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UNIVERSITY OF ILLINOIS

Agricultural Experiment Station

BULLETIN No. 118

BITTER ROT OF APPLES

Botanical Investigations

By THOMAS J. BURRILL

URBANA, ILLINOIS, SEPTEMBER, 1907

SUMMARY OF BULLETIN No. 118

Bitter rot of apples, an exceedingly destructive disease attacking fruit on the tree, is due to a specific fungus called *Glomerella rufomaculans*, which though sometimes found on other fruits, and which can be artificially grown upon many substances, is in Illinois practically confined to apples and to apple-tree limbs. On the latter the affected spots are called cankers. There are two forms of spores, but they appear to be alike in function, neither of them being specialized to survive the winter. It is the mycelium in the cankers and in old infected fruits that does this. Page 555

Limbs of apple trees become infected only in spots where the bark has been previously injured mechanically or by some other parasite. Cankers may originate as late as the time of the apple harvest and in wounds made at this time. Page 561

The spores are very easily destroyed by copper sulphate. It seems impossible to kill the fungus in the limb cankers by any permissible external application. They must be destroyed by cutting off the affected limbs. Page 563

Outbreaks of the disease usually begin in July or August, but may start as early as June 1, in north latitude 38°. Page 571

The spores are to some extent distributed by pomace flies, but no insects are largely instrumental in the distribution or development of the disease. The spores are readily washed from cankers and infected fruits to fresh fruits hanging below in the tree. Light showers most effectively aid infection. Spores and spore-masses are distributed by wind, sometimes to considerable distances. Page 572

New apples are first infected only by spores produced in limb cankers or in infected apples (mummies) of the previous year which have hung during the winter on the trees. Neither the fungus nor its spores live over winter in the ground or in anything upon the ground. Page 580

The absolute eradication of the disease from an orchard is entirely possible by careful collection and destruction of the cankers and mummies, faithfully supplemented by effective spraying with Bordeaux mixture and the prompt removal of early infected fruit. Page 593

Bulletin No. 117 should be consulted for methods and results of spraying.

BITTER ROT OF APPLES

Botanical Investigations

BY THOMAS J. BURRILL, CHIEF IN BOTANY

INTRODUCTION

The fungus to which the decidedly distinctive disease of apple fruits called bitter rot is due, is undoubtedly of American origin and the disease seems to be still confined to our own country. With us its geographical extension is very wide, but offers peculiarities of development which are apparently due solely to climatic characteristics. It attacks apples on the tree during any period of the season when hot, humid weather prevails, and is at its worst enormously destructive,—an estimate of \$10,000,000 a year for the United States cannot be considered an exaggeration.

The disease has long been recognized but where it came from or to what cause it could be attributed was little understood. Since it frequently occurred year after year upon some particular tree or trees it was supposed to result from something inherent, some inside characteristic of the tree itself, or that the poison rose in the sap from some source not explained. An outbreak often seemed exceedingly sudden, and as the origin was mysterious, prevention or cure was entirely beyond reach. There was nothing to do but helplessly to submit to the inevitable. The promise of a fine harvest, perhaps after the fruit was nearly or fully grown, was swept away as by the breath of contagion, blasting in a few days the apparently well-founded hopes of the orchardist for requisite remuneration for his year's labor and expenditures. There was something in it apparently beyond recognition,—something mysterious, something outside of the run of ordinary cause and effect. While apples were grown principally for home use, the losses, though keenly felt, were not counted in dollars and cents; but as orchards were increased in size and apple-growing assumed commercial importance the inroads made seemed still harder to bear and came to be reckoned in financial terms, rising sometimes even in the case of single plantations into the thousands.

It was at this stage that the matter began to receive serious attention on the part of the state agricultural experiment stations most directly concerned, and by the pathologists of the United States

Department of Agriculture. Some direct, successful studies were made during the last quarter of the Nineteenth Century, and certain other important discoveries found afterward helpful had been made, but the knowledge which is at this date relied upon as a guide in the prevention of these losses has mainly resulted from investigations undertaken since the beginning of the year 1900. Thanks to these studies and experiments, it may now be said that what formerly seemed incomprehensible is easily understandable. The cause of the difficulty has been ascertained and a fungus identified as that cause. The time has gone by for any one to cavil in regard to the application of the word "cause" so used. It is true that the parasite must have conditions suited to itself, but conditions are not active agents. It is the fungus as the active agent working under permissible conditions of susceptible fruits and of heat and moisture which must be charged, if there is to be such a charge, with the cause of the effects witnessed. Combative measures to be direct must be upon this basis, and even the control of conditions must be upon the assumption that this means warfare upon the living, injury-working parasite.

Fortunately there is no longer reason to fear great losses from this infectious disease. No one need permit the destruction of a crop of well-grown fruit. The destroyer has not only been identified and its mode of life made known, but means of successful combat have been ascertained. It is hoped this bulletin and the companion one from the department of horticulture will be found to be useful additions to former luminous literature upon the subject. While in this number studies upon the fungus are reported, in Bulletin 117, by Professor Joseph C. Blair, the means of control are clearly and conclusively set forth. Perhaps never before in the history of any parasitic plant disease has there been such elaborate experimentation under circumstances and methods so likely to furnish incontestable results.

Besides the acknowledgments given in the text it should be said Mr. James T. Barrett, First Assistant in Botany, has had a very large part in these investigations since 1903. He has efficiently carried into execution the plans for the various experiments reported and has more or less devised his own methods of procedure. The laboratory and field notes appended under the different subjects are substantially as written out by him. The illustrations were all made by him except as otherwise credited.

For a general citation of literature see Von Schrenk, Hermann and Spaulding, Perley *The Bitter Rot of Apples*, Bul. U. S. Dept. Agr., Bul. Plt. Ind. 44: 46-51, 1903.

PART I GENERAL INFORMATION

The so-called bitter rot of apples owes its name to the taste of the affected tissue, but this varies much from an exceedingly bitter quality to that which can scarcely be so identified. The term ripe rot sometimes applied is much more of a misnomer for the disease is especially one of growing fruits.

The malady is easily recognized by the very characteristic, black, circular, sharply margined spots on fruits varying in size from an eighth to three-fourths or more of an inch in diameter (Pl. III., Fig. 2.) These spots become somewhat depressed by the shrinkage of the affected tissues which are always dry and become leathery and even corky in texture. Properly then the effect is not that of a rot at all. On the contrary the pulpy substance of apples is well preserved from ordinary decay through the action of this infecting agent.

The disease occurs practically throughout the United States, wherever apples are very much grown, from Maine to Texas and from Michigan to Louisiana, but is greatly more injurious in certain large areas, the largest of which seems to be a broad belt extending from Virginia to Oklahoma, roughly between the parallels of 35 and 39 degrees of north latitude. In Illinois it has not been seriously troublesome north of some 30 to 40 miles south of Springfield. Further southward it has often destroyed a million or more dollars worth of fruit a year.

It is well known that the destructive disease of apples just described is due to a specific fungus, which was long called *Glaeosporium fructigenum* Berk., but which has more recently been referred to as *Glomerella rufomaculans* (Berk.) Spauld. and Von Schrenk.¹ This fungus grows on developing apples, beginning at any time during the summer or autumn when the conditions are favorable after the fruits are formed and until and after they are ripe; and it lives as a parasite in the bark of apple-tree limbs, usually in localized areas called cankers. It does not occur upon the leaves.

The same fungus grows in the same way upon several other fruits; such as pears, quinces, peaches, and grapes, and even on tomatoes, peppers, egg-plants, etc. (Pl. III. Fig. 1.) Sometimes it naturally develops on grape berries to a destructive extent; but commonly it is only decidedly injurious to apples, and has never been reported as forming limb-cankers on other trees. It seems, however, to develop as a parasite in some cases on the stems of

1. The fungus referred to in this bulletin is the same as that for which this name was proposed, whatever may be said of the name itself, a matter not here given further consideration.

sweet peas,¹ and what is probably the same fungus causes the so-called "mummy" disease of guavas.² It can readily be grown and fruited in the laboratory as a saprophyte on the most various substances, including all the standard media used for bacterial cultures.

There are two kinds of fruiting or two methods of spore-production. The one upon which the generic term *Glœosporium* is founded is by far the most common, and is that upon which the identification of the parasite is made in the field. This it is which is seen on green or ripe apples, and more commonly this only is found in the cankered spots of the apple-tree limbs. In these and other situations the mycelium which vegetates in the tissues of the host, often penetrating deeply therein, forms in minute specialized areas little cushions of interlaced threads just beneath the surface, and from these cushions numerous, closely associated, erect threads (sporophores) arise, on the apices of which spores (conidia) are produced by abstriction. That is, the terminal portion of one of the filamentous sporophores is separated by the formation, a little below the apex, of a cross partition, and at the same time or closely thereafter the side walls at this place are contracted as though by a strangulating thread. The part above the partition and constriction becomes the conidial spore and when mature this spontaneously separates from its parent filament (Pl. II. Fig. 2 and Pl. VI., Fig. 1). The same process may take place many times, conidium after conidium being formed from the same sporophore. On the apple and other fruits the little localized masses of sporophores forming what are called sori, push up the epidermis from beneath until it ruptures, making a pustule, which may be discerned by the unaided eye. The unopened pustules are black, due to the dark color of the fruiting threads, and not infrequently the dark-olive colored mycelium grows in dense mats or masses, outside the substance in which it derives its nutriment; but the spores issue from the ruptures in pinkish masses and are then still easier seen by unaided vision. Very often the spore-pustules are arranged in several concentric lines best seen towards the borders of the infected spots on the fruit. These spots from a small beginning constantly increase in size until perhaps half or more of the apple is involved; or several spots, originally distinct, coalesce into one large area of infection. These circular spots, blackish in color, somewhat depressed, with their concentric rings of spore-pustules and the pinkish spore-masses, clearly characterize the malady and serve definitely to dis-

1. Sheldon, John L. Concerning the Identity of the fungi Causing an Anthracnose of the Sweet Pea and the Bitter-Rot of the Apple. *Science*, N. S. 22:51. Jan. 1905.

2. Sheldon, John L. Ripe Rot or Mummy Disease of Guavas. *Bul. W. Va. Agr. Exp. Sta.* 94:297-315. Ap. 1906.

tinguish the disease from any other such fruit infection. (Pl. III., Fig. 2.)

The spores as they issue from a pustule are imbedded in an adhesive substance so that they remain in a coherent body, sometimes taking the shape of a curled tendril-like thread, more often of an irregularly rounded globule. After exposure to dry air the cementing substance becomes hard, but is quickly dissolved again in water. When once thus separated in water the spores lose this cohesive peculiarity. Under proper conditions of temperature and moisture they quickly germinate by sending out one or more thread-like tubes, and these, in artificial cultures, soon begin to produce a new generation of spores like those from which the threads originated. All this may occur within 24 to 48 hours in a glass tube or dish.

In such cultures there are frequently seen at the tips of germinal threads peculiar, dark colored, spore-like bodies, the nature and office of which have been considerably discussed. Recently Hasselbring has shown that they are specialized organs, called *appressoria* by Frank of Germany, for adhesion to smooth surfaces like those of fruits, and for aid in the penetration of the epidermis by the germinal tube which each emits.¹ The appressoria are produced on the conidial germ-tubes when these touch a solid body not bathed in nutrient liquid. They seem, therefore, to play an important role in the infection of apples (Pl. X).

While the conidia are protected by the substance which causes them to cohere as they issue from the spore-pustules, they preserve their vitality a long time when they are kept in a dry place; but they very soon lose the power of germination if the readily soluble protecting substance is first washed away. In laboratory experiments fresh spores placed in tap water and at once filtered out and dried for 24 hours fail to germinate when again moistened. As indicated above, the germination of viable conidia is very prompt when conditions are favorable and they were favorable in these tests.

Notwithstanding the ordinary name this fungus does not cause a true rot, as has been stated above. The apple substance does not become soft, but becomes instead tough, and at length dry and hard. Other fungi may simultaneously or subsequently invade the tissues and break down the structure, but the effect of this parasite is a preservative one upon the cell walls. When infected apples dry and harden into the shriveled state usually called mummies, the vegetative threads (mycelium) of the fungus and the cushion of erect,

1. Hasselbring, Heinrich. The Appressoria of the Anthracnoses. Bot. Gaz. 42:135-142. Aug. 1906.

crowded, spore-bearing filaments (sporophores) retain their vitality for a long time—one or more years if kept dry—and pinkish masses of spores are promptly formed again within about three to six days after sufficient moisture is supplied. The spores (conidia) are produced on the tips of the old sporophores as they may have been months before.

In this way certain of the old mummies become sources of infection the season after they are formed, (Pl. VIII., Fig. 2) but it will be shown later that this is true only when the old fruits are not destroyed by other rots; that is, only when they have been mummified by the *Glœosporium* fungus and then have not been subject to the attack of bacteria, molds, or other saprophytic growths.

The fungus lives over winter in wound-like spots called cankers on the limbs of the apple trees, and from these infection may start the succeeding season. Not infrequently the location of a canker can be made out by the spots beneath it on the growing fruit, caused by spores washing with rain water from the former onto the surfaces of the apples. The area of such infection in a well-fruited tree frequently takes the form of a cone with its apex upward at or beneath the limb-canker. The water carrying the spores does not descend perpendicularly because of the interference of the foliage, hence the wider area of infection below.

The fungus in the cankers grows and fruits just as it does in the apples, (Pl. II.) although the spore-pustules do not show much, *i. e.* are not very evident, before the spores exude and they are not arranged in concentric circles, owing in both cases to the nature of the substratum. The method of production of the conidia and the conidia themselves are the same. The latter exude in pinkish, (sometimes showing shades of yellow instead,) adhesive tendrils or irregular heaps, indistinguishable from those on the fruit. There is likewise no difference in the behavior of the conidia in germination, etc. It is to be noted, however, that the spore-masses exude only when the bark has been moistened by water or when the air continues very moist for some days.

