STUDIES ON THE YOUNG FRUIT-ROT OF APPLE-TREE

BY

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(With 7 Plates)

Contents

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INTRODUCTION

The young fruit-rot as well as the blossom-blight is one of the most serious diseases of apple-tree in our country. Several cases of its epidemic outbreaks have been reported during the past four decades from the north-eastern parts of Honshu and from Hokkaido. The epidemics which happened recently (in 1931 and in 1933) are thought indeed to be the most disastrous considering the large acreage damaged.

Although a great number of researches concerning the *Monilia*-diseases of apple and other fruit-trees have hitherto been published, they have leaned toward the mycological field of the study or, in other words, have rather neglected the phytopathological considerations, particularly in respect to prophylaxis.

It is self-evident that the principle of the prevention of parasitic diseases shall be established by clearing up the life history and physiological characters of the causal fungus and the host-parasite relations especially such as the mode of infection and varietal resistance of the host-plant. Notwithstanding that the *Monilia*-diseases are common through the pomaceous

and drupaceous fruit-trees, and their occurrence is extended over various organs of these host-plants, rather a few researches on the mode of infection of the fungus have been published except by WORONIN (49) on Vaccinia-berries and by SCHELLENBERG on quince (106) and medlar (108). Besides them WORMALD (142, 144, 145, 146 and, 147) published many valuable works on *Monilia*, having his interest focused upon the comparison of species or strains of the fungi. He stated in one of his papers (144) that *Monilia*-fungus ceases the development of the blossom or checks the pollen-tubes growing into the style, but he did not show how it occurs. Investigators in our country have studied mostly on the blossom-blight of apple-tree and their opinions concerning the mode of infection seem to have been inconsistent.

Although the application of Bordeaux-mixture has been recommended as the most effective method for the control of these diseases, but it has not necessarily been so in all cases. The results for many years have shown that it is only effective for the blossom-blight, but not for the young fruit-rot no matter what kind of fungicide may be used. It is greatly interesting from the phytopathological point of view to study the reason why spraying is only effective for the blossom-blight, but not for the young fruit-rot, in spite of the same causal fungus, and also it is greatly important to find out the effective controlling measures for the latter case of the disease. This is a motive actuating the present author in undertaking this research work. The present thesis deals whth some characteristics of the *Monilia*-diseases and their mode of infection, especially stigma-infection as well as an anatomical survey of the relation between pollination and infection. In particular, the relation between the results of this research work and practical observation has been considered from the standpoint of apple growing for the purpose of throwing some light upon the control measure.

The field observations and experiments were done at the Aomori Agricultural Experiment Station in Aomori Province and the University orchard in Sapporo since 1924, and the anatomical studies have been executed in the laboratory of the Department of Horticulture, the Hokkaido Imperial University.

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I. HISTORICAL REVIEW

Among the *Monilia*-diseases of fruit-trees, the Brown Rot of ripe fruits has been known in Europe and America for a considerably long time, It was, however, at the end of the 19th century that mycologists and pathologists called attention to the disease since it seriously attacked blossoms and young shoots of cherry trees in the Continent. Consequently a good many researches have been published of which 170 have been enumerated by WORMALD (144).

The first recorder PERSOON (96) discoverd *Monilia* spores on *Pirus communis*, *Prunus domestica*, and *Amygdalus Persica*, and named the fungus *Torula fructigena*. In 1801 he established the genus *Monilia* and named it *Monilia fructigena*. EHRENBERG (34) found a form on the almond tree and named it *Oidium laxum*. BONORDEN (15) described *Monilia cinerea* in 1851. SCHRÖTER (108) anticipated that *Monilia fructigena* must be the conidial stage of *Sclerotinia fructigena*. WORONIN (151, 152) claimed in his noted papers that *Monilia fructigena* can be distinguished from *Monilia cinerea* clearly and that the former attacks the pomes whereas the latter the drupes. SCHELLENBERG (105) described *Sclerotinia Cydoniae* which attacks the quince tree. NORTON (91) proved SCHRÖTER's anticipation by finding apothecia on peaches and pears. ADERHOLD (2) also succeeded in obtaining apothecia of *Monilia fructigena* and *Monilia cinerea*, and pointed out that *Sclerotinia fructigena* (PERS.) SCHRÖTER of NORTON must be *Sclerotinia cinerea* (BON.) SCHRÖTER newly describing the latter. After that he and RUHLAND (4) described *Sclerotinia laxa* (EHRENBERG) ADERHOLD and RUHLAND by the discovery of the apothecia, and here three different species of *Sclerotinia* which attack fruit-trees have been established. Although there were many researches or contentions about the taxonomy of the fungi published up to the present time, it is not necessary to go over them again since former writers have already reviewed them repeatedly. But, since the reports which concern the pathology of *Monilia*-disease on leaves, blossoms, shoots or young fruits especially the stigma-infection, have close connection to the present study, the following citations may be worth while.

In Europe, von THÜMEN (129, 131) for the first time has pointed out

the economical importance of *Monilia* which attacks not only fruits but also blossoms and shoots. HALLIER (47), too, stated this, and thereafter many reports concerning the blossom-blight of cherries and pomes have been published by various authors as follows:—

SORAUER (117, cherry), DRATEN (32, cherry), WITMACK (140, cherry), SORAUER (118, cherry), WITMACK and FRANK (141, cherry), WORONIN (153, cherry), ADERHOLD (1, cherry and pear), WEHMER (135, cherry), FRANK (41, cherry, almond and apple), BEHRENS (13, apple and pear), WORONIN (151, cherry and apple), SCHELLENBERG (106, quince), FRANK and KRÜGER (42, cherry), MÜLLER-THURGAU (85, apple), SORAUER (120, apple), WORONIN (152, apple and cherry), WEISS (136, pear and apple), MOLLIARD (83, almond), ADERHOLD (2, apple), OSTERWALDER (93, quince), SCHELLENBERG (106, medlar), EWERT (35, cherry, apple etc.), VOGES (133, cherry, apple), SORAUER (122, cherry), BROZ (19, fruit-trees), ERIKSSON (37, cherry, apple), CHIFFLOT (23, apricot), ANON (5, pear).

In England there is a note of the apple blossom-blight in the textbook of MASSEE (76). SALMON (103, 104) reported that blossoms or stems of plum, cherry, peach, pear, apple and medlar are damaged by the fungus: ever since then the *Monilia*-disease has been noticed as one of the important apple diseases in that country, especially in Kent. WORMALD (146) has turned his attention to the so-called biologic forms and collected many strains of *Sclerotinia fructigena* and *Sclerotinia cinerea* from several parts of the world. Those strains were compared with each other in pure cultures and by inoculations. He (142) reported that the fungus which attacks apple-stems produces grey acervuli, and it seems to be *Monilia cinerea* BON. being distinguished apparently from *Monilia fructigena*. WORMALD (143) studied 'Wither-Tip' of plum and stated that there exist two biologic forms of *Monilia cinerea*, i.e., *forma Mali* and *forma Pruni*, and also reconized *Monilia americana*, an American strain distinguishing from European strains owing to their different characters. He (146) again compared several *Monilia* strains which had been collected from the European Continent, United States of America, Canada, Australia Japan and Manchuria conculded that *Sclerotinia fructigena* does not exist in the United States, Australia and New Zealand; that *Sclerotinia americana* is not found in Europe except one example from Holland; that there is not reason to distinguish *Monilia laxa* of Manchuria from *Monilia cinerea*; that *Monilia Kenjiana* is quite different from any other strains. In 1930 WORMALD (147) further observed that *Sclerotinia cinerea* and its *forma Pruni* obtained in cherries can attack pear-blossoms and a strain obtained on pear

can attack apple blossoms. He concluded that whether a strain parasitic on pears is an intermediate type between *f. Pruni* and *f. Mali* or whether *f. Pruni* not only able to attack pear-blossoms but also apple-blossoms under favorable conditions must be left as a problem for future solution. In Ireland, BOYLE, MURPHY and CUMMINS (16) have proved the result which had been obtained by WORMALD (144), namely, that *Monilia cinerea* obtained from plums can attack apples and give rise to a typical blossom-wilt.

In North America, ARTHUR (6) recorded blossom-blight of cherries for the first time, and since E. F. SMITH (116) alarmed the grower by stating that *Monilia fructigena* causes peach blossom-blight resulting in serious damage, a number of researches have been published by many authors as follows:—

GALLOWAY (43), HUMPHREY (60, 61), and E. F. SMITH (116) on cherry, peach and other fruit-trees, CHESTER (22) on peach, TAFT (1894) on peach and plum, HALSTEAD (48) on pear, BAILEY (8) on peach, GOFF (45) on plum, STURGIS (123) on peach, CLINTON (24) on plum and cherry, JEHLE (63) on peach, QUAINTANCE (99) and WAUGH (134) on plum, BARSS (10) on drupes and pear, FANT (39) on peach and RUDOLF (102) on apricot.

Most of these studies were related to control measures but as for the causal fungus most workers seem to have referred it to *Monilia fructigena*. Even NORTON himeself who discovered the apothecia of *Monilia cinerea*, the causal fungus of Blossom-Blight in 1902 recognized it incorrectly as *Sclerotinia fructigena*. Thereafter MATHENY (78), COOLEY (27), BROOKS and FISHER (17) and BARTRAM (11) accepted *Monilia cinerea* BON. of Europe. ROBERTS (100) claimed that there is a type which would attack only blossoms and shoots of the drupes, giving it the name *Monilia oregoniensis* BARSS et POSEY. NORTON and EZEKIEL (92) recognized *Monilia cinerea forma americana* of WORMALD (145) and proposed the name *Sclerotinia americana* (WORMALD) *com. nov.*. EZEKIEL (38) stated that formation of the species is genetical, and the characteristics can not be changed by environment or continual culturing, suggesting that differences may be produced through some hyphal anastomosis. ROBERTS and DUNEGAN (101) claimed that the Brown-Rot fungus which generally attacks drupes and pomes must be *Sclerotinia fructicola* (WINT.) REHM. but not *Sclerotinia americana* of NORTON and EZEKIEL. They concluded that *Sclerotinia cinerea* (BON.) SCHRÖTER is a pacific type of Brown-Rot and *Sclerotinia fructigena* (PERS.) SCHRÖTER is not known in the United States.

Monilia-blights of blossoms or shoots of various plants other than fruit-trees also have been reported by a number of workers (148, 149,

150, 115).

The first record of blossom-blight of apples caused by *Monilia* in our country was published by SENGOKU (109). He stated: "This fungus (*Monilia fructigena* PERS.) has never been found in our country, but this year in Sapporo I found the proper disease of apple caused by the fungus; this fungus attacks not only fruits but also blossoms and leaves." HANZAWA (50) reported that the apple blossom-blight in Hirosaki districts is nothing but the *Monilia* disease, stating that the blight of blossoms is a characteristic thereof. Studying the symptom and morphology of the causal fungus he concluded that the fungus is *Monilia fructigena* PERS. and discussed its parasitism, control measures, etc. SHIRAI (115) stated in his book that the fungus (*Monilia fructigena*) kills the shoots penetrating into them from fruits. YAMADA (155) stated in his book: "About May or June blossoms of the apple are killed giving rise to the so-called 'blossom-blight' disease. This is also caused by *Monilia fructigena* PERS." P. HENNING (55) described a new species of *Monilia* which had been found on cherry trees in Tokyo and sent to him by KUSANO. He named it *Monilia Kusanoi* P. HENN.. HANZAWA (50) again said about *Monilia* diseases of fruit-trees that the apple blossom-blight is certainly caused by *Sclerotinia fructigena* (PERS.) SCHRÖTER. Further he claimed that the apple blossom-blight, as well as the Brown-Rot of ripe fruits, are caused by *Monilia fructigena* PERS. and *Monilia cinerea* BON. which attack cherries, pears and Mume-plant are identical with *Monilia Kusanoi* P. HENN.

TAKAHASHI (125) observed that 'Brown-Rot' is caused by *Monilia fructigena*, but 'blossom-blight' by *Monilia cinerea*. Further he (126) compared the mode of infection in the case of the causal fungus of blossom-blight to that of several *Monilia* diseases of rosaceous plants. KASAI (54) concluded that the blossom-blight is caused by the conidial stage of *Sclerotinia Kusanoi* P. HENN having investigated 20 diseased orchards in the suburbs of Sapporo, and performed a mycological survey. MIYABE (80) stated that the causal fungus of the apple blossom-blight is either *Monilia Kusanoi* or one closely related to. TAKAHASHI (125) gave a name *Sclerotinia Mali* TAK. for the causal fungus of the apple blossom-blight, describing it in detail. He pointed out in the discussion of the young fruit-rot that there are various symptoms which would be caused by the single species. HORI (57) described *Sclerotinia phaeospora* as a new species on account of its colored ascospore which he had obtained on the apple blossom-blight in Nagano Prefecture, and soon after he (105) established a new genus *Phacosclerotinia* and named this fungus *Phaeosclerotinia nip-*

ponica HORI. BOKURA (14) reported about an outbreak of the apple blossom-blight in Akita and Aomori. MIURA (79) investigated the apple blossom-blight in Aomori in detail and described *Sclerotinia malicola* M. MIURA, having found that the stalk length of apothecia, diametre of apothecial disc and shape and size of ascospores as well as conidia are different from those of other species. The writer (111) divided the *Monilia* diseases of apple into four classes in convenience according to their various symptoms. He (113) also reported the relation between the climatic condition and the occurrence of apothecia of the apple young fruit-rot fungus, giving information as to the control measure. Further he (114) discussed the relation between low temperature and the outbreak of the disease.

Above is the outline of the history of researches concerning the *Monilia* diseases of blossoms, fruits, leaves and shoots of fruit-trees. Now then the preceding studies concerning the stigma-infection of *Monilia* fungus will be reviewed fully because they have the most intimate connection with the present study.

WORONIN (148) who first proved experimentally the stigma-infection of the *Monilia* fungus reported that *Sclerotinia Padi* WORON. infects blossoms of *Prunus Padus* and *Prunus Cerasus* via stigma, and *Sclerotinia Aucupariae* LUDW. does the same on *Sorbus aucuparia*. He (144) also observed this fact in the *Monilia*-disease of cranberries and traced the behaviour of the hyphae invaded the stigma. MAUL (73) reported the stigma-infection of *Sclerotinia Alni* MAUL in *Alnus incana*. ADERHOLD (2) inoculated the *Monilia* spores on the stigma of cherries and observed that the fungus invaded the pistil and ovary killing the latter readily without causing mummification of the fruits, and infected further the flower stalk or the spur. BEHRENS (13) reported that in an inoculation experiment of conidia of *Oidium fructigenum* LK. (*Monilia fructigena* PERS.) on the stigma of apples and plums, one part of the inoculated blossoms dropped without fertilization, while the others normally developed into young fruits. WEHMER (135) suspected a natural occurrence of the stigma-infection of *Monilia fructigena* in sour cherries, though it might be possible artificially, quoting the results of BEHRENS. WORONIN (152) again observed the stigma-infection of *Monilia fructigena* and *Monilia cinerea* on the pomes and the drupes respectively. SCHELLENBERG (106) confirmed the fact that spores of *Sclerotinia Cydoniae* SCHELL. (*S. Lynhartiana* PRILL. et DELAC.) infect the blossoms of quince via stigma. He published some valuable results proving that the path of the hyphae in the pistil is just the same as the pollen-tubes reaching the ovule at last. Further, SCHELLENBERG

(107) reached the same conclusion in the case of *Sclerotinia Mespili* and *Ariae* which attack medlar and European mountain-ash. FRANK and KRÜGER (42) recognized in inoculation experiments that *Monilia* spores obtained from ripe fruits of plums and pears were able to infect the pistil via stigma in these fruit-trees, but they opposed the opinion of ADERHOLD who regarded stigma as the only entrance of the fungus, though he had observed browning of the infected tissues of style in wihch even mycelia developed, concluding that any parts above ground which have been grown in the current spring or summer should be affected. WORONIN (152) again stated his previous theory and concluded that in cherries *Monilia cinerea* is infectious via stigma, but not *Monilia fructigeua*, while in apples the matter is quite contrary. OSTERWALDER (93) reported that the germ-tubes of *Monilia fructigena* penetrate into the stigma of *Cydonia japonica* resulting in blossom- and stem-blight. MAGNUS (75) reported the stigma-infection of *Sclerotinia Crataegi* P. MAG. in blossom-blight of *Crataegus oxycantha*. ADERHOLD and RUHLAND (4) reported the stigma-infection of *Sclerotinia fructigena* in apples or pears resulting rot of the whole blossom-organs and even stalks with a rich production of conidia, but they concluded that *Sclerotinia cinerea* is less infectious for pomes though such is not the case as WORONIN denied the parasitism of the latter fungus on these fruit-trees. EWERT (35) observed the wintering of spores of *Sclerotinia cinerea* obtained from sweet cherries and the browning of pistils or stalks by inoculating them on the stigma. VOGES (133) succeeded to infect the sour cherries and peaches on the stigma with the spores of *Sclerotinia fructigena*, and he stated that the invasion of the fungus is influenced by atmospheric moisture. ERIKSSON (37) found that conidia of *Monilia cinerea* formed on the lately diseased stems of the apple tree are carried to the stigma by such agencies as insects or wind and cause the blossom-blight; he also reported the same facts of the *Monilia*-disease of cherry. WORMALD (142, 144 and 147) executed several inoculation experiments to determine the fungus species which causes 'Blossom-Wilt', and he recognized the stigma-infection both in field and in laboratory.

In our country HANZAWA (50) recognized the stigma-infection in the case of young fruit-rot of apple caused by *Monilia*, stating that the young fruits are attacked shortly after the petals fell, and by this time the hypha of causal fungus goes down the style from stigma to base. Thereafter, however, he (51) described the symptoms of the infected blossoms as follows: "Change of color starts either from the stigma or from the midrib of leaf gradually enlarging the browned area until at last it reaches to the

petiole." That is, he recognized two ways of infection by *Monilia* fungus in the blossom-blight of apple, but he has not distinguished apparently the blossom-blight from the young fruit-rot. TAKAHASHI (126) stated as follows: "The causal fungus usually infects stalks or leaves directly, and I have not yet observed the evidence of the stigma infection." KASAI (64) stated as follows: "If the conidia formed on leaves of cherry disseminate and infect apple blossoms via stigma, the pistil is killed becoming browned, then the lesion extends to stamens and petals reaching at last to the flower-stalk, or the conidia formed on the cherry-leaf in nature were inoculated on the stigma of apple blossoms and after a week the pistil was brought to death." He recognized the stigma-infection, but not the further development of the disease to young fruit-rot than the browning of the pistil. MIYABE (80) gave a suggestion on the occurrence of stigma-infection in apple blossom-blight in connection with the criticism of TAKAHASHI's report (125) cited above saying that the problem of the stigma-infection must be solved in future by more through observation, although the investigators in Europe and America had reported its occurrence in nature. TAKAHASHI (128), however, did not approve the occurrence of stigma-infection in *Monilia*-rot of apple saying that it is true in some cases of plant diseasses, but the *Monilia* fungus directly attacks the young fruits of apple causing brown lesions on the skin and not through the stigma. MIURA (79) also denied the stigma-infection in apple blossom-blight stating that since 1910 he has observed this point carefully, but he did not meet with any sign which induced him to question about the stigma-infection. He concluded that this fungus does not infect the apple young fruit via stigma of the flower according to the results obtained in his microscopical examinations of stigma and inoculation experiments to the flowers.

It has been the case in our country that the opinions have not been unanimous in regard to the stigma-infection among workers on the blossom-blight or the young fruit-rot of apple. It was approved by HANZAWA and KASAI, but denied by TAKAHASHI and MIURA. The present author was much interested about the possibility of the stigma-infection in consequence of close observations in field for many years, and he has undertaken experiments since 1924. Some results showed that the stigma is the only entrance of *Sclerotinia Mali* in the case of the young fruit-rot. Parts of them have already been reported preliminarily in 1924, and in 1927. The further results obtained in the advanced studies are presented in this paper.

II. GENERAL DESCRIPTIONS OF THE DISEASE

(1) General name

As the same fungus induces various symptoms on the different organs of the same hostplant, the *Monilia* disease on apple caused by *Sclerotinia Mali* is called by different names according to the difference of the organs affected. It seems to be confusable to call the *Monilia* disease of blossom as 'blossom-rot' and the same disease of young fruit as 'young fruit-rot' and so forth.

In the present author's opinion distinction of such names as 'leaf-blight', 'blossom-blight', 'young fruit-rot', or 'axis-blight' is merely based upon the symptoms appeared in different organs; but this is rather important in expressing each phase of the morbid changes. Since the blossom-blight and the young fruit-rot, which are representatives of the apple *Monilia* disease caused by *Sclerotiuia Mali*, not only appear at different times, but also are believed to be different in the mode of infection, if this is the case, they must be controlled by different protecting applications even though the same causal fungus is involved. In such case it would be advantageous to know the pathogenic phases of the disease. Hence, the present author proposes 'Apple *Monilia*-Disease' for the general name and intends to divide this into four kinds, viz., leaf-blight, blossom-blight, young fruit-rot and axis-blight.

(2) Symptoms

The browned disintegration of the affected tissue is the common symptom of the apple *Monilia*-disease, but since it exhibits the characteristic symptom stepwise, each step will be described chronologically.

The **leaf-blight** (the first phase of *Monilia*-disease)

The infection occurs mostly on the rosette-leaves which bear blossoms, in other words, on spurs or fruit-twigs. Leaves which developed from the transitional buds or those on growing shoots are ordinarily seldom attacked excepting in case of a serious epidemic. Sometimes, however, the infection of leaves on suckers is not unusual as WORMALD (144) reported. Regarding the position of a infected leaf former investigators in our country gave no clear description. Both HANZAWA and TAKAHASHI recognized that leaves of a rosette were infected at first. KASAI (64) wrote thus: "In most cases the infection occurs on the nearest leaf toward the blossom-cluster and it

decreases in distal portion". MIURA (79) stated only that the infection takes place on both single-leaf and rosette-leaves. In any case to recognize exactly the kind or the position of the leaf on which the leaf-blight appears aids one to determine whether ascospores or macroconidia are the source of the infection.

It is in the pink stage of flower buds that the leaf-blight appears first. The initial symptom is a small brown fleck on young leaf blade which has developed to a length of 2–3 cm.. A characteristic symptom appears with the development of the leaf, that is, in the center of it and along the midrib of veins, there appears a reddish brown-colored lesion; then that coloration extends towards both the tip and base of the blade in fusiform or in coneshape; at last the mycelium completely invades the petiole and reaches the axis of the blossom-cluster. (Plate III, Fig. 13 b.) The lesion often extends to the tip of the leaf blade along the midrib, but the total area of the leaf seldom affects, because the infected leaf soon becomes wilted and flagged if the fungus invades the petiole, and consequently water circulation stops. In time, browning covers the whole leaf surface and within an average of 4 days dark grey or greyish white acervuli are formed mainly on the midrib or petiole even at 20° C. or so.

Usually only one leaf, not two or more, of a rosette is infected. The position of the diseased leaf in a rosette is not fixed exactly though generally one situated in the middle part of the rosette is attacked. The writer has never seen a case of leaf-blight which had been initiated from the petiole. In a few cases the causal fungus stops its invasion at a certain point on the petiole or at the base of it, or sometimes enters into the axis injuring the tissue only a little, but the mymelium usually makes its way through the axis giving rise to the next phase of the disease.

The **blossom-blight** (the second phase of *Monilia*-disease)

This appears 3 or 4 days after the outbreak of the leaf-blight and the most striking characteristic of the blossom-blight is the wilting of the cluster, and therefore HANZAWA's or WORMALD's "Blossom-Wilt" caused by *Sclerotinia cinerea* corresponds well to the symptom (Plate III, Fig. 13, a and Plate III, Fig. 14). The disease, however, is different from WORMALD's description (142) which reported the actual rotting of pistil or other blossom organs. HANZAWA (50) reported also the rotting of petals, but this is not the case as MIURA (79) described in his studies of the *Monilia* disease of apple caused by the so-called *Sclerotinia Malicola*. In

the blossom-blight axis, pedicel, and a part of the spur are usually affected and the rot of receptacle or leaf-blade excepting the original diseased leaf which falls under leaf-blight is seldom observed. The development of the symptoms of blossom-blight is cleared up by a close examination of the behaviour of mycelium in the affected tissues. As soon as the fungus which caused the leaf-blight invades the axis and decays the tissue, it rises up inversely in other pedicels or petioles running down the axis on the other hand toward the spur or shoot. So, the water supply to the cluster would be discontinued at its base, and consequently the whole cluster itself is forced to wilt before the blossoms and leaves would be affected actually by the mycelium. European workers have generally believed the blossom-wilt to be caused by stigma-infection. The blossom-blight at least in Japan, however, is not caused in such a way as stigma-infection, and it is self-evident, if one recalls the case that the disease appears already before the blossoms open. It is a strong evidence of the blossom-blight coming from the leaf-blight that only a leaf which shows the characteristic symptom produces macroconidia at first is found by an examination of a little older diseased cluster. On the affected cluster dark grey or greyish white acervuli are found in abundance on axes, pedicels or petioles giving out a characteristic smell like that of molasses. WORONIN (149) and SCHELLENBERG (106) reported a strong almond odour of acervuli which allures insects. When all parts of the cluster becomes totally browned, it dries up and falls on the ground finally. When the shoot-canker is caused by an invasion of the mycelium started either from the leaf-blight or from the blossom-blight the lesion sinks down and dries up developing wound callus at the line of demarcation between the diseased and healthy tissues. This point was reported by VOGES (133) and WORMALD (142) in Europe.

The **young fruit-rot** (the third phase of *Monilia*-disease)

When the weather conditions are favorable for the development of the disease, the young fruit-rot prevails and four to five or often all fruits in a cluster are damaged. If one cut in half an appearently healthy but affected fruit 0.5–0.9 cm. in length and 0.4–0.8 cm. in width, there would be found seed, core or a part of flesh which are browned, and the collapsed tissues are crushed easily by fingers (Plate II, Fig. 11). In the earliest stage of the disease the brownig occurs definitely in the funicular region, extending gradually toward the outside. Some specimens show only one seed chamber being diseased while the other four remain normal. It is

often observed that only the funicular part of a seed is browned. Such an infection occurred on seed is generally rare in young fruits which have reached a length of more than 1 cm.. In 1925, however, a fruit of the variety Helm which measured 1.47 cm. in length and 1.03 cm. in width was observed to have a seed infected while other parts were quite normal. Browning of seed body or of funicle is observed also in fruits which abscissed soon after the petals fell and the mycelia are detected in the browned area. As the fruit inwardly infected makes lop-sided growth in general (Plate II, Fig. 10), it can be pointed out of the cluster by the external appearance of the fruit.

