BLACK ROT OF APPLE: SOME FACTORS AFFECTING ITS ETIOLOGY AND CONTROL

JAMES HOLMES JR.

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BLACK ROT OF APPLE: SOME FACTORS AFFECTING ITS ETIOLOGY AND CONTROL

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BLACK ROT OF APPLE: SOME FACTORS AFFECTING ITS ETIOLOGY AND CONTROL

by

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B. S., California State Polytechnic College, 1955

A THESIS

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Department of Botany
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ABSTRACT

BLACK ROT OF APPLE: SOME FACTORS AFFECTING ITS ETIOLOGY AND CONTROL

by

JAMES HOLMES JR.
The objectives of this investigation were to elucidate some of the factors affecting the etiology and control of black rot of apple caused by *Physalospora obtusa*. The main problems in New Hampshire are the cankerling of apple limbs by the black rot fungus and the fruit rot. There is a lack of understanding of the factors affecting the dissemination of *P. obtusa*. The Cortland variety is very susceptible to the frogeye leafspot and fruit rot.

Amount of leafspot, fruit rot, and overwintering of mature apple mummies and immature apple mummies were determined on six trees per variety. Cortland had ten times more leafspot than the McIntosh or Red Delicious varieties. Cortland trees also had the most mature apple mummies in the fall, but retained few over winter. Cortland retained 282 immature apple mummies per tree over winter. The other two varieties had few mummified mature apples in the fall, none remained over winter, and they had no immature apple mummies in the fall. Infected immature apple mummies attached to the branches of McIntosh and Red Delicious trees increased the incidence of frogeye leafspot on the leaves of these varieties. The retention of infected immature apple mummies by the Cortland variety appears to be of primary importance in the overwintering of the organism and for the varietal differences in the amount of frogeye leafspot and apple rot observed.

A study was made of the factors affecting the spore release and dissemination of *P. obtusa*. At five stations
directly under inoculum sources and at two in the open, silicone-coated slides were placed daily to trap spores in 1967 and 1968. The number of spores on 0.5 square cm were counted and correlated with weather data. The number of hours of 100% humidity per day and also the maximum-minimum daily temperature difference showed significant correlation at the 5% level with the quantity of spores released. Amount of rainfall, estimated wet period, and mean, maximum and minimum temperatures had no significant effect on the quantity of spores released. Spore traps in the open had a very small spore deposition on only 3 very windy days. Ladybird beetles (Hippodamia convergens Guerin) were collected in 1968 and 18% were found to be infested with P. obtusa. Thus, spores were disseminated mainly by rain-splash from inoculum sources in the tree and by at least one species of insect and only rarely by wind-blown spray.

The inoculation of apples on three varieties of trees with cultures from single spore isolates of P. obtusa was correlated positively with the number of fruit drops. The number of apple drops was affected significantly by the source of single spore isolates, but all isolates increased the number of drops over the controls.

Over a 3-year period folpet, captan, maneb, thiram and ferbam significantly reduced frogeye leafspot. Folpet and captan gave consistently good control of both frogeye leafspot and apple rot. Difolatan, which was used only in 1968, appeared promising as it significantly reduced frogeye leafspot and completely controlled fruit rot.
In 1968, field tests showed that phenyl mercuric acetate reduced canker elongation by 66% as compared to the control. The period of greatest canker elongation was from early July to the middle of August. This coincided with a dry period suggesting that moisture stress may be responsible for the increase of canker growth. This has also been suggested by the work of other writers. Phenyl mercuric acetate should be evaluated further as a method of reducing black rot cankers.
SECTION I

INTRODUCTION

Black rot of apple is a fungus disease which can cause chronic losses in apple orchards. This disease was once considered to be well under control but it has been observed in several commercial orchards in New Hampshire in recent years. The disease occurs in three forms on the apple tree: canker, leafspot, and fruit rot. The causal organism of this disease is the fungus *Physalospora obtusa* (Schw.) Cooke.

In well managed commercial orchards and old neglected orchards which have been reclaimed the principal problem appears to be the cankering of large limbs and dieback of twigs and branches. In poorly managed orchards and orchards where only a specific spray for apple scab is used without regard for black rot control, all three aspects of this disease may be present.

The resurgence of this disease in New Hampshire may be due to changing cultural practices such as the chipping of prunings instead of burning them, drought, changing use of chemical sprays, or lack of attention to this disease for several years.

With these considerations in mind it was decided to examine the existing conditions in the field by means of surveys and to study the underlying principles of some factors
of the etiology and control of this disease by laboratory and field experimentation. This study should help answer some of the academic and pragmatic questions which are posed by the resurgence of this disease in New Hampshire.
SECTION II

LITERATURE REVIEW

Several common names have been applied to this disease based on the organs attacked. The disease on the fruit is called "black rot", on the leaf "frogeye leafspot", and on the limbs "New York apple tree canker" or "canker". The rot on the fruit has also been called "ring spot", "brown rot", and "blossom end rot". The canker is also referred to as the "black rot canker", "dieback", "twig blight", and "apple canker" (3). The leaf phase is also referred to as "leafspot", "leaf blight", and "brown spot" (29).

The first report of the rot on the fruit was made by Peck in New York in 1879. In that same year specimens sent by French, from Illinois, were identified by Peck as having the same disease (3). Arthur recorded black rot of quince fruit in New York, in 1885, and noted the importance and infectious nature of the disease. In 1890 Scribner noted the disease in New Jersey and described the symptoms and causal pathogen. The same year Beccarini, in Italy, observed the rotting of otherwise sound apples, pears, and peaches. Two years later Halstead discussed the black rot of quince in New Jersey. Sturgis noted the disease on quinces in Connecticut in August, 1892, and in 1894 confirmed the work of Halstead. Kinney, in 1895, described the disease in Rhode Island (32). Frequent references to the rot can be found in various parts of this country and Europe between 1890 and 1900 (3).
The earliest record concerning the leafspot is that of Alwood who reported brown spot of apple foliage in Virginia in 1892 (32). During the period between 1890 and 1900 frequent references were made to the leafspot from various sections of the country, indicating that it had a wide distribution (3). Taylor (63) reported serious problems in the black rot and leafspot forms of the disease in Georgia in 1953.

Control of the leafspot phase of black rot received special attention by Brooks and Demeritt in New Hampshire in 1912 and by Walton in Pennsylvania in 1920 (3). Paddock, working in New York in 1899, first called attention to the canker form of black rot and described it in detail (3). Zeller (73), in 1926, reported work on controlling the canker form of the disease in Oregon, and Swartwout (60), in 1927, reported work done on canker in Missouri.

Losses from black rot result from rotting of the fruit, weakening the tree through defoliation, and loss of limbs by canker (3). The estimated average annual loss caused by black rot of apples for the period 1951-1960 was 1.1% of the total loss caused by fruit diseases in the United States (4).

In common storage, black rot may be serious when the fruit has received a certain amount of wounding from insects and handling, but under modern commercial conditions of insect control and rigid sorting of fruit, the rot is of minor importance in storage (3). Damage to the foliage depends on the extent of the infection. In mild cases of infection injury is not appreciable. In more severe cases, partial or
even complete defoliation may occur from six weeks to two months before the ripening of the fruit, in which case the fruit either drops from the tree or remains small and is of poor quality. In such cases, the fruit buds are weakened so as to decrease the possibility of a crop the following year, and the vitality of the tree is impaired (32).

It is difficult to estimate the injury due to cankers, since many factors are involved. The loss from black rot canker is most serious in winter-injured or neglected orchards (3). Taylor (61) also reported a definite relationship between fire blight and black rot occurrence in Georgia orchards.

Black rot on the fruit usually appears around a worm hole or some other wound. Frequently it originates at the calyx and where splitting or spray injury provides an infection court (3). Usually there is only one spot on a fruit. The skin at first becomes brown in a small area, but later darkens, finally turning black. Often concentric bands of uniform breadth and slightly different shades of color appear about the center of the lesion (32). The rotted tissue is somewhat firm and inclined to be rather leathery when the rot occurs before the apple is completely mature. The taste is not unpleasant, as in the case of bitter rot. After the fruit is completely rotted the tissues collapse, due to drying, and a wrinkled black mummy results (3).

On the leaf the first evidence of the disease is the appearance of from few to many small purple specks with a rather indefinite outline. Ordinarily these are first evident about
1 to 3 weeks after petal fall, although they may appear earlier. The spots enlarge to a diameter of about 1/8 to 1/4 inch (3). Later the lesion is of a yellowish-brown color and the spot assumes a more or less circular shape, while the margin becomes more definite. Still later the margin becomes elevated and the central area sunken. As the spots grow older the center becomes grayish-brown, and the entire lesion presents an appearance which gave it the name of frogeye leaf spot (32). The spot at the time of the leaf fall is a more or less irregular blotch made up of a light gray center, sharply defined, surrounded by many concentric rings of brown with light zones in between (29). The necrotic tissues are soon invaded by secondary fungi such as Phyllosticta and Alternaria (3, 32, 54).

In the earlier stages of the formation of a canker, the bark is slightly sunken and reddish brown in color. The diseased area slowly increases in size and darkens. Some lesions remain very small, measuring only a few cm in length; in such cases the canker ceases to enlarge. Where the infected area is larger, the diseased spot enlarges from year to year for a distance of 1 m or even more. It is often observed that a canker is merely a superficial roughening of the bark. In other cases the bark is killed to the wood and becomes conspicuously cracked (32). The bark clings firmly to the wood in recently killed areas, but after a year it cracks and finally it can be easily removed (3). Large limbs are rarely girdled the first year; the girdling results from the enlargement of the canker the following season. Complete encircling
results in the death of the parts above the canker as evidenced by yellowing and dropping of the leaves and the shriveling of bark and fruit (32).

On the fruit, sooner or later, black, pimple-like pycnidia appear on the surface of the rotted area (3). On leaf-spot, frequently small, black dome-like bodies (pycnidia) are found on the upper surface of the leaf, usually toward the center of the lesion (32). On cankers, minute, black-fruited pustules (pycnidia) may be very abundant over the bark of blighted twigs or in the bark of the localized cankers (29).

The present name of the causal organism is Physalospora obtusa (Schw.) Cooke, 1892 (12, 27, 63, 69). The black rot causal organism has been collected and described under different names from a large number of hosts, both in the imperfect and perfect stages, thus the proper nomenclature is very much confused. Anderson (3) states the following:

The imperfect stage has been described as one or more species of the genera Sphaeria, Diplodia, Melogramma, and Sphaeropsis. In 1879 Peck described the fungus on apple as Sphaeropsis malorum, the name which has been generally used for the imperfect stage since that date. The perfect stage of the fungus was first discovered in America on apple twigs by Hesler in 1913. He determined that it belonged to the genus Physalospora. Arnaud in France in 1912 had described a Physalospora which seemed to agree with this fungus, and consequently Hesler accepted Arnaud's name Physalospora cydoniae. The combination P. malorum was proposed when it was found that Arnaud's P. cydoniae differed in some respects from the perfect stage of the common black rot fungus in America. In 1933 Stevens, as a result of careful study of type specimens, came to the conclusion that the earliest description of the fungus has been made by Schweinitz in 1832, who named it Sphaeria obtusa. In 1892, Cooke in examining specimens from the Schweinitz collection, found the perfect stage of the fungus and transferred
it to the genus *Physalospora*. Thus the correct name would become *P. obtusa* (Schw.) Cooke, provided that the two associated stages belonged to the same fungus. This was proved by Stevens from fresh material collected both in England and America, from which cultures were obtained. On the basis of his findings, Stevens also was of the opinion that two species of *Physalospora* were associated with the black rot of apples. The one common in western Europe and northwestern United States he named *P. mutila* (Fries) N.E.S. The synonymy of *P. obtusa* given by Stevens shows 62 synonyms for the imperfect stage and 4 for the perfect stage, while *P. mutila* has at least 15 synonyms.