The second spore-form or *Glomerella* stage is readily secured in laboratory cultures, but is not very commonly found in nature. It was first discovered by Clinton¹, in 1901, in cultures and then on old, infected apples which were kept for some time in a moist chamber. Hasselbring² (1901-02) made special studies for this

1. Clinton, G. P., Apple Rots in Illinois. Bul. Ill. Agr. Exp. Sta. 69:206.

2. Burrill, T. J., and Blair, J. C. Bitter Rot of Apples. Bul. Ill. Agr. Exp. Sta. 77:354.

purpose of old mummies, but failed to find again this form. It has not been found by the author in numerous examinations on fruits kept out of doors. Spaulding¹ found this asco-fruit in rotting apples kept in the laboratory and in artificially produced limb-cankers, and Scott² reports it from infected apples kept in the laboratory as well as in artificial cultures. From natural cankers cut on July 28, 1905, and again on August 21, same year, Barrett (Pl. I, Fig. 2, and Pl. VI., Fig. 2) found perithecia and asci with ascospores. This suffices to show that the second, perfect, or asco-fruit does develop at times and under certain conditions, but the *Glomerella* stage surely does not play any important role in the seasonal development or dissemination of the fungus, as was at one time supposed.

The ascospores are altogether similar to the conidia in appearance, structure, and reproductive characteristics. They germinate as readily whenever conditions favor and they die as soon under unfavorable circumstances. They have no more resistance to external vicissitudes and are therefore not specialized to carry the fungus over winter. It is the mycelium both in the mummies and the cankers which retains vitality and which upon the recurrence of favorable conditions produces, again and again, crops of conidia. It was Hasselbring³ who first proved this to be true of the mummied fruits, though it was previously very generally assumed that in some way these old infected apples carried the fungus over the winter. That the same thing occurred in regard to the limb-cankers was evident upon the first examination made upon them⁴ and abundant verification for both observations have since been made.

It has, however, been found that the early statements to the effect that the cankers are limited to one year's growth is not always true. Sometimes the mycelium remains alive a second, or even a third year, in the border around the originally infected area, or it may be re-infection takes place due to the wound-like character of the first canker. Plate VIII., Fig. 1, shows a canker two years old which, after the limb was cut, produced spores from the portion which encircles the central area killed by the fungus the first year.

In the case of the mummies it will be seen later that the conclusions commonly reached from the earlier studies need some modification, but our information still points to them and to the cankers

1. Von Schrenk, H., and Spaulding, P., *The Bitter Rot of Apples*. Bul. U. S. Dept. Agr.; Bul. Plt. Ind. 44:24.

2. Scott, W. M., *The Control of Apple Bitter Rot*. Bul. U. S. Dept. Agr.; Bul. Plt. Ind. 93:10 Mar. 1906.

3. Hasselbring, H., *The Bitter Rot and its Control*. Trans. Ill. Hort. Soc. 33:350. 1902, and Bul. Ill. Agr. Exp. Sta. 77:354, 1902.

4. *Ibid*, page 355.

as the sources of infection as the new season arrives, and to the living mycelium in these mummies and cankers as the agent of perpetuation. When the weather conditions become suitable, from June to October, the previously dormant mycelium becomes active again and soon furnishes an abundant crop of conidial spores, in the familiar pinkish, waxy masses. Possibly, too, asci and ascospores may be produced in such successive manner, but if so the occurrences must be far less common and the spores far less numerous. They are not often encountered in nature when direct search is made. It has been shown above, however, that this second or *Glomerella* stage is of small importance as a practical matter. It adds nothing to the persistence of vitality nor to the possibilities of starting a new outbreak of the disease. The two kinds of spores are altogether similar in structure, and in function there is no difference that can be determined.

PART II SPECIAL INVESTIGATIONS

The account given upon preceding pages represents fairly well public information upon the growth and development or life history of the fungus under consideration. More details can be found in the citations, but whether references have or have not been made everything stated is upon the authority of or agrees with the writer's personal observations and studies, except that the office and mode of action of the appressoria are quoted from the writer cited.

This does not mean, however, that everything needful concerning the life history of the parasite had heretofore been ascertained and made known, or that additions of great importance may not be made. There are now to be given the further results of studies upon special points in the developmental history of the fungus, knowledge of which has been deemed necessary before fully intelligent warfare can be prosecuted. In the statement some facts heretofore well known must be recounted, but it will not be difficult for the reader to distinguish these from those now reported for the first time.

ORIGIN OF LIMB CANKERS

Limb infections (cankers) originate in wounds (Pl. I., Fig. 1, Pl. VII., Fig. 2). While cankers are very easily produced artificially by mechanical punctures and the insertion of conidia taken from the pustules of infected fruit and from pure cultures, no positive results have been obtained by placing such conidia on the sur-

face of uninjured bark, whatever the age of the twig or limb. It is often observed that a cankered area has at or near its center a little dead twig, or the evidence of one having been there. This is especially true in regard to those varieties of apples which are more than others subject to twig blight, like the Jonathan and Willow. Sometimes there may be found at the base of the portion of a twig killed by blight a small area infected by the rot fungus, the latter evidently having started in the dead or dying bark and descended in the tissues to the living parts. Here, as elsewhere, the fungus shows its saprophytic proclivities. But it is wide of the mark to infer from such observations that it does not also possess vigorous parasitic powers; on rapidly growing fruits it needs no aid except favorable external conditions either in the penetration of the epidermis or the subjugation of the living tissues. It simply is not able to make entrance through the exterior protecting coating of the uninjured bark of a living branch without mechanical help, but such bruises as made by hail are sometimes sufficient aid. It may be added here that fruit spurs which have scars left by the separation of fruit stem the preceding year, are open to infection and may bear small cankers of the bitter rot fungus (Pl. IV., Fig. 2).

Cankers may be started as late in the season as the weather conditions permit the growth of the fungus. These conditions often exist at or after the time of picking the apples, and limb bruises then made may become cankers to serve as sources of infection the following year. When orchard managers, becoming impatient with the slow process of hand picking, resort to thrashing off rotting apples with the idea of preventing further infection, they are unconsciously arranging for a fine crop of cankers the next season; the bruises thus made on the bark of limbs open the way for the development of the fungus.

It must be admitted, however, that we cannot yet explain why so many more cankers are formed at one time than another, in one season than another, beyond accounting for by the prevalence of the disease on the fruit, the comparison of climatic conditions, the handling of the trees, etc. During the season when the existence of limb-cankers was first made known¹, they were found in large numbers in many orchards of Southern Illinois. Never since that year (1902) have they been so generally abundant. While it has been easy, one year as well as another, to produce them artificially,

1. Burrill, T. J., and Blair, J. C. Prevention of Bitter Rot. Cir. Ill. Agr. Exp. Sta., 58: Jul. 1902. Same, Bitter Rot of Apples. Bul. Ill. Agr. Exp. Sta., 77:354, Jul. 1902. Von Schrenk, H., and Spaulding, P., The Bitter Rot of Apples, Bul. U. S. Dept. Agr., Bul. Plt. Ind. 44:29, Jul. 1903.

(Pl. II., Fig. 1, Pl. IV., Fig. 1,) during some seasons it has been hard to find natural ones containing the living fungus except upon some few trees. In some sections of the country¹ this seems to be always the case.

FIELD AND LABORATORY DATA

July 11, 1903, inoculated a limb of a Willow apple tree with bitter rot spores. August 11 the canker was four inches long and many black sori were being formed under the surface. Three weeks later the canker had grown to be ten and one-half inches long and had encircled more than half of the limb which was seven-eighths of an inch in diameter. This is the canker later used in experiments 2 and 4 under treatment of cankers.

Inoculated on July 27 three more limbs of a Willow apple tree with bitter rot spores. All three inoculations produced cankers.

Inoculated three limbs of a Willow apple on July 30. The bark of one limb was slit, that of the other two was bruised. After inoculation the wounds were wrapped with damp cloths. All developed cankers and produced cushions of hyphae.

August 7, inoculated two limbs of a Willow apple tree with bitter rot spores. Both produced cankers.

July 11, 1904, inoculated limb of young apple, (2 years out) and July 28 a canker 1 $\frac{1}{4}$ inches long had formed. It was dark, very much sunken, and sori were evidently forming under the surface. On same date another limb was inoculated and July 28, a canker one inch long had developed and bore same small elevations. The canker became three inches long and was cut March 11, 1905. Other inoculations made on same date were followed with cankers which on July 28 were in length respectively as follows: 1 inch; 1 inch, with sori; 1 inch, with sori; 1 inch, with sori; 1 $\frac{1}{4}$ inch, with sori; 2 $\frac{1}{4}$ inches, with sori.

July 15, 1904. The bark of 4 limbs of Willow apple tree was bruised and spores inserted, with results as follows: (1) A small canker developed; (2) slight development, but no canker; (3) no canker; (4) a small canker formed.

July 28, 1904. Inoculation on a pear limb produced a slight development, but no canker.

August 12, three limbs of Willow apple tree were inoculated with bitter rot spores. All three inoculations produced cankers.

August 18, three limbs of unknown variety were inoculated with bitter rot spores. All produced small cankers.

September 1, two limbs of a Willow apple tree were inoculated with bitter rot spores. Small cankers were produced.

September 12, two limbs of a Willow apple tree were inoculated. Both developed small cankers.

September 12, inoculated a limb of apple of unknown variety. October 3, there was a small canker.

September 15, inoculated several twigs of Willow apple. October 3, all show considerable development.

September 22, inoculated two Willow apple limbs. October 3, some development.

September 24, inoculated three branches of a Willow apple tree. No development took place.

September 30, inoculated three limbs of an unknown variety with bitter rot spores. No development took place.

1. Alwood, Wm. B., Orchard Studies. Bul. Va. Agr. Exp. Sta. 142:265 N. 1902.

EFFECTS OF CHEMICALS ON SPORES AND ON CANKERS

It was deemed worth while to make some careful tests upon the strength of fungicides which are necessary to kill the spores for such aid as might thereby be furnished for practical work in the field. Since Bordeaux mixture holds in suspension solid particles which interfere with microscopic examinations, solutions in water of copper sulfate were used instead. It is known that the copper is the chief agent that gives Bordeaux mixture its effectiveness as a spray, hence the copper solutions may be taken to apply well to the action of the more commonly used mixture. The results to be reported certainly give abundant reason for the effectiveness of the Bordeaux spray.

The laboratory tests show that solutions of copper sulfate in distilled, or ordinary rain-water, in amounts above 1 to 1,000,000 parts prevent the germination of spores recently matured in all cases when there is considerable of the solution applied. When, however, the quantity of the latter is much reduced the effect is not so certain. Further tests show that the presence of organic compounds in the solutions greatly reduce the fungicidal action. For instance, when copper sulfate is added to weak beef-broth (bouillon one-half standard strength) instead of to water, germination of spores immersed therein is not prevented in a strength of the solution up to 1 part of the copper compound to 1000 of the broth. That is, it requires more than 1000 times as much of the copper in weak broth as it does in water to kill the spores of this fungus. The explanation probably is that some combination takes place between the copper salt and the organic compounds, rendering the former inert. It seems that something similar takes place when quantities of these spores are substituted for the organic matter of the broth, for when approximately the same small quantity of spore-mass is placed in one medium-sized drop of copper sulfate solution the proportion of the copper salt, to be effective, must be not less than 1 to 100,000 parts of water, while in one hundred drops (4 cc.) similar killing effect occurs in a solution containing 1 part of copper sulphate to 400,000 parts of water (see data following, Expts. 7 and 8). (Pl. X). It is plain, therefore, that the liquid used as a spray must be very considerably stronger than the laboratory tests, as commonly conducted, indicate, and a larger quantity of a given solution will be more certain than will a smaller amount to kill the spores with which the liquid comes in contact. The copper compound as dry dust has no effect whatever, but the spores in that case could not germinate for the want of moisture.

Since common salt (sodium chlorid) has been advocated as useful in controlling bitter rot, it was deemed best to determine what its action is upon the spores. Experiment soon showed that a watery solution of this substance does have very considerable effect (see Expts. 12 and 13 following). When fresh spores are immersed in 3 parts of the salt to 1000 of water germination does not take place, but if the proportion is reduced to 2.5 to 1000 the germ tubes are emitted and growth, evidently not quite normal, is continued. This then seems to be the crucial point when there is a considerable amount of the solution. It is probable that the facts found to be true in this case of copper sulfate will hold here too for small quantities of the solution and for a spray or even a wash stronger solutions would be required to prevent spore development, and then serious damage to the foliage may follow.

It would be very helpful if some way could be found to render the limb-cankers innocuous without injuring the tree on which they are found. The first thing to be thought of is a spray or wash of some substance destructive to the fungus, and copper sulfate and Bordeaux mixture naturally first present themselves to the mind of the experimenter.

It is evidently necessary to have some exact knowledge of the existence of the living fungus in the tissues of a canker before any inference can be drawn as to the positive effect of an application to the exterior surface. The plan adopted for the tests herein reported was to submit the cankers to conditions favorable to the formation and exudation of these spores without at the same time encouraging too much growth of molds, etc. After trying several methods the procedure adopted was to soak the cankered limbs freshly cut from the trees for a few hours, or those that had been severed some weeks or months before, for a longer time usually 24 hours, in ordinary tap water and then to keep them, often wrapped in moist paper, in a closely covered tin box placed in a warm room (preferably about 26° to 30° C.) It was found that the pinkish spore-masses usually appeared in from three to six days, giving clear evidence of the activity of the fungus and definitely announcing its identity. (Pl. II., Fig. 1).