The common symptoms of the *Monilia*-rot of young apple fruit are a brown fleck on the green surface at the equator of the young fruit and a drop of light brown colored exudation from the lesion. The exuded juice is of transparent and viscid nature. As this flows down the stalk and dries up, some dirty yellowish brown staining would be seen on the fruit surface or stalk without showing the browned lesion. This droplet of juice would often appear upon the pedicel only and in that condition it dries up without flowing (Plate II, Figs. 10 and 11). The author reported in 1927 that the fresh juice exuded early in the morning is hyaline and changing gradually from brownish yellow to brown (112). Following the occurrence of the exudation the browned lesion appears enlarging its area and at last the fungus makes its way into the stalk which becomes blackish brown in color and collapses. A daily increase of the area of browned lesion can be observed; in some cases the fungus does not invade the stalk and the lesion remains as a greyish brown depression on one side of the fruit which consequently makes a long and lop-sided growth. Such a fruit soon falls down. In every case the affected fruits become 'mummified' having dried out. A few of them are left hanging on the tree even until the next spring, but they mostly fall down after some time. On the stalk of a diseased fruit a few acervuli are formed when wet weather prevails. The fungus which has invaded the whole length of the stalk advances to the axis and gives rise to the next phase of the *Monilia*-disease.

The **axis-blight** (the fourth phase of *Monilia*-disease)

If the fungus which caused the young fruit-rot invades the tissue of the axis having passed through the stalk, then the axis-blight may appear (Plate III, Fig. 15). The relation between the young fruit-rot and the axis-blight is analogous from the pathogenic point of view to that of the leaf- and blossom-blight. It is an only difference in the comparsion

of the symptoms of blossom-blight and axis-blight that in axis blight the wilting of the fruit cluster does not occur quickly in consequence of slow girdling of the axis, and the fungus infects other normally growing fruits in the cluster. The fungus, by this time, inversely invades those fruits from the stalk to the receptacle. The petioles of rosette leaves also are affected by the fungus in quite the same way. The whole axis is collapsed in the case of serious infection, but the tissues grown in the foregoing season i. e., spurs, are not commonly attacked. If the axis is girdled, both fruits and leaves wilt as in the case of the blossom-blight. The affected fruit-cluster soon dries up and hardens. The greyish white acervuli are formed on the stalk or the axis several days after the symptom of the disease has appeared, but the production of conidia is not always observed as in the case of the blossom-blight. Callus tissue is developed along a line of demarcation between the diseased and normal tissues unless the whole axis is completely collapsed, then the collapsed portion separates and a vigorous shoot would grow out from the lower portion of the axis. These conditions are characteristics of the axis-blight which has healed over. The writer (111) ascribed the rot of fruit-axis to the category of axis-blight in 1924. Former workers seem to have attached no importance to this phase of *Monilia*-disease.

(3) Causal Fungus

Three species of fungi are recorded at present in the apple *Monilia*-disease in question, i, e., the blossom-blight and the young fruit-rot, namely, *Sclerotinia Mali* TAKAH. of Hokkaido, *Sclerotinia malicola* M. MIURA of Aomori Prefecture and *Phaeosclerotinia nipponica* HORI. Among them the last fungus is quite different from the former species by the brownish coloration of the conidia and ascospores. Two species of the *Sclerotinia* have been separated by a considerable difference in the length of apothecial stalk, diameter of disk and shape or size of ascospore and conidium. But since there is usually a large fluctuation among these morphological characters even within a species, it seems to be doubtful whether such differences can be regarded as the distinct characters to distinguish the species.

As the writer did not undertake a mycological study he refrains from entering a discussion of species determination, but he did not find any difference between *Sclerotinia Mali* and *S. malicola* having compared the symptoms, the life history and the pathogeny of the blossom-blight in every part of the apple districts (Hokkaido, Aomori and Akita Prefecture etc.),

and also having considered from the view point of control measures. He will prefer *Sclerotinia Mali* TAKAHASHI to *S. malicola* M. MIURA as the causal fungus of the disease in question by reason of the priority.

(4) Life History

At first the causal fungus attacks the young leaves from the late April to early May resulting in the leaf-blight. The fungus invades the axis through the petiole and gives rise to the blossom-blight. Two types of conidia (macroconidia and microconidia) are produced on midribs, petioles or axis in four days or five; the macroconidia are disseminated by an agency such as wind or insects (bees and flies) and reach the blossoms which have just opened. It has been a disputed point as to what part of the blossoms the fungus attacks first. Young fruit-rot occurs about ten days after anthesis and subsequently axis-blight. With the drying out of the collapsed tissues of leaves, sclerotia are formed in young fruits and axes and pass the winter the sclerotium which fell on the ground and hibernated lying half-buried in the soil germinates forming apothecium in late April or early May. Ripe apothecia soon disseminate ascospores which reach the leaves just evolving and infection occurs resulting in the leaf-blight again. Thus the causal fungus concludes its life history.

Macroconidia formed on the fruit-stalk or the cluster axis of course are infectious. Abroad it has been reported that the ascospore is able to infect blossoms (122), but in our country it has been proved experimentally. In England and the European Continent it seems to be very common that the overwintered mycelia in the cankered tissue produce conidia on the affected shoot during the period from winter to spring. The writer has never met such a case of conidia formation, though TAKAHASHI (126) had suggested its possibility.

III. EXPERIMENTAL STUDIES ON STIGMA-INFECTION

The present studies are divided into two parts, i. e.,
(1) inoculation experiments to determine stigma-infection and
(2) experiments verifying several factors related to the stigma-infection.

(1) Inoculation Experiment to determine Stigma-Infection.

a. *Materials and Methods*

The most part of this experiment was excuted in the orchard of the Aomori Agricultural Experiment Station from 1924 to 1927. Healthy apple

trees, nine to fifteen years old, were selected in the orchard and no covering was used during the experiment. Sometimes, trees planted in pots were used. Two or four blossoms per cluster were left to be inoculated, each being tagged with a number. Fresh macroconidia produced on the infected blossom-clusters which had been gathered beforehand and preserved in Petridish, were used for the source of inoculum. Macroconidia formed in the pure potato bouillon were also used. The inoculation experiments with natural spores have already been executed by EWERT (35), VOGES (133), ADERHOLD and RUHLAND (4) and WORMALD (147). Each stigma of the blossom was inoculated with the macroconidia by means of a steel needle after the stigma had been smeared with 10 % sugar solution using a hair pencil. All inoculations were conducted before anthesis, opening the petals except in particular cases. The results were exmained 10 or 15 days after inoculation.

b. *Results*

In 1924 three varieties of apple, i. e., Jonathan. A. S. Pearmain and Ralls were selected and their blossoms were artificially inoculated by the fungus on May 22 and examined the infection on June 1-2. In the case of Jonathan thirty blossoms were inoculated on the stigma but all except No. 15 cluster were lost accidentally by thinning manipulation. One fruit of No. 15 cluster showed the symtom on the toral surface and core two others being injured by insects. The results on the latter two varieties are given in Table one.

TABLE I

Results of inoculation on stigma and receptacle in 1924

Apple varieties	No. of inflorescence (tested)	No. of blossoms (tested)	Organ inoculated	No. of diseased fruits					No. of fruits injured by other cause	No. of healthy fruits	
				Toral surface only affected	Torus and core affected	Core only affected	Total	%		No.	%
A. S. Pearmain	10	30	stigma	0	5	22	27	89.99	3	0	0.00
Ralls	10	30	control	0	0	0	0	0.00	3	27	89.99
Ralls	10	30	stigma	0	1	27	28	93.32	2	0	0.00
Ralls	10	30	receptacle	0	9	0	9	30.01	15	6	19.99

The results of the experiment given in the Table I show a higher percentage of occurrence of diseased-fruits in the stigma inoculation than in the receptacle-inoculation, but the latter is inconclusive because care was not taken to prevent natural infection. Another inoculation on the receptacle was excuted with young fruits of Jonathan and Ralls which had grown about 1 cm. in diameter. Inoculation was made with macroconidia by means of a steel needle penetrating into the torus. The conidia obtained from Jonathan were inoculated on 32 Jonathan and 10 Ralls fruits, and the conidia from Ralls were inoculated on 27 Jonathan and 10 Ralls, but all showed negative results.

In 1925 the experiments were repeated using McIntosh, Jonathan and Ralls. Their stigmas or receptacles were inoculated on May 20 and examined on June 1-2. The results were as follows:

TABLE II

Results of inoculation on stigma and receptacle in 1925

Apple varieties	Organs inoculated	No. of inflorescence (tested)	No. of blossoms (tested)	No.of diseased fruits					No. of fruits injured by other cause	No. of healthy fruits	
				Toral surface only affected	Torus and core affected	Core only affected	Total	%		No.	%
A.S. Pearmain	control	10	30	0	0	1	1	3.33	a 0	29	96.67
	stigma	10	30	0	0	4	29	96.67	0	1	3.33
	receptacle	10	30	0	2	1	3	9.99	1	26	86.68
Jonathan	control	10	30	0	0	0	0	0.00	2	28	93.32
	stigma	10	30	0	12	13	25	83.33	4	1	3.33
	receptaele	10	30	0	0	0	0	0.00	0	30	100.00
McIntosh	control	5	15	0	2	2	4	26.66	1	10	66.68
	stigma	5	14	0	11	3	14	100.00	0	0	0.00
	receptacle	—	—	—	—	—	—	—	—	—	—

a- Insect injury and abscission.

The results of the experiment given in the Table II show clearly the prevalence of stigma-infection. In A. S. Pearmain the receptacle-inoculation gave nearly 10 % infection of the fruits, but this is inconclusive because care was not taken to prevent natural infection; besides, no diseased-fruit which was infected only at the receptacle but not at the core was found in every case as is seen in both Tables I and II. The fact that infection through toral surface occurs rarely in general case is shown also in the following Tables III and IV. These data obtained by inspecting normal appearing fruits which had been gathered at random on June 3–4.

TABLE III

Location of infection and number of diseased fruits among thinned fruits (1925)

Apple varieties	Position of fruit in cluster	No. of inspected fruit	Seed and core infected	Toral surface infected	Core and pulp infected	Size of fruit		Remark
						maximum cm	minimum cm	
Jonathan	middle	291	1	0	1	1.45 × 1.06	0.56 × 0.44	
do	do	81	4	0	3			abscissed
do	side	265	2	0	9	1.08 × 0.88	0.52 × 0.43	
do	do	251	2	0	10			abscissed
do	mixed	228	10	1	8			
total		1116	19	1	31			
A. S. Pearmain	middle	126	1	1	1			
do	do	18	1	0	2			
do	side	172	3	0	0			abscissed
do	do	117	11	0	2			
do	mixed	260	48	0	7			abscissed
total		693	64	1	12			
Helm	middle	45	2	0	0			
do	side	100	7	0	0			

do	do	142	6	0	1			
total		287	15	0	1			abscissed
McIntosh	middle	230	3	0	0			
do	do	36	2	0	2			abscissed
do side	side	171	4	0	3	1.41 × 1.07	0.76 × 0.56	
do	do	211	6	0	4			
total		648	15	0	9			
Ben Davis	middle	191	0	0	1	1.59 × 1.02	0.78 × 0.55	
do	do	40	1	0	1			abscissed
do	side	216	2	0	3	1.25 × 0.93	0.59 × 0.50	
do	do	104	1	0	5			abscissed
total		551	4	0	10			

On June 10, and 11 and 12, all the young fruits borne on three lower main stems of a tree of Ralls were gathered again for inspection. The fruits abscissed or inclined to absciss which were collected to be examined were measured 0.4 × 0.3 cm or so, while the other fruits in the same cluster had already grown to 1.70 × 1.41 cm or so and the fruits of another lot which appeared normal were measured 0.54 × 0.42 cm or 0.70 × 0.65 cm. The results of the examination are given in Table IV.

TABLE IV

Location of infection and number of diseased fruits among gathered fruits (1925)

Experimental lot number	Variety	Position of fruit in a cluster	No. of fruit examined	Number of diseased-fruit				Remark
				Seed and core infected	Toral surface infected	Core and pulp infected	Total	
1)	Ralls	middle	64	5	0	3	8	
	do	do	67	3	0	8	11	abscissed
	do	side	204	10	0	9	19	

	do	side	251	11	0	0	17	abscissed
	total		586	29	0	26	55	
(2)	Ralls	middle	20	8	1	2	11	
	do	do	28	0	0	2	2	abscissed
	do	side	202	24	0	16	40	
	do	do	249	4	0	4	8	abscissed
	total		499	36	1	24	16	
(3)	Ralls	side	97	6	0	2	8	
	do	do	76	4	0	2	6	abscissed
	total		173	10	0	4	14	
(4)	Ralls	side	200	44	0	17	61	
	do	do	125	16	0	1	17	abscissed
	total		325	60	0	18	78	
(5)	Ralls	side	94	27	0	5	32	
	do	do	51	1	0	1	2	abscissed
	total		145	28	0	6	34	
(6)	Ralls	side	214	40	0	32	73	
	do	do	82	3	0	2	5	abscissed
	total		296	43	0	35	78	
(7)	Ralls	side	258	27	0	0	27	
	do	do	57	5	0	25	30	abscissed
	total		325	32	0	25	57	
	sum		2349	238	1	138	377	

In the lot (1) total fruits of 5 trees, in the lot (4) total fruits of 3 trees, and in the other lots total fruits of 2 trees were examined. From the results of the examination shown in Tables III and IV, it is conceivable that the diseased fruit which had been infected from the toral surface are very few.

In 1926 using Jonathan and Ralls, stigma-base (neck of the style) and stalk in addition to stigma and receptacle were inoculated with fresh conidia in the spring, and also with preserved ones from the previous year. Jonathan was inoculated on May 24 (fine in morning and cloudy in afternoon) or May 25 (fine in morning and cloudy in afternoon) and Ralls on May 26 (fine). The results observed, Jonathan on June 2–3 and Ralls on June 5 respectively are given in Table V.

TABLE V

Results of inoclation experiments on stigma, receptacle, stigma-base and stalk (2926)

Variieties	Organ inoculated	No. of inflorescence (tested)	No. of blossoms (tested)	Number of diseased fruits					Number of fruits injured by other cause	Healthy fruits	
				Total surface only affected	Torus and core affected	Core only affected	Total	%		No.	%
Jonathan	control	25	100	0	0	0	0	0.00	1	99	99.00
	stigma (a)	25	100	0	11	32	93	93.00	7	0	0.00
	stigma (b)	25	100	0	0	0	0	0.00	2	98	98.00
	stigam-base (a)	25	100	0	10	18	28	28.00	6	66	66.00
	recepta-cle (a)	25	100	0	0	0	0	0.00	1	99	99.00
	do (c)	25	100	0	8	5	13	13 00	8	79	79.00
	stalk (a)	25	100	0	5(d)	2(d)	7	7.00	2	91	91.00
	control	25	100	0	3	4	7	7.00	10	83	83.00
Ralls	control	25	100	0	1	2	3	3.00	0	97	97.00
	control	25	100	0	4	4	8	8.00	0	92	92.00
	stigma (a)	25	100	0	63	26	89	89.00	8	3	3.00
	do (b)	25	100	0	1	0	1	1.00	0	99	99.00
	stigma-base (a)	25	100	0	7	13	20	20.00	1	79	79.00
	rccepta-cle (a)	25	100	0	2	11	13	13.00	0	87	87.00
	do (e)	25	100	0	0	1	1	1.00	0	99	99.00
	do (c)	25	100	0	2(f)	9	11	11.00	0	87	87.00
	stalk (a)	25	100	0	1(d)	1	2	2.00	0	98	98.00

a- Inoculated with fresh conidia, b- inoculated with hibernated conidia, c- pricked with steel needle, d- no lesion on stalk, e- preparatory treatment with aq. dis. instead of sugar solution, f- browing around prick wound.

The results of the inoculation experiment given in Table 5 show that the highest percentages of infection, i. e., 93 % and 89 % respectively in Jonathan and Ralls were obtained in the case of stigma-inoculation with new conidia, on the contrary receptacle-inoculation after the treatment with sugar solution showed a negative result in Jonathan, agreeing with the result of the previous year. Although 13 % of infection were obtained in the receptacle-inoculation in the case of Ralls, it is inconclusive because most of the diseased-fruits is affected only in the core of them. Stalk-inoculations in both Jonathan and Ralls resulted in a very small percentage of the occurrence of diseased fruits, but these must be resulted by some other mode of infection because no lesion was found on the stalk itself. Hibernated conidia showed almost negative results in infection.

In 1927 the previous inoculation experiments were repeated in the field using both natural and pure-cultured conidia (a), besides a pot-experiment, inoculation being made on style-base and cutting surface of the style. The varieties used were A. S. Pearmain, Jonathan and Ralls. The inoculation were made in the interval between May 16 and June 1, examination between May 26 and June 7. The results are given in Table VI.

TABLE VI

Results of inoculation experiments on stigma, style-base, receptacle, stalk and cutting surface of styles (1927)

Test number	Varieties	Organ inoculated	No. of blossoms (tested)	Number of diseased-fruit					No. of fruits injured by other cause	No. of healthy fruits	
				Total surface only affected	Torus and core affected	Core only affected	Total	%		No.	%
1	A.S.P.	control	100	0	0	0	0	0.00	11	89	89.00
2	do	stigma	100	0	30	15	45	45.00	6	49	49.00
3	do	do	60	0	40	14	54	90.00	5	1	1.70
4	do	do (p)	11	0	0	6	6	54.50	0	5	45.50
5	do	style-base	100	0	0	3	3	3.00	5	92	92.00

6	do	receptacle	100	0	0	0	0	0.00	4	96	96.00
7	do	stalk	100	0	0	0	0	0.00	8	92	92.00
8	Jonathan	control	100	0	8	2	10	10.00	21	69	69.00
9	do	stigma	100	0	42	20	62	62.00	25	13	13.00
10	do	cut surface of styles	92	0	2	24	26	28.30	19	47	51.09
11	do	stigma (a)	18	0	0	0	0	0.00	0	18	100.00
12	Ralls	stigma (a)	21	0	0	0	0	0.00	0	21	100.00
13	do	do (a)	13	0	0	0	0	0.00	0	13	100.00
14	do	do (a)	21	0	0	12	20	95.20	0	1	4.80

a) Conidia formed on potato-bouillon were inoculated on May 18 by courtesy of Mr. W. YAMAMOTO, to whom the writer expresses thanks. p) Pot.

As seen in Table IV stigma-inoculation gave the higher percentage of the diseased fruit while receptacle- and stalk-inoculation failed. Inoculation on the basal part of style resulted only 3 % diseased. In the cases of stigma-inoculation in variety Ralls, the results of tests 12 and 13 were entirely negative, when they were examined a week after the inoculation, while those of test 14 showed so high percentage of infection as 95.2 % when they were examined 2 weeks after the inoculation. It is infered from these facts that the incubation period in stigma-infection of *Monilia* is longer than a week, accordingly by too early examination the result of inoculation experiment appears apparently negative. The same may be said about test 11 in variety Jonathan. In test 10 the infection of the fungus through cut-surface of styles is indicated to be possible. This experiment was repeated in 1932 using McIntosh. In that case, however, cutting of the style was made very near the stigma or at the base and either inoculation or pollination with the pollens of Yellow Transparent were done on May 25. The results examined on June 6 are given in the following table.

TABLE VII

Results of inoculation experiments on cut-surface of styles (1932)

Location of inoculation	No. of blossoms (tested)	Number of diseased-fruit						No. of healthy-fruit		No. of abscission	
		Toral surface only affected	Core only affected	Torus and core affected	Seed only affected	Total	%	No.	%	(+)	(−)
cut-surface of upper part of style	42	0	1	0	21	22	52.38	20	48.58	6	10
cut-surface at style-base	42	0	0	0	0	0	0.00	42	100.00	0	42

(+) Those which became infected and abscissed; (−) those which abscissed but were not infected.

The data show that the infection through cut-surface of upper portion of the style is quite possible. The failure of the infection on cut-surface at the base of style will be discussed later.

It is evident from the above described results of the inoculation experiments for five years that the young fruit-rot is caused by the stigma-infection of *Sclerotinia Mali.*

(2) Experiments verifying several Factors related to stigma-infection

Since it has been proved in the previous experiments that the young fruit-rot is caused by the stigma-infection of the *Monilia* fungus, now in this chapter the results of inoculation experiments in relation to several factors influencing stigma-infection will be described. Materials and methods are quite the same as those stated above.

a. *Relation to age of blossoms*

Pre- and post-anthesis inoculation experiments were executed to know how the age of blossoms, that is whether the age of the stigma influences the infection. The pre-anthesis inoculation was made by opening the petals and exposing the stigma, on the other hand the post-anthesis inoculation was made by selecting the blossoms completely opened, stigmatic surface of them being still uncontaminated. Table VIII gives the results obtained during three years from 1925 to 1927 at the apple experiment orchard of the Aomori Agricultural Experiment Station.

TABLE VIII

Results of pre- and post-anthesis inoculation experiment

Years	Varieties	Time of inoculation	No. of blossoms (tested)	Number of diseased-fruit					No. of healthy fruit		No. of fruit injured by other cause
				Toral surface only affected	Torus and core affected	Core only affected	Total	%	No.	%	
1925	McIntosh	control	15	0	2	2	4	26.70	10	66.70	1
		pre-anthesis	14	0	11	3	14	100.00	0	0.00	0
		post-anthesis	15	0	13	1	14	93.30	0	0.00	1
1926	Jonathan	control	100	0	0	0	0	0.00	99	99.00	1
		pre-anthesis	100	0	11	82	93	93.00	0	0.00	7
		post-anthesis	100	0	24	51	75	75.00	14	14.00	11
		post-anthesis	100	0	7	63	70	70.00	17	17.00	13
1927	A.S. Pearmain	control	100	0	0	0	0	0.00	89	89.00	11
		pre-anthesis	100	0	30	15	45	45.00	49	49.00	16
		post-anthesis	100	0	0	0	0	0.00	86	86.00	14
1927	A.S. Pearmain (a)	pre-anthesis	11	0	0	6	6	54.50	5	45.50	0
		post-anthesis	8	0	0	7	7	87.50	1	12.50	0
1927	Jonathan	control	100	0	8	2	10	10.00	69	69.00	21
		pre-anthesis	100	0	42	20	62	62.00	13	13.00	25
		post-anthesis	100	0	74	14	61	61.00	5	5.00	34

a- pot.

From Table VIII it is apparent that the stigma in the pre-anthesis stage is a little more susceptible than in the post-anthesis stage, although differences in the actual inffection occurred in the two cases of inoculations are small except in the case of A. S. Pearmain (1927).

b. *Relation to Pollination*

When the stigma-infection occurred the hyphal growth in the conducting tissue of the style can not of necessity avoid some dependency upon the pollentube growth. In this connection the interrelation between stigma-infection and pollination may have two important meanings. Firstly

the stigma-infection may become a cause of abscission of blossoms or young fruits in consequence of the hyphal attack on ovules hindering fertilization. Secondly, the influence of pollen-tubes on the invading mycelia must act in various ways dependent upon whether the stigma-infection or the pollination precedes the other regardless of whether the pollination is self or cross. If it should be that such influence of pollen-tubes is different according to the kind of pollens the pollination problem will play a more important role in the disease.

Since 1929 for the purpose of ascertaining the interrelation above mentioned, the five following inoculation experiments accompanied with self- and cross-pollination were executed: (1) pollination and inoculation at the same time; (2) inoculation 24 hours before pollination; (3) inoculation 24 hours after pollination; (4) pollination at a definite interval after inoculation; (5) inoculation at a definite interval after pollination. All the blossoms in use were castrated and covered with paraffin paper bags, and the inoculating procedure was similar to that described above. These results are shown in Tables IX, X, XI, XII and XIII.

TABLE IX

Results of inoculation experiment accompanied by self- and cross-pollination (1929). Variety inoculated Cox's Orange, pollinizer Ben Davis, castrated June 8, inoculated and pollinated June 9, examined June 19

Kind of pollination	No. of blossoms (tested)	Number of diseased fruits					No. of healthy fruits		No. of abscission	
		Toral surface only affected	Torus and core affected	Core only affected	Total	%	No.	%	(+)	(−)
self-pollination	14	0	0	12	12	85.70	0	9.00	2	0
cross-pollination	18	0	6	4	10	55.60	4	22.22	4	0

TABLE X

Results of inoculation experiment accompanied by self- and cross-pollination in pre- and post-anthesis respectively (1930). Variety inoculated Summer Champion, pollinizer Ben Davis, castrated May 26, inoculated and pollinated May 26–28, examined June 10

Treatments	No. of inoculated blossoms	Number of diseased fruits					Normal fruits		No. of abscission	
		torus and core affected	core only affected	seed only affected	total	%	No.	%	(+)	(−)
1. Inoculation immediately after castration	a 15	8	6	1	15	b 100.00	0	0.00	9	0
2. Inoculation 2 days after castration	15	6	3	5	14	93.33	1	6.67	13	1
3. Inoculation 24 hours before self-pollination	23	5	13	1	19	82.61	4	17.39	14	4
4. Inoculation 24 hours after self-pollination	19	1	4	0	5	26.32	14	73.68	4	14
5. Inoculation 24 hours before cross-pollination	24	14	1	0	15	62.50	9	37.50	0	2
6. Inoculation 24 hours after cross-pollination	16	10	0	0	10	62.50	6	37.50	0	1
7. Inoculation pre-anthesis, open-pollination	11	10	0	0	10	90.90	1	9.10	0	0
8. Inoculation post-anthesis, open-pollination	15	15	0	0	15	100.00	0	0.00	0	0

a– without pollination, b– abscissed fruits included, (+) positive, (−) negative.

TABLE XI

Results of inoculation experiment accompanied by pollination in a definite interval before and after anthesis (1931). Vatiety inoculated Jonathan, pollinizer McIntosh, castrated June 4, inoculated June 8–10, pollinated June 8–10, examined June 18–20

Treatments	No. of inoculated blossoms	Number of diseased fruits					Normal fruits		No. of abscission	
		torus and core affected	core only affected	seed only affected	total	%	No.	%	(+)	(−)
1. Inoculation 2 days before self-pollination	36	25	8	1	34	b 94.44	2	5.56	32	2
2. Inoculation 2 days after self-pollination	45	33	2	0	35	77.78	10	22.22	33	10
3. Inoculation 2 days before cross-pollnation	45	33	9	1	43	95.56	2	4.44	43	2
4. Inoculation 2 days after cross-pollination	42	10	16	1	27	64.29	15	35.71	10	14
5. Inoculation 2 days after castration	a 39	14	19	0	33	84.62	6	15.38	33	6
6. Self-pollination only (control)	42	0	0	0	0	0.00	4	c 9.52	0	38
7. Cross-pollination only (control)	36	0	0	0	0	0.00	32	c 88.89	0	4

a– without pollination, b– abscissed number included, c– percentage set given, (+) positive, (−) negative.

