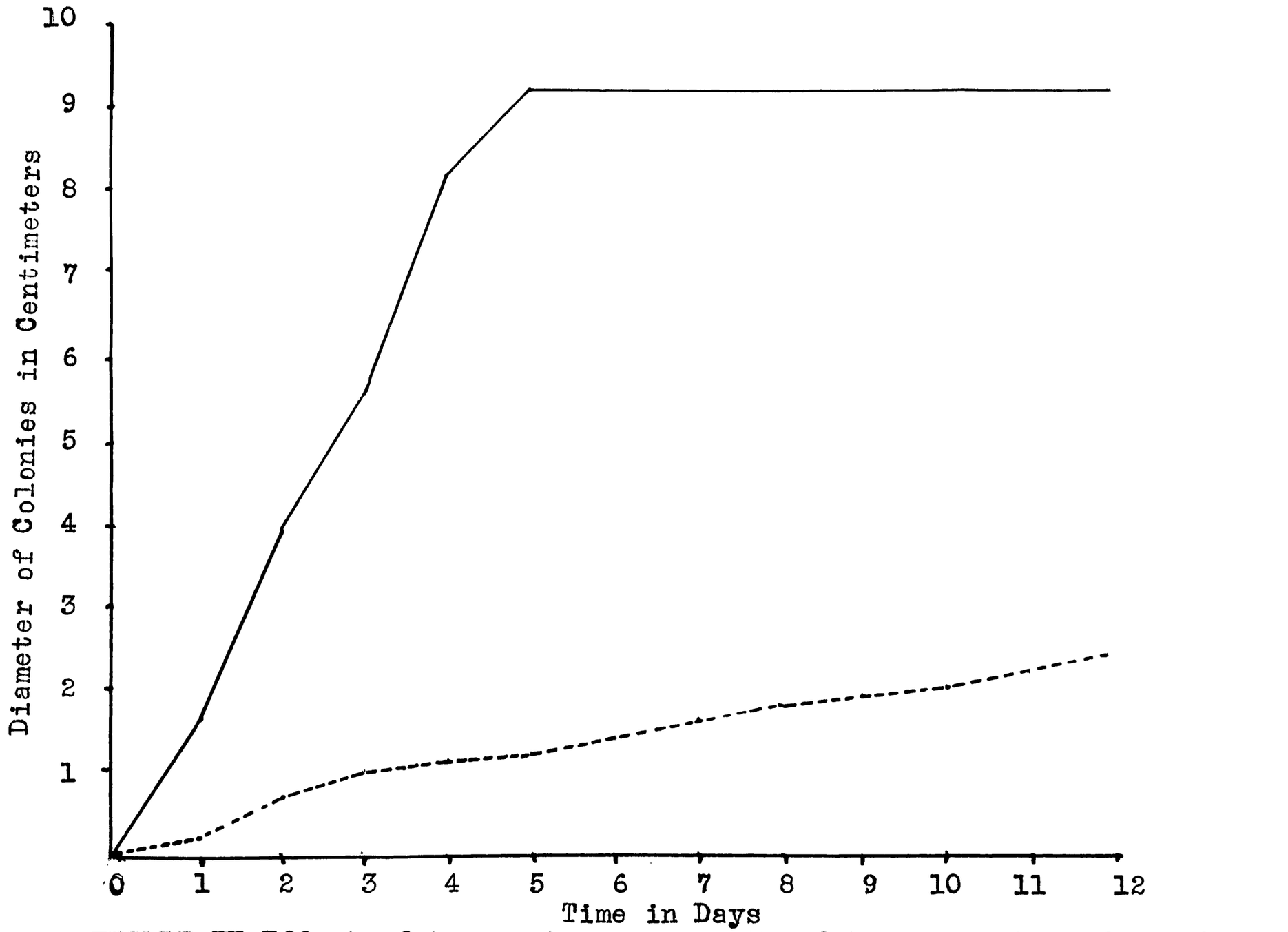


FIGURE IX. Effect of temperature on growth of tomato-rotting fungi. Grown at 30-34°C. Botrytis sp. (————); Cladosporium fulvum (---------).

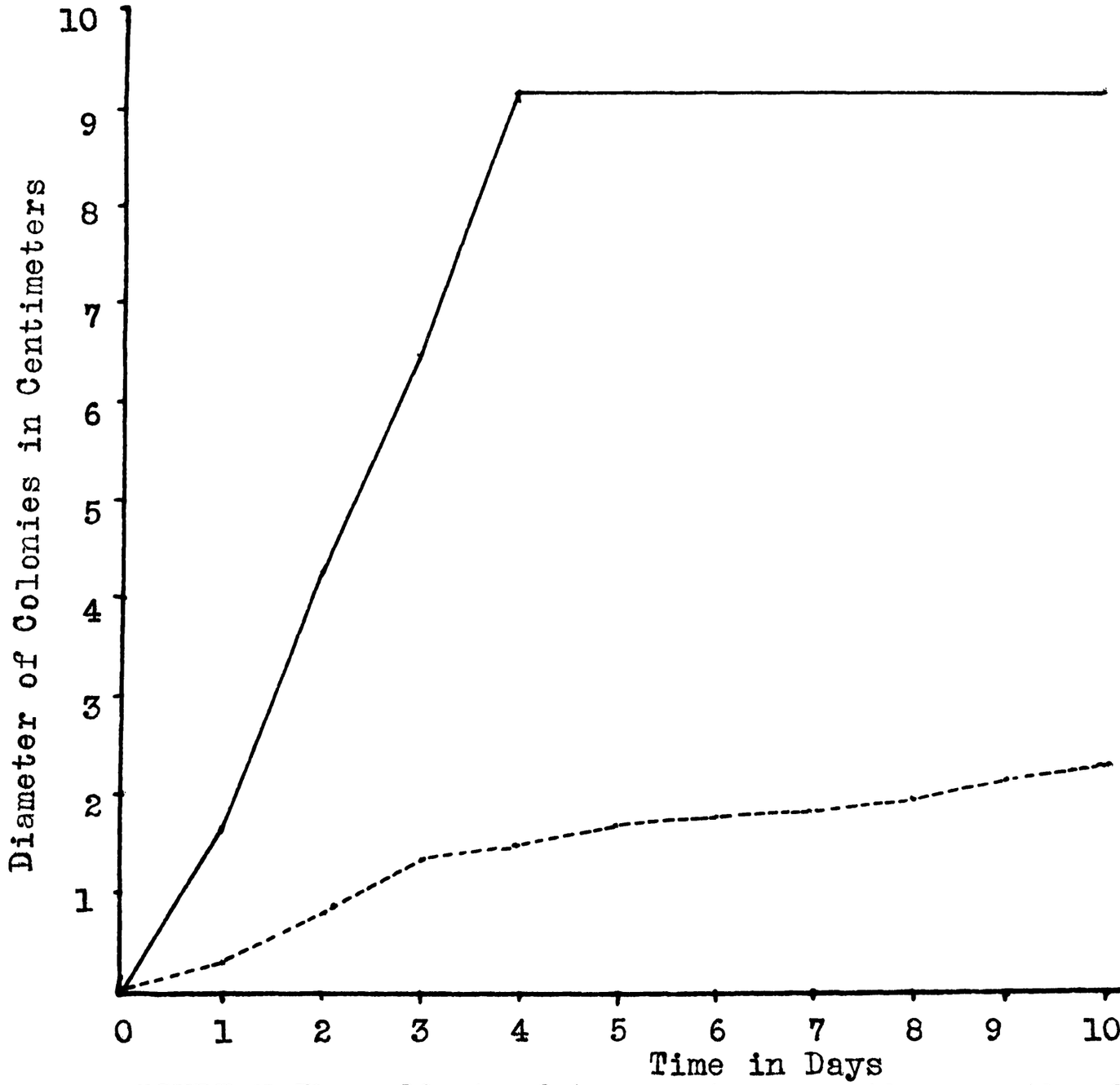


FIGURE X. The effect of temperature on the growth of tomato-rotting fungi. Grown at 20-24°C. Botrytis sp. (———); Cladosporium fulvum (-------).

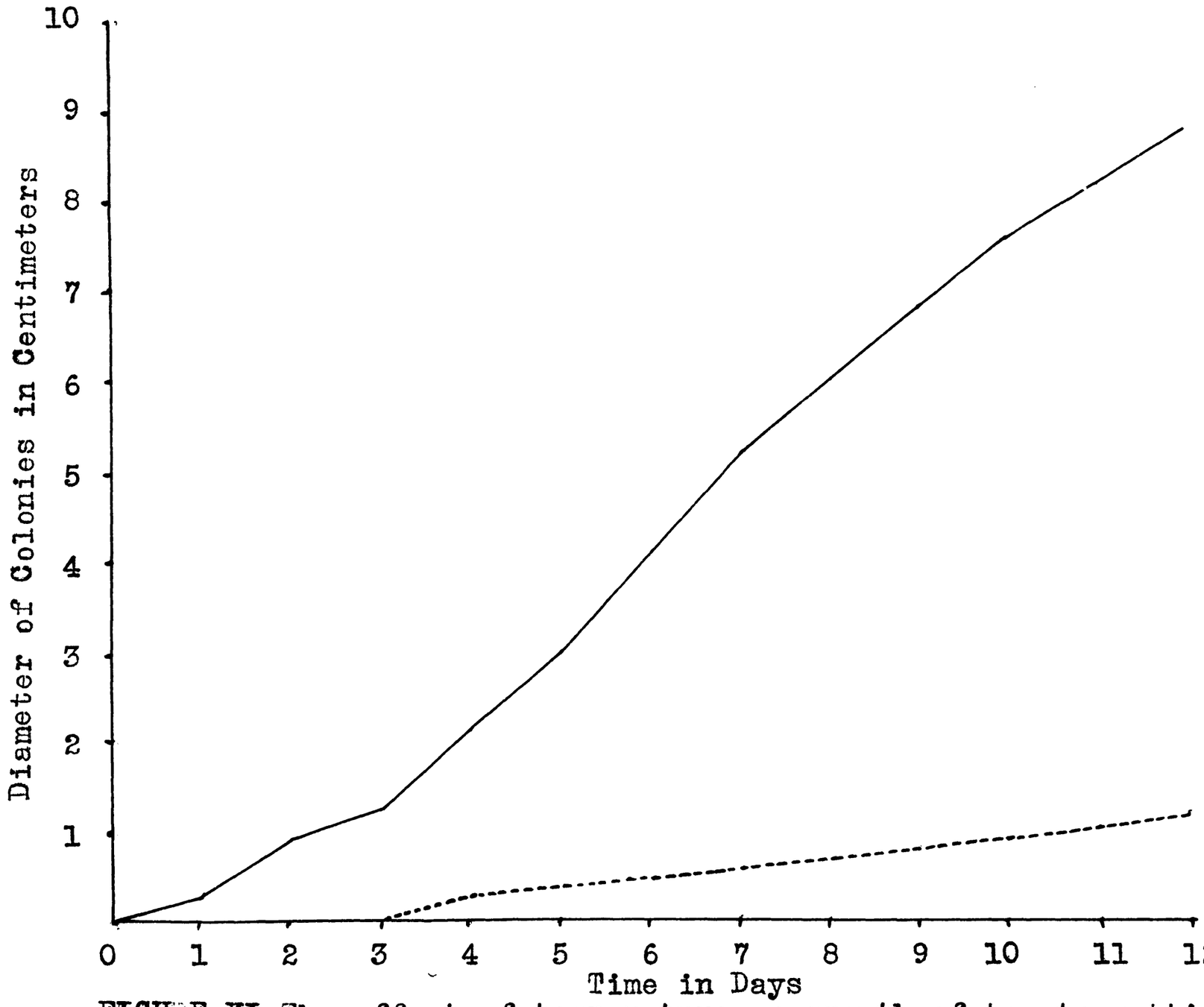


FIGURE XI. The effect of temperature on growth of tomato-rotting fungi. Grown at 4-8°C. *Botrytis sp.* (————); *Cladosporium fulvum* (---------)

few spores.There was no pigment production by the mycelium.The growth of Cladosporium fulvum was slightly greater than at the higher temperature.

At 4-8°C. growth of Botrytis sp. was comparatively slow.The mycelium was closely appressed to the medium.No spore or pigment production was observed.Cladosporium fulvum was retarded considerably in germination,so that the colonies did not become visible until after four days had elapsed from the time of inoculation.

The results of the above experiment are given in graphic form in Figures IX,X,and XI.

Association of phytopathogens at varying temperatures was best illustrated when the above pathogens were cultured on liquid media.Richard's modified solution was used,distributed as in other experiments,that is fifty cubic centimeters of the medium,adjusted to pH 6.5,per small flask.The flasks were divided into three series,each series being placed at a different temperature and consisting of a duplicate set of culture flasks inoculated as follows.

As in this experiment it was possible to combine two separate experiments,one dealing with the effect of temperature,and the other with spore number,on association.A double series of data could be obtained from the same experiment.A more detailed account of the method of preparing the inoculum will be given later. However,it was found that in general the results agreed very well with those obtained from the preceeding experiment,where the fungi were grown on potato-dextrose agar instead of the liquid medium.

At 30-34°C. Penicillium expansum grew poorly,but the other

organism grew very well.Sporulation was decreased in each case. In mixed cultures it was found that when at 20-24°C. there was a tendency for the former organism to grow well and suppress the growth of the latter.even though the spore numbers were equal,at 30-34°C.the latter organism was able to grow more effectively in the mixed cultures,even though the concenctration of Penicillium spores may have been greater than in the first case when the limit for the growth of Sclerotinia fructicola was reached.It is plainly a case where the high temperature had decreased the physiological activities of the inhibiting organism,and had allowed the other to grow better at a temperature to which it was tolerant.

At 20-24°C.,both Penicillium expansum and Sclerotinia fructicola grew well.Sporulation of the latter fungus was considerably less than that obtained from the culture on potato-dextrose agar.In mixed cultures,there was a distinct tendency for the former organism to suppress the growth of the latter,unless the spore number of the latter was considerably in excess of the number of spores in the former.A more detailed account of this tendency to inhibit growth will be given below.

At 4-8°C.,there was practically no growth of Sclerotinia, while Penicillium grew very slowly.In mixed cultures Penicillium expansum grew well at all spore concentrations,due to its resistance to the low temperature,and also to the inability of Sclerotinia fructicola to grow under the same conditions.

The effect of temperature on the association of the above fungi is shown in chart form in Figure XII.

In the case of the onion-rotting fungi,Botrytis allii and Botrytis byssoidea,there was no suppression at any spore concentration.

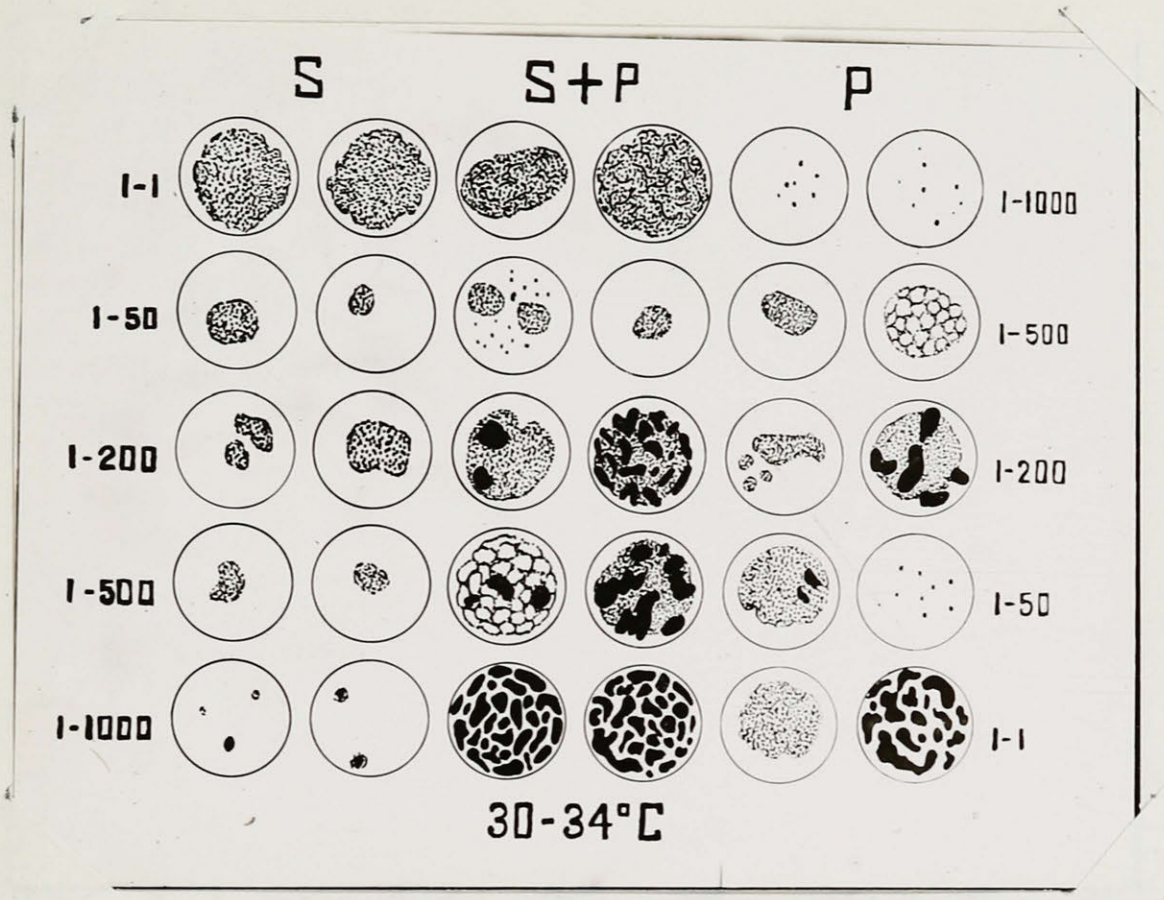


FIGURE XII,(a).Growth of Penicillium expansum (P)and Sclerotinia fructicola(S) in pure culture and in association at 30-34°C.Heavy shading shows colonies of P.expansum,light shading colonies of S.fructicola,except in cultures where P.expansum alone is present,where it indicates submerged colonies of this fungus.The figures at the sides indicate the dilution of the standard spore suspension containing 10,000 spores per cubic centimeter.