Anderson (3) suggests, therefore, using the name *S. malorum* when referring to the fungus in its relation to the black rot disease, and *P. obtusa* for the saprophytic perfect stage. It is the writer's observation, however, that this recommendation does not seem to have been followed in most of the recent publications. According to the International Rules of Botanical Nomenclature prior to 1950, a member of the Plant Kingdom may have but one valid name, therefore *Physalospora obtusa*, being the perfect stage of the fungus, is the valid name. But, since the name *Sphaeropsis* indicates the precise type of conidial stage produced in the bark, in rotting fruit, and more rarely in the leafspots and on fallen leaves, mycologists find it more convenient to say that *Physalospora obtusa* has a *Sphaeropsis* imperfect stage than to describe the conidia and conidiophores in many words. The naming of the conidial stages is so convenient and has been so widely adopted that the International Botanical Congress decided at its Stockholm meeting in 1950 to legalize the use of form names for conidial stages, still recognizing, of course, the name of the perfect stage as the official name for the entire organism (2).
The present classification of the black rot causal organism appears to be as follows (2, 14, 51, 43, 44):

Kingdom Plant
Division Mycota
Subdivision Eumycotina
Class Ascomycetes
Subclass Loculoascomycetidae
Order Pseudosphaeriales (Pleosporales)
Family Pleosporaceae (Pseudosphaeriaceae)
Genus Physalospora
Species obtusa

The subclass Loculoascomycetidae was described by Luttrell (41) in 1955 as Loculoascomycetes. In this subclass the asci are bitunicate and the ascocarps are ascostromata in which the asci are borne in locules. Alexopoulos (2) states that some Euascomycetidae have bitunicate asci and some others bear unitunicate asci in ascostromata, but when the two characters (bitunicate ascus and ascostroma) are combined, the fungus is placed in the Loculoascomycetidae.

Miller (44) first pointed out the two distinct developmental types of the Pseudosphaeriales and Dothideales which had previously been combined. In the Dothideales the centrum is composed of pseudoparenchyma which disintegrates as the asci develop. This results in the formation of a locule occupied by a cluster of aparaphysate asci. In the Pseudosphaeriales the centrum is composed of pseudoparaphyses among which the asci develop later. Bessey (14) reports that the Pseudosphaeriales appear to develop as follows:
Within a pseudoparenchymatous stromatic structure arise branching ascogenous hyphae, probably—in many cases, if not in all—from an ascogonium. These hyphae grow out into the stromatic tissue, dissolving it so that eventually each terminal ascus lies in a cavity of the original sterile tissue. These asci may be separated rather widely or the separating tissue may be but a thin sheet of cells. The developing asci may arise in a fan shaped cluster destroying the stromatic tissue as they enlarge.

Luttrell (41) feels that Miller's separation of these orders has not been generally accepted due to a false conception of the nature of pseudoparaphyses. He states that pseudoparaphyses do not represent remnants of interthecial stromal tissue but originate as separate paraphysis-like hyphae prior to the formation of the asci. Luttrell, however, accepts Miller's definition of the Dothideales and Pseudosphaeriales, but he proposes the new name Pleosporales for the order Pseudosphaeriales as delimited by Miller. The name Pleosporales is used by Alexopoulos (2). He states that the Pleosporales are characterized by the Pleospora type centrum, in which the bitunicate asci develop among pseudoparaphyses and grow upward between them. The pseudoparaphyses are attached both to the roof and the floor of the locule. They originate in the upper wall and grow downward. The ascostroma is either a pseudothecium or a multiloculate cushion-shaped stroma.

Bessey (14) and Miller (43, 44) use the family name Pleosporaceae. Luttrell (41) uses Pseudosphaeriaceae as the family name. Alexopoulos (2) uses both under the older family heading of Pleosporaceae.
In 1933 Stevens (59) listed 75 host genera on which *Physalospora obtusa* has been found. At present there are 55 families represented in the host range (6, 21, 25, 59). It would seem that the fungus will grow saprophytically and fruit on almost any plant having a woody texture if conditions are favorable. As a parasite, however, it seems to be limited largely to the pome fruits—apple, pear, and quince. The leafspot is known only from the apple (3).

Considerable variation has been noted in the susceptibility of varieties of the apple (22, 26, 29, 31, 63, 68). The fruit rot phase is more severe on early varieties previous to maturity, while late or winter varieties are likely to suffer in storage. The frogeye leafspot phase is reported as severe on Ben Davis in Nebraska, on York Imperial and Stayman Winesap in Pennsylvania and Chenango Baldwin, Rhode Island and Twenty Ounce in New York (29).

Taylor (63) found in Georgia in 1951 that the number of fruit drops infected with *P. obtusa* varied with the variety—about 73% of Golden Delicious, 60% of Red Delicious, and 30% of Stayman Winesap. In New Hampshire in 1965 the Cortland variety was found to be the most severely attacked and McIntosh moderately attacked (A.E. Rich, unpublished data). Azpeitia (13) found the variety "Rheineta del Canada" was susceptible to the disease in Spain in 1965. Heald (29) reports that Ben Davis and Northern Spy were severely affected in Ontario. Swartwout (60) says Ben Davis and Gano varieties are moderately susceptible to black rot canker in Missouri. Physiological
strains of the fungus have not been definitely established, but different isolations have been shown to vary greatly in rapidity of rot produced and in behavior in culture (29).

With its wide natural host range the disease occurs in most humid areas in the temperate zones of the world (3, 37, 38). It is widely distributed in America from the Gulf States northward to Ontario, Quebec, and Nova Scotia. It has been reported from California, Oregon, and Washington on the West Coast (29). Leafspot seems to be the most prevalent and destructive in sections of the Eastern States, while the canker form is prevalent in New York (3). Black rot occurs in Europe from Italy and France northward to Germany and England, and extends eastward into southern Russia (29). Recently it has been reported in Spain (13). It occurs also in Australia, South Africa, and New Zealand (29). The most likely methods for distribution of the disease from one area to another today are probably the shipment of infected apples, and the transportation of apple seedlings from one area to another (32).

The pathogen may be carried over the winter in the form of dormant mycelium, immature pycnidia, mature pycnidia, developing perithecia and possibly by pycnospores that have been set free and are lodged on the surface of the bark (32). Anderson (3) states that the fungus may overwinter in the mycelial form in dead bark, dead twigs, or mummified fruit. In 1951 Groves (28) found that partially developed fruits which would normally have fallen in the "June drop" or earlier, remained attached to the Rome variety sprayed with naphthalene
acetic acid and subsequently became infected with P. obtusa. The pycnidia containing viable pycnospores are very common both on the mummied fruit and on cankers and dead twigs early in the spring. Also the fungus may overwinter in the perfect stage in the bark or blighted twigs and may account for considerable initial infection (3). Heald (29) states, however, that because of the rarity of the perithecia, it seems that these play a very minor part in the life history of the pathogen, the main reliance being placed on the pycnospores for the dissemination of the fungus. The pycnospores are prevalent and ready to produce infections when conditions are favorable. The retention of their vitality is an important feature in increasing the chances for infection (52). Hesler (32) states that pycnospores two years old or older are still capable of germination.

With such a wide wild host range as exists for this disease it becomes increasingly important to know the methods and factors affecting the local dissemination of the spores of this organism. This is especially true in New Hampshire where, in many cases, forests adjoin apple orchards, and changing cultural practices such as chipping instead of burning prunings occur. Three main methods of dissemination are generally discussed in the literature: rain-splash, wind and wind-blown mist, and insects (3, 29, 32). Dissemination by rain-splash and possibly by insects is generally accepted, whereas wind and wind-blown mist present an area of disagreement among writers on the subject (1, 3, 29, 32). Anderson (3) states
that spore dissemination seems to be due largely to washing and wind-blown mist, although miscellaneous insects may play a part, since the gelatinous nature of the spores would cause them to adhere to the bodies and legs of any insect crawling over the discharging pycnidia. Hesler (32) reports:

It is clear that the behavior of the pycnosporoes on being discharged places them at the disposal of wind, rain, and possibly insects, depending largely on the conditions of moisture. Halsted (1892) states that "the germs pass . . . through the air or by means of the various insects that visit the fruits, especially those with broken surfaces due to partial decay." A similar opinion is expressed by Sturgis (1894). Lamson (1902:76) states that the spores are easily floated in slight currents of air, while Bethune (1909:29) and McCready (1910) attribute dissemination to the wind.

Heald (29) disagrees on this subject by stating:

Rain and insects may bring about their further dissemination, but evidence of wind dissemination is lacking, since spores were not collected in spore traps set in orchards in which black rot was prevalent (Wolf, 1910). Their liberation in tendrils is also opposed to wind transport, and the great frequency of the pycnidia on dead twigs and the work of Walton (1920) showing that: froséeye infection is correlated with periods of rainfall make it unnecessary to assume any extensive wind dissemination.

Wolf (71), however, trapped spores only from September to May (through the winter). Thus wind and wind-blown mist remain controversial and possibly important methods of spore dissemination. Hesler (32) found spores on the feet of the rosy apple aphis (Aphis sorbi Kaltenbach), but it is his opinion that insects are of little importance in carrying the spores. He thinks their role in making openings in the fruit and bark is probably much more important. Anderson (3) states
that the prevalence of black rot around worm holes may be due in part to the fact that flies and ants often visit the freshly wounded areas on the apple.

Germination of the pycnospores is rapid under favorable temperature and moisture conditions. Recently matured spores will germinate within 5 or 6 hours, although overwintering ones may require longer periods. The most favorable temperature for spore germination is about 23 - 27 C (26). The incubation period is from 2 - 4 days in the case of leaf-spot, from 2 - 9 days for bark lesions, and about 48 hr for the fruit rot (32).

Epiphytotics of this disease occur rarely. Instead, it is characteristic of the disease to take a toll year after year. In estimating disease losses so incurred, one must also consider the cost of growing tree replacements. In many cases diseased limbs die, resulting in the cost of their removal and destruction (32). The serious increase in black rot canker and limb dieback in recent years in New Hampshire and other Northeastern States has probably been caused by the long drought experienced in this region. Palmiter (50) reported in 1966 that at one time apple cankers were considered a major problem in New York. Then for several years, they caused little trouble, but recently, because of drought conditions, the canker has again gained prominence as a disease problem in orchards. Taylor (62) reported that during the unusually dry 1951 growing season in Georgia black rot caused more loss to
the growers of Georgia than all other diseases and insects combined. Bier (15) related tissue age, dormancy and turgor pressure as the factors affecting bark susceptibility to cankers. Landis (40), while working on the cankers of ornamental crabapples associated with *P. obtusa*, found that attempts to inoculate two year old grafted plants failed until the trees were subjected to water stress, after which *P. obtusa* produced cankers. It was stated that the ability of a tree to wall off a pathogen depends in part upon the relative turgidity of the host and varies among hybrids of the same species. Thus the lowering of the vigor, that is, reducing the relative turgidity of the 2-year old plants by placing them on water stress, allowed infection to occur. Palmiter (49) found that all flush pruning cuts on apple trees made in March, April, May and June showed good callus formation by September. Flush cuts made in July showed little callus formation by November.

Black rot is especially serious as a leafspot where wet weather prevails during and immediately following the bloom. Wet weather, optimum temperatures for fungus growth, sufficient light to stimulate production of pycnidia, and the presence of other factors, such as wounds and insects, affect the seriousness of the disease in an apple orchard (3, 27, 29, 32).

