# STUDIES ON SOME MARKET DISEASES OF TOMATOES WITH SPECIAL REFERENCE TO ANTHRACNOSE ( *COLLETOTRICHUM PHOMOIDES* (SACC.) CHESTER).

---

Réal L. Pelletier

Department of Plant Pathology

---

A THESIS

submitted to McGill University in

partial fulfilment of the requirements for

the degree of

MASTER OF SCIENCE

---

September, 1944

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

# INTRODUCTION

Anthracnose of tomatoes is a fungus disease that manifests itself on ripe fruits. A survey of fruit diseases on the Montreal market showed it to be one of the most important of the tomato diseases. Since it is essentially a rot of ripe fruits, the principal losses occur in the retail stores. Losses caused by anthracnose have also been sustained, however, by field growers picking ripe tomatoes for the local market and for canning (McNew,1943a). A survey of the literature showed that, even though the disease was recorded as early as 1881, comparatively little was known about the life history of the causal organism, the factors affecting the development of the disease, or the control measures that might be employed. This investigation was therefore undertaken in an attempt to gain some information on these points.

## HISTORICAL REVIEW

The first descriptions of tomato anthracnose were those of Plowright in England and Saccardo in Italy in 1881. The former named the causal fungus Shphaeronema Lycopersici and the latter describing what was apparently the same organism, named it Gloeosporium phomoides. The disease was first described, on the American continent, as a "ripe rot of tomatoes", by Arthur in the United States in 1885. He accepted the name Gloeosporium phomoides Sacc. In 1891, Chester again described the disease in the United States and applied the name "ripe rot or anthracnose" to it. He first named the causal organism Colletotrichum Lycopersici later changing this to Colletotrichum phomoides, and finally to Colletotrichum fructigenum. Further studies centering mainly around the classification of the causal organism were made by Stoneman (1898) in the United States, Guéguen (1902) in France, and Edgerton (1908) and Taubenhaus (1912) in the United States. Seymour (1929) in his "Host index of the fungi of North America" summarized the resulting synonymy as follows:

Ascomycetes:

Glomerella cingulata (Stoneman) Spauld & von Sch
" rufomaculans Spauld & von Sch.

Melanconiales:

Colletotrichum gloeosporioides Penz.
Gloeosporium fructigenum B.
" versicolor B & C.
Glomerella rufomaculans Spauld & von Sch.

Colletotrichum Lycopercisi Chester, 1891.
" " Ell & Ev. 1893.

Colletotrichum phomoides (Sacc.) Chest.
Gloeosporium phomoides Sacc.

The name of the causal organism most frequently adopted appears to be Colletotrichum phomoides (Sacc.) Chester and it is therefore used in this paper.

The first author to study the disease from an economic stand point was Rosenbaum (1918) in the United States. He was principally concerned with the spread of tomato diseases in transit and found in this connection, that anthracnose did not spread through paper wrappers or infect healthy adjacent fruits. In 1931, Ocfemia reported that anthracnose caused five percent field loss in Los Banos (Philippines) tomatoes. Ramsey and Link (1932) in studying the market diseases of tomatoes, found that anthracnose was of considerable importance on fruits, ripened on the vines, for the local market or canning. It was not as serious on green wrapped export tomatoes, although it was occasionally found on fruits ripening in transit and in ripening rooms at the receiving markets. They emphasized that very little was known about the overwintering

of the causal organism or the relation of environmental factors to infection and disease production. Wardlaw and McGuire (1932), investigating the possibilities of shipping tomatoes from the British West Indies to Canada, found that Trinidad-grown tomatoes could be held in cold storage for 20 days, followed by a distribution period of ten to fourteen days, without undue wastage. The common molds mainly responsible for the rotting of ripe tomatoes in temperate countries were entirely absent in their storage trials and anthracnose was not very important when compared to the rot caused by Phomopsis spp. and Phoma destructiva. Nightingale and Ramsey (1936) in temperature studies on the causal organism, Colletotrichum phomoides, found that it would grow over a wide range of temperatures on both green and ripe puncture-inoculated tomato fruits as well as on media of an acidity corresponding to these stages of fruit maturity. They concluded that the temperature ordinarily used in the storage of tomatoes would not satisfactorily prevent the development of anthracnose in previously infected fruits. More recently, Baker (1938) described a type of infection occurring early in the life of the fruit but manifesting itself only at the time of ripening. He termed this type of infection "latent infection", and found it to be an important characteristic of the

anthracnose diseases of fruits, including the one occurring on tomatoes. Latent infections were found to be of particular significance when fruits were picked green for shipment to distant markets, because apparently healthy fruits became badly spotted as they ripened in transit and on the market. Jones (1940) and Parris and Jones (1941) working on methyl bromide fumigation found that it delayed ripening from three to six days and caused latent infections to resume activity in unripe papayas, beans and avocadoes. Similar treatment, when applied to tomato fruits, delayed their ripening but usually did not increase the amount of anthracnose on immature fruits.

More recently, McNew (1943a) reported that the three most important diseases of tomatoes, namely, anthracnose, stem-end rot and fruit blight, caused up to 30% fruit rot in the fields of New York. Of these, anthracnose was the most serious and objectionable because the small fruit spots present in the early stages of the disease were easily overlooked. Particular importance was attributed to the occurrence of these spots, since in the canning process a few infected fruits increased the mold count of the finished product and rendered it unsalable under government regulations. He also found that from 5% to 15% of the tomato fruits were ordinarily affected by anthracnose and as

many as 70% might be affected in some instances. In field spraying trials which included a number of different copper fungicides and a new material named "Fermate" (ferric-dimethyldithiocarbamate), it was found that only the latter gave satisfactory control of anthracnose. Wilson (1943 and 1944) concluded that from the standpoint of the canning trade, anthracnose was the most important disease of the tomato in Ohio. It was of third importance for the growers, being less severe than the early blight and the Septoria blight. He obtained results similar to those of McNew (1943a) in his field spray trials, which included Fermate and other fungicides.

## DESCRIPTION OF THE DISEASE

Tomato anthracnose is a disease whose symptoms usually do not appear until the fruit is well ripened. In the early stages it is characterized by small circular slightly sunken spots on the fruit. These spots are of about the same colour as the healthy surrounding tissue. As the disease progresses the spot takes on a transluscent water-soaked appearance and the tissue becomes a slightly darker red than that of the rest of the tomato. Typical lesions are illustrated in Plate I. Sometimes the spot has a slight abrasion in its center that resembles an insect sting. Signs of the causal fungus appear as the spot enlarges. These signs seem to be of three distinct types: a mass of cream-coloured or salmon-pink spores in the center of the spot becoming brownish-black with age, a darkening of the lesion brought about by the production of dark fruiting bodies in concentric rings under the epidermis, or the production of spores and setae from a white stromatic fungal layer beneath the epidermis.

Lesions increase rapidly in size after their initial appearance. A fruit may be symptomless on picking but develop large lesions in 24 hours. The tissue in the

PLATE I

Typical Anthracnose Lesions

on John Baer Tomatoes.

lesions is felty and yellowish. Ordinarily the lesions do not extend into the fruit to a very great depth during transit or marketing, but they offer a channel of entrance for several organisms which soon destroy the whole fruit. In overripened fruits and in fruits ripened on the vine however, the spots may become over three centimeters in diameter and extend deeply into the pulp. When several lesions occur on a fruit they may coalesce to cover a considerable portion of the surface.

## THE CAUSAL ORGANISM

## *Colletotrichum phomoides* (Sacc.) Chester.

*Colletotrichum phomoides* (Sacc.) Chester is an imperfect fungus characterized by the production of one-celled, hyaline conidia in acervuli. Setae are usually associated with the conidia in these acervuli but their presence or absence seems to depend somewhat on the environmental conditions. During the course of this work numerous isolations of *C. phomoides* were made from tomato fruit lesions. These isolates appeared to be divisible into three distinct types or strains (Plates 2 and 3) which retained their characteristics through continuous subculturing. These types have been designated as Isolate A, Isolate B and Isolate C. Isolate A, when planted on a potato dextrose agar plate, produced a colony characterized by fluffy white mycelial growth (Plate 2,A), the absence of setaegenous structures, and the production of pink spore masses. Concentric rings, conspicuous on the underside of the plate, generally occurred. In contrast to this, Isolate C, (Plate 2,C) when grown on potato-dextrose agar, produced scant mycelial growth and numerous dark perithecia-like bodies with setae in concentric rings. Isolate B, (Plate 2,B) appeared to be intermediate in characters

PLATE II

Three Isolates of Colletotrichum phomoides

on Potato-dextrose Agar Plates.

Colonies are 12 days old.

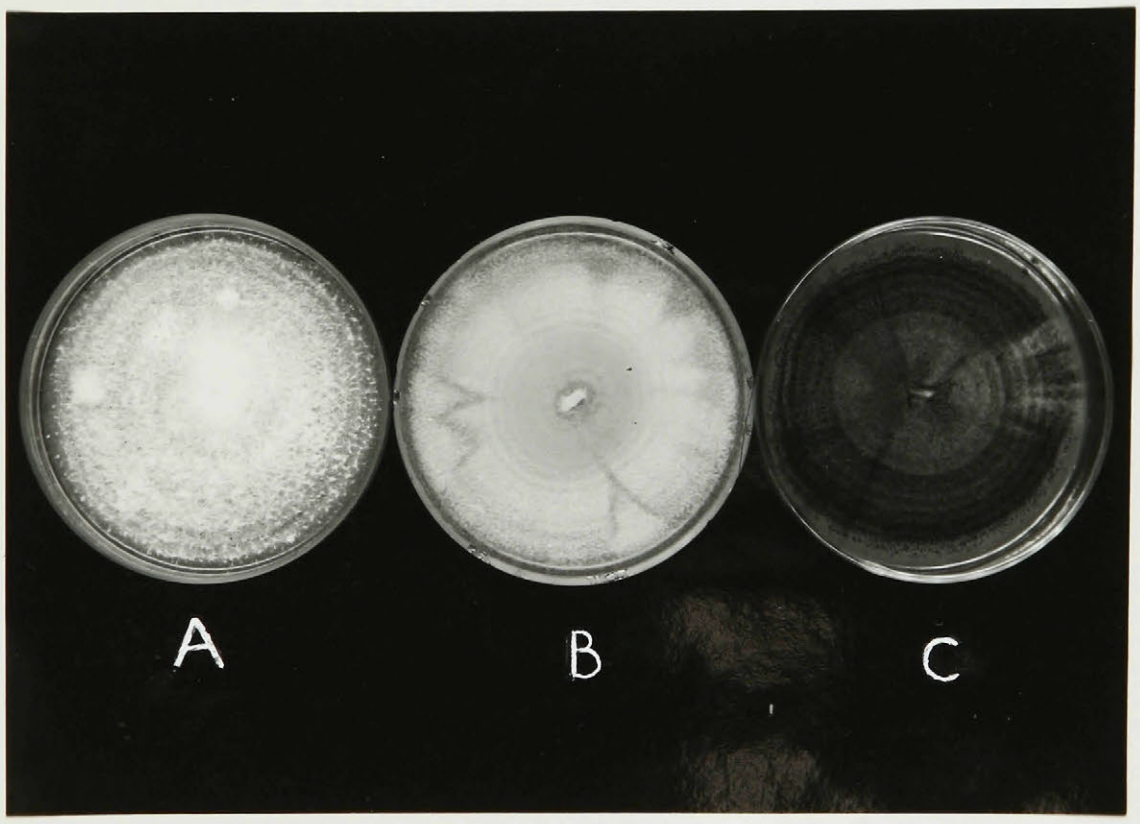
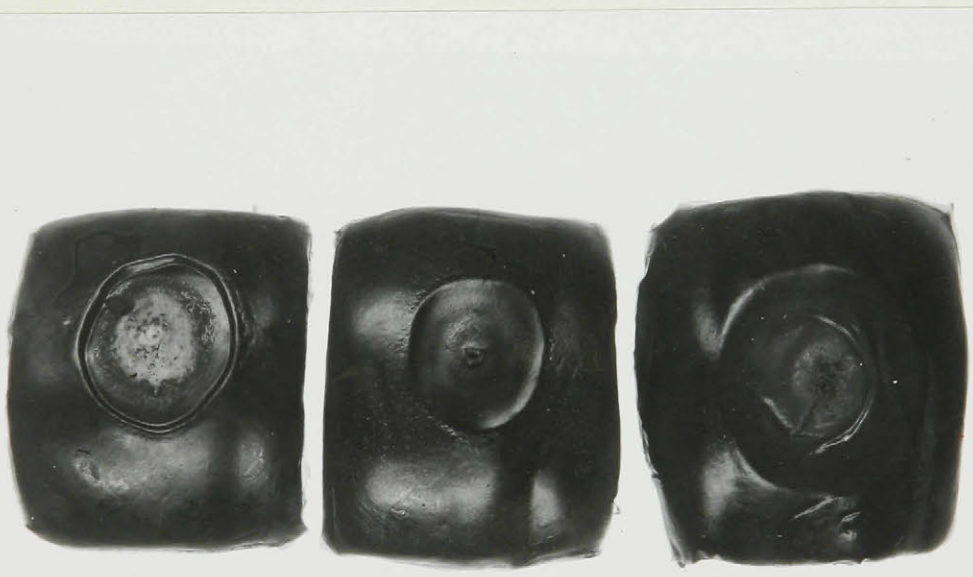


PLATE III

Anthracnose Lesions Produced by Isolates of *Colletotrichum phomoides* on a Tomato Fruit.



A B C

between Isolates A and C.

Early symptoms on puncture-inoculated tomato fruits were similar with all three isolates and consisted of small water-soaked slightly sunken spots. As the lesions enlarged however a divergence of symptoms occurred. When inoculation was done with Isolate A, cream to salmon-pink spore masses appeared in the center of the lesion at about the time it attained a diameter of one centimeter (Plate 3,A). These spore-bearing areas usually became brownish-black with age. When inoculation was done with Isolate C, the stromatic tissue under the epidermis often gave rise to perithecia-like bodies bearing dark setae. These bodies imparted a roughened appearance to the center of the lesion (Plate 3,C). In some instances they failed to form. In these cases, the lesion had a flat smooth rather than concave rough appearance. Lesions resulting from inoculation with Isolate C enlarged more rapidly than those produced by either Isolate A or B. The lesion developing from inoculation made with Isolate B showed a roughened surface as the fungus began to produce dark fruiting structures (acervuli) in concentric rings beneath the epidermis. This lesion was generally brownish-black in its early stage (Plate 3,B) in contrast to the lighter colour of those produced by Isolates A and C.

Isolations from these three types of lesions developed in plate culture, colonies showing the characteristics of the three isolates already described. Reinoculation on tomato fruits of these isolates produced lesions indistinguishable from the parent lesion. This fact suggests that the three types are actually three distinct physiologic forms. Some workers (Stoneman 1898 and Edgerton 1908) consider the fungus causing tomato anthracnose to be a distinct species while others (Chester 1891 and Taubenhaus 1912) consider it to be identical with the fungus causing bitter rot of apple. In order to check this point, puncture inoculations with the three isolates were made in ripe apples. It was found that Isolate C failed to grow but Isolates A and B grew readily on the apple fruit and produced symptoms indistinguishable from those commonly ascribed to bitter rot. It would seem, therefore, that there are at least two distinct physiologic forms of C. phomoides with the possibility of a third and that the disagreement between previous workers might be explained by the occurrence of physiologic forms with differing host range. Further work on this point is necessary, however, before definite conclusion can be drawn.

---

## LIFE HISTORY IN RELATION TO PATHOGENESIS

Accounts of the life history of *C. phomoides* appearing in the literature have been based largely on conjecture. A knowledge of the period of susceptibility of tomato fruits and of the method of overwintering of the fungus is necessary in an intelligent study of control measures. Tests were therefore undertaken in an attempt to obtain definite information on these points.

### Period of Susceptibility of Tomato Fruits

A test to determine the period during which tomato fruits were susceptible to infection by *C. phomoides* was conducted in the greenhouse. First Picking tomatoes planted in a soil-bed were used. Fruits of different known ages were obtained by tagging flowers at weekly intervals for a period of eight weeks, by the end of which time most of the fruits developing from the first tagged flowers had ripened. Tagging was discontinued and all plants were inoculated by spraying with an aqueous spore suspension of *C. phomoides*. A temperature of 32°C and a relative humidity of a 100% was maintained for a period of 24 hours, following inoculation, in order to encourage fruit infection.

After this incubation period the temperature was lowered to approximately 20°C and the humidity allowed to return to that ordinarily prevailing in the greenhouse. Fruits were picked as they ripened. All picked fruits were incubated in the laboratory and notes were taken on the number developing anthracnose. The results are summarized in Table I.

Table I. Effect of Age, of First Picking Tomato Fruits, at Time of Inoculation with Colletotrichum phomoides, on the Development of Anthracnose Following Ripening.

| Age of fruit at inoculation (weeks) | Percent of fruit with anthracnose. |
|---|---|
| 8 | 61.8 |
| 7 | 15.9 |
| 6 | 14.3 |
| 5 | 4.7 |
| 4 | 11.1 |
| 3 | 3.2 |
| 2 | 0 |
| 1 | 0 |
| Flower | 0 |

The high percentage of anthracnose resulting from inoculation of the 7 and 8-week old fruits can be

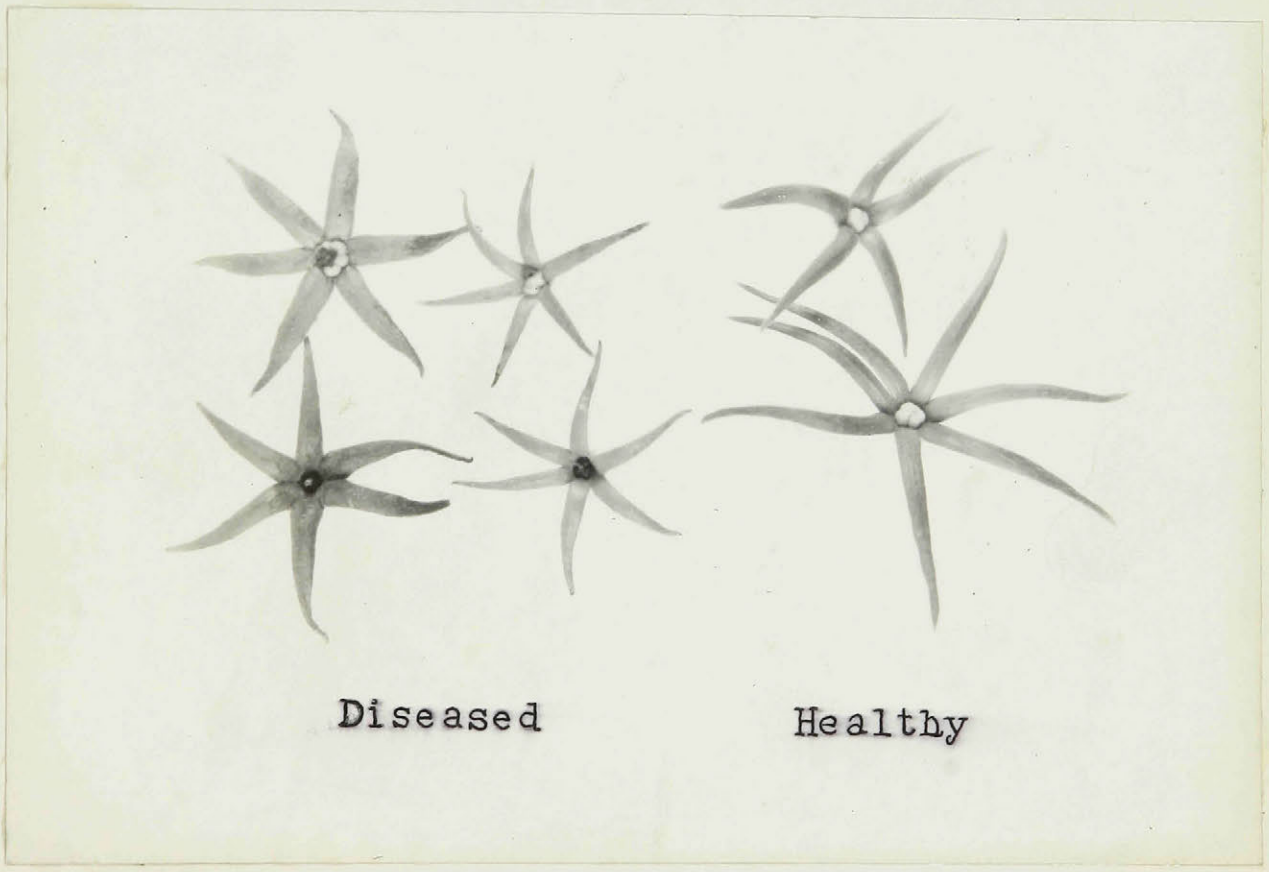
attributed to the fact that cracks formed on many of these fruits under the conditions of high humidity maintained during the incubation period. The resulting cracks provided an easy channel of entrance for the fungus. Following inoculation, growth of C. phomoides was observed on the stigmas and the stamens of many flowers. This resulted in the rapid blighting of the young ovary as illustrated in Plate 4 and in the abundant production of spores. Blossom infection usually resulted in abscission of the fruits while they were very small. For some unknown reason First Picking tomatoes inoculated one and two weeks after flowering did not develop anthracnose.