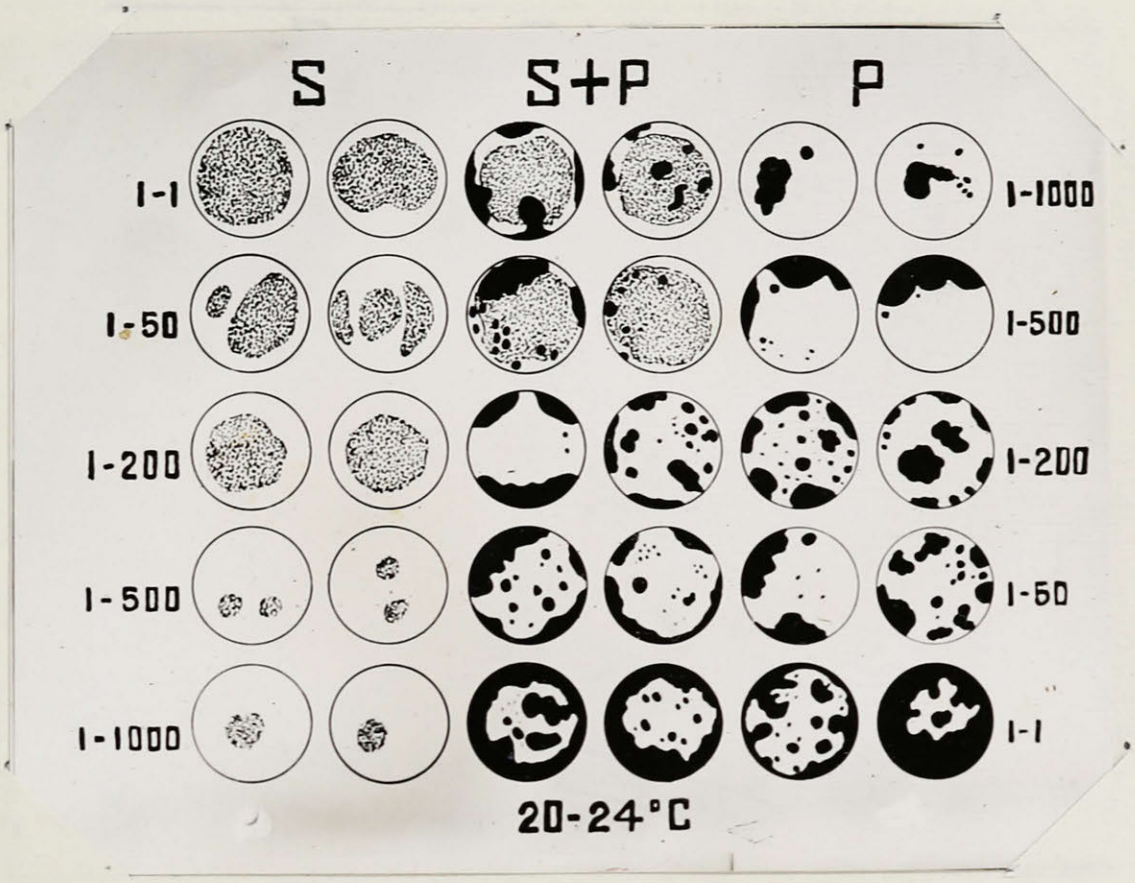


FIGURE XII,(b) Growth of Penicillium expansum (P), and Sclerotinia fructicola (S) in pure culture and in association, at 20-24°C. Heavy shading shows colonies of P. expansum while light shading shows colonies of S. fructicola. Figures on sides indicate spore concentration of each fungus. Standard spore suspension contained 10,000 spores per cubic centimeter. The figures indicate the amount of dilution of this standard suspension.

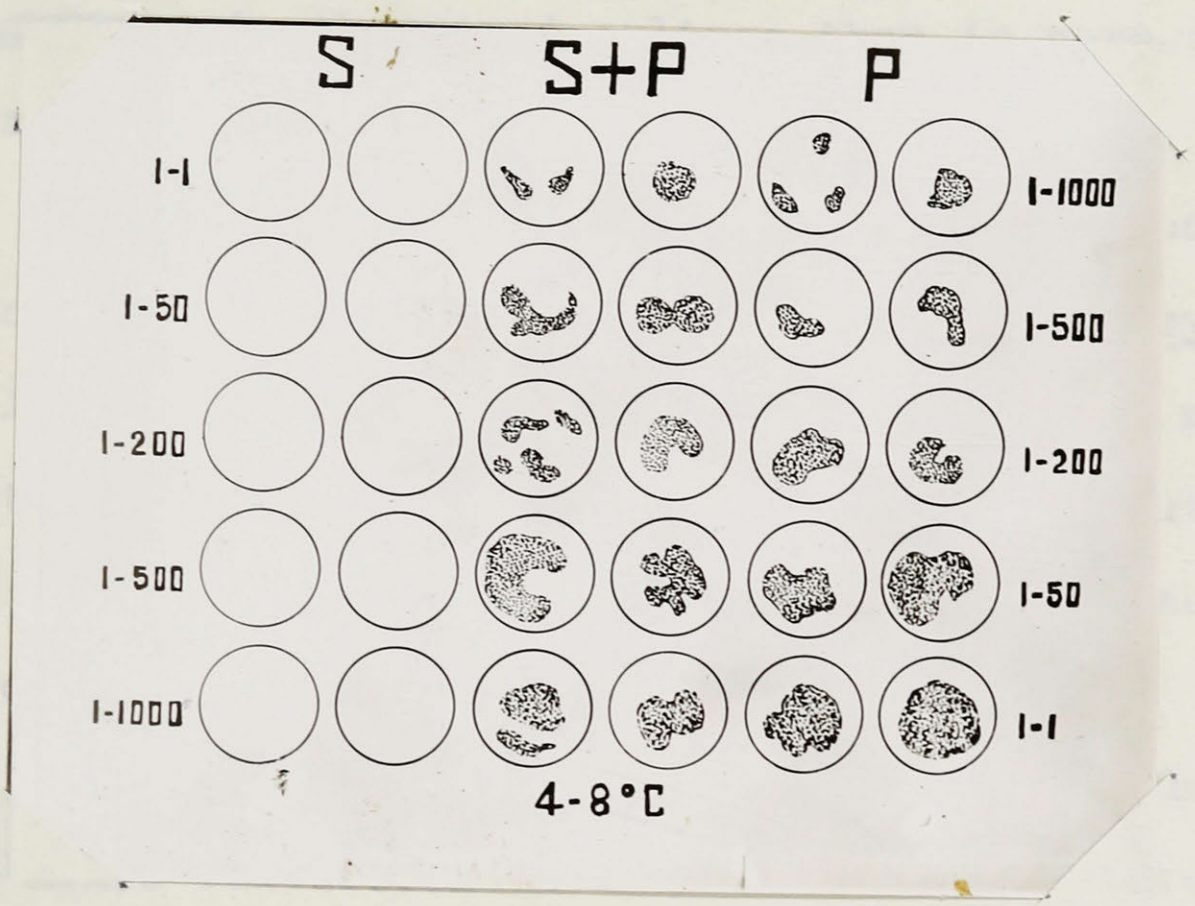


FIGURE XII.(c)Growth of Penicillium expansum (P),and Sclerotinia fructicola (S) in pure cultures and in association at 4-8°C.Heavy shading shows colonies of P.expansum .No growth of S.fructicola.Figures on sides indicate spore concentration of each fungus.

At 30-34°C. growth of both fungi was abundant. Botrytis allii predominated in the mixed culture, but the other was not completely suppressed. The predominance of the former organism appeared to be due chiefly to the high temperature, as was observed when the same fungi were grown on potato-dextrose agar. There was less growth of both organisms in the mixed culture than in pure culture, but the total growth in both cases was about the same.

At 20-24°C. growth of both organisms was good. Sporulation was abundant in Botrytis allii, but slight in Botrytis byssoidea. Some sclerotia were formed on the surface of the medium. In association growth was abundant for both fungi, but not as much as when the fungi were grown in pure culture. No suppression of either organism occurred.

At 4-8°C. growth was much reduced in both fungi, either in pure or mixed culture. In the mixed cultures, however, Botrytis byssoidea predominated in all the flasks.

In the case of the tomato-rotting fungi, Botrytis sp. and Cladosporium fulvum, it was found that the former organism grew well at low temperatures, while the latter grew best at high temperatures. The results concerning the growth at each temperature are given below.

At 30-34°C. the growth of both organisms was somewhat less than that produced at 20-24°C. Botrytis sp. appeared to be more inhibited at this high temperature than Cladosporium fulvum, which sporulated freely and grew well. The former organism did not sporulate much, but more so than at any other temperature used. In combination the former organism was completely suppressed

where the spore concentration was high,and the suppression was more complete than at 20-24°C.The cultures at this temperature are shown in graphic form in Figure XIII (a).

At 20-24°C.both fungi grew very well.Abundant mycelial growth and spore production was found in all cultures,of Cladosporium fulvum,while abundant myeelial growth,but almost no spores were found in the case of Botrytis sp.Suppression of the latter organism only occurred where the spore concentration of the former organism was at the maximum.

At 4-8°C.Botrytis sp.grew moderately well,but no spores were produced.Cladosporium fulvum did not grow very well,due to the effect of low temperature.In mixed cultures the former organism predominated,but the latter fungus appeared only in the cultures containing the maximum number of spores in the inoculum.The cultures are shown in graphic form in Figure XIII(c).

From the above results the writer concluded that the temperature at which the above pathogenic fungi grew,was a dominant factor which determined the amount of growth of each fungus,for each definite temperature,and also determined which fungus appeared to grow best when the fungi were grown in combination.As was found in later experiments,the temperature variation produced the same changes in the development of the above fungi in their host as in artificial culture,and the temperature at which the host plants were stored largely determined which fungus was responsible for the rot.

The Influence of the Amount of Inoculum on Association.

During preliminary investigations on association between Sclerotinia fructicola and Penicillium expansum,it was observed

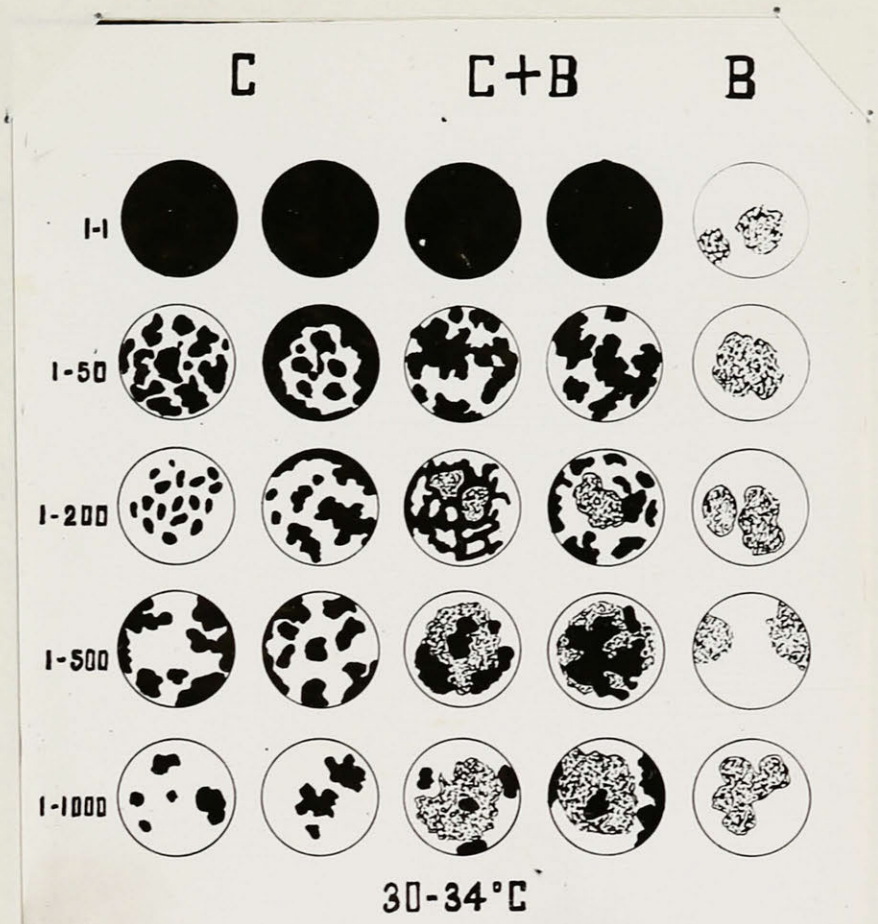


FIGURE XIII (a). Growth of Botrytis sp. and Cladosporium fulvum (C) in pure culture and in association at 30-34°C. Heavy shading represents growth of Cladosporium fulvum, light shading that of Botrytis sp. The figures on the side indicate spore concentration of the former organism (C). The amount of Botrytis inoculum was the same throughout. The standard spore suspension was used. The figures indicate the amount of dilution of this suspension.

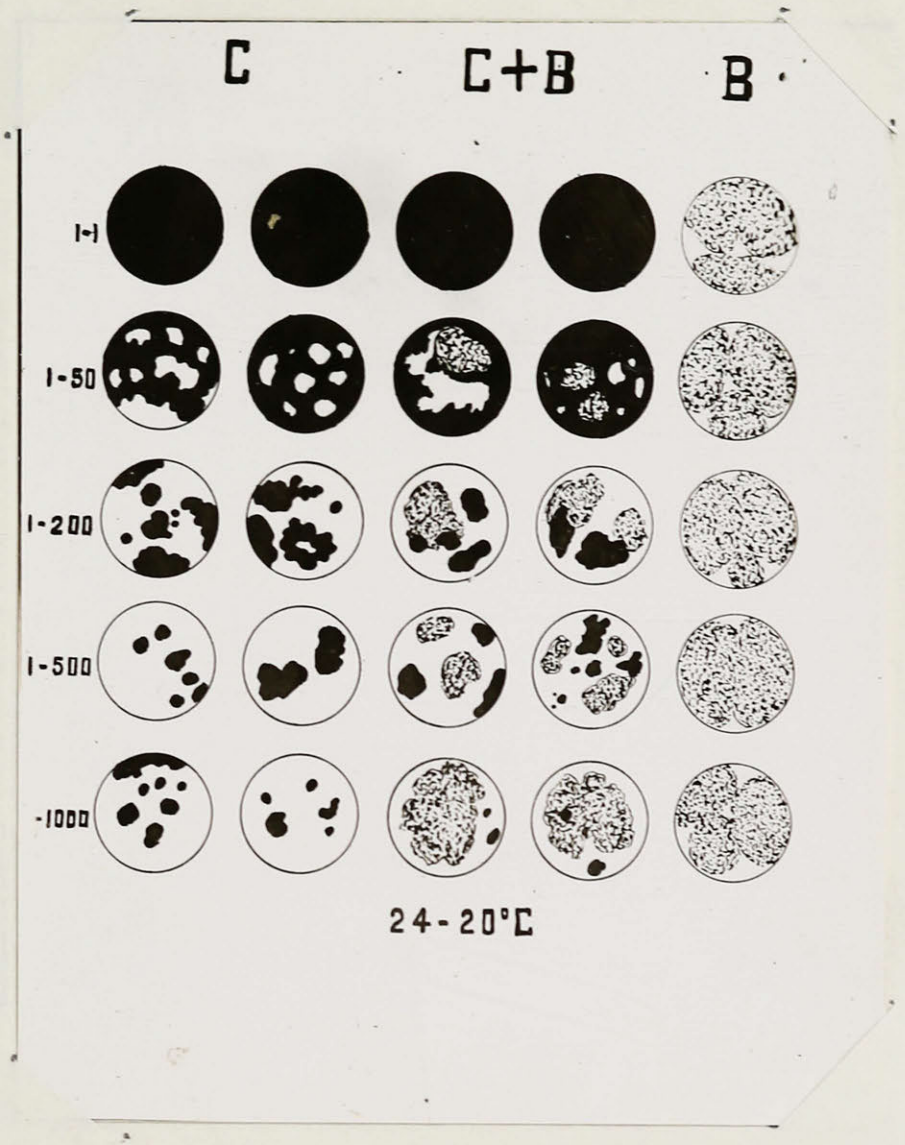


FIGURE XIII(b).Growth of Botrytis sp.and Cladosporium fulvum, in pure cultures and in association,at 20-24oC.Heavy shading represents growth of Cladosporium fulvum (C),light shading that of Botrytis sp.(B).The figures at the side indicate the spore concentration of the former (C) organism.The amount of Botrytis inoculum was the same in each case.