Control by exclusion of the black rot disease was attempted by the British Government at Cape Colony in 1908. Importers were warned that, under a government notice of 1908, all consignments of pomaceous fruits found infected with this
The eradication of the black rot disease is difficult since the pycnospores are almost omnipresent and they remain viable over a period of at least a year. The fungus is found on so many wild host plants that there is no assurance that removal of dead wood in the orchard would be of practical significance. However, mummified fruit, dead twigs and cankers in the tree are responsible in many cases for the heavy primary leaf infection (3).

Walton (68) recommended pruning and burning of all dead twigs, and brush around the orchard. As a general rule, cankers on large productive limbs should be removed. The destruction of black rot mummies that remain on the tree or ground, and very close pruning to remove all dead wood in which the fungus may hibernate are also commendable practices (28, 29, 49, 50). All tree prunings and diseased bark, whether killed by black rot or from other causes, should be destroyed by burning (29). The practice of chipping prunings, which are then left on the orchard floor and often contain infected twigs and branches, may be contributing to the increased incidence of this disease (10). Either clean cultivation or plowing under the leaves previous to the blossoming period has been suggested (18, 29). Since insects cause wounds and carry the spores to them they should be controlled by the use of spray materials in the orchard (3, 32).
Swartwout (60) recommends treatment of cankers by removal of cankered wood and treating with mercuric chloride (corrosive sublimate). Agrios and Dockerty (1) recommended that cankered limbs be removed at least 6 to 12 inches below the visible canker. Large cuts should be covered with a wound dressing such as orange shellac, asphaltum paint, grafting wax, house paint, bordeaux paste or any of the commercial tree paints. The dressed surfaces should be inspected periodically and recoated once or twice a year, when the dressing blisters, cracks or peels.

Taylor (61) reported that there appears to be a definite relationship between fire blight and black rot occurrence in Georgia orchards, and recommended the control or removal of fire blighted limbs to help prevent a possible build up of black rot.

Since fruit rot progresses very slowly at temperatures below 10 C immediate refrigeration after packing is a good practice (17, 34). Anderson (3) says that maximum infection of leaves occurs during periods of scab infection. Special fungicide applications for leafspot control are not necessary except in those cases where the regular schedule for scab control is not followed. Rich (A. E. Rich, unpublished data), however, observed in 1965 that Cyprex, which controls apple scab, does not control black rot of apple or frogeye leafspot. Taylor (63) reported that (2-4-100) Bordeaux mixture and ferbam proved effective. Walton (68) and Wolf (72) recommended Bordeaux, (4-5-50), for control of leafspot. Bordeaux mixture is not ordinarily recommended today, since fruit russetting may result (3).
Later writers gave recommendations for newer materials such as: captan \((4, 9, 10, 56, 64, 65, 66)\), folpet \((9, 24, 65)\), ferbam \((4, 9, 42)\), thiram \((9, 10)\), zineb \((4, 9, 10, 33, 57)\), organic mercury \((4)\), Glyodin \((4)\), maneb \((33)\), Thiolutin \((33, 51)\), and Streptomycin \((33)\). One recent publication reports that sanitation is the primary control measure, as none of the present-day fungicides appears very effective against black rot \((7)\).
SECTION III

MATERIALS AND METHODS

1. **Field Observations**

   Observations and notes were taken on the progress of the black rot disease in the springs and summers of 1967 and 1968. This was done to compare the descriptions and observations of earlier writers with what actually took place in New Hampshire.

2. **Disease Survey of Cankered Limbs**

   For the purpose of determining the extent of the canker problem in commercial and neglected orchards in New Hampshire, cankered limbs were collected from six orchards in five townships in the fall of 1965. Samples were taken from the basal end of the cankers, surface sterilized with a 10% solution of sodium hypochlorite for 2 - 5 min, washed three times in sterile, distilled water, and transferred to potato dextrose agar. *Physalospora obtusa* was identified by spore size and shape.

3. **Physalospora obtusa Isolates from Parts of Marked Cankers**

   To obtain more information on the life cycle of *P. obtusa* in relation to canker dieback, 100 cankered limbs (1 - 8 cm in diam) from Cortland, McIntosh and Red Delicious trees, were tagged in a local, neglected apple orchard. Samples were then obtained from various parts of these cankers (terminal end, center, basal end) and from tissue immediately behind the
basal end of the cankers (2.4 cm and 15 cm behind the basal end). These samples were surface-sterilized, cultured, and identified as described above.

4. **Infected Plant Parts**

Samples of plant parts (leaves, immature apple mummies, decayed fruit spurs, and prunings from the brush pile and the ground) were obtained from both commercial and neglected apple orchards in southern New Hampshire. The samples were surface sterilized, cultured, and identified as described under disease survey of cankered limbs.

5. **The Overwintering of P. obtusa on Apple Trees as Influenced by Variety, Apple Mummies and Dead Fruit Spurs**

In the local neglected apple orchard differences were visually observed in the amount of frogeye leafspot and apple rot on Cortland, McIntosh and Red Delicious varieties. To check on this observation the amount of leafspot per 100 leaves (checked six times, summer 1967), the percent of fruit rot, and the overwintering of mature apple mummies, immature apple mummies (retained June drops) and decayed fruit spurs were determined on six trees per variety. Cyprex was used to control apple scab.

6. **The Effect of Increased Inoculum in McIntosh and Red Delicious Trees**

To determine if varietal differences in the amount of frogeye leafspot were caused by the amount of inoculum in the tree or by resistance of the variety to the pathogen, the following experiment was performed. Twenty-five immature
apple mummies (obtained from Cortland trees) were attached with plastic coated wire ("twistums") to the branches of six McIntosh and Red Delicious trees (Figure 1). Six branches per variety were also used as a control. The amount of leafspot per 100 leaves was checked six times during the summer of 1968. Cyprox fungicide was used to control apple scab.

7. The Infection of Immature Apples by P. obtusa

Six trees each of Cortland, McIntosh and Red Delicious varieties were used in this experiment in the local neglected orchard. Ten immature apples per tree, known as "June drops", were readily selected by a slight yellowing of their stems. Prior to dropping from the tree these sixty samples per variety were surface sterilized, cultured, and P. obtusa was identified as described under disease survey of cankered limbs.

8. The Number of Fruit Drops as Influenced by Single Spore Isolates and Variety

Four trees for each of three varieties (Cortland, McIntosh and Red Delicious) in the local neglected orchard were used in this experiment. Single spore isolates were obtained (55) from four sources: a Cortland canker, Cortland mummy, McIntosh canker, and Red Delicious canker. A single spore isolate suspension was made using the method developed by Miller (45) whereby the mass of mycelium is diluted 1:5 by volume with distilled water, chopped in a blender for 2 min, and strained through a double thickness of cheese cloth.
Figure 1. Immature apple mummy infected with *P. obtusa* attached to an apple branch with plastic coated wire ("twistum"), and the resulting pattern of frogeye leafspots on the leaves.
Ten apples per tree (one repetition) were inoculated with inoculum from each single spore isolate, four repetitions being made (40 apples total). Each apple was cut in a "V" shape (approximately 2 cm wide at the hinge end) with a knife previously dipped in 70% alcohol. The "V" was then flipped back using the skin of the apple as a hinge and the single spore isolate suspension was sprayed into the cavity with a hand operated spray bottle. The cut was sealed with silicone grease. The control was treated in a similar manner, but distilled water was used instead of the spore suspensions. The apples were inoculated August 1, 1967 and the number of apple drops checked 10 days later on August 11, 1967. The data were treated statistically as a 3 x 4 factorial experiment and the analysis of variance derived (58).

9. Spore Release and Dissemination Experiment

Some inconsistencies have been found in other writers' descriptions of the methods of release and dissemination of the spores of *P. obtusa* (1, 3, 29, 32). Compounding this problem is the wide host range of this fungus and modern cultural practices such as chipping the prunings instead of burning. In order to resolve these problems spore collection stations were set up in the local neglected orchard from April 15, 1967 to October 20, 1967, and from April 1, 1968 to August 1, 1968.

Microscope slides coated with silicone (48) were used at all stations as spore traps. These slides were collected
daily and examined under a microscope to ascertain the number of spores deposited during the 24-hr period in a 0.5 sq cm area of the slide. A battery-operated Kramer-Collins spore sampler, designed for automatic quantitative collection of fungus spores every 15 min over 24-hr periods, was used at one station in 1967. At the remaining stations, exposed microscope slides were set on plastic covered boxes approximately 50 cm above the ground.

A. Station Location:

April 15, to October 20, 1967

Station A. Kramer-Collins spore sampler operated from April 15, 1967 to August 3, 1967 (Fig. 2). Located directly under the canopy of a large apple tree showing heavy infection of black rot. Since no spores were collected from April 15, 1967 to June 12, 1967 infected limbs were placed 15 cm above stations A and B from June 12, 1967 to October 20, 1967.

Station B. Silicone coated microscope slide. Located 25 cm from station A to check on the efficiency of the Kramer-Collins spore trap.

Station C. Silicone coated microscope slide. Located in an open area approximately 10 m from the nearest apple tree and approximately 20 m from the brush pile at the edge of the orchard.

Station D. Silicone coated microscope slide. Located 15 cm under infected pruned limbs in the brush pile (Fig. 3).
Figure 2. View of the Kramer-Collins automatic spore sampler, (Station A). A continuous temperature recorder (normally covered) is shown at the side.
Figure 3. View of spore trap station D. The silicone coated microscope slide is placed on a plastic covered box directly under limbs infected with *P. obtusa* in the brush pile.
Station E. Silicone coated microscope slide. Located approximately 2 m from the brush pile on the east side. (The prevailing wind is from the northwest.)

B. Station Location:

April 1 to August 1, 1968

Station E. Silicone coated microscope slide. Located similar to station C (in the open area). The slide was placed under a wire cage and 25 immature apple mummies placed on the wire mesh 6 cm above the slide.

Station G. Silicone coated microscope slide. Located the same place as Station D (under the brush pile).

Weather data were collected with a temperature recorder at the spore station area, and a recording hygrothermograph and a rainfall recorder located at a weather station approximately 100 m north of the spore trap area. Weather information was checked with the University of New Hampshire weather station which was located approximately 1 km north of the orchard. The instruments were calibrated once every 2 weeks with a thermometer and a sling sychrometer. Spore deposition and weather data were treated as a linear regression and the correlation coefficient was derived (57).

The ladybird beetle (Hippodamia convergens Guerin) was chosen to check the presence and dissemination of spores on the bodies of insects in the local neglected apple orchard. It is a bark beetle that is present from early to late spring during the time of primary and secondary spore release of P. obtusa. It crawls and hides in the bark and flies from one
tree to another. When touched it releases its hold and falls to the ground to escape.

Beetles were collected from early March 1968 to late May 1968. They were collected individually in a sterilized test tube with a cotton plug. The test tube was pressed gently to the body of the insect and when it dropped it rolled into the tube which was then immediately plugged. The insects were placed on potato dextrose agar in petri plates and incubated for 2 weeks.

10. **Perfect Stage Data**

It was observed that cankered limbs and prunings in the brush pile were infected with what resembled the perfect stage of this fungus. It appeared to be so plentiful in the neglected orchard that it was decided to study this stage in more detail.

Microscope slides were prepared by fixing the wood with Formalin-Aceto-Alcohol fixative (F. A. A.) and infiltrated with celloidin (70). The wood was sectioned 10 - 12µ thick using a sliding microtome. The sections were stained using the safranin-hematoxylin method (70). Photomicrographs were made of these sections using Ectachrome X film.