## Overwintering of Colletotrichum phomoides in Soil and on Tomato Refuse.

To test the winter survival of C. phomoides in the soil, 200 grams of moist greenhouse soil were placed in each of eight one pint fruit jars. These jars were then plugged with cotton and four of them were sterilized by autoclaving. A second similar series were prepared in which 10% of corn meal by weight was added to the soil. On October 23rd. 1943, all jars were inoculated by placing a 10 mm. disk cut from a potato-dextrose agar plate

PLATE IV

Blight of Young Tomato Fruits Resulting
from Blossom Infection by
Colletotrichum phomoides.



Diseased Healthy

culture of C. phomoides, on the top of the soil. Following a three-day incubation period in the laboratory, two jars of each treatment were placed outside. The other two jars of each treatment were maintained at room temperature. On April 3rd. 1944, soil from each jar was plated on potato-dextrose agar. In every instance, C. phomoides was recovered, proving that it had survived in the soil under both cold and warm conditions on sterilized and unsterilized soil, up to this date. Since tomato plants do not bloom until July under field conditions further tests for survival will be made at that time.

A similar experiment was designed to test for survival of C. phomoides on crop refuse. Short lengths of tomato stem were inoculated with C. phomoides by needle punctures and placed in test tubes containing about 3 ml. of water. These test tubes were plugged and placed outside on September 23rd. 1943. The fungus was successfully reisolated from these stem pieces on April 6th. 1944.

## Summary

C. phomoides overwinters in the soil, on tomato refuse and possibly on other hosts as indicated by the capacity of this organism to infect apples. There is every reason to believe that spores from these sources cause

blossom infection. The fungus growing on the stigmas and stamens produces a new supply of spores. These spores, spread by insects and other disseminating agents, will cause further floral and fruit infections. Once fruit infection occurs the fungus becomes dormant or latent and its presence can not be detected until the fruit ripens. At this time the dormancy ceases, the fungus develops rapidly and the characteristic fruit spots are produced.

## FACTORS AFFECTING THE APPEARANCE OF ANTHRACNOSE SYMPTOMS ON TOMATO FRUIT CARRYING LATENT INFECTIONS OF *COLLETOTRICHUM PHOMOIDES*.

Tomato anthracnose, as has already been pointed out, is typically a disease of ripe fruit. Preliminary observations on imported tomatoes indicated, however, that under certain conditions, the stage at which the spotting appeared, was advanced relative to the maturity of the fruit causing spoliage before the fruit could be consumed. The resulting loss presented a serious problem to the growers, wholesalers and retailers. Published information on latent infections deals largely with their demonstration and their prevention by field spraying. Little is known about the conditions that cause the latent infections to resume active growth. A study of a number of factors affecting the growth and development of *C. phomoides* on artificial media and in tomato fruit was therefore undertaken.

### Effect of Temperature on Growth of *Colletotrichum phomoides* in Culture and on Fruit.

The effect of temperature on growth of *C. phomoides*

in plate culture and in puncture-inoculated fruit was studied by Nightingale and Ramsey (1936). They concluded from their results that the storage temperature generally recommended for tomatoes was ineffective in controlling anthracnose. It was felt desirable to carry on an experiment similar to the one conducted by these workers, in order to check their results and to obtain exact information on the behavior of the strain of C. phomoides used in these studies.(1)

Methods - A graded series of temperatures, ranging from 9 to 38°C., were obtained by the use of a differential thermostat. Ripe tomato fruits were inoculated by puncturing the epidermis with a needle previously dipped in a water suspension of mycelium and spores of C. phomoides. Each fruit was inoculated in three places. Three such fruits were placed at each temperature. In order to compare fungus growth on artificial media with that on fruit, four plates, each poured with 15 ml. of potato-dextrose agar and inoculated in the center with a 7 mm. disk of mycelium, were placed at each temperature. A pan of water was kept in each compartment throughout the experiment in order to

(1) Isolate A of Colletotrichum phomoides, previously described under the heading "THE CAUSAL ORGANISM" was used throughout these studies.

maintain a high relative humidity. Daily records were kept of colony diameter in the plate cultures and lesion diameter on the fruits. After six days, plates and fruits were transferred to room temperature and the measurements continued.

Results - The effect of temperature on the growth of C. phomoides in culture, and on puncture-inoculated fruits, is given in Tables 2 and 3 respectively and illustrated in Figure 1. As can be seen from these data the optimum for growth, both in culture and on fruit lies between 24 and 29°C. The fungus has a slightly wider growth range when grown in culture than it has on tomato fruit. For growth in culture the minimum was between 9 and 12°C., and the maximum was between 32 and 38°C., while on fruit the minimum was between 12 and 14°C. and the maximum between 29 and 32°C. The optimum temperature for growth in culture was approximately 22 to 29°C., and for growth in fruit was 24 to 32°C. These results are in agreement with those of Nightingale and Ramsey (1936) except for the fact that their minimum temperature is lower than that found in this experiment. The cardinal points as given by these authors were: minimum 1.7°C., optimum 26.7°C., maximum 35°C., for growth in

Table 2 - Effect of Temperature on the Growth of *Colletotrichum phomoides* in Culture.

| Temperature °C. | Colony diameter in mm. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Day at indicated temperature | | | | | Day at 22°C.(1) | | |
| | 1st | 2nd | 3rd | 4th | 6th | 1st | 2nd | 3rd |
| 9 | 7(2) | 7 | 7 | 7 | 7 | 8 | 14 | 20 |
| 12 | 7 | 7 | 7 | 8 | 10 | 15 | 24 | 32 |
| 14 | 7 | 7 | 11 | 16 | 26 | 34 | 42 | 50 |
| 19 | 7 | 11 | 18 | 25 | 40 | 46 | 54 | 60 |
| 22 | 7 | 13 | 20 | 26 | 42 | 50 | 60 | 66 |
| 24 | 7 | 16 | 24 | 34 | 53 | 60 | 72 | 77 |
| 29 | 7 | 13 | 19 | 25 | 43 | 49 | 54 | 63 |
| 32 | 7 | 7 | 7 | 9 | 11 | 12 | 17 | 28 |
| 38 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |

(1) at the end of 6 days all plates were moved to a laboratory temperature of approximately 22°C.

(2) diameter of the disk of inoculum.

Table 3 - Effect of Temperature on the Growth of Colletotrichum phomoides in Puncture-inoculated Tomato Fruits.

| Temperature °C. | Lesion diameter in mm. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Day at indicated temperature | | | | | Day at 22°C.(1) | | |
| | 1st | 2nd | 3rd | 4th | 6th | 1st | 2nd | 3rd |
| 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 |
| 12 | 0 | 0 | 0 | 0 | 0 | 6 | 9 | 12 |
| 14 | 0 | 0 | 0 | 3 | 6 | 10 | 14 | 17 |
| 19 | 0 | 0 | 0 | 7 | 13 | 19 | 22 | 24 |
| 22 | 0 | 0 | 6 | 8 | 14 | 20 | 23 | 25 |
| 24 | 0 | 0 | 5 | 12 | 20 | 25 | 30 | 33 |
| 29 | 0 | 0 | 5 | 13 | 26 | 27 | 28 | 40 |
| 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 38 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

(1) at the end of 6 days all fruits were moved to a laboratory temperature of approximately 22°C.

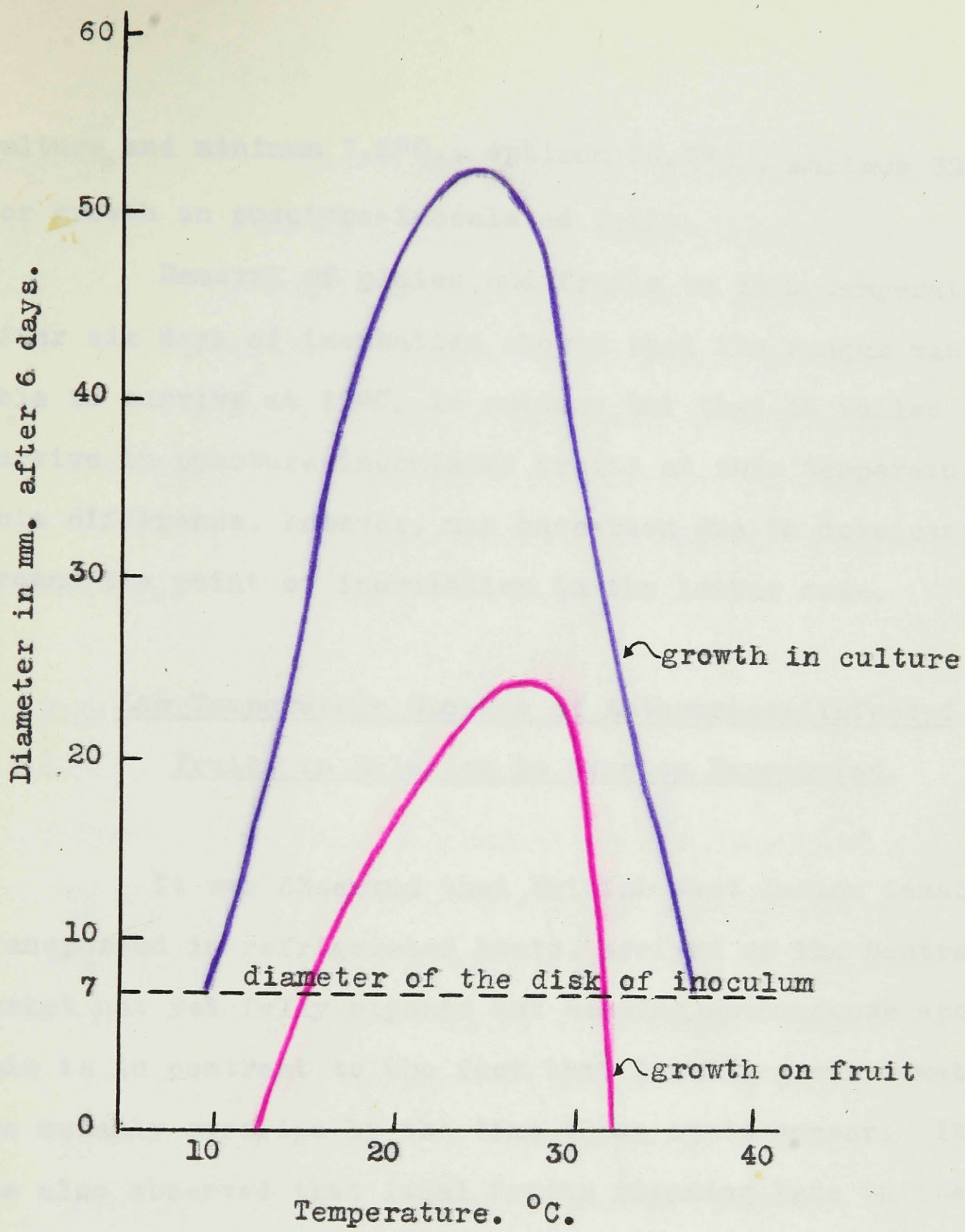



Figure 1 - Effect of temperature on the growth of Colletotrichum phomoides in culture and on tomato fruit.

culture and minimum 7.2°C., optimum 26.7°C., maximum 30°C., for growth on puncture-inoculated fruit.

Removal of plates and fruits to room temperature after six days of incubation showed that the fungus was able to survive at 32°C. in culture but that it failed to survive in puncture-inoculated fruits at this temperature. This difference, however, may have been due to dessication around the point of inoculation in the latter case.

## Low Temperature Storage of Anthracnose-infected Fruits in Relation to Symptom Expression.

It was observed that British West Indian tomatoes, transported in refrigerated boats, arrived on the Montreal market not yet fully ripened but bearing anthracnose spots. This is in contrast to the fact that locally grown tomatoes are usually overripe by the time these spots appear. It was also observed that local fruits ripening late in the season, when the nights were cold, developed anthracnose symptoms at a less mature stage than those ripening early in the season when the nights were warmer. Similar observations were made by Wardlaw, Baker and Crowdy (1939) who found that certain factors induced C. phomoides infections to lose their latent character. Among these factors were

exposure to low temperature, injury during cold storage and certain chemical treatments. This greatly complicated the problem of tomato storage. Further temperature studies were, therefore undertaken in which infected fruits were exposed to a cold storage temperature, corresponding to shipment under refrigeration, followed by an incubation period at room temperature, corresponding to marketing conditions.

Methods - The tomato fruits used in this test were of the John Baer variety. They were obtained from a field experiment (described in a later section) designed to test the value of various spray treatments for the control of C. phomoides. A separate record was kept of lots of fruits receiving different field spray treatments. Degree of fruit maturity was evaluated on the basis of colour and firmness, the following six classes being established:-

overripe - fruit deep red, lacking firmness, unmarketable.

ripe - fruit red and firm, marketable.

pink - fruit pink and firm, marketable.

turning - fruit yellowish with pink showing at blossom end, marketable.

mature-green - fruit yellow-green, marketable.

green - fruit green,unmarketable.

Ripe, turning and mature-green fruits were selected, for this experiment, from a field picking. Two similar samples, each of approximately 72 fruits, 24 at each stage of maturity, were selected from the fruits receiving each spray treatment. The tomatoes in one of each pair of samples were spread out on a laboratory table in a single layer. The temperature of the room ranged from 21 to 24°C. The fruits from the second sample were packed in a standard 30 pounds tomato box, set in a large refrigerator at 10 to 12°C., for 13 days and then spread out on the laboratory table beside the first sample (21-24°C.). Notes on fruit maturity and on anthracnose development were taken at intervals.

Results - The results of this experiment are presented in Tables 4 and 5. An examination of the figures giving the total percentage of anthracnose shows that the amount developing in fruit after a period of refrigeration is much higher than that found in fruit allowed to ripen at room temperature. Six days after removal, refrigerated fruit had reached approximately the same degree of maturity as the fruit held continuously at room temperature for thirteen days, yet the amount of anthracnose-infected fruits in the two lots were 87% and 41% respectively. After thirteen days

Table 4 - Effect of Field and Storage Treatment on the Appearance of Anthracnose Symptoms on Fruit Held Continuously at 21-24°C.

| Field spray treatment | Initial condition of fruit | Number of fruit | 6 days after picking | | 9 days after picking | | 14 days after picking | |
|---|---|---|---|---|---|---|---|---|
| | | | General condition of fruit | Percent of fruit with anthracnose | General condition of fruit | Percent of fruit with anthracnose | General condition of fruit | Percent of fruit with anthracnose |
| Bordeaux | Ripe | 24 | Ripe | 8 | Overripe | 17 | Overripe | 54 |
| | Turning | 24 | Ripe | 0 | Ripe | 8 | Overripe | 58 |
| | Mat.green | 30 | Turning | 0 | Pink | 0 | Ripe | 13 |
| Copper A | Ripe | 24 | Ripe | 17 | Overripe | 41 | Overripe | 75 |
| | Turning | 24 | Ripe | 8 | Ripe | 21 | Overripe | 37 |
| | Mat.green | 28 | Turning | 0 | Pink | 3 | Ripe | 11 |
| Fermate I | Ripe | 24 | Ripe | 8 | Overripe | 12 | Overripe | 29 |
| | Turning | 22 | Ripe | 0 | Ripe | 0 | Overripe | 9 |
| | Mat.green | 20 | Turning | 0 | Pink | 0 | Ripe | 5 |
| FermateII | Ripe | 23 | Ripe | 13 | Overripe | 51 | Overripe | 73 |
| | Turning | 22 | Ripe | 0 | Ripe | 5 | Overripe | 36 |
| | Mat.green | 20 | Turning | 0 | Pink | 0 | Ripe | 40 |
| Check | Ripe | 24 | Ripe | 17 | Overripe | 58 | Overripe | 83 |
| | Turning | 20 | Ripe | 5 | Ripe | 15 | Overripe | 65 |
| | Mat.green | 26 | Turning | 0 | Pink | 8 | Ripe | 30 |
| Total % | Ripe | 119 | Ripe | 13 | Overripe | 38 | Overripe | 64 |
| | Turning | 112 | Ripe | 22 | Ripe | 10 | Overripe | 41 |
| | Mat.green | 124 | Turning | 0 | Pink | 2 | Ripe | 19 |
| | All stages | 355 | - | 5 | - | 17 | - | 41 |

Table 5 - Effect of Field and Storage Treatment on the Appearance of Anthracnose Symptoms on Fruit Held for 13 Days at 10-12°C. and then at 21-24°C.

| Field spray treatment | Initial condition of fruit | Number of fruit | 13 days after picking | | 14days after picking | | 15days after picking | | 19days after picking | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | General condition of fruit | % of fruit with anthracnose | General condition of fruit | % of fruit with anthracnose | General condition of fruit | % of fruit with anthracnose | General condition of fruit | % of fruit with anthracnose |
| Bordeaux | Ripe | 24 | Ripe | 79 | Overripe | 100 | Overripe | 100 | Overripe | 100 |
| | Turning | 24 | Pink | 41 | Pink | 79 | Ripe | 92 | Overripe | 100 |
| | Mat.green | 30 | Turning | 17 | Turning | 37 | Pink | 67 | Ripe | 70 |
| Copper A | Ripe | 24 | Ripe | 71 | Overripe | 88 | Overripe | 88 | Overripe | 88 |
| | Turning | 24 | Pink | 50 | Pink | 88 | Ripe | 100 | Overripe | 100 |
| | Mat.green | 28 | Turning | 14 | Turning | 25 | Pink | 54 | Ripe | 64 |
| Fermate I | Ripe | 24 | Ripe | 42 | Overripe | 79 | Overripe | 92 | Overripe | 96 |
| | Turning | 22 | Pink | 27 | Pink | 36 | Ripe | 64 | Overripe | 73 |
| | Mat.green | 20 | Turning | 0 | Turning | 10 | Pink | 40 | Ripe | 55 |
| FermateII | Ripe | 23 | Ripe | 74 | Overripe | 78 | Overripe | 96 | Overripe | 100 |
| | Turning | 22 | Pink | 68 | Pink | 90 | Ripe | 100 | Overripe | 100 |
| | Mat.green | 20 | Turning | 20 | Turning | 55 | Pink | 65 | Ripe | 75 |
| Check | Ripe | 24 | Ripe | 92 | Overripe | 96 | Overripe | 96 | Overripe | 96 |
| | Turning | 20 | Pink | 70 | Pink | 85 | Ripe | 90 | Overripe | 100 |
| | Mat.green | 26 | Turning | 46 | Turning | 81 | Pink | 85 | Ripe | 92 |
| Total % | Ripe | 119 | Ripe | 71 | Overripe | 88 | Overripe | 94 | Overripe | 96 |
| | Turning | 112 | Pink | 52 | Pink | 72 | Ripe | 89 | Overripe | 95 |
| | Mat.green | 124 | Turning | 20 | Turning | 42 | Pink | 63 | Ripe | 72 |
| | All stages | 355 | - | 45 | - | 68 | - | 82 | - | 87 |

of refrigeration the percentage of fruit showing anthracnose lesions increased from 45% to 68% within 24 hours, and to 87% within six days.

The time at which anthracnose symptoms appeared was also advanced in relation to the stage of fruit maturity by a period of refrigeration. After refrigeration a high proportion of the fruit developed anthracnose symptoms while still in a marketable condition. In contrast to this the fruit held continuously at room temperature had reached an advanced stage of ripeness and was unmarketable before the appearance of symptoms,

Field spray treatments, with the exception of Fermate, did not influence the development of anthracnose symptoms. Four field applications of Fermate, as compared to the other treatments, lower the percentage of fruit showing the disease after thirteen days either at room temperature or in the refrigerator. Six days after removal from the refrigerator, however, the percentage of anthracnose in Fermate-sprayed fruit had increased and was not significantly different from that in fruit receiving other spray treatments. It appears then that spraying in the field with Fermate delays the appearance of anthracnose symptoms on the fruit but does not prevent infection by C. phomoides.

## Effect of Various Carbon and Nitrogen Sources and pH on the Growth and Development of *Colletotrichum phomoides* in Culture.

The loss of latency by *C. phomoides* in tomato fruits has been attributed to changes in the chemical composition of the fruit accompanying ripening (Simmonds, 1941; Wardlaw, Leonard and Barnell, 1939). The most obvious of these are the qualitative and quantitative changes in carbohydrate and nitrogen content and the increased pH of the fruit. Comparative chemical analysis of green and ripe tomato fruit were made by Sando (1920), Rosa (1925), and Saywell and Robertson (1932). Their results are summarized in Table 6.