Then to test the efficiency of external applications, selected cankers were cut transversely through the middle and one-half was kept for a check without treatment, while on the other half the fungicidal wash under test was applied, after which both parts were treated alike for the development of spores. In some cases the division of the cankers was made before the soaking, in some cases

subsequently. The substances used were copper sulphate in the proportion of 6 pounds to 50 gallons of water; thick Bordeaux mixture; copper carbonate $12\frac{1}{2}$ ounces (with ammonia water $7\frac{1}{2}$ parts) to 50 gallons of water; and Oregon wash after the formula: sulfur 15 pounds, copper sulfate 20 ounces, slacked lime 15 pounds to 50 gallons of water.

None of the experiments tried (see Expts. 1-17 following under "Treatment of Cankers," etc.) gave encouragement for the hope that any such application to the tree can be of any practical importance in destroying the cankers or in controlling the disease. This is disappointing, for it must be acknowledged that it is impossible to find and remove by the knife or other pruning implement all of the infected spots on the limbs, shoots, and fruit-spurs. After the position of the canker is announced by the spots on the fruit, when this evidence does sufficiently show, it is too late for best results and the cone-shaped figure in the tree is not always traceable. The preventive method of cutting out the cankers is not, therefore, altogether effective; but nothing better has been devised towards suppressing the pernicious part they play in starting infection upon the fruit.

The same may be said concerning any mummied fruit which serve as a wintering place for the fungus. Their collection by hand seems to be the only way to secure the destruction of the infecting agent.

FIELD AND LABORATORY DATA

Copper sulfate. For the most part the following described cultures were made in Syracuse watch glasses and kept at room temperature, about 24 C:

Experiment 1. This experiment consisted of four cultures of the following proportions of copper sulfate to water: 1:125, 6:1000, 1:200, and 1:250. Each culture was inoculated with as nearly as could be estimated the same quantity of bitter rot spores. At the end of 48 hours no germination had taken place in any of the cultures. A duplication of these cultures with newly formed spores gave a similar result.

Experiment 2. In experiment 1 distilled water was used in making up the solutions. In this experiment the same percentage solutions were made up with rain-water and inoculated with spores. After 24 hours aside from the swelling of a few spores in the weakest solution, there was no indication of a tendency to germinate. Germination was good in a rain-water check culture.

Experiment 3. The following copper sulfate solutions were made up and inoculated with bitter rot spores: 1:10,000, 1:100,000, 1:1,000,000, and 1:10,000,000. A check culture was made with distilled water and one with half rain-water and half bouillon. In three days no spores had germinated in the two strongest solutions but a few spores in the 1:1,000,000 solution had sent out very short, weak germ tubes which were apparently dead and the protoplasm somewhat disintegrated. The few germinated spores in the 1:10,000,000 solution were still alive, but very little growth was taking place. They eventually died. The spores which had germinated in the two last named cultures were mostly spores floating on the surface of the liquid. A few appressoria were produced by the sub-

merged, germinated spores, but none by those on the surface. Germination in distilled water was fair and here it was particularly noticeable that the spores in contact with the bottom of the dish produced appressoria. Germination in the bouillon and rain-water was very good.

Experiment 4. This experiment was made for the purpose of determining what effect a nutrient solution in the presence of copper sulfate solutions would have on the germination of the spores. Standard bouillon diluted with an equal amount of water was selected as the nutrient solution, and the following copper sulfate solutions were made up: 1:1000, 1:10,000, 1:100,000, 1:1,000,000, and 1:10,000,000. The check was standard bouillon. After inoculation the cultures were kept at 26° C. In 24 hours germination was pretty good in each culture, slightly better in the weaker solutions and in the check. Appressoria were common except with the floating spores. Bacteria were present in all cultures, a fact which seemed to indicate that the copper had entered into combination with some of the substances of the bouillon, probably the proteids, and formed an insoluble compound. Tests for soluble copper made with thin slices of potato and potassium iodide showed it to be present in slight quantities in the 1:1,000, and 1:10,000 solutions only. On the following day there was a more apparent difference in the various cultures, particularly in the growth of the germ tubes. After germination practically no growth took place in the 1:1,000 culture, while in the 1:10,000 solution a number of germ tubes which had originated from a few masses of spores were growing well and a few were producing new spores.

Experiment 7. This experiment and the following one were made to determine, if possible, whether or not the amount of the solution influenced the toxic effect on the germinating spores. In other words, have the spores or newly formed germ tubes an accumulative action on the copper in solution? In this experiment 4 c.c. of each of the following copper sulfate solution was placed in a watch glass and as nearly as could be judged the same amount of spores added to each: 1:100,000, 1:200,000, 1:400,000, 1:1,000,000, 1:2,000,000, 1:4,000,000, and 1:10,000,000.

In 24 hours the condition was as follows:

1:100,000, no germination, some spores dying.

1:200,000, germination slight, no continued growth, and the short germ tubes soon became hyaline at the tip, or formed an irregular shaped appressoria.

1:400,000, germination slight. A few germ tubes produced a length equal to or a little longer than the length of the spore and then produced appressoria or soon died.

1:1,000,000 germination fair. Some of the germ tubes attained a length two to three times that of the spore, some produced branched tubes while others germinated at both ends. Most of the tubes developed appressoria.

1:2,000,000, germination very fair. Some germ tubes were five to seven times the length of the spore, and although somewhat slender, were apparently healthy. Appressoria were common.

1:4,000,000, germination very fair. The germ tubes in this culture were about as those in the preceding culture; if any different the former were slightly shorter as a whole.

1:10,000,000, percent of germination not quite so high as the last named culture, but the growth of the germ tubes better.

Distilled water. Germination and growth slightly better than the 1:10,000,000 culture.

In all of the above cultures there was little germination or increase in growth after the second day. After appressoria were formed growth ceased, as not one of the latter was seen to germinate.

Experiment 8. In this experiment only 1-25 of 1 c.c. of each solution was used. The solutions were of the same strength as those used in experiment 7, and approximately the same amount of spores was used. After 24 hours the following condition existed:

1:100,000. A few spores had pushed out short germ tubes which were slender and somewhat abnormal. No appressoria.

1:200,000. Germination very fair. The germ tubes were three to four times the length of the spores, apparently healthy, and some of them branched. Many appressoria were produced, and some had germinated and formed a second appressorium. An occasional new spore had been cut off.

1:400,000. Germination good. Germ tubes fifteen to twenty times the length of the spores and many of them had produced one and some two appressoria. Newly formed spores were not uncommon.

1:1,000,000. Germination very good. In this culture the germ tubes had become somewhat branched and some of the short lateral branches were cutting off spores. Appressoria were present but not so abundant as in the above mentioned cultures.

1:2,000,000. Germination pretty good. Both germination and growth not so good as the two preceding cultures. Appressoria were rather abundant. In a few cases as many as four were produced on the same tube.

1:4,000,000. Percentage of germination pretty good. Germ tubes considerably branched but short, not much longer than the spore. Many appressoria present, and an occasional one germinated.

1:10,000,000. Percentage of germination a little less than the 1:4,000,000 solution, but growth somewhat better. Germ tubes were sparsely branched and terminated in poorly developed appressoria.

Distilled water. Germination pretty good, but growth poor. Germ tubes three to five times the length of the spores, slender, and many of them bearing one to two appressoria.

The results of the two experiments, 7 and 8, show that the germination and subsequent growth is considerably poorer in the cultures containing the larger amounts of the copper sulfate solutions. When 4 c.c. of the liquid is used a 1:400,000 strength is sufficient to prevent the germination of most of a large number of spores and to kill those which do have any tendency to grow. If a smaller amount of the solution is taken, for instance, one small drop (1-25 c.c.), the toxic effect is much less on approximately the same number of spores as was used with the larger amount. A weaker solution than 1:100,000 is unsafe when very small quantities have access to very many spores. In 4 c.c. of a 1:400,000 solution of copper sulfate there is .0000025 gram of pure copper. In 1-25 c.c. of a .001 percent solution of copper sulfate there is .0000001 gram pure copper.

Experiment 9. On August 31 some Bordeaux mixture was washed from a number of leaves on a tree which had been sprayed on August 12. On August 27 there was a heavy shower, but aside from dews there was no other moisture on the leaves. Some bitter rot spores were added to the mixture in a watch glass, and as a check spores were added to clear rain-water. In three days no germination had taken place in the Bordeaux mixture washed from the leaves, but the check showed both good germination and growth.

Experiment 10. Some fresh 1 percent Bordeaux mixture was prepared and inoculated with bitter rot spores. No germination took place.

Experiment 11. Some Bordeaux mixture was placed on two cover glasses and allowed to dry 24 hours when they were remoistened and again allowed to dry a few hours. After remoistening a second time, bitter rot spores were added and the cover glasses inverted over Van Tieghem cells. In three days no germination had taken place.

Experiment 12. Six cultures were instituted with the following sodium chlorid solution: 1:200, 1:400, 1:1,000, 1:2,000, 1:10,000, 1:20,000. These were inoculated at 10 a.m. with spores of bitter rot. 5 c.c. of each solution was used in each culture. At 4 p.m. there was no germination in the 1:200 and 1:400 solutions but was good in the next two weaker solutions and fair in 1:10,000 and 1:20,000 strengths. Many appressoria were produced. At 9:30 a.m. on the following day no germination had taken place in the 1:200 solution and only a few spores had started to germinate in the next weaker strength. Germination was good in the 1:1,000 and 1:2,000, fair spores of the 1:20,000 solution. The floating spores of this culture had produced long germ tubes.

Experiment 13. In order to determine more nearly the minimum strength of sodium chlorid which would injure the spores the following cultures were made and 5 c.c. of each inoculated with bitter rot spores: 3:1,000, 1:400, 1:500, 3:2,000, 1:1,000. After 24 hours a few floating spores had germinated, but none of the immersed ones in the 3:1,000 strength. In the second strength the percentage of germination was small, but growth was pretty good; no appressoria had formed. Germination was fair in the 1:500 solution as was also the growth. A few appressoria had formed. In the 3:2,000 solution germination was good,

growth rather poor, and the germ tubes short and bearing appressoria. The percentage of germination in 1:1,000 solution was good, growth poor, and the germ tubes very short and bearing appressoria. At the end of 48 hours the following condition existed:

3:1,000. No germination of the submerged spores.

1:400. A small percent of the submerged spores had germinated but the germ threads were long and somewhat branched and in some cases bearing appressoria on the branches. The floating spores had formed a thin film of mycelium.

1:500. Germination fair, growth not quite as good as the 1:400 solution, but appressoria more numerous.

3:2,000. Germination pretty good, growth rather poor, germ tubes short and almost all bore appressoria. The floating spores had rather long tubes.

1:1,000. Germination good but growth very poor.

TREATMENT OF CANKERS AND MUMMIES TO SHOW VITALITY OF THE FUNGUS, AND TO DESTROY IT

To determine the persistence of vitality of the fungus in cankers they were first soaked in water, when deemed necessary, about twenty-four hours, then wrapped in moist paper and put away in a warm, moist place, usually in a rather tightly closed tin box. These culture experiments were made with many cankers from various sources. Some of them were artificially inoculated, but by far the most were naturally formed. Only a few details of experiments need be given.

Experiment 1. July 28, 1905, a canker was taken from a tree just above two infected apples. The canker was treated as stated above and on the fourth day a few very small masses of pink spores were seen. An examination revealed the fact that the spores, or at least some of them, were coming from perithecia, in which asci were found. Inoculations with these ascospores produced typical bitter rot. At the same time three other cankers, one taken above two infected apples, were treated, but there was no apparent development of the fungus.

Experiment 2. In this case three cankers were used that had been taken from a Ben Davis tree directly above an infected area. They were soaked and treated, as already stated, and at the end of ten days all three were producing pink spores of bitter rot. On one, however, several perithecia were found containing mature asci and ascospores.

Experiment 3. July 28, a canker was cut from a Ben Davis tree above an infected apple and treated as above. On August 5, after eight days, quite a number of small masses of spores appeared.

Experiment 4. On August 21, twenty-one cankers representing fifteen infected areas were removed from about fifteen trees. Seven of the most typical of these were treated as explained. After five days two were producing bitter rot spores. A number of dead, and broken twigs, some bearing wounds, small cankers, etc., were treated at the same time, but no development was apparent.

Experiment 5. A large canker, apparently three years old was taken from a Willow apple tree above an infected area, and treated as all others. In a few days a number of pustules of pink spores appeared and inoculations proved them to be those of bitter rot.

Experiment 6. On July 28, one spur and one canker were taken from a Ben Davis tree above an infected area. After ten days of culturing the spur produced a number of spore masses which proved to be of bitter rot.

Experiment 7. A number of cankers, produced by inoculation during July, August, and September, were cut from trees late in November, and the most of them were made to produce spores after a few days culture.

Experiment 8. March 11, 1905, two small cankers were cut from a small tree in the University orchard and brought to the laboratory. The inoculations had been made August 30, 1904, on small branches and only a slight development had taken place, about $\frac{1}{2}$ inch in length. After a few days' culturing, however, spores were produced on both cankers Nos. 214 and 222.

Experiment 9. On August 21, 1905, a canker one and one-half inches long and on a limb one inch in diameter was cut and cultured. The canker was grow-

ing when brought into the laboratory. At the end of three days both sori and perithecia were found.

Experiment 10. A number of inoculations were made on limbs of Willow apple tree, without breaking the epidermis. The spores were placed on the moistened, smooth bark, and the limb wrapped with a moist cloth. The latter was moistened from time to time, but no development took place.