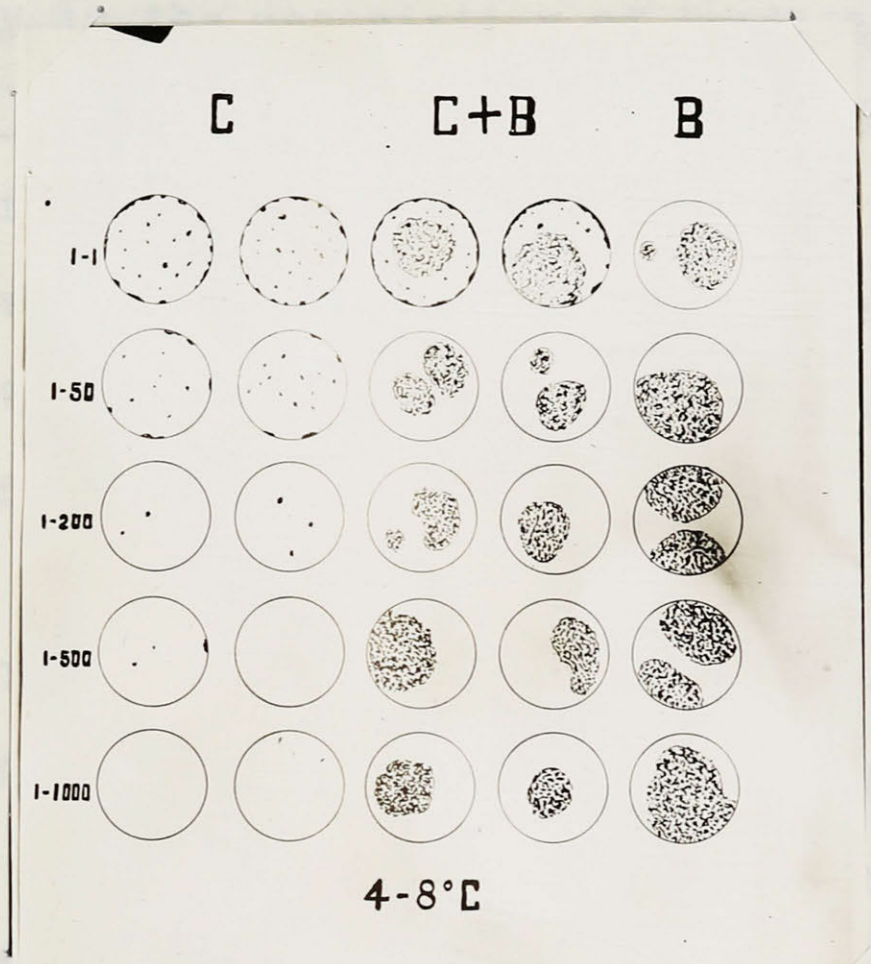


FIGURE XIII(c).Growth of Botrytis sp. and Cladosporium fulvum, in pure culture and in association,at 4-8°C.Heavy shading indicates growth of Cladosporium fulvum (C),light shading growth of Botrytis sp.(B).The figures at the side indicate the concentration of spores of the former (C) organism.The amount of the Botrytis inoculum was the same in each case.

that when equal numbers of spores of some fungi were used to inoculate mixed cultures of these fungi, there was a tendency for some of these fungi to suppress the others. This was noticed particularly in the association of Penicillium expansum with Sclerotinia fructicola. The writer, having in mind the possible influence of spore number on the presence or absence of a fungus in a rot, investigated the problem, and after dealing with several fungi, came to the conclusion that the amount of inoculum had a direct influence on presence or absence of an organism in culture.

The cultures of the various fungi were all grown on Richard's modified nutrient medium adjusted to pH 6.5 and placed in fifty cubic centimeter lots in small Erlenmeyer flasks. The media were then autoclaved and after cooling inoculations were made as follows.

A heavy suspension of spores, where they were formed, of the different fungi was prepared. These spores were suspended in sterile tap-water, and diluted according to need. The standard suspension of spores contained about 10,000 spores per cubic centimeter of the suspension, and the dilutions made from this standard suspension contained approximately 200, 50, 20, and 10 spores per cubic centimeter. All five spore concentrations were used in making the inoculations.

When inoculation was performed, flasks of media were inoculated with each suspension to determine the amount of growth produced from the varying number of spores in the inoculum. One cubic centimeter of suspension was used in making the inoculation. In the case of mixed cultures only half the amount of each suspension was used, so that the total number of spores in all cases would

approximately the same. In making the inoculations in the mixed cultures, the highest concentration of spores of one organism was mixed with the lowest concentration of spores of the second fungus, and the further inoculations had gradually decreasing numbers of spores of the first fungus, while the number of the second was increasing, until finally the least concentrated spore suspension of the first was mixed with the most concentrated suspension of the second fungus.

After inoculations were complete, the three series of cultures were placed at three different temperatures; incubator temperature, 30-34°C.; room temperature, 20-24°C.; and cold storage temperature, 4-8°C. The cultures were kept at these temperatures for ten days, after which the amount of growth was charted as shown in Figures XII, XIII, and XIV. The observations made were as follows.

At 30-34°C. abundant growth was found in all cultures, except in the pure cultures where spore concentration was low. Evidently the high temperature did not allow the fungi to form large colonies, as shown by the mycelial development in these latter cultures. In all the other cultures the surface of the medium was largely occuppied by the colonies, which though small, were numerous. In the apple-rotting fungi, Penicillium expansum and Sclerotinia fructicola, sporulation was noticeably lessened in the latter, and only slightly reduced in the former. It was found that at this temperature the latter fungus was suppressed by the former unless the spore concentration was greater than 1-500, or 20 spores per cubic centimeter of the inoculum.

At 20-24°C. growth was better in all cultures than above. Growth and sporulation was best where the fungi were grown in pure culture. The colonies were larger than above. However, in the

mixed cultures of the above apple-rotting fungi,complete suppression of Sclerotinia fructicola occurred unless the spore concentration exceeded 1-200 or 50 spores per cubic centimeter of the inoculum.Where the spore concentration of the above fungus exceeded the above limit,abundant growth occurred;but it was foundthat Penicillium expansum was also able to grow well in these cultures,even though the Sclerotinia predominated.The results are shown in diagrammatic form in Figure XII (b).

At 4-8°C.Sclerotinia fructicola did not grow very well,even after the ten-day period of growth used in these experiments,so that the effect of the spore concentration could not be judged as it could be at the higher temperatures.However,in the mixed cultures,after the above period had elapsed,it was seen that Penicillium expansum had caused almost complete suppression of the other fungus at all spore concentrations.Results are shown in Figure XII (c).

In order to determine more accurately the effect of spore concentration on the growth of the above apple-rotting fungi, a more elaborate experiment was prepared.The apparatus used was similar to the one above,but the gradation in spore concentrations was more gradual.The range of spore numbers varied from 10,000 to 10,in the following series,10,000;1,000;500,250,125, 62,33,20,and 10.In the mixed cultures the combinations of the different spore suspensions of the two fungi were made as above, but the temperature used was 20-24°C. only.The cultures were grown at this temperature for ten days,after which the growth of the fungus colonies was charted as shown in FigureXIV.

The results obtained were almost similar to the results shown in Figure XII (b).Where the spores of Penicillium expansum

exceeded the number of spores of Sclerotinia fructicola complete suppression of the latter organism occurred.Where the number of spores of the latter organism was greater than that of the former, then the latter fungus was able to appear in the mixed culture.

The Penicillium appeared likewise and grew almost as well as in pure culture,though at a slower pace.

In the onion-rotting fungi,Botrytis allii and Botrytis byssoidea,it was impossible to obtain spores of the latter fungus in sufficient quantities to be used for inoculation purposes in cultures, such as in the case of the apple-rotting fungi mentioned above.However,in previous and subsequent experiments, where various amounts of inoculum was used,it had been observed that the two fungi exhibited a distinct tolerance for each other when in mixed cultures,even though sometimes the amount of inoculum of one exceeded the other considerably.There was never any definite suppression of either organism in mixed culture, unless the temperature was distinctly unfavourable to the growth of one of them.

With two tomato-rotting fungi,Cladosporium fulvum and Botrytis sp.somewhat similar results were obtained as in the case of the apple-rotting fungi.An abundance of spores was formed by the former organism,so no difficulty was encountered in making the various spore suspensions.In the latter organism the number of spores formed was considerably less,so instead of using the spores,thereby increasing the need for a larger number of pure cultures ofthe organism and likewise increasing the danger of contamination when the suspensions were made,the writer

decided to use the mycelium of Botrytis sp. in making the inoculations, using equal quantities of mycelium for each flask. Therefore, in Cladosporium only, the number of spores in the inoculum was varied.

Three identical series of cultures of the above fungi were prepared. They were placed at the three different temperatures used in the study of temperature relationships of the apple-rotting fungi. After ten days the cultures were examined, and the growth of the colonies charted. The results are shown in chart form in Figure XIII.

At 30-34°C. both fungi grew well, but sporulation was reduced. Cladosporium fulvum formed fewer spores than at lower temperatures, but Botrytis sp. produced more spores in some cultures, and less in others. As shown in Figure XIII (a), the growth of the latter fungus was suppressed altogether in the mixed cultures where the two highest concentrations of the associated fungus were found. In other mixed cultures both organisms were able to grow well.

At 20-24°C. abundant growth of both fungi occurred in pure culture. Both grew better than at the higher temperature, though spore production was greater in Cladosporium and slightly less in Botrytis. In mixed cultures the growth of Botrytis sp. was found suppressed by the highest spore concentration of Cladosporium fulvum only. The growth of the latter fungus was somewhat greater in proportion as the spore number of the associated organism was decreased. Therefore, at 20-24°C. the growth of Botrytis sp. appeared to be favoured more than at the higher temperature, as it was better able to overcome the inhibition caused by the greater amount of inoculum of the other fungus.

At 4-8°C. the growth of Cladosporium fulvum was very much reduced,and only a few spores were produced.Botrytis sp.grew moderately well,and in mixed cultures no inhibition of growth or suppression was suffered by it,no matter what the spore concentration of the other fungus happened to be.However,at this temperature,the Botrytis was able to show a detrimental influence on the growth of Cladosporium,for outside of the greatest spore concentration of the latter fungus,in all other mixed cultures complete suppression of the latter organism was found.

Therefore,from the above experiments,it has been concluded that not only the spore concentration but also the temperature may determine the presence or absence of an organism in mixed cultures.All fungi do not possess the power to grow at similar ranges of temperature,for one will grow well at a temperature which is inhibitive to the growth of another .A fungus which may be completely suppressed at a certain temperature may be able to appear if the temperature is favourable to its growth, but not to the growth of the fungus which suppresses it.

Similarly,the amount of inoculum has been shown to influence the appearance of a fungus in a mixed culture.It has been shown that in a case where one fungus is strongly inhibited by another fungus,as with Sclerotinia fructicola and Penicillium expansum, the former organism is suppressed completely only when the spore number of the latter exceeded the number of the former,or where the spore numbers are equal.At other spore concentrations the Sclerotinia is able to grow well for a time,but eventually is slowed down in its growth by the growth of the Penicillium.In the tomato-rotting fungi,Cladosporium fulvum and Botrytis sp.,

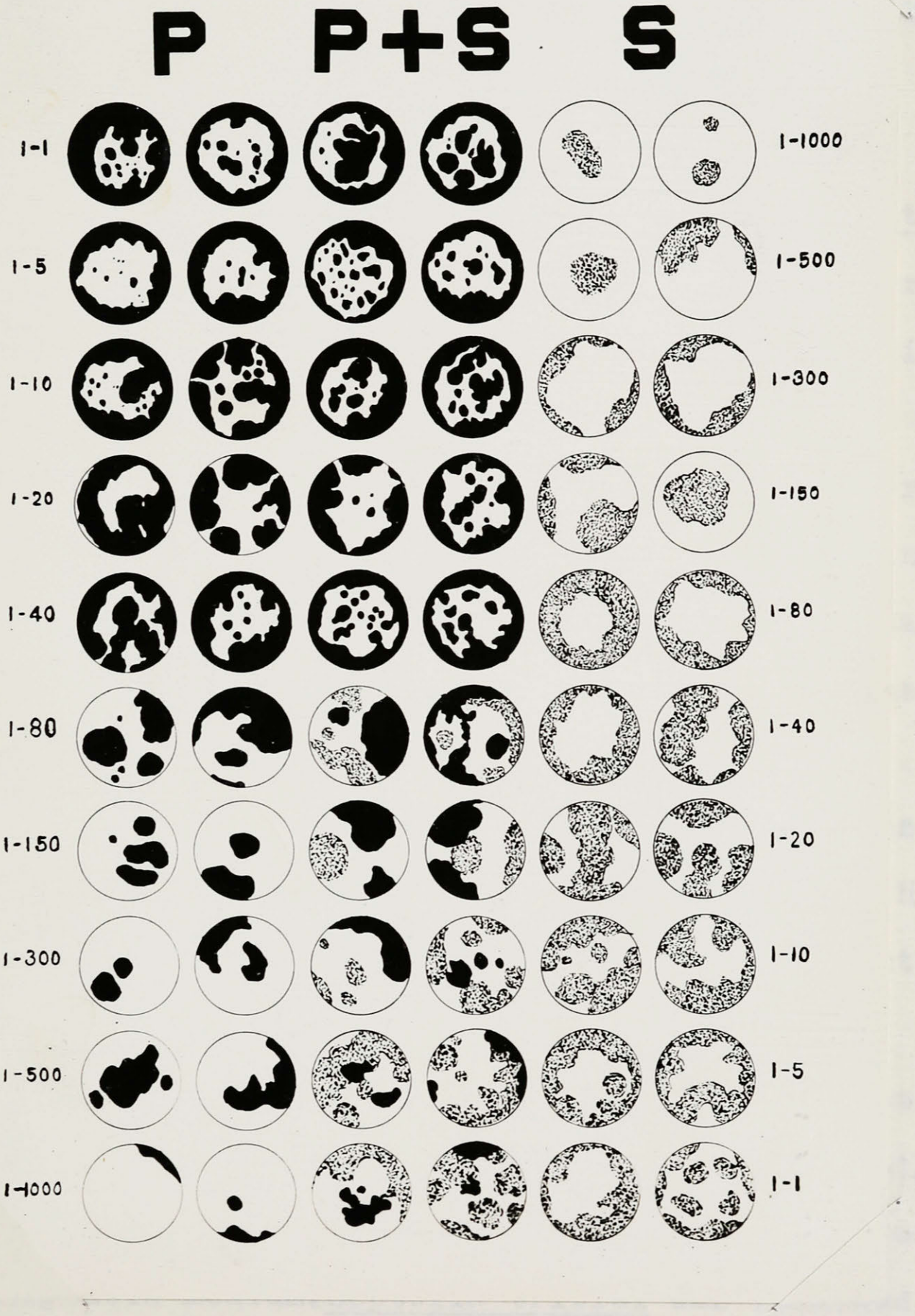


FIGURE XIV. The effect of spore concentration of Penicillium expansum (P) and Sclerotinia fructicola (S), on growth and association. The figures at the sides indicate dilution of original suspension, which contained 10,000 spores per c.c.

the former also exhibits a tendency to suppress the latter,but can do so only when the amount of inoculum appears to be very much in excess of that of the associated organism.In the case of onion-rotting fungi,neither organism appears to be able to inhibit the growth of the other,although more conclusive results can be obtained only by more detailed experiments.

The Effect of Hydrogen-ion Concentration on Germination and Growth.

A survey of the literature on the subject has revealed that a considerable amount of work has been done by previous workers on the relation between the H-ion concentration and spore germination,fungus growth,and plant metabolism.The results of these workers have agreed in some respects but differed in others.This difference appears to be largely due to the fact that there was little uniformity in the methods,media,and organisms employed by these previous workers.

Boyle (8) found that a certain Fusarium species caused a decrease in acidity when grown on Richard's medium.He found that in general,the amount of change in the H-ion concentration was relative to the amount of mycelium produced.

Weber (85) found that Leptosphaera avenaria was retarded in growth when grown on a medium whose H-ion concentration was above pH 7.0 and below pH 3.8. Therefore,the best growth occurred in a slightly acid medium.Septoria tritici was retarded below pH 3.8 and above pH 8.0.

Johnson (46) found that temperature,either below or above room temperature,tended to shift the limiting pH value towards neutrality when the cultutes were grown on Czapek's nutrient

solution.He found that above room temperature it was very difficult to get exactly the same end point for pH relationships for a number of cultures with the same inoculum and the same nutrient solution.In some cases this end point was sharp,in others indefinite.In different media the total acidity due to a certain acid may vary greatly,but the pH values for the critical point of growth are usually the same.This shows that it is the Hydrogen-ion concentration that is a significant factor in such cases.

Johnson also observed that the molds he worked with showed two widely separated optima with respect to the H-ion concentration of the Czapek's solution,but he was not able to determine this very accurately,since the poorly buffered solution he used changed in pH very rapidly.The optima were shown to be wide zones and somewhat on the acid side.All the molds studied changed the reaction of the media in which they grew,the acid solutions becoming less acid in most cases,and the alkaline ones less alkaline.This has been found in many organisms where the original reaction is somewhat removed from the neutral point,but the molds apparently will survive more acid and more alkali than other organisms,and so the magnitude of the changes produced were greater than would have been found for other organisms.The change is not necessarily towards an optimum.