TABLE XII

Inoculation experiment in definite serial intervals before and after cross-pollination (1931). Variety inoculated Jonathan, polliniser Ben Davis, first inoculation and pollination June 9, examined June 23

Treatments	Date	No. of inoculated blossoms	Number of diseased fruits					Normal fruits		No. of abscission	
			torus and core affected	core only affected	seed only affected	total	%	No.	%	(+)	(−)
1. Inoculated and pollinated at the same time	9/6	12	3	8	1	12	a 100.00	0	0.00	12	0
2. Pollinated 2 days after inoculation	11/6	15	6	9	0	15	100.00	0	0.00	15	0
3. Pollinated 3 days after inoculation	12/6	15	2	10	1	13	86.67	2	13.33	13	2
4. Pollinated 4 days after inoculation	13/6	12	0	5	0	5	41.67	7	58.33	5	7
5. Pollinated 6 days after inoculation	15/6	12	1	2	0	3	25.01	9	74.99	3	7
6. Inoculated 1 day after pollintion	10/6	15	4	1	1	6	40.00	9	60.00	3	0
7. Inoculated 2 days after pollination	11/6	12	0	0	0	0	0.00	12	100.00	0	2
8. Inoculated 3 days after pollination	12/6	15	0	0	0	0	0.00	15	100.00	0	3
9. Inoculated 5 days after pollination	14/6	14	0	3	3	3	21.43	11	78.57	3	0

a– abscissed number included, (+) positive, (−) negative.

TABLE XIII

Results of inoculation experiment in definite serial intervals before and after cross-pollination (1932). Variety inoculated Jonathan, pollinizer Yellow Transparent, first inoculation and pollination May 27, examined June 6

Treatments	Date	No. of blossoms (tested)	Number of diseased fruits						Healthy fruits		No. of abscission	
			Toral surface only affected	Torus and core affected	Core only affected	Seed only affected	Total	%	No.	%	(+)	(−)
1. Pollinated 1 day after inoculation	27/5	8	0	1	5	1	7	a 87.50	1	12.50	0	1
2. Pollinated 2 days after inoculation	28/5	8	0	0	2	4	6	75.00	2	25.00	0	2
3. pollinated 3 days after inoculation	29/5	7	0	0	5	1	6	85.70	1	14.30	1	0
4. Pollinated 4 days afrer inoculation	30/5	8	0	1	7	0	8	100.00	0	0.00	0	0
5. Inoculated 1 day after pollination	27/5	7	0	0	2	4	6	85.70	1	14.30	0	0
6. Inoculated 2 days after pollination	28/5	8	0	0	0	5	5	62.50	3	37.50	0	0
7. Inoculated 3 days after pollination	29/5	7	0	0	0	2	2	28.50	5	71.50	0	0
8. Inoculated 4 days after pollination	30/5	7	0	0	0	0	0	0.00	7	100.00	0	0

a- abscissed number included, (+) positive, (−) negative.

According to above results obtained during four years, it is impossible to see easily which, self- or cross-pollination, is favorable to the infection of the fungus. In 1929 when inoculation and pollination occurred at the same time the cross-pollination showed evidently favorable. In 1930 and 1931, however, the results are quite reversed according as pollination took place either before or after the inoculation. From Tables XII and XIII it is evident that the percentage of the occurrence of diseased fruits decreases decidedly as inoculation is delayed after pollination, but in the case of the reversed condition the results are inconsistent. These relations of pollen kind and time of pollination to inoculation are considered to be very complicated; henceforth, they will be discussed later in detail.

c. *Relation to fungicide*

It is important to ascertain whether the application of fungicides to such a special organ as stigma is effective or not, as the stigma-infection is an established fact. Granting the stigma-sterilization to be effective it is important to know the effects of fungicide on pollens.

To ascertain these points the influence of Bordeaux-mixture and Lime-sulfur upon the germination of pollen was observed experimentally in 1924. A drop of Bordeaux-mixture (0.8%) or Lime-sulfur (Beaume 0.5) was put on thin agar-plate one centimeter square placed on slide-glass, and pollen grains were sown. The number of germinated pollen grains was counted two hours after the plate had been left in moist chamber (18–22 C). In general pollen grains in contact with Bordeaux-mixture became contracted deepening their yellowish color and showing a few pollens germinated, but their short germ tubes were bursted soon. Most of those in contact with Lime-sulfur did not germinate showing no change though plasmolysis was observed rarely and the germinated short tubes, if any, also bursted soon. The percentage of germinated pollen grains is presented in Table XIV.

TABLE XIV

Influence of fungicides upon germination of pollens (1924)

Varieties	Bordeaux-mixture			Lime-sulfur			Control			Remark
	No. of pollen grains	No. germinated	%	No. of pollen grains	No. germinated	%	No. of pollen grains	No. germinated	%	
Jonathan I	68	1	1.47	62	2	3.23	36	35	97.22	original liquid
do II	26	2	7.69	18	4	22.22	55	55	100.00	do
Ben Davis I	49	6	12.24	27	2	7.41	33	30	90.91	do
do II	—	—	—	20	2	10.00	27	3	11.11	do
McIntosh I	24	0	0.00	31	3	9.68	19	11	50.89	powder
do II	31	2	6.45	34	0	0.00	11	10	90.91	do
Ben Davis I	34	11	26.85	24	11	45.83	36	36	100.00	do
do II	29	1	3.45	39	8	20.51	34	34	100.00	do
A.S. Pearmain I	46	4	8.70	15	1	6.67	25	14	56.00	do
do II	28	1	3.57	21	3	14.29	18	13	72.22	do

These results show evidently the toxic action of both fungicides toward pollen grains. In the case of practical application of these fungicides there may be some certain influences of the stigmatic fluid. Henceforth, it is important to ascertain the influence of the fungicides upon pollination and fertilization by the application of fungicides on the stigma. In 1925 the experiment was conducted, applying the undermentioned four kinds of spray-materials on the stigma immediately after the blossoms opened leaving matters to natural inoculation and pollination, namely, (1) 0.8 % Bordeaux-mixture (2) same as 1, but calcium caseinate was added, (3) Beaume 0.5 lime-sulfur, (4) same as 3, but calcium caseinate was added. Table XV shows the results.

TABLE XV

Influence of fungicides applied on stigma upon fruit-setting and fungus-infection (1925)

Varieties	Treatments	No. of blossoms (tested)	No. of fruit set	Percentage set	No. of diseased fruits
McIntosh	B	169	107	63.31	1
	B+C.c	323	178	55.11	2
	L.s	249	183	73.49	0
	L.s+C.c	227	116	51.10	0
	Control	225	185	82.22	1
Jonathan	B	119	97	81.51	1
	B+C.c	116	91	78.45	1
	L.s	140	98	70.00	3
	L.s+C.c	116	91	78.45	1
	Control	138	116	81.40	0
Ralls	B	104	94	90.38	4
	B+C.c	202	162	80.15	3
	L.s	99	85	95.96	7
	L.s+C.c	108	72	66.67	5
	Control	148	114	77.03	1

B– 0.8 % Bordeaux-mixture, C.c– Calcium caseinate, L.s– Beaume 0.5 lime-sulfur.

From Table XV it will be seen that the application of fungicides on the stigma does not always reduce the percentage set in comparison to the control, and the number of diseased fruit is rather small.

In 1926 the similar experiment was repeated and a further inoculation experiment was done in parallel, conidia being stuck one hour after the application of fungicides. The results are presented in Tables XVI and XVII.

TABLE XVI

Influence of fungicides applied on stigma upon fruit setting (1926)

Varieties	Treatment	No. of blossoms (tested)	No. of fruit set	Percentage set
A.S. Pearmain	B	285	230	81.27
	B+C.c	253	204	80.27
	L.s	128	92	71.88
	L.s+C.c	164	120	72.17
	Control	184	160	86.96
McIntosh	B	205	76	37.07
	B+C.c	222	95	42.79
	L.s	231	119	51.52
	L.s+C.c	307	117	38.11
	Control	484	362	74.59
Ralls	B	461	367	79.61
	B+C.c	622	387	62.22
	L.s	677	534	78.80
	L.s+C.c	911	789	86.39
	Control	455	398	87.47
Jonathan	B	902	469	52.00
	B+C.c	634	322	50.79
	L.s	395	184	46.58
	L.s+C.c	416	215	51.68
	Control	254	182	71.65

Calculating from Table XVI the average percentage set of each variety is 42.37 of McIntosh, 76.74 % of A.S. Pearmain, 50.26 % of Jonathan and 76.78 % of Ralls respectively. Such percentage of setting may be tolerable economically under the practical condition comparing to the control.

TABLE XVII

Effect of fungicides upon artificial inoculation on stigma (1926)

Varieties	Treatment	No. of blossoms (tested)	Number of diseased fruits					Healthy fruits		Questionable	No. of fruits injured by other cause
			Toral surface only affected	Torus and core affected	Core only affected	Total	%	No.	%		
Jonathan	B	100	0	6	43	49	49.00	46	46.00	3	2
	L.s	100	0	0	44	44	44.00	46	46.00	9	1
Ralls	B	100	0	63	23	86	86.00	13	13.00	0	1
	L.s	100	0	65	26	91	91.00	8	8.00	1	0

TABLE XVIII

Effect of fungicides upon artificial inoculation on stigma (1927). Treatment and inoculation on June 1, examination on June 7

Plot	Treatments	No. of blossoms (tested)	Diseased Fruits		Healthy Fruits		Abscissed fruits	
			No.	%	No.	%	No.	%
1	B, inoculated 1 hour after	83	65	78.30	11	13.30	7	8.40
2	L.s., inoculated 1 hour after	89	56	62.90	29	32.60	4	4.50
3	Co_3Cu, inoculated 1 hour after	100	46	46.00	3	3.00	51	51.00
4	D.L s., inoc. 6 hours after	95	52	54.70	0	0.00	43	45.30
5	Ls., inoculated 6 hours after	100	37	37.00	2	2.00	61	61.00
6	L.s., inoculated 6 hours after	99	67	67.70	0	0.00	32	32.30
7	Control I	100	1	1.00	74	74.00	25	25.00
8	do II	100	62	62.00	13	13.00	25	25.00
9	do II	100	61	61.00	5	5.00	34	34.00

D.L.s.,- dried lime-sulfur, Control I- treatment and no inoculation, Control II- pre-anthesis inoculation, Control III- post-anthesis inoculation, but not treated.

Comparing Table XVII with Table V (1926) it will be seen that the application of fungicides reduced the percentage of disesed fruit to about 50 % for the Jonathan, but had not effected on the Ralls. In 1927 a similar experiment was repeated. At this time, powder of dried lime-sulfur and copper carbonate besides the two fungucides mentioned above were used and inoculated one or six hours after the treatment of the stigma under open pollination. The data are given in Table XVIII.

The data given above do not show conclusive evidence of the effect of the fungicides except those which had been inoculated 6 hours after the application of copper carbonate, dried lime-sulfur and Bordeaux-mixture, but it must be noticed that the latter three gave a higher percentage of abscission.

d. *Relation to ringing and defoliation*

Ringing on spurs is often practised for the purpose of increasing the percentage of setting of fruit or for inducing parthenocarpy. If the vigor of blossom-cluster is influenced by such treatments as ringing or defoliation it is important to ascertain whether these treatments may effect the infection as well, or not, as one intends to prevent the disease rather by nutritional conditions than by fungicidal application. In 1931 three blossoms left per cluster of the variety Hatsuwarai were inoculated before anthesis and the corresponding spurs were ringed or defoliated at the same time. The results are given in Table XIX.

TABLE XIX

Influence of ringing or defoliation upon stigma-infection (1931).
Treated and inoculated on May 31, examined on June 17.

Treatments	No. of blossoms (tested)	Number of diseased fruits						Healthy fruits		No. of abscission	
		Toral surface only affected	Torus and core affected	Core only affected	Seed only affected	Total	%	No.	%	(+)	(−)
Ringing	45	0	12	15	11	38	84.44	2	4.45	10	5
Defoliation	45	0	15	11	14	40	88.88	0	0.00	13	5
Control	45	0	17	9	10	36	81.81	3	6.83	11	5

(+) positive (−) negative

In these results the largest percentage of diseased fruit were obtained in the defoliation plot, while the smallest in control. These are not conclusive since the difference is very small.

e. *Relation to varieties*

Opinions concerning the resistance of the apple varieties have not always been agreed. The writer has not only observed in the apple orchard but also made the stigma-inoculation experiment. The results are given in Tables XX, XXI and XXII.

TABLE XX

Results of stigma-inoculation determining resistance of variety (1927). Inoculated on June 1–2, examined on June 12

Varieties	No. of blossoms (tested)	Nuber of diseased fruits						No. of fruits injured by other cause	No. of healthy fruits
		Toral surface only affected	Torus and core affected	Core only affected	Seed only affected	Total	%		
Ralls	60	0	19	36	1	56	93.29	1 [a]	3
Champion	60	0	10	21	11	42	66.66	9	9
Giant Geniton	60	0	41	12	0	53	88.29	4	3
Allington Pippin	60	0	17	27	7	51	84.96	9	0
Bismark	60	0	45	3	0	48	79.96	12	0
Lawver	60	0	31	0	0	31	51.64	29	0
Texas Red	60	0	55	4	0	59	98.29	1	0
Rivers	60	0	59	1	0	60	100.00	0	0
Ortley	60	0	24	31	3	58	89.29	4	3
Ben Davis	79	0	10	16	0	26	32.91	48	5
Red Pearmain	54	0	38	14	6	48	88.88	6	0
Ingram	54	0	6	48	0	54	100.00	0	0
McIntosh	100	0	24	10	0	38	38.00	62	0
Hay's Midseason	60	0	3	2	0	5	8.33	55	0
Winter Majetin	60	0	19	37	0	56	93.32	2	2
Winter Maybinden	60	0	35	24	0	59	98.32	0	1
Daver	58	0	58	26	0	48	82.75	8	2
Porter	60	0	2	43	5	50	83.33	10	0
Malus prunifolia	40	0	0	0	0	0	0 00	4	36

a– mainly insect injury.

TABLE XXI

Reaults of stigma-inoculation determining resistance of variety (1928). Inoculated on May 25-31, examined on June 5-12

Varieties	No. of blossoms (tested)	Number of diseased fruits						No. of fruits injured by other cause	No. of healthy fruits
		Toral surface only affected	Torus and core affected	Core only affected	Seed only affected	Total	%		
Jonathan	30	0	0	1	14	15	50.00	0	15
Control	38	0	0	0	0	0	0.00	0	38
Fameuse	11	0	2	6	0	8	72.72	0	3
Control	16	0	0	0	0	0	0.00	0	16
Ralls	18	0	5	6	3	14	77.77	1	3
Control	20	0	0	0	0	0	0.00	0	20
Tallman's Sweet	20	0	4	10	1	15	75.00	1	4
Control	31	0	1	1	0	2	6.45	0	29
Willaim's Favorite	35	0	5	30	0	35	100.00	0	0
Control	36	0	1	1	2	3	8.33	0	33
Pumpkin Sweet	28	0	2	23	2	27	96.42	0	1
Control	43	0	0	0	0	0	0.00	0	43
McMahon's White	44	0	4	8	5	17	38.63	3	24
Control	45	0	1	0	1	2	4.44	2	41
Texas Red	34	0	1	21	2	24	70.58	0	10
Control	40	0	0	0	0	0	0.00	0	40
Summer Champion	40	0	0	11	3	14	35.00	1	25
Control	46	0	0	0	0	0	0.00	2	44

From Tables XX and XXI it will be seen that all 25 varieties showed positive results. A species of apple (*Malus prunifolia*) gave negative results. This species, however, was observed to be always susceptible later. Hay's Midseason, Ben Davis und McIntosh showed a lower percentage of diseased fruit but it is doubtful whether they are truly resistant to Monilia or not since they yielded many waste fruits injured by insects.

In 1931 there happened a serious epidemic and henceforth there was good chance to observe the disease widely. The data recorded at that time are presented in Table XXII.

TABLE XXII

Varietal susceptibility of apple to young fruit-rot and axis-blight observed in 1931

Varieties	Susceptibility	Varieties	Susceptibility
Wealthy	+	St. Jacinth	+ + +
Blue Pearmain	+	Summer Sweet	+ +
Red Astrachan	+ +	York Imperial	+ +
Yellow Transparent	+	Northern Spy	+
Ortley	+ +	St. Lawernce	+
McIntosh	+	Mita No. 90	+
Tallman's Sweet	+ +	Baldwin	+
Sops of wine	+ +	Peacegood	+
Alexander	+ + +	Ben's Red	+
Yellow Bellflower	+ +	Wagener	+
Hubbardston	+	Ribston Pippin	+
Santa Clara King	+	Hatsuwarai	+
Grimes	+ +	Beauty of Bath	+
Mansaku	+	Golden Russet	+
William's Favorite	+	Rhode Island Greening	+
Duchess	+	Ko Shojo	+
Yellow Newtown	+	Pumpkin Sweet	+
Texas Red	+ +	Early Harvest	+
King	+	White Winter Pearmain	+ +
King David	+ +	Black Gilliflower	+
Ingestrie	+ +	Fanny	+
Black Ben Davis	+ + +	Keswick Codlin	+ +
White Pippin	+	Roman Stem No. 1	+ +
Helm	+	Iowa Beauty	+ +
Carolina	+ +	Evelyn	+ +
Ralls	+ +	Canada Baldwin	+
Maiden Blush	+	Giant Geniton	+ +
Ben Davis	+ +	Whitney No. 20	+ +
Porter	+ + +	Anisim	+ +
Boskoop	+	Eastman (a)	−
Rivers	+	Monstrous Pippin	+

Oregon	+	Winter Banana	+
Fameuse	+	Chenango Strawberry (a)	−
Bismark	+	Beauty of Kent	+
Winter Goldparmane	+	Benoni	+
Konishiki	++	A.S. Pearmain	+
Givens	+	Boiken	+++
Lowel	+	Dominie	+
Cox' Orange	+	Dees	+
Erly Melon	++	Fameuse Sucree	++
Winesap	+	Skelton	+
Champion	+	Summer Champion	+
Doyle	+	Rombo	+
Early Joe	++	Henry Clay	+
Bauman's Reinette (a)	−	Gravenstein	++
Terry	+	Transcendent Crab	−
Spring Dale	+	Peck's Pleasant	+
Delicious	+	French No. 1 (a)	−
Senator (a)	−	Red Bellflower	+
Benful	+	Grieve	++
Landsberger Reinette	++	Garcoin	+
McMahon's White	+	Cooper's Early	++
Esopus	++	Borodavka	++
Indo	+	Jonathan	+++
White Astrachan	++	Stark Florence	−
Willow Twig (a)	−	Doctor	−
Christmas Pearmain	+		

+ light, ++ heavy, +++ heavy and axis-blight, − no disease found, a- very few or no blossoms.

Only 6 varieties out of 113 showed no diseased fruit in the writer's observation. It is, however, questionable whether these six varieties are resistant or not, for, though the blossoms of these trees were free from infection, the number was too few to determine their susceptibility or resistance to the *Monilia* disease.

f. *Inoculation experiments with ascospores*

Since the period of ascospore-discharge occurring in apothecia corresponds to that of leaf-bud oppening of apple trees when the blossoms

are yet unopened, it is evident that the spores have chance to attack only the leaves which are just developing. It is interesting, however, to know whether ascospores can commit stigmatic infection or not, and henceforth in 1932 inoculation experiments were excuted in the greenhouse using some potted apple trees. The ascospores used were obtained from apothecia which had been gathered and preserved previously. Suspension of ascospores in 10 % of sugar solution in which apothecia were immersed in proportion of one disc per 100 cc. of the solution was applied on the stigma with a fine brush. The varieties used were Red Astrachan and McIntosh. Blossoms to be inoculated were all castrated. The experiment was done in the following four plots: (1) inoculation without pollination, (2) inoculation and self-pollination at the same time, (3) inoculation and

TABLE XXIII

Results of inoculation experiment on stigma with ascospores (1932)

Treatments	No. of blossoms (tested)	Number of diseased fruits						No. of fruits injured by other cause	No. of fruirs abscissed	No. of healthy fruits
		Toral surface only affected	Torus and core affected	Core only affected	Seed only affected	Total	%			
1. Inoculated without pollination	10	0	4	2	0	6	60.00	a 2	b 1	2
2. Inoculated and self-pollinated	10	0	0	0	0	0	0.00	5	5	5
3. Inoculated and cross-pollinated	10	0	3	0	0	3	30.00	4	0	3
4. Inoculated 1 day after cross-pollinated	10	0	0	0	0	0	0.00	4	0	6
5. Inoculated 2 days after cross-pollinated	10	0	0	0	0	0	0.00	3	0	7
6. Inoculated 3 days after cross-pollinated	10	0	0	0	0	0	0.00	1	0	9

a– injured mostly by leaf-roller (*Cacoecia sp.*), b– diseased fruit not included.

cross-pollination at the same time, and (4) inoculation in a definite interval after cross-pollination. First, second and third plots were inoculated and pollinated on May 14, while the fourth plot was pollinated on May 16, and inoculated on May 17, 18 and 19 respectively. Since young fruit-rot appeared in the first and third plots as early as May 25, all plots were examined on May 26. The results are given in Table XXIII figures being total of both varieties.

From Table XXIII it is evident that the ascospore is able to infect via stigma resulting in the young fruit-rot. That the negative results were obtained in the second and fourth plots may perhaps be due to the improper preparation of the inoculum.

g. *Relation to other fruit-trees*

WORMALD (143) recognized *Sclerotinia cinerea* which is parasitic on plums attacks also apple-blossoms, and he (147) observed that *Monilia*-fungus obtained from cherries attacks pear-blossoms, one obtained from pears attacks apple-blossoms and one which causes apple-blossom-blight attacks pear-blossoms. In Japan, KASAI (64) has given a report, and said that the conidia of *Sclerotinia Kusanoi* produced on cherry leaves can infect apple blossoms and cause the blossom-blight. The author intended to ascertain whether the apple *Monilia*-fungus, *Sclerotinia Mali*, can infect other fruit-trees via stigma, taking the facts in consideration that the cross infection of *Monilia*-fungi to different kinds of fruit-trees takes place in foreign countries. Macroconidia obtained from diseased apple blossom-

TABLE XXIV

Results of inoculation experiment on staigma of pears with macroconidia of *Sclerotinia Mali* (1931)

Kind of fruits	No. of blossoms (tested)	Number of diseased fruits						No. of fruits abscissed	No. of healthy fruits
		Toral surface only affected	Torus and core affected	Core only affected	Seed only affected	Total	%		
European pear	33	0	0	0	5	5	11.12	0	28
Japanese pear	51	0	0	0	0	0	0.00	12	51

clusters were inoculated on the stigma of an European pear (Flemish Beauty) and a Japanes pear (Taihaku). The results are shown in Table XXIV.

The results of the experiment were positive for the European pear, but not for the Japanese pear. As it will be described in Chapter V, however, the mycelium was found in the ovules of both pears on microscopic examination. Accordingly the writer believed its ability to penetrate into the pear-styles, and the same inoculation experiment was repeated in 1932. Macroconidia or ascospores were inoculated on the stigma of the following fruit-trees: *Pirus communis* (Flemish Beauty), *Pirus serotina* (Ichiwara Wase), *Cydonia vulgaris* (variety not identified), *Mespilus germanica* (variety unknown) *Pruuus avium* (Black Tartarian) and *Prunus salicina* (variety not identified).

Inoculations were made in cherry on May 19, in Japanese plum and pear on May 20, in European pear on May 21, in quince on June 3 and medlar on June 13. Examination was made from May 29 to June 23. The data are given in Table XXV.

TABLE XXV

Results of inoculation experiment on stigma of four pomes and two drupes with spores of *Sclerotinia Mali* (1932)

Kind of fruits	No. of blossoms (tested)	Number of diseased fruits					No. of abscission		No. of healthy fruits
		Torus and core affected	Core only affected	Seed only affected	Total	%	(+)	(−)	
Prunus avium	c 17	0	0	0	0	0.00	0	12	17
	a 17	0	0	0	0	0.00	0	11	17
Prunus salicina	a 27	0	0	0	0	0.00	0	1	27
Pirus communis	c 42	0	6	31	37	88.09	2	0	5
	a 18	0	0	4	4	22.22	2	1	14
Pirus serotina	43	1	14	25	40	93.02	9	3	3
	a 30	0	10	4	14	46.66	1	0	16
Cydonia vulgaris	c 28	0	1	12	13	46.42	14	7	15
Mespilus germanica	c 19	0	0	10	10	52.60	0	0	9

a- ascospore, c- macroconidia, (+) positive, (−) negative

The results of the inoculation experiments were negative to both drupe-fruits, while positive to all the pomes. It must, however, be noticed that the cases of cherries which were inoculated with ascospores or conidia gave over 60 % abscissed fruits. Japanes pear gave the largest percentage of disease among the pomes, producing, evidently the young fruit-rot which differs from the symptoms on apple fruit only in color of the lesion, i. e., deep black instead of deep brown of the apple. The ascospore proved also to be infectious though the percentage of diseased fruit was lower.

V. BEHAVIOUR OF *MONILIA*-HYPHAE AND POLLEN-TUBES IN THE TISSUES OF PISTIL

(I) Materials and methods

In 1930 and 1931 blossoms and young fruits of apple trees were fixed at a definite interval after inoculation and pollination and which had been treated in the same manner as shown in the inoculation experiments described in Chapter IV. The plan and the interval of fixation are as follows.

In 1930 inoculation and pollination were done as shown in Table XXVI, choosing the Summer Champion apple for inoculation and the Ben Davis as pollinizer.

TABLE XXVI

Plot	Treatments	Date of inoculation	Date of pollination	Remark
IA	Inoculated immediately after castration, but not pollinated	May 27	——	bagged
IB	Inoculated 1 day after castration, but not pollinated	May 28	——	do
IIC	Inoculated 1 day after self-pollination	May 29	May 28	do
IID	Inoculated 1 day before self-pollination	May 28	May 29	do
IIIE	Inoculated 1 day after cross-pollination	May 29	May 28	do
IIIF	Inoculated 1 day before cross-pollination	May 28	May 29	do
IVG	Inoculated pre-anthesis open-pollination	May 27	——	do
IVH	Inoculated post-anthesis open-pollination	May 28	——	do

Materials inoculated and pollinated according to above table were collected and fixed at the following hours after inoculation:

Plot	Hours fixed after inoculation							
	I	II	III	IV	V	VI	VII	VIII
IA	6	31	48	96	120	144	172	196
IB	6	24	72	96	120	144	192	—
IIC	6	48	72	96	120	168	192	—
IID	24	72	96	120	144	192	216	—
IIIE	6	48	72	96	120	168	192	—
IIIF	24	72	96	120	144	192	216	—
IVG	6	31	48	96	120	144	168	216
IVH	6	24	72	96	120	144	192	216

Further, blossoms of Jonathan were inoculated before anthesis on May 27 between 9 and 10 A.M. and exposed to open-pollination. These materials were used for the comparison and fixed at a definite interval after inoculation on stigma indicated by abbreviation as follows: AA- 5 and 31 hours, BB- 48 and 96 hours, CC- 120 and 144 hours, DD- 168 and 216 hours, and EE- 240 hours.

In 1931 three varieties of apple, i. e., Rhode Island Greening, Ortley and Jonathan as well as an European pear (Flemish Beauty) and a Japanes pear (Taihaku) were used. Rhode Island Greening and the pears were inoculated in the open, while in the case of Ortley and Jonathan cut branches with many blossom-clusters were used being put in a beaker placed in the greenhouse (about 25 C.) or in an incubator of 20 C. These are tabulated in Table XXVII.