11. **Frogeye Leafspot and Apple Rot Control by Fungicide Spray Treatment**

In the local neglected orchard 12 trees of the Cortland variety were selected for the experiment. Cyprex was used to suppress apple scab. The trees were paired and the limbs and treatments numbered from a random number table and assigned.
Each limb was color tagged for each treatment. In 1966 the effect of eight fungicides and a control were compared on these trees using four replicates per treatment. In 1967 five fungicides and a control were evaluated using six replicates per treatment. In 1968 seven fungicides and a control were compared using six replicates per treatment. Spray materials, formulations, rates of application and spray dates for 1966, 1967, and 1968 are shown in Table 1. The leafspot results from 1966 were analyzed statistically as an incomplete block design using analysis of variance (2) and Duncan's new multiple range test (58). The leafspot results from 1967 and 1968 were analyzed statistically as a completely randomized block design using analysis of variance and Duncan's new multiple range test (58). The incidence of frogeye leafspot was determined by counting the number of spots per 100 leaves. These counts were repeated six times during the summer.

<table>
<thead>
<tr>
<th>Year</th>
<th>Material</th>
<th>Commercial formulation</th>
<th>% active ingredient</th>
<th>Rate of application (lb. per 100 gal)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Folpet</td>
<td>50</td>
<td></td>
<td>2 lb.</td>
</tr>
<tr>
<td></td>
<td>Captan</td>
<td>50</td>
<td></td>
<td>2 lb.</td>
</tr>
<tr>
<td></td>
<td>Thiram</td>
<td>65</td>
<td></td>
<td>2 lb.</td>
</tr>
<tr>
<td></td>
<td>Maneb</td>
<td>80</td>
<td></td>
<td>2 lb.</td>
</tr>
<tr>
<td>1966</td>
<td>Zineb</td>
<td>65</td>
<td></td>
<td>2 lb.</td>
</tr>
<tr>
<td></td>
<td>Ferbam</td>
<td>76</td>
<td></td>
<td>2 lb.</td>
</tr>
<tr>
<td></td>
<td>Polyram</td>
<td>80</td>
<td></td>
<td>2 lb.</td>
</tr>
<tr>
<td></td>
<td>Glyodex (Glyodin+ Dodine)</td>
<td>50</td>
<td>16</td>
<td>.5 lb.</td>
</tr>
<tr>
<td></td>
<td>Folpet</td>
<td>50</td>
<td></td>
<td>2 lb.</td>
</tr>
<tr>
<td></td>
<td>Captan</td>
<td>50</td>
<td></td>
<td>2 lb.</td>
</tr>
<tr>
<td>1967</td>
<td>Ferbam</td>
<td>76</td>
<td></td>
<td>2 lb.</td>
</tr>
<tr>
<td></td>
<td>Thiram</td>
<td>65</td>
<td></td>
<td>2 lb.</td>
</tr>
<tr>
<td></td>
<td>Maneb</td>
<td>80</td>
<td></td>
<td>2 lb.</td>
</tr>
</tbody>
</table>
Table 1. (cont.)

<table>
<thead>
<tr>
<th>Year</th>
<th>Material</th>
<th>Commercial formulation % active ingredient</th>
<th>Rate of application (lb. per 100 gal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1968</td>
<td>Thiram</td>
<td>65</td>
<td>2 lb.</td>
</tr>
<tr>
<td></td>
<td>Maneb</td>
<td>80</td>
<td>2 lb.</td>
</tr>
<tr>
<td></td>
<td>Difolatan</td>
<td>80</td>
<td>.8 lb.</td>
</tr>
<tr>
<td></td>
<td>Phenyl mercuric acetate</td>
<td>22</td>
<td>.25 lb.</td>
</tr>
</tbody>
</table>

* Spray dates:

1966: 5-21, 5-26, 6-2, 6-9, 6-24, 6-30, 7-7, 7-14, 8-2, 8-17.

1967: 6-9, 6-16, 6-24, 6-30, 7-6, 7-20, 8-3, 8-17.

1968: 5-15, 5-23, 5-31, 6-7, 6-14, 6-24, 7-8, 7-22, 7-31.
Apple rot was counted six times during the summer also, thus assuring the inclusion of those apples that might drop prematurely from the tree. Apple rot was calculated as percent rot per total number of apples.

12. **Imbibition and Initial Growth Inhibition Experiments**

A. General

The rate of imbibition and initial growth inhibition were measured on four distinct parts of the apple tree:

1. Decayed fruit spurs (.6 cm diam x 1.2 cm long)
2. Immature apple mummies (1.2 cm diam)
3. Old cankered limbs (.6 cm diam x 5 cm long)
4. New cankered limbs (.6 cm diam x 5 cm long)

The ends of the old and new cankered limbs were dipped in paraffin to prevent entry of the solutions through the cut ends of the limb samples. The solutions tested for imbibition rate were:

- Control (distilled water)
- Folpet 50 WP (1.12 g / .473 liter)
- Captan 50 WP (1.12 g / .473 liter)
- Ferbam 76% (1.12 g / .473 liter)
- Thiram 65% (1.12 g / .473 liter)
- Maneb 80% (1.12 g / .473 liter)
- Difolatan 80% (.48 g / .473 liter)
- Phenyl mercuric acetate 22% (.14 g / .473 liter)

Five samples were used for each plant part in each separate solution. (i.e. five decayed fruit spurs were used to check the imbibition rate of distilled water etc.)
B. Imbibition Experiment

Plant part samples were obtained from the field and identified visually as infected with \textit{P. obtusa}. Initially they were dried at room temperature (approximately 27°C overnight. The plant part samples were distributed into 250 mL beakers in a random manner. Dry weights of the five samples combined for each plant part were obtained prior to soaking them in the individual solutions. The sample plant parts were then soaked in the various solutions and weighed after 2, 4, 6, and 12 hr of soaking. The percent increase in weight of the plant parts was calculated for the 2, 4, 6, and 12 hr periods.

C. Experiment on the Initial Inhibition of Growth of \textit{P. obtusa} \textit{in vitro}

Plant part samples of the imbibition experiment were allowed to continue to soak for a total of 48 hr. They were then removed and placed individually on potato dextrose agar in petri plates 9 cm in diam and incubated at room temperature (approximately 27°C) by a window facing south to provide sunlight. Fungus growth was measured and recorded for a total of 17 days. The results were calculated on the basis of the number of days growth of the fungus was totally inhibited during that period.

Each petri plate was examined to determine the actual presence of \textit{P. obtusa}. \textit{P. obtusa} was not always present in the cultures probably due to the inhibition of growth caused by fungicide treatment. The ratio of positive identifications
of *P. obtusa* to the total number of plant part samples was recorded.

13. **Limb Canker Control**

An experiment on the control of limb cankers was conducted in the local neglected apple orchard using 46 McIntosh trees in the late dormant period (early March 1968). The limbs used were from 1 - 8 cm in diam and all had exhibited canker growth the previous year. The cankers were color tagged and alternately either treated with phenyl mercuric acetate or used as a control. The phenyl mercuric acetate slurry (14 g per liter) was painted on the canker and also on an area extending approximately 1 m behind the basal end of the canker.

The increase in canker growth was measured by placing a thumbtack 5 cm inside the cankered area at the basal end of the canker and measuring the increase of canker elongation from this point minus the 5 cm (Fig. 4). The canker growth was measured in early July, mid-August, and mid-September 1968.
Figure 4. A color tagged limb canker infected with *P. obtusa*. The thumbtack is 5 cm behind the basal end of the canker and is used to measure the canker elongation.
SECTION IV

RESULTS AND DISCUSSION

1. Field Observations

It was observed that frogeye leafspot appeared on the apple leaves approximately 19 days after the first large spore release (data obtained from spore traps) in the spring of both 1967 and 1968. The first large spore release occurred at approximately the middle of the blossom period in 1967 and 1968. The exact dates of the spore release varied due to the occurrence of an early or late spring. In 1967 the first large spore release occurred on May 25th, and in 1968 on May 11th.

It was also observed that a "shotgun" pattern of frogeye leafspot occurred on leaves located below immature apple mummies (Fig. 5 and 6) and dead fruit spurs (Fig. 7). Leafspot in the summer and fruit rot in the fall occurred more frequently on the Cortland variety than on the McIntosh or Red Delicious varieties. There also appeared to be more immature apple mummies (retained June drops) on the Cortland variety. This was a confused issue at first since many immature apples (potential June drops) were present on all of the varieties until about the middle of June. Then it appeared that many were retained on the Cortland variety whereas they normally dropped from the McIntosh and Red Delicious varieties.

Some difficulty was encountered at first in recognizing the fruiting bodies of P. obtusa on apple mummies and
Figure 5. Immature apple mummy and the associated pattern of frogeye leafspots on the leaves of an apple tree.

Figure 6. Close-up view of immature apple mummy and the associated frogeye leafspot pattern on apple leaves.
Figure 7. Pattern of frogeye leafspot on the leaves of an apple tree under a decayed fruit spur infected with *P. obtusa*.
cankered limbs. By accident it was discovered that after about 1 hr of rain the infected mummies, twigs and limbs were covered with a glistening, jet-black mass of fruiting bodies. This color lasted about 5 min after the rain stopped whereupon the color again returned to a dull gray black. Thus, during a rain it is very easy to pick out the fruiting bodies of the fungus.

2. Disease Survey of Cankered Limbs

The percent of infection of cankered limbs caused by P. obtusa was determined in two neglected orchards and four commercial orchards in New Hampshire. The results of this survey are presented in Table 2. Both neglected orchards had a large percentage (66%, 82%) of cankers infected with P. obtusa. Of the four commercial orchards surveyed, three had a very low incidence of black rot among the cankered limbs, and one had 34% of the cankered limbs infected with P. obtusa. It was discovered that the latter one was an old orchard that had once been abandoned, then later reclaimed 10 years prior to the time of the survey. During this 10 year period the grower had no problem with cankers until recently. It was suspected that a lack of vigor, as exhibited in neglected orchards and in reclaimed orchards of older trees, possibly predisposed the trees to canker infections during a drought period. This was suggested by Bier (15) when he mentioned tissue age, dormancy and turgor pressure as affecting the susceptibility of a tree to canker formation.
Table 2. Disease survey on cankered apple limbs in New Hampshire orchards.

<table>
<thead>
<tr>
<th>Orchard location (township)</th>
<th>Number of isolates per total cankers</th>
<th>% Infection of <em>P. obtusa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Stratham</td>
<td>24/70</td>
<td>34</td>
</tr>
<tr>
<td>Gilford</td>
<td>33/50</td>
<td>66</td>
</tr>
<tr>
<td>Durham</td>
<td>82/100</td>
<td>82</td>
</tr>
<tr>
<td>Londonderry</td>
<td>0/50</td>
<td>0</td>
</tr>
<tr>
<td>Londonderry</td>
<td>1/50</td>
<td>2</td>
</tr>
<tr>
<td>Hampton Falls</td>
<td>0/50</td>
<td>0</td>
</tr>
</tbody>
</table>
3. *Physalospora obtusa* Isolates from Parts of Marked Cankers

The results of a survey of 100 marked cankers in a neglected apple orchard is presented in Table 3. While 82% of the cankers examined were infected in some part either parasitically or saprophytically with *P. obtusa*, probably only 34% were initiated by this fungus. The fungus has been isolated 15 cm behind the basal end of the canker which indicates that the limb should be cut at least 30 cm (1 ft) behind the basal end of the canker. Thus, any chemical treatment for black rot canker must extend behind the basal edge of the canker for at least 30 cm as recommended by Agrios (1).
Table 3. *Physalospora obtusa* isolates from various areas of marked cankers.