Experiment 11. A large number of mummied apples were collected from trees in various orchards for examination. In no case was a mummied fruit found which showed pink spores of bitter rot, although by washing with a little water some spores were found. It was found after culturing many of these that the hard, very compact mummy was the one which usually contained the fungus in the living condition and which produced the spores. The mummied fruits were soaked a day or more in water and either wrapped in moist paper or put in tight wooden boxes, such as cigar boxes, and frequently sprayed with water. A considerable number of culture experiments were made and the majority of the hard dried mummies produced bitter rot spores. A number of infected areas were traced to such mummied fruits.

Many culture experiments were also made with mummied fruits gathered under trees on the ground. Most of the trees were known to have had bitter rot the previous year. These mummies came from various Southern Illinois orchards and were cultured as those taken from the trees. In most cases they were more or less broken, soft and attacked by saprophytic fungi. In no case was there any development of the bitter rot fungus on a mummied fruit collected from the ground.

On November 22, 1906, about 520 apples infected with bitter rot in various degrees, which had been recently collected under trees in Richland county, Illinois, were used in an experiment, the purpose of which was to test the effect on the vitality of the fungus of outdoor winter conditions. Of these apples 200 were put on the ground under an apple tree in the Horticultural variety orchard and covered over with a wire net frame $\frac{1}{2}$ -inch mesh; 200 were enclosed in a similar wire net frame, and placed near the ground on 2x4 inch pieces. In both cases the apples were but one layer deep. The remainder of the apples were suspended from a tree on wires; some,—about 20,—were suspended individually and the others on three long wires stretched from limb to limb. During May, 1907, a few of the best preserved mummies from each of the lots mentioned above were cultured in the laboratory, but no evidence was obtained that the bitter rot fungus was alive in any of the specimens. In June, 1907, a second lot was brought in and given cultural treatment with the same result.

None of the mummied fruits, if such they could be called, were in the hard, corky condition that characterizes the typical bitter rot mummy which naturally hangs on the tree. In most cases saprophytic fungi, yeasts, and bacteria had attacked the only partially rotted fruits, and as a result left the decayed apples in a more or less soft, (on ground) or spongy, (on tree) condition. This is the same condition that usually prevails in bitter rot mummies which have fallen in summer or autumn from the trees and lie on the ground over winter.

Experiment 12. A bitter rot canker seven inches long was soaked in water one-half hour January 1, 1904. It had been cut from the tree in Southern Illinois late in November, 1903, and was a result of artificial inoculation. In the meantime it had been kept in a cold room where it had dried but little. After the soaking it was suspended over a steam exhaust pipe, where it was kept warm and moist. On January 14 many pustules of bitter rot spores were exuding through the ruptured places in the bark. The canker was cut transversely into halves, and one-half was treated with a solution of copper sulfate, six pounds to fifty gallons of water. The two halves were again placed in the green-house over the exhaust steam. On February 2, there being no further development, the cankers were wrapped in moist paper, and placed in a tin box. After ten days both pieces of the canker were producing pustules of spores and almost as many on the treated as on the untreated half.

Experiment 13. On February 2, two large cankers were taken from the cold room and soaked in water over night. One canker, ten inches long, produced by inoculation, was cut transversely into halves and one-half was treated with copper sulfate solution of the same strength as that used in experiment 12.

This and the other untreated half with the other whole canker were wrapped in moist paper and put in a tin box. Six days later all were producing bitter rot spores, only a few, however, on the treated half. The canker cut into halves was on a limb seven-eighths of an inch in diameter, and the wood was darkened down to the center. When the examination was made it was found that a considerable amount of mycelium was coming from the wood of the cut ends.

Experiment 14. A small canker, about three inches long, was cut into halves. One piece was soaked in water a few hours, the other was placed on end in a copper sulfate solution. The solution was two inches or more below the canker proper. The first piece was wrapped in moist paper, and after three days the second piece was removed from the copper sulfate solution and treated as the first. The solution had been taken up by the limb and was oozing from different parts of the canker. Three days later, March 8, both halves of the canker were producing bitter rot spores.

Experiment 15. On July 18, the two halves of the canker used in experiment 13 were put to soak and left until the following morning. The previously untreated half was wrapped in moist paper and placed in a tin box, the other half was sprayed thoroughly with Cu CO_3 solution of the strength below: Copper carbonate $12\frac{1}{2}$ ounces, ammonia (weak) $7\frac{1}{2}$ pints, water 50 gallons. It was then wrapped as the first half. Seven days later both pieces were producing bitter rot spores in about equal quantities.

Experiment 16. In this experiment eight cankers were used, four were used as checks and four treated with Oregon wash made up according to the following formula: water 100 gallons, sulfur 30 pounds, copper sulfate $2\frac{1}{2}$ pounds, slacked lime 30 pounds. After the wash had dried all cankers except one were soaked six hours in water and then wrapped in moist paper. Five days later, April 2, two untreated cankers and one treated had developed a few pustules of spores. April 7, all cankers were resoaked, but no additional spores were produced. April 23, they were soaked over night again and five days later all of the untreated and three of the treated cankers were producing a few pustules of spores.

Experiment 17. On April 5, the two halves of the canker used in experiments 13 and 15 were soaked in water and the previously treated half was coated with Oregon wash and with the other put into the moist box. Two days later there was no development of spores, and the cankers were again soaked in water. April 11, only the untreated piece was producing spores.

WHEN DOES INFECTION BEGIN?

It is well known that the development of bitter rot of apples depends greatly upon climatic conditions. The fungus grows well only during hot weather and moisture is essential to the germination of the spores. Frequent light showers, with the prevailing temperature above 80° Fahr., contribute much to make the conditions favorable for rapid spread of the malady and to create an epidemic. Too commonly little attention is given the matter until the spotted fruit shows the disease is already well under way. It is even erroneously claimed by some investigators that the fungus is so saprophytic in character that it only grows upon fruit that has nearly or quite reached maturity and then offers little resistance.

Now whatever may be said about the survival over winter, it is clearly evident that the abundant production of spores on the apples first attacked furnishes the principal source of subsequent infection. It has been determined that under favorable circumstances the be-

ginning of a new crop of spores may follow within five or six days after the seed-spores are placed on the fruit, and even in a shorter time if the skin is mechanically punctured. As innumerable spores are produced upon one apple-spot, abundant infection may follow its development. The date when this latter occurs is, therefore, a matter demanding careful attention.

The earliest record made by ourselves for southern Illinois of the appearance in the field of the disease was for June 13, 1905, when a tree of Chenango (Sherwood's Favorite) in Richland county was found badly infected. This variety is among the earliest to mature its fruit and at the time stated the apples were nearly full grown. The rot-spots in some cases covered one-half of the surface. Many of the spotted fruits had fallen. The evidence, therefore, that at least thirteen days had elapsed since the beginning of the infection seemed conclusive and the date of June 1 may be put down for this beginning. Other observations make the middle of this month not too soon to anticipate the earliest spots on some fruit in Illinois between 38 and 39 degrees of north latitude. If, upon this date only a very few apples show the characteristic marks of the disease, there may be enough to start infection in great numbers of young fruits and to provide the way for serious trouble later.

FIELD AND LABORATORY DATA

On July 2, 1903, bitter rot was found in two different orchards at Kell, Illinois. In one case Lowell apple was infected, in the other Northern Spy. The size and appearance of the affected spots indicated that the first outbreak was not less than ten days earlier. The Lowell trees, four in number, bore the infected apples mostly on the lower limbs which touched the ground in a number of places. The trash and leaves under the trees were more or less moist all the time. By July 17 of the same year fully fifty percent of the Ben Davis trees—a large number—in the orchard containing the Lowell trees were infected.

The first report of bitter rot in 1904 came from Tamaroa, Ill., about July 15. One Ben Davis tree was infected. Bitter rot was found in a Kell, Ill., orchard on a Northern Spy tree on July 20. Some of the apples found were almost half involved. This tree was the same Northern Spy tree on which bitter rot was first found in this orchard in the summer of 1903.

The first records of the occurrence of bitter rot during the summer of 1905 were made on June 13. Many bitter rot apples, some of them half rotted, were found in an orchard west of Olney, Ill., on four Chenango trees. The apples were approaching maturity and were evidently very susceptible to this disease. July 12, the rot was found on a Northern Spy tree at Kell, Ill., and July 15 on two Ben Davis trees in Olney, Ill., orchard No. 3, one infected apple on each tree.

In 1906 the first report of bitter rot came from Olney orchard No. 3 on June 29. July 26 it was reported from orchard No. 1 at Flora, Ill., and by July 27 seventeen Ben Davis trees in Olney orchard No. 3 were infected.

THE DISSEMINATION OF SPORES BY INSECTS

The mucilaginous substance exuded with the spores prevents them in this state from carriage as dust by the wind. The fact that this substance together with the spores dries into a hard cake seems further to prevent wind distribution. While adhesive, the spores may stick to the limbs or bodies of insects and thus be carried about and sometimes deposited on the surface of fruits. That this actually occurs was early shown by Clinton¹, and both before and since his experiments were made, this method of distribution has been assumed in various publications. The adhesive character of the spores, and the fact that so-called pomace flies (*Drosophila*) especially are everywhere visitors to decaying apples, makes the supposition almost a certainty that considerable distribution of the spores takes place by this means. It not infrequently happens that the first affected apples on a tree are upon the lowest limbs, those perhaps which sweep the ground, and explanations have been made that these slow-moving flies would, after their visits to the old mummies lying upon the ground, most likely deposit spores on these lowermost fruits.

We shall see that the premises here are not sound, whatever the inference may be. But it was deemed worth while further to test the general matter by experiments, the details of which are reported later (see data following). Clinton's experiments were repeated with apples producing spores of the bitter rot fungus in one end of a box fourteen inches long and sound apples in the other end, and with some pomace flies shut in to serve as distributing agents. These experiments showed results similar to those before reported. The sound apples soon became spotted with rot evidently carried by the flies. On making microscopic examinations of these flies it was further found that they not only carry spores attached to their hairy legs, etc., but that they feed upon them. The spores still whole and apparently sound can be easily seen through the semi-transparent bodies in the intestines, and in considerable numbers. It was not satisfactorily made out that any of these spores were actually voided in a condition permitting germination, but fruit inoculations with crushed flies were often followed by the development of the disease. At all events the fact that the flies eat the spores strengthens the supposition that the insects play some rôle in the spore distribution in nature, since they must be attracted to infected fruits when spores are exuding and also to those upon whose sur-

1. Clinton, G. P., Apple Rots in Illinois. Bul. Ill. Ag. Exp. Sta. 69:197.

faces spore-masses previously produced have dried and remain somewhat adhesively attached. It has not been shown that other insects are agents in a similar way in this distribution, and from what is now known, it is not at all likely that any insects are largely instrumental in aiding infection through punctures of the epidermis of growing fruits, or of the bark of susceptible limbs. On the fruits the spores do not need such aid when the weather conditions favor their germination. In this case all that is necessary is that the spores while still possessing vitality shall be carried to and lodged upon the unbroken surface of the apples, although punctures such as may be made with a needle facilitate the fungous attack.

Upon the whole, it seems pretty certain that in the orchard, insects have small part in the dissemination of the disease. The little flies mentioned do sometimes carry the spores and may sometimes deposit them on the surfaces of fresh fruits in such way that new infection results, but there is little evidence in support of this from direct observation or from anything really known which lends suggestiveness to the proposition. The greater infection sometimes observed upon the lower limbs must surely be explained in some other way, because (to be shown later) the infection does not arise from the ground. Insect agency need not now be considered at all to explain satisfactorily the distribution of the disease in an orchard.

FIELD AND LABORATORY DATA, 1903

Experiment 1. Twenty *Drosophila* flies collected about bitter rot apples were crushed and examined one at a time under the microscope for bitter rot spores, and fourteen of the twenty contained such spores in the alimentary tract. Twenty inoculations were made with these crushed flies, by inserting them under the skin of apples and six of them developed bitter rot.

Experiment 2. At 5 p.m., July 10, twelve flies were collected about infected apples and three placed in each of four vials. At the end of fifteen hours, July 11, 8 a.m., the flies of vial Nos. 1 and 2 were examined and two in No. 1 were dead. Only the living fly contained bitter rot spores. All three flies in vial No. 2 were living and only one contained spores of bitter rot. A little water was added to each vial and after shaking well four apples were inoculated with the water from each. Three of the inoculations from vial No. 1 developed bitter rot. At the end of twenty-two hours the flies of vial Nos. 3 and 4 were examined. All flies were dead and all three flies of vial No. 3 and one of No. 4 contained bitter rot spores. Inoculations were made with both the flies and the wash water from each vial. One inoculation, a fly from vial No. 3 produced bitter rot.

Experiment 3. In one end of a box fourteen inches long, ten inches wide, and eight inches high were placed several bitter rot apples producing many spores. In the other end were six sound apples, part of which were punctured in many places with a fine needle. After a number of flies had entered the box a glass plate was placed over the top. By varying the position of the box with reference to the light causing the shadow to fall on one then in other end of the box, the flies being positively heliotropic (going toward the light) could be kept for a time on or about the infected apples then changed, with somewhat less success, to the sound ones. After six days the sound apples were removed to moist

conditions at room temperature. Four of the six apples developed bitter rot. On July 24, more sound apples were placed in the box and at the end of seven days removed to a moist chamber where two of the six apples developed bitter rot. A third set was left in the box five days then removed as the others. One apple became infected.