Scott (75) found that acid solutions became less acid with Fusarium lycopersici and alkaline solutions became less alkaline. He suggests the changes may be due to secretions of the fungus which reduce acidity,and where acid material such as carbonic

acid and organic acids are present, the changes may be due to the selective or unequal absorbtion of ions. Sporulation was limited to the more acid media. Growth occurred from pH 2.4 to pH 9.4 .

Webb and Fellows (84) grew Ophiobolus graminis on potato-dextrose decoction and on potato-dextrose agar. They found that the range of pH values in the former extended from pH 3.6 to pH 7.7 , while in the latter the range was from pH 3.2 to pH 10.3 . Two maxima were observed, one at pH 5.8 and the other at pH 9.6 . All cultures were grown at 24°C.

White (89) tested twenty-four strains of Fusarium lycopersici. He found that the limits of pH values were from 3.1 to 10.4 , this depending on the strain of fungus used. Two maximum points were observed, one at pH 4.5-5.5 , while the second was about pH 7.0, this also depending on the strain used.

Hopkins (41) found that Gibberella saubinetti would tolerate a wide range of acidity, from pH 2.5 to pH 8.4 and showed that there was a minimum in growth at pH 6.0 . This gave two maxima in the growth curve. This same phenomenon was found in three series with striking similarity.

Sideris (76) made an extensive study of the pH changes induced by a species of Fusarium which he grew. When this fungus was grown on different media and different pH values, he found that in some media there was a tendency to make some pH values greater and others less, so that there would be a constant final approximate reaction. To this final pH value the term "isometabolic point" has been assigned. Sideris found this to be variable with different media. In dextrose it was pH 3.8; in amygdalin, 5.0; in peptone, 8 plus; in potato-starch solutions, 5.2; in pectin, 6.4; in beef broth, 7.4.

When the culture was grown with the original reaction near the isometabolic point there was very little change in the reaction. Sideris concludes that,(1) the different organisms may or may not produce the same effect on the same or different media,(2) the tendency in the majority of cases is to increase the pH value of the culture medium containing available carbohydrates,and decreasing it in those media containing available proteins,and (3) the reactions produced in certain vegetable and meat decoctions depend to a considerable extent upon the initial H-ion concentration and the ratio of the available carbohydrate and protein in the particular decoction.

Robbins (67) and his co-workers have done work with what they term the "isoelectric point" of plant tissues.They used one of the standard methods for the determination of the isoelectric points of proteins.They placed pieces of plant tissue in a series of buffer solutions and then determined the change in pH undergone by the buffer.The pH value of the buffer solution that remained unchanged after a period of contact was called the isoelectric point.For Fusarium lycopersici this was found to be 5.4 . Their explanation is that the fungus tissue behaves like an amphoteric substance which can neutralize both acid and base, that is,assimilate both anions and cations,depending upon which is in excess.Hence the tissue seems to behave in a manner analogous to a protein.They offer this as an important point in the explanations of the shift of pH values in cultures of fungi.

Youden and Denny (97) believe the method used by Robbins to be unsatisfactory as they can get the same shifts in pH values from watery extracts of plant tissues which contain no colloids whatever.

Webb (83) used Botrytis cinerea, Aspegillus niger, Penicillium cyclopium, P.italicum, Puccinia graminis, Lenzites speciaria, Fusarium sp. and Colletotrichum gossypii in his studies. Germination was found to be a process strikingly supported by conditions of active acidity. Usually relatively low ~~percentage~~ germination percentages were obtained with alkaline media. With Penicillium and Fusarium a second maximum occurred near the neutral zone. He found that while germination may be favoured by high acidity, growthh may not, for the germ-tubes of Botrytis cinerea were found to disintegrate at pH 2.1 .

Matsumato (56) found that Rhizoctonia collected from widely separated geographical stations always produced acid, and the acid and growth were proportional.

The writer observed somewhat similar results in his experiments on the effect of the H-ion concentration on germination and growth. The particular phases studied included, (1) the effect of the H-ion concentration on spore germination, (2) the effect on growth and association, (3) the study of the "isometabolic point" of several rot-producing fungi, and (4) the effect of changing the used media by autoclaving, neutralizing, etc.

In the study of the effect of the H-ion concentration on spore germination, the following procedure was followed. Clean Van Tieghem cells were attached to clean, glass slides with melted paraffin. Glass cover-slips, sufficiently large to cover the cell, were cleaned and flamed. Then a small droplet of the spore suspension used in this experiment, and containing about 8,000 spores per cubic centimeter, was placed on the center of the cover-slip, and another drop, of sterile distilled water adjusted to the desired pH value, was added to the droplet of spore sus-

pension.A drop of the distilled water was also placed in the bottom of the cell,in order to compensate for vapour pressure and evaporation from the droplet containing the spores.The cover-slips were then inverted over the cells and sealed to the edges of the cell with melted vaseline.The range of pH values used here was from pH 1.5 to pH 7.0.The inoculations were all made in triplicate.After the cells were ready,the entire series was placed under bell-jars.The hanging drops were then examined at eight-hour intervals for two days,to mark the changes in germination.

In the case of fungi which did not produce enough spores to use in making a large quantity of a uniform suspension,as in the case of Botrytis byssoidea,small amounts of mycelium were placed in the drops of water hanging from the cover-slips.In this case the effect of the H-ion concentration could not be judged by spore germination counts to determine the amount of germination, but the effect could be determined by measuring the growth of the mycelium from the inoculum.This was easily observed,for the growing mycelium radiated away from the inoculum.Where spores were available,the amount of germination could be determined,For making these counts of germinated spores,the average of five fields,using the high power objective of a microscope,was taken as the index of the germination for a particular time and the particular pH value of the medium in which the spores were germinating.The data obtained are given in percent,where spores were available,or by means of a symbol where no spores were used.The symbols used to show the amount of growth are as follows;slight growth (+),fair growth (++),Good growth (+++),medium growth(+++),

very good growth (++++),no growth (0).By following the above procedure the time of germination as well as the amount could be determined.All the cells were kept at room temperature,20-24°C.

The first experiment performed was on the influence of the H-ion concentration on spore germination in the two fungi, Penicillium expansum and Sclerotinia fructicola.Two media were used in this case,one being sterile distilled water,and the other Richard's modified nutrient solution,both adjusted to various pH values ranging from pH 1.5 to pH 6.5 .The results of the experiment are shown in Table VI.

From data given in Table VI it is seen that there was a lesser percentage of spore germination in the distilled water than in Richard's modified solution.There was a distinct effect of acid on the germination in the hanging drops,for the count of the germinated spores revealed the fact that acidity not only can decrease the amount of germination but can also retard it when the H-ion concentration is high enough.Penicillium expansum was better able to germinate with high acidity than Sclerotinia fructicola,in respect to both the amount of germination and the time.Germination was best at pH 4.2 to 5.3 in the former fungus,but slightly higher for the latter.

A similar experiment was performed using the two fungi, Botrytis allii and Botrytis byssoidea.The latter fungus did not produce enough spores which could be used in making an adequate suspension to be used in making the inoculations,so small portions of the mycelium had to be used instead.Therefore,in the latter fungus the amount of growth is given by the use of the previously described symbols,but in B.allii the germination is given in percentage.

TABLE VI. EFFECT OF H-ION CONCENTRATION ON SPORE GERMINATION OF SOIL APPLE-ROTTING FUNGI.

pH of media	Percentage of spore germination at eight-hour intervals.							
	8 hours		16 hours		24 hours		32 hours	
	dist. water	Rich. med.	dist. water	Rich. med.	dist. water	Rich. med.	dist. water	Rich. med.
Penicillium expansum								
1.5	0	0	0	0	0	0	0	0
2.0	0	0	6	0	16	3	27	10
2.5	8	0	27	0	29	8	33	15
3.3	0	30	0	38	6	43	17	47
4.2	0	42	0	52	16	56	25	58
4.9	0	25	0	49	0	58	8	61
5.3	0	34	9	63	28	69	33	73
5.9	0	283	0	50	0	63	17	65
6.5	0	24	0	42	0	48	38	55
Sclerotinia fructicola								
1.5	0	0	0	0	0	0	0	0
2.0	0	0	0	0	0	0	0	0
2.5	0	0	0	6	0	9	3	15
3.3	0	6	14	32	19	45	27	49
4.0	0	9	17	45	34	53	48	58
4.9	7	21	29	31	44	57	55	63
5.3	20	34	32	47	51	59	62	62
5.9	23	28	31	36	42	49	47	56
6.5	18	23	26	38	42	47	45	67

TABLE VII. EFFECT OF H-ION CONCENTRATION ON SPORE GERMINATION OR GROWTH OF SOME ONION-ROTTING FUNGI.

Initial pH of medium	B.allii Percent spores germinated at 8-hour intervals				B.byssoidea Growth of mycelium at 8-hour intervals			
	8 hrs.	16 hrs.	24 hrs.	32 hrs.	8 hrs.	16 hrs.	24 hrs.	32 hrs.
1.5	0	0	0	0	0	0	0	0
2.0	0	0	18	23	0	0	+	+
2.5	14	27	48	63	0	+	++	+++
3.0	34	44	48	62	+	++	++++	++++
3.5	33	41	49	53	++	+++	++++	++++
4.0	29	34	50	53	++	++++	++++	++++
4.5	47	58	61	67	++	++++	+++++	+++++
5.0	35	51	59	64	++	+++	++++	+++++
5.5	42	49	62	73	+	+++	++++	+++++
6.0	38	45	52	59	++	+++	+++++	+++++
6.5	35	47	58	67	++	+++	++++	++++
7.0	37	52	66	69	++	+++	++++	+++++

Conversely, the spores of S.fructicola were used to inoculate the flasks containing the medium following the growth of P.expansum. With Botrytis sp. and Cladosporium fulvum the above cross inoculation was also employed.

The H-ion concentration was determined for each flask before and after adding the staled media. It was found that the addition of the staled medium did not appreciably change the pH value.

After inoculations were completed, the cultures were incubated at 20-24°C. for ten days. Observations were made daily, but the final data dealing with growth and H-ion concentrations were not taken until after the end of the culture period. The results are shown in Table VIII.

The results of the above experiment show that in every case there was a decrease in the amount of growth, when the flasks containing the cultures prepared by the above method were compared with the checks. There was a distinct change in the H-ion concentration in all flasks, the change being towards the acid side. In all cases, except Botrytis sp., the change was considerable. It was noted that the small quantities of the staled medium added to the different flasks were in some fungi able to cause a considerable inhibition of growth. It appears, then, that some other substance besides acid is able to limit the growth of some fungi, for the increase in acidity due to the addition of the staled medium was only slight, but the inhibition of growth was considerable. It appears that this toxic agent is a powerful one, for the inhibition of growth could not have been due to neither acidity nor to lack of food materials in the medium.

As small amounts of used media appear to have such a marked effect on the growth of some fungi, the writer decided to test

TABLE VIII. EFFECT OF SMALL AMOUNTS OF USED MEDIA ON GROWTH OF SOME FUNGI

Fungus	med.used	pH of medium	pH of used med.#	pH after 10 days	amount of growth
1) P.expansum	Rich.med.	6.1(#)		3.1	++++
2) S.fructicola	" "	6.1(#)		3.5	++++
3) Botrytis sp.	" "	6.1(#)		3.7	++++
4) C.fulvum	" "	6.2(#)		4.2	++++
P.expansum	Rich.med. plus med. from (2)	6.0	3.5	3.05	++++
S.fructicola	Rich.med. plus med. from (1)	6.0	3.1	3.15	++
Botrytis sp.	Rich.med. plus med. from (4)	6.0	4.2	5.3	++++
C.fulvum	Rich.med. plus med. from (3)	6.1	3.7	3.1	++

#. These figures are derived from H-ion determinations of media on which the four fungi have grown. Into the cultures designated with the symbol (#) no staled medium have been added.

the effect of the above staled media on spore germination in certain fungi.As the media following the growth of Penicillium expansum and Botrytis sp.appeared to be most powerful in causing the reduction of growth of the other fungi used in the above experiment,they were used in the study of spore germination.The two fungi named above were grown on Richard's modified solution for ten days,when the mycelial mats completely covered the surface of the medium in the flasks.After this period,the staled media were filtered through sterile Berkefeld filters as before and used in making the hanging drops on cover-slips in Van Tieghem cells.Three series of these cells were prepared,one series containing fresh Richard's modified solution,and the other two the staled media following the growth of Penicillium and Botrytis. The four organisms used in the experiment shown in Table VIII were used in making the inoculations.The hanging-drop cultures were kept under bell-jars for thirty-two hours,examinations of the cultures being made at eight-hour intervals.Table IX shows the results obtained.

It is noted from the above table that both the staled media had an inhibitive influence upon spore germination.The staled medium following the growth of Penicillium appeared to be most powerful,although germination of the spores of the same fungus occurred in it.The medium following the growth of Botrytis was less powerful,for some germination occurred in all cultures where it was used,although in a few cases the germination of spores was retarded for a time.It appears,from an examination of the data in Table IX,that the staled medium of either fungus used in the experiment was best suited for the germination of its spores,but in each case the germination was less than in the unused medium.

TABLE IX. THE EFFECT OF STALED MEDIA ON GERMINATION OF THE SPORES OF SOME FUNGI. GROWN AT 20-24°C.

Fungus	Unused Richard's mod.medium				Botrytis-staled medium				Penicillium-staled medium			
	Time in hours											
	8	16	24	32	8	16	24	32	8	16	24	32
P.expansum	0	38	73	79	4	39	52	67	16	35	42	55
S.fructicola	85	88	95	95	18	22	34	42	0	0	0	0
Botrytis sp.	76	84	91	93	48	81	87	90	0	0	18	21
C.fulvum	0	43	94	95	0	12	21	34	0	0	0	4

It has been seen from the above experiments that the growth of fungi is reduced when these fungi are grown in staled media, and that this inhibiting factor is possibly some toxic substance other than acid. It is not probable that acidity is the factor in question, for, as learned from a previous experiment, the addition of a small amount of the staled medium caused only a negligible change in the H-ion concentration while there was a distinct reduction in growth of fungi cultured on the medium to which the staled medium had been added. However, to decide further if acidity was a factor in determining the amount of growth the writer performed the following experiment.

Pure cultures of Penicillium expansum were grown in 500 c.c. Erlenmeyer flasks containing 200 c.c. of Richard's modified solution. The cultures were grown at 20-24°C. for ten days, after which period the mycelial mats were removed, and the staled medium passed through a sterile Berkefeld filter (size N) into sterile flasks, and treated in the following manner. The staled medium was separated into four parts; one part of this being left for a check, while the others were used for cultural experiments. One part was autoclaved at 15 pounds for 15 minutes; another part was changed in H-ion concentration until the medium was equal in acidity to the unused medium. The third portion was left without being changed.

After the above staled media were prepared as described, 50 c.c. lots were poured into 150 c.c. Erlenmeyer flasks and inoculated with both Sclerotinia fructicola and Penicillium expansum. These fungi were grown on these media either in pure culture of in association. The same amount of inoculum was added to each flask, approximately 10,000 spores. The cultures were then

grown at 20-24°C. for ten days, after which time the growth in each flask was charted as shown in Figure XV.

The chart shows that the above fungi grew very well on unused Richard's modified solution, but not so on the other media. Penicillium expansum grew better than Sclerotinia in all the cultures, and when the two fungi were grown in association, the former totally suppressed the latter. Penicillium grew well on all the staled media, though less growth was obtained than on fresh medium. Growth of Penicillium was about equal on either the unchanged staled medium, or where the H-ion concentration had been changed to that of the fresh medium. On the autoclaved medium less growth occurred than on the other types of media used.

Sclerotinia fructicola grew moderately well on the fresh Richard's modified solution, but only slightly on any others. Of these, the autoclaved medium appeared to be most favourable to the growth of this fungus, while the unchanged used medium was distinctly very unfavourable to its growth.

There was very little change in the H-ion concentration of the media used in the experiment, except where fungi were growing. In all the cultures the media became more acid, though in varying degrees. In the unused medium the check changed from pH 6.5 to pH 6.3. Penicillium changed it to pH 3.1, while Sclerotinia changed it to pH 4.3. In the used but adjusted medium, the check changed from 6.5 to 6.4. The change wrought by Penicillium expansum brought it down to pH 3.4, and by Sclerotinia fructicola to 4.2. The used and autoclaved medium check did not change. P. expansum changed it from pH 3.0 to 2.4, while S. fructicola caused no change. In the unchanged used medium there was no change in the check, but P. expansum changed it from pH 3.2 to pH 2.6.