Materials inoculated and pollinated according to Table XXVII were collected and fixed at the following hours after inoculation: RIBP- 72, 120, 168 and 216. RPBI- 48, 96 and 144. RIP- 72, 120, 168 and 216. TOJ- 18, 42, 66, 90 and 114. TJO- 18, 42, 66, 90 and 114. TJJ- 18, 42, 66, 90 and 114. GJS- 24 48, 120, 144, 168 and 192. GJC- 24, 48, 120, 144 and 168. T and F- 24 and 72.

TABLE XXVII

Varieties and Treatments	Abbreviation	No. of blossoms (tested)	Date of inoculation	Date of pollination
R.I.G. × McIntosh inocu. before pollination	RIBP	18	9/6	10/6
R.I.G. × McIntosh inoculated after pollination	RPBI	18	10/6	9/6
R.I.G. × McIntosh inocu- and polli. at the same time	RIP	18	9/6	9/6
Ortley × Jonathan, inocu. and polli. at the same time	TOJ	18	11/6	11/6
Jonathan × Ortley, do	TJO	15	do	do
Jonathan × Jonathan, do	TJJ	51	do	do
Jonathan × Jonathan, do	GJS	18	7/6	7/6
Jonathan × Red Astrachan, do	GJC	18	do	do
Taihaku, inocu. and polli. before anthesis open-pollination	T	15	27/5	—
Flemish Beauty, do	F	15	29/5	—

Besides these, materials fixed in 1929 were also used, and three to four blossoms were fixed each time. The fixers in use were CARNOY's fluid in 1930 and FLEMING's weak solution in 1931. There was little to choose between the two fixers as far as the present experiment concerned. Some of the fixed materials were inbedded into paraffin after an ordinary treatment and remainders were preserved in 80 % alcohol for occasional use. Sections about 12–15 microns thick gave most satisfactory results. Cotton blue stain was used in almost every case, while DELAFIELD's Haematoxylin and Saffranin were seldom employed. ASAMI (7) recommended cotton blue for staining of pollen-tubes of Japanese pear. The writer found that the *Monilia* hyphae as well as the apple pollen-tubes were stained well by the 0.1 % soiution of cotton blue pigment in lactic acid or lactic-carbolic acid, and the latter was convenient for long use. Staining from 18 to 20 hours was enough to give differential effect.

(2) Experimental Results

a. *Anatomical survey of fixed materials*

Under this topic the writer intends to compare the relation between infection of the fungus via stigma and pollination giving the anatomical survey of every material collected and fixed in the years 1930 and 1931.

IA. This group contains some preparates of the materials which were inoculated with the spores immediately after castration.

IA (1). Fixed 6 hours after inoculation: the tissues are stained as follows: stylar epidermis blue, vascular system greenish blue and conducting tissue deep blue. The conducting tissue remains homogeneous and not yet slitted. Papillate cells of stigmatic surface maintain original shape. There are a few germinated conidia, but no penetration occurs.

IA (2). Fixed 31 hours after inoculation: staining of tissues is similar to IA (1). Penetration of a few hyphae is observed but no change in their passage.

IA (3). Fixed 48 hours after inoculation: many conidia are seen attached to the outer surface of the style, but few germinated. Some well-stained hyphae are observed in the upper portion of the conducting tissue. In some specimens the slit is observed in the conducting tissue under the stigma. Some specimens show the segregation of nucellus and internal integument, though the ovules remain normal.

IA (4). Fixed 96 hours after inoculation: hyphae reached the junction of styles or the base of them, but they are not seen in the seed cavity. Ovules are not yet flaccid.

IB. This group comprises some preparates which were made of the materials inoculated with the spores after the stigma had ripened.

IB (1). Fixed 6 hours after inoculation: a few conidia germinated, but the germ tubes not yet penetrated the tissues.

IB (2). Fixed 24 hours after inoculation: a few hyphae which penetrated into the upper portion of the style are seen.

IB (3). Fixed 72 hours after inoculation: the mycelial passage is shown in the upper portion of the conducting tissue and a few hyphae arrived at the junction of styles.

IB (4). Fixed 96 hours after inoculation: the hyphae entered the seed cavity and some reached the funicle. Ovules are not flaccid.

IB (5). Fixed 120 hours after inoculation: a characteristic mycelial passage is shown in the cross-section at the junction of the styles and

the penetration of 6–17 hyphae is seen there.

IB (6). Fixed 192 hours afrer inoculation: the tissue of the funicle is attacked by the mycelium and a thick hyphae is seen in the middle of the nucellus (Pl. VIII, Fig. 34).

IIC. This includes the preparates of materials inoculated with the spores one day after self-pollination.

IIC (1). Fixed 6 hours after inoculation: this corresponds the just 30 hours after the pollination. The pollen-tubes are seen at the distance of 1 mm. from the stigma. Tubes in which protoplasm has already moved away toward the tip are also observed. A few conidia on the stigma have germinated, but not penetrated.

IIC (2). Fixed 48 hours after inoculation: the hyphae are penetrating, but the mycelial passage is not yet shown. Neither hyphae nor pollen-tubes are seen in the seed cavity.

IIC (3). Fixed 72 hours after inoculation: the hyphae arrived at the junction of styles showing the mycelial passage. Some pollen-tubes are seen among the mycelia being distinguished by their well-stained narrower body. Neither hyphae nor pollen-tubes enter the seed cavity yet.

IIC (4). Fixed 96 hours after inoculation: neither hyphae nor pollen-tubes are seen either at the base of styles or in the seed cavity, and ovules begin to become flaccid. The whole tissues of the ovary begin to contract.

IIC (5). Fixed 120 hours after inoculation: neither hypha nor pollen-tube is seen either at the base of styles or in the seed cavity.

IIC (6). Fixed 168 hours after inoculation: some pollen-tubes reached the funicle, while hyphae are seen neither at the base of styles nor in the seed cavity. A few ovules remain normal.

IIC (7). Fixed 192 hours after inoculation: same as IIC (5).

IID. This comprises the materials inoculated with the spores one day before self-pollination.

IID (1). Fixed 24 hours after inoculation: the hyphae have already penetrated and are seen at the diatance of 0.9 mm. from the stigma.

IID (2). Fixed 72 hours after inoculation: the hyphae and pollen-tubes are seen in the conducting tissue showing a diametre of about 12 μ in the former and about 4.5 μ in the latter. The mycelial passage[a)] is appearing, neither of them reaches either the style-base or the seed cavity.

IID (3). Fixed 96 hours after inoculation: a characteristic mycelial passage is shown and many hyphae have arrived at the style-base. Some

(a). This term will be commented upon in the next section of this paper.

of the hyphae are about to enter the seed cavity. In the conducting tissue in which the hyphae are less easily seen there are many of the pollen-tubes passing through. In a certain specimen both are passing side by side at the style-base. The hyphae takes stains as well as the pollen-tubes, but the former has a narrow tip whereas the latter has a swollen one.

IID (4). Fixed 120 hours after inoculation: the conducting tissue has contracted, showing large slits and staining only the periphery of styles. Neither hypha nor pollen-tube is seen either at the style-base or in the seed cavity.

IID (6). Fixed 192 hours after inoculation: the hyphae have entered the nucellus. A very thick branched hypha is approaching the synergids. The pollen-tube is seen neither in the style nor in the seed cavity.

IID (7). Fixed 216 hours after inoculation: the hyphae have spread over or through the funicle and the nucellus, while ovules have become flaccid. The passing of the hypha through the micropyle is clearly observed. The pollen-tube is not seen anywhere except near the stigma.

IIIE. This includes the preparates of the materials inoculated with the spores one day after the cross-pollination.

IIIE (1). Fixed 6 hours after inoculation: conidia do not germinate, while pollen-tubes have arrived at a distance of 2.8 mm. from the stigma.

IIIE (2). Fixed 48 hours after inoculation: there are many pollen-tubes in the conducting tissue. The leading one of them has already reached the funicle, while many are passing the style-base. The hyphae do not yet reach even the junction of styles.

IIIE (3). Fixed 72 hours after inoculation: a great number of the pollen-tubes are approaching to the seed cavity and a vigorous one is found near the funicle. No hypha is found in the seed cavity.

IIIE (4). Fixed 96 hours after inoculation: the pollen-tube has entered the embryo sack passing the microphyle. No hypha is found in the seed cavity.

IIIE (5). Fixed 120 hours after inoculation: the hyphae have entered into the seed cavity. A number of pollen-tubes are seen near the funicle. The hypha with scanty protoplasm is running down the placental wall. The styles have contracted irregularly, showing slits but not well differentiated mycelial passage.

IIIE (6). Fixed 168 hours after inoculation: the hyphae have entered into the seed cavity and many are running down the placental wall.

Several pollen-tubes are seen at the funicle and at the entrance of the nucellus.

IIIE (7). Fixed 192 hours after inoculation: the hyphae are seen in seed cavity while the pollen-tube is found at the funicle.

IIIF. This comprises the preparates of the materials inoculated with the spores one day before the cross-pollination.

IIIF (1). Fixed 24 hours after inoculation: the hyphae are penetrating but do not show their passage.

IIIF (2). Fixed 72 hours after inoculation: the pollen-tubes have reached the junction of styles or the funicle and the hyphae too have entered into the seed cavity. The mycelial passage is shown in the upper portion of the conducting tissue while in the lower parts the pollen-tubes is stained much deeper than the mycelium. In the middle portion of the style it appears as if the hypha has been disturbed in its growth by the pollen-tube.

IIIF (3). Fixed 96 hours after inoculation: on the stigma many pollen-grains are germinating (62 hours after the pollination) and the pollen-tubes passing through the conducting tissue are numbered as many. The leader of them has arrived at the micropyle or the egg-cell. Many hyphae are found at the junction of styles.

IIIF (5). Fixed 144 hours after inoculation: the hyphae are found on the funicle and some of them are about to enter the embryo sack. The hyphae are also entering the bottom tissue of the seed cavity. Some pollen-tubes are seen near the funicle. Some ovules have become flaccid.

IIIF (6). Fixed 192 hours after inoculation: at the funicle only pollen-tubes are found. Both hypha and pollen-tube are running down the placental wall. They are also observed at the style-base passing side by side. On the stigma pollen grains or pollen-tubes are often observed being surrounded by the hyphae, but no particular phenomenon is recognized.

IVG. This includes the preparates of the materials inoculated with the spores before anthesis in open-pollination.

IVG (5). Fixed 120 hours after inoculation: the pollen-tube is found at the funicle, but no mycelium.

IVG (6). Fixed 144 hours after inoculation: the hyphae entered into the seed cavity, while the pollen-tubes seemed to have entered into the embryo sack.

IVG (7). Fixed 168 hours after inoculation: the hyphae are shown at the base of styles and some are running down the placental wall. Pollen-

tubes are not found in the seed cavity.

IVG (8). Fixed 216 hours after inoculation: the hyphae attacked the funicle and some have entered into the nucellus. Pollen-tubes are not found in the seed cavity.

IVH. This includes the preparates of the materials inoculated with the spores after anthesis in open-pollination.

IVH (1). Fixed 6 hours after inoculation: a pollen-tube arrived at a distance of 1 mm. from the stigma. Some conidia germinated.

IVH (2). Fixed 72 hours after inoculation: no mycelium is found at the style-base or in the seed cavity. The pollen-tubes are seen in the style.

IVH (4). Fixed 96 hours after inoculation: the hyphae entered into the seed cavity, and the pollen-tubes are seen at the style-base or on the placental wall.

IVH (5). Fixed 120 hours after inoculation: the hyphae reached the funicle and the pollen-tubes are running down the placental wall or entering into the micropyle.

IVH (6). Fixed 144 hours after inoculation: the mycelia filled the tissue of funicle and some hyphae are entering into the micropyle. The pollen-tube is also seen on funicle. In the seed cavity hyphae and pollen-tubes are observed to be attached to each other.

AA–EE. These lots comprise the preparates of the materials inoculated with the spores before anthesis in open-pollination.

AA (1). Fixed 5 hours after inoculation: the inoculated conidia have not yet germinated.

AA (2). Fixed 31 hours after inoculation: the hyphae arrived at a distance of 2.6 to 3.5 mm. from the stigma, though the mycelial passage is not yet clear. Some pollen-tubes are found at the junction of styles.

BB (1). Eixed 48 hours after inoculation: The hyphae entered into the nucellus, whereas the pollen-tube is found only at the style-base (Pl. IX, Fig. 41).

BB (2). Fixed 96 hours after inoculation: a number of hyphae are seen on the funicle and the pollen-tube has entered into the seed cavity or reached the funicle. The mycelial passage is shown in the conducting tissue. On the surface of the funicle some hyphae are seen following after the pollen-tube (Pl. VI, Fig. 22).

CC (1). Fixed 120 hours after inoculation: the hyphae are found in the micropyle or the nucellus. Some pollen-tubes are seen among the mycelia in the central cavity, or both of them are running down the

placental wall.

CC (2). Fixed 144 hours after inoculation: the hyphae have entered into the nucellus and a hypha is seen in the micropyle pushing its tip into the embryo sack. In style-base or in funicle some of the pollen-tubes are found among mycelia.

DD (1). Fixed 168 hours after inoculation: the hyphae attacked the nucellus and a thick hypha is seen in the micropyle. Both hyphae and pollen-tubes are passing side by side at the base of styles. Some of the pollen-tubes are found on the funicle also.

DD (2). Fixed 216 hours after inoculation: in the subepidermal portion of the style immediately under the stigma a hypha is observed to penetrate that tissue longitudinally. This phenomenon is never seen in the other preparates. The ovules are destroyed having been occupied with mycelia. The hyphae spread through tissues of the placental wall, especially of the bottom of seed cavity. Some of these tissues stained a greenish color. The hyphae are breaking through the ovarial region from the placental tissue heading radially in all directions for the toral region. A part of the mycelia is running downward through the primary vascular bundle. All hyphal threads in this stage are thick measuring about 7.5 μ to 11.25 μ in diametre. In the tissues where the hyphae are well developed large fissures are produced owing to the shrinkage of dissolved cell wall. The mycelia become tangled in the nucellus and some of them are pushing out through the external integument. The hyphal threads near the funicle are poor of protoplasm, but those in the ovules are rich of it. The pollen-tubes were not observed anywhere except in the upper portion of styles.

EE (1). Fixed 240 hours after inoculation: the hyphae have at last reached the toral skin. Thus, it is shown that the time needed for the fungus to get to the skin after the conidia have been inoculated on the stigma corresponds with that needed from time of stigma-infection until the external symptom will appear. In the diseased tissues many fissures are formed and these communicate with each other ending at the periphery. The hyphae seem often to invade into the vasucular bundles (Pl. IX, Fig. 37). In the cross-section it can be seen that the hyphae invade into the tissues in the order of funicle, placenta and carpel. It is observed that the diseased portion removes radially from the infection center (placenta) as the hyphae grow especially quickly toward the primary vascular bundles. That is, the diseased tissue has a depth in the distal portion of the placenta (Pl. VIII, Fig. 34). These facts show that the hyphae easily invade into

the mesocarp from the placental tissue, whereas they are incapable of penetrating directly through the carpellary epidermis from the seed cavity. The hyphae which entered into the tissues of mesocarp or the torus attain about 15 μ in diametre. Most tissues through which the hyphae have passed do not stain well with cotton blue being colored a dark yellowish green.

RIBP. This class inculdes the preparates of the materials inoculated with the spores before pollination.

RIBP (1). Fixed 72 hours after inoculation: the hyphae are found at a distance of 1.6 mm. and the pollen-tube at 0.6 mm. from the stigma, the diameter being 2.5-4.7 μ in the former and about 7.5 μ in the latter. The passage of the hyphae is forming a little split. Some hyphae have a clamp near their septa which have stained a deep color. Hyphae about 2.35 μ in diameter have a swelled tip measuring about 3.5 μ. As soon as the hyphae penetrate into the stigma their tips swell nearly doubling their diameter. The pollen-tube seems to run along the splits of the conducting tissue.

RIBP (2). Fixed 120 hours after inoculation: the hyphae attacked the funicle or entered into the embryo sack, while ovules have become flaccid with browned color. The hyphae in the funicle or the nucellus stain mostly less and only those in the embryo sack are deeply stained. In a cross-section six hyphal threads are seen in the tissue at the ventral side of the placental wall.

RIBP (3). Fixed 168 hours after inoculation: from near the stigma the characteristic mycelial passage starts and most of the hyphal contents are finely granulated. The hyphae in the conducting tissue at the stylebase have generally a diametre of about 15 μ, but those in the seed cavity are narrower than the former measuring 7.0-7.5 μ. The hyphae have entered into the nucellus, while the ovules have become flaccid. A number of hyphae are found in the tissue of the under central cavity.

RIBP (4). Fixed 216 hours after inoculation: the fungus has infected a half of the nucellus while the pollen-tube is not found even at the style-base.

RPBI. This class includes the materials inoculated with the spores after pollination.

RPBI (1). Fixed 48 hours after inoculation: both hyphae and pollen-tubes penetrated the tissues of styles, but both of them are not found in the seed cavity.

RPBI (2). Fixed 96 hours after inoculation: both hyphae and pollen-tubes reached the style-base running side by side, but latter are more numerous. The pollen-tubes have already entered into the seed cavity and the leader of them is found near the funicle, while the hyphae are not seen there-about. In a cross-section at the style-base no mycelial passage is observed, showing that the hyphae have not yet passed. Some ovules have become flaccid as shown by their staining less.

RPBI (3). Fixed 144 hours after inoculation: a few hyphae are observed on the funicle, while many are passing the style-base. Most of the ovules have become flaccid.

RPBI (4). Fixed 192 hours after inoculation: both hyphae and pollen-tubes are seen at the entrance of the seed cavity running in parallel into it. Some hyphae have entered into the nucellus, but generally speaking the hyphae which entere into the nucellus are less in number and ovules are shrinked. The pollen-tube is found only on the funicle.

RIP. This includes the materials inoculated with the spores and pollinated at the same time.

RIP (1). Fixed 72 hours after inoculation: the hyphae are found near at the style-base, but not the pollen-tube.

RIP (2). Fixed 120 hours after inoculation: the hypha entered into the embryo sack, while ovules became flaccid. In a cross-section there are as many hyphae as 20–22 in number passing through the conducting tissue.

RIP (3). Fixed 168 hours after inoculation: the hyphae are seen in the seed cavity or the micropyle, while a few pollen-tubes are found near the style-base.

RIP (4). Fixed 216 hours after inoculation: the nucellus became flaccid separating the cells of integuments from each other. A few hyphae are seen in the seed cavity or the funicle. The hyphae are seldom found in the nucellus in some specimens, but in others the shrinked ovule occupied by the mycelia are observed. The well-developed hyphae are seen in the style, but not the pollen-tube.

TOJ. This group comprises the materials inoculated with the spores and cross-pollinated at the same time in the incubator maintained at 20 C.

TOJ (1). Fixed 18 hours after inoculation: the papillate cells of the stigma are held in their original shape. Both hyphae and pollen-tubes penetrated the conducting tissue.

TOJ (2). Fixed 42 hours after inoculation: neither hyphae nor pollen-tubes get even to the style-base.

TOJ (3). Fixed 66 hours after inoculation: there are many conidia on the stigma which have not yet germinated, while the hyphae grown from the germinated ones are penetrating through the conducting tissue and the leader of them has arrived at the style-base. The pollen-tubes were not found in the style.

TOJ (4). Fixed 90 hours after inoculation: the number of pollen-tubes is larger than that of the hyphae. The leader of the pollen-tubes has arrived at the funicle.

TOJ (5). Fixed 114 hours after inoculation: the ovules became flaccid showing some hyphae at the entrance of the seed cavity.

TOJ (6). Fixed 138 hours after inoculation: the hyphae running down the style-base and an abnormally shaped pollen-tube are seen (Pl. IX, Fig. 39). Both of them are also detected on the funicle and the placental wall. The ovules either entirely shrinked or undeveloped.

TJO. This class comprises the materials inoculated with the spores and cross-pollinated at the same time in the incubator maintained at 20 C.

TJO (1). Fixed 18 hours after inoculation: most of the germinated pollen-tubes have penetrated the stigma and some are seen at a distance of 1.4 mm from it. The papillate cells are held in shape. The hyphae too are penetrating the tissue of stigma. In general the quantity of both conidia and pollens attached to the stigma in these materials is greater than in the field experiments.

TJO (2). Fixed 42 hours after inoculation: the pollen-tube is stained with clear differentiation in the conducting tissue. The hyphae, however, are more in number than the pollen-tubes showing the beginning of the mycelial passage.

TJO (3). Fixed 66 hours after inoculation: the hyphae reached the style-base and some of them entered into the seed cavity showing anastomosis. The pollen-tubes are not found in the seed cavity.

TJO (4). Fixed 90 hours after inoculation: the hyphae entered into the embryo sack. Most of them are found at the style-base or in the seed cavity running in parallel with the pollen-tubes. In a certain specimen the hyphae are entering into the seed cavity forming a bundle and spreading over the funicle. At the style-base some shriveled pollen-tubes are seen among the mycelia.

TJO (5). Fixed 114 hours after inoculation: it is difficult to detect pollen-tubes by means of differential staining in the conducting tissue in which a large number of hyphae are found. On the other hand some of the pollen-tubes may be detected in the style if no hypha exists. The embryo sack is occupied by strongly stained mycelia. The hyphae which are found in the organs other than ovule are weakly stained or even hyalin. Cell walls of the external integument or those of the internal integument, especially about the micropyle, are yellowish. The nucellus is separating from the integument though the ovules have not yet shrinked. Some hyphae are found to take their way for the chalaza between the two integuments instead of entering through the micropyle. In some specimens no hypha is found in the seed cavity while the ovules have already withered.

TJO (6). Fixed 138 hours after inoculation: the nucellus is occupied by mycelia which are stained intensely, ovules withered and the external integument browned. The carpellary epidermis and other tissues thereabout do not stain except the tissue in which the hyphae have passed. The hyphae in the nucellus or the funicle are thick. In the cross-section only the nucellus and the border of the central cavity are seen to have stained. Well-stained hyphae are also seen in the shriveled ovule (Pl. IX, Fig. 40).

TJJ. This class comprises the materials inoculated with the spores and self-pollinated at the same time in the incubator maintained at 20 C..

TJJ (1). Fixed 18 hours after inoculation: pollen-tubes and hyphae are found respectively at a distance of 1.2 and 1.4 mm. from the stigma. Much of the pollen has germinated. The penetrated hyphae are about 2.2 μ in diametre.

TJJ (2). Fixed 42 hours after inoculation: there are pollen-tubes and hyphae in the style running in parallel, the leader of the latter having already reached the style-base. The papillate cells of the stigma mostly withered, though a few have retained their original shape.

TJJ (3). Fixed 66 hours after inoculation: the hyphae entered into the seed cavity.

TJJ (4). Fixed 90 hours after inoculation: the hyphae are crowding about the funicle and some are found to have entered into the nucellus or to have usurped their way between the integuments. Ovules either begin to wither or completely shriveled. The hyphae are not found in the nucellus or even in the seed cavity in the case of the ovule which has wholly shriveled. The stigmas in most cases are inhabited by some bacteria.

GJC. This class comprises the materials inoculated with the spores and cross-pollinated in the greenhouse maintained at about 25 C..

GJC (1). Fixed 2 hours after inoculation: the papillate cells of the stigma are retained holding many pollen grains or conidia. Some pollen grains are germinating and penetrating the tissue. The hyphae also have penetrated the tissue of stigma, some of them measuring 7.05 μ in length.

GJC (2). Fixed 48 hours after inoculation: many pollen grains germinated and the grains became empty. The conidia showed vigorous germination and some germ tubes penetrated the tissue of stigma. The mycelial passage is shown in the cross-section of the style. Neither pollen-tubes nor hyphae reach the style-base.

GJC (3). Fixed 120 hours after inoculation: the hyphae have entered into the embryo sack and some are found also on the funicle or at the entrance of the embryo sack. Ovules show a sign of shrivelling having attained the size of 622.5×210 μ. The hyphae are passing actively through the style and pollen-tubes do not reach even the base of it.

GJC (4). Fixed 144 hours after inoculation: the nucellus is occupied by them. Further, the hyphae are found abundantly near the funicle. There are many pollen grains which have fallen into the calyx-disc, and they have germinated

GJC (5). Fixed 168 hours after inoculation: some hyphae reached the chalaza spreading through the nucellus, and on the other hand some of them attacked the funicle at the same time. Most ovules do not stain being shriveled, while the hyphae invaded and developed in their tissue are intensely stained. Some hyphae have already spread over the bottom tissue of the seed cavity. All the styles are found to have been infected.

GJS. This comprises the materials inoculated with the spores and self-pollinated at the same time in the greenhouse maintained at about 25 C..

GJS (1). Fixed 24 hours after inoculation: the papillate cells of the stigma retain their normal shape. A few conidia are germinating and penetrating through the tissue. Some pollen-tubes are seen on the stigma.

GJS (2). Fixed 120 hours after inoculation: most of the papillate cells of the stigma are withered. Both pollen-tubes and hyphae reached the style-base.

GJS (3). Fixed 120 hours after inoculation: the hyphae entered into the embryo sack though many are crowding on the funicle. The development of the hyphae is generally good. Some ovules are withered and no pollen-tube is seen in the seed cavity.

GJS (4). Fixed 144 hours after inoculation: the hyphae are spread over the embryo sack while no pollen-tube is found in the seed cavity.

F. This class includes the materials of Flemish Beauty inoculated with the spores before anthesis left to open-pollination.

F (1). Fixed 24 hours after inoculation: the pollen-tubes and hyphae are found to have penetrated the tissue.

F (2). Fixed 72 hours after inoculation: a similar mycelial passage to that in the case of the apple is shown in the conducting tissue and the pollen-tube is seen intermingled with the mycelia. In the nucellus intensely stained thick hyphae are seen.

T. This comprises the materials of Japanese pear Taihaku inoculated before anthesis left to open-pollination.

T (1). Fixed 24 hours after inoculation: both pollen-tubes and hyphae are found about the middle of the styles.

T (2). Fixed 72 hours after inoculation: the mycelial passage is recognized in the conducting tissue as in the case of the apple, and a few hyphae are seen in the ovules.