### Methods

The medium used consisted of a solution of the following salts:

| | |
|---|---|
| Monopotassium phosphate ($KH^2PO^4$) | 1.00 g. |
| Magnesium sulphate ($MgSO^4,7H^2O$) | 0.50 g. |
| Potassium chloride (KCl) | 0.50 g. |
| Ferrous sulphate ($FeSO^4$) | 0.01 g. |
| Distilled water | 1000.00 ml. |

Table 6 - Composition of the Tomato Fruit at Different Stages of Maturity with Respect to Carbohydrate and Nitrogen Content, and Hydrogen Ion Concentration.

| Fruit constituent | Stage of maturity | | | | | |
|---|---|---|---|---|---|---|
| | Green | Mature green | | Ripe | | |
| | Sando (1920) | Sando (1920) | Rosa (1925-26) | Sando (1920) | Rosa (1925-26) | Saywell & Robertson (1932) |
| Total sugar (1) | 1.743 | 2.37 | 2.48 | 2.66 | 3.075 | - |
| Reducing sugars (1) | 1.724 | 2.30 | 2.36 | 2.63 | 3.035 | 2.32 to 3.76 |
| Total (1) carbohydrate | 3.64 | 3.62 | - | 3.44 | - | - |
| Total nitrogen (1) | 0.199 | 0.140 | 0.186 | 0.116 | - | - |
| pH | 4.7 | - | 4.3 | 6.01 | 4.5 | - |

(1) expressed as a % of fresh weight.

In the carbohydrate studies, nitrogen was supplied by adding 0.2% sodium nitrate while in the nitrogen studies, carbohydrate was supplied by adding 3% dextrose. In the pH studies both these constituents were added in the above concentration. All media were solidified by the addition of 1.5% agar-agar. Plates were inoculated by placing in their center a circular disk, 7 mm. in diameter, cut from a colony of C. phomoides growing on potato-dextrose agar. Six plates were poured with each medium, 10 ml. being put in each plate. All plates were incubated at 24°C. in a constant temperature oven. Notes were taken on growth, as measured by colony diameter and density, and on spore production.

Colony diameter was measured with a millimeter ruler, two measurements at right angles to one another being taken on each occasion.

Density of growth was measured by means of the apparatus shown in Figure 2. In this apparatus a General Electric photoelectric light meter was inserted in the end of a black box. At the other end of the box a circular hole, 10 mm. in diameter, was made. It was located directly opposite the center of the ground glass of the photoelectric light meter. A beam of light from a projection lantern

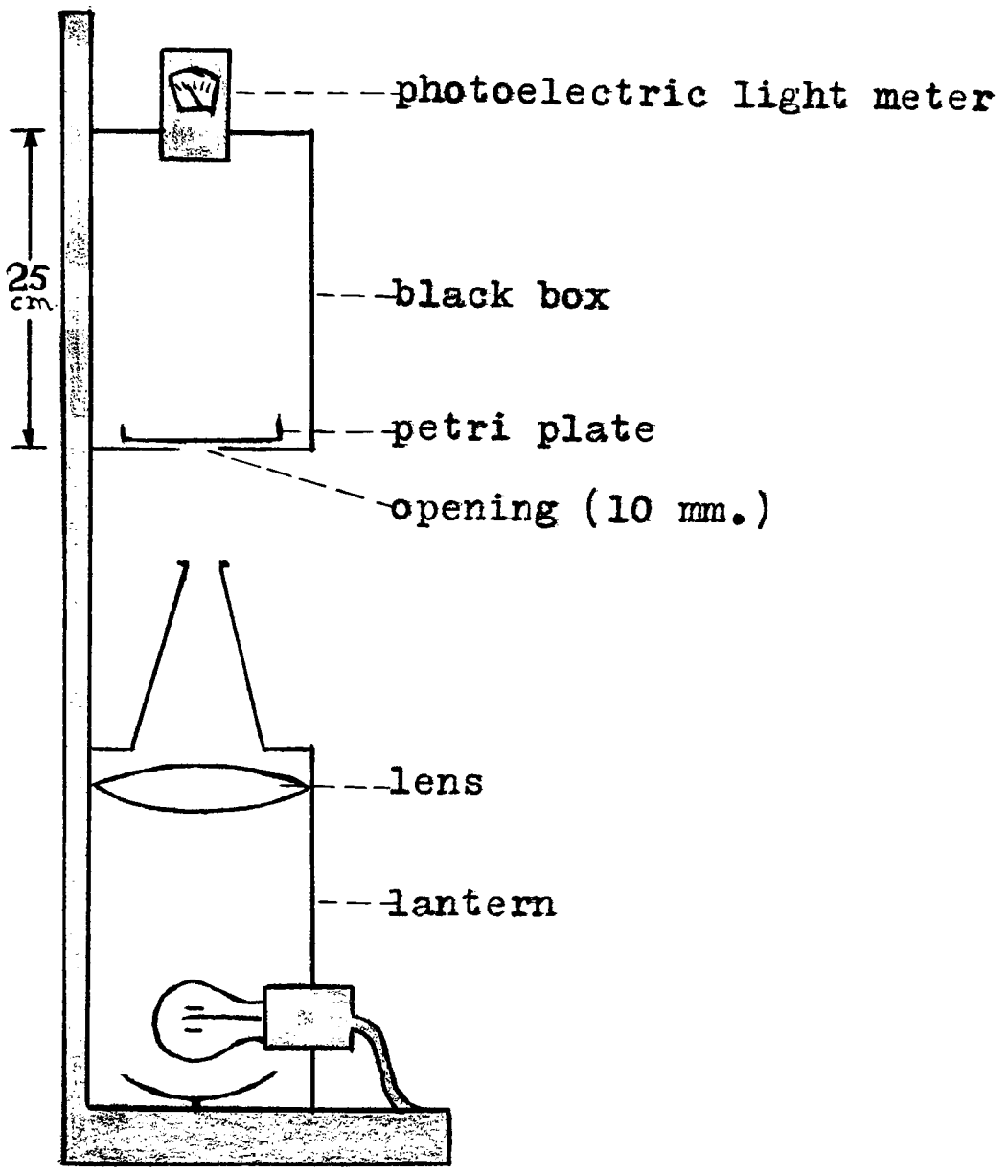



Figure 2 - An apparatus for the measurement of the growth density of fungal colonies.

was directed through this hole and impinged on the photo-electric cell. The apparatus was standardized by placing an uninoculated Petri dish, containing the medium on which growth was being studied, over the hole in the box, as indicated in the diagram. The box was then closed and the reading of the light meter adjusted to 70 foot candles (the maximum reading of the meter) by varying the distance of the light source from the box. The Petri plate was then removed and replaced by one of those on which a measurement was to be taken. The light value in foot candles was recorded. (The readings obtained of course were only relative, since they apply only to the particular apparatus used.) Eight such readings were taken in various places over the surface of the colony. An average of these readings was substracted from the original 70 foot candles to give the amount of light obstructed and this was used as an index of growth density.

Spore production was determined by a dilution method. Eight disks, each 10 mm. in diameter were cut, at random, with a sterile cork-borer, from the colony on which a determination was being made. These disks were put in a test tube, containing 10 ml. of water, thoroughly crushed with a glass rod, and spore counts made with the aid of a haemocytometer. From these spore counts the number of spores per square millimeter of colony surface

was calculated.

Effect of sugars on the growth and development of Colletotrichum phomoides.

A comparison was made of the effect of varying concentrations of galactose, dextrose, levulose, lactose, maltose and sucrose, on the growth and development of C. phomoides. In each instance the medium consisted of the basic medium to which the sugar had been added. The concentrations used were 0.25, 0.5, 1.0 and 2.0%.

The effect of the various concentrations of these sugars on the daily growth of C. phomoides, as measured by colony diameter, is presented in Table 7. These data show that dextrose, levulose, maltose and sucrose, at concentrations up to 2% do not influence the rate of diameter increase, while with lactose and galactose, diameter decreased with increasing concentration. The data presented for a sugar concentration of 2% in Table 7 are presented graphically in Figure 3. Colony diameter varied with the kind of sugar, being greatest with maltose and least with galactose. Table 8 gives the colony diameter, density and spore production after nine days of growth. This table shows that colony density increased with increasing

Table 7 - Effect of Various Sugars on the Growth of Colleto-trichum phomoides as Measured by Colony Diameter.

| Number of days | Sugar concentration % | Colony diameter in mm. | | | | | |
|---|---|---|---|---|---|---|---|
| | | Dextrose | Galactose | Levulose | Lactose | Maltose | Sucrose |
| 1 | 0.0 | 7 | 7 | 7 | 7 | 7 | 7 |
| | 0.25 | 8 | 7 | 8 | 8 | 9 | 8 |
| | 0.5 | 8 | 7 | 8 | 7 | 9 | 8 |
| | 1.0 | 8 | 7 | 8 | 7 | 10 | 8 |
| | 2.0 | 7 | 7 | 8 | 7 | 9 | 7 |
| 2 | 0.0 | 17 | 17 | 17 | 17 | 17 | 17 |
| | 0.25 | 14 | 9 | 15 | 14 | 17 | 13 |
| | 0.5 | 14 | 8 | 15 | 14 | 18 | 14 |
| | 1.0 | 15 | 9 | 15 | 14 | 19 | 14 |
| | 2.0 | 14 | 11 | 15 | 12 | 18 | 14 |
| 3 | 0.0 | 26 | 26 | 26 | 26 | 26 | 26 |
| | 0.25 | 25 | 12 | 25 | 23 | 26 | 24 |
| | 0.5 | 24 | 11 | 25 | 22 | 29 | 25 |
| | 1.0 | 23 | 12 | 24 | 21 | 28 | 24 |
| | 2.0 | 24 | 13 | 23 | 21 | 28 | 28 |
| 4 | 0.0 | 30 | 30 | 30 | 30 | 30 | 30 |
| | 0.25 | 30 | 16 | 29 | 29 | 35 | 30 |
| | 0.5 | 30 | 13 | 28 | 29 | 36 | 31 |
| | 1.0 | 40 | 13 | 29 | 26 | 36 | 31 |
| | 2.0 | 30 | 14 | 29 | 26 | 34 | 31 |
| 5 | 0.0 | 38 | 38 | 38 | 38 | 38 | 38 |
| | 0.25 | 40 | 18 | 37 | 36 | 44 | 39 |
| | 0.5 | 40 | 15 | 36 | 32 | 46 | 39 |
| | 1.0 | 40 | 14 | 38 | 33 | 46 | 39 |
| | 2.0 | 39 | 15 | 38 | 33 | 45 | 38 |
| 6 | 0.0 | 47 | 47 | 47 | 47 | 47 | 47 |
| | 0.25 | 48 | 22 | 48 | 45 | 53 | 48 |
| | 0.5 | 48 | 20 | 46 | 39 | 57 | 48 |
| | 1.0 | 47 | 18 | 46 | 37 | 56 | 48 |
| | 2.0 | 46 | 18 | 46 | 37 | 53 | 46 |
| 7 | 0.0 | 53 | 53 | 53 | 53 | 53 | 53 |
| | 0.25 | 56 | 25 | 57 | 50 | 63 | 55 |
| | 0.5 | 56 | 22 | 50 | 41 | 67 | 55 |
| | 1.0 | 56 | 20 | 53 | 42 | 64 | 55 |
| | 2.0 | 54 | 19 | 54 | 42 | 63 | 54 |
| 8 | 0.0 | 64 | 64 | 64 | 64 | 64 | 64 |
| | 0.25 | 64 | 28 | 63 | 53 | 72 | 63 |
| | 0.5 | 63 | 25 | 58 | 49 | 76 | 63 |
| | 1.0 | 62 | 22 | 60 | 47 | 73 | 64 |
| | 2.0 | 62 | 20 | 61 | 46 | 72 | 62 |
| 9 | 0.0 | 71 | 71 | 71 | 71 | 71 | 71 |
| | 0.25 | 71 | 31 | 70 | 60 | 79 | 70 |
| | 0.5 | 71 | 27 | 70 | 54 | 84 | 70 |
| | 1.0 | 69 | 23 | 67 | 51 | 81 | 71 |
| | 2.0 | 69 | 21 | 68 | 51 | 79 | 69 |

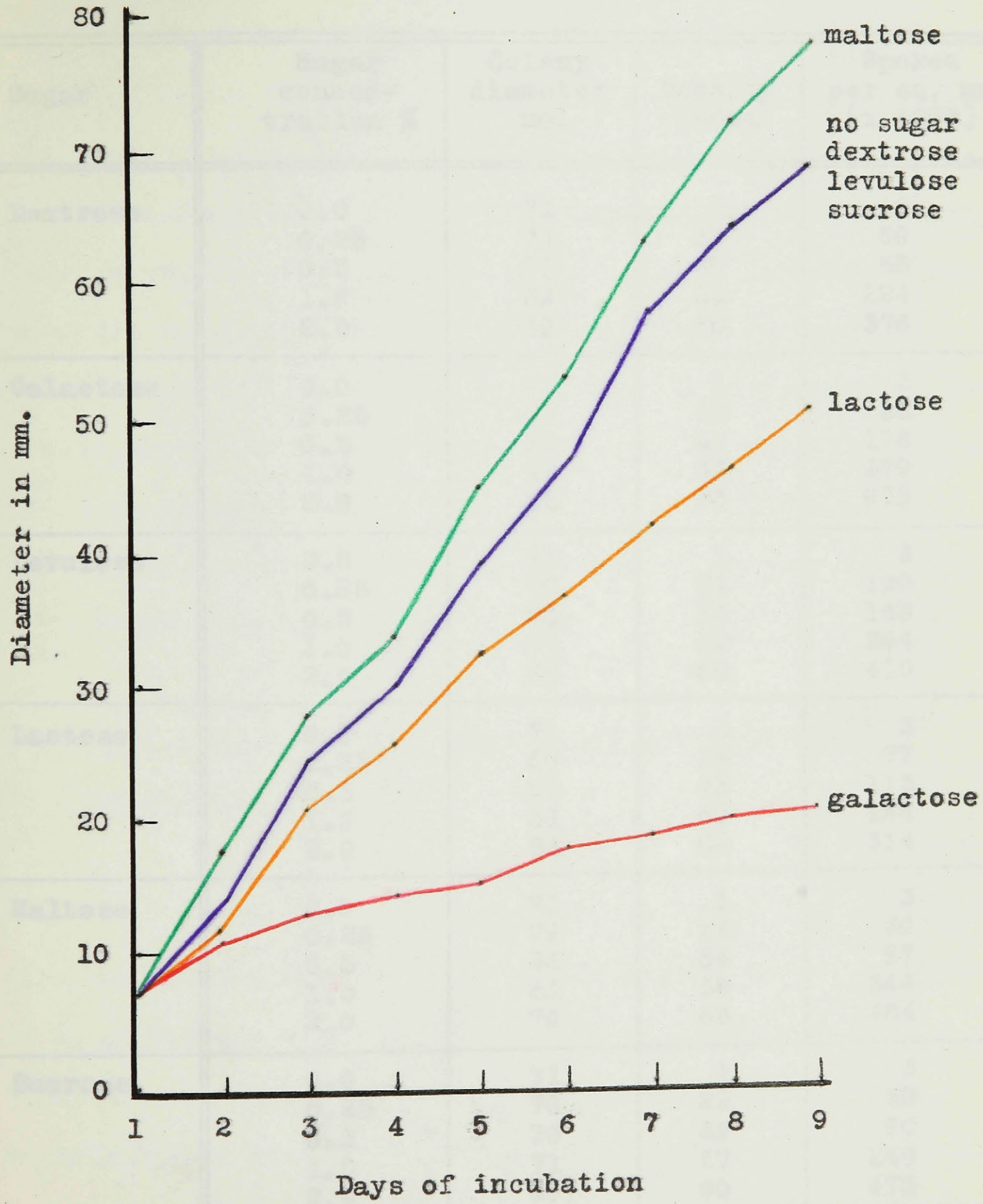



Figure 3 - Effect of various sugars on the growth of Colletotrichum phomoides as measured by colony diameter.

Table 8 - Effect of Various Sugars on the Growth and Sporulation of Nine-day Old Colonies of Colletotrichum phomoides.

| Sugar | Sugar concentration % | Colony diameter mm. | Density index | Spores per sq. mm. (x 1000) |
|---|---|---|---|---|
| Dextrose | 0.0 | 71 | 1 | 3 |
| | 0.25 | 71 | 17 | 60 |
| | 0.5 | 71 | 39 | 88 |
| | 1.0 | 69 | 53 | 224 |
| | 2.0 | 69 | 66 | 376 |
| Galactose | 0.0 | 71 | 1 | 3 |
| | 0.25 | 31 | 43 | 86 |
| | 0.5 | 27 | 47 | 178 |
| | 1.0 | 23 | 66 | 379 |
| | 2.0 | 21 | 68 | 975 |
| Levulose | 0.0 | 71 | 1 | 3 |
| | 0.25 | 70 | 24 | 100 |
| | 0.5 | 70 | 28 | 168 |
| | 1.0 | 67 | 62 | 294 |
| | 2.0 | 68 | 65 | 410 |
| Lactose | 0.0 | 71 | 1 | 3 |
| | 0.25 | 60 | 18 | 77 |
| | 0.5 | 54 | 43 | 115 |
| | 1.0 | 51 | 63 | 166 |
| | 2.0 | 51 | 64 | 314 |
| Maltose | 0.0 | 71 | 1 | 3 |
| | 0.25 | 79 | 14 | 30 |
| | 0.5 | 84 | 34 | 97 |
| | 1.0 | 81 | 56 | 344 |
| | 2.0 | 79 | 63 | 484 |
| Sucrose | 0.0 | 71 | 1 | 3 |
| | 0.25 | 70 | 25 | 52 |
| | 0.5 | 70 | 42 | 90 |
| | 1.0 | 71 | 57 | 240 |
| | 2.0 | 69 | 60 | 475 |

sugar concentration. The data showing the effect of kind and concentration of sugar on colony density are presented graphically in Figure 4. As can be seen from this figure, the kind of sugar had little effect on colony density. Plate V illustrates the colonies produced on media containing various sugars, while Plate VI illustrates colonies produced on media containing various concentrations of sucrose. Spore production, per unit area (Table 8 and Figure 5) was greatest on a medium in which carbohydrate was supplied in the form of galactose. Since galactose colonies were relatively small the total spore production was, however, much lower than with other sugars.

Effect of nitrogen on the growth and development of Colletotrichum phomoides.

The effect of sodium nitrate, ammonium sulphate, glycine, asparagine and urea on the growth and development of C. phomoides was compared. Each of these nitrogen sources was added to the basic medium in amounts calculated to give a nitrogen content of 0.004, 0.02, 0.1 and 0.5 grams per liter. The pH of each medium was found to be 5.8 so that adjustment of acidity was unnecessary.