<table>
<thead>
<tr>
<th>Area</th>
<th>Number of isolates per samples</th>
<th>% <em>P. obtusa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminal end</td>
<td>26/100</td>
<td>26</td>
</tr>
<tr>
<td>Center</td>
<td>43/100</td>
<td>43</td>
</tr>
<tr>
<td>Basal end</td>
<td>33/100</td>
<td>33</td>
</tr>
<tr>
<td>2.5 cm behind basal end</td>
<td>34/100</td>
<td>34</td>
</tr>
<tr>
<td>15 cm behind basal end</td>
<td>2/23</td>
<td>9</td>
</tr>
<tr>
<td>Total number of cankers</td>
<td>82/100</td>
<td>82</td>
</tr>
</tbody>
</table>
4. **Infected Plant Parts**

The results of a survey of plant parts for infection with *P. obtusa*, other than cankered limbs, in commercial and neglected apple orchards are shown in Table 4. The most interesting result of this survey is that 88% of the immature apple mummies (retained June drops) and 46% of the decayed fruit spurs were infected with *P. obtusa* in neglected orchards. In commercial orchards only 4% of the immature apple mummies and 14% of the decayed fruit spurs were infected with *P. obtusa*. Thus, it is thought that the immature apple mummies and decayed fruit spurs are potential sites of inoculum for *P. obtusa* when sanitation and chemical control are neglected. The infection of prunings from brush piles varied from year to year and ranged from 24% to 58%. In commercial orchards the incidence of *P. obtusa* on plant parts other than cankered limbs appears to be negligible. This is probably due to extensive sanitation and chemical control measures used in commercial orchards.

5. **The Overwintering of *P. obtusa* on Apple Trees as Influenced by Variety, Apple Mummies and Dead Fruit Spurs**

The incidence of fruit rot, decayed fruit spurs, frogeye leafspot and percent leaf infection as influenced by the variety of apple tree is shown in Table 5. The overwintering of mature and immature apple mummies (Fig. 8) on these varieties of apple trees is shown in Table 6. It was found that the Cortland variety had approximately ten times more leafspot than the other two varieties as indicated by the
Table 4. *Physalospora obtusa* isolates from various parts of apple trees from neglected and commercial orchards.

<table>
<thead>
<tr>
<th>Part</th>
<th>Number of isolates per total orchard samples</th>
<th>% <em>P. obtusa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neglected orchard</td>
<td>Commercial orchard</td>
</tr>
<tr>
<td>Frogeye leafspot</td>
<td>33/70</td>
<td>41</td>
</tr>
<tr>
<td>Frogeye leafspot</td>
<td>1/50</td>
<td>2</td>
</tr>
<tr>
<td>Immature apple mummies</td>
<td>88/100</td>
<td>88</td>
</tr>
<tr>
<td>Immature apple mummies</td>
<td>2/50</td>
<td>4</td>
</tr>
<tr>
<td>Decayed fruit spurs</td>
<td>32/70</td>
<td>46</td>
</tr>
<tr>
<td>Decayed fruit spurs</td>
<td>11/76</td>
<td>14</td>
</tr>
<tr>
<td>Decayed fruit spurs adjacent to leafspot</td>
<td>29/38</td>
<td>76</td>
</tr>
<tr>
<td>1967 Prunings-brush pile (Isolated Sept. 1967)</td>
<td>29/50</td>
<td>58</td>
</tr>
<tr>
<td>1966 Prunings-brush pile (Isolated Sept. 1966)</td>
<td>12/50</td>
<td>24</td>
</tr>
<tr>
<td>1966 Prunings-brush pile (Isolated Sept. 1966)</td>
<td>0/25</td>
<td>0</td>
</tr>
<tr>
<td>1965 Prunings-brush pile (Isolated Sept. 1966)</td>
<td>14/50</td>
<td>28</td>
</tr>
<tr>
<td>1966 Prunings-orchard floor (Isolated Sept. 1966)</td>
<td>11/50</td>
<td>22</td>
</tr>
</tbody>
</table>
Table 5. Incidence of fruit rot, decayed fruit spurs, frogeye leafspot, and percent leaf infection as influenced by variety of apple tree. (Six trees per variety) September 1967.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Fruit rot per total apples</th>
<th>Decayed fruit spurs per fruit branch</th>
<th>Number of leafspots per 100 leaves</th>
<th>% of leaves infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortland</td>
<td>4/97</td>
<td>1-3/branch</td>
<td>185</td>
<td>86</td>
</tr>
<tr>
<td>McIntosh</td>
<td>0/80</td>
<td>1-3/branch</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Red Delicious</td>
<td>0/174</td>
<td>1-3/branch</td>
<td>16</td>
<td>13</td>
</tr>
</tbody>
</table>

Table 6. The overwintering of mature and immature apple mummies on three varieties of apple trees.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Number of trees</th>
<th>Mature apple mummies per tree Fall 1967</th>
<th>Mature apple mummies per tree Spring 1968</th>
<th>Immature apple mummies per tree Fall 1967</th>
<th>Immature apple mummies per tree Spring 1968</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortland</td>
<td>19</td>
<td>11.1</td>
<td>.4</td>
<td>319</td>
<td>282</td>
</tr>
<tr>
<td>McIntosh</td>
<td>40</td>
<td>.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Red Delicious</td>
<td>7</td>
<td>3.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 8. A comparison of the size of mature and immature apple mummies.
number of frogeye leafspots per 100 leaves. This is also substantiated by the percent leaves infected which showed Cortland as having about five times more leaves infected than the other two varieties. Cortland also had the most mummified mature apples in the fall, but few were retained on the tree over winter. Cortland retained an average of 282 immature apple mummies per tree over winter. The McIntosh and Red Delicious varieties had few mummified mature apples in the fall, and none remained over winter. These two varieties also had no immature apple mummies in the fall, and so none were retained on the tree over winter.

Dead fruit spurs (1-3 per fruit branch) were present and were retained over winter equally on all three varieties. Infected fruit spurs probably produced a small amount of inoculum for McIntosh and Red Delicious varieties as revealed by the small number of leafspots in the absence of immature apple mummies. The fungus was isolated from 88% of the immature apple mummies and 46% of the dead fruit spurs examined.

The retention of immature apples (retained June drops) by the Cortland variety appears to be of primary importance in the overwintering of the organism and the subsequent varietal differences in the amount of frogeye leafspot and apple black rot observed. Murneek (47) in 1933 made a study of the nature of shedding of immature apples and described four waves of apple drops in Missouri. He gave the following factors as possibly responsible for the shedding of immature
fruit: (a) genetic constitution of the plant, (b) the physiological condition of the tree, (c) type of pollination, (d) external environmental factors, (e) diseases and insect pests. Murneek (47) also stated that distinct varietal traits are exhibited by apples in the abscission of their immature fruit. He suggested that the behavior is determined by the genetic constitution of the clone and hence is deep seated and hereditary.

Childers (19) says that horticulturists usually lump the first and second drops into the "first drop" and the third and fourth drops into the "June drop". No mention has been found in the literature of the fact that in some apple varieties the "June drops" are retained in nature on the trees as was observed by this investigator on the Cortland variety. Groves (28) in 1951 did mention the retention of immature apples (that normally dropped) on Rome trees which were sprayed with naphthalene acetic acid.

Many writers have noted the association of apple mummies with the black rot disease, but whether or not the mummies are retained immature apples or are mummified mature fruit is not mentioned. The results shown in Table 6 leave little doubt as to the importance of naturally retained immature apple mummies (June drops) on the tree and their potential for providing inoculum for primary infection in the spring. It was observed that although the average number of immature apple mummies overwintering per tree was 282, the count ranged from 55 to 477 for individual trees.
This variation was probably due to genetic variation within the Cortland variety. Thus, it is suggested that the selection or breeding of apple varieties that do not retain June drops might reduce the incidence of frogeye leafspot and apple black rot in apple orchards.

6. The Effect of Increased Inoculum

In McIntosh and Red Delicious Trees

The effect of attaching immature apple mummies from Cortland trees to the branches of McIntosh and Red Delicious trees on the quantity of frogeye leafspot is shown in Table 7. The inoculum increased the number of leafspots per 100 leaves from 25 to 108 (4 X) in the McIntosh variety, and from 10 to 93 (9 X) in the Red Delicious variety. Thus, although genetic factors might have been partly the cause of the previously observed difference between the number of frogeye leafspots in the three varieties tested, the presence in the spring of inoculum in the form of overwintering immature apple mummies definitely increases the primary infection.

7. The Infection of Immature Apples by P. obtusa

The percent infection of immature apples by P. obtusa prior to dropping from the tree is shown in Table 8. This table shows that in the Cortland variety 83% of the immature apples that would normally be "June drops", except for their retention on the tree, are infected with P. obtusa in early June prior to dropping from the tree. The McIntosh variety
Table 7. The effect on frogeye leafspot of attaching immature apple mummies from Cortland trees to the branches of McIntosh and Red Delicious trees. (Six trees per variety)

<table>
<thead>
<tr>
<th>Variety</th>
<th>Number of leafspots per 100 leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Cortland</td>
<td>185</td>
</tr>
<tr>
<td>McIntosh</td>
<td>25</td>
</tr>
<tr>
<td>Red Delicious</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 8. The infection of immature apples (June drops) by P. obtusa prior to dropping from the apple tree as influenced by variety. (Six trees per variety)

<table>
<thead>
<tr>
<th>Variety</th>
<th>Number of isolates per samples</th>
<th>% P. obtusa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortland</td>
<td>50/60</td>
<td>83</td>
</tr>
<tr>
<td>McIntosh</td>
<td>16/60</td>
<td>27</td>
</tr>
<tr>
<td>Red Delicious</td>
<td>9/60</td>
<td>15</td>
</tr>
</tbody>
</table>
is 27% infected and the Red Delicious variety is 15% infected. Thus, even before the normal "June drop" would have taken place, the immature apples in the Cortland variety are already heavily infected with _P. obtusa_, again indicating the probability of a greater source of primary inoculum in the Cortland variety than in the McIntosh and Red Delicious varieties.

8. **Number of Fruit Drops as Influenced by Single Spore Isolates and Variety**

The number of fruit drops as influenced by inoculation with single spore isolates of _P. obtusa_ and apple variety is shown in Table 9. It was found that the means of the apple drops per variety (regardless of isolate) are significantly different at the 5% level. It was also found that the means of the apple drops per isolate (regardless of variety) are not significantly different at the 5% level. There was no significant interaction between variety and isolate. The McIntosh variety had the highest number of apple drops, followed by Cortland, and Red Delicious the lowest.

The infection of apples with _P. obtusa_ greatly increased the dropping of apples as compared to the control (no drops). While the isolates varied in the rate at which they rotted the apples, no significant difference occurred in the drop count of infected apples at the 5% level.

This experiment substantiates Taylor's (63) observations in 1953, in which he found that the percent of
Table 9. Number of fruit drops as influenced by inoculation with single spore isolates of *P. obtusa* and by apple variety, summer 1967.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Source of single spore isolates</th>
<th>Control (Distilled water)</th>
<th>Means per variety*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>McIntosh canker</td>
<td>Cortland canker</td>
<td>Cortland mummy</td>
</tr>
<tr>
<td>Cortland</td>
<td>7.75</td>
<td>3.50</td>
<td>3.75</td>
</tr>
<tr>
<td>McIntosh</td>
<td>9.25</td>
<td>10.00</td>
<td>8.75</td>
</tr>
<tr>
<td>Red Delicious</td>
<td>7.25</td>
<td>1.50</td>
<td>1.75</td>
</tr>
<tr>
<td><strong>Means per isolate</strong></td>
<td><strong>8.08</strong></td>
<td><strong>5.00</strong></td>
<td><strong>4.75</strong></td>
</tr>
</tbody>
</table>

* The means of the apple drops per variety (regardless of isolate) are significantly different at the 5% level.