Based upon the facts observed in the above materials the time required for pollen-tubes and hyphae to reach the funicle and the ovule may be summarised as follows: —

TABLE XXVIII

Time required for hyphae and pollen-tubes to reach funicle and embryo sack (1930 and 1931)

Lot	Hypha and pollen-tube	Hours required to		Remark
		Funicle	Embryo sack	
GJC	Hypha	96	120	
	Pollen-tube	?	?	They are clearly recognized in the stlye (3 days after pollination)
TJO	Hypha	66?	90	
	Pollen-tube	?	?	Same as GJC (4 days after pollinatioln)
GJS	Hypha	96?	120	
	Pollen-tube	?	?	They are recogniztd to the junction of the style (2 days after pollination)

TJJ	Hypha Pollen-tube	66? ?	90 ?	Same as GJS (3 days arter pollination)
TOJ	Hypha Pollen-tube	138 90	? ?	
RPBI	Hypha Pollen-tube	144 120–216	192 ?	
RIBP	Hypha Pollen-tube	96 ?	120 ?	They reached the style-base (192 hours after pollination)
RIP	Hypha Pollen-tube	96? ?	120 ?	They reached the style-base (168 hours after pollination)
IA	Hypha Pollen-tube	? —	? —	They reached the style-base (96 hours after inoculation)
IB	Hypha Pollen-tube	96 —	120? —	
IIC	Hypha Pollen-tube	? 192	? ?	They are not found in the seed cavity (144 hours after inoculation)
IID	Hypha Pollen-tube	144? ?	192? ?	They are recognized to the style-base (3 days after pollination)
IIIE	Hypha Pollen-tube	? 72	? 96	They entered into the seed cavity 5 days after inoculation
IIIF	Hypha Pollen-tube	96? 48	144 72	
IVG	Hypha Pollen-tube	144 ?	168 ?	On June 1 (perhaps 3 days after pollination) they are found at funicle and micropyle
IVH	Hypha Pollen-tube	120 ?	144? ?	Same as IVG
AA-EE	Hypha Pollen-tube	? ?	48 ?	On May 31 (perhaps 3 days after pollination) they are found at funicle

Since the figures given in Table XXVIII are nothing but time of fixation the actual hours required may be less. It is quite possible from the data given above that the *Monilia* hyphae can grow from the stigma to the embryo sack within 48 hours and pollen-tubes within 72 hours at the minimum. The behaviour of the pollen-tube is not generally clear either in the case of self-pollination or of any other pollination accompanied by inoculation at the same time, while on the contrary the behaviour of the *Monilia* hyphae seems to be somewhat restricted if they are accompanied by crossed pollen-tubes.

It has not been known before whether *Sclerotinia Mali* attacks pears in nature or not, but in the writer's experiment there is evidence that the *Monilia* hyphae also invade into the nucellus of pears through the same process as in the apple.

b. *General characters and behaviours of the apple pollen and pollen-tube and the causal fungus.*

Apple pollen grains are spherical in shape, greyish yellow in color measuring 30.0–34.2 μ in diametre. They have rich contents which stain deeply with cotton blue. The pollen-tube penetrates the special cell tissue of the stigmatic surface and many tubes crowd there. The tip of a pollen-tube has a tapered club-like form. Concomitant with the movement of protoplasm toward tip of the pollen-tube the pollen-spheres or the basal part of the tubes become hollowed and stained very slightly or none showing only their wall. The diametre of pollen-tubes is quite variable measuring on an average from 7 to 13 μ. They are thicker on the stigmatic surface than in the conducting tissue. DORSEY (31) also recognized the same fact stating that it appears to be due principally to the stage of growth, though MOORE (1917) attributes this difference in diametre to food supply. The pollen-tube does not grow straight at first when it is in the stigmatic tissue but in the conducting tissue it runs down with an approximately straight elongation. It is difficult to differentiate hollow tubes which have lost their protoplasm in the tissue for they mostly degenerate and no more maintain their original shape.

Each of the five styles communicates respectively to each of five seed cavities and the conducting tissue is constructed with long slender prosenchymatous cells even after that tissue has entered into the ovarial region from the style-base. The conducting tissue ends at the junction of two placentae adjoining each other and opens in the seed cacity at this point just like an inverted funnel. Most pollen-tubes which have passed through

the conducting tissue enter into the seed cavity at this point, and grow down along the placental surface. Having reached the funicle the pollen-tube creeps further downward along the side of the funicle to reach the micropyle and at last enters into it to meet the egg cell. A hollow pollen-tube is found seldom in the tissue other than the stigma and most tubes degenerated seem to be buried in the tissue. On the contrary, a portion of the pollen-tube can be often observed in the seed cavity, but the lateral, the central and the stylar cavities are occupied with thick pubescence and accordingly it is not only difficult to distinguish the remains of hollow tubes exposed in those cavities, but also a slender hair is often taken for the tube. Nevertheless, the former shows two thick membranes distinctly, whereas that of the latter is very thin with a obscure line.

Macroconidia are mostly short ellipsoidal lemon-shaped, obtusely papillate, hyaline, 11.75–7.05 × 9.40–4.70 μ in size, with typical disjunctors. On germination the protoplasmic contents contract toward a germ pore which stains deeply. The diametre of the germ-tube is variable measuring 1.80–2.35 μ. The hyphae are well differentiated being very fine in the stigmatic tissue, but they broke often on sectioning (Pl. VI, Fig. 23). The hyphae in the conducting tissue, however, increase their diametre gradually, growing straight without branching. Many germinated conidia are seen on the epidermal surface of the style other than the stigma, but the hyphae never penetrate the tissue through its epidermis. The penetration occurs always between the papillate cells of the stigmatic surface. After they have advanced into the conducting tissue they proceed down between the prosenchymatous cells increasing their diametre gradually and attaining to 4.7–7.0 μ at the style-base. Neither hyphae nor pollen-tubes are easily discriminated in the materials fixed 24 hours after inoculation. There are, however, several distinctions between the fungus-hyphae and pollen-tubes existing in the tissues of apple blossoms. The hyphae stain lighter compared to pollen-tubes and their outline is not distinct: septa present in the hyphae, whereas the callose plug of the pollen-tube is not well differentiated: only the tip portion of the pollen-tube, length of which is about 112.5–150 μ, is deeply stained, usually the pollen-tube tapering backward and going out of sight (Pl. VI, Fig. 21); the hyphae are isodiametric through their whole length growing very straight (Pl. V, Fig. 17): there is no visible compression of cells adjacent to the path of pollen-tubes, on the contrary the hyphae dissolve and compress the cells adjacent to their path as if they widen it; the hyphae have large vacuoles in their cells which contract usually by dehydration, consequently the passage

of the hyphae often appears as if it were a streak (Pl. VII, Fig. 25); the hyphae never branch after they have entered the stigma nor do they traverse the stylar tissue cross wise.

The writer should like to call the peculiar streaks described already above by the name 'mycelial passage' for convenience of interpretation. This 'mycelial passage' is shown distinctly rather in the cross section than in the longitudinal one (Pl. V, Fig. 18, Pl. VI, Figs. 19, 20, Pl. VII, Fig. 26).

The browning of styles for some distance from the stigma after infection is often observed, but on microscopic examination it is found that the hyphae keep on their straight growing only in the conducting tissue but they are never seen in other tissues which do not stain by cotton blue showing only yellowish brown colour. In the tissue of stigma or the upper portion of style long after anthesis several saprophytic fungi are found penetrating through the tissue and prolific colonies of certain bacteria are also found on the stigmatic surface.

Of the writer's experiments the most interesting point is that the behaviour of the hyphae in the pistil ever after it has penetrated by way of the stigma is quite the same as that of the pollen-tubes. That is, the hyphae proceed down straight through the conducting tissue forming the characteristic mycelial passage, and even after having passed the style-base they continue their growth through following just the same route as the pollen-tubes choose. Having thus arrived at the junction of the suture of carpel margins they proceed into the seed cavity, then creep down the placental wall for the funicle. Here the hyphae go down along the side of the funicle or penetrate through its loose tisue to reach the micropyle and at last enter the embryo sack.

The hyphae change their forms gradually as they have arrived at the tiny space under the epidermal cap of nucellus passing through the micropyle (Pl. VII, Fig. 28). This change of forms occurs particularly after they have entered the embryo sack or the nucellus (Pl. VII, Fig. 29), i.e., the hyphae become shortened and corpulent staining very deep in consequence of being full of a rich protoplasmic content without vacuoles. Further the hyphae appear themselves to be about to branch and propagate, giving off processes here and there on their bodies.

The branching of hyphae begins for the first time after they have exposed themselves in the seed cavity (Pl. VII, Fig. 30), but most of them further continue straight growth creeping down along the surface of the placental wall. Then having reached the funicle they branch vigorously.

The hyphae once invade into the embryo sack branch and propagate

as time elapses, and spread over the nucellus. Subsequently, integuments are attacked and at last the hyphae appear again in the seed cavity breaking the outer wall of ovule. Whether the hyphae entering into the embryo sack do attack the egg cell at first or not is uncertain. By the time when the mycelium spreads over the embryo sack or the nucellus the funicle tissue is also invaded by the hyphae (Pl. VIII, Fig. 31), and the inner integuments which build up the micropyle are destroyed too (Pl. VIII, Fig. 32).

The hyphae having entered the small cavity under the epidermal cap of nucellus invade into the embryo sack as stated above, but there are a few of them which grow up without branching along the path between nucellus and internal integument towtrd the chalaza (Pl. VII, Fig. 29). There are some hyphae which proceed directly to the ovule after they have appeared in the seed cavity (Pl. VIII, Fig. 33), but they never infect the ovule penetrating through the epidermal layer of it, whereas they grow downward along the surface of the ovule to reach the micropyle. In the case of the tissues of the micropyle, i. e., that of the internal and external integment, destruction of the hyphae begin at the micropyle or the small cavity under the epidermal cap of nucellus, proceeding gradually outward. The writer could not seen even one example of a specimen in which the hyphae are questioned to have peretrated through the wall of integument near the micropyle. It is without exception that the mycelium stained deeply with cotton blue is already found in the nucellus, notwithstanding no change has been shown in the outward tissue of the ovule (Pl. IX, Fig. 40).

From the above mentioned observations it should be concluded that the *Monilia* hyphae invade the embryo sack invariably passing first through the micropyle.

If the evironment turns favorable at the time when the hyphae have infected the embryo sack or the nucellus, they begin to destroy the ovule and the funicle, subsequently they invade the placental tissue upward or downward, one part of them spreading along the under layer of the carpel epidermis (Pl. VIII, Figs. 34, 36) and the others traversing toward the torus radially through the ovarial tissue (Pl. VIII, Fig. 35) until at last they reach the outer surface of the young fruit (Pl. IV, Fig. 10). Thereafter some of the hyphae proceed inversely toward the style-base and the others down to the stalk. The hyphae of this stage greatly increase their diametre measuring often from 15.75 to 22.50 μ. But the hyphae usually make a straight growth almost without branching. The hyphae tend to enter the

vessels of the vascular bundles, but the parenchymatous tissue only is destroyed so that the cells contract and are dissolved forming large cavities here and there in the tissue. Of these cavities one formed near the opening on the toral surface is larger and seems to be made of mechanically broken epidermis due to the pressure produced by the solution which had been filled in those cavities, but seems not to be due to the dissolution of epidermal cells by the hyphae themselves, for a part of them expose temdelves in the air while the others invade along under the subepidermal layer without dissolving the cuticle of the epidermis. SCHELLENBERG (107) observed similar fact in the case of quince. As stated above in the chapter on symptoms a characteristic symptom of the young fruit-rot is the secretion of brown-colored juice on the toral or pedicel surface. It is evident that juice is collected in the cavities obove mentioned according as cells are dissolved and at last it flows out from the necrotic lesion on the toral surface. The *Monilia* hyphae are not in general capable of dissolving epidermal and vascular cells.

The hyphae which have infected the ovarial or toral region further invade into the stalk under favorable condition. In this case too the hyphae go straight between cells of the cortical parenchym without infecting the epidermal cells. The hyphae in the stalk tissue usually measure 15.75–22.5 μ in diametre. Some hyphae are often found penetrating up and down through the inside of the spiral vessel. Most of the hyphae proceed down through the starch layer and other parenchymatous tissues. Maceration of the cells occurs even in advance of the hyphae and the attacked cells become flattened tangentially showing the positive reaction to ruthenium red. The fact that a certain parasite kills the host cells by secreting enzymes or toxins in advance of hyphal bodies was set forth by DE BARY (30) for the first time. According to SCHELLENBERG (107) it is an evident fact observed in all *Sclerotinia*-fungi that the hyphae can grow only in the tissue which already has been browned. This can be interpreted only assuming that a certain particular enzyme kills the cells in advance of the hyphae. He reported that vascular tissue is more resistant than the parenchymatous tissue showing the browning three or four days later. COOLEY (27) studying on the physiology of *Sclerotinia cinerea* reported that the hyphae on coming in contact with cell wall, secrete first enough enzyme to dissolve the cell walls in marking their way through, leaving the walls of the host cells surrounding hyphae entirely normal, i. e., without swelling or disorganization. Whether or not the host cells are killed by the secreted substances of the hyphae extended over a wide range is questionable, but

in the case of the *Monilia* fungus it is not uncommon to see browning and dissolving of the host cells near the hypeae except in the case of the invasion of ovule.

The hyphae generally enter into the vascular tissue, but do not dissolve the vessel itself. A depressed cavity which is formed by the dissolution of the cortical parenchym adjacent to the bast fiber is often observed. In this case it is shown evidently that the fibers act as a barrier of the hyphae. Any discontinuous arrangement of the bast fibers in the young stalk seems to admit the hyphal invasion cross wise into the pith-part although the hyphae, of course are able enter into the pith directly from the base of the receptacle.

The tissue disorganized by the *Monilia* hyphae is usually attacked by the multiplication of some saprophytic fungi. VOGES (133) reported the invasion of *Chladosporium, Fusicladium* or *Fusarium* in the subepidermal tissue of diseased twig.

The behaviour of the fungus in the tissues of axis of the young blossom-cluster axis is the same as in the stalk. In the well-developed axis, however, the invasion of the fungus is limited to the subepidermal layer and a crack is produced between the diseased and the normal part by gradually developing the callus tissue. Since the axis-blight is commenced only while the tissue is young, it may be stated that the resistance of the host-tissues to the hyphal invasion is closely dependent on the development of the mechanical elements. WEHMER (135) reported that in "Zweigdürre" of sour cherry the causal fungus multiplies in the phloem-region without penetrating xylem or bark portion, and hyphae are seen in xylem occasionally owing to their traversing into the pith through a medullary ray. He observed also that the hyphae go down rapidly to the long shoot passing through the cortical tissue of the spur, but he withheld his interpretation for the future why the infection does not always occur in such a shoot as well as even in a wounded shoot as ADERHOLD (1) had shown. Further he reported that "gum"- secretion from the infected cortex is not uncommon. In the apple axis-blight infection of spurs rarely occurs, much less frequently does it occur on a long shoot bearing a spur, and "gum"-secretion is not known. The hyphae, however, attack the cortex of those shoots which bear lateral fruit-clusters if the latter are diseased. In this case the lesion is limited only in the cortex nothing remaining but fibers and callus tissue develops soon.

V DISCUSSION

(1) Inoculation experiments

The results of inoculation experiments for nine years have proved that the young fruit-rot is caused by the stigma-infection of the causal fungus, and it is questionable that the fungus does penetrate into the young fruit through other parts than the stigma at least in natural condition. In Table I the higher percentage of infection on the receptacle in Ralls may be attributed to the fact that conidia accidentally dispersed by wind or transmitted by insects resulting in stigma-infection because these diseased fruits had always shown decay of core. The frequencies of occurrence of diseased fruits in the case of Ralls inoculated with the fungus on receptacles and those in the case of control of Ralls and McIntosh shown in Table II are almost the same. The writer has examined a great many fruits more than those shown in Tables III and IV, but he could not ever find a diseased fruit with the lesion showing on the surface of the receptacle, while the core was not yet decayed or only the stalk was infected. Infection at the style-base, i. e., on the calyx-disc has been found in only one example. For inoculation with wound on the receptacle 13 % of Jonathan and 11 % of Ralls have been recorded positive, but as these also had the infection in the core, the possibility of infection via stigma can not be overlooked. There is no diseased fruit of which the toral surface only was affected all over the inoculation experiments. The higher percentage of the occurrence of the infection at stigma-base is quite doubtful because the location is too near the stigma. Browning around the prickwound where the inoculation was made is also ambiguous because the flesh of fruits may be browned easily by wound. Inoculation on the stalk gave 7 % of Jonathan and 2 % of Ralls positive infection, but it must have been a natural infection via stigma for there was no indication of infection on the stalk itself. Overwintered conidia which had been preserved in Petridish under laboratory conditions failed to infect except 2 or 3 % positive results on Ralls which were caused perhaps by natural infection. It can be said safely that the hibernated conidia have no viability.

The fact that the stigma-inoculation with pure-cultured conidia showed negative results in Jonathan No. 14, Ralls No. 15 and 16 (Table VI), seems to have happened through the too early examination, because they were inoculated on May 26 and were cut in half for examination on June 2, i.e., just a week after the inoculation. As for Ralls No. 17, therefore,

the experiment was delayed a week longer till June 9 and 95.2 % positive infection were found. In this view the pure-cultured conidia are also infectious.

It was shown that penetration of the fungus through cut surface of the style was quite possible, for instance, 28.3 % positively infected of Jonathan and 52.38 % of McIntosh (Table VI) were obtained while only in the case of inoculation on cut-surface at the style-base the infection failed. This failure in the latter case may have been due to imperfect technic.

According to the pollination experiment done in parallel to inoculation it was proved that pollens also can germinate on the cut-surface of style and the pollen-tubes enter into the tissue. Thirty blossoms of McIntosh were castrated, bagged and pollinated with the pollens of Yellow Transparent after smearing with 10 % sugar solution on both apical or basal cut-surface of the style. In the case of pollination on the apical cut-surface of the style 26 out of 30, that is, 85.65 % set, while on the basal cut-surface 7 out of 30, that is, 23.33 % set. The fact that the pollination on cut-surface of the style is effective has also been reported in apple by NAMIKAWA (89). KATS (65) who had experimented with the pollination on cut-surface of the style of *Cestrum elegans* and other plants, however, reported that stigmatic fluid is necessary for penetration of pollen-tubes through cut-surface of the style.

Having considered that above results, it must be assumed that the entrance of both hyphae and pollen-tubes into the conducting tissue through the cut-surface occurs readily if the very tissue of the style was exposed, and existence of the stigma is not always necessary. A conducting tissue seems after all to be the best qualified medium for the development of hyphae or pollen-tubes.

About the results of all the inoculation experiments one thing to be noticed is that the blossom-blight and young fruit-rot belong to entirely different categories from the view point of infection. Had the causal fungus infected the stigma, it would attack the core at first and appear in about ten days on the surface of young fruit causing the young fruit-rot, but never the blossom-blight. Had the young fruit ceased early to grow, the fungus would attack only the seeds or core of the affected fruit which sooner or later would absciss, resulting in no wilting of blossom-clusters.

The opinions which most European writers have held, that is, that the so-called "blossom-blight" or "Blütendürre" are caused by fungal infection via stigma, should not be possible as far as *Sclerotinia Mali* is concerned.

Of the several factors related to the stigma-infection the blossom-age-

relation, i. e., pre-anthesis and post-anthesis inoculation showed generally a higher percentage of diseased fruits in the former than in the latter. These differences are considered to be caused by the direct or indirect influence of pollen tubes upon the mycelia and not by the conditions of the tissues of stigma or the style, for the penetration of pollen-tubes would occur soon after the anthesis. It has been shown in the case of inoculation unaccompanied by pollination that a larger percentage of infection was given either in the inoculation immediately after the castration or in that of two days later, though the former gave a little higher percentage of diseased fruits than the latter (Table X). On the other hand, in the inoculation experiments accompanied by pollination, the inoculation before pollination also showed more infection than the one after it (Tables XI, XII and XIII). The influence of the pollen-tube, therefore, may be regarded as the more important factor than the age of blossoms in producing different results of infection between pre- and post-anthesis inoculation. The influence of pollen-tubes upon the growth of hyphae in the tissues of style may be different according as the kind of pollen is different. As to the relation between infection and self- or cross-pollination, the results showed that the cross-pollination influences more unfavorably in general to the hyphal development in the tissue of style excepting the result in 1930 (Table X). The averaged percentages of the affected young fruits obtained in the experiments were 85.75 % and 86.11 % in the case of self-pollination, 55.60 % and 79.72 % in the case of cross-one, respectively in 1929 (Table IX) and 1931 (Table XI). In 1930 (Table X), on tha contrary, the averaged percentage was rather lower (54.47 %) in the self-pollination and this diminution of percentage happens due to very low value (26.32 %) obtained in the case of post-inoculation. But, as for this decreases the larger number of abscissed fruits must be noticed in this class. Although the self-pollination my be a reason of these abscission, the infection itself is also considered to be another imortant reason, because the fertilization will not occur if the fungus attacks ovules in advance of it. It is unknown in this case how the hyphae and pollen-tubes influence each other, but the pre-existence of the hyphae or the pollen-tubes in the conducting tissue must yield unfaverable conditions for the growth of each reciprocally, considered the fact that the percentage of affected fruits is lowered according as the inoculation is delayed after the pollination, and that is higher in all the cases of pre-inoculation. Then it can be said that the mutual influence to which the hyphae and pollen-tubes are subjected is naturally variable according as the qualitative- (the kind of pollen) or quantitive-

(the number of pollen-tubes or hyphae) relation vary in the style. In every inoculation experiment, therefore, somewhat of inconsistency may be seen in the percentage of affected fruits, number of abscissed fruits and extent of the disease.

That the apple is self-incompatible in most varieties is a well known fact and accordingly self-pollination results in generally abundant abscission lowering the percentage set. In the data of Table XII it is shown that the self-pollinated blossoms gave barely 9.52 % set while the cross-pollinated ones set 88.89 % indicating that the abscission was severer in the former case. Since the growth rate of the pollen-tube is in general believed to be larger in case of cross-pollination than in self there may be handicap against the hyphal growth in the former case even when pre-inoculation was made not to speak of the pre-pollination. However, even in the case of cross-pollination, if inoculation has occurred very early, the disease can not be avoided as the pollen-tube would have less effect upon the development of mycelium. The result as given in the Table XI, 3 (inoculated two days before cross-pollination) seems to have come from such reasons mentioned above. Then, what is the reason for the case given Table X, 4 (inoculated 24 hours after self-pollination) ? It may be rational to consider that, in this case, the diseased fruits were reduced rather a priori owing to abscission resulting from self-pollination rather than from the disturbance which the pollen-tube would cause on the hyphae, because the self-pollinated blossoms would generally absciss due to the decrement of nutrition so far as they do not grow parthenocarpically. In this connection it must be noticed that there are different phases of pathogeny, i.e., each infection of seeds, core and torus respectively. Such differences in the pathogenic phase are thought to depend closely upon the degree of development of the host, that is, the young fruit.

As already stated, the percentage of the disease is lower in the cross-pollination, but the most advanced phase of the disease, i.e., lesion on the toral surface (torus and core included) is observed rather more frequently in the case of cross-pollination. For instance, in 1929 and 1930 the percentage of diseased fruits which showed lesion on the toral surface averaged respectively 0 % and 25 % in the case of self-pollination, but 60 % and 96 % in the case of cross one. In 1931, though this relation was reversed showing 84.05 % in the self-pollination, but good many of 61.42 % are given in the cross one.

That the abscission and differences in the pthogenic phase might have depended on the relation of the development between the hyphae and the

pollen-tube may be interpreted by assuming the following four cases, that is:

(a) the mycelium attacks all the ovules at the same time with fertilization, or in advance of it,

(b) some ovules are fertilizaed while the others are infected,

(c) the hyphae infect the ovules some time after all or most of them have been fertilized,

(d) the receptacle (including ovary) develops parthonocarpically without fertilization and early infection takes place.

Such a case as (a) or (b) would usually occur if there were pre-inoculation, faster growth of the hyphae than the pollen-tube, or partial infection and pollination, i.e., some styles are penetrated only by the pollen-tube, while the others only by the fungus, and (c) would occur only when pollination goes ahead of inoculation and (d) in the case of well nourished blossoms which are inoculated early but not pollinated. In the case of (a) most young fruits tend to absciss showing the lesion in seeds or core, while in the case of (b) the lesion woud appear on the toral surface or in the core. These relations are seen in the results shown in Table XII, Pl. III. Figs. 1–5, and Pl. IV. Figs. 7–9, that is, the blossoms pollinated after earlier inoculation (Table XII, 1, 2) or those inoculated earlier after pollination (Table XII, 6) show a larger number of the toral- or core-lesions and those which have not shown the symptoms were abscissed. From these data it may be concluded that the case of (b) is brought about when the interval between pollination and inoculation is short, whereas (c) occurs when it is long. Since the hyphae seem to invade with difficulty in the case of (c), the infection can not occur or is reduced at least according to the delayed inoculation. Good examples are shown in (Table XII, 7, 8, 9) and (Table XIII (6, 7, 8). As already mentioned in Table X (1, 2) and Table XI (5) it is evident that infecting the toral surface is possible if the receptacle (ovary) develops parthenocarpically without being abscissed. Thus the case of (d) may be interpreted.

The severe occurrence of abscission was presumed to be the reason of the diminution of disease happened in the case of self-pollination (see Table X (4)). This just correponds to the case of (a). The disease, however, seems to be generally reduced in the compatible cross-pollination. In this case the growth of hyphae may be checked by the vigorous growth of pollen-tube, or otherwise a higher pathogenic phase (affected torus or core) may occur, the case of (c) being presumd. The pollination after inoculation gradually reduced the disease according as the former had been delayed, in 1931 (Table XII). On the contrary, in the same experiment

in 1932 (Table XIII), it was rather increased, but accounting for that since there was more abscission in the former than in the latter, the result in 1931 may be after all interpreted as corresponding to (a), while that of 1932 to (d). In 1932, indeed, the blossoms seem to have developed parthenocarpically being well nourished as there were both higher temperatures and more sunshine than in 1931 during the blossoming period. Since the growth rate of pollen-tubes is said to be greater in higher temperature the much less interference of the pollen-tubes with the hyphae may be expected in the delayed pollination after inoculation than in the earlier pollination, and accordingly the percentage of the disease seems to have increased in the former case, as the pollen-tube intervention has become less. That the disease decreases according as the inoculation has been delayed after the pollination, is indicated both in Table XII (1931) and Table XIII (1932). For an exception No. 9 in Table XII showed 21.43 % of the affected fruit in spite of the inoculation being done five days after the pollination, this perhaps may be due to imperfect pollination because the infection ocurred only in seeds and all affected fruits were abscissed.

If the stigma is the main entrance for the fungus some applications of protecting treatments to the stigmatic surface should be necessary. If such is applied, however, it is not only spores that are affected by the spray-materials, but also the pollen grains. BEACH (12) reported Bordeaux-mixture added to culture medium in proportion to 200 : 1000 injured the germination of pollens. NIETAMMER (90) who carried out experiments on the toxic action of arsenic, sulfur and other insecticides which were added to sugar solution in the drop-culture upon the pollen-germination of 27 species reported that the preparates were partly without influence, partly more or less injurious and that no stimulating effect was observed. He, however, gave notice that the practical spraying should be avoided at the time when pollens are ripe and germinating. HOWARD (58) reported that the spraying of Bordeaux-mixture or lime-sulfur in bloom of the apricot yielded no injury. From the data given in Table XIV it is evident that Bordeaux-mixture and lime-sulfur are injurious to pollen germination. These spray materials applied on the stigma reduced the percentage set compared to the controls as shown in Tables XV and XVI. According to Table XVI, however, the average set of McIntosh, A. S. Pearmain, Jonathan and Ralls is 42.37 %, 76.74 %, 50.26 % and 76.78 % respectively, and yet these are thought to be economically tolerable even with the minimum of McIntosh. As shown in Tables XVII and XVIII the fungicidal effect is not so potent but that all except one plot (Table XVIII, No. 5) showed

about 50 % diseased fruits. It is, nevertheless, questionable if that minimal yielding was caused by the fungicidal effect, for many of the treated blossoms fell according to some unknown cause. The same may be said about Numbers 3 and 4 in Table XVIII. Bordeaux-mixture is believed to become gradually effective after its application contrary to lime-sulfur which is effective without delay. The reason why these toxic materials are less active on the stigma may be perhaps due to the influence of the stigmatic secretion. ISTVANFFI (62) observed that Bordeaux-mixture diluted ten times with grape juice was unable to inhibit the germination of spores of *Botrytis* and other fungi. He suggested that this must be important in practice.