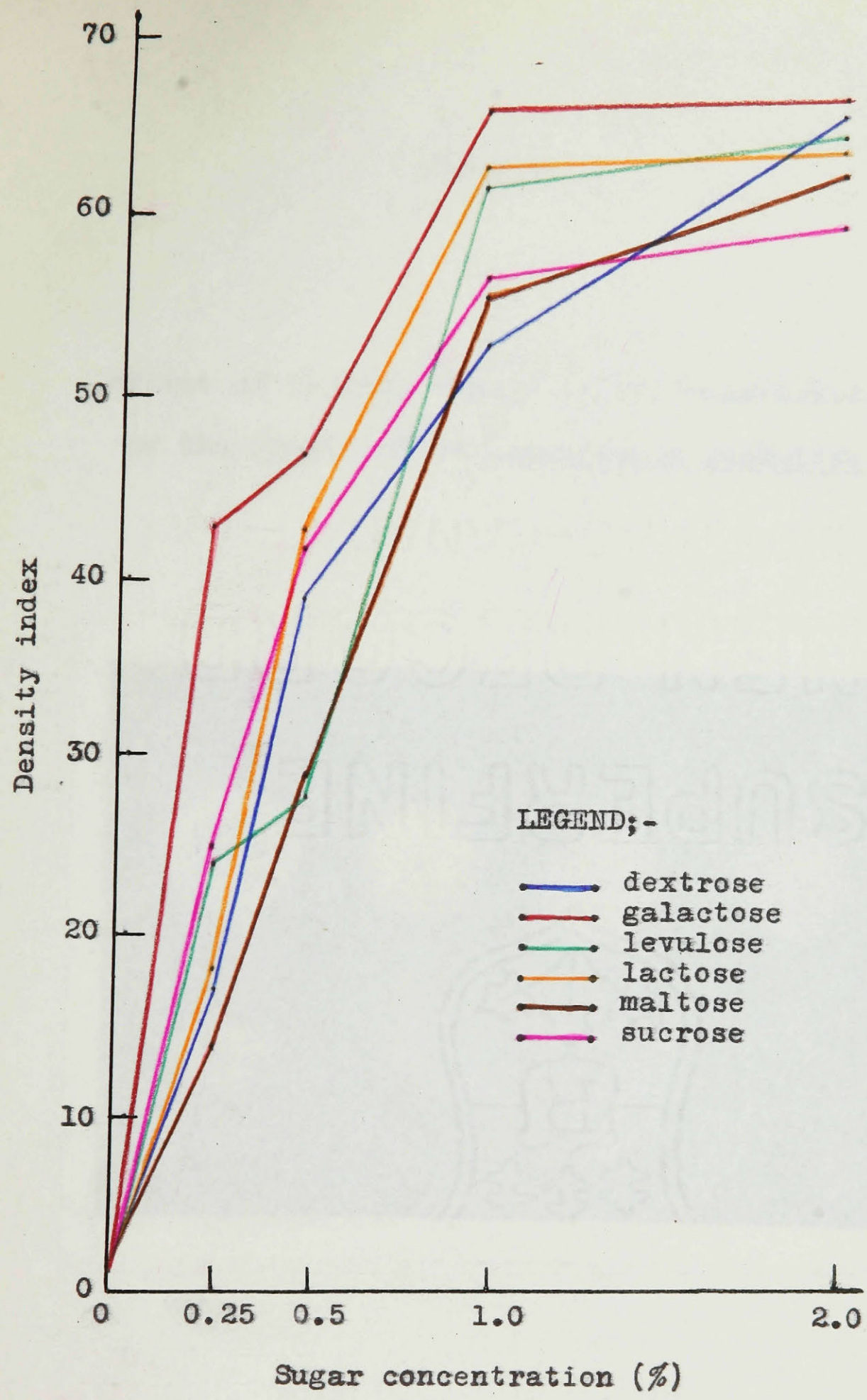
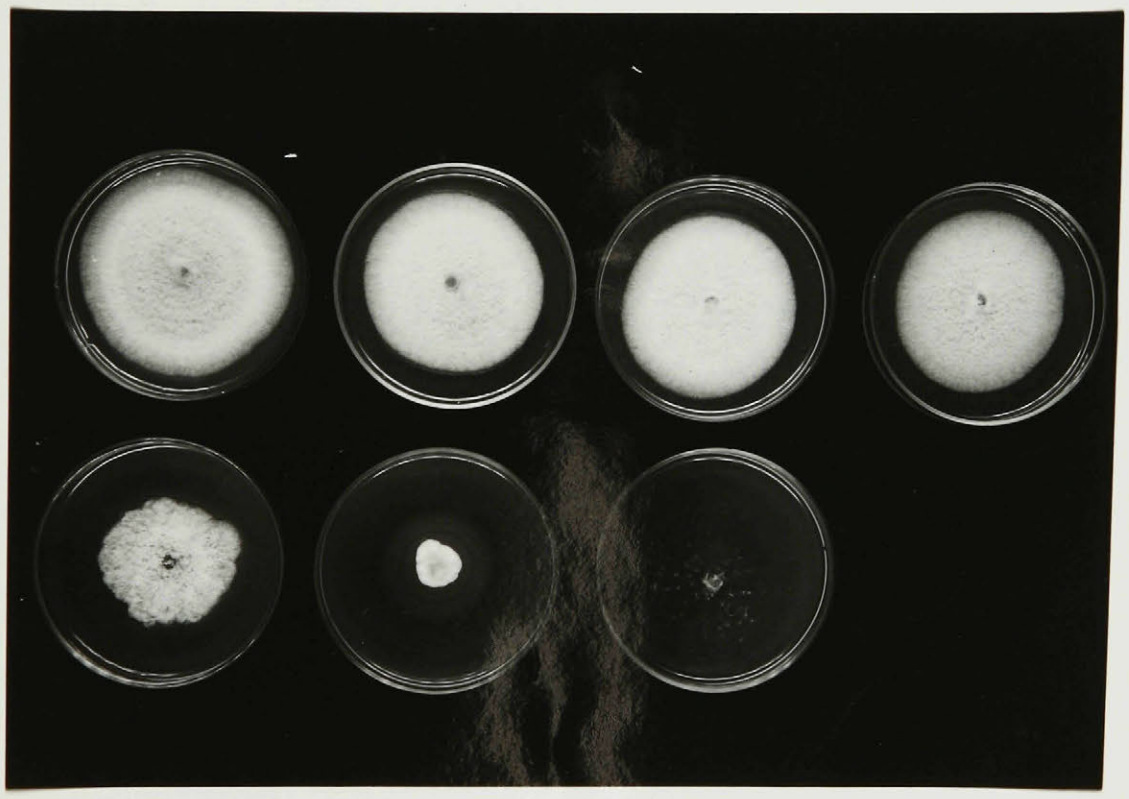



Figure 4 - Effect of various sugars on the growth of nine-day old colonies of Colletotrichum phomoides as measured by colony density.

PLATE V

Effect of Various Sugars in 2% Concentration on the growth of Colletotrichum phomoides.



From left to right,
upper row - maltose, sucrose, dextrose and levulose.
lower row - lactose, galactose and no sugar.

PLATE VI

Effect of Various Concentrations
of Sucrose on the Growth of
Colletotrichum phomoides.

Left to right,
upper row - 2% and 1% sucrose.
lower row - 0.5% and 0.25% sucrose.

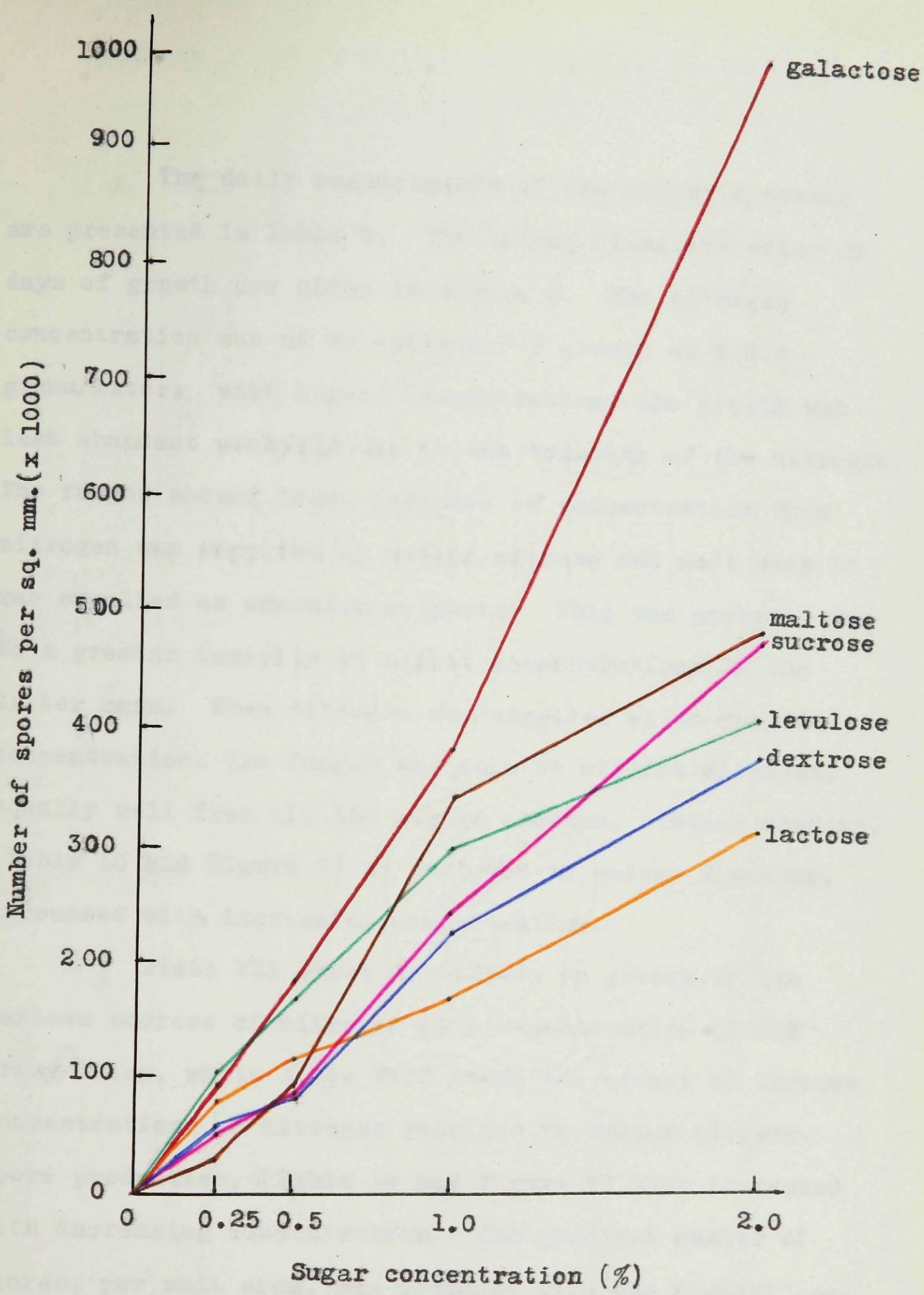



Figure 5 - Effect of various sugars on the spore production of nine-day old colonies of Colletotrichum phomoides.

The daily measurements of the colony diameter are presented in Table 9. The colony diameters after 10 days of growth are shown in Figure 6. The nitrogen concentration was at an optimum for growth at 0.004 grams/liter; with higher concentrations the growth was less abundant probably due to the toxicity of the nitrogen. The fungus showed least response to concentration when nitrogen was supplied as sodium nitrate and most when it was supplied as ammonium sulphate. This was probably due to a greater toxicity at higher concentrations in the latter case. When nitrogen was supplied at an optimum concentration, the fungus was able to utilize nitrogen, equally well from all the tested sources. Colony density, (Table 10 and Figure 7) in contrast to colony diameter, increased with increasing concentration.

Plate VII shows the effect on growth of the various sources of nitrogen at a concentration of 0.5 grams/liter, while Plate VIII shows the effect of various concentrations of nitrogen supplied as sodium nitrate. Spore production, (Table 10 and Figure 8) also increased with increasing concentration. The greatest number of spores, per unit area, was produced with the highest concentration of urea. At lower concentration, very little difference existed between the various nitrogen sources.

Table 9 - Effect of Various Sources of Nitrogen on the Growth of *Colletotrichum phomoides* as Measured by Colony Diameter.

| Number of days | Nitrogen concentration grams/liter | Colony diameter in mm. | | | | |
|---|---|---|---|---|---|---|
| | | Nitrate | Ammonium | Glycine | Asparagine | Urea |
| 1 | 0.0 | 7 | 7 | 7 | 7 | 7 |
| | 0.004 | 7 | 7 | 7 | 8 | 7 |
| | 0.02 | 7 | 7 | 7 | 7 | 7 |
| | 0.1 | 7 | 7 | 7 | 7 | 7 |
| | 0.5 | 7 | 7 | 7 | 7 | 7 |
| 2 | 0.0 | 14 | 14 | 14 | 14 | 14 |
| | 0.004 | 15 | 11 | 14 | 14 | 15 |
| | 0.02 | 14 | 10 | 13 | 14 | 14 |
| | 0.1 | 13 | 9 | 11 | 13 | 13 |
| | 0.5 | 12 | 9 | 12 | 12 | 13 |
| 3 | 0.0 | 22 | 22 | 22 | 22 | 22 |
| | 0.004 | 22 | 19 | 21 | 22 | 23 |
| | 0.02 | 21 | 18 | 20 | 22 | 21 |
| | 0.1 | 19 | 11 | 18 | 19 | 19 |
| | 0.5 | 20 | 9 | 18 | 16 | 18 |
| 4 | 0.0 | 31 | 31 | 31 | 31 | 31 |
| | 0.004 | 35 | 29 | 31 | 30 | 30 |
| | 0.02 | 30 | 25 | 29 | 30 | 31 |
| | 0.1 | 28 | 12 | 26 | 28 | 26 |
| | 0.5 | 28 | 10 | 25 | 27 | 23 |
| 5 | 0.0 | 40 | 40 | 40 | 40 | 40 |
| | 0.004 | 42 | 37 | 40 | 39 | 39 |
| | 0.02 | 38 | 34 | 36 | 39 | 39 |
| | 0.1 | 35 | 15 | 33 | 37 | 33 |
| | 0.5 | 36 | 10 | 32 | 35 | 28 |
| 6 | 0.0 | 47 | 47 | 47 | 47 | 47 |
| | 0.004 | 50 | 44 | 47 | 45 | 47 |
| | 0.02 | 45 | 40 | 43 | 45 | 48 |
| | 0.1 | 43 | 16 | 40 | 44 | 38 |
| | 0.5 | 45 | 10 | 39 | 43 | 32 |
| 7 | 0.0 | 55 | 55 | 55 | 55 | 55 |
| | 0.004 | 58 | 52 | 55 | 54 | 56 |
| | 0.02 | 53 | 45 | 51 | 53 | 56 |
| | 0.1 | 50 | 19 | 49 | 51 | 45 |
| | 0.5 | 51 | 11 | 45 | 48 | 36 |
| 8 | 0.0 | 61 | 61 | 61 | 61 | 61 |
| | 0.004 | 62 | 59 | 60 | 62 | 63 |
| | 0.02 | 58 | 49 | 58 | 60 | 64 |
| | 0.1 | 58 | 20 | 55 | 58 | 51 |
| | 0.5 | 58 | 11 | 52 | 55 | 41 |
| 9 | 0.0 | 67 | 67 | 67 | 67 | 67 |
| | 0.004 | 70 | 66 | 66 | 68 | 72 |
| | 0.02 | 67 | 55 | 66 | 67 | 71 |
| | 0.1 | 63 | 22 | 61 | 65 | 57 |
| | 0.5 | 63 | 13 | 57 | 62 | 44 |
| 10 | 0.0 | 74 | 74 | 74 | 74 | 74 |
| | 0.004 | 76 | 73 | 75 | 75 | 80 |
| | 0.02 | 74 | 60 | 73 | 73 | 79 |
| | 0.1 | 71 | 24 | 66 | 71 | 65 |
| | 0.5 | 70 | 13 | 62 | 68 | 49 |

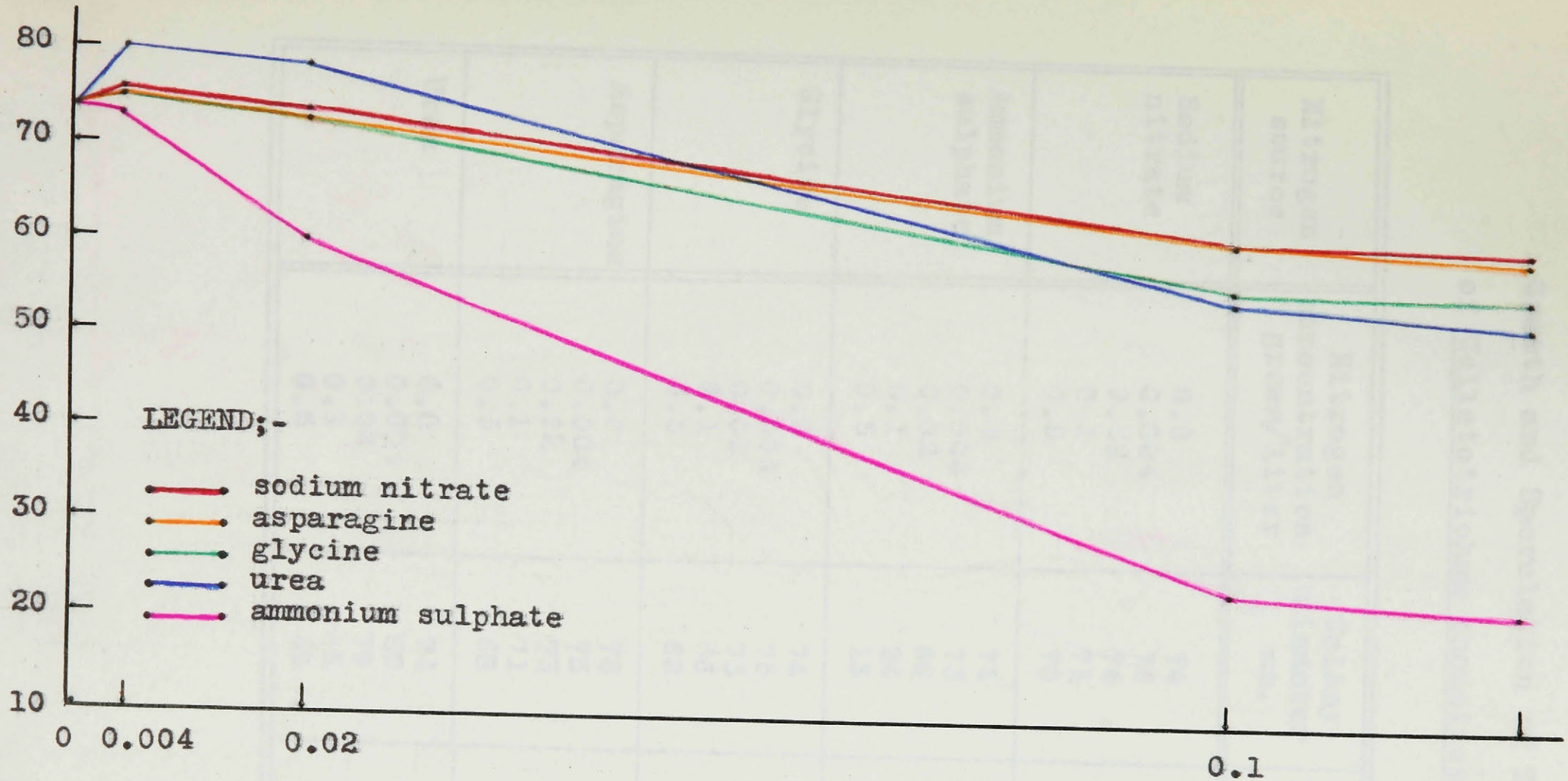



Figure 6 - Effect of various sources of nitrogen on the growth of ten-day old colonies of Colletotrichum phomoides as measured by colony diameter.