** The means of the apple drops per isolate (regardless of variety) are not significantly different at the 5% level, and there is no significant interaction between variety and isolate.
affected apple drops varied with the variety. He reported that about 73% Golden Delicious, 60% Red Delicious, and 30% Stayman Winesap drops showed this disease. Hence, an equally severe infection might appear to be causing more fruit rot on one variety than another. It is possible that the variety thought to be least affected, because of few visibly rotten apples on the tree, might in fact be more severely affected due to the early dropping of infected apples. As can be seen from Table 9, the McIntosh variety had the highest rate of apple drops when the apples were infected with *P. obtusa*. In the orchard, however, the Cortland variety has more visible apple rot on the trees, due to the abundance of inoculum, than does the McIntosh.

9. **Spore Release and Dissemination Experiment**

The results from this experiment are given in Tables 10, 11, 12, and 13. Stations A and B in 1967 collected little data the first months until inoculum was placed directly above them on June 12, 1967. This was attributed to the fact that the slides at these two stations were directly under the canopy of leaves and most rain was deflected from the scattered sources of inoculum. Stations C and E were not represented in table form due to the very low spore deposition present at these locations.

The number of hours of 100% humidity, and also the difference between the maximum and minimum daily temperatures were significantly correlated (5% level) with the
Table 10. Hours of 100% humidity and mean spore deposition for days when spore deposition occurred.*

<table>
<thead>
<tr>
<th>Hours of 100% Humidity per Day</th>
<th>Mean Spore Deposition May 1 - July 31, 1968</th>
<th>Station F</th>
<th>Station G</th>
<th>Combined Stations</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-6</td>
<td>18</td>
<td>-</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>7-12</td>
<td>82</td>
<td>17</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>13-18</td>
<td>373</td>
<td>158</td>
<td>265</td>
<td></td>
</tr>
<tr>
<td>19-24</td>
<td>461</td>
<td>211</td>
<td>366</td>
<td></td>
</tr>
</tbody>
</table>

* The correlation between hours of 100% humidity and the number of spores deposited is significant at the 5% level.
Table 11. Maximum-minimum daily temperature difference and mean spore deposition for days when spore deposition occurred.*

<table>
<thead>
<tr>
<th>Maximum-minimum daily temperature difference °C.</th>
<th>Station A</th>
<th>Station B</th>
<th>Station D</th>
<th>Station F</th>
<th>Station G</th>
<th>Mean for combined stations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0-2.9</td>
<td>870</td>
<td>373</td>
<td>1464</td>
<td>681</td>
<td>271</td>
<td>732</td>
</tr>
<tr>
<td>3.0-4.9</td>
<td>130</td>
<td>234</td>
<td>183</td>
<td>669</td>
<td>384</td>
<td>320</td>
</tr>
<tr>
<td>5.0-6.9</td>
<td>31</td>
<td>19</td>
<td>88</td>
<td>550</td>
<td>117</td>
<td>161</td>
</tr>
<tr>
<td>7.0-8.9</td>
<td>-</td>
<td>31</td>
<td>260</td>
<td>5</td>
<td>1</td>
<td>59</td>
</tr>
<tr>
<td>9.0-10.9</td>
<td>225</td>
<td>58</td>
<td>125</td>
<td>62</td>
<td>8</td>
<td>96</td>
</tr>
<tr>
<td>11.0-12.9</td>
<td>-</td>
<td>5</td>
<td>174</td>
<td>76</td>
<td>9</td>
<td>53</td>
</tr>
<tr>
<td>13.0-14.9</td>
<td>-</td>
<td>-</td>
<td>87</td>
<td>38</td>
<td>18</td>
<td>29</td>
</tr>
<tr>
<td>15.0-16.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>137</td>
<td>160</td>
<td>59</td>
</tr>
<tr>
<td>17.0-18.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>158</td>
<td>27</td>
<td>37</td>
</tr>
<tr>
<td>19.0-20.9</td>
<td>-</td>
<td>-</td>
<td>315</td>
<td>167</td>
<td>40</td>
<td>104</td>
</tr>
</tbody>
</table>

* The correlation between maximum-minimum daily temperature difference and the number of spores deposited is significant at the 5% level.
Table 12. Mean daily temperature and mean spore deposition for days when spore deposition occurred.*

<table>
<thead>
<tr>
<th>Mean daily temperature</th>
<th>Station A 5-1-67 to 7-31-67</th>
<th>Station B 5-1-67 to 7-31-67</th>
<th>Station D 5-1-67 to 7-31-67</th>
<th>Station F 5-1-68 to 7-31-68</th>
<th>Station G 5-1-68 to 7-31-68</th>
<th>Stations D, F, &amp; G combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0-10.9</td>
<td>-</td>
<td>-</td>
<td>848</td>
<td>409</td>
<td>286</td>
<td>514</td>
</tr>
<tr>
<td>11.0-15.9</td>
<td>1422</td>
<td>623</td>
<td>1080</td>
<td>382</td>
<td>103</td>
<td>522</td>
</tr>
<tr>
<td>16.0-20.9</td>
<td>33</td>
<td>143</td>
<td>270</td>
<td>363</td>
<td>174</td>
<td>269</td>
</tr>
<tr>
<td>21.0-25.9</td>
<td>139</td>
<td>118</td>
<td>97</td>
<td>39</td>
<td>18</td>
<td>51</td>
</tr>
</tbody>
</table>

* The correlation between mean daily temperature and the number of spores deposited is not significant at the 5% level.
Table 13. Inches of rain and mean spore deposition for days when spore deposition occurred.*

<table>
<thead>
<tr>
<th>Inches of rain</th>
<th>Station A (6-15-67 to 7-31-67)</th>
<th>Station B (6-15-67 to 7-31-67)</th>
<th>Station D (5-1-67 to 7-31-67)</th>
<th>Station F (5-1-68 to 7-31-68)</th>
<th>Station G (5-1-68 to 7-31-68)</th>
<th>Combined stations</th>
</tr>
</thead>
<tbody>
<tr>
<td>.10-.39</td>
<td>640</td>
<td>170</td>
<td>409</td>
<td>139</td>
<td>44</td>
<td>280</td>
</tr>
<tr>
<td>.40-.69</td>
<td>225</td>
<td>108</td>
<td>489</td>
<td>823</td>
<td>243</td>
<td>378</td>
</tr>
<tr>
<td>.70-.99</td>
<td>69</td>
<td>250</td>
<td>121</td>
<td>360</td>
<td>124</td>
<td>185</td>
</tr>
<tr>
<td>1.00-1.29</td>
<td>-</td>
<td>-</td>
<td>1515</td>
<td>1426</td>
<td>468</td>
<td>1136</td>
</tr>
<tr>
<td>1.30-1.59</td>
<td>329</td>
<td>588</td>
<td>98</td>
<td>411</td>
<td>1424</td>
<td>570</td>
</tr>
</tbody>
</table>

*The correlation between rainfall and the number of spores deposited is not significant at the 5% level.
number of spores released. The highest spore releases were obtained with 20 hr of 100% humidity per day and a maximum-minimum daily temperature difference of 2 C (Tables 10 and 11). While not statistically significant at the 5% level, the highest spore releases for stations D, F, and G (1000-4000 spores deposited) occurred in conjunction with the following: 1 inch of rain, 14 hr of estimated wet period, a mean daily temperature of 11.24 C, maximum daily temperature of 12.64 C, and a minimum daily temperature of 9.84 C. Rain was needed for spore release but was not significantly correlated with the number of spores released.

The highest single spore release obtained (Tables 11 and 12, Station D), had a mean daily temperature of 7.22 C (not significant at the 5% level) with a maximum-minimum temperature difference of 1.1 C. Walton's (67) data in 1920 indicate that his first large infection probably occurred at a mean temperature of 7.22 C with a maximum-minimum daily temperature difference of 1.62 C. Foster (26), in 1937 showed that frogeye leafspot infection caused by P. obtusa isolates was optimum at 20 C for a 24 hr interval. The temperature for the germination of spores and growth of germ tubes ranged from 8 - 32 C, with 23 - 27 C as most favorable. Foster (26) added, however, that when inoculated Yellow Transparent and Northwestern Greening trees were kept in the moist chamber for 48 hr at 8 C, considerable leaf infection developed. It was suggested that spore
germination may occur at 8 C, although more time is required for germ tube development, and for penetration to take place.

Anderson (3) did not consider this time-temperature relationship when he stated that the most favorable temperature for spore germination is about 23 - 27 C, while at a temperature of 15 C they germinate so slowly that infection usually failed. Hesler (32) reports that in most cases a temperature of 15.5 C is unfavorable for germination, and that below this point germination fails, as indicated by laboratory tests. Ingold (35) reported that with most fungi, a rise in temperature between the ranges of 5 C and 35 C tends to increase the rate of spore liberation.

What actually appears to occur in nature is that the greatest number of spores are released at a relatively low mean daily temperature range from 6 - 16 C (Table 12) with a very low (1.1 - 4.9 C) maximum-minimum daily temperature difference (Table 11). This means that major spore releases usually occur during relatively low daily mean temperatures that hold relatively stable for long periods of time, due in part to the presence of 100% humidity (Tables 10 and 11). Thus the humidity, besides providing moist conditions for spore release, also helps provide the long periods of stable temperature probably required for the heavy spore releases.

It is also of interest that the mean temperature for the days of major spore release varied monthly as follows: May - 9 C, June - 15 C, July - 20 C. The number of spores
released were the highest in May, followed by June and July. The largest spore release in May occurs during blossom time which has long been identified as the time of greatest primary infection (3, 5, 29, 32, 68).

Station C, located in the open 10 m from the nearest apple tree, had a very small spore deposition of 5 spores during 2 successive days of very high winds in June 1967. Station E, located 2 m from the brush pile in the open, had 37, 64, and 2 spores deposited on 3 successive days of very high winds during the same time period. These two stations show that only a very limited amount of spore dissemination by wind and wind-blown mist occurs and that this is limited to rainy periods of high wind velocity. However, the possibility of a large spore dissemination from a brush pile or the surrounding forest during an infrequent hurricane can not be discounted. This very infrequent deposition of spores, even close to a major source of inoculum such as a brush pile, could explain why Wolf (71) failed to trap P. obtusa spores in an apple orchard in 1910.

Walton (67) in 1920 used 3-day periods of infection in bagging experiments correlated with rainfall and temperature. He stated that frogeye leafspot infection is correlated usually with periods of rainfall when the temperature is sufficiently high for spore germination. By observing Walton's weather and percent infection data it was found similar to that presented in this section. His 3-day exposure of the leaves to infection was too great a period,
however, to correlate and pinpoint the factors affecting spore release.

While correlating spore release with weather data, it was discovered that spore release was 13 times greater when rains occurred at night than during the daytime. Since the number of hours of 100% humidity was significantly correlated (5% level) with the number of spores released, (Table 10) nights would logically be the period of largest spore release. Ingold (36) suggested that it must be borne in mind that each of four major factors influencing the release of spores (temperature, humidity, light, and wind) tends to exhibit a daily cycle of behavior. In the day-time light, temperature, and wind velocity is usually maximal, while humidity is usually high at night and associated with lower temperature and decreased atmospheric turbulence. Walton's (67) meteorological data in 1920 also appeared to show a similar increase in infection following rains which occurred at night.