Although the relation between vigour of tree and the disease has been already recognized among growers, such treatments as ringing or defoliation which directly control the nutrition of blossom-clusters neither increase nor decrease the percentage of disease (Table XIX). Since the effect of these treatments may be different according to the time of application this experiment must be repeated.

The results of inoculation experiment and field observation disproved the existence of resistant varieties. A. S. Pearmain, Ben Davis and Fameuse formerly have been said to be resistant, but both A. S. Pearmain (Table VI, No. 2) and Fameuse (Table XXI) showed considerable susceptibility. Ben Davis also is not resistant according to the writer's experience. In *Malus prunifolia* a negative result was observed as shown in Table XX, but afterward it has been observed to be susceptible. The observation made of 113 varieties and 9 species on the occasion of the epidemic in 1931 indicated that there was no resistant variety.

As shown in Tables XXIII and XXV the ascospore can also infect the stigma resulting in the young fruit-rot though the percentage was low or semetimes negative, but it has not been determined whether this was attributable to the imperfect technic of inoculation or due to some unsuitableness to stigma-infection of the spore.

From the data given in Tables XXIV and XXV it is evident that *Sclerotinia Mali* is able to infect certain pomes other than the apple, e.g., *Pirus communis*, *Pirus sinensis*, *Cydonia vulgaris* and *Mespilus germanica* by artificial inoculation on the stigma. It is, however, not easy to say why in our country the young fruit-rot has not been known among these pomes in natural condition. EWERT (35) states that *Monilia cinerea* alone is not able to infect blossoms or stems of the drupes, but also that *Monilia fructigena* is parasitic on them if it is inoculated artificially, the disease caused by the latter being unknown because the new spores are not yet

formed during the blossom-period of the hosts. He concluded that this is the reason why blossom- or stem-blight caused by *Monilia fructigena* is not found in natural condition, notwithstanding the possibility of its artificial infection. In Aomori Prefecture or Hokkaido leaves or blossoms open in the succession of cherries, plums, Japanes pears, European pears, apples quinces and medlars. Since either the leafing or the blossoming of those fruit-trees other than the apple, the quince and the medlar are already over just at the time when the ascospores are ejected, or the conidia are disseminated respectively, and further since the quince and the medlar open their blossoms far later than the apple, it might be taken that this sequence is the reason why the disease caused by *Sclerotinia Mali* in those fruit-trees other than the apple has not been found in natural condition.

(2) Anatomical studies

a. *Behaviour of pollen-tubes*

The writer's observation of the course of pollen-tubes from stigma to ovule agree with the records of OSTERWALDER (94) and ASAMI (7). Contrary to the general belief NAMIKAWA (89) observed with the Yellow Bellflower apple that the pollen-tube goes along the outer surface of the style and after reaching the base of the style, the pollen-tubes enter into the cavities which extended between the insertion of the styles, and next further into the central cavity. Subsequently the pollen-tubes turn the direction of growth toward the suture of the carpel margins, where loose tissue exists. The pollen-tubes grow through this tissue into the ovary core. This author stated also that he had observed partially and completely isolated cells adjacent to the path of the pollen-tubes. The writer has never seen such pollen-tubes penetrating into the stylar tissue nor those tubes exist in the stylar cavity which is made of five styles communicating with the central cavity, nor also, the isolated cells along the path of the tube.

In the writer's experiments the rate of pollen-tube growth was observed to be a little slower in self-pollination, but it reached the style-base as well as in cross-pollination. In many specimens of cross-pollination (e. g. IIIE) the pollen-tubes were early near the funicle, whereas in the self-pollination (e. g. IIC) few pollen-tubes were found in the seed cavity. These facts suggest that the growth rate of pollen-tubes might be generally less in the latter case as EAST (36) recognized. Abnormal shape of the tip of the pollen-tube in the self-pollination was observed only in one

example (Pl. IX, Fig. 39). Swelling of the tip of the pollen-tube is observed in both cases and this may be cosidered as a shape necessary for tubes in order to get some mechanical force when they split down between cells of the conducting tissue (Pl. VI, Figs. 21 and 24).

The writer often treated the conducting tissue with ruthenium red, but no distinct pectine reaction was observed along the path of pollen-tubes, nor was any morphological change also. In the case of certain styles long after pollination the tissue become less stained, the cortical parenchym is filled with brown oily droplet-like substances and the conducting tissue itself becomes withered giving rise to large longitudinal splits. In brief the pollen-tube may secrete some enzymes for the absorption of nutrients from surrounding tissues to grow downward but it would be water mainly to be absorbed.

b. *Behaviour of hyphae*

The stigmatic infection of the *Monilia* fungi has been hitherto reported by a number of workers as already mentioned, but the reports that have described the anatomical researches of this problem are not known except those of WORONIN on *Vaccinium* (149) and SCHELLENBERG on *Cydonia* (166) and *Mespilus* (108). According to WORONIN both pollen-tubes and hyphae of *Sclerotinia Vaccinii* are found in the style proceeding in parallel two or three days later when the stigma is inoculated and pollinated at the same time. The pollen-tube searches for the micropyle and enters the ovule as soon as it proceeds into the ovarial cavity, while the hyphae soon begin to propagate vigorously contacting fast to the placenta or creeping on it, then they intrude between the ovules or often penetrate through them branching, tangling and anastomosing. SCHELLENBERG (106) showed that the hyphae of *Sclerotinia Cydoniae* which have penetrated into the style via the stigma usually attack the egg cell at first along the same path as the pollen-tubes go down and subsequently spread over integuments, ovary walls and receptacle. Similar observations were made in the case of *Sclerotinia Mespili* which attacks the blossoms of medlar (107). The writer's observation in the apple *Monilia* agrees exactly with SCHELLENBERG's but the latter has not written concerning the mycelial passage. The fact that *Monilia* hyphae often anastomose each other has been reported (149). In the preparates of cross sections of the conducting tissue into which pollen-tubes alone have penetrated it is observed that there is merely a round, deep stained, solid mass of protoplasmic contents, if sectioning has been done at the tip portion of the pollen-tube. We, therefore, can know whether

the hyphae have penetrated or not in the conducting tissue simply by the presence or absence of such mycelial passage. The reason why such a mycelial passage apperes as the characteristic streaks in the conducting tissue of style may be understood under the hyphae dissolve the middle lamella secreting some enzymes and absorb water or nutrients, consequently the cells adjacent to their path become compressed and on the other hand the hyphae themselves enlarge their vacuoles as they grow up. If the inoculated style is treated with ruthenium red pectine is shown along the mycelial passage in a comparatively deep red stain, while in the case of pollen-tubes that reaction is not distinct excepting in the tube wall. Although it should not be considered that the hyphae secrete only an enzyme such as pectinase, it may be admitted by the phenomena as seen in the so-called mycelial passage that the hyphae exert some destructive action against the cells adjacent to the path of them. However, as we consider how the hyphae behave in the conducting tissue, it should be noticed that they proceed down straight through the tissue without any branching after they have entered the stigma exerting no destructive action against the tissue beside their passage. From this point of view the *Monilia* hyphae in the style may just correspond to 'Anpassungstypus' which, according to KÖHLER (69), grows either passing or staying as 'Euphagen'. Then, the *Monilia* fungus in this case may be said to manifest higher parasitism because it grows without directly killing the host. WORMALD (142) and others reported that the styles of apple blossoms infected by *Monilia cinerea* BON. become browned in 7 or 8 days after inoculation and this browning proceeds gradually from the stigma until at last it reaches the pedicel or the axis, of course passing through the toral (ovarial) part. They, nevertheless, do not give anatomical evidence. It is, accordingly, obscure from their experiments, too, whether the hyphae have proceeded killing the whole tissue of styles from the stigma to the pedicel, or whether they have attacked only the mycelial passage. The hyphae of apple *Monilia* as stated above never do branch and attack the tissue beside the mycelial passage till they enter the seed cavity, as far as the writer observed. Even after they have entered the seed cavity the hyphal branching is not profuse they go down along the placental surface without attacking the adjacent cells. As SCHELLENBERG stated that one may observe the hyphae passing through the micropyle and attacking the egg cell in certain ("glückliche") specimens, the writer also has observed the hyphae passing through the micropyle without exception and entering the nucellus. On the contrary direct penetration of integuments has never been observed. Thus it must be concluded

that the behaviour of the apple *Monilia* hyphae in the tissues of pistil agree completely with that of the pollen-tubes. Although VEH (132) reported that he had certainly found chalazogamy in the Schöner von Boskoop apple, porogamy is common in apples and the writer has never recognized chalazogamy or other such fertilization.

Having observed the state of the mycelia spread over the tissues of funicle or micropyle as shown in Figures 31 and 32 it may be assumed as if the hyphae would infect in due succession of the funicle and micropyle without always first invading into the embryo sack or the nucellus. In the writer's many sections, however, the mycelium is observed already in the nucellus before it has spread over the tissue of the funicle or micropyle. That is, in the sections made three or four days after inoculation, the mycelium is always seen in the micropyle or the nucellus, while the tissues of the funicle or other parts are not yet destroyed. Therefore one can not but recognize that the fungus proceeds aiming at the nucellus (embryo sack).

The behaviour of the fungus in the fruit stalk and the cluster axis is evidently limited by the development of mechanical elements in the tissues, but the relation is quite complicated.

There is also evidence that the apple *Monilia* fungus attacks the nucellus of European and Japanese pears passing through the same course as in the apple, though the development of mycelium was slight. From the results obtained in both anatomical and inoculation researches, it may be suggested that a certain strain of *Sclerotinia Mali* would be able to become parasitic on these pomes under favorable conditions.

c. *Relation between hyphae and pollen-tubes*

Anatomical studies on the behaviour of the hyphae and pollen-tubes after they have entered the stigma shows that the two reach the ovule in the first place through exactly the same course. It should be, henceforth assumed that the prosperity of each of the two has to do closely with the outbreak of the disease and the abscission of blossoms and young fruits. Whether the hyphae grow up further or not after they have entered the embryo sack or the nucellus, depends on the development of the host tissues (torus and ovary). Now, having traced the hyphal behaviour after they have entered into the tissues of pistil the observation may be divided of itself into two categories. It comes under the first category that the hyphae stop their growth without spreading beyond the infection of embryo sack, nucellus, funicle or placental tissue, while under the second category

that the hyphae further continue their growth spreading over the toral tissue until at last the symptom of the young fruit-rot is shown outside.

In the first category, on account of the destruction of the ovule itself, or of the disturbance of fertillization, the hyphae stop their growth since the ovarial and toral parts cease to develop, as the supply of water and nutrients is discontinued. In this case young fruits fall down usually as the abscission layers develop. That is, one can not make a distinction simply by the outward appearance between this condition and the common abscission phenomena of young fruits.

In the second category the hyphae continue to grow accompanied by young fruit development where fertilization or parthenocarpic growth must have occurred. The young fruit of Janathan BB (1) collected 48 hours after inoculation on May 29, 1931 measured 5 × 3 mm. in dimension, while that of Jonathan EE (1) collected 192 hours after inoculation on June 6 attained the size of 6 × 4 mm., therefore, 1 mm. increase in diametre was shown in the latter which, however, had been invaded by the hyphae through the ovarial and toral tissue showing characteristic symptom on the fruit skin. Since some of the ovules of EE (1) were forming embryos without infection, the normal portion of the ovary and the receptacle was yet developing. It may be assumed that the fertilization of ovules other than affected by *Monilia* should be necessary in order to occur the typical young fruit-rot of apple, because the outbreak of the symptom can not be conceived unless it is presumed that the causal fungus grows only in the developing fruits which was already fertilized.

It is a known fact that, the apple normally wants pollination and fertilization for fruit setting while in a very few cases parthenocarpy is occasionally possible. As the result of development of young fruits, supply of water and nutrient should be continued in favour of the fungus development. SCHELLENBERG (107) reported that the sclerotium of *Sclerotinia Mespili* are formed only in the affected fruits which were infected after fertilization, but no conidium on them. When the infection had taken place in advance of fertilization, the fructification is contrary to the former case. He concluded from these observations that the nutrient supply for young fruits must be regarded as the limiting factor in sclerotium formation. In the writer's view, however, either conidia or sclerotia of *Sclerotinia Mali* are formed on the affected young fruits regardless of pre- or post-inoculation on the stigma, though the sclerotia formation in the case of pre-inoculation is less frequent. When the infection takes plae in advance of fertilization, most young fruits would absciss without showing any outward

lesion, both sclerotia and conidia being seldom formed. On the contrary, in the case where even one ovule has fertilized without infection or if all the ovules have been attacked very soon after the fertilization,[a] the development of young fruits will continue as well as that of the hyphae until at last the symptom will appear on the surface of young fruits.

In brief, the above connection may occur because of the difference between the growth-rate of the hyphae and the pollen-tubes, and it is evident that this difference is based upon both environmental and their mutual influence. The growth-rate of pollen-tubes is generally greater at first and gradually becomes slower, whereas that of the hyphae tends to be reciprocal. WORMALD (147) reported the same fact about the hyphal growth of *Sclerotinia cinerea* weich attacks pear, referring it to the supply of nutrients. Even in the normal material of apples usually the number of the pollen-tube which assemble to the seed cavity or the funicle are comparatively few in spite of the fact that many are found in the style.

As already proved in the inoculation experiments the disease percentage in the case of inoculation 24 hours after pollination differs from that of the reciprocal cace. For instance, comparing RPBI, RIBP, and RIP in Table XXVIII it will be found that the time needed for the hyphae to reach the funicle or the nucellus varies. That is, the time required in RPBI (post-inoculation) is twice that in RIBP (pre-inoculation) and even in RIP (simultaneous inoculation) the time required is less than that in RPBI. The time required for the pollen-tube to reach the funicle or the nucellus could not be ascertained excepting in RPBI, but this was due to the fact that no pollen-tube had been found beside the style in RIBP and RIP. Accordingly it may be considered in these last two that the pollen-tube were impeded their growth by the hyphae. In the case of pre-inoculation and simultaneous inoculation generally in most sections pollen-tubes have not been found in the seed cavity, funicle and micropyle, while quite the reverse was true of the hyphae. Although in the case of inoculation accompanied by self, cross and open pollination, i.e., IIC, IID, IIIE, IIIF, IVG, IVH and AA-EE (Table XXVI), the pre-pollination (IIC, IIIE and IVG) generally showed a longer time required for the hyphae to reach the funicle or the ovule. AA-EE showed the shortest time (48 hours), but this lot must be regarded as pre-inoculation because the blossom had been inoculated pre-anthesis and exposed to open pollination. On the other hand, however, IID, IIIF and IVH which are pre-inoculation required 96-

a) The writer was unable to determine the limit of that time. It is obscure from what stage of the embryonal development the hyphae is unable to enter the embryo sake.

144 hours, i.e., over twice that of AA–EE. These differences in the growth rate of the hyphae seem to depend on several factors such as variety or weather condition.

The growth rate of pollen-tubes is higher in cross pollination as stated above. In most sections of pre- cross-pollination (Table XXVIII, IIIE) good development of the ovule or other tissue is observed, while few hyphae are found in the nucellus. A few hyphae which reached the funicle develops poorly and they are stained weakly.

It is conceivable that all developments of the pollen-tube whether self or cross pollination are affected by temperature, moisture or other conditions (EAST and PARK (36), DORSEY (31), KNOWLTON and SEVY (67)). There was found a considerable difference of temperature and precipitation during the blossoming period comparing 1930 and 1931 when the materials were gathered. Having compared RPBI (1931, Table XXVII) with IIIE (1930, Table XXVI) the growth of the pollen-tube of the former is 48–144 hours later than the latter, while on the contrary the growth of the hyphae in RIBP and RIP (1931) is average 96 hours against average 128 hours in IID, IIIF and IVH (1930) provided that the time required to reach the funicle is considered. That is, the growth rate of the pollen-tube and the hyphae is quite reverse in 1930 and 1931 respectively. In general any pollen-tubes are scarcely found in parts other than the style in sections when inoculation accompanied pollination: this is true particularly in 1931. For example, in IIIE earlier germination of pollens is found and also more pollen-tubes are seen in both styles and funicles. In the section of 72 hours after pollination the pollen-tubes are found at the funicle while the hyphae which proceed down the style are very few, having entered the seed cavity 120 hours after inoculation for the first time; none was seen in the embryo-sack.

In RPBI the pollen-tube entered the seed cavity about 120 hours after pollination, some ovules having shrinked. A few hyphae also reached the style-base 96 hours and the funicle 144 hours after inoculation. In certain preparate the pollen-tube was found at the funicle 216 hours after pollination, while the hyphae had entered the nucellus already 192 hours after inoculation, but there were very few hyphae entering the nucellus in these materials according perhaps to the retardation of hyphal growth due to the withering of the ovule. Further, comparing IIIF (1930) with RIBP (1931), in IIIF the hyphae entered the seed cavity 72 hours, reached the funicle 96 hours, and the embryosack 144 hours after inoculation respectively, but in this case the hyphae found in the embryosack were rare

even after 168 hours had elapsed from inoculation. On the contrary the pollen-tubes reached the funicle 48 hours and the nucellus 72 hours after pollination drawing near the egg cell. Many more tubes than hyphae are found in the style or at the funicle in these sections. Pollen-tubes and hypae proceeding in parallel on the placental surface or the funicle are also found. In RIBP the hyphae advanced a distance of 1.6 mm. from the stigma in 72 hours, spreading over the ovule in 120 hours. It may be perhaps 96 hours after inoculation that the hyphae entered the embryo-sack, though the ovule had shown a slight shrinkage. The pollen-tubes reached the average distance of 0.6 mm. from the stigma in 72 hours and it does not reach even the style-base in 216 hours after pollination. Many more hyphae are generally found in the style and the seed cavity showing good development. Many infected ovules are found in most of these sections.

From the comparison mentiomed above it is known that the growth rate of the pollen-tubes and hyphae are closely related to the climatic coditions, i. e., low temperature considerably retards the process of the pollen-tubes, while it effects the hyphae less.

Further the participation of tree vigor, i.e., nutritional condition of the host tissue upon the development of the pollen-tubes and hyphae will be considered. As stated above TJO, TJJ, GJC, GJS and TOJ (Table XXVII) were experimented in the incubator (20 C) and the greenhouse (about 25 C).

Having compared TJJ and TJO (20 C) with GJS and GJC (about 25 C), it is seen that the growth rate of the pollen-tubes, however, is always the same in both cases, showing the minimum time required to reach the funicle to be 66 hours (TJJ, TJO) and the maximum 138 hours (TOJ). In every section the pollen-tubes are found clearly up to the five styles or their base, but their behaviour after that is obscure, the pollen-tube being found in the seed cavity only in the case of TOJ. Since the materials used in these experiments are cut branches put in the beaker, somewhat abnormal nutrition could not be avoided. In fact, most of the ovules in these materials were already shrinked and every tissue showed less staining within 72 hours after the treatment. Accounting for these facts the slow rate of the pollen-tubes in these materials seems to be effected by either the decrement of the function of ovules necessary to draw pollen-tubes or that of the conducting tissue due to the difficulty in the normal supply of water and nutrients rather than to the temperature relation. It can not of course be said that fertilization does not occur in the case of cut branches, but so far as these experiments concern the writer could not find any

setting of young fruits on the cut branches used in spite of the adequate pollination. If the growing of the pollen-tube is caused by chemotropism the shrinkage of the ovule which has lost its normal function may of necessity result in an unfavorable condition for the growth of the pollen-tube. As EAST and PARK (36) suggested if the growth of the pollen-tube is caused by the mutual reaction existing between the pollen-tubes and adjacent cells, but not by parasitism simply, then supply of water and nutrients for the conducting tissue may act unfavorably upon their growth. It can be said safely that the condition of the pistil in cut branches compared with the normal one becomes unfavorable for the growth of the pollen-tube with the lapse of time from cutting. The hyphal growth, on the contrary is relatively faster than the pollen-tube even in the cut branches, for example, the hyphae entered the nucellus in advance of the shrinkage of ovules earlier than 72 hours after inoculation. But the hyphae themselves also were found to stop their growth without spreading over other tissues according as the ovule became shrinked (TJO 5, Pl. VII, Fig. 33). In some preparates which showed delayed shrinkage of the ovule the hyphae reached the chalaza having broken the nucellus, and on the other hand they invaded to the bottom-tissue of the seed cavity or spreading over the funicle (GJC 5, see Chapter IV, 2). Generally, however, the hyphae present in the tissues other than the nucellus or the embryosack are less developed, and they are stained weakly. It is evident that the hyphal growth will be checked gradually, being starved by the shrinkage of the ovules and of the conducting tisssue.

From the observation described above it may be accepted that not only the fertilization is difficult but also the growth of the hyphae is retarded when the tree vigour, and accordingly the vitality of blossom-clusters, is abnormally weak. But since the hyphal growth is relatively faster than the pollen-tube as stated above, the hyphae are able to invade into the ovule in advance of its shrinkage and give rise to the disease so far as the condition of the surrounding tissues is favorable for the growth of them. For the outbreak of the disease two reasons may be suggested, that is, first that the hyphal invasion has some stimulative effect upon the ovule like that of normal fertilization, and second that the blossom grow up parthenocarpically. The second case may be possible though the first is questionable. In the inoculation experiments with cut branches the typical young fruit-rot and axis-blight are often observed about ten days after inoculation. It is, however, hardly known whether the disease was aroused by the fertilized ovules which would stimulate the tissues around them to

grow or as the result of the young fruit having developed parthenocarpically.

The growth of the pollen-tube and the hyphae is not only effected by internal or external factors, but also it may be checked mutually. It has hot been determined whether or not the hyphae attack pollen-grains or tubes, but as seen in the preparate of BB (2) (see Chapter IV, 2) they often attach themselves to the pollen-tube. Granting that pollen-tubes and hyphae conflict directly, it is conceivable that they do interfere each other indirectly. Since the conducting tissue will become dried and contracted losing moisture as a great number of pollen-tubes or hyphae pass there the forerunner should leave an unfavorable field for the afterrunner regardless which of the two precedes ahead. But the hyphae in particular seem to impede the pollen tube-growth, the mycelial passage being easily built up. The hyphae become more vigorous and increase their growth rate as they run down the conducting tissue, consequently, though a few of the pollen-tubes go ahead, the hyphae often run over the former which would become rather disturbed in their own passage. For example, in RPBI (Table XXVIII) which is pre-pollination the hyphae reached the funicle in 144 hours although of the pollen-tube spent 216 hours to pass the same course. On the contrary, the slow rate of the hyphal growth in the case of IIC, IIIE and IVG seems to be the effect of the vigorous pollen-tubes which went ahead, that is to say, the pollen-tubes are also capable of blocking the path of the hyphae provided they exist in a considerable number under favorable conditions.

VI THEORETICAL AND EXPERIMENTAL CONSIDERATIONS ON PREVENTION OF YOUNG FRUIT-ROT AND AXIS-BLIGHT

It is important not only from the phytophathological view point, but also from the pomological one to review several factors closely related to the outbreak of the disease and its mode of infection. A certain control measure becomes necessary after all which depends particularly on general cultural methods when the research work in diseases extends beyond the scope of mycology and pathology, and in such a case studies on those factors or mode of infection must be as conclusive as possible in order that those cultural methods may be proper. Henceforth, these points entering into the control measure will be discussed in this chapter.

(1) Factors related to outbreak of the disease

a. *Blooming period*

In Europe it is mentioned in many reports that sour cherries are more susceptible to *Monilia cinerea* than sweet cherries. EWERT (35) suggested in this connection, that the pure timely moment plays an important role (see page 215). The writer accounts for the relation between the blooming period and the young fruit-rot from a somewhat different standpoint.

It is conceivable that the coincidence of the period of ascospore discharge of *Sclerotinia Mali* with the opening period of buds of the host plants has an important relation to the outbreak of leaf-blight. The blooming period also coincides with the period of stigma infection of the fungus which causes the young fruit-rot. It has been reported by MIURA (79) that the varieties whose buds open early like Red Astrachan or Jonathan are in general more susceptible to the blossom-blight than to the young fruit-rot, while those like Rawls which is a late bloomer are more susceptible to the young fruit-rot than to the blossom-blight. The writer's observations are in agreement with his opinion.

Concerning the young fruit-rot the extent of damage is different by year even in the same variety. For example, in 1923 the damage to Rawls was severe in some districts (Kuroishi Aomori Prefecture). On the other hand, in the suburbs of Hirosaki city both Jonathan and Rawls were severely damaged, while generally less damage was observed in the autumn varieties like A.S. Pearmain, Fameuse and McInsosh. In 1924, however, the damage was severer to Jonathan and McIntosh all over the province than to Rawls in which much less disease was observed. In 1925 the outbreak of both the blossom-blight and the young fruit-rot was observed less in every variety, but the latter was found to be somewhat heavy in the Rawls. A most interesting fact is that the damage is different according to the position of fruits in an inflorescence, i.e., whether fruit is in the middle or at one side. In 1924 this connection was examined in the orchard of the Aomori Agricultural Experiment Station. The data are given in Table XXIX.

TABLE XXIX

Relation between the young fruit-rot and position of fruit in the inflorscence (1924)

Varieties	Tree No.	Date	No. of diseased fruits	
			middle	side
Yellow Transparent	224	June 3	3	18
do	225	do	8	33
do	215	do	5	32
Total			16	84
McIntosh	777	June 3	7	37
Total			7	37
Jonathan	22	June 3	22	13
do	231	do	16	10
do	78	do	14	4
do	236	do	11	15
Total			44	42
Rawls	216	June 5	4	2
do	222	do	1	4
do	226	do	1	0
do	217	do	12	4
Total			18	10

In apple trees the number of blossoms in an inflorescence is 5 or 6 (one middle and 4 or 5 side), hence the side-blossoms are four or five times as numerous as the middle, and accordingly the more chance of infection should be expected in the former than in the latter. Therefore the following theoretical numbers were calculated for comparison as it is not appropriate to compare directly the above figures.

Varieties	Observed number		Calculated number		
	Middle	Side	Middle	Side	
				4 times	5 times
Yellow Transparent	16	84	16	64	80
McIntosh	7	37	7	28	35
Jonathan	44	42	44	176	220
Rawls	18	10	18	72	90

Looking over the above data, it will be seen that Yellow Transparent (early variety) and McIntosh (intermediate variety) had more chance of infection in the side fruits than in the middle, since the calculated number is approximately the same as the observed, whereas for Jonathan and Rawls which are both late varieties the matter is quite reversed, that is, the middle fruits are rather frequently infected. It is a well known fact that apple blossoms being to open at the center of the inflorescence. It is, henceforth, conceivable that the middle blossoms would be infected more in some instances or the side ones more in the others.