Table 10 - Effect of Various Sources of Nitrogen on the Growth and Sporulation of Ten-day Old Colonies of Colletotrichum phomoides.

| Nitrogen source | Nitrogen concentration grams/liter | Colony diameter mm. | Density index | Spores per sq. mm. (x 1000) |
|---|---|---|---|---|
| Sodium nitrate | 0.0 | 74 | 26 | 3 |
| | 0.004 | 76 | 37 | 6 |
| | 0.02 | 74 | 51 | 16 |
| | 0.1 | 71 | 65 | 134 |
| | 0.5 | 70 | 66 | 328 |
| Ammonium sulphate | 0.0 | 74 | 26 | 3 |
| | 0.004 | 73 | 32 | 5 |
| | 0.02 | 60 | 48 | 30 |
| | 0.1 | 24 | 68 | 146 |
| | 0.5 | 13 | - | 0 |
| Glycine | 0.0 | 74 | 26 | 3 |
| | 0.004 | 75 | 40 | 9 |
| | 0.02 | 73 | 47 | 12 |
| | 0.1 | 66 | 63 | 88 |
| | 0.5 | 62 | 69 | 845 |
| Asparagine | 0.0 | 74 | 26 | 3 |
| | 0.004 | 75 | 34 | 5 |
| | 0.02 | 73 | 38 | 12 |
| | 0.1 | 71 | 51 | 21 |
| | 0.5 | 68 | 65 | 134 |
| Urea | 0.0 | 74 | 26 | 3 |
| | 0.004 | 80 | 48 | 13 |
| | 0.02 | 79 | 48 | 22 |
| | 0.1 | 65 | 63 | 142 |
| | 0.5 | 49 | 68 | 1350 |

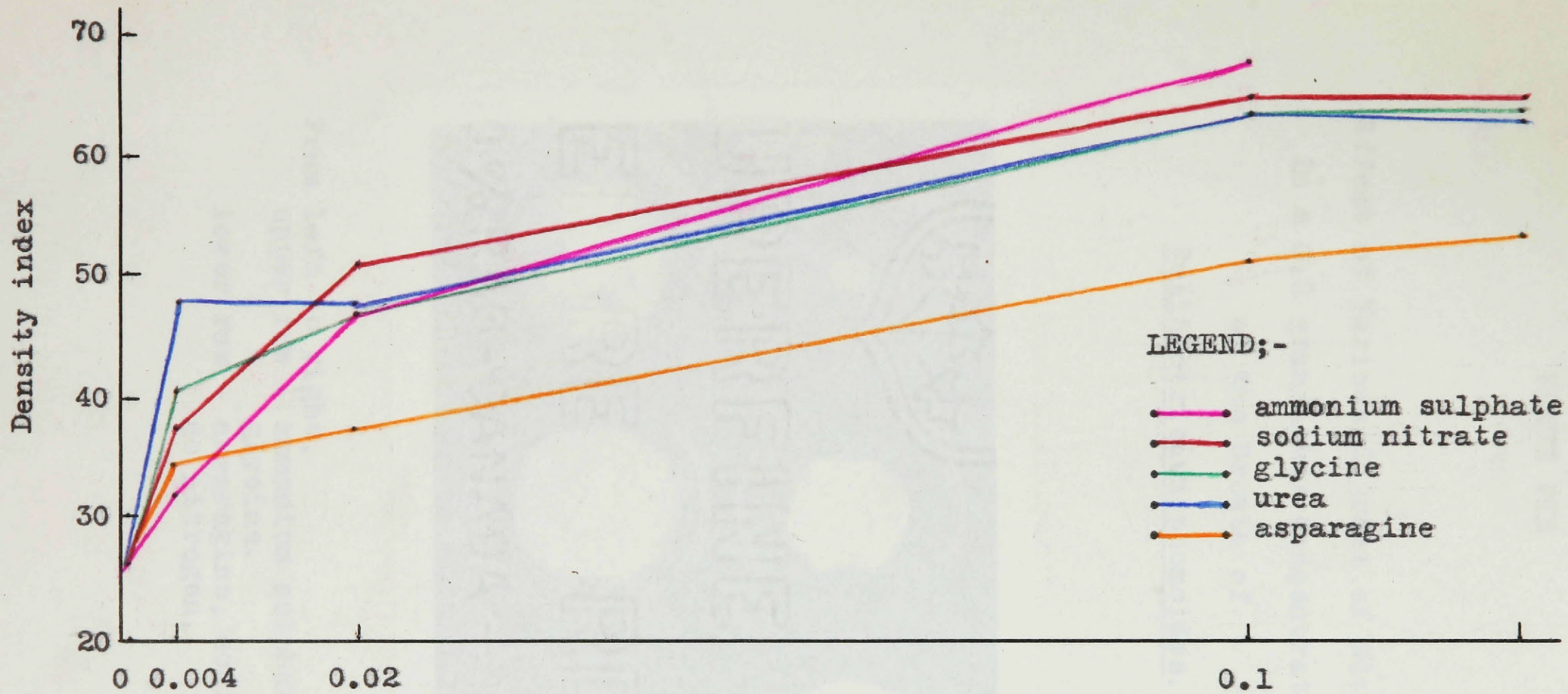
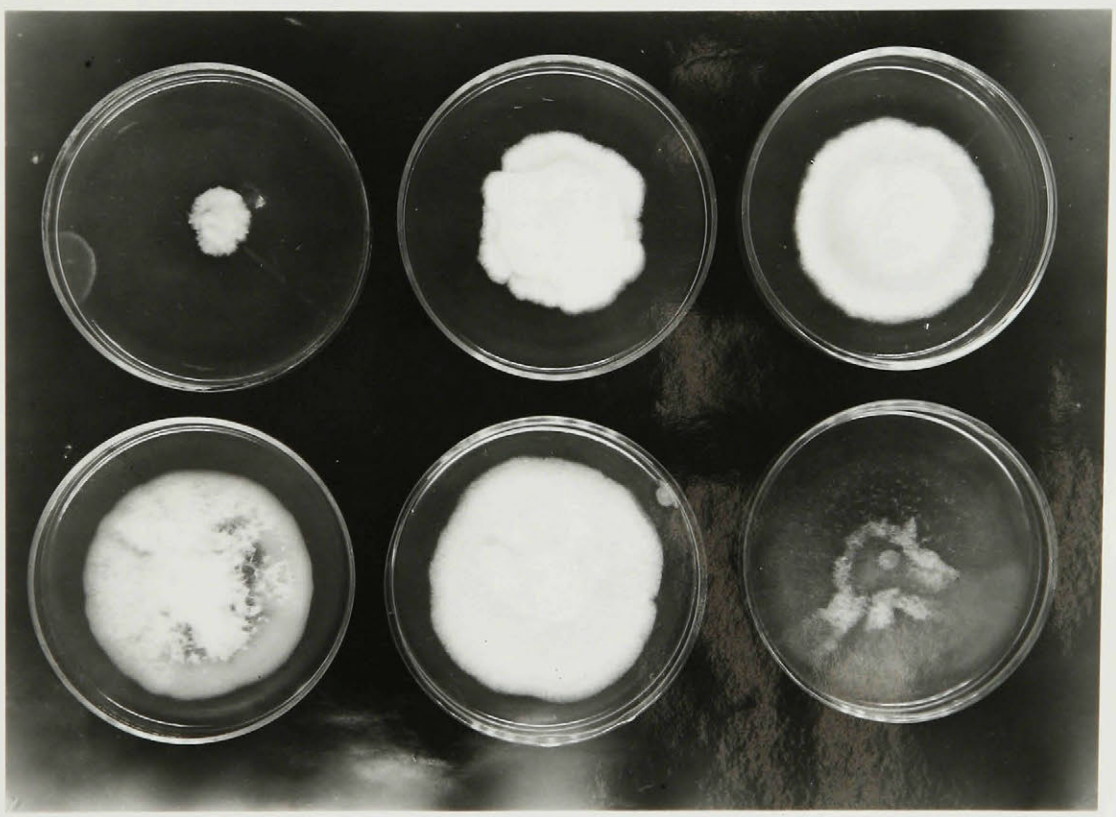



Figure 7 - Effect of various sources of nitrogen on the colony density of ten-day old colonies of Colletotrichum phomoides.

PLATE VII

Effect of Various Sources of Nitrogen
in a 0.5 gram/liter Concentration,
on the Growth of
Colletotrichum phomoides.



From left to right,
upper row - ammonium sulphate, urea, glycine.
lower row - asparagine, sodium nitrate, no nitrogen.

PLATE VIII

Effect of Various Concentrations of Sodium Nitrate on the Growth of Colletotrichum phomoides.

From left to right,
upper row - 0.1 and 0.5 grams/liter.
lower row - 0.02 and 0.004 grams/liter.

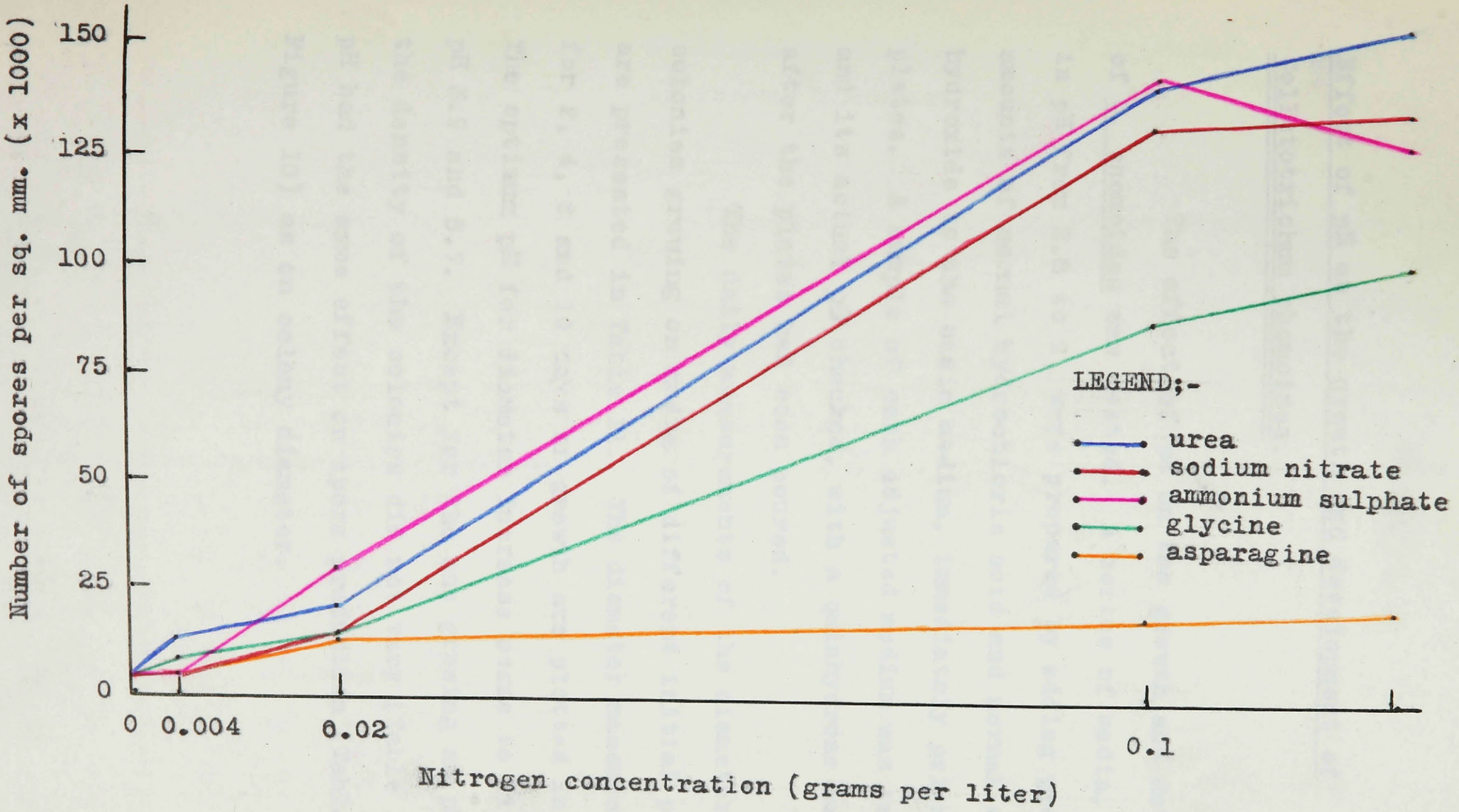



Figure 8 - Effect of various sources of nitrogen on the sporulation of ten-day old colonies of Colletotrichum phomoides.

## Effect of pH on the growth and development of *Colletotrichum phomoides*.

The effect of pH on the growth and development of *C. phomoides* was tested. A series of media, ranging in pH from 2.8 to 9, were prepared by adding predetermined amounts of normal hydrochloric acid and normal sodium hydroxide to the basic medium, immediately prior to pouring plates. A sample of each adjusted medium was retained and its actual pH checked, with a quinhydrone half-cell, after the plates had been poured.

The daily measurements of the diameter of colonies growing on media of different initial pH values are presented in Table 11. The diameter measurements for 2, 4, 6 and 10 days of growth are plotted in Figure 9. The optimum pH for diameter increase seems to lie between pH 3.9 and 5.7. Except for the one growing at pH 2.8, the density of the colonies did not vary (Table 12). The pH had the same effect on spore production (Table 12, Figure 10) as on colony diameter.

Table 11 - Effect of pH on the Growth of
<u>Colletotrichum phomoides</u> as Measured
by Colony Diameter.

| Number of days | Colony diameter in mm. | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Initial pH | | | | | | | | | | | | | | | | |
| | 2.8 | 3.4 | 3.9 | 4.4 | 4.9 | 5.4 | 5.7 | 5.9 | 6.3 | 6.5 | 6.7 | 6.9 | 7.2 | 7.3 | 7.9 | 8.4 | 9 |
| 2 | 7 | 9 | 14 | 14 | 14 | 14 | 14 | 13 | 14 | 13 | 13 | 13 | 12 | 12 | 12 | 11 | 10 |
| 3 | 7 | 16 | 24 | 24 | 24 | 23 | 24 | 23 | 22 | 21 | 23 | 21 | 21 | 21 | 19 | 20 | 18 |
| 4 | 7 | 25 | 32 | 33 | 33 | 30 | 32 | 31 | 30 | 29 | 31 | 28 | 29 | 29 | 25 | 27 | 24 |
| 5 | 7 | 33 | 40 | 39 | 38 | 37 | 40 | 38 | 38 | 37 | 38 | 36 | 36 | 36 | 33 | 33 | 30 |
| 6 | 7 | 40 | 48 | 48 | 47 | 47 | 48 | 46 | 46 | 45 | 47 | 44 | 44 | 44 | 42 | 40 | 38 |
| 7 | 8 | 49 | 57 | 58 | 55 | 54 | 57 | 55 | 54 | 54 | 56 | 54 | 53 | 53 | 50 | 51 | 48 |
| 8 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 9 | 12 | 67 | 73 | 73 | 72 | 72 | 73 | 71 | 71 | 71 | 72 | 71 | 70 | 71 | 67 | 66 | 64 |
| 10 | 14 | 74 | 81 | 80 | 78 | 77 | 80 | 78 | 80 | 79 | 79 | 78 | 78 | 77 | 75 | 73 | 70 |

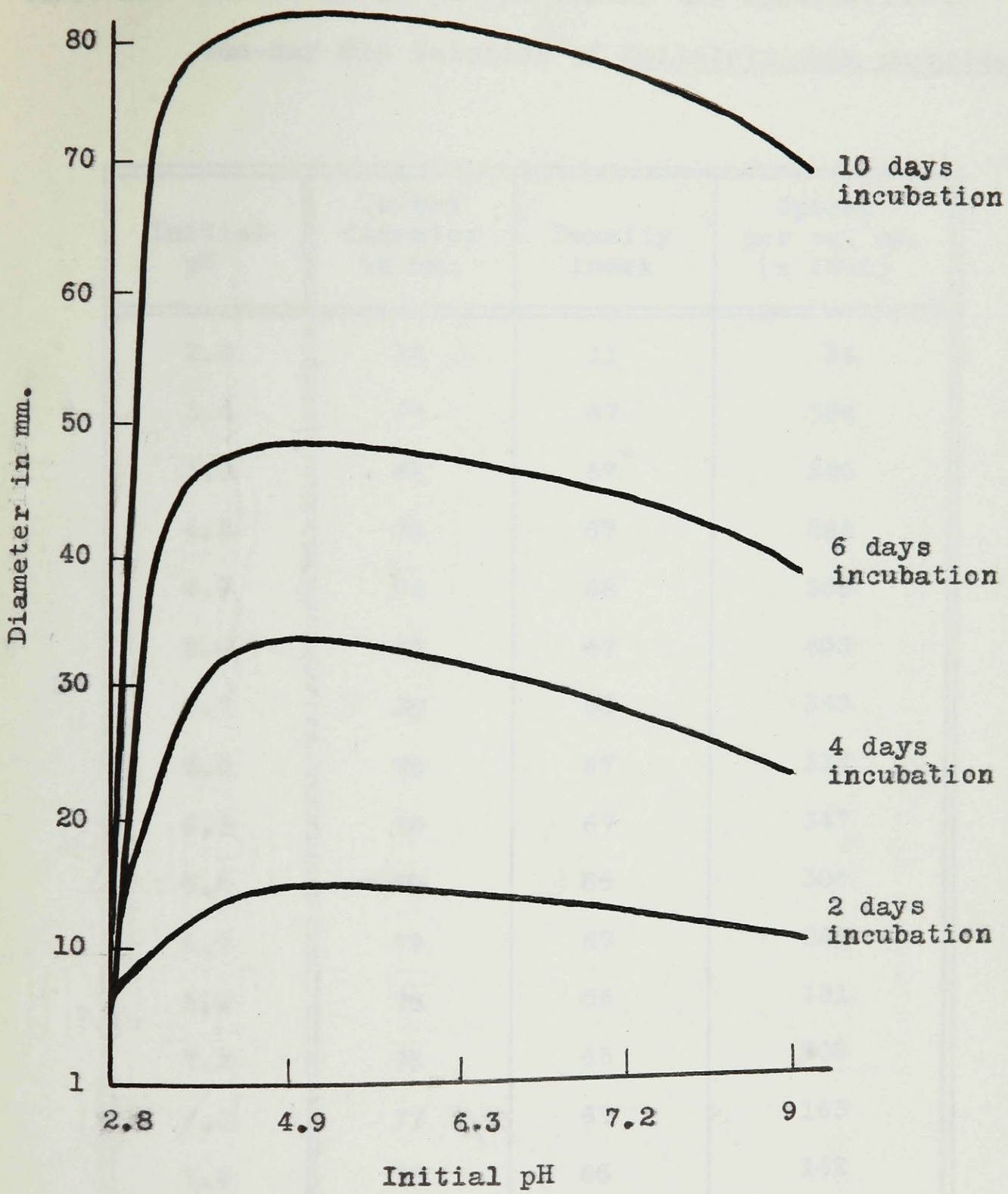



Figure 9 - Effect of pH on the growth of Colletotrichum phomoides as measured by colony diameter.

Table 12 - Effect of pH on the Growth and Sporulation of Ten-day Old Colonies of *Colletotrichum phomoides*.

| Initial pH | Colony diameter in mm. | Density index | Spores per sq. mm. (x 1000) |
|---|---|---|---|
| 2.8 | 14 | 11 | 34 |
| 3.4 | 74 | 67 | 324 |
| 3.9 | 81 | 67 | 265 |
| 4.4 | 79 | 67 | 298 |
| 4.9 | 78 | 68 | 308 |
| 5.4 | 77 | 67 | 403 |
| 5.7 | 80 | 68 | 340 |
| 5.9 | 78 | 67 | 317 |
| 6.3 | 80 | 67 | 347 |
| 6.5 | 79 | 66 | 306 |
| 6.7 | 79 | 67 | 266 |
| 6.9 | 78 | 65 | 181 |
| 7.2 | 78 | 65 | 208 |
| 7.3 | 77 | 67 | 165 |
| 7.9 | 75 | 66 | 142 |
| 8.4 | 73 | 67 | 200 |
| 9 | 70 | 67 | 164 |

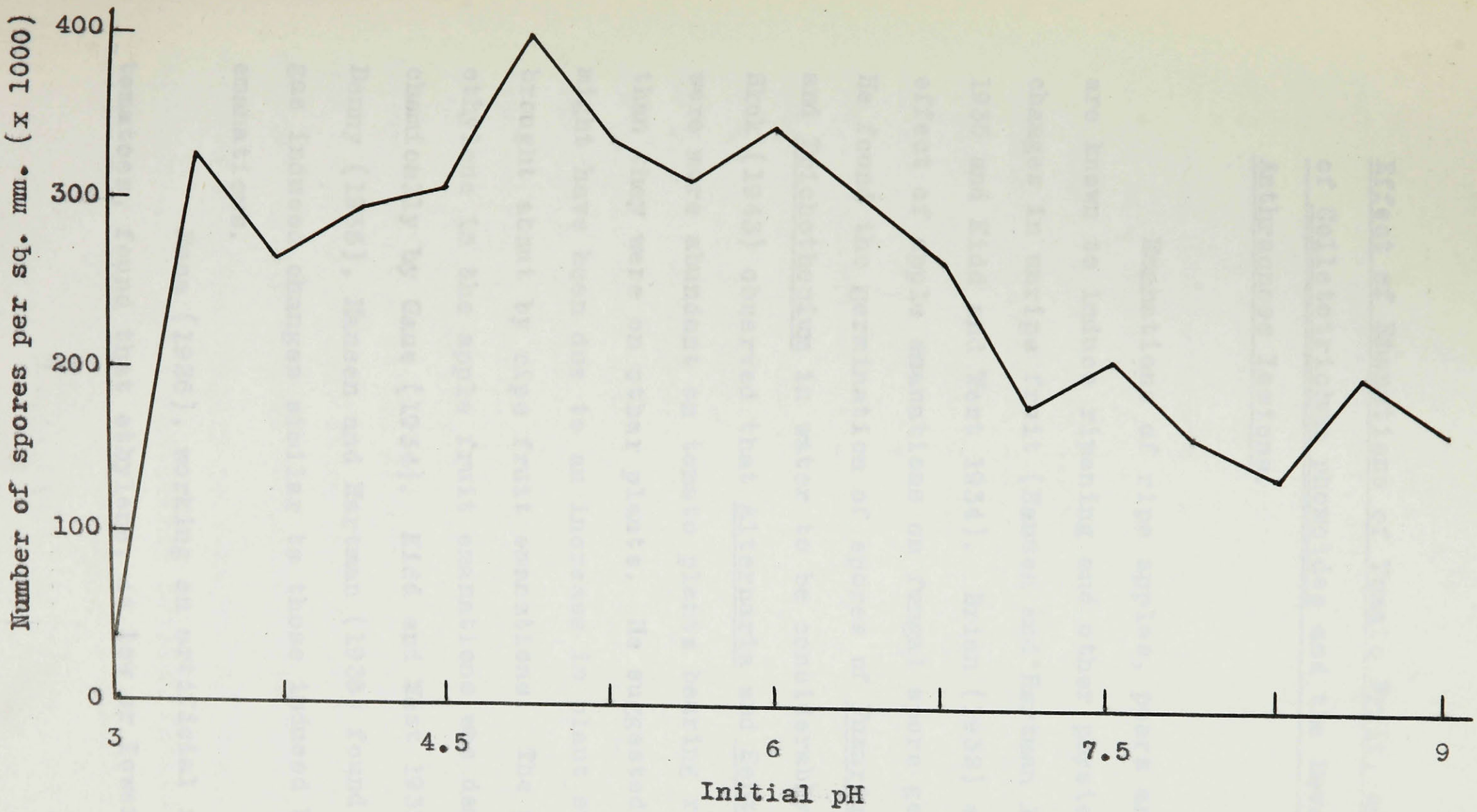



Figure 10 - Effect of pH on the sporulation of ten-day old colonies of Colletotrichum phomoides.