During the fall it was observed that even with heavy rains and seemingly favorable temperatures and humidity, there were very few spores released. It was thought that possibly the pseudoperithecia and pycnidia had exhausted their supply of spores and had few left to release. Upon examination of the fruiting bodies in the laboratory it was discovered that an estimated 25% of the spores were still present in the fruiting bodies. Spore collections were
continued until October 20th in 1967; by this time all apples had been picked and stored. This failure to release spores in the fall at a time when the apple is exposed to injury and infection, during the process of picking and storage, is thought to be of critical importance. If spore release did occur at this time, black rot of stored apples would probably be a much greater problem than it is at present. The exact reason for the retention of spores in the fruiting bodies in the fall under seemingly favorable conditions for spore release is not understood. It may possibly be a physiological mechanism to preserve a quantity of spores in the fruiting bodies to insure a supply in the spring for the initial spore release. The retention of spores in the asci of \textit{Venturia inaequalis} was also reported by Miller and Waggoner (46) who stated that the appearance of ascospores in the air ceased in late spring while an estimated 25\% of the spores still remained in the asci.

Initial spore release occurred as early as March 31st in 1968 which was an early spring. The earliest spore release in 1967 was April 23rd. It was found that the first major spore release from infected apple mummies (pycnidia present) occurred one week earlier in the spring than the first major spore release from infected prunings (pseudo-perithecia present). Thus the immature apple mummies in the trees, besides being in strategic positions close to the leaves and fruit, would provide the inoculum for the primary infection of the leaves and fruit.
Ladybird beetles (Hippodamia convergens Guerin) were collected in 1968, and 18% were found to be infested with P. obtusa. These beetles are prevalent during pollination of the apple flowers, and the only way to prevent the local spore dissemination is to minimize the amount of inoculum present in and around an apple orchard, as suggested by other writers (3, 16, 23, 29, 32, 53). Thus, it was shown that spores were disseminated mainly by rain splash from inoculum sources in the tree and by at least one species of insect, and only rarely by wind-blown mist.

10. Perfect Stage Data

The photomicrographs in Fig. 9 and 10 show the pseudoperithecium of P. obtusa. These compare favorably with the camera lucida drawing by Hesler (30) made in 1913 during his study of the perfect stage of this fungus. No other photomicrographs have been found in the literature, and so these are a substantiation of Hesler's work. Aycock (11) suggested in 1949 that the sexual stage in North Carolina has a more important etiological role than is generally recognized. The observations in New Hampshire appear to corroborate this, particularly in older neglected apple orchards.

11. Frogeye Leafspot and Apple Rot Control by Fungicide Spray Treatments

The effects of fungicide treatments on frogeye leafspot are presented in Tables 14 for 1966, 15 for 1967 and 16 for 1968. In 1966 folpet was the best fungicide treatment
Figure 9. Pseudoperithecium of the fungus *Physalospora obtusa* (Schw.) Cooke (200 X).

Figure 10. Slightly flattened view of pseudoperithecium (200 X).
used. Folpet, captan, ferbam, thiram and maneb significantly reduced (5% level) frogeye leafspot in 1966. Zineb, Glyodex and Polyram were not significantly different from the control in 1966. These last three fungicides were dropped from the test after 1966, partly due to their poor showing against frogeye leafspot and partly due to their failure to reduce fruit rot (Table 17). In 1967 folpet again was the most effective material tested. Folpet, captan, maneb, thiram and ferbam all significantly reduced (5% level) frogeye leafspot in 1967. In 1968 phenyl mercuric acetate and Difolatan were added to the list of fungicides tested. Phenyl mercuric acetate was the most effective material tested in 1968. Phenyl mercuric acetate, maneb, folpet, captan, Difolatan, thiram and ferbam all significantly reduced frogeye leafspot in 1968.

Some of the five materials tested for the 3-year period were consistently better than others, although all these materials significantly reduced frogeye leafspot. Folpet and captan placed first and second in effectiveness in 1966 and 1967 and second and third in effectiveness in 1968. Maneb placed fifth in 1966, third in 1967 and first in 1968. Thiram placed fourth all three years. Ferbam placed third in 1966, and fifth in 1967 and 1968.

The effect of fungicide treatments on fruit rot is shown in Table 17 for 1966, 1967 and 1968. Glyodex, zineb and Polyram were discontinued after the first year of study, all three permitting over 3% fruit rot. In 1968 phenyl
Table 14. Effect of fungicide treatments on frogeye leafspot, 1966. (Trees sprayed once a week with Cyprex to suppress apple scab.)

<table>
<thead>
<tr>
<th>Treatment (Commercial formulation lbs. per 100 gal.)</th>
<th>Number of leafspots per 100 leaves*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 lb. Folpet</td>
<td>18 a</td>
</tr>
<tr>
<td>2 lb. Captan</td>
<td>31 a</td>
</tr>
<tr>
<td>2 lb. Ferbam</td>
<td>50 abc</td>
</tr>
<tr>
<td>2 lb. Thiram</td>
<td>64 abc</td>
</tr>
<tr>
<td>2 lb. Maneb</td>
<td>76 abc</td>
</tr>
<tr>
<td>2 lb. Zineb</td>
<td>80 abcd</td>
</tr>
<tr>
<td>½ lb. Glyodex</td>
<td>95 bcd</td>
</tr>
<tr>
<td>2 lb. Polyram</td>
<td>103 cd</td>
</tr>
<tr>
<td>Control</td>
<td>135 d</td>
</tr>
</tbody>
</table>

♦Means within a column not followed by the same letter(s) are significantly different at the 5% level according to Duncan's new multiple range test.
Table 15. Effect of fungicide treatments on frogeye leaf-spot, 1967. (Trees sprayed once a week with Cyprex to suppress apple scab.)

<table>
<thead>
<tr>
<th>Treatment (Commercial formulation lbs. per 100 gal.)</th>
<th>Number of leafspots per 100 leaves*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 lb. Folpet</td>
<td>23  a</td>
</tr>
<tr>
<td>2 lb. Captan</td>
<td>39  ab</td>
</tr>
<tr>
<td>2 lb. Maneb</td>
<td>46  ab</td>
</tr>
<tr>
<td>2 lb. Thiram</td>
<td>89  bc</td>
</tr>
<tr>
<td>2 lb. Ferbam</td>
<td>124 bc</td>
</tr>
<tr>
<td>Control</td>
<td>185  d</td>
</tr>
</tbody>
</table>

*Means within a column not followed by the same letter(s) are significantly different at the 5% level according to Duncan's new multiple range test.
Table 16. Effect of fungicide treatments on frogeye leaf-spot, 1968. (Trees sprayed once a week with Cyprex to suppress apple scab.)

<table>
<thead>
<tr>
<th>Treatment (Commercial formulation lbs. per 100 gal.,)</th>
<th>Number of leafspots per 100 leaves*</th>
</tr>
</thead>
<tbody>
<tr>
<td>.25 lb. Phenyl mercuric acetate</td>
<td>14  a</td>
</tr>
<tr>
<td>2 lb. Maneb</td>
<td>18  a</td>
</tr>
<tr>
<td>2 lb. Folpet</td>
<td>22  ab</td>
</tr>
<tr>
<td>2 lb. Captan</td>
<td>25  ab</td>
</tr>
<tr>
<td>2 lb. Difolatan</td>
<td>39  bc</td>
</tr>
<tr>
<td>2 lb. Thiram</td>
<td>46  bc</td>
</tr>
<tr>
<td>2 lb. Ferbam</td>
<td>64  bc</td>
</tr>
<tr>
<td>Control</td>
<td>142  d</td>
</tr>
</tbody>
</table>

*Means within a column not followed by the same letter(s) are significantly different at the 5% level according to Duncan's new multiple range test.
Table 17. Effect of fungicide treatments on fruit rot. (Trees sprayed once a week with Cyprex to suppress apple scab.)

<table>
<thead>
<tr>
<th>Treatment (Commercial formulation lbs. per 100 gal.)</th>
<th>1966</th>
<th>1967</th>
<th>1968</th>
<th>3 year mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 lb. Folpet</td>
<td>.19</td>
<td>1.52</td>
<td>.72</td>
<td>.81</td>
</tr>
<tr>
<td>2 lb. Ferbam</td>
<td>1.84</td>
<td>.91</td>
<td>1.06</td>
<td>1.27</td>
</tr>
<tr>
<td>2 lb. Captan</td>
<td>.18</td>
<td>1.67</td>
<td>3.44</td>
<td>1.76</td>
</tr>
<tr>
<td>2 lb. Thiram</td>
<td>3.66</td>
<td>0</td>
<td>2.08</td>
<td>1.91</td>
</tr>
<tr>
<td>2 lb. Maneb</td>
<td>3.34</td>
<td>1.67</td>
<td>3.55</td>
<td>2.85</td>
</tr>
<tr>
<td>2 lb. Zineb</td>
<td>5.64</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2 lb. Polyram</td>
<td>3.32</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>.5 lb. Glyodex</td>
<td>3.53</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>.8 lb. Difolatan</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>.25 lb. Phenyl mercuric acetate</td>
<td>-</td>
<td>-</td>
<td>2.13</td>
<td>-</td>
</tr>
<tr>
<td>Control</td>
<td>3.61</td>
<td>4.12</td>
<td>2.89</td>
<td>3.54</td>
</tr>
</tbody>
</table>
mercuric acetate and Difolatan were tested. Phenyl mercuric acetate had 2.13% fruit rot, and Difolatan controlled the fruit rot completely during this 1-year test. Considering the five materials tested over the entire 3-year period, the order of effectiveness in controlling fruit rot caused by *P. obtusa* is: folpet, ferbam, captan, thiram and maneb. All these materials, except maneb, had less than 2% fruit rot while the control had a mean of 3.54% fruit rot for the 3-year period.

Thus, in both the frogeye leafspot and the fruit rot tests it can be concluded that over the 3-year period folpet and captan were two of the consistently best materials used. All five materials (folpet, captan, ferbam, maneb and thiram) used for the 3-year period significantly controlled the frogeye leafspot and appeared to control the fruit rot to a certain degree. Of the materials evaluated in 1958, Difolatan appeared promising for further tests as it significantly reduced both the frogeye leafspot and the fruit rot. While phenyl mercuric acetate significantly controlled frogeye leafspot and slightly controlled fruit rot, it is considered to be more of academic than pragmatic interest.

The recommendations of many writers are thus confirmed in that folpet (9, 24, 65), captan (4, 9, 56, 64, 65, 66), maneb (33), thiram (9, 10) and ferbam (4, 9, 42) have been found to significantly control frogeye leafspot. Zineb (4, 9, 33, 51) was not confirmed as a good control
fungicide in the 1 year it was tested. Thus the comment by one writer (7) that none of the present day fungicides appears very effective against black rot should be modified.

12. Imbibition Experiment

The results for this experiment are shown in Tables 18 and 19. In Table 18 the results of all four plant parts analyzed (decayed fruit spurs, immature apple mummies, old cankered limbs and new cankered limbs) were combined for each treatment. The control (distilled water) showed the greatest imbibition rate as expressed by the percent increase in weight of the sample over 2 hr periods. The fungicidal material that exhibited the highest imbibition rate was phenyl mercuric acetate. The material that exhibited the slowest imbibition rate was folpet. This appeared to indicate that phenyl mercuric acetate would be imbibed more readily than the other materials into the plant parts known to be infected with P. obtusa.

In Table 19 the imbibition rates of the different parts of the apple tree tested were measured for the control (distilled water). The highest imbibition rates occurred during the first 2 hr of soaking. Decayed fruit spurs exhibited the highest percent increase in weight. Immature apple mummies showed the next highest value, followed by old cankered limbs. New cankered limbs exhibited the lowest percent increase in weight of the four plant parts tested. Thus, it can be concluded that old fruit spurs and immature
Table 18. Imbibition rates of fungicides by combined plant parts. (Five samples each of: decayed fruit spurs, immature apple mummies, old cankered limbs, and new cankered limbs.)