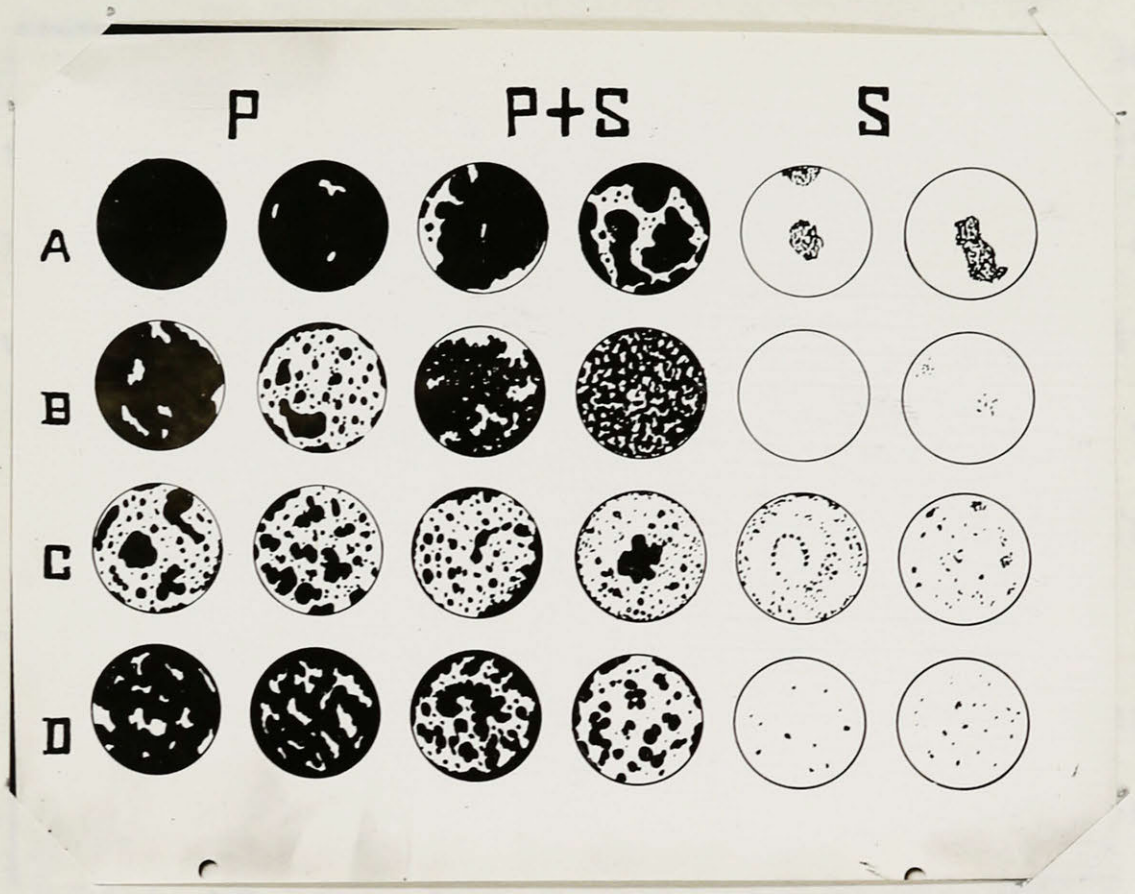


FIGURE XV. The effect of staled media on growth of Penicillium expansum (P), and Sclerotinia fructicola (S), grown in pure culture and in association. (A) The unchanged fresh Richard's modified nutrient medium. (B) The unchanged used medium. (C) The autoclaved used medium. (D) The used medium whose H-ion concentration has been adjusted to that of the fresh medium. Heavy shading shows growth of Penicillium; light shading the growth of Sclerotinia.

Sclerotinia produced no change whatsoever.

The conclusion that can be drawn from the above experiment seems to indicate that acidity is not the determining factor in the inhibition of fungus growth, although it appears to play a distinct part when the culture media are too acid, as will be shown in further experiments. There appears to be one or more toxic agents which cause this inhibition of growth, and this effect can be only partially removed by autoclaving. It is very probable that several factors combine to cause a decrease of fungus growth in the staled media.

After the above preliminary experiments were completed, an attempt was made to discover the effect of H-ion concentration on the growth of some rot-producing fungi. These fungi were grown on Richard's modified medium of varying H-ion concentration either in pure culture or associated in pairs, consisting of two organisms causing the rot of the same host. These fungi were: from the apple, Sclerotinia fructicola and Penicillium expansum; from onions, Botrytis allii and Botrytis byssoidea; from the tomato, Cladosporium fulvum and Botrytis sp.

The acid range used in the growth of these fungi varied from pH 1.5 to pH 7.0. The medium used was Richard'S modified solution adjusted to the various H-ion concentrations. 50 c.c. of the medium was placed in each culture flask of 150 c.c. capacity, and then inoculation was carried out by the addition of about 10,000 spores per flask, when the spores were available, or by the addition of small squares of growing mycelium on a potato-dextrose agar plate, if no spores were formed. The cultures were all made in duplicate. Some uninoculated medium served to check up on any change in the H-ion concentration of the medium itself.

After growth for ten days at 20-24°C. the flasks were examined to determine the characteristics of the cultures. The amount of growth of each fungus was charted, and the final H-ion concentration determined for each flask. The charts of the growths of the three pairs of fungi are shown in Figures XVI, XVII, and XVIII. The changes in H-ion concentrations are shown in Tables X, XI, and XII. The results of the above experiments can be summarized as follows.

When Penicillium expansum and Sclerotinia fructicola were grown in association, it was found that the former fungus was able to suppress completely all growth of the latter, no matter what the H-ion concentration proved to be. The former fungus grew at all pH values above and including pH 1.9, while the latter grew at pH 2.5 or above. The growth of both fungi was small and sporulation was negligible at these high acidities. P. expansum exhibited a double optimum of acidity, for best growth occurred at pH 3.1 and also near the neutral point. S. fructicola grew best in almost neutral media, the growth decreasing in proportion to the increasing of the acidity.

It was noticed that the isometabolic point in the case of P. expansum was between pH 3.5 and pH 4.0 . For S. fructicola this point was somewhat lower, centering at pH 2.0. These points are probably subject to a good amount of variation, for the figures obtained have not always been the same with consecutive experiments.

The data and charts referring to this experiment are shown in Figure XVI and Table X.

An experiment similar to the one above was performed with two onion-rotting fungi, Botrytis allii and Botrytis byssoidea.

TABLE X. EFFECT OF H-ION CONCENTRATION ON GROWTH OF APPLE-ROTTING FUNGI

Initial pH	Check	Penicillium expansum		P. expansum and S. fructicola		Sclerotinia fructicola	
		Final pH	Growth	Final pH	Growth	Final pH	Growth
1.5	1.5	1.5	0	1.5	0	1.5	0
1.9	1.9	1.9	+	2.4	+	1.7	0
2.5	2.5	2.5	++	2.5	++	2.5	+
3.1	3.1	4.2	++++	3.0	++++	1.9	++
3.5	3.5	3.1	+++	3.1	+++	1.9	+++
3.9	3.8	3.8	+++	3.1	+++	1.9	+++
4.5	4.4	4.0	+++	3.9	+++	1.95	++++
5.0	4.8	4.1	++++	3.2	++++	1.9	++++
6.2	6.0	4.1	++++	3.5	++++	3.15	++++
7.0	6.7	3.9	++++	3.6	++++	4.0	++++

The following symbols are used to describe the amount of growth

No growth.....	0	Good growth.....	++++
Slight growth..	+	Very Good growth	+++++
Fair growth....	++		
Medium growth..	+++		

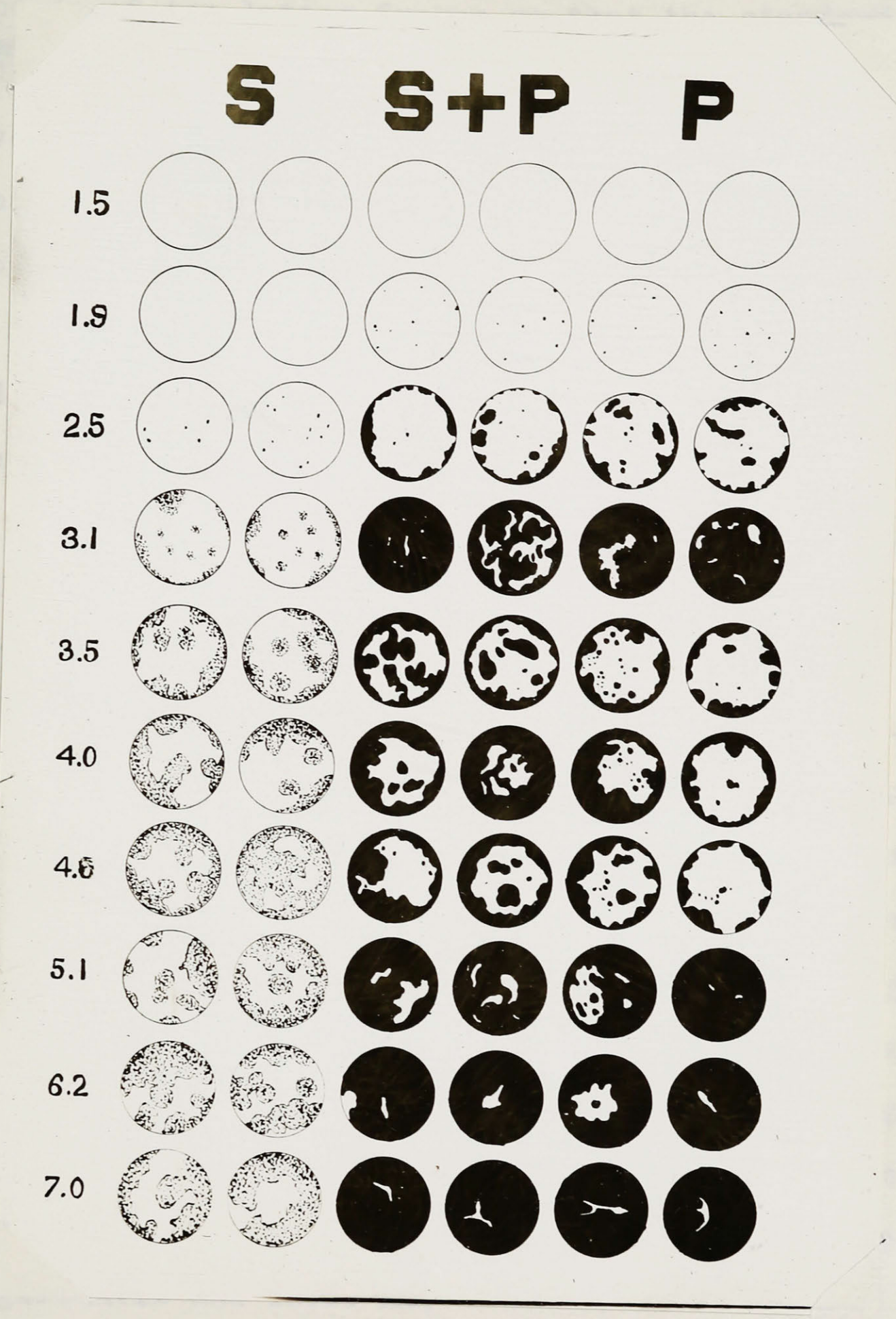


FIGURE XVI. The effect of Hydrogen-ion concentration on the growth of Penicillium expansum (P) and Sclerotinia fructicola (S), in pure culture and in association. Heavy shading shows growth of P. expansum, light shading that of S. fructicola. Cultures grown at 20°- 24°C.

Unfortunately it was impossible to obtain sufficient quantities of spores of the latter fungus so that the standard number (10,000) could be used for the inoculation of each culture flask. In order to overcome this difficulty, uniform squares of mycelium (25 sq. mm.) on potato-dextrose agar in Petri dishes were used for inoculation purposes, one square being placed in each flask. Abundant spores were available in the former fungus, so a good suspension of spores could be prepared. Otherwise the experiment was the same as with the cultures of S.fructicola and P.expansum.

The following results were obtained. Botrytis byssoidea grew in almost the same range of pH values as B.allii, but was able to grow slightly in a medium more acid than B.allii would tolerate. Both fungi preferred a slightly acid medium for their growth. Sporulation was abundant in cultures of B.allii at pH 5.0 and above. B.byssoidea did not form many spores, but produced them at the same range of H-ion concentrations as B.allii. There was a moderate formation of sclerotia in cultures of B.byssoidea at the same pH range as spore production occurred. In cultures where the two fungi were grown in association, both fungi grew well, though less than in pure culture, and there was no tendency for one fungus to be suppressed by the other. The results are shown in Figure XVII and Table XI.

It was noticed that there was a tendency for the fungus to render the culture media more acid where the initial acidity was low, and the amount of change was roughly correlated with the amount of growth of the fungus. The uninoculated medium changed only slightly in H-ion concentration, the tendency being to render the medium more acid.

TABLE XI. THE EFFECT OF H-ION CONCENTRATION ON GROWTH OF ONION-ROTTING FUNGI

Initial pH	Check	Botrytis allii		B. allii and B. byssoidea		Botrytis byssoidea	
	Final pH	Final pH	Growth	Final pH	Growth	Final pH	Growth
1.5	1.5	1.5	0	1.5	0	1.5	0
2.0	2.0	2.0	0	2.0	+	2.0	+
2.4	2.4	2.5	+	2.5	++	2.4	++
2.9	2.9	2.6	++	2.7	++	2.7	++
3.4	3.4	2.6	+++	2.5	+++	2.5	+++
3.9	3.7	2.6	+++	2.4	+++	2.5	+++
4.5	4.2	2.4	+++	2.2	+++	2.7	+++
5.0	4.9	2.6	+++	2.2	+++	2.6	+++
5.5	5.3	3.2	+++	2.4	+++	2.4	+++
6.3	6.0	3.1	+++	3.1	+++	2.7	+++
7.0	6.8	3.1	+++	3.1	+++	2.7	+++

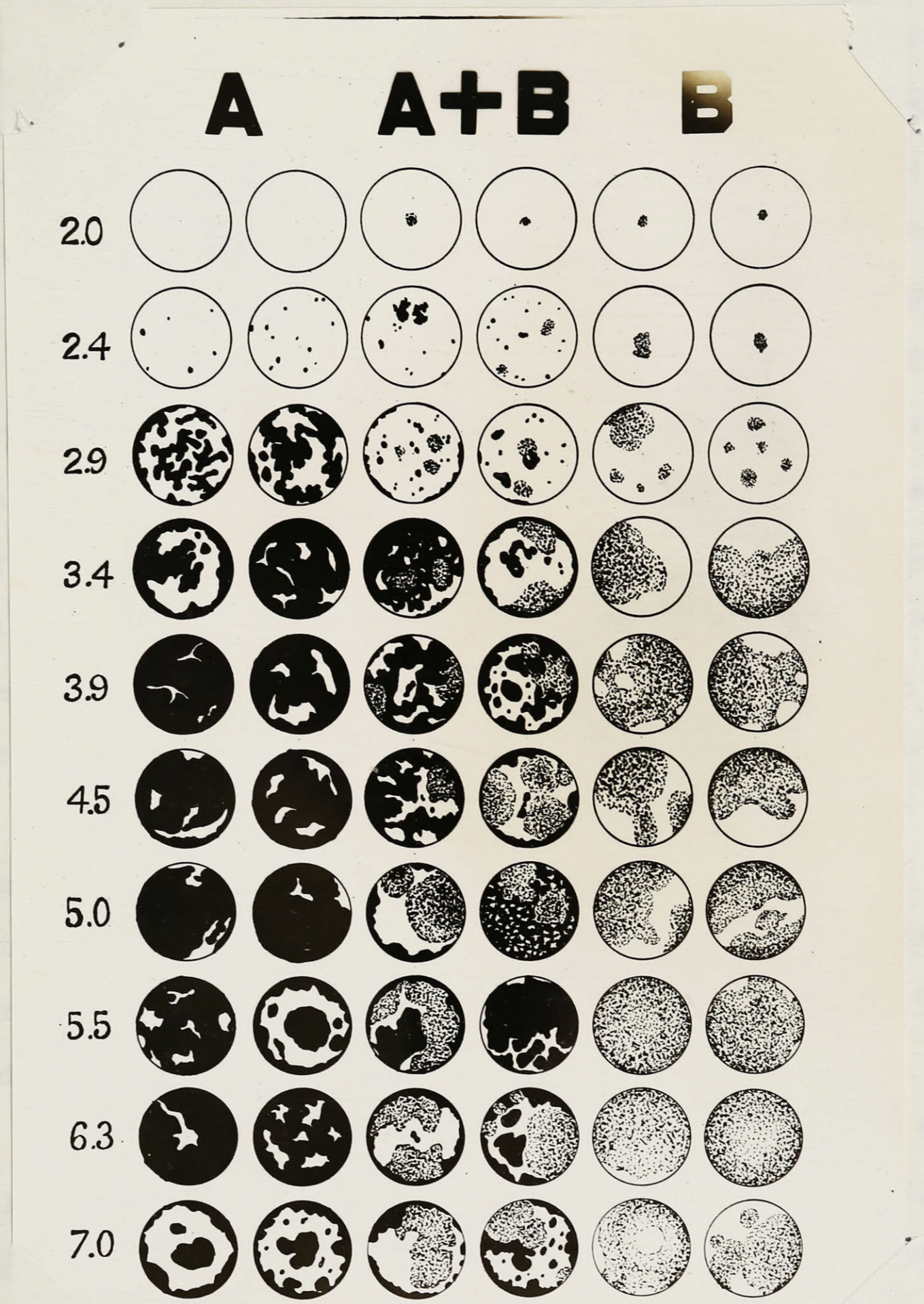


FIGURE XVII. The effect of H-ion concentration on the growth of Botrytis allii (A) and Botrytis byssoidea (B), in pure culture and in association. Heavy shading represents growth of B. allii, light shading that of B. byssoidea. Cultures grown at 20°- 24°C.