## Effect of Emanations of Tomato Fruit, on the Growth of Colletotrichum phomoides and the Development of Anthracnose Lesions.

Emanations of ripe apples, pears and tomatoes are known to induce ripening and other physiological changes in unripe fruit (Hansen and Hartman 1935, Denny 1935 and Kidd and West 1934). Brian (1932) studied the effect of apple emanations on fungal spore germination. He found the germination of spores of Fusarium, Gloeosporium and Trichothecium in water to be considerably stimulated. Skok (1943) observed that Alternaria and Septoria spots were more abundant on tomato plants bearing ripe fruits than they were on other plants. He suggested that this might have been due to an increase in plant susceptibility brought about by ripe fruit emanations. The presence of ethylene in the apple fruit emanations was demonstrated chemically by Gane (1934). Kidd and West 1932a and 1934). Denny (1935), Hansen and Hartman (1935) found that this gas induced changes similar to those induced by ripe fruit emanations.

Rosa (1926), working on artificial ripening of tomatoes, found that ethylene, as low or lower than one

part in 4300 parts of air, greatly accelerated red pigment development, destruction of starch and organic acids, and conversion of insoluble nitrogen to soluble nitrogen. He concluded, that ethylene, a reducing agent, acts on the tomato as a stimulus to the normal oxidative processes in the fruit. When fruit ripens in a chamber where the emanations accumulate, it results in an increase not only of ethylene, but also of carbon dioxide. Brooks, Bratley and McCollock (1936) found that at temperatures of 10 to 25°C., atmospheres containing 25% or more carbon dioxide caused a definite checking of growth of several fungi including C. phomoides, in puncture-inoculated fruits. The rate of development in the carbon dioxide atmosphere was usually about half of that in normal air at the same temperature and similar to that in normal air at temperatures 3 to 6°C. lower. Within a temperature range of 10 to 25°C. the inhibition due to carbon dioxide was somewhat greater at the higher than at the lower temperatures. Kidd and West (1932b) found that at 12°C. or lower, exposure to atmospheres containing more than 5% of carbon dioxide increased injury to tomatoes by fungi. Published information on the relationship between tomato fruit emanations and disease susceptibility contains no reference

to the effect of these emanations on the development of fungi present in a latent form. A series of experiments were, therefore, undertaken to test the effect of tomato emanations on the growth of C. phomoides and the expression of anthracnose symptoms.

Effect of a confined atmosphere containing ripe tomato fruits on the growth of Colletotrichum phomoides.

This experiment was designed to test, whether or not, the confined gases emanating from ripe tomato fruits affected the growth of C. phomoides. Four puncture-inoculated turning tomato fruits and six potato-dextrose agar Petri plate cultures were enclosed in a large dessicator containing 25 ripe tomato fruits. A similar number of inoculated fruits and plates were enclosed in a second empty dessicator to serve as a control. A piece of sterile string was placed between the lid and bottom of each Petri plate, in order to raise the lid slightly and allow free gas exchange between the atmosphere of the dessicator and that inside the plate. The control dessicator was opened from time to time to prevent gas accumulation.

The average colony diameter in plates incubated

for 10 days in the dessicator with ripe tomatoes was 14.5 mm., while the average colony diameter in plates incubated for a similar period in the empty dessicator was 68.5 mm. Plate IX illustrates these results. Three of the plates from the dessicator with tomatoes were transferred to the dessicator without tomato fruit, at the end of 10 days. Similarly, three plates from the control dessicator were transferred to the dessicator with tomato fruits. The plates removed from the dessicator with tomato fruits resumed normal growth, while growth was checked in those moved from the control dessicator to the dessicator with tomato fruits.

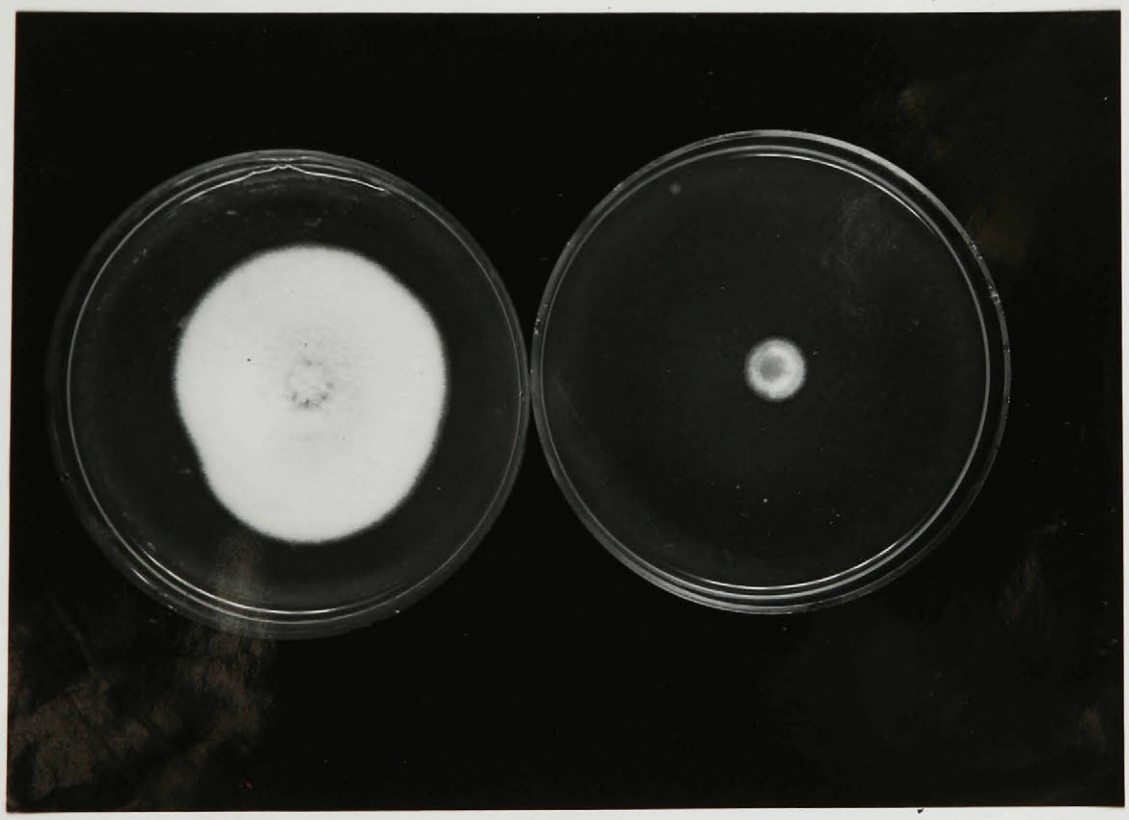
The puncture-inoculated fruits which were in the control dessicator developed characteristic lesions in four days, while those in the dessicator with other fruits did not. The 25 ripe tomato fruits used in this experiment did not show anthracnose after 14 days even though they were a sample from a heavily infected crop and in an advanced stage of ripeness.

Effect of a stream of air which had passed over ripe tomato fruits on the growth of Colletotrichum phomoides.

The effect of a restricted atmosphere containing

PLATE IX

Effect of a Confined Atmosphere Containing Ripe Tomato Fruit on the Growth of *Colletotrichum phomoides*.



left - in empty dessicator.
right - in dessicator with tomato fruits.

ripe tomato fruits in checking growth of C. phomoides on an artificial medium and in fruits might possibly be explained by the accumulation of the carbon dioxide of respiration. In order to test this possibility three bell-jars were connected in series with glass tubing and air drawn through them by means of a suction pump. The central bell-jar was filled with ripe tomatoes known to carry latent infections of C. phomoides. Twelve Petri plates containing potato-dextrose agar were inoculated with C. phomoides and six placed in each of the other bell-jars. In this way the plates farthest from the pump were provided with normal air and those nearest to the pump were in a stream of air containing the tomato emanations. Carbon dioxide could not accumulate in any of the three bell-jars. A record of the colony diameter on the Petri dishes for each bell-jar was kept. No difference was apparent in seven days. The fruits in the central jar first showed anthracnose lesions after two days and the severity of the disease increased steadily until the termination of the experiment. The experimental set up did not allow fruit emanations to accumulate anywhere within the system. This may have accounted for the lack of any evidence of any effects from such emanations

on the growth and development of the fungus in the fruits and in the cultures. The effect of a confined, carbon dioxide-free atmosphere containing ripe tomato fruits on the growth of C. phomoides was, therefore, tested in order to gain some evidence as to whether the retardation in growth noted in the previous test was due to carbon dioxide or some other emanation.

Effect of a confined, carbon dioxide-free atmosphere, containing ripe tomato fruits, on the growth of Colletotrichum phomoides.

In this experiment 25 ripe, naturally infected, tomato fruits and two inoculated potato-dextrose agar Petri plates were enclosed in a dessicator in the bottom of which was placed a normal solution of potassium hydroxide, to absorb the accumulating carbon dioxide. An inlet tube extending to the bottom of the dessicator and dipping in the potassium hydroxide allowed air to replace the absorbed carbon dioxide without allowing other gases to escape. Fruits and plates were also spread on the table around the dessicator to serve as a control. The diameters of anthracnose lesions on fruits and the diameters

of colonies on plates were measured at the beginning of the experiment and again at the conclusion of the experiment seven days later.

The results are presented in terms of colony and lesion area in Table 13:

Table 13 - Effect of Carbon Dioxide-free Emanations of the Growth of Colletotrichum phomoides in Culture and on Fruit.

| | Average initial area (sq. mm.) | Average final area (sq. mm.) | Relative area increase |
|---|---|---|---|
| Colony - In tomato emanations | 255 | 1590 | 6.2(1) |
| - In air | 284 | 3850 | 13.5 |
| Lesions - In tomato emanations | 63 | 255 | 4.0 |
| - In air | 20 | 132 | 6.6 |

(1) Final area devided by initial area.

These results show that the emanations, without their carbon dioxide, had an inhibiting effect on the fungus growing both in culture medium and in tomato fruit.

Effect of an atmosphere, containing ethylene, on the growth of Colletotrichum phomoides.

The effect of ethylene gas in air on the growth of C. phomoides was tested. The gas was prepared according to the method described in Gatterman (1937) and used in concentrations of 50%, 1%, 0.2%, 0.1%. These atmospheres were established in bell-jars each of which contained four inoculated Petri plates. For purposes of comparison, four similar plates were exposed to carbon dioxide-free tomato emanations, collected from a dessicator in which ripe tomato fruits had been enclosed over potassium hydroxide for four days. Four additional plates exposed to the room atmosphere served as a control.

Measurement of colony diameter was made after ten days. The results are summarized in Table 14. Tomato emanations and ethylene concentrations as low as 0.2% seemed to cause some inhibition of fungus growth.

Relative effect of complete, carbon dioxide-free and ethylene-free tomato fruit emanations on the growth of Colletotrichum phomoides.

The previous experiments may not be directly

Table 14 - Effect of Ethylene Gas and Tomato Emanations Free of Carbon Dioxide on the Growth of Colletotrichum phomoides in Plate Culture.

| Treatment | Average colony diameter in mm. (after 10 days incubation) |
|---|---|
| Air | 83 |
| Carbon dioxide-free emanations. | 69 |
| 50% ethylene in air | 71 |
| 1% " " " | 75 |
| 0.2% " " " | 74 |
| 0.1% " " " | 80 |
| 0.01% " " " | 79 |

comparative since they were conducted at different times and with different lots of fruits. This experiment was designed in order to make the treatments more comparative.

Four similar samples, of 20 fruits each, were prepared from Bahamas tomato fruits. Each sample weighed about 200 grams and was composed of six turning and fourteen pink to ripe fruits. These fruits were inoculated by spraying them with a spore suspension of C. phomoides. In addition, five fruits in each sample were puncture-inoculated in two places. Petri plates containing potato-

dextrose agar were also inoculated with C. phomoides and exposed to the treatments. One sample of fruit and four Petri dishes were placed in each of four dessicators. The atmosphere in the first dessicator was changed continuously by drawing a stream of air through it with a suction pump. This stream of air was humidified by bubbling it through water before entering the dessicator. A carbon dioxide-free atmosphere was maintained in the second dessicator. This was accomplished by enclosing potassium hydroxide with the fruits as previously described. The third dessicator contained an ethylene-free atmosphere. This was accomplished by enclosing charcoal in which bromine had been absorbed to remove the ethylene (Smith and Gane, 1932). Complete fruit emanations were allowed to accumulate in the fourth dessicator. A beaker of water was enclosed in the last two dessicators to maintain the humidity. After eight days at room temperature (24°C.) the four dessicators were opened and notes were taken on the growth of the fungus on plates and in fruit. Notes were also taken on the stage of maturity of the fruits.

Table 15 shows the effect of various treatments on fruit ripening. These results show that ripening of the fruit was retarded in both ethylene-free and in complete emanations.

Table 15 - Effect of Tomato Fruit Emanations on the Rate of Ripening.

| Treatment | Condition of fruit in % | | |
|---|---|---|---|
| | Overripe | Ripe | Pink |
| Emanations in a stream of air | 95 | 5 | 0 |
| Emanations without $CO^2$ | 70 | 20 | 0 |
| Emanations without ethylene | 0 | 70 | 30 |
| Complete emanations | 5 | 65 | 30 |

Table 16 shows the effect of various treatments on the growth of C. phomoides and development of anthracnose symptoms. The action of the treatments on C. phomoides in culture was similar to that on ripening. Inclusion of inoculated plates in complete and ethylene-free emanations resulted in a definite checking of the fungus growth. Carbon dioxide-free emanations also checked the growth of C. phomoides but to a lesser degree. The lesion area on puncture-inoculated fruit was largest where the fruit was held in a stream of air. Growth was checked by the other three treatments, the greatest checking occurring in complete emanations. On the spray-inoculated fruit, a greater number of lesions developed when the fruit was in a stream of air. Fewer lesions developed in ethylene-free emanations than in carbon dioxide-free emanations. Complete, ethylene-free and carbon dioxide-free emanations repressed lesion area.

Table 16 - Effect of Tomato Fruit Emanations on the Growth of Colletotrichum phomoides and Development of Anthracnose Symptoms.

| Treatment | Plate culture | Puncture-inoculated fruit | Spray-inoculated fruit | | | |
|---|---|---|---|---|---|---|
| | Average colony area (sq.mm.) | Average lesion area (sq.mm.) | Number of(1) fruits with anthracnose | Number of lesions per fruit | Average lesion area (sq.mm.) | Total area of all lesions (sq.mm.) |
| Emanations in a stream of air | 3110 | 615 | 7 | 2 | 176 | 2464 |
| Emanations without $CO^2$ | 1660 | 176 | 3 | 3 | 13 | 127 |
| Emanations without ethylene | 452 | 113 | 1 | 1 | 13 | 13 |
| Complete emanations | 201 | 20 | 1 | 1 | 13 | 13 |

(1) samples of 20 fruits each.

## TOMATO FIELD SPRAYING WITH SPECIAL REFERENCE TO ANTHRACNOSE CONTROL.

In recent years a great deal of work has been done on the control of fungi such as the anthracnose fungi which cause latent infections of fruits. It was felt, up to 1943, that control could be obtained only by keeping the fruits constantly covered with a fungicide (Baker 1938, Wardlaw et al 1939, McKee 1940 and Simmonds 1941). Attention was focused chiefly on the use of copper containing fungicides. Since it was demonstrated (Baker 1938) that latent infection occurred early in the life of the fruit, it followed that spraying would have to be commenced early and continued throughout the growing season. This was born out by the work of Wardlaw, Baker and Crowdy (1939) who showed that, with Bordeaux, the degree of control obtained was proportional to the number of sprays made. Even with frequent applications, however, the control obtained was far from being perfect and was not practical from an economic standpoint. McNew (1943a) while testing fungicides for the control of anthracnose found that only four applications of a new material called Fermate (ferric-dimethyldithiocarbamate) were required to decrease the severity of anthracnose on tomato fruits from 32% to 1.4%. He suggested that two late

applications might be sufficient. Fermate was not as effective as the copper sprays in controlling leaf blight of tomato. A partial control of anthracnose was obtained with copper oxychloride-sulphate, Copper A, yellow cuprocide, Bordeaux, Spergon and Tennessee Tribasic. Later work (McNew 1943b) confirmed these results, control of anthracnose being obtained with five applications of Fermate during July and August. Wilson (1943) obtained results similar to those of McNew (1943a) with three applications of Fermate made in August. He also found (1944) that Fermate applied in an early spray schedule was more effective than when applied in a late one. He concluded "it seems likely that the most satisfactory results should be obtained by spraying (2-100) or dusting (10-90) with Fermate on a schedule of five treatments at 10-day intervals in which the first application is made when the fruits of the first cluster are approximately two-third developed."

In the light of these findings and recommendations an experiment designed to test the effect of tomato field spraying was conducted during the summer of 1943.

## Methods

Six-weeks old John Baer tomato plants were trans-

planted in the field from greenhouse flats on June 2nd. They were spaced four feet apart in the rows which were five feet apart. The plants were then divided into twenty plots of ten plants, each one of which had five plants in its length and two plants in its width. Five treatments, each replicated four times were used. Plots were arranged in four blocks and the treatments were randomized so that one replicate of each treatment fell in each block. In an effort to establish epiphytotic conditions for early blight and anthracnose, all plants were inoculated with Alternaria solani on July 15 and with A. solani and Colletotrichum phomoides on July 20th. by spraying with a water suspension of spores and mycelia of the fungi prepared from agar cultures. The spray treatments were begun on July 29th. The spraying was done with a hand sprayer(1) capable of delivering spray at 150 pounds per square inch. The spray treatments used and the time of application are given in Table 17.

A weak Bordeaux was applied at the beginning of the season because of the findings of Horsfall and Huberger (1942) who suggested that the increased number

(1) Paragon sprayer manufactured by "The Campbell-Hausfeld Co." Harrison, Ohio.

Table 17 - Rates and Dates of Spray Applications for the Field Control of Anthracnose and other Diseases of Tomatoes.

| Date of application | Rate of application gals/acre | Spray treatments | | | | |
|---|---|---|---|---|---|---|
| | | Bordeaux(1) | Copper A(2) | Fermate I | Fermate II | Check |
| July 29 | 80-90 | 2-2-32 | 4-80 | 2-80 | - | - |
| August 12 | 80-90 | 2-2-32 | 4-80 | 2-80 | - | - |
| August 25 | 140-150 | 2-2-32 | 4-80 | 2-80 | - | - |
| September 6 | 140-150 | 4-4-32 | 4-80 | 2-80 | 2-80 | - |
| September 15 | 140-150 | 4-4-32 | 4-80 | 2-80 | 2-80 | - |
| October 6 | 80-90 | 4-4-32 | 4-80 | 2-80 | 2-80 | - |

(1) In the first three applications "Dry Bordeaux Mixture" (manufactured by Canada Paint Co.) was used. The last three applications were made with Bordeaux mixture prepared as follows: Copper sulphate crystals were dissolved in a small amount of water. This was poured into the sprayer tank and the lime was added gradually as the rest of the water was being poured in.

(2) Copper A (manufactured by the Niagara Lake Co.) is also called Compound A and copper oxychloride.

of green fruits on sprayed plants when killing frost occurs in the fall was due to blossom killing by strong early Bordeaux sprays rather than to a delayed ripening effect. Two Fermate treatments were applied; one of these was an all season and the other a late season spray. For purposes of convenience these are referred to in the text as Fermate I and Fermate II respectively. The plants were left unstaked. The fruits from each plot were harvested from the turning to the "just ripe" stage. A record was kept of the number and total weight of fruits harvested at each picking. Notes were also made at each picking on the health condition of all fruits and later, on a sample of the fruits from each plot that had been incubated in the laboratory at room temperature (24°C.). In the latter case the sample was assumed to be representative of the whole plot and the amount of disease was recorded on the basis of the total number of fruits harvested.

The amount of defoliation by leaf spotting diseases was determined on September 4th. according to the method described by Horsfall and Huberger (1942). Immediately after the first damaging frost, all green fruits were harvested, counted and weight.