The fact that the damage is locally different based on the difference of soils, topography or weather condition is generally known. The year 1931 had very unfavorable weather conditions to healthy growth of apple and the outbreak of the *Monilia* disease was one of the severest. The serious epidemic in 1931 worked greater damage in the slope orchards than in the plain in Hokkaido as well as in Aomori Prefecture. Distinct difference was also shown in kind of soils, on sandy or gravelly soil the apple being attacked less than on clay or humus one. Beside these the writer observed in this year that the effect of planting distance, manuring and pruning likewise was discernible. It is interesting to note that in the usual year the small difference in the cultural conditions will not influence greatly the outbreak of the disease and even the quantities or qualities of fruits, but in an emergency of unfavorable weather conditions striking variations of the matter are presented. Although there may be various reasons for the local differences of the damage, the blooming period whether earlier or later is still one of the essential factors.

The relation of the blooming period to the *Monilia* epidemics is shown statistically in Table XXX, comparing the extent of damage with the date of full bloom which indicates the earliness of blooming and days in bloom

of three leading varieties.

TABLE XXX

Comparison of date of full bloom and days in bloom and extent of damage observed in Kuroishi from 1908 to 1925 (figures in parentheses show days in bloom)

Year	Date of full bloom and days in bloom			Blooming earlier or later	Extent of damage	Remarks
	A.S. Pearmain	Jonathan	Rawls			
	May	May	May			
1908	12	—	—	—	heavy	
1909	12	15	16	early	severe	
1910	11	11	18	early	light	
1911	11	11	18	early	light	
1912	20	22	24	late	heavy	
1913	21	25	26	late	heavy	locally
1914	8	10	18	early	heavy	
1915	26	28	31	late	severe	
1916	24	26	30	late	heavy	
1917	18	22	27	late	heavy	
1918	20	23	25	late	heavy	
1919	18(11)	17(9)	22(10)	early	light	
1920	19(13)	19(9)	21(7)	early	light	
1921	13(10)	13(9)	16(9)	early	heavy	locally
1922	18(10)	18(13)	22(11)	early	slignt	A.S.P. late Jonathan early
1923	18(14)	19(12)	24(7)	late	severe	
1924	18(19)	23(20)	29(14)	late	severe	
1925	17(12)	20(11)	23(11)	early	slight	Jonathan late
average	17	19	23			

The extent of damage mentioned in the above table is concerned mainly with the young fruit-rot. In Aomori Prefecture the blossom-blight has decreased in the orchards since 1920 or so and only the former disease

or the axis-blight has broken out in several epidemics. In Hokkaido there have been few records taken and accordingly a comparison through long years is impossible. But the epidemic seems to break out simultaneously as well as in Aomori Prefecture. For instance in 1908 the apple orchards near Sapporo were attacked severely (65), and the recent epidemic in Hokkaido in 1931, which has already been mentioned was observed in such a great extent as the present writer had not experienced before. The comparison of the extent of damage with the date of full bloom of the three leading varieties in Sapporo is shown in the following table.

TABLE XXXI

Comparison of extent of damage with date of full bloom observed in Sapporo from 1907 to 1909 and from 1926 to 1932

Year	Date of full bloom			Blooming early or late	Extent of damage
	A.S. Pearmain	Jonathan	Rawls		
1907	—	5/25	—	early	?
1908	—	6/ 1	—	late	severe
1909	5/27	5/26	5/30	early	?
—					
1926	5/31	6/ 1	6/ 3	late	?
1927	5/24	5/26	5/28	early	light
1928	5/23	5/24	5/25	early	light
1929	5/30	6/ 1	6/ 3	late	heavy
1930	5/26	5/25	6/ 1	early	slight
1931	6/ 8	6/ 9	6/14	late	severe
1932	5/26	5/28	6/ 2	late	heavy
average	5/27	5/27	5/31		

From the data given in Tables XXX and XXXI, it will be seen that the extent of damage is generally larger in the season of late blooming. After all, the reason for this may be assigned to the effect of low temperature during the blooming time as will be stated in the next article.

b. *Weather*

The relation between the weather conditions and the outbreak of various *Monilia* diseases has been discussed by many writers. Rains were said to be favorable for the growth of *Monilia fructigena*, but not for the dissemination of spores (135) and experience on *Monilia cinerea* indicates that much rain favors the outbreak of epidemics (1). Frost during bloom is said to make the infection of *Monilia cinerea* easy by weakening the host tissue (85, 119). In our country also KASAI (64) recognized the relationship between frost injury and the blossom-blight, and TAKAHASHI (128) stated that the damage caused by the young fruit-rot in 1913 was heavy in Sapporo and Yoichi because the low temperature in June favored the spread of the causal fungus. MIURA (79) recognized the promoting effect of aeration, temperature, and atomospheric moisture on the outbreak of the blossom-blight, but denied that of frost. Looking over the opinions of these authors they laid stress on the effects of meteorological conditions except frost injury mainly to the part of the fungus and not to the part of the host plant. The meteorological factors, however, influence not only the development of the fungus directly but also the disease-resistance of the host plant, and the synthesized effects act on the outbreak of the disease. In the present case, accordingly, the effects of weather condition must be considered from both sides, that is, one of which is upon the fungus itself, while the other is on the host plant.

The present author considers especially this point as to the effect of temperature. In the case of the young fruit-rot such effect seems particularly delicate. In the present the author considers that temperature has more influence upon the host plant than upon the fungus. As mentioned above there is trend towards increasing damage according to the delayed blooming. Such delay after all is brought about by low temperature. It is conceivable that the chances of infection may be increased by the prolonged blooming period, but the low temperature seems to effect rather greatly the setting or development of young fruits by disturbing pollination or fertilization.

Low temperature would often limit the activity of insect resulting in hindrance to the cross pollination, and consequently the fruit-growth is retarded, while the hyphal invasion is made easy. Since the causal fungus can attack only the young tissue of the host in nature, the infection would be checked if tissues become rapidly differentiated. In warm and sunny weathers the infection appears to be checked in consequence of high dif-

ferentiation of the cluster tissue being stimulated by the prompt accomplishment of the fertilization, while in cold and rainy weathers the matter is just reversed and these facts would usually be observed in the field evidently. MIURA (79) stated: "Air-temperature averages 10°–16° C. during late May and early June when this disease (the blossom-blight) breaks out" and "The fact that the infection would be retarded for a while if there comes cold or warm weather beyond the range of the above mentioned temperature after the disease had already appeared, was observed in 1911 and 1913, and I recognized this disease would be reduced at the temperature above 20°–30° C." TAKAHASHI observed likewise the activity of the *Monilia*- hypha which has been kept down for a while recovers again by low temperature and invades into the stem. The causal fungus, however, grows well in such a high temperature as 25°–30° C. as MIURA himself recognized, too. The writer found that both the young fruit-rot and the axis-blight occur at 20° C.. Retardation of the outbreak of apple blossom-blight by high temperature seems to be the result of the rapid differentiation of the tissue, but not the direct effect of the change of temperature. In every case, of course, the check of the infection should not be referred only to the high differentiation of the tissue, also the water deficiency due to excess transpiration from the tissue may be responsible. Rain and fog during the time of bloom have long been known as an important factor affecting the outbreak of the disease. Apple-trees are particularly susceptible to the *Monilia* disease in foggy districts (111). SCHELLENBERG (107) reported in medlar that even the fruit stalk become infected in wet weather. Similarly it is observed in the apple young fruit-rot. Some opinions have been given that too much rain rather checks the spread of the disease (135, 79), but in general the damage seems rather to increase when the precipitation is heavy. The comparion of the extent of damage with precipitation and its frequency as observed at Kuroishi and Sapporo showed that there is trend of decreasing damage generally in less precipitation, especially in less frequency, though the extent of damage does not always coincide proportionally with precipitation and its frequency. It is evident that the meteorological factors have synthesized effect. For example, in 1931 there were comparatively few rainy days, but there was a great quantity of precipitation together with low temperature (10.9° C average) throughout the blooming period. These latter two factors must have been responsible for the outbreak of the serious epidemic in that year. Frost in the blooming period of the apple-tree is not uncommon, but the view that it injures the tissue and facilitates the penetration of the fungus

may not be accepted so far as the data concern. Lowering of temperature near the frost point often accompanies a dense fog and retards the development of blossom organs without hindering the fungus growth, consequently a great damage is often resulted.

Wind also plays an important role in the outbreak of the disease by favoring the dissemination of the spores or by lowering temperature especially when the north-eastern wind prevails. It is known that the damage is light near buildings or windbreaks, whereas it is heavy in open orchards on the slope.

The relation between meteorological factors and the disease can not be well understood by considering only their effects on the causal fungus but not that on the host, because the latter being rather important.

c. *Tree vigor*

Certain growers often anticipate whether the apple tree in hand will be susceptible or not in the coming season according to the vigor of the shoot which they prune. In general the decrease of tree vigor due to deficiency in drainage or manuring is considered to reduce resistance to *Monilia* disease and this fact has been actually reported (50, 79). In accordance with the present author's observations, the trees which gave excessively heavy crops in the preceding season, or which have too dense crowns by insufficient pruning, or the trees planted too closely are mostly apt to be affected by the disease. Over-bearing of fruits weakens the vigor of the tree, and too close planting and too dense crown obstruct the penetration of sunlight and aeration. It is evident that these conditions are favorable for the fungus growth and unfavorable for the growth of apple tree.

A number of workers have recognized that fruit trees want a great deal of water and nutrients at the time of fruit setting (21). Whether the young fruit-rot will at last result in the axis-blight or not partly depends on whether the affected fruits absciss or not, and partly on the condition of cluster-axis after the infection. In a strong cluster not only the fruit setting is certain but the growth of healthy young fruits is accelerated, and since struggle for existence among young fruits of a cluster usually occurs, abnormal ones (unfertilised, less nourished or injured by disease or insects) are forced to absciss sooner or later. Though the hyphae have invaded into the axis in some cases further infection may be inhibited if the cluster vigor is strong enough. Although deficiency in pollination and fertilization may be the essential condition for abscission of blossoms or

young fruits, but even when no deficit has existed the abscission phenomenon occurs and lowers the percentage set if the nutrition does not suffice, especially if nitrogen is lacking (68, 54, 70, 71 and 9). The present author has often observed that available nitrogen which was given to the tree for the purpose of getting a high percentage of set resulted in preventing the axis-blight on the other hand at the same time.

It has been suggested that the abscission of blossoms in various plants is also caused by the water deficit (72, 25 and 53). CHANDLER (20) has given evidence suggesting that since the leaves have higher osmotic concentration, the water deficit should be greater for the young fruits, or the blossoms, than for the leaves. In sunny days at the blooming time when the young shoots and blossoms are flourishing, the wilting occurs more apparent than at any other time of the year. The present author has not made any special experiment about the range of water quantity of which the development of the fungus and also that of blossoms or young fruits are controlled, but in some orchards suffering from excessive drying the retardation or young fruit growth probably due to the water deficit has been observed. MIURA (79) found that at the time of the disease-outbreak if the atomosphere is excessively dry and the decrease of tree vigor is evident, the dressing of a small quantity of water is effective and reduces the damage, the tree vigor being recovered. The present author also experimented similarly and obtained the same result. Nevertheless, the interrelationships among the young fruit-rot, axis-blight and tree vigor are complicated, not being the subject to be interpreted simply by considering the respect of nutrition (see Chapter IV). As to the outbreak of axis-blight, therefore, the time elements in the abscission of the affected fruits and in the growth of the fungus should also be considered, that is, which occur antecedently, the abscission of the affected fruit or the invasion of the hyphae to the tissues of the axis. These circumstances are thought to be intimately related not only to nutritional but also to wearher conditions. These complicated points will be discussed in this chapter under section (4).

d. *Varietal resistance*

The facts about varietal resistance of apples to *Monilia* disease have been reported by several investigators. (142, 104, 37, 85, 50, 64, 80 and 79). The present author does not believe as stated above the existence of any resistant variety of apple judging from his field observation to the *Monilia* disease and from the results of inoculation experiments.

(2) Mode of infection of the causal fungus

The writer has commented in Chapter III upon the necessity of classifying the disease into two categories, one of which contains the leaf-blight and the blossom-blight while the other young fruit-rot and the axis-blight. The writer proved experimentally the stigma-infection of *Monilia* fungus, and he has revealed the behaviour of the hyphae after they have infected the stigma by anatomical studies. The pathological nature of the young fruit-rot has been made clear, differing entirely from the blossom-blight in the mode of infection. The protecting spray method applied generally to the *Monilia* diseases of apple in the past was effective only for the diseases belonging to first category but of no use for those of the second category.

As mentioned in Chapter II, no worker has ever described the symptoms of the blossom-blight and young fruit-rot in detail observing that each of them belong to a different category in the mode of infection. It is evident that the European blossom-wilt differs from our own in the mode of infection notwithstanding they have common symptoms. Beside most workers do not make a distinction between the blossom-blight (or wilt) and the young fruit-rot except WORONIN and SCHELLENBERG who have given the anatomical description of the young fruit-rot. MÜLLER-THURGAU (85) reported that 'Zweigdürre' breaks out nearly concomitant with anthesis while the infection may occur at or before the anthesis. ERIKSSON (31) stating that 'Blütendürre' became a common disease of the apple in Sweden quoted an observer's communication as follows: "In the variety Red Astrachan abundant blossoms were apparently very sound about one week before, on the contrary some spur clusters here and there on the branches began to wither suddenly just after petals fall, showing most of them having been killed." The state of matters shown in this communication is nothing but that of the axis-blight. ADERHOLD and RUHLAND (4) observed the formation of acervuli in laboratory 10 days after, in field apple trees 14 days after and in field pear trees 8 days after the stigma-infection respectively. WORMALD (142, 147) studying on the blossom-blight[a] of apples and pears reported that in the Prince Bismark apple blossoms inoculated on the stigma April 1st. needed 10 days for appearing the browning of 4 mm. of the style and 20 days after that the whole cluster was killed showing canker on the spur at last on May 5. Hence about one month was required from inoculation till the killing of the whole cluster. He also reported that the

a) Wilting of cluster and formation of conidia are consistent, but browning of stigma, style, receptacle and stalk in succession are never seen in our own blossom-blight.

pear blossoms inoculated on the stigma showed the death of the whole cluster in 12 days, adding that in nature this disease appears 2 weeks after anthesis.

In Japan, however, the blossom-blight appears 3 to 4 days after the leaf-blight has appeared without browning of blossoms themselves nor of the leaves. For the young fruit-rot the outward symptom does not appear within ten to fourteen days after stigma-inoculation, still less the wilting of the cluster, though browning of the stigma and a stylar part is already observed. The blooming period of the apple covers two weeks in the normal year during which interval the petals of most blossoms fall, which either develop into young fruits attaining size of 0.5–1.0 cm. in diametre, or abscis in various stage of development. The blossom-blight as WORMALD described, therefore, can not be observed at about two weeks after anthesis. BEHRENS (13) who obtained negative results in the experiments of stigma-infection of apples and plums reported that one part of blossoms abscissed owing to no fertilization while the other developed normally. This abscission might have been caused by failure of fertilization due to ovular infection. Again SCHELLENBERG, as quoted above, reported that in the medlar, killing of the whole cluster occurs if the stigma is infected before anthesis, whereas the clusters are safe if the infection occurred later after anthesis. But there is no reason in natural condition why the stigma-infection should occur before anthesis. According to the writer's view, therefore, these foreign workers seem rather to have confused the young fruit-rot and axis-blight as they are attributed in our country to the so-called blossom-blight in the gross, regardless of the pathogenesis. WORMALD himself (142) thought the disease of fruits to be rare, but in Wye, Kent, he observed infected young fruits just after setting. VOGES (133) reported the presence of a number of hyphae in the pulp of young fruits on May 16, which had been inoculated with the conidia of *Monilia* on the stigma of Schattenmorelle on May 4. SALMON (104) stated that the morbid changes of the wood proceeds sometimes via fruit, and sometimes and perhaps more frequently, from the blossoms. From these observations it is evident that the young fruit-rot exists in Europe, consequently the axis-blight also. Hence, considering the diagnosis of the disease and its time relation, the so-called Blossom-Wilt in Europe does not fall under blossom-blight as known in this country, rather it probably includes two more diseases, i.e., the young fruit-rot and axis-blight besides the blossom-blight proper. In brief, WORMALD and others without discriminating each phase of the continuously developing blossoms might consider various diseases under blos-

som-wilt regarding them as caused uniformly by the process of stigma-infection. Although the writer does not care whether thses diagnoses are proper or not, he can neither accept that the European writers attributed the main entrance of the *Monilia* fungus to the stigma nor that the investigators in our country believed incorrectly the epidermal infection in most cases.

The fact that the blossom-blight does not occur by direct infection other than the leaf-blight is shown by the results obtained in the inoculation experiments and is fully discussed in Chapter V already.

As far as the writer knows there is no report which claims the leaf as only entrance of the causal fungus in the case of blossom-blight. There is a consistent opinion concerning the infection of spurs or shoots that the hyphae invade into them through a petiole or cluster axis. In former researches of the *Monilia* diseases the problem concerning the mode of infection was laid aside and consequently a definite study in which each phase of the *Monilia* diseases is included has not been published. This must be the reason why the control measure for the disease has not been thoroughly worked out.

What kind of spores is responsible for the outbreak of the leaf-blight which is the forerunner of the *Monilia* diseases ? In this connection two kinds of spores may be considered, viz., (1) ascospores and (2) macroconidia[a]). SORAUER (122) stated that the primary infection always takes place by the ascospores. The apothecia appear ordinarily on the ground, but sometimes they are formed in the spaces between clods which had been heaped in a mound with weeds. According to BROOKS and FISHER (18) the *Monilia* fungi of almonds or cherries in the Pacific Northwest lift their apothecia to the surface of the soil from a depth of 12 cm., but the present writer does not know such a case. The sclerotia half buried or buried at least 1 cm. deep in soil seem to germinate more commonly. Growers in general are not acquainted with the apothecia of the causal fungus because not so many of them are found on the orchard soil every year. Several reasons for this can be considered. It may be one of the reasons that the formation of apothecia is greatly influenced by the moisture relations. DANDENO (29) reported that the *Monilia* fungus of almonds forms apothecia if wintered in grasses, while they are formed with difficulty if buried in soil being decayed or if exposed on earth being dried up. KASAI (64) has obtained the apothecia of *Sclerotinia Kusanoi* which he considered to

a) Concerning microconidia nothing is known except that HUMPHREY (61) succeded in germination of them.

be the causal fungus of apple *Monilia* disease from mummied cherries toward the end of May in Sapporo. TAKAHASHI (128) reported as follows: "On May 4, 1914 Mr. KOICHI KATSUFUJI has found in Yoichi several dozens of sclerotia under some apple trees wich suffered from the young fruit-rot, and the apothecia of the causal fungus were found for the first time." and "subsequently he also collected them in the vicinity of Sapporo, and again in 1915 several hundreds of apothecia have been collected in Yoichi." MIURA (79) succeeded in obtaining apothecia emerged from sclerotia formed in wintered mumnied fruits in the laboratory, but he might have no chance of finding them in the field. On April 27, 1931 the writer found the apothecia in the university orchard following the finding of Mr. KATSUMI KAWAI. In the year, 1932, the writer looked for them continuously under the diseased apple tree from early April, however, they did not appear even until April 30, so the ground surface was watered once a day because the germination of the sclerotia was retarded due to the dry weather. Thus on May 2 a dozen apothecia appeared in the watered plot. There was precipitation measuring 1.7 mm. after the midnight of April 30 and a few apothecia were also found in the control plot. The effect of watering in proper time may be quite favorable to the formation of apothecia. It was true that in 1931 precipitation toward end of April measured 42.7 mm. and heavy apothecium fromation was met with, while on the contrary in 1932 there was only 9.8 mm. rain during the same period and only far less apothecia were found. The number of apothecia thereafter collected in the above mentioned plot was 20 on May 3 and 4, and one or two daily from May 5 to 16. It is then concluded that the period of the apothecium formation is about two or three weeks between late April and mid May showing the peak at the end of April or early May. Since the leaf-blight was found for the first time on May 15 (1930), May 12 (1931) and May 14 (1932), it should be said that the period of spore-dissemination and of the disease outbreak are in close accord.

The apothecium ripens and becomes whitish in color two or three days after emergence. Ascospores are ejected from asci and dispersed by slight air current like white smoke in mass being visible to the naked eye. This phenomenon is often experienced by picking up clods, or by uncovering the Petri dish which contained some apothecia. The ascospore being coated with mucilagenous membrane sticks easily to other bodies. Concerning the infection caused by ascospores of Sclerotinia spp., WORONIN (149) reported that the hyphae germinated from ascospore penetrates through the epidermis of *Vaccinia* leaves or enters the host tissue penetrating betwen

two epidermal cells but never through the stomata. SCHELLENBERG (106) reported also that the germinated hyphae enters the epidermal cells of quinces penetrating the cuticle. The epidermal penetration of the hyphae, however, seems to be possible only with the younger leaf which mechanical tissues have not developed enough, as WORONIN pointed out. The writer has set up a plot where the trees are not sprayed for the purpose of causing an outbreak of the disease in the university apple orchard. Accorrding to every year's observation the nearer to the ground the branches are, the more severely they are attacked by *Monilia* disease, and this is shown in particular about suckers, leaves of which being almost diseased. In an extreme case even very young seedlings of the *Malus* plant are killed by the fungus, their every part except cotyledons being attacked. On May 2, 1932 the writer covered some developing leaves of suckers and spurs with paraffin paper bags and examined these on the 19th of the same month. The covered suckers or spurs showed very little leaf-blight while all the uncoverd were killed. Infection caused by the ascospore was also successful, that is, on May 5, 1932 the following inoculations were executed using the McIntosh apple in the greenhouse and *Malus Sieboldii* in the field: (1) on just opened leaves, (2) on the surface of developed leaves 1 cm. in length and (3) on the under surface of leaves the same as above. Infection occurred in (1), but not in (2) and (3). In view of these facts it may be said that the ascospore having germinated almost at the same time as buds open, the germ-tube enters the leaf tissue, and a characteristic symptom is exhibited according to the leaf-development. The reason why the leaf-blight is usually seen only on the spurs which have inflorescence as most workers have observed, is that on the apple tree the compound fruit buds open the earliest, being followed by transitory buds and the leaf buds being the last.

As to the wintering of conidia, GALLOWAY (43) reported that those of *Monilia fructigena* kept viability for two years. EWERT (35) studied the relation of the viability of spores to low temperature by infection experiment and concluded that conidia of *Monilia fructigena* lack the wintering power whatever the host may be, whereas those of *Monilia cinerea* are viable through the whole winter, but the hibernation of the latter has no significance in itself, because the conidia are formed newly in the next spring. According to MIURA (79) conidia of apple *Monilia* fungus are able to survive two winters becoming thick-walled and light brown in color when the environments are favorable. KASAI (64), on the contrary, recognized that even in August or September in the current year conidia of

Sclerotinia Kusanoi lose their germinating power. The writer often preserved conidia produced in nature on diseased cluster in Petri dish under dry condition and inspected their germination after one year at room temperature in 10 % sugar solution. The conidia were observed always to retain their original shape without showing any abnormality and they germinate. Inoculation experiments were also executed (Table XI), but no infection occurred.

The fact that the perennial mycelia wintered in fruits or twigs become the source of the disease by forming macroconidia newly under moist conditions in the following spring has been reported in Europe and America (116, 114, 77, 35, 13, 135, 42, 119, 152, 37 and 142). In this country TAKAHASHI (126) stated that although he has hot yet witnessed the conidia of the *Monilia* fungus produced on the diseased apple twig in the spring, he expects the discovery of them in the future. MIURA (79) also stated that in the spring many microconidia are produced in properly moist conditions. The writer does not know any other worker having recognized the new formation of the macroconidia on old diseased twigs. It is generally known that the macroconidia production of hibernated mycelia is frequent in Europe, whereas it is rare in America and Japan. Concerning this point the writer is much interested in ROBERTS and DUNEGAN's paper (101) which states that the American strain of *Monilia* cinerea would rarely form conidia on spurs or shoots, while the European one seldom produces apothecia, and these circumstances may be related to the environmental factors. According to WORMALD (142) cankers which had produced *Monilia* pustules in 1915 were labelled and all proved to be barren in 1916, no pustule being formed. Since the problem whether the hibernated mycelia in the diseased twig in the previous year would produce the macroconidia again or not is very important in view of the control measure, the writer has endeavored to ascertain this very fact but without success. The writer has examined several times the diseased fruit-axes or spurs which had been sterilized with 0.1 % corrosive sublimate solution, washed with sterilized water, and put on wet filter paper in Petri dish placed in laboratory or in an incubator at 20°C. No macroconidia formation of the *Monilia* has been observed but new growth of Fusarium, Alternalia, and other saprophytes was seen. FRANK and KRÜGER (42) reported that the twigs infected by *Monilia* are ordinarily contaminated by some yeasts, *Cladosporium* or *Phoma* and in case of mummied fruits it is contaminated by *Cladosporium*. VOGES (132) recognized *Cladosporium*, *Fusarium* or *Fusicladium* in the epidermal tissue of the twigs infected by *Monilia*. MAC-

CUBBIN (74) reported that the peach stem-canker is produced primarily by the *Monilia* and secondarily by *Leucostoma Persooni* TOGASHI (*Valsa leucostoma*). It is not uncommon to find the diseased axes or spurs in which other fungi or bacteria besides *Monilia* hyphae are spread. It may be, henceforth, reasonable to assume that new production of the macroconidia on the old diseased twigs is hindered either by the secondary saprophytes or by the climatic conditions in the early spring of northeastern Japan.

On the ground of the above discussion the writer should like to insist upon the opinion that the ascospore is responsible for the source of infection in the case of the leaf-blight. As to why the apothecia are not found so commonly in proportion to the extent of the disease, various reasons may be suggested. The number of the apothecia may be reduced by the sclerotia being buried deep in the soil on cultivation, being carried away with weeds or buried with fallen leaves, and still more, the dry weather brought out by less precipitation and strong dry wind in April and May hinder the germination of the sclerotia.

The macroconidia are formed on veins, petioles, blossom stalks and axes three to four days after the outbreak of the blossom-blight. The macroconidia are easily blown off by wind and perhaps also disseminated by bees or other insects in some cases as they are attracted by a characteristic smell, and the conidia attach to the stigma[a] which is the only entrance of the fungus in the period of blooming. Since the anthesis of certain varieties corresponds to the time of production of the macroconidia, they are carried to the stigma, germinating within a few hours, proceeding straight down the conducting tissue of the style and at last they enter the embryo sack along the same path of the pollen-tube. The fungus which disorganizes ovules, ovarial and toral parts invades at last into the axis through the stalk resulting in the occurrence of axis-blight. Thus the infection which belongs to the second category is to be concluded.

The writer made the hereinafter described observation in 1925 for the purpose of knowing the process of the transition from the young fruit-rot to the axis-blight. These results will be good reference to the control measures. Those are shown in Table XXXII.

a) The ascospores also can artificially give rise to the young fruit-rot as shown in Chapter III, but they seem to have fewer chances than the macroconidia because their formation takes place long before the anthesis.