Experiment 4. A microscopic object slide smeared with glycerine was placed on a plate with a number of bitter rot apples about which were many *Drosophila* flies. After three days the glycerine was washed off with a little water into a small dish and examined. Quite a number of bitter rot spores were present in the water. Twenty-four hours later a number of the spores had germinated. July 14, this test was repeated with five slides instead of one. At the end of three days the slides were washed and an examination showed that spores were on all of the slides. Some of these spores germinated later. Again the test was repeated on July 21, and spores were found on each slide.

DISTRIBUTION BY WATER

The cohering spore-masses dissolve immediately when placed in water, whether or not drying has previously taken place. The agglutinating substance is readily soluble in rain or other water. It is easy, therefore, for the spores to be washed down from an infecting source to the lower parts of a tree by rain water. All observations, confirmed by actual tests, show that infection is spread in this way, and much more by drizzling showers than by heavy rains. The spores are washed down in both cases, but in the latter they have little chance to remain on any exposed surfaces—they are washed entirely off and seem to fail in getting back. The well-known cone of infection on the limbs of a tree with a canker or earlier affected apple at its apex, is thus best brought about by very moderate showers or even by dew, if that is heavy enough to cause dripping, and this is no doubt why such well marked areas of infection are sometimes so much easier observed than at other times; in some seasons, perhaps, than in other seasons. They were exceedingly common and well marked the year when the cankers were first discovered (1902), but they had often been observed and described before that date.

DISTRIBUTION BY WIND

That the spores are actually washed down in trees by rain was early shown from experimental tests by Hasselbring (1902), and the fact has been abundantly verified by similar experiments since that time. It is also clear enough that rain with heavy winds may sometimes carry the spores from tree to tree with droplets and splashes of water, because the latter are certainly so carried. But again these conditions are not the most favorable for the lodgment of spores, on the surfaces of fruits, and the spread of the disease in orchards still seemed mysterious. There is undoubtedly much dif-

ference during different seasons as to this dissemination in the orchard. Sometimes a thoroughly infected tree stands alone with no others harmed even though these others are of the same variety and evidently equally susceptible; at other times, doubtless more often, the neighboring trees in gradation by proximity show the effects of the situation. It may be easy to surmise why such difference appears, but we have no other exact published data to quote, and nothing to offer except what follows. Since the discovery of the limb-cankers it is well understood why some one tree continues to be infected year after year, but why there should be so much difference in the spread of infection from such a tree has been the puzzle.

It has everywhere been assumed in our own and other publications that the wind can play little part in the dissemination of the spores, because of the mucilage in which they are embedded as they issue from the spore-pustules, and the hardened crust this forms when dried. It is indeed impossible for air currents to move the spores from surfaces to which they are glued when the attachment is at its best. When the substance with the spores is of the proper consistency to flatten down, as would a droplet of thick syrup, into an even, convex, closely adherent body—and this is frequently the case—surely it cannot be broken in fragments or become detached by the action of the wind unaided by anything else. Whenever the spore-mass is in the condition of serviceable paste or when like hardened gum, removal by wind cannot occur.

It is a fact, however, that the consistency of the spore-masses varies much. When the fungus is in very active development and the air is fairly moist, the issue from the pustules is in the shape of slender threads which become variously curved or coiled (Pl. V., Fig. 1). Bits of these threads may be easily separated and carried away by air in rapid motion. Under certain atmospheric and perhaps other conditions the spore-masses have little viscosity; they readily crumble, and bits containing a considerable number of spores and particles of the spore-masses may thus be separated and then can be removed and carried off in a stiff breeze. Further there is more or less rubbing of the surface of apples on the tree by the leaves and branches through the movements caused by the wind, and particles of the spore-masses may thus be separated and then borne away in the air. The conditions are really not such as must necessarily prevent dissemination of spores by the air in motion. Practical experiments show that they are so contributed and sufficiently to explain phenomena not heretofore understandable.

Whether the dust-like particles have one or numerous more or less coherent spores in them may not be important; so long as they are carried in any shape while retaining their vitality, infection at a distance must be possible, and under favoring conditions probable.

In order to test the matter experimentally, glass slips 3 by 1 inch, used in ordinary work with the microscope, smeared with glycerine, were placed from 14 to 24 inches from infected apples having spores issuing from pustules. Movements of air were caused to pass the rotting apples towards the glass slips, by means of a small bicycle pump, or at other times the natural breeze through an open window or in the field was utilized for the purpose (see data following, Expts. 1-9). In numerous instances microscopical examinations of the smeared slides showed the presence of spores of the bitter rot fungus, either singly or in pellets. Further to test this in the orchard, a tin funnel was constructed 16 inches across its wide end and 2 inches at the exit. At the latter tincups—with end of fine wire screen—filled with glass wool, moistened with a mixture of alcohol and glycerine, were made to fit so as to be readily removable. The funnel was fixed upon a 7-foot stake which could be thrust into the ground and was then used in various positions in the orchard with the open mouth to the wind. After exposure, the glass wool was washed in a little water and this was then examined for spores. In no case as tried were all the conditions favorable and but few spores of this fungus were ever found in the wash water, but there were enough to show again that they are sometimes distributed by the wind. Undoubtedly further tests of this kind would be more convincing (see data following, Expts. 10-13).

It was shown that spores which had been carried by the wind were capable of germination by direct culture and microscopic tests. There can be no doubt now but that infection spreads to some extent from tree to tree in an orchard, and possibly though to a much less extent, from orchard to orchard, by means of wind-carried spores derived either from the limb-cankers or much more likely, because of the greater production, from diseased fruit. If this method of distribution in a given tree is comparatively ineffective, and it is sometimes shown to be so by the cone-shaped area of spotted fruit under a single spore-producing body, it nevertheless offers explanation of observed facts which have been otherwise puzzling, and it emphasizes anew the prime importance of destroying the contagion at its source. Any diseased tree is a menace to others nearby and to some degree to those widely distant whether or not insects

are actively carrying the spores. Whether or not the spores may be subject to wind-dissemination depends much upon the conditions under which they are produced, and this again offers explanations for observed peculiarities in this respect. They may at one time be widely distributed and may not be movable by wind at all at some other time, owing to the state and peculiarities of exudation from the producing pustules, and to the peculiarities of the weather.

Much importance is placed upon these experiments and their results. They not only clear up difficulties heretofore existing in studies of geographical progress of the disease, but they emphasize anew the importance of thorough work in exterminating the contagion when first discovered and of keeping a sharp outlook for it during the season of its development. Any stamping out process is futile unless absolute extermination is accomplished. The main service of these studies is to tell when and where to strike most effectively and most economically. In this there is evident need of coöperation when different owners or managers have adjoining or nearly adjoining orchards, and there may be need of legislation making effort compulsory.

FIELD AND LABORATORY DATA

Experiment 1. Three wet microscopic object slides were placed on end fourteen inches from four infected apples bearing exuding pustules of bitter rot spores. After passing a current of air from a small bicycle pump over the infected apples toward the glass slides for a few seconds, the slides were examined and bitter rot spores were found on each.

Experiment 2. Four wet glass slides were placed eighteen inches from an infected apple bearing many tendrils of bitter rot spores. The air current from the bicycle pump was passed over the infected apple in the direction of the slides a few seconds. An examination of the slides showed that not only single spores, but small masses had been blown to the slides.

Experiment 3. A wooden box was constructed 8 inches wide, 8 inches deep, and 24 inches long with one removable side and a small hole in one end. By means of small nails driven through the box sound apples were arranged on the inside walls of one end of the box. On the bottom of the opposite end of the box were placed four apples bearing many pustules of bitter rot spores. By means of the small bicycle pump a current of air was passed through the hole in the box and over the infected apples toward the sound ones. Along with the sound apples was a wet slide on which, after passing the air current, one hundred fifteen bitter rot spores were counted. Two of the six sound apples used became infected with bitter rot.

Experiment 4. Five apples were punctured with a fine needle and placed in one end of the wooden box used in experiment 3 and that experiment was repeated. After three days several spots of bitter rot on three apples had developed.

Experiment 5. Thinking that perhaps the current of air from the bicycle pump was stronger than would ordinarily be met with in the orchard, some tests were made with the natural breeze. An apple bearing many tendrils of bitter rot spores was held before the open window permitting the incoming breeze to pass over it and onto a wet slide some eighteen inches behind. This test was repeated three times with three different apples, and in each case bitter rot spores were found on the slide. The same test was tried, but with three dry instead of one wet slide. Bitter rot spores were found on two of the slides.

Experiment 6. In this test the breeze that passed into the open window was quite strong. It was permitted to pass over an infected apple bearing spores and onto a wet object slide. This was repeated several times and in most cases spores were found on the slide.

Experiment 7. Six infected apples bearing some pustules of bitter rot spores were taken from a Northern Spy tree and brought into the laboratory for testing. The spots on the apples were large and the spore-masses somewhat dried down. Five of the apples were subjected to currents of air from both the bicycle pump and the open window. In every case spores were found on the wet object slides.

Experiment 8. Two apples punctured in many places with a needle were moistened and a current of air from the bicycle pump passed over infected apples toward them. At the end of three days the two apples showed a total of 38 spots of bitter rot.

Experiment 9. By means of the air current from the bicycle pump bitter rot spores were blown from an infected apple into a small dish containing weak beef broth. Within five hours twenty-five percent of the spores had germinated and at the end of twenty-four hours seventy-five percent showed activity.

Experiment 10. In order to test whether the wind that passed through the trees and over the infected apples in the orchard carried bitter rot spores, a number of experiments were made with a large tin funnel. This funnel was sixteen inches in diameter of mouth, and sixteen inches in length, and to the small end were attached tin cups two inches in diameter and four inches long. Over the outer end of the cup was fastened a piece of fine wire netting which held in place a small amount of glass wool. After saturating the glass wool with a mixture of alcohol and glycerine and adjusting the cup, the funnel supported on a seven-foot pole was set up facing the wind and a tree infected with bitter rot. This tree, a Northern Spy, had but a few bitter rot apples on it. After two days the cup was removed and the glass wool washed with water. An examination of the water showed the presence of many pollen grains, some spores of several kinds, but none of bitter rot.

After the funnel had remained before a second tree two days, an examination revealed one bitter rot spore in the wash water. Showers during the interval that the funnel was up made the spores unsuitable for wind dissemination.

Experiment 11. The large funnel was taken to Kell orchard No. 1, where there were more trees infected with the rot and set up before a Ben Davis tree bearing a few infected apples. At the end of three days no bitter rot spores were discovered in the glass wool.

Experiment 12. The funnel was set up in the open, facing the wind, and twelve inches before it were suspended a number of bitter rot apples bearing many pustules of spores. After two days no bitter rot spores were found. New apples were placed before the funnel and after two days more an examination showed the presence of bitter rot spores in the wash water. During the last two days the wind was quite mild. After leaving a second cup on the funnel as it stood in the last test for twenty-four hours, three washings of the glass wool were made and examined. Spores were found in all three, but the most in the first wash water. Two days later a third cup was examined and many bitter rot spores were found suspended near the bottom of the dish in the mixture of glycerine and water. It is quite probable that in former observations spores were overlooked, thinking at the time that they all settled to the bottom of the dish.

Experiment 13. The apples used in the last test of experiment 12 were replaced by old apples, almost mummies, but bearing many dried pustules of spores. At the end of twenty-four hours the glass wool was washed and the water examined. A few bitter rot spores were found in each of the first three washings.

On July 27, the above apples were replaced by newly infected apples bearing many exuding tendrils of spores. After two days a cup from the funnel was examined and a few spores were found, more in the first washing than in the second and third. Heavy dews rendered the spores unfit for wind dissemination. A new lot of apples was placed before the funnel and after three days the cup was examined. Very few bitter rot spores were found.

THE ORIGIN OF EPIDEMICS

It is now possible to undertake an answer to the question: "How does the disease start in any season?" or "What is the seasonal beginning of an epidemic?" Here is the problem upon which these investigations were hoped especially to throw some light. It has been well shown elsewhere that by proper spraying the greater proportion of loss can be prevented. This is upon the understanding that the spray material protects the fruit from the spores of the fungus which are deposited upon their surfaces. Is there any way to strike more nearly at the root of things? Can the formation and dissemination of these spores be prevented? May an epidemic be controlled by heading it off entirely?

We understand fully that neither this nor any other disease of transmissible character affecting animals or plants can occur without the presence and successful action of a definite or specific parasite which is the active agent of the trouble. There very certainly can be no bitter rot disease of apples without the bitter rot fungus. The contagion cannot start at the beginning of a season without the successful passing of the winter by the agent constituting the contagion. This latter must retain its vitality and must start again in its own growth before any new effect is possible on the tree or fruit.

We know that there are what seem to be inscrutable differences in outbreaks of the disease. Though history in this case more commonly repeats itself year after year in different orchards, it is at times decidedly variant. Without apparent reason one orchard of a given number may suffer severely in comparison with others one season, and escape the next while another bears the chief burden of injury. Effects follow causes. If such things are mysterious, it is only because we are ignorant of ascertainable facts not because the matter is beyond our full comprehension. What we do not know is no credit to us; the more we can find out the better fixed we shall be to understand whatever remains to be found out. It is in this spirit that work upon the special problem in hand has been undertaken and prosecuted. Beginning with the well grounded idea that all mystery connected with the seasonal outbreaks of the disease may become real knowledge, we have undertaken to bring this to pass. Fighting in the dark is unprofitable if by any means we can turn on the light; the latter is our task.