Preliminary analysis of the data showed that the

same trends occurred when yield was expressed on the basis of number of fruit per plot as when it was expressed on a weight basis. As the former figures were smaller than the latter they were used in a statistical analysis of the results. These data were treated according to the analysis of covariance (Paterson, 1939).

## Results

The cumulative yield data, presented in Table 18 show the number of fruits per plot, weight of fruit in pounds per plot and weight of fruit in pounds per acre. These data are illustrated graphically in Figure 11. It is obvious that, as judged by any of these criteria the total yields from treated plots, up to the time of freezing, did not differ significantly either between each other or from the yield of the check plot. This is not only true for the final yield but also for the yield up to any time during the picking season. The amount of fruit remaining on the Bordeaux, Copper A and Fermate I plots, after the first killing frost, however, was greater than that remaining on either the Fermate II or check plots. There appears to have been a direct correlation with the control of defoliation by the spray treatments as shown in Table 19.

Table 18 - The Effect of Spray Treatments on the Yield of John Baer Tomatoes Up to Various Dates.

| Fruit harvested up to | Yield categories | Treatment | | | | |
|---|---|---|---|---|---|---|
| | | Bordeaux | Copper A | Fermate I | Fermate II | Check |
| August 20th. | Number per plot | 8 (1) | 8 | 5 | 6 | 7 |
| " " | Weight lbs/plot | 2.2 | 2.2 | 1.5 | 1.5 | 2.0 |
| " " | Weight lbs/acre | 484 | 465 | 352 | 358 | 457 |
| August 30th. | Number per plot | 39 | 41 | 31 | 42 | 43 |
| " " | Weight lbs/plot | 11.2 | 10.1 | 10.1 | 11.8 | 12.5 |
| " " | Weight lbs/acre | 2495 | 2248 | 2230 | 2597 | 2759 |
| Sept. 17th. | Number per plot | 186 ± 16 | 181 ± 16 | 163 ± 16 | 173 ± 16 | 179 ± 16 |
| " " | Weight lbs/plot | 64.2 | 60.1 | 51.6 | 55.7 | 57.4 |
| " " | Weight lbs/acre | 14034 | 13218 | 11255 | 12173 | 12514 |
| Oct. 7th. | Number per plot | 330 ± 24 | 335 ± 24 | 338 ± 24 | 320 ± 24 | 352 ± 24 |
| " " | Weight lbs/plot | 99 ± 7.7 | 97 ± 7.7 | 101 ± 7.7 | 924 ± 7.7 | 106 ± 7.7 |
| " " | Weight lbs/acre | 21562 | 21125 | 22086 | 20164 | 23042 |
| After frost Oct. 11th. | Number per plot | 477 | 498 | 486 | 421 | 438 |
| " " | Weight lbs/plot | 140.8 | 145.4 | 141.5 | 105.8 | 108.2 |
| " " | Weight lbs/acre | 30758 | 31728 | 30864 | 23163 | 23616 |

(1) mean of 4 plots.

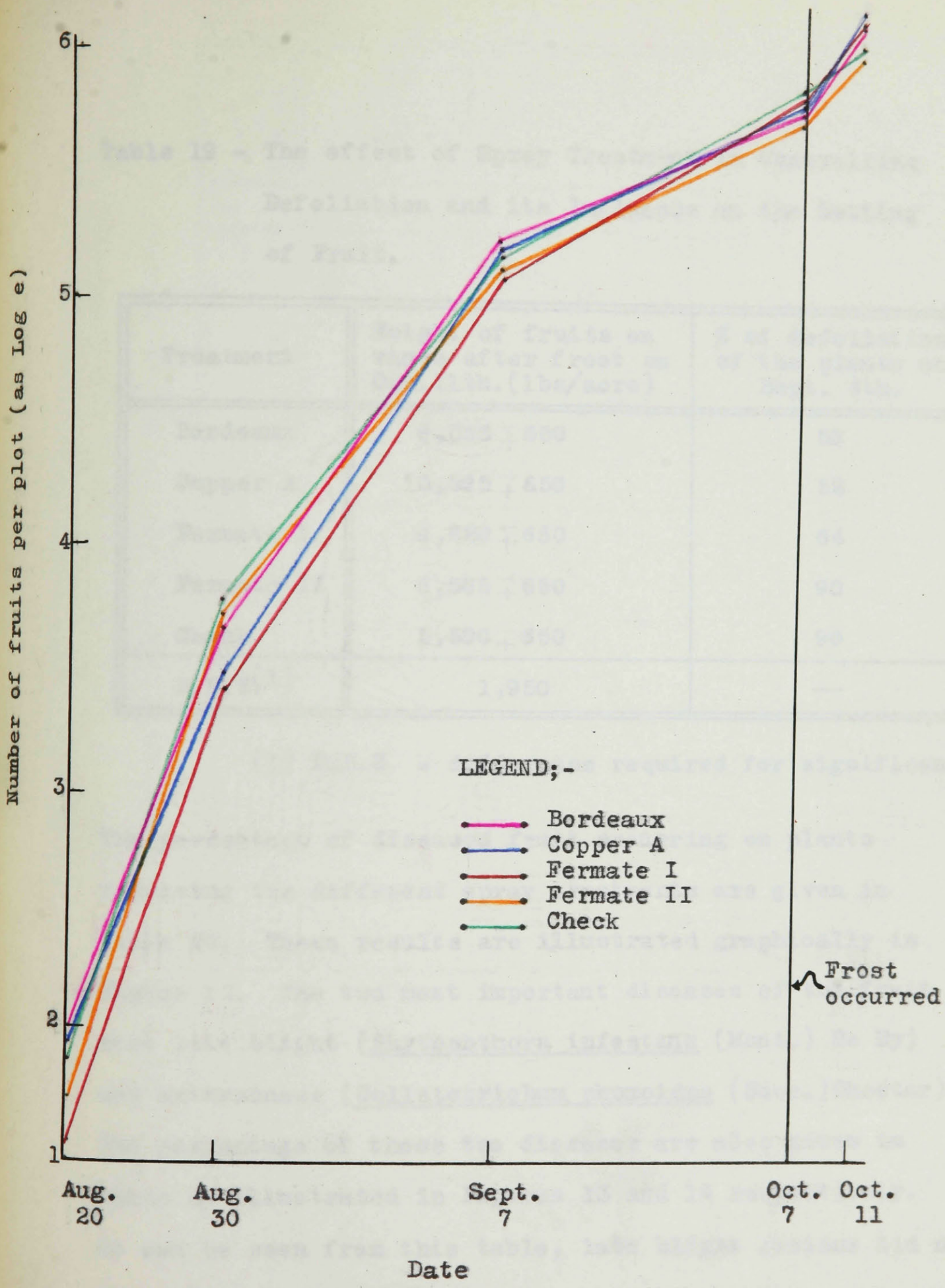



Figure 11 - The effect of spray treatments on the cumulative yield of John Baer tomatoes.

Table 19 - The effect of Spray Treatments in Controlling Defoliation and its Influence on the Setting of Fruit.

| Treatment | Weight of fruits on vines after frost on Oct.11th.(lbs/acre) | % of defoliation of the plants on Sept. 4th. |
|---|---|---|
| Bordeaux | 9,555 ∓ 650 | 50 |
| Copper A | 10,595 ∓ 650 | 52 |
| Fermate I | 9,620 ∓ 650 | 64 |
| Fermate II | 6,565 ∓ 650 | 90 |
| Check | 5,590 ∓ 650 | 90 |
| D.R.S.(1) | 1,950 | — |

(1) D.R.S. = difference required for significance.

The percentage of diseased fruit occurring on plants receiving the different spray treatments are given in Table 20. These results are illustrated graphically in Figure 12. The two most important diseases of the fruit were late blight (Phytophthora infestans (Mont.) De By) and anthracnose (Colletotrichum phomoides (Sacc.)Chester). The percentage of these two diseases are also given in Table 20 illustrated in Figures 13 and 14 respectively. As can be seen from this table, late blight lesions did not increase markedly in number after picking while anthracnose

Table 20 - Effect of Spray Treatments on the Percentage of Diseased Tomatoes for the Entire Season.

| Diseases | Time of examination | Treatment | | | | | $\frac{\sigma}{-}$ + | $\frac{-}{+}$ D.R.S.(1) |
|---|---|---|---|---|---|---|---|---|
| | | Bordeaux | Copper A | Fermate I | Fermate II | Check | | |
| All diseases | At picking | 5.2'' | 12.7 | 34.4'' | 14.6 | 16.1 | 2 | 6 |
| | 4 days after | 9.8'' | 18.5 | 42.3'' | 23.5 | 27.7 | 3 | 10 |
| | 11±2 days after | 36.7'' | 49.5' | 60.2 | 53.7' | 68.0 | 4 | 14 |
| Anthracnose | At picking | 0 | 0 | 0 | 0 | 0 | 7 | 22 |
| | 4 days after | 1.8 | 2.4 | 0.3 | 3.3 | 5.1 | 4 | 13 |
| | 11±2 days after | 26.8' | 28.0' | 7.1'' | 25.3' | 36.1 | 2 | 7 |
| Late blight | At picking | 4.4'' | 9.5' | 33.1'' | 13.7 | 15.2 | 2 | 6 |
| | 4 days after | 4.4'' | 10.7' | 36.8'' | 14.0 | 17.9 | 2 | 7 |
| | 11±2 days after | 4.8'' | 11.0' | 40.5'' | 14.9 | 20.2 | 2 | 7 |

(1) D.R.S. = difference required for significance at P = 0.05
'' = different from the check at P = 0.01, ' at P = 0.05
D.R.S. between values of any two lines = $D.R.S._x = \sqrt{D.R.S._y^2 + D.R.S._z^2}$

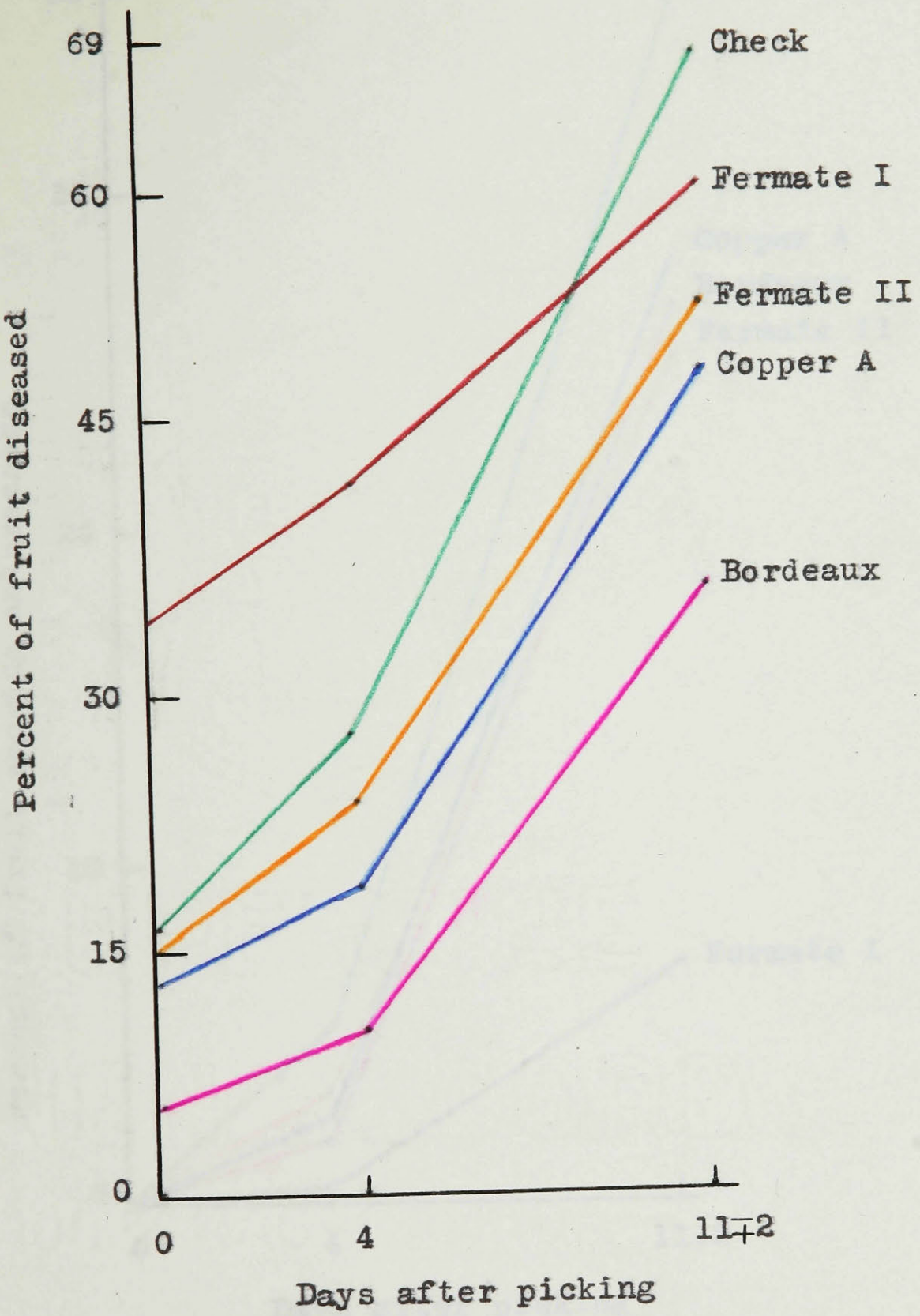



Figure 12 - Effect of spray treatments on the percentage of diseased fruit.

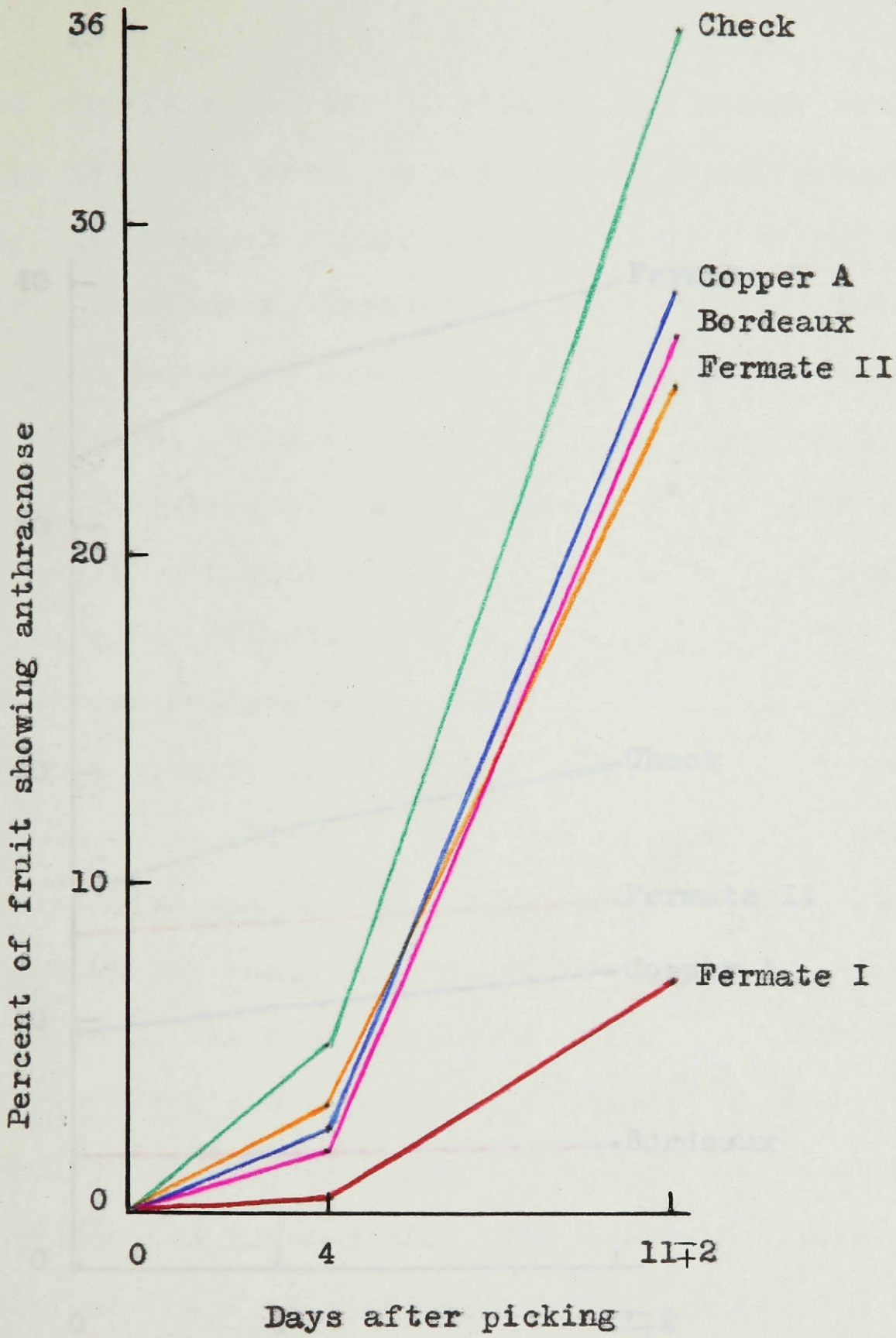



Figure 13 - Effect of spray treatments on the percentage of fruit with anthracnose.

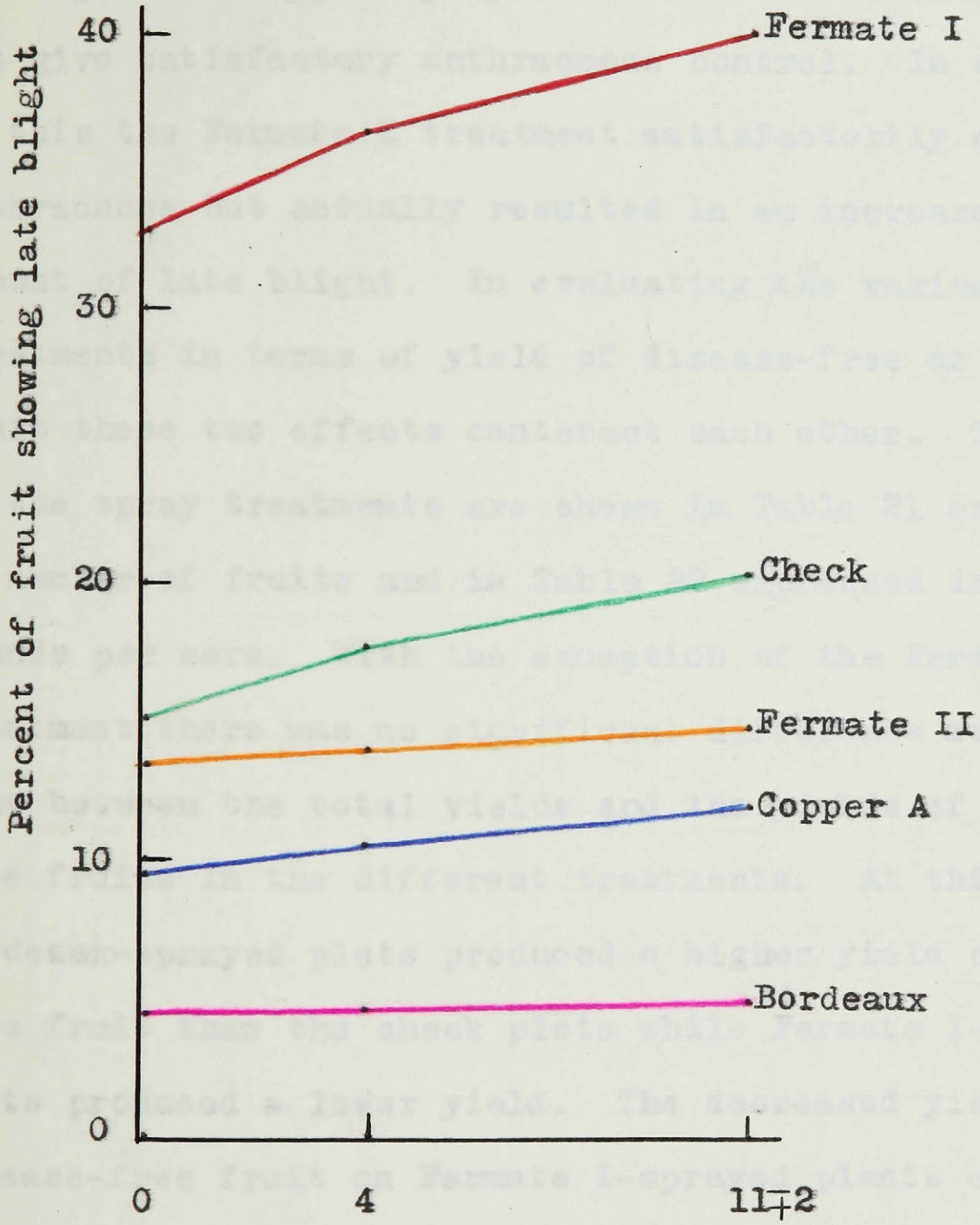



Figure 14 - Effect of spray treatments on the percentage of fruit with late blight.

lesions were rare at time of picking and became abundant when the fruit was held for a period of time following picking. The copper sprays controlled late blight but did not give satisfactory anthracnose control. In contrast to this the Fermate I treatment satisfactorily controlled anthracnose but actually resulted in an increase in the amount of late blight. In evaluating the various spray treatments in terms of yield of disease-free or marketable fruit these two effects conteract each other. The effects of the spray treatments are shown in Table 21 expressed in terms of number of fruits and in Table 22 expressed in terms of pounds per acre. With the exception of the Fermate II treatment there was no significant difference at picking time between the total yields and the yields of disease-free fruits in the different treatments. At this time, Bordeaux-sprayed plots produced a higher yield of disease-free fruit than the check plots while Fermate I-sprayed plots produced a lower yield. The decreased yield of disease-free fruit on Fermate I-sprayed plants can be explained by an increase in the amount of late blight as a result of treatment (Table 20). Four days after picking the results remained essentially the same while eleven days after picking the only treatment that failed to result in

Table 21 - Effect of Spray Treatments on the Yield of Tomatoes

Expressed as Number of Fruits Per Plot for the Entire Season.

| Fruit categories | Time of examination | Treatment | | | | | $\sigma \pm$ | $\overline{\pm}$ D.R.S.(1) |
|---|---|---|---|---|---|---|---|---|
| | | Bordeaux | Copper A | Fermate I | Fermate II | Check | | |
| All fruits | At picking | 330(2) | 335 | 338 | 320 | 352 | 24 | 74 |
| Disease-free fruits | At picking | 318'' | 297 | 223'' | 286 | 281 | 6.5 | 20 |
| | 4 days after | 302 | 273 | 193'' | 256 | 242 | 11 | 34 |
| | 11±2 days after | 212'' | 169' | 133 | 155' | 107 | 15 | 47 |
| Anthracnose-free fruits | At picking | 330 | 335 | 338 | 320 | 352 | 24 | 74 |
| | 4 days after | 329 | 327 | 334 | 324 | 318 | 15 | 48 |
| | 11±2 days after | 245' | 241' | 311'' | 250' | 214 | 8 | 26 |
| Late blight-free fruits | At picking | 320'' | 303' | 224'' | 289 | 284 | 6 | 19 |
| | 4 days after | 320'' | 299' | 212'' | 288 | 275 | 8 | 24 |
| | 11±2 days after | 319'' | 298' | 199'' | 285 | 267 | 8 | 26 |

(1) D.R.S. = difference required for significance at P = 0.05
'' different from the check at P = 0.01, ' at P = 0.05
D.R.S. between the values of any two lines = $D.R.S._x = \sqrt{D.R.S._y^2 + D.R.S._z^2}$

(2) average of the 4 replicates.