In the case of the tomato-rotting fungi, Botrytis sp. and Cladosporium fulvum, the procedure used in the above experiments was followed. Here also, as in the case of Botrytis byssoidea, squares of the mycelium of Botrytis sp. were used to inoculate the cultures. Cladosporium fulvum formed an abundance of spores, so no difficulty was found in making an adequate spore-suspension.

It was observed in this experiment that Cladosporium grew very well in markedly acid media, more so than Botrytis, although the latter fungus was found able to grow in more acid than the former. Sporulation was abundant in the cultures of C. fulvum at all H-ion concentrations which allowed the growth of the fungus. There was a tendency on the part of this fungus to form small masses of white aerial mycelium in acid media, pH 4.2 and below.

Botrytis sp. grew well at nearly all the H-ion concentrations used, though the amount of growth was approximately in inverse ratio to the degree of acidity. Sporulation was only slight, but formation of small sclerotia occurred in cultures of pH 5.2 and above.

In mixed cultures, Botrytis sp. was completely suppressed at all H-ion concentrations less than pH 3.2, but it appeared in media which were more acid than this point. It appeared better able to resist the high acidity, and so was partly able to overcome the inhibitory action of C. fulvum. The results of this experiment are shown in Figure VIII and Table XII.

From the above experiments it is concluded that different fungi do not respond in the same way when grown at different degrees of acidity, but that there is a distinct influence of the acid on association of some of these fungi, especially where the inhibited organism is favoured by extremes of acidity.

TABLE XII. THE EFFECT OF H-ION CONCENTRATION ON GROWTH OF TOMATO-ROTTING FUNGI.

Initial pH	Check	*Cladosporium fulvum*		*C.fulvum* and *Botrytis sp.*		*Botrytis sp.*	
	Final pH	Final pH	Growth	Final pH	Growth	Final pH	Growth
1.5	1.5	1.5	0	1.5	0	1.5	0
2.2	2.2	2.2	0	2.2	+	2.2	+
2.7	2.7	2.7	++++	2.8	++++	2.8	++
3.2	3.1	2.8	++++	2.9	++++	3.2	+-
3.7	3.6	2.6	++++	2.8	++++	3.2	+++
4.2	4.0	2.7	+++	2.7	+++	3.4	+++
4.7	4.5	2.6	+++	2.7	+++	3.0	++++
5.2	5.0	2.5	+++++	2.6	+++++	3.6	++++
5.7	5.4	2.8	+++++	2.6	+++++	3.8	++++
6.2	6.1	2.9	++++	2.8	+++++	3.7	++++
6.7	6.5	2.6	+++++	2.8	+++++	3.8	++++

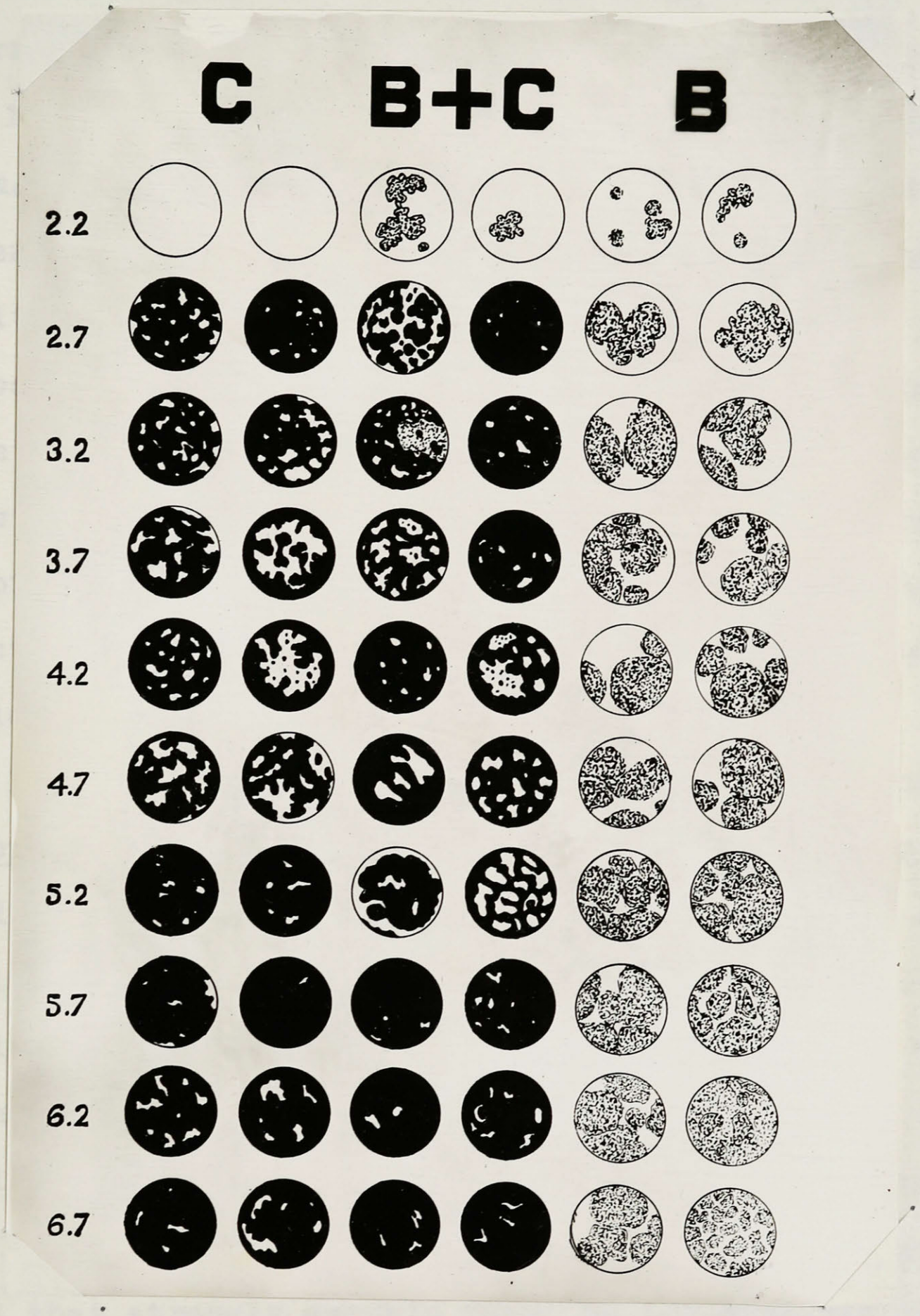


FIGURE XVIII. The effect of H-ion concentration of growth of Botrytis sp. and Cladosporium fulvum, in pure and mixed cultures. Heavy shading represents growth of C. fulvum (C), and light shading the growth of Botrytis sp. (B). Cultures grown at 20-24°C.

Anaerobiosis and Association of Phytopathogens.

Pasteur (63) made the notable observation that various plants liberate carbon dioxide in an oxygen-free atmosphere,and he found that here fermentation had been substituted for ordinary respiration.Seed plants are able to form the primary products of alcoholic fermentation,i.e.ethyl alcohol and carbon dioxide,under anaerobic conditions.In the yeasts this fermentation is more obvious than in most fungi.Pasteur was also able to establish the fact that there were among fungi transition forms between the yeasts and the strictly aerobic forms such as Penicillium.The Mucors are in part aerobic,but in part they are organisms of fermentation.Sometimes strictly aerobic fungi form small quantities of carbon-dioxide and alcohol under anaerobic conditions.

Diakonow (21) carried out some experiments with Penicillium glaucum,Aspergillus niger,and Rhizopus nigricans.He grew the moulds on various organic substances as sources of carbon.Carbon dioxide was formed only when the fungi were grown on saccharine nutrients,while on other nutrients the fungus mats soon died out. On the basis of these results the author concluded that anaerobic respiration of moulds is only alcoholic fermentation which can take place in the absence of free oxygen.In later experiments he found that strongly aerobic fungi do not give off any carbon dioxide when grown in the absence of free oxygen,even when supplied with fermentable sugars,and the cultures died after one or two hours.

Kostychev (48) was able to show that Penicillium glaucum liberates carbon dioxide regularly,though free xygen was excluded. He repeated Diakonow's experiment and clarified his results.The results of Kostychev's experiments seem to show that the so-called

anaerobic respiration is peculiar to all aerobic plants.Diakonow's results may be traced to the poisoning of some of the aerobic moulds by products of imperfect metabolism.He found that there was very little difference between the sugars and non-sugars as sources of carbon for anaerobic growth.

Thoytta and Avery (81) discovered that aerobic bacteria can grow under anaerobic conditions if raw,sterile vegetable tissue is added to the medium on which the organisms are growing.The reason for this phenomenon appears to be that in aerobic bacteria an enzyme,a peroxidase,is lacking.This peroxidase is possessed by anaerobic bacteria,who through its use,can obtain combined-oxygen from the medium,and use it in their respiration.If this deficiency of oxygen-freeing peroxidase is overcome,by supplying it to the medium from an outside source,then strictly aerobic bacteria can grow in the medium,even though atmospheric oxygen is excluded.The peroxidase can be most easily supplied through the addition of raw,sterile plant tissue from which the peroxidase can diffuse into the medium and become available for use by bacteria in the medium.

An attempt has been made to determine if fungi respond to a similar treatment;and also to see if there is any growth of plant pathogens under anaerobic conditions.The experiment that was performed was not an elaborate one,but it was sufficient to show any growth of the plant pathogens in the absence of free oxygen,or any effect due to the addition of fresh,sterile,plant tissue on the growth and association of these plant pathogens.

The experiment was performed as follows.Small test-tubes (1.5 X 15 cm.),each containing 10 c.c. of Richard's modified

nutrient solution,were autoclaved at fifteen pounds for fifteen minutes.After the media had cooled,small cubes of raw,sterile potato-tuber tissue were added.These cubes were prepared by washing and paring the tubers,and sterilizing the surface of the tubers with 1-500 strength corrosive sublimate ($HgCl_2$).After sterilization for five minutes,the tubers were washed in sterile distilled water,and then sectioned in a sterile dish into cubes. The side of each cube measured approximately five millimeters. Care was taken throughout the experiment to eliminate contamination of the medium and tuber sections as much as possible.

Four fungi were used for inoculation purposes,Penicillium expansum,Sclerotinia fructicola,Botrytis sp.,and Cladosporium fulvum.Two series of cultures were prepared,those containing Richard's modified medium only,and those containing the same medium with the addition of the potato cubes.The inoculum used was made as uniform as possible,consisting of a spore suspension in each case.

After inoculations were complete,the culture tubes were placed upright in larger tubes containing crystalline pyrogallic acid in the closed end of the tube,the crystals being held in place by a plug of absorbent cotton.Just before the culture tubes were inserted,5 c.c. of concentrated caustic potash (KOH) solution were poured into the larger tubes,and then the opening of the large tubes were sealed by inserting a cork impregnated with melted paraffin.This provided an air-tight container for each culture tube,from which all free oxygen had been removed by the pyrogallic acid and caustic potash.

The cultures were incubated at 20-24°C. for fifteen days,

after which period final observations were made.

It was found that in pure cultures of Penicillium expansum practically no growth took place.What growth occurred,took place during the first three days of the culture period.No difference was found in the cultures containing the raw potato tissue.

In pure cultures of Sclerotinia fructicola some aerial mycelium but no spores were formed.No stimulus to growth was observed in the cultures containing the potato-cubes.

In mixed cultures of the above organisms very little growth occurred.The Penicillium dominated in all the cultures.The addition of raw potato tissue did not produce any increased growth.

In the cultures of Botrytis sp.growth of mycelium was slight, and no spores were formed.There was no stimulus to growth given by the addition of the raw potato-tissue.

In pure cultures of Cladosporium fulvum very little growth occurred.The potato cubes produced no visible effect on growth.

In mixed cultures Botrytis predominated,but the growth was no better than in pure cultures of that organism.The addition of potato tissue did not produce any visible effect on growth.

After the above observations were made,the culture tubes were exposed to atmospheric oxygen.The effect was seen at once. After one day there was a distinct increase in growth in all the cultures,especially those of Penicillium.Apparently spore germination had occurred in the cultures after they had been placed under anaerobic conditions,but growth soon ceased.When air was admitted, growth took place at once.The presence of the raw potato cubes did not produce any visible effect on growth,either in the anaerobic conditions or after exposure to air.

From the above results the writer concluded that the fungi described are obligate aerobes.It is possible that some respiration took place while the cultures were kept in anaerobic conditions,for growth occurred as soon as they were exposed to atmospheric oxygen.This result would not have been observed so soon if germination had not begun while the tubes were still sealed. There may have been a temporary inhibition of growth on account of the absence of atmospheric oxygen,but the cultures were not killed.As there was no visible effect on growth caused by the addition of the potato cubes,it is possible that the peroxidase, so useful to aerobic or anaerobic bacteria,can not be used by fungi;and hence the addition of fresh,vegetable tissue is of no value for the growth of fungi in the absence of free Oxygen.

The results of this experiment are shown in Table XIII.

The Association of Phytopathogens in the Host.

The Effect of Host Variety.

In the study of apple rots,the varieties MacKintosh Red, Fameuse,and McMahon White were used.All the varieties were susceptible to both Penicillium expansum and Sclerotinia fructicola,but the amount of rot was least in the ~~another~~ variety, Russet,which was not used for experimental purposes.The most damage in storage was done by the Penicillium,and,except in the last variety named,there did not appear to be any marked difference in the amount of rot.

Penicillium expansum is a parasite which is confined almost entirely to fruit rots in storage,usually when the fruit is ripe. No investigations have been made by the writer to determine the amount on green fruit.A survey of the literature revealed no

TABLE XIII. EFFECT OF ANAEROBIC CONDITIONS ON GROWTH OF SOIL FUNGI

Fungus	anaerobic cultures 15 days		aerobic cultures 10 days	
	Rich.mod. med.alone	Rich.mod. med.plus potato	Rich.mod. med.alone	Rich.mod. med.plus potato
Penicillium expansum	+	+	++++	++++
Sclerotinia fructicola	++	++	+++	+++
S.fructicola and P.expansum	+	+	++++	++++
Botrytis sp.	+	+	++++	++++
Cladosporium fulvum	+	+	++++	++++
Botrytis sp. and C.fulvum	+	+	+++	+++

reports giving positive results.Further experimental work is necessary to determine the effect on unripe fruit.

The Brown Rot fungus,Sclerotinia fructicola,appears to be able to attack the fruit in any stage of its development.Heald (36) reports that all summer varieties of apple are susceptible to the disease,but the varieties Yellow Transparent,Chenango,and Genet are especially so.Data on the subject of varietal susceptibility are as yet very meager.

In the onion rots,a similar lack of data exists.Munn (62) finds that white and yellow varieties of onions are especially susceptible to the attacks of Botrytis allii,and the red varieties are reported to be somewhat resistant.The experiments of Munn have been confirmed by the writer.The yellow varieties have been found most susceptible to invasion by Botrytis allii and B.byssoidea,but the white varieties were attacked by B.squamosa.

In the case of tomato rots only one variety,Livingstone Globe, was used.The amount of infection appeared to be correlated with the maturity of the plant ,the ripeness of the fruit,and the amount of shade in which the plants were growing.Neither Cladosporium fulvum nor Botrytis sp. attacked the vines prior to fruit formation,although young plants when grown under a bell-jar at room temperature are susceptible to the attacks of both fungi. Under green-house conditions,Cladosporium did not appear until after fruit formation had taken place for some time,but Botrytis was found to cause infection as soon as the young fruit had begun to form.The former fungus did not produce an extensive rot in the diseased fruit,confining its activities to the superficial layers,but cracking sometimes followed the attacks of the fungus on the fruit,and through the wounds Botrytis was able to enter

and complete the rot. Botrytis sp. was found able to attack green fruit much more readily than the ripe fruit, for here the fruit was either free from the rot, or if the fungus had already entered the progress of the rot was slow.

The Effects of Host Acidity.

As mature specimens of the different host plants were used for these experiments, only the data concerning the acidity of these plants at maturity were obtained. However, numerous accounts dealing with the acidity of some of the hosts were read, and an attempt has been made to correlate the amounts of acidity and infection.

Magness and Diehl (53) have shown that the varieties of apples with which they have worked underwent a steady decrease in acidity during the time the apples were kept in storage at 32°F. The rate of decrease was nearly the same for all varieties, regardless of the original acid content. Delicious, a variety with an initial acid content of 3 c.c. of N/10 acid per 10 grams of wet tissue lost acid until after six months only 2 c.c. of N/10 acid were required to neutralize the same weight of pulp, a loss of 30 to 40 percent. Rhode Island Greening changed from 9 to 8 cc. of the N/10 acid. The varieties in order of decreasing acid content were Rhode Island Greening, Ben Davis, York, Imperial, Winesap, Rome Beauty, and Delicious. The percentage of acid lost during the six months in storage is in reverse order, showing that the varieties with lowest acidity lost the most.

Patrick, another worker with acidity of apples, found that there appeared to be no relation between acidity and sugar content. The range of acid, however, was considerable in the different varieties.

The results of Patrick are shown in Table XIV.

However, as Heald (36) states, there is very little difference in the amount of rot in apple varieties, and it is probable that acidity is not an important factor in determining the presence or absence of the rot in ripe fruit. It is possible that the high acidity of immature fruit may be responsible for the exclusion of some rot-producing fungi.

An experiment was performed to determine the acidity of some plants before and after rotting. The acidity of the healthy specimens was determined by finding the pH value of the juice from ten uniform apples, onions, or tomatoes. After this has been done, other similar specimens were inoculated with rot-producing fungi. After inoculation and sealing of the wounds with melted paraffin, the different hosts were incubated at 20-24°C. for some time, depending on the host, after which period the amount of rot was determined.