The discovery of the limb-cankers was a notable event. Its importance has never been exaggerated. The facts announced have remained facts and the accounts as published are still true. But the cankers do not tell the whole story concerning the seasonal origin

of epidemics. As herein before stated there are great seasonal and apparently geographical variations in the formation of cankers and in relation to their existence and numbers compared with the extent of infection. There is something else to be taken in account besides peculiarities of weather and the presence of the living fungus in localized spots on the limbs, though abundant evidence exists that in many cases these cankers do constitute the source from which the malady starts and are themselves in these cases sufficient explanation of all that follows. It is as important today as it was deemed to be when directions were first made in regard to the process, that these infected limbs should be carefully removed or in some way rendered sterile. There is really nothing now of importance to add to the early publications, as there is nothing to retract or modify, concerning the bitter rot cankers and their relation to the spread of the disease, except that we know more about the difficulties attending their complete removal and of killing the fungus by external washes.

But fuller recognition exists that outbreaks of the disease must be sometimes attributed to something else, to some other means of passing the winter on the part of the parasite. Renewed attention has been given to the mummies. Cone-shaped areas of infection on trees have been traced to one of these old shriveled apples hanging above, in numerous instances. Such old fruits have also in numerous instances produced an abundance of spore-masses when brought from the trees in May or later into the laboratory and submitted to conditions suitable for the renewal of activity of the fungus (Pl. VIII., Fig. 2). The case is as well made out that an outbreak of bitter rot may be due to old mummies as it has been in regard to the cankers, and sometimes and in some places this seems to be the more common occurrence.

However, it was soon learned that mummies, undoubtedly originated by the bitter rot fungus the year before, were not all fertile. The fungus does not always remain alive over winter in these old infected apples. In this respect there is certainly a marked difference between them and the cankers. In the latter there is apparently no condition or circumstance when the fungus fails to survive at least one winter, and it may do so for two or even more seasons in their natural positions. Why the relative unreliability of the mummies?

Studies upon the cankers have shown that the bitter rot fungus usually has in this infection the field to itself. No other fungous parasite or saprophyte commonly interferes, although black rot,

Sphaeropsis malorum, is sometimes associated in the same canker, usually in Illinois as a later intruder. While the fungus of bitter rot has a remarkably preservative effect upon the cell-walls of apple fruits, causing the substance to assume a corky character, the invasion of bacteria, yeasts, and saprophytic fungi of many kinds is more than not liable to happen, as the fruits remain exposed to the weather out of doors. With sufficient moisture these saprophytes are certain to develop and in their development they destroy the dormant parasite. They, or some of them, thrive at much lower temperatures than does the bitter rot plant, and thus during the winter and spring they have very decidedly the advantage. The old fruits lose their firm, cork-like consistence and become soft or friable. In this state they lose or have lost their infective characteristics. The bitter rot fungus is dead whenever the old fruit substance becomes soft or easily broken, and it has left behind no spores capable of renewing its existence.

Let us see now under what circumstances in nature the fungus is likely to retain its vitality in the old apples upon which it developed the preceding year. The date and conditions of this development will evidently have some deciding influence. After the fungus has gained entrance to the tissues of growing fruits, its own growth proceeds without much reference to outside moisture conditions. If the atmosphere continues dry other soft rots are not so likely to follow before the entire fruit is involved in the action of the parasite. This latter undoubtedly has its own way better without competition of other fungi when the fruit is attacked while comparatively young. The texture assumes the cork-like character described above and if the shrunken fruit is now little exposed to soft rot agents, the fungus easily survives. If on the other hand, the bitter rot infection is late in the season, when the maturity of the fruit and the external conditions are more favorable for saprophytic growth, the less chance there must be that the bitter rot fungus can continue alive. There must also be the possibility of decisive difference as to whether after the formation of the mummy most suitable for the perpetuation of the fungus, the old fruit continues to hang upon the tree, or drops to the ground. We know that drying does not kill the parasitic mycelium; it does tend to prevent the action of soft-rot fungi. The mummy hanging over winter on the tree is more likely to remain dry and hard, those on the ground to be moist and to become the habitat of numerous kinds of fungi,—fermentation and decay producers. The bitter rot fungus should then be much more likely to live over winter in the mummies which

remain attached to the fruit-spurs than in those that lie upon the ground.

To determine the truth in this matter, extended studies were made on old apples in the field and in the laboratory, and the results were abundantly conclusive. Sometimes those remaining on the trees until May, or June, or later, of the succeeding year show gradual breaking down with other rots, but this is somewhat uncommon. When it does occur the old fruits soon become easily detached from the limb, while those continuing firmly adherent are the ones in which the bitter rot fungus is more often found in a living state. Out of hundreds of specimens (certainly known to have been originally affected with bitter rot) remaining on the ground over winter and picked up after May 1, during three successive seasons, none have produced spores of the fungus when placed in moist chambers and kept under conditions favoring the process, or have given other indications of the vitality of this fungus. Numerous specimens, again numbering in the hundreds, spotted with the rot collected at the time of the apple harvest and placed on the ground soon afterward in various situations have always shown decay the next spring and have failed to give any indications of the vitality of the mycelium of *Gloeosporium*. Plate VII, Fig. 1 shows one set of experiments of this kind.

A barrel full of characteristically spotted apples was received in November from Richland county and on the 22d, 520 of these were placed in the orchard upon the University grounds. Some of the infected fruits were strung upon threads and hung upon the trees, others were placed directly upon the ground, and others were put into trays made of wooden frames with wire screen bottoms. These were then securely supported at varying distances from the ground to test what effect this distance might have. None of these apples produced spores or showed other evidence of the vitality of the fungus in the spring. All were softened by water and various rot-producing agents. This was true, let it be understood, of those hung upon the trees as well as those on or near the ground. The explanation is that these apples were not dried out when so hung up, and at that season of the year with the conditions which prevailed they did not become dry enough to be preserved from other rots.

The evidence seems abundant and conclusive. It cannot be said that the fungus never lives over winter in old infected fruit lying on the ground, but if it does occur in nature, it must be in very rare cases. The only exception which has been witnessed was in

regard to a few hardened specimens which were placed while they were dry in a muslin sack after the ground was frozen and laid under the shelter of a Norway spruce hedge. This is really no exception to the statement as applied to the apples as they naturally fall from the trees. It may, however, occur that some mummies which have hung through the winter on the trees fall or become knocked off so late in the spring that the fungus can survive long enough to perpetuate itself in new infection. This in a practical sense appears to be the only danger from the old apples found on the ground. Plate VIII., Fig. 2, shows a mummified Willow apple which was picked up with many others under a tree in Clay county, Illinois, on June 24, 1907. The fruit on this tree was practically all destroyed by bitter rot occurring late the preceding season. The apple shown was still firm in texture and upon incubating produced spores which in the photograph print show as small white dots or spots. No others among the 250 specimens picked up with this gave any evidence, under similar treatment, of spore production, while 9 out of 18 taken from the tree where they had hung over winter gave forth spores just as did this particular specimen. An examination of the stem of this apple revealed clearly enough that it had only recently fallen, for the marks of recent separation were plainly seen. The photographic reproduction, (Pl. IV., Fig. 3,) shows this, but not so satisfactorily as did the specimen itself.

May separate spores survive in or on the earth so as to carry the infective agent over from season to season?

From laboratory experience it cannot be considered possible that spores of the bitter rot fungus washed from infected fruits to the ground may long retain their vitality. If the temperature permits, they quickly germinate and in this way exhaust themselves and then die from want of nourishment. Deprived of the protecting coating, they soon die by drying, if they are not favored by conditions conducive to germination.

But to leave nothing undone attempts were made to determine the facts by experimental methods. Direct search was first made by microscopic examinations in June for the spores in the dirt and among the trash under trees upon which there had been the year before a great amount of bitter rot. It was ascertained that the spores of this fungus can be found again after they have been recently mixed with such dirt by treating the earth with a small quantity of water, stirring well, allowing slight settlement, and examining under the microscope the material remaining on or near the surface of the water. After considerable practice with earth in

which spores had been purposely placed just previously, and by methods determined by such practice, careful and extended search was made of the earth and trash under previously infected trees. Many spores of various kinds were seen, but in no case were there any *Gloeosporium*. It was confidently believed they could be found, at least occasionally, if they were present in any considerable number, in the materials examined. A long search gave only negative results.

Trial was made by inoculations in fruit half or more grown. Earth in which a few fresh spores were mixed was placed under the skin of the apples by means of a scalpel and in a few days after proper incubation the characteristic spots of the rot appeared. Numerous similar inoculations into the same kind of apples of earth from under trees badly infected the year before were never followed by a similar result, though the tests were very numerous.

Still another method, believed to be more conclusive, was thoroughly tried—to the extent of several hundred tests. The best apples for the purpose which could be obtained in June and July were used in these experiments. They were Maiden Blush in the market from the South; a very early variety, nearly ripe, locally known as Britton's Early, and Chenango (Sherwood's Favorite), also quite mature, both taken from the trees. Tin pans about 8 inches in diameter and 3 inches deep were used in pairs, one as a dish and one as a cover, to form a culture box. The test apples, some of which were punctured in many places with a needle to favor infection, were buried in moistened earth placed in these pans, sometimes mixed with fresh bitter rot spores, in other cases from under trees on which the fruit had badly rotted the year before. Temperature conditions were made favorable for the development of bitter rot.

The results showed characteristic infection in about 75 percent of the tests in which the spores were added to the soil, but in no case not under suspicion when earth without such spore addition was used. In fact, however, there were two positive results when no spores were placed in the earth, one upon a Maiden Blush and one on a Chenango apple; but the latter was taken from a tree on which there were at the time apples spotted with the disease, and the other had been in the same paper sack with it. Since these proved to be so exceptional among the large number it is reasonable to infer that these two apples were infected or had spores on them when they were put into the dirt. It was a fault in the experiment that it was permitted to be open to this suspicion, but could not be remedied after the test was made, and the facts demand reporting.

Having found, as it was believed, that earth under trees having an abundance of diseased apples, the previous year, carried no infection, an attempt was made to determine by the same method how long such infection continues after fresh spores are added to the earth. As has been stated, positive results very commonly followed when fresh spores were added to the earth just as the test was to be made. What would result should apples be placed in this moistened earth at different intervals of time after the spores were added? Would there soon come a change in the infectiveness of the earth due to the death of the spores which had been mixed with it? If so, when?

Many experiments were tried. After one set of apples had been buried two or three days, under conditions all the time favorable to the germination of the spores, they were removed and another set of apples were placed in the same dirt; then after a similar period others were tried. If such tests should be made again, the procedure would be somewhat modified, but the results (see data following, Expts. 26-29) satisfactorily show that in the soil so situated the vitality of these spores is of short duration,—it is a question at most of four or five days. In none of the tests was there evidence that it is more than four days. While the rot spots were numerous on the apples of the first set left in the inoculated soil three days, they were few on the set which immediately followed, *i. e.* upon those put into the soil three days (72 hours) after the spores had been stirred into the earth, and then none on the apples of subsequent sets.

The only explanation of these results is that the spores die, through germination or otherwise, when placed in moist soil in the summer time within a period not much more than seventy-two hours. This entirely corresponds with what is known otherwise concerning the behavior of *Glœosporium* spores. If they are kept dry in the condition otherwise than they are in when exuding from the pustules, they do live for months; but this can never be the case while they are in the ordinary soil of orchards in our part of the country. We may confidently conclude there is nothing in such soil over winter and through the spring season which can give origin to an outbreak of the disease the succeeding summer.

Does the fungus in any stage or in any manner live from season to season on anything else besides the apple tree and its fruit? It is well known that it does grow as a parasite on other plants or their products, and it does lead very readily a saprophytic existence, as is sufficiently shown by experiments. There are scarcely no

fruits on which it may not be artificially grown. It has been found spontaneously growing on the stems of sweet peas. There is nothing, therefore, to suggest the impossibility of a first seasonal infection of apples by the fungus grown on something else.

Careful and long continued search has been made in and about the orchards of the area of the State subject to the disease without finding the least evidence that the fungus does develop in this region on anything but apples and apple-tree limbs, so as to start or to perpetuate orchard infections. Neither does there seem to be anything unexplainable on the supposition that the disease on apples always comes from preceding apple or apple-limb infections. The first apple-infections in any season comes from spores produced that season and only shortly before from mycelium that has survived the winter in the cankered spots on the limbs or in bitter-rot mummies and exclusively or nearly so from those that have dried while hanging to the tree and have there passed the winter.

If it is borne in mind that "a little leaven leaveneth the whole lump" in this case quite as well as that first so described, and if it is remembered too that the spores can be carried at certain times and under certain conditions to a considerable distance by the wind, nothing further is necessary to account for the beginning of rot wherever it has been and probably wherever it may be observed. This ought both to simplify matters and to give renewed encouragement in the warfare which should be waged in the manner herein suggested. If, during the winter, the sources of infection—cankers and mummies—are absolutely removed from the trees and exclusive attention given to these, the battle will be won. If in regard to the cankers—for there is no similar "if" in regard to the mummies—the work in perfection is sometimes impossible, much can be done towards preventing their formation by keeping the twigs and limbs free from bruises and by preventing the disease on the fruit by careful hand-picking and especially at first by the thorough application of Bordeaux mixture at the proper time. That is, cankers need not be allowed to form—at all events they need not be aided in formation by ignorant or careless management—and if formed, most of them can be seen by close looking and can be removed.

FIELD AND LABORATORY DATA

Experiment 1. On June 15, 1905, some dirt and trash was collected in Olney orchard No. 3, under trees known to have been badly infected with bitter rot the previous summer, and taken to the laboratory at Olney for examination.

About a tablespoonful of earth with some trashy vegetable matter was shaken up with a half-pint of water, and after two minutes settling, the liquid

was carefully poured off. After two minutes more no spores were found in the surface layer, but below, $\frac{1}{2}$ inch from the surface, there were several spores of at least four kinds suspended in the water. Among these were *Fusarium*, *Macrosporium*, numerous spindle-shaped, rather small, probably, uniseptate forms, and still more numerous smaller, short, cylindrical ones, with several small oval ones. All were white except the second and the last, which were slightly tinted.