Table 22 - Effect of Spray Treatments on the Yield of Tomatoes in Pounds per Acre for the Entire Season.

| Fruit categories | Time of examination | Treatment | | | | | $\sigma \pm$ | $\overline{+}$ D.R.S.(1) |
|---|---|---|---|---|---|---|---|---|
| | | Bordeaux | Copper A | Fermate I | Fermate II | Check | | |
| All fruits | At picking | 21450 | 21775 | 21970 | 20800 | 22880 | 1560 | 4810 |
| Disease-free yield | At picking | 20670'' | 19305 | 14495'' | 18590 | 18265 | 475 | 1300 |
| | 4 days after | 19630'' | 17745 | 12545'' | 16640 | 15730 | 715 | 2200 |
| | 11$\pm$2 days after | 13780'' | 10985' | 8645 | 10075' | 6955 | 994 | 3055 |
| Anthracnose-free yield | At picking | 21450 | 21775 | 21970 | 20800 | 22880 | 1560 | 4810 |
| | 4 days after | 21385 | 21255 | 21710 | 21060 | 20670 | 1007 | 3120 |
| | 11$\pm$2 days after | 15925' | 15665' | 20215'' | 16250' | 13910 | 520 | 1560 |
| Late blight-free yield | At picking | 20800'' | 19695' | 14560'' | 18785 | 18460 | 403 | 1235 |
| | 4 days after | 20800'' | 19435' | 13780'' | 18720 | 17875 | 500 | 1540 |
| | 11$\pm$2 days after | 20735'' | 19370' | 12935'' | 18525 | 17355 | 546 | 1690 |

(1) D.R.S. = difference required for significance at P = 0.05
'' = different from the check at P = 0.01, ' at P = 0.05
D.R.S. between values of any two lines = $D.R.S._x = \sqrt{D.R.S._y^2 + D.R.S._z^2}$

an increase of marketable fruit was Fermate I.

Since the copper and Fermate treatments produced different results with respect to anthracnose and late blight control, yields of anthracnose-free fruit and late blight-free fruit were taken and are also presented in Tables 21 and 22.

At picking time the yield of anthracnose-free fruits was identical with the total yield and consequently the yields of fruit from plots receiving different spray treatments did not differ significantly from the yield of unsprayed plots.

The results are essentially the same four days after picking. After eleven days the anthracnose-free yield was significantly lower than at picking time with all treatments except Fermate I. As compared to the anthracnose-free yield of the control at this time, an increase was produced by all treatments. The only really satisfactory increase, however, was given by the Fermate I treatment.

At picking time the yield of the late blight-free fruit on the copper-sprayed plots was significantly increased. At this time a significant increase over the yield of the control was produced by copper sprays and a

significant decrease by the Fermate I spray. Since the amount of late blight did not increase after picking time, this result also holds true for data taken after a period of fruit incubation.

---

## GENERAL DISCUSSION

Experimental work prior to the time this study was initiated had indicated that Colletotrichum phomoides gains entrance into immature tomato fruits where it remains in a latent condition until time of ripening. A possible explanation for the latency of C. phomoides in green fruit and its resumed activity in ripe fruit is that the fruit is rendered a more suitable medium for fungus growth by changes in composition during ripening. My studies on the effect of carbohydrate source, nitrogen source and pH on the growth of C. phomoides in culture do not support this hypothesis. The fungus was found to be able to grow on a wide range of concentrations of a variety of carbohydrate and nitrogen sources. The range of concentrations used was much greater than any change occurring during the development of the tomato fruit. Even on media in which either a carbohydrate or nitrogen source was omitted, the fungus maintained itself and the colonies increased considerably in diameter. Good growth occurred over a pH range of 3.0 to 8.5 on artificial media. According to the literature, the changes occurring in the pH of the fruit during its development, cover a range from pH 4.3 to 6.1. Other causes for the latency of C. phomoides must there-

fore be sought.

Since anthracnose spotting usually does not appear in the field until the later part of the summer it is not apparent how the inoculum of the fungus spreads and builds up. A study of the life history of C. phomoides showed that the fungus is able to overwinter in the soil and on crop debris and that, from these sources of inoculum, infection of pistils and stamens of tomato flowers can occur. The fungus did not remain latent on these floral organs but grew profusely producing masses of spores. These findings are of significance since they indicate the initial source of inoculum and the means by which the fungus builds up in the field. They suggest the possible value of crop rotation as a means of control and give some information of value in determining the timing of a spray schedule. The situation however may be further complicated by the presence of several physiologic forms, some of which may be capable of attacking other hosts such as apple.

It was found that C. phomoides could grow in artificial media and develop in the fruit over a wide range of temperatures. The growth of the fungus in the fruit is affected directly by the temperature which also affects the ripening and maturing of the fruit. Since

C. phomoides does not normally develop on green fruits and as the resistance of the fruit, to the growth of the fungus, seems to decrease with ripening, the growth of the fungus will be determined to a large extent by the effect of temperature on fruit ripening. These findings are essentially the same as those previously reported by Nightingale and Ramsey (1936). In the naturally infected fruit, two factors are involved. There is firstly, the effect of temperature in overcoming the latency of the fungus, and secondly, the effect of temperature on growth once latency has been overcome. Studies on the effect of a period of low temperature storage showed that this treatment reduced the number of infections remaining latent, hence increased the number of actively developing lesions and increased fruit wastage. Not only was there an increase in the number of lesions developing but the time at which these lesions appeared was advanced in relation to fruit maturity. Mature-green fruit, bearing latent infections when ripened at room temperatures may pass the marketable stage before developing symptoms, while the same fruit subjected immediately to a period of refrigeration following picking may become unmarketable because it develops anthracnose symptoms while still turning.

From the above results, it is concluded that the temperatures commonly recommended for the storage of tomatoes are not satisfactory for anthracnose control. This difficulty may, however, be partly overcome by field spraying with Fermate since this treatment delayed the time at which anthracnose symptoms appeared in relation to fruit maturity. This was true for fruit held continuously at air temperature and also for fruits subjected to a period of refrigeration.

Maximum lesion development occurred under conditions of good aeration. When a quantity of naturally-infected tomato fruit was stored in a closed container, anthracnose symptoms did not develop. Growth in plate cultures and lesion development in puncture-inoculated ripe fruits enclosed in the same container, were also inhibited. Normal growth was, in all cases, resumed on removal from the container. These results suggest that emanations given off by the fruit were responsible for the inhibition. Further studies showed that the inhibition was largely due to an accumulation of the carbon dioxide of respiration, although in a confined carbon dioxide-free atmosphere slight inhibition, possibly due to ethylene, also occurred. Since carbon dioxide can cause this inhibition it may well be that changes in the carbon

dioxide content of the internal atmosphere of the tomato fruit determine whether or not C. phomoides will remain latent. This hypothesis is supported by the fact that a decrease in respiration rate occurs as fruit matures (Gustafson 1929). It is further supported by the fact that refrigeration which depresses the respiration rate also increases the number of anthracnose lesions and advances the time at which anthracnose lesions appear in relation to fruit maturity. When the ethylene emitted by the fruit was removed from the surrounding atmosphere by absorption with bromine adsorbed in charcoal, anthracnose development was increased. This suggests that the accumulation of ethylene given off by the fruit in storage may be a factor in determining the latency of the fungus.

The results of a field experiment showed that spray treatments did not significantly change the yield of ripe fruit. This seems to be in contradiction with the findings of Wilson and Runnels,(1937), Jolivette and Walker (1942), who reported a diminution in the yield of ripe fruits on copper-sprayed plants. The lack of difference in the yields may have been due to the weakness of the copper solutions at the beginning of the season. Such diluted solutions do not reduce the fruit setting by

killing of the blossoms according to Horsfall and Heuberger (1942). After freezing more green fruits remained, however, on Bordeaux and Copper A-sprayed plots. These plots would have, eventually, outyielded the other plots, had the weather continued favorable for growth. A correlation seems to exist between the amount of green fruits on the plants after frost and the control of defoliation by the spray treatments. The less defoliated plants gave the highest amount of fruit setting. Fermate did not control defoliation; this result is in agreement with those reported by Wilson (1943-1944) and McNew (1943a and 1943b). Bordeaux and Copper A effectively controlled late blight but gave only limited control of anthracnose. Six applications of Fermate satisfactorily reduced the amount of anthracnose occurring in otherwise marketable fruit but actually increased the amount of late blight. Three late applications of Fermate gave an anthracnose control comparable to six applications of the copper sprays but were without effect on late blight. As has been already indicated anthracnose control by Fermate seems to be due to an inhibiting rather than a toxic effect since Fermate-sprayed fruit, when held to overripeness, developed as much anthracnose as the control. No explanation can be

advanced for the increase in amount of late blight on the plots sprayed all season with Fermate.

---

## SUMMARY

1 - A detailed investigation of the development of anthracnose (Colletotrichum phomoides (Sacc.) Chester) and its control was made in connection with a study of market diseases of tomatoes.

2 - It was found that C. phomoides can overwinter in the soil and on crop refuse, and that it can infect and grow actively on stamens and pistils of tomato flowers.

3 - C. phomoides normally gains entrance into immature fruits. Once inside the fruit it remains latent, causing no fruit symptoms, until the fruit begins to ripen. At this time, it resumes active growth and produces characteristic spots.

4 - Carbohydrate, nitrogen and acidity changes in tomato fruit during ripening do not satisfactorily explain the lack of development of C. phomoides in green fruit and its rapid development in ripe fruit.

5 - Aeration increased the percentage of fruit showing anthracnose symptoms. Storage of fruit in a closed chamber prevented the development of disease in naturally infected fruits and inhibited growth on puncture-inoculated fruit and in artificial media.

This effect seems to be due primarily to the accumulation of the carbon dioxide of respiration and apparently to a lesser extent to the ethylene given off by the fruit. The behavior of C. phomoides in green and ripe fruits may be explained by difference occurring in the carbon dioxide content of their internal atmospheres.

6 - The ordinary tomato storage temperatures were unsatisfactory for anthracnose control. A period of storage at 10-12°C., besides greatly increasing the amount of anthracnose appearing on the fruit, advanced the time at which symptoms appeared in relation to the stage of fruit maturity.

7 - Bordeaux and Copper A applied as field sprays controlled late blight (Phytophthora infestans (Mont.) De By) but failed to control anthracnose. Fermate failed to control late blight but satisfactorily controlled anthracnose. The effect of Fermate was to inhibit the development of anthracnose symptoms rather than to prevent infection.

ACKNOWLEDGMENTS

The author expresses his thanks to the following members of the Department of Plant Pathology, Macdonald College; to Professor John G. Coulson, Head of the Department, for supervising this investigation, and to Ralph A. Ludwig, Demonstrator, for assisting in the preparation of this manuscript.

## LITERATURE CITED

ARTHUR, J.C. 1885. Rot in ripe tomatoes. N.Y. State, Exp. Sta. Ann. Rept. 3; 380-382.

BAKER, R.E.D. 1938. Studies in the pathogenecity of tropical fungi. II- The occurrence of latent infections in developing fruits. Ann. Bot., N.S. vol. 2; 919-931.

BRIAN, P.W. 1932. Effect of volatile substances arising from apples on the germination of mould-spores. Dept. Sci. & Ind. Res. Rept., Food Inves. Board 1932.

BROOKS, C. BRATLEY, C.O. and MCCOLLOCH, L.P., 1936. Transit and storage diseases of fruits and vegetables as affected by initial $CO^2$ treatments. U.S.D.A., Tech. Bul. 519.

CHESTER, F.D. 1894. The ripe rot or anthracnose of tomato. (Colletotrichum phomoides, Sacc.) - Del. Agr. Expt. Sta. Ann. Rept. 6; 111-115.

_____ 1891. The ripe rot or anthracnose of tomato. (Colletotrichum Lycopersici) - Del. Agr. Expt. Sta. Ann. Rept. 4; 60-62.

DENNY, F.E. 1935. Testing plant tissue for emanations causing leaf epinasty. Boyce Thompson Inst. Cont., vol.7; 341.

EDGERTON, C.W. 1908. Physiology and development of some anthracnoses. Bot Gaz., vol.45; 367-409.

GANE, R. 1934. Identification of ethylene among the volatile products of ripe apples. Dept. of Sci. & Ind. Res., Rept. Food. Inves. Board. 1934; 122.

GATTERMAN, L. 1937. Laboratory methods of organic chemistry MacMillan and Co. Ltd. London.

GUEGUEN, M.F. 1902. Recherche anatomique et biologique sur Gloeosporium phomoides, Sacc. parasite de la tomate. Bull., Soc., Myc. France, 18; 312-327.

GUSTAFSON, F.G. 1929. Growth studies on fruits. Respiration of tomato fruit. Plant Phys. 4; 349-355.

HANSEN, E. and HARTMAN, H. 1935. Occurrence in pears of metabolic gases other than $CO^2$. Ore. Agr. Exp. Sta. Bul. 342.

HORSFALL, J.C. and HEUBERGER, J.W. 1942. Causes effects and control of defoliation on tomatoes. Conn. Exp. Sta. Bul. 456.

JOLIVETTE, R.T. and WALKER, J.T. 1942. Spraying tomatoes doesn't pay in Wisconsin, Wisc. Agr. Exp. Sta. Bul. 445.

JONES, W.W. 1940. Methyl-bromide fumigation of papaya and tomato. Hawai Agr. Exp. Sta. Cir. 17.

KIDD, F. and WEST, C. 1934. The effect of ethylene on apples at low temperatures. Evidence for the production of ethylene by unripe, immature fruit. Dept. Sci. & Ind. Res., Rept. Food Inves. Board 1934; 119.

_____ 1932a. Effect of ethylene and of apple vapours on the ripening of fruits. Dept. Sci. & Ind. Res., Rept. Food Inves. Board 1932; 55.

_____ 1932b. Gas-storage of tomatoes. Dept. Sci. & Ind. Res., Rept. Food Inves. Board 1932; 209-212.

MC KEE, R.K. 1940. On the control of mango anthracnose by spraying. Trop. Agr., vol XVIII, no.6; 115-117.

MC NEW, G.L. 1943a. New spray controls anthracnose of tomato fruit. U.S.D.A. Farm Research, vol. IX, no.1; 667.

_____ 1943b. Use of fungicide during war. XII-Control of anthracnose on cannery tomatoes. Canner 96-; 26-28.

NIGHTINGALE, A. and RAMSEY, G.B. 1936. Temperature studies on some tomato pathogens. U.S.D.A. Tech. Bul. 520.

OCFEMIA, G.O. 1931. Notes on some economic plant diseases new in Philippine Islands (II). Philipp. Agri XIX, No. 9.

PARRIS, G.R. and JONES, W.W. 1941. The use of methyl bromide as a means of detecting latent infection by Colletotrichum spp. Phytopath. 31; 570.

PATERSON, D.D. 1939. Statistical technique. Agricultural research. McGraw-Hill.

PLOWRIGHT, M. 1881. On the fungoid diseases of tomato. Gardiner's Chronicle.

RAMSEY, G.B. and LINK, G.K. 1932. Market diseases of fruits and vegetables. Tomatoes, peppers and egg-plants. U.S.D.A. Miscell. Pub. 121.

ROSA, J.T. 1926. Ripening and storage of tomatoes. Proc. Am. Soc. Hort. Sci. 23; 233-240.

_____ 1925. Ripening of tomatoes. Proc. Am. Soc. Hort. Sci. 22; 315-322.

ROSENBAUM, J. 1918. The origin and spread of tomato fruit rot in transit. Phytopath. 8; 572-580.

SACCARDO, P.A. 1882. Gloeosporium phomoides, Michelia II: 540. 1881. Gloeosporium phomoides. Fungi Italici.

SANDO, C. 1920. The process of ripening in the tomato, considered especially from the commercial standpoint. U.S.D.A. Bul. 859.

SAYWELL, L.G. and ROBERTSON, D.P. 1932. Carbohydrate content of tomato fruits. Plant Physiology 7; 705-710.

SEYMOUR, A.B. 1929. Host index of the fungi of North America.

SIMMONDS, J.G. 1941. Latent infections in tropical fruits discussed in relation to the part played by species Gloeosporium and Colletotrichum. Proc. Royal Soc. Queensland for 1940, vol. 104, no.3; 486-489.

SKOK, J. 1943. Defoliation of tomato plant as a response to gaseous emanations from the fruit. Bot. Gaz. vol. 104, no.3; 486-489.

SMITH, A.M. and GANE, R. 1932. Influence of gaseous products from apples on the germination of seeds. Dept. Sci. & Ind. Res. Rept., Food Inves. Board 1932; 156.

STONEMAN, B. 1898. A comparative study of the development of some anthracnoses. Bot. Gaz. vol. XXVI, no.2; 69-120.

TAUBENHAUS, J.J. 1912. A further study of some Gloeosporium and their relation to sweet pea disease. Phytopath. 2; 152-161.

WARDLAW, C.W., BAKER, R.E.D. and CROWDY, S.G. 1939. Latent infections in tropical fruits. Trop. Agr. vol.16, no. 12; 275-276.

WARDLAW, C.W. LEONARD E.R., and BARNELL, HR. 1939. Metabolic and storage investigations on the bananas. Imp. Coll. Trop. Agr. Low Temp. Res. Sta. Memoire No.11.

_____ Mc GUIRE, L.P. 1932. The storage of tropically grown tomatoes. E.M.B. 59.

WILSON, J.D. 1944. Further observations on the control of the tomato anthracnose. Ohio. Agr. Exp. Sta. Bimonth. Bul. 226; 56-63.

_____ 1943. Preliminary results on the control of tomato anthracnose. Ohio, Agr. Exp. Sta. Bimonth. Bul.28; 34-38.

WILSON, J.P. and RUNNELLS, E.R. 1937. Five years of tomato spraying. Ohio, Agr. Exp. Sta. Bimonth. Bul. 22; 184.