In the case of the apples, the varieties MacKintosh Red, Fameuse, and McMahon White were used for inoculation purposes. Only uniform, sound apples of each variety were selected. After inoculation had been made with either Penicillium expansum or Sclerotinia fructicola spores the rots progressed quickly, and in about ten days the apples were almost completely rotted. The H-ion concentration was determined before and after rotting, and it was found that in each case, no matter which fungus had been used for inoculation, the acidity of the juice had been increased. The acidity of the checks was found to decrease slightly, this probably being due to the raising of the temperature after being brought from storage, to effects of enzymes.

TABLE XIV. SUGAR AND ACID CONTENT OF SOME APPLE VARIETIES.

Variety	Percent acid	Percent sucrose	Percent glucose	Total sugar
Soulard Crab	1.124	3.11	5.15	8.26
Scott's Winter	.960	3.06	6.07	9.13
Silken Leaf	.837	1.46	7.87	9.33
Willow Twig	.683	3.87	6.79	10.66
Wythe	.646	2.05	6.83	8.88
Ostrokoff's Glass	.622	1.78	7.68	9.46
Winsted Pippin	.527	1.30	8.33	9.63
Cross	.423	2.12	6.80	8.93
Borsdorf	.418	2.82	7.48	10.30
Fameuse	.394	3.29	8.31	11.60
Iowa Greening	.117	1.25	7.87	9.12

The above data were obtained from the report on the analysis of apples by G.E.Patrick (Bull 3.Iowa Agric.Exp.Sta. 1888)

The results of this experiment are shown in Table XV.

In the inoculated onions there was a similar change. The varieties Red Weatherfield, Yellow Globe, and Silverskin were used, and there was only a slight difference in the pH value of the juice. The change in H-ion concentration was directly proportional to the initial pH value. During the experiment the checks did not change, but in the decaying specimens there was a slow increase im acidity. The results are shown in Table XVI.

In the tomato practically no change in H-ion concentration was observed although the specimens were almost completely rotted. Cladosporium fulvum produced only a slight rot, which did not affect the acidity of the fruit. Botrytis sp., on the other hand, rotted the fruit very quickly, but contrary to the results obtained in the rots of the apple and onion, the change was towards less acidity. The actual change was from pH 3.8 to pH 3.9; these figures giving the average of the determinations made on ten healthy and ten rotted specimens of the variety Livingstone Globe.

From the above results, acidity does not appear to be a limiting factor for tne production of a rot in the host by the various pathogenic fungi used. As seen from previous experiments these same fungi are able to grow well at H-ion concentrations greater or less than those found in the healthy hosts, and so it is not probable that the host acidity could limit the development of the fungus in the host.

Association of Phytopathogens in the Host.

The apple-rotting fungi, Penicillium expansum and Sclerotinia fructicola have been only rarely found growing together in

TABLE XV. THE EFFECT OF ROTTING ON THE ACID CONTENT OF SOME APPLE VARIETIES

Variety	pH of healthy apples		pH of apples rotted by P.expansum		pH of apples rotted by S.fructicola	
	initial	final	initial	final	initial	final
Fameuse	3.1	3.1	3.1	2.8	3.1	2.95
McMahon White	3.2	3.9	3.2	2.9	3.2	3.0
MacKintosh Red	3.15	3.3	3.15	2.8	3.15	2.9

TABLE XVI. THE EFFECT OF ROTTING ON THE ACID CONTENT OF SOME ONION VARIETIES.

Variety	pH of healthy onions		pH of onions rotted by *B.allii*		pH of onions rotted by *B.byssoidea*	
	intial	final	initial	final	initial	final
Red Weathersfield	5.1	5.1	5.1	5.0	5.1	4.95
Yellow Globe	5.2	5.2	5.2	4.8	5.2	4.7
Silverskin	5.5	5.5	5.5	5.3	5.5	5.1

nature. By means of artificial inoculation of the apple fruit it is possible to secure association of the two organisms, but only if inoculation with Penicillium spores was done after Sclerotinia fructicola had already rotted a considerable amount of the host tissue.

When spores of both fungi were placed into the same wound at the same time, it was found that both organisms grew and formed the typical rot. The Brown Rot, caused by Sclerotinia fructicola, usually appeared first, and the growth of the fungus was equal to that produced by the inoculation of apples with pure cultures of the organism. After a few days it was possible to distinguish the lesion caused by the growth of Penicillium expansum. It was easy to differentiate the two rots. The lesion caused by the latter fungus is sunken, the epidermis wrinkled, and coloured pale brown. The rotted tissue is soft and watery. The lesion caused by the growth of Sclerotinia is brown at first, black later, but there is no shrinking of the epidermis as the rotted tissue is firm and the apple retains its shape. The rot in this case spreads considerably faster than that caused by the Penicillium. This fungus appeared to have the property of changing the colour of the epidermis, so that finally it is the same as when Penicillium alone is responsible for the rot.

A similar change was observed when an apple partially rotted by the Brown Rot fungus had been inoculated with P. expansum. It was noted, moreover, that when P. expansum followed in the wake of Sclerotinia fructicola its progress was slower than when growing alone.

In apples which had been rotted by Penicillium expansum and then inoculated by Sclerotinia fructicola no secondary rot caused by the latter fungus was developed. It is possible a case of complete suppression of the latter by the former organism and is probably similar to the suppression obtained when the two fungi were grown in artificial media.

When each apple had been inoculated by both fungi, but each one placed separately in different wounds on opposite sides of the fruit, the fungi produced their characteristic rot around the points of infection. There was no reduction in the rate of growth until the borders of the lesions approached one another. When the lesions merged with one another, further growth of Sclerotinia was inhibited completely, but the Penicillium was able to continue its growth, although at a diminished rate. The growth of the lesion could be followed by making a surface marking along the borders of the lesions at regular periods.

The above phenomena were somewhat modified by temperature. It was found that at 30-34°C. no rot was developed by the fungus Penicillium expansum, but Sclerotinia fructicola was able to advance very well. At 20-24°C. both fungi grew well, and were able to rot the apples in a short time. At 4-8°C. the growth of both fungi was much reduced, and the amount of rot reduced in proportion. A more detailed account of the effect of the temperature on growth and association will be given below.

In the onion-rotting organisms, Botrytis allii and Botrytis byssoidea, the former grew faster at the high and medium temperatures which are stated above, than the latter. At the lowest temperature the latter fungus grew best. When growing together

in the same host it was found that B.allii usually produced its own characteristic rot,while the other fungus did not develope. This result was not modified by changes in temperature,though B.byssoidea was able to produce a rot of the onion through its own activity.The color of the onion did not make any difference in the growth of the associated organism.

In tomatoes it was impossible to obtain large lesions by inoculations ofthe fruit with Cladosporium fulvum,even though rotting has been observed in the green-house.The inoculated fruit was kept at 20-24°C. for a long period,and though rots produced by Botrytis sp. soon developed,and caused a rapid rot of the tomato fruit,Cladosporium rots developed slowly.This is contraty to the results when the two fungi were grown in artificial culture.(See Figure XVIII.)

Effect of Temperature on Association

As temperature has a distinct influence on the development of phytopathogens in artificial culture,it is probable that similar effects are produced on rot-producing fungi when growing on their hosts.To see if the above is true,the following experiments were performed.

In the study of apple rots,apples of the same size,maturity, and variety were selected.The volume of the apple in each case was determined by the water-displacement method.After the end of the incubation period,the amount of healthy tissue was found by the same method when the rotted part was scraped out.It was found that a small amount of shrinkage occurred,so checks were

used to determine the amount.These checks were treated in a similar manner to the inoculated apples but no inoculum was introduced into the wound.

In a preliminary experiment the varieties MacKintosh Red and McMahon White were used.Six apples of each variety were inoculated with spores of Penicillium expansum or Sclerotinia fructicola or both.After incubation at 20-24°C.for seven days the rotted tissue was scraped out from each apple,and the amount of rot measured.It was found that the rots developed well in both varieties,but in McMahon White the percentage of rot was greater than in the MacKintosh Red.In each case S.fructicola rotted more of each apple than did P.expansum.The results are shown in Table XVII.

In a second experiment,only the variety MacKintosh Red was used.The same fungi were used as above,but the rots were incubated at 30-34°C.;20-24°C.;and 4-8°C.Sixteen apples were placed at each temperature,four being inoculated with P.expansum, four with S.fructicola,four with the two fungi in association, and the remaining four serving as checks to determine the amount of shrinkage.The period of incubation extended over ten days,after which the amount and type of rot was determined. The data are given in Table XVIII and Figure XIX.

In a third experiment the variety Fameuse was used instead of MacKintosh Red,but other wise the experiment was similar to the above.The data are shown in Table XIX.

In the study of onion rots the procedure used above could not be adopted,as measurements were difficult to obtain on account of the inability of the writer to determine the

TABLE XVII. AMOUNTS OF TISSUE ROTTED BY APPLE-ROTTING FUNGI IN TWO VARIETIES

Fungus	Initial volume	Volume healthy after 10 days	Volume rotted	Percentage rotted.
	Variety McMahon White			
S.fructicola	240	115	125	52.4
P.expansum	225	150	75	33.3
	Variety MacKintosh Red			
S.fructicola	77	43	34	44.3
P.expansum	103	80	23	22.5

In the checks the average shrinkage in volume was from five cubic centimeters in the McMahon White variety to two cubic centimeters in the MacKintosh Red, or a change from 226 cc. to 221 cc. in the former variety, and from 92 to 90 cc. in the latter.

All volumes are given in cubic centimeters.

TABLE XVIII. EFFECT OF TEMPERATURE ON AMOUNT OF ROT IN THE APPLE VARIETY MACKINTOSH RED

Fungus	Initial vol. before inoculation	Final healthy volume	Amount rotted	Percentage rotted.
	INCUBATOR TEMP.(30-34°C)			
S.fructicola	147.5	62.5	85.0	57.6
S.fructicola and P.expansum	140	102.5	37.5	26.7
P.expansum	137.5	133.0	0.0	0.0
	ROOM TEMP.(20-24°C.)			
S.fructicola	135.0	0.0	135.0	100.0
S.fructicola and P.expansum	130.0	25.0	105.0	80.7
P.expansum	125.0	62.5	62.5	50.0
	COLD STORAGE TEMP.(4-8°C.)			
S.fructicola	142.5	140.5	0.0	0.0
S.fructicola and P.expansum	111.0	108.0	3.0	2.7
P.expansum	147.5	142.5	5.0	3.4

In the checks the following shrinkage was observed;

At 30-34°C. shrinkage from 139 to 135 cc. or 4 c.c.

At 20-24°C. " " 137-133.5 c.c. or 3.5 c.c.

At 4-8°C. shrinkage from 134 to 132 c.c. or 2.0 c.c.

All volumes are given in cubic centimeters.

TABLE XIX. EFFECT OF TEMPERATURE ON AMOUNT OF ROT IN THE APPLE VARIETY FAMEUSE.

Fungus	Initial vol before inoculation	Final healthy volume	Amount rotted	Percentage rotted
	INCUBATOR TEMP. (30-34°C.)			
S. fructicola	120.0	19.0	101.0	84.1
S. fructicola and P. expansum	121.0	13.0	108.0	89.2
P. expansum	123.0	120.0	3.0	2.4
	ROOM TEMP. (20-24°C.)			
S. fructicola	103.0	10.5	92.5	89.8
S. fructicola and P. expansum	109.5	15.0	94.5	86.3
P. expansum	111.0	66.5	44.5	40.1
	COLD STORAGE TEMP. (4-8°C.)			
S. fructicola	114.0	111.0	3.0	2.6
S. fructicola and P. expansum	116.0	102.5	13.5	11.6
P. expansum	124.0	120.0	4.0	3.2

In the checks the following shrinkage was observed:

At 30-34°C. shrinkage from 119.0 to 113.0 or 6.0 c.c.

At 20-24°C. " " 104.0 to 101.5 or 2.5 c.c.

At 4-8°C. shrinkage from 113.5 to 112.0 or 1.5 c.c.

All volumes are given in cubic centimeters.

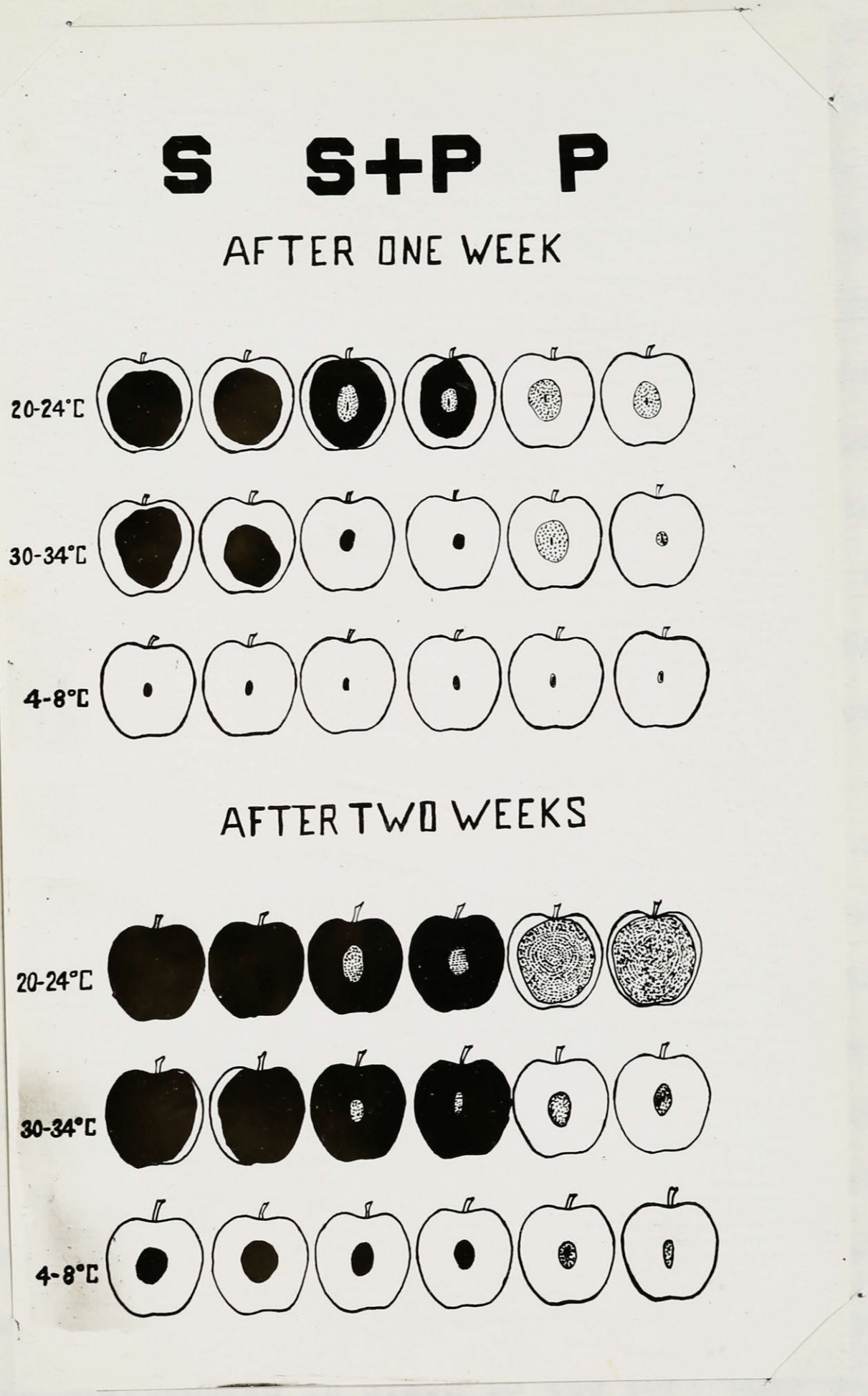


FIGURE XIX. The influence of temperature on the development of apple rots caused by Penicillium expansum and Sclerotinia fructicola grown in pure culture or in association. Heavy shading shows growth of S. fructicola (S), light shading that of P. expansum. Note dominance of former in mixed culture.

exact line of demarcation between the healthy and diseased tissue,and also because the diseased tissue was not easily separated from the healthy.As the amount of rot in each scale of the bulb varied considerably,the volume of the rotted tissue could not be accurately determined.The relative amounts of rot caused by Botrytis allii and Botrytis byssoidea could therefore be best determined by external examination,and so this method was used in obtaining the data.The development of each rot is shown in Figure XX,where the growth of both of the above fungi singly and in association at the different temperatures can be seen.The growth of the rot in all cases was slow,for nearly four weeks had elapsed after inoculation of the bulbs, before the first of them was completely rotted.

For the tomato rots charts similar to those showing the onion rots were prepared.A difficulty in determining the amount of rot was encountered here also.It appears that though externally a small lesion is produced by Botrytis sp.yet the entire interior portion of the tomato fruit is softened through its activities, and so the exact limits ofthe rot could not be determined. Therefore,the increase of the lesion on the surface of the fruit was used as an index of growth.The results are shown in Figure XXI.

Again,attention is drawn to the fact that temperature appears to be a limiting factor which determines the amount and kind of rot that developes when the rot-producing fungi are growing separately or in association on the host.Practically similar results are obtained when the fungi are grown on the host or in artificial media ,with several important exceptions.It has been seen that Cladosporium fulvum dominates Botrytis sp. in artificial media,but the reverse is true on the host.