After pouring from one vessel to another five times, allowing each time about a half-minute for the heaviest material to settle, and decanting, the liquid was placed in a 100 c. c. graduate and allowed to settle 36 hours. The following day the precipitate was examined and but few spores of any kind were found. Some dark colored kinds had not germinated, but most of the hyaline ones had thrown out long, slender germ tubes. No bitter rot spores were found.

After many trials it was learned that most spores of various kinds were to be found by treating the dirt and trash with a small amount of water, just enough to saturate the solid material and leave a little free water, then after allowing to settle about one minute, to take a drop from the surface for examination.

On dissolving some spores of *Gleosporium* from a Chenango apple and allowing to stand two minutes in a bottle in which the fluid was two inches deep, most of the spores were found near the surface.

Samples of dirt from orchards located at Flora, Clay City, Carbondale, and Ashley, and known to have had much bitter rot the previous summer, were treated as above, and although various kinds of spores were found in every case, no *Gleosporium* spores were seen.

Experiment 2 (a). On June 15, 1905, two Maiden Blush apples were inoculated by inserting dirt into punctures. The dirt was collected in Olney orchard No. 2 under a tree that had been badly infected with bitter rot the past two years. (b) Two Maiden Blush apples were inoculated as above with similar dirt collected in Olney orchard No. 3. (c) Some soil collected under a maple tree in the Olney school-yard was sprayed, until moist, with water containing fresh bitter rot spores. This dirt was used to inoculate, as in (a) and (b) two Maiden Blush apples.

All the inoculated apples were placed in a moist chamber and occasionally sprayed with water to keep them moist. After four days, an examination revealed that all inoculations in (c) showed a marked development of bitter rot, and on the sixth day the characteristic pink spores were oozing through the epidermis. After eleven days one spot of bitter rot appeared on one apple of (a) and one on one apple of (b).

Experiment 3. On June 16, four sound apples, and two with epidermis punctured in many places with a fine needle, were covered in a tin-pan with moistened dirt and trash collected in Olney orchard No. 3 under a Ben Davis tree. The pan was covered and kept at room temperature. After three days the apples were removed from the dirt and placed in a moist chamber. No bitter rot developed.

Experiment 4. June 17. Two punctured and two unpunctured apples were covered in a pan with some of the same dirt as used in experiment 16, moistened with water containing bitter rot spores. The pan was covered. After two days the apples were removed from the dirt and put in a moist chamber. Seven days later both punctured apples showed a number of bitter rot spots. The sound apples were not infected.

Experiment 5. June 17. Two Britton's Early and two Maiden Blush apples, one of each punctured, were covered in a tin pan with dirt moistened with water containing fresh bitter rot spores. June 19, the apples were removed from the dirt and kept in a moist chamber. June 26 one unpunctured Maiden Blush had developed bitter rot and one punctured apple had a soft rot.

Experiment 6. June 28. Four Chenango apples, two punctured and two sound, were placed in a tin pan and covered with moistened dirt and litter collected in Olney orchard No. 2, under an apple tree known to have had bitter rot the previous year. The pan was covered and placed in an extemporized incubator which registered about 36 C. during the day, gradually falling during the night to 24 C. by 7 a.m. After two days the apples were removed from the dirt and placed in a moist chamber at room

temperature. July 7 the two punctured apples had bitter rot and one unpunctured one showed infection of some kind. July 11 these apples had bitter rot. It was found later that the apples used in the experiment had been taken from a Chenango tree that was much infected with the bitter rot. The apples were sound when used in the experiment, as were those which remained in the sack from which they were taken. Later, however, several apples in the bag developed bitter rot and it is probable that the apples which developed the rot after treatment with the soil were already infected with the spores of bitter rot fungus. The Maiden Blush apples used in experiment 1 were taken from the same bag as the Chenango.

Experiment 7. June 29. Four Britton's Early apples, two of them punctured, were covered in a tin pan with moistened dirt and trash collected in Olney orchard No. 4, under a Ben Davis tree. After incubating twenty-four hours the apples were removed from the dirt and placed in a moist chamber at room temperature. No bitter rot developed.

Experiment 8. June 29. Four Britton's Early apples, two punctured, were treated as those in experiment 6 with moistened dirt and trash collected in Olney orchard No. 3, under a Ben Davis tree known to have bitter rot the previous year. On July 21 no bitter rot had developed.

Experiment 9. June 29. Treated four Britton's Early apples, two punctured, as those in experiment 7 with dirt and trash collected in the Olney orchard No. 3, under a Ben Davis tree known to have had the rot the previous year, and which developed it later the same season. No bitter rot developed.

Experiment 10. June 29. A duplicate of experiment 8. No bitter rot developed.

Experiment 11. June 29. Four apples were treated as those in experiment 7 with dirt and trash collected in Olney orchard No. 3, under a Ben Davis tree which had bitter rot the previous year and which developed it later the same season. No development of bitter rot took place.

Experiment 12. June 29. Four Red Astrachan apples were treated as those above with moistened dirt and litter collected in an orchard at Ashley, Ill. No bitter rot developed.

Experiment 13. (a) June 29. Four Red Astrachan apples were treated as above with moistened dirt from the Olney school-yard. July 21 one apple had decayed with a soft rot. No bitter rot developed. (b) June 29. Four "Britton's Early" apples were treated as above with some of the same kind of dirt used in (a). No bitter rot developed. (c) June 29. Four Red Astrachan apples were treated as those in (a) and (b) with some of the same kind of dirt moistened with water containing bitter rot spores. July 7 one punctured apple had fifteen spots of bitter rot, the other one ten. July 17 all four apples had developed bitter rot.

Experiment 14. July 7. Four Red Astrachan apples, two punctured and two sound, were placed in a tin pan and covered with moistened dirt and trash collected in an orchard at Clay City, Ill., in an orchard which suffered very much from bitter rot the previous year. After incubating sixty hours the apples were removed and kept in a moist chamber at room temperature. After fourteen days three apples were infected with black rot. No bitter rot developed.

Experiment 15. July 7. Four apples, two Britton's Early and two Early Harvest, one of each punctured, were treated as those in experiment 13. No bitter rot developed.

Experiment 16. July 7. Four Red Astrachan apples, two punctured, were treated as those above with dirt and trash collected in an orchard at Carbon-dale, Ill., and said to have been badly infected with bitter rot the previous year. No bitter rot development took place.

Experiment 17. July 7. Four apples, two Early Transparent and two Early Harvest, one of each punctured, were treated as those in experiment 15. Bitter rot failed to develop.

Experiment 18. July 7. Two Early Harvest and two Red Astrachan apples, one of each punctured, were treated as those in experiment 16, with dirt from the same orchard, but collected under a different tree. After fourteen days no bitter rot developed.

Experiment 19. July 7. Two Early Harvest and two Early Transparent apples, one of each punctured, were treated with dirt collected under a third tree in the last mentioned orchard. July 21 no bitter rot had developed.

Experiment 20. July 7. This experiment was made as a check to the six preceding experiments. Four Britton's Early apples, two punctured, were treated as above with dirt from the Olney school-yard moistened with water containing bitter rot spores. July 11 one unpunctured apple had a soft rot. No bitter rot developed.

Experiment 21. This experiment was made in order to determine whether, while exposed in the laboratory for several days, the dirt and trash used in experiments 1 and 5 had become infected with spores of bitter rot fungus. (a) July 10 four Britton's Early apples, two punctured, were placed in a tin pan, covered with some of the moistened dirt and trash mentioned above, and incubated 48 hours. August 5 no bitter rot had developed. (b) A duplicate of (a). No development of bitter rot was apparent.

From these results one would judge that the infection of bitter rot in experiments 1 and 5 was due not to spores in the dirt and trash used but to those that were present on the apples.

Experiment 22. July 10. Four Britton's Early apples, two punctured, were covered with moistened dirt and trash collected in Olney orchard No. 3 under Ben Davis tree X, which is known to have had bitter rot the two preceding years and which developed it later the same season, and incubated twenty-four hours. At the end of this time they were placed in a moist chamber at room temperature. There was no development of bitter rot.

Experiment 23. July 10. A duplicate of experiment 22 except that the apples were treated with dirt from Ben Davis tree next north of X. Bitter rot failed to develop.

Experiment 24. On July 10 four Britton's Early apples, two punctured, were treated as above with dirt and trash collected under a Ben Davis tree not far from the one mentioned in experiment 23, and known to have had bitter rot the preceding year. No bitter rot developed.

Experiment 25. July 10. Four Maiden Blush apples, two punctured, were treated as those in experiment 24 with dirt and litter collected in Clay City orchard No. 1, an orchard badly infected the previous two or three years with bitter rot. There was no development of bitter rot.

Experiment 26. This and the two following experiments were made for the purpose of determining how long, after inoculation with spores, dirt would be capable of producing an infection of bitter rot on apples buried in it and subjected to different temperatures. The dirt used in these experiments was some of the same kind of dirt used in check experiments already given, being free from bitter rot spores. July 11. (a) Four Britton's Early apples two punctured, were covered with dirt moistened with a suspension of spores in water, and incubated forty-eight hours. At the end of this time the apples were removed and kept in a moist chamber at room temperature. On July 21 two apples had bitter rot and a third developed it a day later. (b) Two Britton's Early apples, one punctured, were put into the same dirt used in (a) on July 13, and incubated four days. Both apples rotted with a soft rot, aided quite probably by a too long period of incubation. (c) On July 21 four days after the removal of the apples in (b) from the dirt, two Britton's Early apples, one punctured, were placed in the same, remoistened, and incubated three days. July 24 the apples were removed from the dirt and both were found to be infected with what afterwards proved to be a soft rot. No bitter rot developed. (d) As a check four Maiden Blush apples were treated with the same kind of soil, but without the spores. No bitter rot developed.

Experiment 27. In this experiment, begun July 11, the apples were always kept at room temperature, otherwise they were treated about as the above. But two apples were used in (a). (a) July 21 the punctured apples had developed bitter rot and by July 29 the other showed infection. (b) The apples were put into the dirt July 13 and were removed July 15. Six days later one apple had developed bitter rot, and the other a soft rot. (c) Two apples, one punctured, were placed in the same dirt used in (b) on July 21, and removed July 24 when they were placed under moist conditions. No bitter rot developed.

Experiment 28. This experiment is practically a repetition of experiment 27 but with this difference that the apples were kept in a moist condition under a tree in the open. (a) On July 11 two Britton's Early apples, one punctured, were covered with the moistened dirt containing the spores of bitter rot. July 13 the apples were removed from the dirt. Eight days later both apples showed development of bitter rot. (b) July 13 two apples, one punctured, were placed in the dirt of (a) and left for four days. July 21 both apples had developed a soft rot but no bitter rot. (c) Two sound apples, one punctured, were placed in the dirt used in (b) on July 21. After three days they were removed and kept moist. No bitter rot developed.

Experiment 29. This series of tests, quite similar to experiments 26, 27, and 28, was made further to test the point in question in the preceding experiments. The soil was of the same kind as that used in the three preceding experiments and was moistened with water containing bitter rot spores.

There were two sets of five pans each, carried on at the same time. Each pan contained two apples, one of them punctured. Set one was incubated, while set two was always kept at room temperature. After three to four days the apples were removed from the pans and kept in moist chambers at room temperature, and fresh apples were put in the same dirt to be subsequently treated in same way. Shown in tabulated form the results were as follows:

SET 1.	Pan.	Started.	Removed from dirt.	Apples infected.	Started.	Removed from dirt.	Apples infected.	Started.	Removed from dirt.	Apples infected.	Started.	Removed from dirt.	Apples infected.
	1	July 14	July 17	1	July 17	July 21	O	July 21	July 24	O	July 27	July 31	O
	2	"	"	1	"	"	O	"	"	O	"	"	O
	3	"	"	1	"	"	O	"	"	O	"	"	O
	4	"	"	1	"	"	O	"	"	O	"	"	O
	5	"	"	O	"	"	O	"	"	O	"	"	O

SET 2.	1	"	"	O	"	"	1	"	"	O	"	"	O
	2	"	"	1	"	"	O	"	"	O	"	"	O
	3	"	"	2	"	"	O	"	"	O	"	"	O
	4	"	"	2	"	"	1	"	"	O	"	"	O
	5	"	"	2	"	"	O	"	"	O	"	"	O

The pans with the apples in dirt of the first set were placed in the incubator—30 to 36 degrees C.—and remained there 24 hours, after which they were kept at room temperature two more days. Those of the second set were kept from the beginning at the temperature of the room. It will be seen that of the 40 apples tried 13 became infected, all but two of which were among those first

put into the dirt and these two were among those that next followed. The spores evidently lived longer in the set kept at room temperature where we know they would be somewhat slower in germinating.

Experiment 30. The purpose of these tests was to determine whether the spores of the bitter rot fungus in the dirt and trash under infected trees, if any are present, can be washed out with water and used in suspension to infect apples. Including the check ten tests were made. The dirt and trash used in the nine tests was collected under trees known to have had the bitter rot, and some of which had been tested in former experiments. (a) Check. July 11 some dirt from the school-yard was sprayed with spores in solution, thoroughly stirred and mixed, and allowed to settle. After adding water until it stood an inch or more above the dirt, it was again stirred and mixed until every portion of the dirt and trash had been washed. After settling the water was poured off and in it were placed two apples, one punctured, and the whole incubated for twenty-four hours. At the end of this time, July 12, the apples were removed from the incubator and two days later from the water. They were kept in a moist chamber at room temperature. Bitter rot developed on the punctured apple. (b) July 11 dirt and trash collected in Olney orchard No. 3 under Ben Davis tree XI was treated as that in (a) with the exception that no spores of bitter rot were added. After thirteen days one apple developed a spot of bitter rot.