<table>
<thead>
<tr>
<th>Materials</th>
<th>% Increase in weight of samples by hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 hr.</td>
</tr>
<tr>
<td>Folpet</td>
<td>53</td>
</tr>
<tr>
<td>Ferbam</td>
<td>58</td>
</tr>
<tr>
<td>Thiram</td>
<td>57</td>
</tr>
<tr>
<td>Difolatan</td>
<td>43</td>
</tr>
<tr>
<td>Maneb</td>
<td>52</td>
</tr>
<tr>
<td>Captan</td>
<td>59</td>
</tr>
<tr>
<td>Phenyl mercuric acetate</td>
<td>67</td>
</tr>
<tr>
<td>Control</td>
<td>74</td>
</tr>
</tbody>
</table>

Table 19. Imbibition rates of parts of the apple tree. (Water controls only, five samples per control.)

<table>
<thead>
<tr>
<th>Plant part</th>
<th>% Increase in weight of samples by hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 hr.</td>
</tr>
<tr>
<td>Decayed fruit spurs</td>
<td>145</td>
</tr>
<tr>
<td>Immature apple mummies</td>
<td>74</td>
</tr>
<tr>
<td>Old cankered limbs</td>
<td>44</td>
</tr>
<tr>
<td>New cankered limbs</td>
<td>30</td>
</tr>
</tbody>
</table>
apple mummies (retained June drops) would imbibe and hold water rapidly in the same way as a sponge while cankered limbs would require a longer period of wetting to imbibe the same amount of moisture (percent increase in weight). It then could be expected that decayed fruit spurs and immature apple mummies would provide a good medium as regards moisture supply for the growth and fruiting of *P. obtusa*.

13. **Experiment on the Initial Inhibition of Growth of *P. obtusa* in Vitro**

The results of soaking various plant parts for 48 hr in seven different fungicidal solutions, and the subsequent initial inhibition of growth on potato dextrose agar are shown in Table 20. The plant parts were those used in the preceding experiment: decayed fruit spurs, immature apple mummies, old cankered limbs and new cankered limbs. The initial inhibition is the suppression of growth from the plant parts on potato dextrose agar as expressed in the number of days it was completely inhibited during a total of 17 days. The control (distilled water) showed the least initial growth inhibition. Phenyl mercuric acetate showed the greatest initial inhibition by inhibiting growth during the entire 17 days of the test.

Difolatan, folpet, mane, captan, ferbam and thiram, in that order, showed a decreasing ability to inhibit
Table 20. Initial inhibition of the growth of *P. obtusa* from various parts of the apple tree. (Number of days inhibited when inoculated on potato dextrose agar.

<table>
<thead>
<tr>
<th>Material</th>
<th>20 Samples combined plant parts</th>
<th>5 Samples decayed fruit spurs</th>
<th>5 Samples immature apple mummies</th>
<th>5 Samples old cankered limbs</th>
<th>5 Samples new cankered limbs</th>
<th><em>P. obtusa</em> growths per 20 combined samples*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>18/20</td>
</tr>
<tr>
<td>Thiram</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>15/20</td>
</tr>
<tr>
<td>Ferbam</td>
<td>0</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>14/20</td>
</tr>
<tr>
<td>Captan</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>13/20</td>
</tr>
<tr>
<td>Maneb</td>
<td>3</td>
<td>5</td>
<td>8</td>
<td>3</td>
<td>4</td>
<td>12/20</td>
</tr>
<tr>
<td>Folpet</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>17</td>
<td>10/20</td>
</tr>
<tr>
<td>Difolatan</td>
<td>6</td>
<td>17</td>
<td>6</td>
<td>7</td>
<td>17</td>
<td>9/20</td>
</tr>
<tr>
<td>Phenyl mercuric acetate</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>0/20</td>
</tr>
</tbody>
</table>

* The number of *P. obtusa* growth identified per 20 combined samples at the end of the experiment (17 days).
initial growth of _P. obtusa_ from the samples. There was variation in the inhibition of initial growth between various plant parts for the same treatment. New cankered limbs had the greatest initial inhibition of the four plant parts. On the basis of this experiment it would appear likely that phenyl mercuric acetate would probably give the best results in a field trial on the inhibition of canker growth caused by _P. obtusa_.

14. **Limb Canker Control**

The results from this experiment are given in Table 21. Treatment of the cankered limbs with phenyl mercuric acetate inhibited the mean canker growth to approximately one-third (11.02 cm), while the mean canker growth exhibited by the untreated limbs was 34.59 cm. Not only was the mean canker growth inhibited by the treatment, but also the number of cankers with no elongation (12 cankers out of 23) was double that of the untreated limbs (6 cankers out of 23). In both the treated and the untreated cankered limbs the greatest canker growth developed from early July to the middle of August during a very dry period. This finding seems to agree with the work of Palmiter (49) on pruning where he reports that all flush cuts made in March, April, May and June showed good callus formation by September. Flush cuts made in July showed little callus formation by November. Landis (40) also reports that all
Table 21. The inhibition of black rot canker growth by treatment with Phenyl mercuric acetate (Phix) at the end of the dormant period 1968. (McIntosh variety: 23 limbs treated, 23 limbs control.)

<table>
<thead>
<tr>
<th></th>
<th>Treated with Phix</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7-4-68</td>
<td>8-17-68</td>
</tr>
<tr>
<td>2.54</td>
<td>2.54</td>
<td>2.54</td>
</tr>
<tr>
<td>.64</td>
<td>.64</td>
<td>.64</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.27</td>
<td>1.91</td>
<td>1.91</td>
</tr>
<tr>
<td>12.70</td>
<td>14.61</td>
<td>14.61</td>
</tr>
<tr>
<td>0</td>
<td>121.92</td>
<td>132.08</td>
</tr>
</tbody>
</table>
Table 21. (cont.)

Increase in the length of canker growth in centimeters after March 14, 1968

<table>
<thead>
<tr>
<th>Treated with Phix</th>
<th>Control</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-4-68 8-17-68 9-19-68</td>
<td>7-4-68 8-17-68 9-19-68</td>
<td></td>
</tr>
<tr>
<td>0 0 0</td>
<td>68.58 76.20 76.20</td>
<td></td>
</tr>
<tr>
<td>0 55.88 55.88</td>
<td>1.27 1.27 1.27</td>
<td></td>
</tr>
<tr>
<td>3.81 4.45 5.08</td>
<td>0 0 0</td>
<td></td>
</tr>
<tr>
<td>3.18 20.32 20.32</td>
<td>4.45 116.84 208.28</td>
<td></td>
</tr>
<tr>
<td>0.64 0.64 1.27</td>
<td>5.08 22.86 22.86</td>
<td></td>
</tr>
<tr>
<td>0 0 0</td>
<td>19.05 111.76 116.84</td>
<td></td>
</tr>
<tr>
<td>15.24 17.15 17.78</td>
<td>3.18 19.05 19.05</td>
<td></td>
</tr>
<tr>
<td>0 0 0</td>
<td>.64 93.98 93.98</td>
<td></td>
</tr>
<tr>
<td>0 0 0</td>
<td>0 1.27 1.27</td>
<td></td>
</tr>
<tr>
<td>1.27 1.27 1.27</td>
<td>52.07 78.74 78.74</td>
<td></td>
</tr>
<tr>
<td>0 0 0</td>
<td>3.81 3.81 3.81</td>
<td></td>
</tr>
</tbody>
</table>

Mean 1.80 10.49 11.02 6.99 30.15 34.59
attempts to inoculate ornamental crabapples with \textit{P. obtusa} failed until the trees were subjected to water stress, after which cankers were produced.

Thus, it seems logical to conclude on the basis of the findings of this experiment, and those of other researchers, that the limb canker problem in New Hampshire was correlated with a long drought period. This drought period probably subjected the trees to water stress and opened the way for the rapid development of cankers. It can also be concluded that phenyl mercuric acetate reduces canker elongation by two-thirds when applied as a slurry during the late dormant period (early March). While perhaps a slurry would not be completely economical, perhaps a concentrated spray with phenyl mercuric acetate in early March would accomplish the same objective more economically. Further experimentation is needed to determine the best time of application. A time prior to bloom appears logical due to present recommendations for use of this material in apple scab control (9).
SECTION V

SUMMARY

The objectives of this investigation were to elucidate some of the factors affecting the etiology and control of black rot of apple caused by Physalospora obtusa. Factors which were previously misunderstood, merely surmised, or of a controversial nature were studied. The main problems from this disease in New Hampshire are the fruit rot and canker ing of apple limbs by the black rot fungus. There is also a lack of understanding of the factors affecting the dissemination of P. obtusa. The Cortland variety is very susceptible to the frogeye leafspot and fruit rot form of the disease.

Frogeye leafspot appeared on apple leaves approximately 19 days after the first large spore releases. The inoculum came mainly from the infected immature apple mummies and decayed fruit spurs. Isolation of the fungus from various plant parts showed that these two plant parts had a high percentage of infection. Observations and studies on overwintering showed that infected immature apple mummies (retained June drops) and a few infected mature apple mummies were retained over winter on the Cortland trees.

The incidence of frogeye leafspot on the McIntosh and Red Delicious varieties was related to the amount of inoculum available. The retention of infected June drops by the
Cortland variety appears to be of primary importance in the overwintering of the organism. The Cortland variety was found to have more immature apples infected with *P. obtusa* prior to the normal time of dropping from the tree than either McIntosh or Red Delicious.

The inoculation of apples on three varieties of trees with cultures from single spore isolates of *P. obtusa* correlated positively with the number of fruit drops. The number of apple drops was affected significantly by the variety of tree. The number of apple drops was not influenced significantly by the source of single spore isolates, but all isolates increased the number of drops over the controls.

The controversy over wind dissemination of spores was partially resolved with the discovery that dissemination is mainly by rain-splash because wind-blown spores were found only rarely in spore traps during a normal season. Dissemination of spores by the ladybird beetle (*Hippodamia convergens* Guerin) was established. The main climatological factors affecting the release of *P. obtusa* spores were found to be the number of hours of 100% humidity and the maximum-minimum daily temperature difference during a 24-hr period. Most of the spore releases were found to occur between 6 and 16 C. Pycnidia released a large number of spores one week earlier than pseudoperithecia in the spring. Fruiting bodies (pycnidia and pseudoperithecia) also retained an estimated 25% of their spores in the fall.
Over a 3-year period folpet, captan, maneby thiram and ferbam significantly reduced frogeye leafspot (in a descending order of effectiveness) and gave partial control of fruit rot. Folpet and captan gave consistently good control of both frogeye leafspot and apple rot. Difolatan, which was used only in 1968, appeared promising for further tests as it significantly reduced frogeye leafspot and completely controlled apple rot.

A survey of cankered limbs in neglected orchards revealed that over 66% of the cankers were infected with *P. obtusa* whereas commercial orchards varied from a trace to 33% infection. *P. obtusa* was found parasitically or saprophytically on 82% of 100 marked cankers and probably was the cause of at least 33% of the cankers. Laboratory experiments showed that, of the fungicides that inhibited the initial growth from inoculum sources on potato dextrose agar, phenyl mercuric acetate was the most effective material employed.

In 1968, field tests showed that phenyl mercuric acetate reduced canker elongation by 66% as compared to the control. The period of greatest canker elongation was from early July to the middle of August. This coincided with a dry period suggesting that moisture stress may be responsible for the increase of canker growth. This has also been suggested by the work of other writers. Phenyl mercuric acetate should be evaluated further as a method of reducing black rot cankers.
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