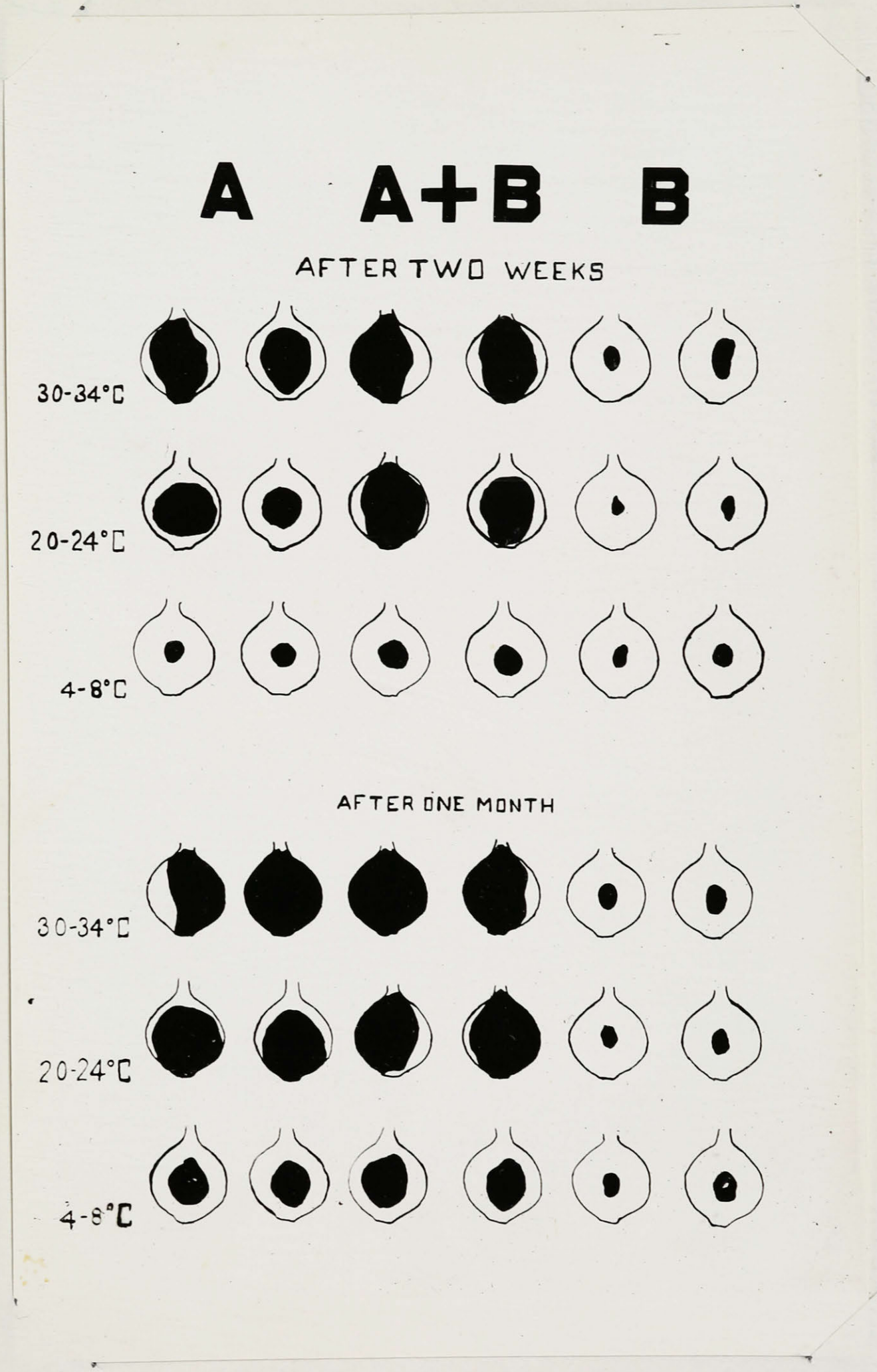


FIGURE XX. The influence of temperature on the development of onion-rots caused by Botrytis allii(A) and Botrytis byssoidea(B) when grown in pure culture and in association.

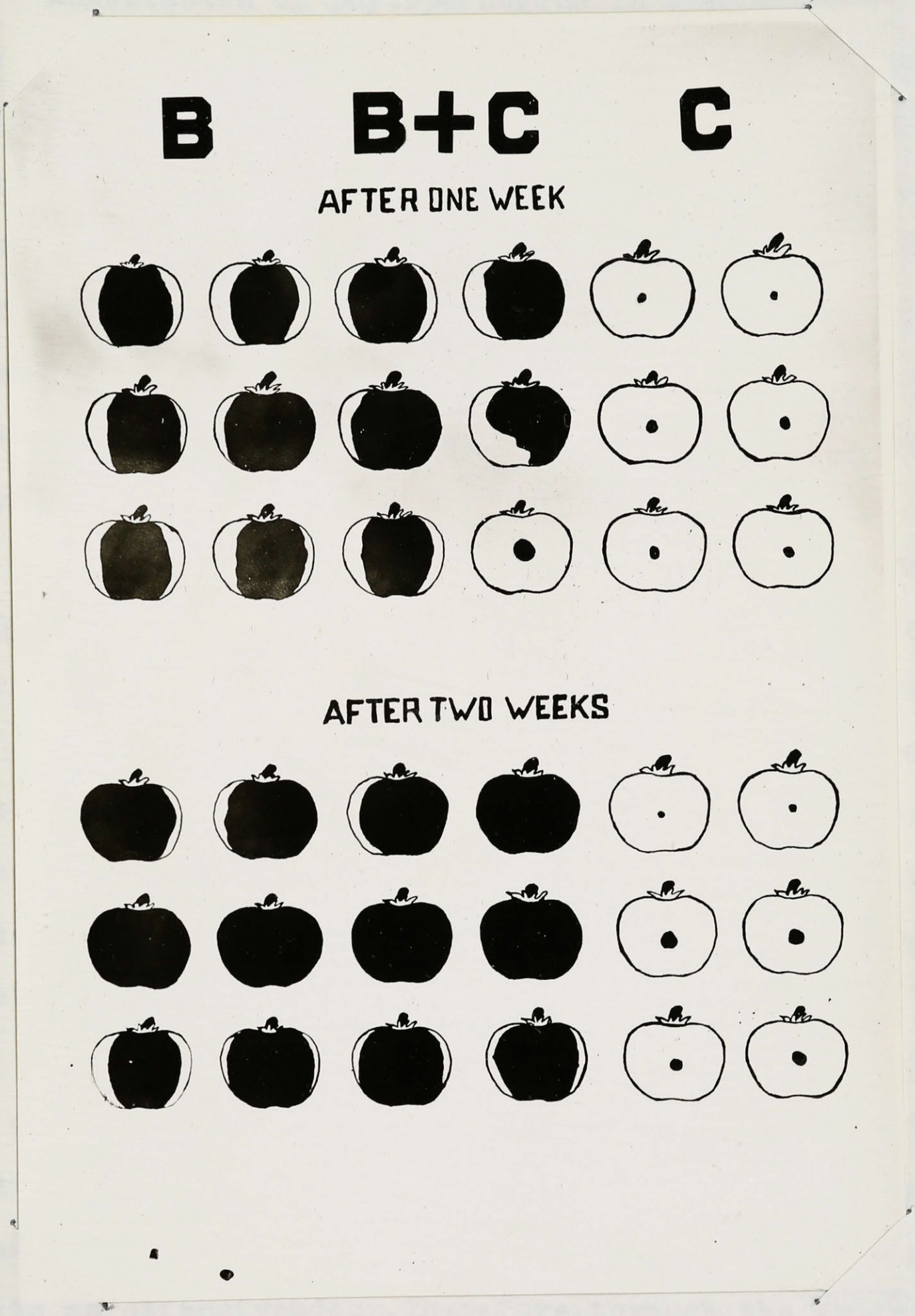


FIGURE XXI. The influence of temperature on the development of tomato rots caused by Botrytis sp.(B) and Cladosporium fulvum (C) when grown in pure culture and in association.

Discussion

Association of phytopathogens in storage rots, at least in early stages, appears to be limited to one or two pathogenic organisms and some saprophytes on each host. In only a few instances were more than two of these pathogens associated in a single lesion, for by far the most common association appeared to be between parasite and saprophyte. Association occurred between various classes of organisms: fungi, yeasts, and bacteria. The yeasts were always strictly saprophytic, but the other two groups included important pathogens.

It was found that in a few cases a surface parasite, such as Venturia inaequalis, or Cladosporium fulvum was able to produce a condition in the host that enabled the rot-producing organism to enter. The surface parasites did not always produce any definite rot themselves, but they were responsible for the development of cracks or other wounds in the epidermis of the apple, so that the secondary parasites then could enter easily and complete the destruction of the host.

A characteristic rot is usually the result of the activities of only one pathogen in the host. This original character may be modified later by the invasion of saprophytes. The results are that a hard or spongy rot may become soft and watery, and often emits an offensive odour. Therefore, through the activities of secondary parasites or saprophytes, the original rot may be masked. When the decaying specimens are only slightly rotted, there is little danger that the primary rot is changed, due to contaminating organisms. Cook(19) has shown that there is a distinct succession of fungi on culture media, and observations made by the writer and others have confirmed this finding.

Therefore,if the rot is far advanced,it is possible that the original pathogen may be suppressed completely in most of the rotted portions,by other organisms,and these organisms can take its place.

The species of pathogens found in any one host appeared to be only slightly influenced by the variety and species of this host, though there has been a tendency for a definite group of fungi to be confined to one variety of host.In the case of apple rots, though all common varieties appear to suffer equally from the attacks of Penicillium expansum and Sclerotinia fructicola, yet the variety Russet appears to be immune to outside infection. Penicillium expansum is confined to pomaceous fruits,but Sclerotinia fructicola can be found to cause rots of both pomaceous and stone fruits.In the case of onion-rotting fungi,Walker (82) has found that Botrytis squamosa is confined almost exclusievly to white onions,while Botrytis allii and B.byssoidea can be found on either yellow or red varieties,usually on the former.

Temperature appears to be a determining factor in the development of a specific rot.Fungi have definite temperature optima; and these optima are often widely separated,the fungi which have the ability to grow together,due to temperature effects,may not always be found in association.Some fungi are favoured by high temperatures,others by low;and at any temperature,when two fungi are associated,the organism favoured by the given temperature will dominate in the culture.Therefore,it is to be expected that in the host the fungus best suited to grow at the given temperature will produce its own characteristic rot.With only two exceptionns, the fungi studied have followed the above rule,these exceptions being Botrytis byssoidea and Cladosporium fulvum.The former fungus

is adapted for growth at a low temperature, more than its associate, Botrytis allii, which predominated; and at these temperatures an abundant rot was not obtained. Similarly, though Cladosporium fulvum is distinctly able to grow well at a high temperature, yet it failed to produce an extensive rot in the host kept at this temperature. In mixed cultures on the fruit, Botrytis sp. predominated.

The H-ion concentration of the culture medium is not always a limiting factor to growth or spore germination. Most fungi are able to grow and sporulate at a considerable range of pH values, but they are unable to do so when the medium is too acid or too alkaline. The limit of acidity tolerated by fungi appears to be in the neighbourhood of pH 1.5. Not all fungi are able to grow well in the same range of H-ion concentrations, but in all cases the fungi were able to grow at H-ion concentrations different from that of the host tissue. There may be some tendency for the apple-rotting fungi to attack the ripe fruit because the acidity of the unripe fruit is too high to allow the fungus to grow well. It is possible that immature fruit is firm and not so readily injured as the ripe fruit. If, according to Heald (36) the amount of rot on mature apples exceeds that of immature apples when the rot is caused by Sclerotinia fructicola, and if, according to the findings of Magness and Diehl (53) the amount of acid in apples is inversely proportional to the length of time in storage, there may be an indirect relation between total acidity and the amount of infection. However, Penicillium expansum attacks the fruit only when mature, although it is able to grow well on more acid media than can the Sclerotinia. On the other hand, Botrytis sp., when growing on tomatoes is able to rot green fruit much faster than the ripe, but the H-ion concentration remains practically unchanged.

In mixed cultures on artificial media plant pathogens sometimes exhibit a tendency to suppress the growth of associated fungi.This has been observed in cultures on artificial media of Penicillium expansum and Cladosporium fulvum which fungi were able to cause almost complete inhibition in the growth of Sclerotinia fructicola and Botrytis sp.,respectively.In the association of Botrytis allii and B.byssoidea, there did not appear to be any inhibition of either fungus,if the environment was favourable to the growth of both.The factors of acidity,temperature,and the amount of inoculum,however,are not always optimum for the growth of both organisms,so that the one more favoured by any of these factors is better able to grow in the mixed culture on artificial media,while the growth of the other isless,though not necessarily.

The inhibition of the growth of these fungi,which is at times reduced under an environment apparently favourable to the growth of fungi,is probably due to the influence of some toxic substance other than acid.Acidity of the medium influences the growth of fungi considerably,but in the above fungi inhibition of growth may have been due to the presence of toxic substances or to the lack of available foods,though it has been shown by experiment that the former factor is rresponsible for the inhibition.Even though small amounts of the toxic medium were added to large amounts of fresh medium,a definite decrease in growth occurred with some fungi which were tested.Autoclaving or neutralizing the medium did not induce any increased growth,and in no case did such treatment completely restore the medium.

The addition of a growth accesory substance in the form of onion extract did not cause any increase in growth of Botrytis allii in Richard's nutrient solution,though various amounts of the

extract were added to the culture flasks.Similar quantities added to cultures of Erwinia caratovora caused a noticeable increase in growth.Similar results were obtained when various plant extracts were added to cultures of Azotobacter chroococcum and Rhizobium radicicolum growing in Asby's Nitrogen-free medium.

The experimental work performed has been sufficient to show that association of phytopathogens commonly occurs in nature, but that the combinations of the factors involved are very complex. The type of association is found to vary with differnt organisms and differant environmental conditions.This involves a considerable expenditure of time and labor,even though associations of two organisms at a time are studied in detail.It was found that an increased number of these organisms in a mixed culture would render the analysis of the relations a very difficult one. More work is necessary to determine the effects of environmental influences,for these appear to be the salient ones.

Summary and Conclusions.

The phytopathogens used in the experimental work during this investigation were those causing rots of stored fruits and vegetables,and of green-house tomatoes.

Association of phytopathogens has been observed in a large number of hosts.These pathogens may be associated with others, or with saprophytic organisms which usually follow the pathogens in their advance through the host tissues.The relations in all cases are complex.

Even though the pathogens may produce a certain result in artificial media, a totally different result may occur on the host.

Association of phytopathogens in artificial media may produce different results when different organisms are associated,those results being controlled by several factors,such as temperature, H-ion concentration,the amount of inoculum;When in the host,the results are controlled by the temperature,and the order in which infections take place.

Several types of association exist:(1) when one organism causes complete inhibition of its associate in artificial media; (2)when there is mutual tolerance between the associated organisms on culture media;(3)when one organism may inhibit the second in artificial culture,but when the same fungus is on the host it may be slow in growth and follows in the second's wake.

The H-ion concentration has no marked influence upon association,although sometimes the inhibited organism can grow in a culture medium of which the H-ion concentration is such as prevents the growth of the inhibiting organism.

Temperature is a limiting factor in association of phyto-pathogens. Usually the results are similar when two organisms are grown together on the host or in artificial culture media. The temperature can sometimes be favourable to the inhibiting organism, so that it may grow well in mixed culture, when at ordinary temperatures (20 - 26°C.) it may be totally suppressed. Therefore it may be expected that the rate of rot can be compared to the rate of growth in culture, and where two pathogens are associated in the host the one most suited to grow at a given temperature will dominate and produce its own characteristic rot.

The amount of inoculum in artificial culture is another limiting factor. One organism may become completely suppressed if the amount of the inoculum of the second is in excess of the first, or in other words, an inhibited organism is able to grow well if the inoculum of that organism is in excess of the inoculum of the inhibiting organism. The latter can also grow in the mixed culture and eventually prevents further growth of its weaker associate.

The inhibition of one organism by another is found to be due to some toxic substances other than acid. An analysis is required to show the nature of these toxic substances. Some of the inhibition of growth may be due to acidity of the medium; or to the unequal assimilation of available foods from the media by the organisms growing on it, causing the starvation of one organism.

The addition of vegetable extract does not cause any visible increase of fungous growth, although a more positive result is obtained in bacterial cultures to which this extract has been added.

The phytopathogens studied in the course of the above investigations were unable to grow well in the absence of free-oxygen. The addition of peroxidase in the form of sterile, raw potato-tuber

tissue did not change this condition, though in cultures of aerobic bacteria the addition of peroxidase from this source allowed growth to take place.

Spore germination has been found to be affected by temperature, H-ion concentration, and the type of medium. Spore germination is decreased when the above factors are extreme.

Acknowledgements.

The writer is indebted to Dr. B.T.Dickson and Prof. J.G. Coulson for many helpful suggestions,and for their kindness in affording laboratory facilities;to Dr. J.R.Sanborn,Dr. H.D.Brunt, Dr. F.C.Harrison,and Mr.T.C.Vanterpool for their constructive criticism;and to the Department of Horticulture,Macdonald College,for the free access to their store-rooms.

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