There was no bitter rot development in any of the remaining eight tests.

Experiment 31. July 17. The dirt and trash used in this experiment was collected July 15, under an apple infected with bitter rot hanging on a Ben Davis tree in Olney orchard No. 3. (a) As in previous tests, the dirt was moistened and into it were placed four sound apples, two punctured. They were incubated for twenty-four hours and on July 21 removed from the dirt and placed in a moist condition at room temperature. One apple developed bitter rot.

Two other tests (b) and (c) were made as (a) with no development of bitter rot.

Experiment 32. The dirt used in this experiment was collected in two orchards at Kell, Ill., July 18. (a) Dirt collected in Kell orchard No. 1 under a Lowell tree bearing many infected apples. After moistening the soil four Britton's Early apples were covered with it and incubated thirty hours. No bitter rot ever developed. (b) This test was carried on as (a) but with the difference that dirt and trash from under another tree was used. Bitter rot failed to develop. (c) The dirt and trash used in this test was collected under a Northern Spy tree in Kell orchard No. 2. No development of bitter rot.

Experiment 33. Since one apple in (b) experiment 30 developed bitter rot, two additional tests of some of the same soil were made. (a) To the water extract of the dirt and trash two apples, one punctured, were added and incubated twenty-four hours. One spot of bitter rot developed. (b) Duplicate of (a). No development of bitter rot.

Experiment 34. July 25. This experiment consisted of three tests, two with dirt from the school-yard and which had been exposed sometime in the laboratory and one with fresh dirt from the same source, to which was added spores of bitter rot fungus in water. (a) To some of the former dirt, four apples two punctured, were added and incubated twenty-four hours. After a number of days no bitter rot had developed. (b) Duplicate of (a). No bitter rot appeared. (c) The treated fresh dirt from the school-yard to which four apples, two punctured, were added, was incubated twenty-four hours. One apple developed bitter rot.

Experiment 35. Not feeling satisfied with the results of experiment 31, this experiment consisting of two parts of five tests each, was made. Two checks were used.

PART I.

(a) In the water extract of some of the same dirt as that used in experiment 31 were placed two Red Astrachan apples, one punctured. After incubating twenty-four hours the pan was removed, and two days later the apples were taken from the water and kept in a moist chamber at room temperature.

Tests b, c, d, and e, were duplicates of (a). No bitter rot developed in any of the tests.

(f) The check consisted of a water extract of dirt from the school-yard, to which spores of bitter rot fungus had previously been added, with two apples, one punctured. They were incubated twenty-four hours and two days later removed from the water. Both apples developed bitter rot.

PART II.

These tests were made with some of the same dirt as that used in Part I, moistened and covered over the apples. All five tests were made at the same time and alike. There was no bitter rot development in any case.

(c) The check was made with inoculated school-yard dirt. Bitter rot failed to develop.

Experiment 36. This experiment was made to determine, if possible, how long bitter rot spores would remain in a living condition in the soil in the orchard and be capable of producing bitter rot of apples which were buried in this infected soil. The experiment was made in the orchard of the Horticultural Department of the University of Illinois, and consisted of a series of five tests. After the apples had remained in the soil three or four days they were replaced with sound ones and the former were brought to the laboratory for further development. (a) An area of soil eighteen inches square was selected under an apple tree and the first four inches was well stirred and moistened with water containing many spores of the bitter rot fungus. After burying ten apples, five punctured, in the dirt, it was again sprinkled with the infected water. Three days later the apples were removed to the laboratory. Four apples, three punctured and one unpunctured, developed bitter rot. (b) Duplicate of (a) under tree No. 2. Eight apples developed bitter rot. (c) Duplicate of (a) under tree No. 3. Seven apples developed bitter rot. (d) Duplicate of (a) under tree No. 4. Three apples developed bitter rot. (e) Check. No spores were added to dirt in this test made under tree No. 5, but otherwise treated as the other tests. No development of bitter rot took place.

On July 25 when the apples of the tests just mentioned were removed from the soil sound ones were put in. Fourteen days later these were removed to the laboratory. The soil was rather dry the first eight days, but on September 2 a heavy shower came and the soil was in excellent condition for the germination of any spores that might be there. (a) Under tree No. 1. Five apples developed bitter rot. (b) Tree No. 2. Two apples became infected with bitter rot. (c) Tree No. 3. Two apples infected. (d) Tree No. 4. No bitter rot. (e) Apples were not put in the soil this time.

The experiment was unavoidably ended at this time. There was, however, a considerable decrease in the number of bitter rot apples in the last test, and judging from this fact and from former results obtained in the laboratory, the life of bitter rot spores in soil is quite short and incapable of perpetuating itself any length of time.

ABSOLUTE ERADICATION

The entire eradication of the disease from an orchard is certainly possible. Notwithstanding the facts made known herein and elsewhere concerning the persistence of vitality on the part of the fungus and concerning the dissemination of its spores, the fuller studies make this statement less hazardous than it might have been previously. We have seen that the spores can be carried while capable of germination by the wind, and it cannot be doubted that the disease is sometimes by this means widely distributed. But instances of this are evidently exceptional, for it is well known that the malady often persists in a remarkable manner in some special portion of an orchard—in one corner, along one side, near the pack-

ing shed, etc., while for a long time it does not appear on the trees elsewhere in the orchard, though these are of the same kind and are in every way as susceptible as are those affected. This means that, as a rule, the disease does not spread rapidly into new territory. We now know it may occasionally take long leaps, but the occasions are not commonly frequent. It is easy enough to understand that when such leaps occur the living spores must not only be carried to a distance, but they must fall on fruits and must there find conditions suitable for their germination and the penetration of the epidermis. While there may be spores enough and wind enough, there may readily be failure otherwise. That there is such failure is sufficiently shown by abundant observation.

It is no longer doubtful that a crop of apples can be saved by spraying with Bordeaux mixture. Let it be remembered that this work thoroughly well done does more than to save the crop. It helps also to save subsequent crops. The disease is to be fought to a finish, and for success must be attacked from all quarters. Less development on the fruit means less chance for winter survival. Restriction of the latter means less liability of fruit infection. Summer spraying and winter inspection of the trees should both be prosecuted and should be energized with the reasonable hope founded in ascertained facts that complete and permanent victory is attainable; and is attainable by ways and means now well understood. Other information of much value may hereafter be added, but no one should wait for it; neither is there any excuse for spending strength and time upon methods or processes which have not the support of well worked out and conclusive experimental tests. It is idle to shoot without first taking true aim; it is worse than useless to fire with ammunition which from its nature makes efficiency impossible. It is exceedingly desirable to experiment if one understands what he is doing; it is well-nigh shameful to draw conclusions from the supposed results of irrelevant tests. It never can be too well understood that not only are effects produced by causes, but that certain and definite effects are due to certain and definite causes. We cannot mix cause and effect and get true explanations. "One cannot make a silk purse out of a sow's ear." One cannot pack apples in a barrel by means of a sewing machine. The bitter rot fungus is the only thing that makes the spots of bitter rot on apples. Hail cannot do it. Hot weather cannot do it. Copper sprays cannot do it. Evil minded persons cannot do it. Nor can neglect and shiftlessness be the direct agents in the production of these well-recognized and much dreaded tokens of great fi-

nancial loss. This agent is the fungus, and it is with this that we must deal, pointedly, directly, purposely; and we must fight with weapons suitable to this particular warfare. If it were folly to try to catch wolves with birdlime, or to take fish in pitfalls, it is no less so to try to head off bitter rot with a plow or with poison well suited to stop the attacks of codling moths.

The first thing to do is clearly to recognize the enemy, then strike when and where and how he may be reached. In this case the foe is now well known; there is no longer any room for doubt as to what the agent of injury is, nor as to the depredator's mode of life and action; neither should there be hesitation concerning methods of combat. Some very sensible new way of procedure may be discovered, but the time has gone by for any sort of hit and miss attempts not founded on existing, and any possible further, actual knowledge.

Putting down in a few words what all this means it may be said: The fungus, *Glomerella rufomaculans*, is the active agent in the apple injury known as bitter rot. It lives over winter in limb-cankers and in mummies that have not been much subjected to other rots and this mostly means those that have hung on the trees from the preceding season. Newly formed spores begin to issue as early as the weather conditions permit, sometimes by the 1st of June in South-central Illinois from cankers and tree-hanging mummies, and they may be produced within one week's time after infection on new fruits. Spores are distributed by insects (especially by so-called pomace flies), by water as it drips from above in a tree, or is splashed from tree to tree in storms, and by wind; but with all these means distribution is very irregular and not usually rapid or extensive compared with what occurs in connection with many other plant diseases due to fungi, as for instance apple scab, and black rot of grapes. Spores can germinate and cause infection of fruit only when there is sufficient moisture on their surfaces; when the air is continuously dry infection cannot occur; this always takes place when it does occur from outside and in no other way than by germ-tubes of spores (including appressoria). There are two methods of directly combating the fungus, viz., (1) cutting off and hand-picking the cankers and old mummies; and (2) spraying with Bordeaux mixture. The first is best done when the leaves are off and it is abundantly worth while to do it thoroughly. If it were possible to remove every limb and twig infection (which may not be possible) and then take off all old infected fruits (which is possible) the disease would be controlled in ordinary cases. The spray must

be used so as to prevent infection; that is, so as to *prevent* the penetration of the germ-tubes of the spores. This means actual coating of the apples with the germicidal substance before these germ-tubes get started on the fruit; and the formation of cankers can be prevented by similar coating of the limbs. Again, if this could be so effectually done that no fruit or limbs become infected one year, control would be accomplished for the next year as well, save that some cankers contain the living fungus through more than one year. Especially in the earlier part of the season a sharp out-look should be kept up for the first fruit infections and prompt removal made. At this time careful search should be made, too, for cankers from which the infection may have been derived and every thing, even suspicious, removed.

Now all this is practicable so far as cost is concerned. While the latter cannot be estimated beforehand, an extreme amount need be but the merest fraction of the possible loss when nothing is done. And the results from proper procedure are assured. Great losses from bitter rot should be charged up to the orchardist, not to the parasitic fungus, much less to the latitude or the weather. With other things nearly equal it is, however, still best to choose varieties not so much subject to the disease.

DESCRIPTION OF PLATES

Plate I. Fig. 1.—Bitter rot canker formed where the limb was broken. Fig. 2.—Natural cankers produced by the bitter rot fungus and in which perithecia were found.

Plate II. Fig. 1.—A large artificially produced canker producing masses of pink spores. This canker was divided and a section removed and cultured. Grayish white mycelium can be seen growing from the wood of the cross-section. Fig. 2.—Pustule of bitter rot fungus on mummified apple. Spores in the opening above. (Hasselbring).

Plate III. Fig. 1.—Bitter rot on apples, pear, and green tomato, produced by artificial inoculation. Fig. 2.—An artificially inoculated apple showing well developed concentric rings of bitter rot spores.

Plate IV. Fig. 1.—Artificial canker producing many pustules of spores. Fig. 2.—An apple spur bearing a small bitter rot canker which was induced to produce bitter rot spores. Fig. 3.—The end of the stem of a bitter rot mummy showing the marks of recent separation from the limb. Though the apple was taken from the ground it gave forth spores when kept in a warm, moist condition. This is very unusual. The mummy is shown on Plate VIII., Fig. 2.

Plate V. Fig. 1.—Tendrils of bitter rot spores oozing from the infected portion of an apple $\times 7$. Fig. 2.—Pustules of bitter rot spores forming on the surface of a canker $\times 7$. The most evident spore-masses are near the margin above lower right.

Plate VI. Fig. 1.—Section of a pustule magnified from a bitter rot canker on limb. (Hasselbring). Fig. 2.—Section of a canker showing perithecia of the bitter rot fungus with asci, some of which are escaping.

Plate VII. Fig. 1.—An out-door experiment with bitter rot mummies. Mummies were suspended on wires from the limbs of a tree (see left-center of cut), placed just above the ground, and directly on it, and left over winter. The fungus died in all cases. Fig. 2.—A bitter rot canker formed where the limb was broken.

Plate VIII. Fig. 1.—An artificial canker on living apple limb showing continued growth the second season after inoculation. Perithecia of the bitter rot fungus were found on this canker. Fig. 2.—Bitter rot mummy bearing pustules of bitter rot spores. This mummy was taken from the ground but apparently had not been there very long c. c. $1\frac{1}{2}$.

Plate IX. Fig. 1.—The old way of fighting bitter rot in Illinois.

Plate X. Effect of copper sulfate solutions on the germination of bitter rot spores. Figs. 1 to 7 inclusive were germinated in 4 c. c. of the solution while Figs. 8 to 15 inclusive were germinated in $\frac{1}{2}$ 5 c. c. of the solution. The solutions were as the following designated proportions of copper sulfate to water; Figs 1 and 8, 1:100,000; figs. 2 and 9, 1:200,000; figs. 3 and 10, 1:400,000; figs. 4 and 11, 1:1,000,000; figs. 5 and 12, 1:2,000,000; figs. 6 and 13, 1:4,000,000; figs. 7 and 14, 1:10,000,000; fig. 15, distilled water. The dark colored bodies are appressoria.

PLATE I

Fig. 1

Fig. 2

Fig. 1 (upper)

Fig. 2 (lower)

Fig. 1 (upper)

Fig. 2 (lower)

Fig. 1

Fig. 2

Fig. 3

PLATE V

Fig. 1

Fig. 2

Fig. 1

Fig. 2

PLATE VII

Fig. 1

Fig. 2

Fig. 1

Fig. 2

PLATE IX

PLATE X

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