TABLE XXXII

Rate of progress of disease and condition of affected fruits (1925); figures shown under dates are distance (cm.) from stalk-base to lesion

Variety	No. of inflorescence	No. of fruit	Size	Fruit	Observation		
					June 5	June 6	June 8
King david	A	1	0.65×0.51	diseased	2/3 of fruit affected	whole fruit	dried and fell
		2	0.24×0.43	healthy			developing
		3	0.46×0.42	do			abscissed
		4	0.46×0.42	do			do
		5	0.46×0.34	do			do
		6	0.51×0.36	do			do
	B	1	0.85×0.74	diseased	a part of toral surface affected	2.23	1.23
		2	0.92×0.80	healthy			developing
		3	0.58×0.53	do			do
		4	0.50×0.36	do			abscissed
		5	1.29×1.05	do			developing
	C	1	1.28×0.91	do			do
		2	0.70×0.49	do			do
		3	0.50×0.33	diseased	half of fruit affected	no change	no change
		4	0.83×0.67	healthy			developing
		5	0.81×0.65	do			do
		6	0.73×0.48	diseased	1.09	0.67	0.29
	D	1	0.79×0.63	do	0.24	no change	dried, fell
		2	0.96×0.77	healthy			developing
		3	0.95×0.73	do			do
		4	0.43×0.37	do			do
		5	0.52×0.43	do			do
		6	0.45×0.54	do			do

Rawls	A	1	1.33 × 1.01	diseased			half of fruit affected
		2	1.27 × 0.93	healthy			developing
		3	0.97 × 0.79	do			abscissed
		4	1.09 × 0.83	do			do
		5	0.85 × 0.54	diseased	2.15	1.72	1.00, fell
		6	0.81 × 0.56	do	1.44	1.26. fell	
	B	1	1.19 × 0.90	healthy			developing
		2	1.12 × 0.83	do			do
		3	0.98 × 0.74	do			do
		4	0.91 × 0.70	do			do
		5	0.55 × 0.35	diseased	1.15	0.98	reached the base, fell
		6	0.61 × 0.35	do	0.50	0.49	fell
	C	1	0.68 × 0.45	do	0.69	0.03	reached the base, fell
		2	0.88 × 0.63	healthy			developing
		3	0.88 × 0.69	do			do
		4	0.89 × 0.70	do			do
		5	0.86 × 0.70	do			do
	D	1	0.63 × 0.35	diseased	fruit, stalk and a part of		fell
		2	0.78 × 0.65	do	axis no change	no change	no change: but half of fruit affected on June 9.
		3	0.63 × 0.54	do	no change	stalk-base	half of stalk
		4	0.76 × 0.57	healthy			developing
		5	0.76 × 0.51	diseased	no change	no change	0.50, fell
	E	1	0.83 × 0.50	diseased	0.90	0.60	fell
		2	0.78 × 0.62				do
		3	1.09 × 0.88	do			developing
		4	1.06 × 0.80	do			do
		5	0.94 × 0.77	do			do
		6	0.74 × 0.62	do			do
	F	1	1.10 × 0.85	do			do
		2	0.46 × 0.33	do			do
		3	0.85 × 0.63	do			do

		4	0.82×0.64	do			do
		5	0.73×0.61	do			do
		6	0.72×0.59	diseased	1/3 of fruit	2.96, juice secreted	1.68
	G	1	0.73×0.49	do	reached the axis	dried, fell	
		2	0.88×0.59	healthy			developing
		3	0.80×0.60	do			do
		4	0.66×0.56	do			do
		5	0.79×0.59	do			do
		6	0.70×0.60	do			do
Jonathan	A	1	1.01×0.83	healthy			developing
		2	0.69×0.67	do			do
		3	0.77×0.62	do			do
		4	0.71×0.59	do			do
		5	0.93×0.75	do			do
		6	0.70×0.63	diseased	1.95	1.59	0.84, fell

Fron Table XXXII the average speed with which the lesion proceeds down the stalk per day was calculated as given in Table XXXIII.

TABLE XXXIII

Growth rate of hyphae

Varieties	Gluster	No. of fruit	Growsh rate per day
			cm
King David	B	1	1.00
do	C	6	0.40
Rawls	A	5	0.58
do	A	6	0.18
do	B	5	0.17
do	C	1	0.39
do	E	1	0.30
do	F	6	0.95
Jonathan	A	6	0.56
Average			0.503

As shown in Table XXXIII the fungus proceeds down the stalk at the rate of minimum 0.17 cm. and maximum 1.00 cm, a day, average being 0.503 cm. The axis-blight, henceforth, will be produced five or six days after the young fruit-rot was found when the stalk length averages 2.5–3.0 cm. Practically speaking, in Aomori the utmost outbreak of the young fruit-rot occurs between June 1 and 10, and the axis-blight occurs most seriously on June 8 or so, while in Sapporo these are each experienced about 10 days later. It has been said formerly that short-stalked varieties such as McIntosh or Jonathan are particularly susceptible to the axis-blight. The results given in Table XXXII, on the other hand, indicate that the affected fruits sooner or later absciss in spite of the hyphae proceed down the stalk, if vigorously growing fruits exist in the very same cluster. Therefore, in spite of the hyphae reaching its tissues, sometimes the axis-blight does not take place, depending mostly on the vigor of cluster. Since the weather condition in 1925 was good for the growth of blossoms or young fruits the growth rate of the hyphae mentioned above in Table XXXIII seems to be rather small. The hyphae would grow faster than this under favorable conditions for them.

According to the above consideration it would be understood that the writer's views concerning the mode of infection of the apple *Monilia* disease are quite different from those of other home workers. In consequence of this also some deficiencies in the control measure which have been practised formerly can be pointed out. The blossom-blight is easily controled by such chemical methods as spraying while in the cases of both the young fruit-rot and axis-blight the matter is different.

(3) Prophylaxis for the leaf and blossom-blight

Since the leaf-blight is caused by infection during the youngest stage of leaf growth and the ascospore is the main source of infection thereof, its prophylaxis wants naturally two sorts of treatment, that is, (1) the hygenic treatment of sclerotia and (2) the spraying with fungicides, while the blossom-blight will be controlled when the leaf-blight is destroyed.

a. *The treatment of sclerotia*

It is true that the selerotium-formation of the apple *Monilia* fungus is mainly recognized in affected young fruits, but the apothecia are often formed on leaves, petioles or stalks (Pl. IV, Fig. 12). The sclerotia are not always formed only in the mummied fruits remaining long on the tree which are very few in number. It is the safest way, therefore, to collect

all the diseased parts for burning. This treatment has of course been long recommended by most investigators.

As a considerable amount of moisture is required for the germination of sclerotia in producing the apothecia, only the sclerotia half buried or shallowly buried in soil are capable of germinating. Accordingly, cultivation of orchard soil in early spring or late autumn should be effective, in burying sclerotia deep in the soil (3). FRANK and KRÜGER (42) have recommended soil sterilization with lime. The present author once practised this but a conclusive result was not obtained.

b. *The spraying of fungicides*

Although such research workers as Aderhold, who supported the idea of stigma-infection only, denied the effect of fungicide sprayed during winter or at the time of bud-opening, others as FRANK and KRÜGER who believed in leaf-infection, reported that the spraying of Bordeaux mixture, milk of lime or petroleum-emulsion is effective at the time of bud-opening, but not in winter. All of the research workers in Japan seem to have recommended the spraying of Bordeaux mixture at the time of bud-opening and pre- or post-anthesis as the fittest chance, because they believed in the versatile mode of infection (epidermal infection).

The practical application of the spray-calendar that characterizes modern fruit growing has developed in Japan since 1918. One of the greatest benefits brought about by the application of the spray-calender to the control of diseases and insect pests is indeed the prevention of blossom-blight. In the days when spraying was not practised commonly, serious damage on apple culture due to blossom-blight was in no case rare, but nowadays its economical damage has been markedly reduced by the pervasion of the protective spraying, though the young fruit-rot and the axis-blight have yet been often serious. Since the blossom-blight is brought about only through the leaf-blight, the sterilization of the leaf surface is evidently rational, but this is possible only for the younger tissue at the time when buds open. Accordingly, the spraying must be practised early in the season, i. e., in the spray-calendar now in use the first (dormant or green-tip) and the second (delayed dormant) sprays are most effective. It has been clearly observed that the outbreak of blossom-blight depends on whether the first spraying (concentrated lime sulfur, Beaume 4.5°–5.0°) is applied or not. The reason why this spray is important is that it sterilizes not only the green-tip of just opening buds, but slso the soil surface where the apothecia germinated from which the ascospores are ejected. In the second

spraying the lime sulfur (Béaume 0.5°–0.8°) or the Bordeaux mixture (0.82%) are used. Since the young leaves opening just at this time are the most susceptible to the disease, it is self-evident that the spray is very effective. MIURA (79) reported that the blossom-blight was reduced to one third by spraying 0.6 % Bordeaux mixture after the leafing and at the pink stage. The present author obtained the following results of the spray experiment in Aomori Prefecture.

TABLE XXXIV

Preventive effect of fungicides for control of blossom-blight (1919)

Lot	Fungicide	Time of application				Damage	Remark
		1	2	3	4		
A	Lime sulfur (B. 5)	greentip					
	Sapporo-mixture		delayed dormant	pink	petals fall	almost nil	Jonathan, Ralls, Smith's Cider A.S. Pearmain
B	Control					above 80%	do
C	Lime sulfur (B. 5)	greentip					
	Sapporo-mixture		delayed dormant	pink	petals fall	almost nil	Jonathan
D	Lime sulfur (B. 3)	dormant					
	do (B. 1)			pink		80%	Jonathan
E	same as C					10%	Jouathan
F	Control					90%	Jonathan
G	Lime sulfur (B. 5)	greentip				10%	Red Astrachan
H	Control					above 80%	do

N.B.: Sapporo-mixture is 0.8 % Bordeaux-mixture to which sodium arsenite was added. A– the rented experiment orchard, Nishi Tsugaru County; B–a certain orchard at the same place as A; C– the leading orchard, Nozawa, Minami County; D– Mr. MAEDA's orchard; E– Mr. TANAKA's orchard; F– a certain orchard in Nozawamura; G– Mr. TSUSHIMA's orchard; H– a certain orchard, D, E, F, G and H are in the same place as C.

On May 24, 1919 the writer calculated the actual number of clusters attacked by the blossom-blight at Shirogane, Nozawamura, Minami Tsugaru County, the following is the data.

Tree No.	Jonathan, 11 years old, sprayed	Jonathan, 9 years old, not sprayed
1	64 a)	76
2	18	86
3	20	109
4	6	116
5	18	139
Total	126	526

a) Number of the diseased clusters calculated about three main branches situated in the under part of the crown. The number of healthy clusters was not calculated, but the damage was presumed as 10 % in sprayed trees and 90 % in the non-sprayed ones.

From the data given above the prophylactic effect of fungicides is evident. The writer, however, does not lay stress on the third spraying (pink stage), for the new infection would not occur at this time when the rosette-leaves are already well developed and the leaf-blight or even the blossom-blight is appearing. It is proper that the effect of spray in this stage should be considered to be rather indirect, that is, the invasion of the fungus from petiole to axis to be hindered by some reason.

It need not to be interpreted why the fourth spraying (petal fall) is meaningless for the control of the blossom blight having considered the mode of infection. In brief, the leaf-blight and blossom-blight may be controlled sufficiently in the economical sense, but the available period for control measures is limited within only two weeks from the bud opening to the leafing.

It is, of course, a valuable control method to cut off the old diseased shoots or spurs on which the hibernate mycelium forms the macroconidia newly, as European writers have reported. In Japan, however, such conidia formation is rare just as in the United States of America perhaps due to the climatic condition. Cutting off the diseased shoots or spurs, therefore, is thought to be not important for that purpose. But since the pruning method in Japan is to cut off the old spurs usually regardless of the disease, the above recommendation is naturally practised.

As for the relation between the blossom-blight and the weather or the tree vigor, it is very important as in the case of the young fruit-rot and the axis-blight. It has been often observed that the blossom-blight

will not occur, the infection being checked at the petiole if the cluster is vigorous, though the leaf-blight appeared, and similary in the case of warm and dry weather conditions the blossom-blight does not appear.

(4) Prophylaxis for the young fruit-rot and the axis-blight

Since the young fruit-rot is usually caused by the stigma-infection of the macroconidia which are produced from the blossom-blight or the leaf-blight, the hygenic elimination of the latter two must be a control measure for the former. It is the reason why the picking of the diseased parts and the spraying of fungicides are effective. As stated above the pink spray has no direct effect upon the blossom-blight, but it may devitalize the macroconidia or hinder their maturation. At first when the stigma-infection was recognized by SHIMA (1924), after having considered the stigma-sterilization he revised "the spray calendar" practised in Aomori Prefecture substituting "spraying in bloom" instead of "petal fall spraying" for the fourth spray. This has been thought generally effective, consequently it is now adopted by some growers. For the present consideration, however, spraying "in bloom" means nothing but the destruction of macroconidia which are formed rampantly during bloom. Some workers thought the spraying in bloom to be dangerous for the reason of lowering the percentage set (12), while others observed no practical injury (58). From the data given above (Chapter III, Tables XIV and XV) it is evident that to spray in bloom would not reduce the percentage set to an economically intolerable degree. One may recognize that once or even twice spraying in bloom would not disturb the pollination resulting in the abscission if he is familiar with the actual condition of the blossoming.

It may be possible theoretically to sterilize the stigmatic surface, but not practically. Henceforth, the control of the young fruit-rot and the axis-blight must depend on some means other than the spraying. The most valuable data for this purpose is provided by the progress of the young fruit-rot which is usually observed in the orchard (see Table XXXIII). The young fruit-rot and the axis-blight show marked differences in damage according to the weather- and cultural conditions. If comparatively low temperature and a high degree of mositure prevail during the blooming time the axis-blight would occur, since the fungus passes the fruit stalk promptly invading into the cluster axis, while if it is comparatively warm and dry the growth of the fungs is retarded in various stages, consequently most of the diseased fruits drop or even if the fungus has reached the

axis it affects only a small area of the tissue never resulting in the axis-blight. Since the axis-blight kills all the fruits of a cluster the economical loss from it is rather greater than from the young fruit-rot. The latter may not be feared theoretically in apple growing where one fruit per inflorscence is the fundamental principle in so far as it does not attack all the young fruits of the cluster. Considering from the fact that the young fruit-rot becomes the cause of the axis-blight and of the lowered percentage set the value of its prevention never falls behind that of the axis-blight, but since the control of the young fruit-rot seems to be difficult from the results described in Chapters III and IV, one would logically conclude that for the prevention of the damage the control measures should be rather focused at the axis-blight which from observations of its pathogenesis is thought to be comparatively easy of control.

If the fungicidal application is directly less effective for the control of the young fruit-rot, a mean which is reliable may be the overwhelming of the hyphal growth by the development of pollen-tube. EWERT (35) experimenting the relation between the age of blossoms or the fertilization reported that there is no relation, but it must be noticed that it was observed in self-pollination in his case. Planting of various pollinizers to facilitate the cross pollination is not only rational for the promotion of the fruit setting, but also for the prevention of the disease, because the fact that the young fruit-rot is markedly reduced if it is warm and sunny during the blooming time corresponds well to the fact that there is much more chance for cross pollination in such a case and pollen-tubes grow faster resulting in early fertilization. After all, however, whether pollination or inoculation will precede and whether the fertilization by pollen-tube or the infection by fungus hypha will follow are subject to chance, hence the effectiveness of control measures can not always be confidently expected.

On the contrary the writer finds more possibility for the control of the axis-blight, that is, all the measures for hardening the cluster, promoting the fruit-growth, developing the disease-escaping in the axis-tissue and making the diseased fruit itself to absciss, can be obtained in cultural practise. Although the meaning[a]) of these measures seem to be complicated apparently inconsistent, they may be simplified to the problem of "fruit-seting".

Nothing is more important for the grower than to obtain rational fruit setting (44, p. 483) and this question long has been the subject of research

a) It has been stated in Chapter IV that the fertilization, accordingly the development of young fruits is necessary for the disease to reach the toral surface.

work in connection with the pollination problem. MOLISCH (82, p. 223) stated that in quinces, apples, plums and other fruit-trees the abscission of young fruits is due partly to poor nutrition and partly to non-fertilization. The parthenocarpous apple can be obtained experimentally and it is found often in nature but it is evident generally from the former researches that fertilization is the most important factor for the fruit setting. There are some local or soil differences concerned in self unfruitfulness, for example, in Australia the Jonathan apple tends to be self unfruitful in rich soils but self fruitful in poor soils (40), or in the United States the same variety is said to be self fruitful everywhere (44, p. 509). But in general the cross pollination insures the fruit crop and some workers indicated that the number of seeds contained in developing fruits and in abscissed ones is different (52, 88). It is, however, evident from DORSEY'S (31) so-called third abscission (June drop) that fertilization does not always insure fruit setting.

There are various factors to insure fruit setting other than fertilization, but nutritional condition should be first cited. MÜLLER-THURGAU (84) indicated the need of carbohydrates having recognized increased fruit setting as a result of ringing. It has been reported in the apple that early leaf-fall markedly influences the percentage set (110). KRAUS and KRAYBILL (68) reported that each excess or deficiency of nitrogen in proportion to carbophydrates checks the fruit setting. MURNEEK and HARVEY (86) stated that the condition of nitrogen favorable for fruit-bud differentiation is different from that for fruit setting. It has also been reported that the fruits do not set well in vigorous young trees compared to old trees (98). It is true, however, as CHANDLER (21, p. 185) stated that under practical orchard condidions there seems to be no danger of getting enough nitrogen into bearing fruit trees to cause the blossoms to fall without setting fruit. MURNEEK (87) reported that the contents of nitrogen as well as carbohydrates increases markedly in the apple tree during anthesis. HOWLETT (59) working with the periodical change of carbohydrates and nitrogen content in the apple blossoms and young fruits recognized that the absolute quantity of nitrogen increases from blossom opening to petal fall. After that the fruits that set increased very markedly in size and withdrew pregressively greater amounts of the total nitrogen and of the various types of carbophydrates, while the flowers that were to absciss showed a decided loss of nitrogen after petal fall. This decrease consistently ranged from 28 to 49 % of the total amounts present in the flowers just after the petals fell.

Water deficiency is likewise responsible for the abscission of blossoms

or young fruits. COIT and HODGESON (25) reported about the fall of Washington Navel Orange caused by severe transpiration. HAAS (46) studying the abscission phenomena of the same fruit tree indicated that small fruits are more susceptible to water deficiency than the larger ones since the former have a comparatively greater surface area. HEINICKE (53) found that a young apple fruit which is cut in half soon abscisses, but it does not if water is supplied by means of suction pump or if a drop of sugar solution is put on its cut surface. On the contrary, he (52) earlier stated that young fruits absciss even if they are put in an atmosphere of excessive humidity. Practically, however, a case of excessive moisture is rare and generally water deficiency is much more often experienced. In Aomori Prefecture or in Hokkaido a dry south-western wind prevails during the apple blooming period and soils often become dry. Abscission phenomena have been recognized to be serious in such cases. In brief, it is evident that both organic and inorganic substances greatly influence the fruit setting in each phase from anthesis to the young fruit development, and both fertilization and normal nutrition must be regarded as important factors in the fruit setting.

What connection, then, may there be between fruit setting and the outbreak of axis-blight? The axis-blight would not occur according to whether the affected fruits (young fruit-rot) absciss or the cluster axis becomes resistant to the disease. In the former case it is evident that the axis-blight does not break out regardless of what stage of the pathogenic progress the young fruit-rot belongs to. As described above, however, since the fungus grows vigorously equally with the development of the toral and ovarial tissue if a part of the ovules is affected while the other part is fertilized. The occurrence or absence of axis-blight depends really on the earliness of the absciss-layer development. As there are five or six flowers in one inflorescence of the apple each being a member of the same cluster axis, the struggle for existence may there be expected among these flowers or young fruits. Therefore, if some fruit is early fertilized, but not infected it will grow vigorously monopolizing most of the nutrition which is sent to the cluster; consequently weak fruits or the infected fruits should be abscissed sooner or later through being starved. For the mechanism of abscission in such a case, NAMIKAWA (88) pointed out the change of osmotic pressure in fruit and axis-base as the direct cause of the young apple abscission indicating that in the normal fruit osmotic pressure is higer in the fruit and lower in the stalk-base while in the abscissed fruits the condition is quite reversed. CHANDLER (20) researching the relation between

the osmotic pressure and the incipient drying in apple leaves and fruits reported that since the osmotic strength in the leaf is greater than that of the fruit, when there is incipient drying of the leaves a strong suction towards them develops and a tendency for the water to move from the fruit to the leaves results. Since the difference of water contents among the diseased fruits, healthy fruit and leaves would become larger with the good nutrition or warm and sunny weather or both, it is conceivable that the abscission may be promoted, just agreeing with the observations in natural condition. However, although the tendency of abscission is great likewise in the poorly nourished cluster, the formation of absciss layer is only delayed; consequently the fungus after all invades into the axis. As a result of a period marked by low temperature and high humidity there is much deficiency in pollination and fertillization and even healthy fruits grow slowly; on the other hand the formation of absciss layer is more delayed while the fungus growth is less checked. Accordingly, then, the chance of the fungus invasion into the axis must be greater.

In the case when the axis becomes resistant to the fungus the blight will not occur as pathogenecity is no more retained though the fungus entered the axis tissue breaking over the barrier of absciss layer. Concerning the problem of the inherent nature of such a diseasse escaping development in the axis tissue must be treated as well as the problem of host resistance in future studies. Although a considerable number of researches regarding the disease resistance have been reported, the change of circumstances in occurrence of disease escaping in the same host has not been often reported. A considerable number of researches on the disease resistance of ripe fruits, however, have been published, especially concering the mode of infection observed from the morphological or physiological viewpoint (99, 26, 95, 131, 27, 28, 135, 138, 139 and 157).

It should be first considered whether the phenomenon of the retardation of fungus-growth is due to only the external influence or simply to the internal. First of all the effect of high temperature may be assumed as the cause of a check of the disease because the time when the axis-blight breaks out just corresponds to the period of increasing temperature generally in North-eastern Japan. As stated elsewhere, however, the writer believes that the effect of high temperature rather favors the host, but not the fungus, that is, the high differentiation itself of the host tissue is responsible for the disease escaping.

What is, then, the essence of the internal condition, i.e., of the so-called aquired resistance? Such substances as oxidase, tannin or oxalic

acid which have been considered as somehow related to the resistance, but as CURTIS (28) stated it is thought to be questionable that the qualitative or quantitative difference of these common constituents of the plant body is really an important factor in the resistance. WILLAMAN and SANDSTROM (138) found that in resistant varieties the contents of ash, CaO, nitrogen, ether extracts and titratable acidity were comparatively low while specific gravity, H-ion concentration and oxalic acid were comparatively high. But they stated that these were not sufficiently so as to constitute the limiting factors in the nutrition of the parasite. On this point DE BARY (30) made this statment: Wenn wir in der Constitution der Zellwände einen oder den Hauptgrund der Empfänglichkeit für Pilzinvasion resp. die Entwicklungusmöglichkeit des Pilzes finden, so ist damit nicht gesagt, dass nicht auch andere in der stofflichen Zusammensetzung der Nährpflanze gelegene differenzen fördernd oder hindernd mitwirken können, insofern sie die Qualität und Menge der Nährstoffe für den Pilz modificieren. Gerade für die individuellen Unterschiede der Empfänglichkeit durften dieselben jedoch wenig ins Gewicht fallen, zumal wenn man sich erinnert, dass der Pilz in sehr verschieden artigen Nährlösungen gedeiht."

According to the modern researches, however, it is considered that the fungus-growth is influenced most sensitively by the change in water amount or H-ion concentration among the various physiological conditions at least in the culture media. The following results of the measuring water content and H-ion concentration in the tissue of the apple blossom organs during its development were obtained. Jonathan and Rawls were mainly used for measuring. The blossom cluster collected in a definite stage was separated into styles, receptacles (ovaries). stalks and axes. Water contents were measured as usual, 15 gr. of each material were ground in mortar, adding 50 cc. of destiled water to it and quickly suctioning and filtering through filter paper fibers. The potential difference of the filtrate was at once measured by means of the chinhydron chain method.

TABLE XXXV

Water contents in each portion of the Jonathan cluster (1929 and 1930)

Year	Date of Measuring	Stage	Portion	%
1929	May 17	full bloom	receptacle (styles included)	85.7
	do	do	stalk	71.8
	do	do	axis	72.5
	May 23	young fruit	receptacle (styles included)	85.8
	do	do	stalk	77.0
	do	do	axis	71.7
1930	May 24	pink	styles	76.6
	do	do	receptacle	76.4
	do	do	stalk	78.4
	do	do	axis	77.0
	May 29	full bloom	styles	70.5
	do	do	receptacle	77.2
	do	do	stalk	71.7
	do	do	axis	74.4
	June 3	young fruit	receptacle (styles included)	75.2
	do	do	stalk	76.8
	do	do	axis	73.3

TABLE XXXVI

Change of pH value in each portion of blossom cluster (1930)

Varieties	Date of measuring	Stage	Portion	pH
Jonathan	June 7	full bloom	receptacle and stalk	7.62
do	June 8	do	do	6.06
do	June 9	do	do	5.80
average				6.49
Jonathan	June 10	young fruit	receptacle and stalk	5.31
do	June 17	do	do	4.06
average				4.68
Porter	June 7	full bloom	receptacle and stalk	5.71
average				5.50
Porter	June 10	young fruit	receptacle and stalk	4.12
do	June 17	do		4.81
average				
Malus 'Sieboldii var. arborescens	June 10	pink	receptacle and stalk	6.01
do	June 12	fullbloom	do	6.04
do	June 17	petal fall	do	5.70
do	June 23	young fruit	do	5.23
Jonathan	June 21	young fruit	receptacle	4.41
do	do	do	stalk	5.93
do	do	do	axis	6.33
do	June 23	do	receptacle	3.95
do	do	do	stalk	6.05
do	do	do	axis	6.40
Rawls	June 21	young fruit	receptacle	4.34
do	do	do	stalk	5.73
do	do	do	axis	5.80

The data given in Table XXXV show two facts concerning water contens, that is, (1) among the portions of the blossom cluster the receptacle (ovary) contains a comparatively large amount of water while the axis a small amount and (2) the axis and the stalk show the maximum amount of water in pink stage decreasing gradually after that and this tendency is much stronger in the axis. Considering from the fact that the causal fungus is only able to penetrate the young tissue particularly and proceeds by choice along the leaf-veins, the stalk, the axis and other vascular systems, it may be assumed that the water supply is an important limiting factor to the growth of the fungus. The reduction of water content in the axis may be regarded as a reason of checking the fungus-growth. DE BARY (30) indicated that as a cause of stonger resistance to the cell maceration in the old twing than in the young one the imbibed water contained in the cell wall in various quantities may be considered. He further stated that a swollen starch grain with water is more quickly attacked by diastase and even the resistant portion becomes at last infected by the fungus if it is continuously wet.

Partial and chronic change of hydrogen-ion concentration given in Table XXXVI showed two tendencies, that is, (1) the pH value is smaller in the young fruit stage than in the blossom stage, and (2) the pH value increases gradually in succession of the receptacle, the stalk and the axis. The fact that the materials collected without separating each portion showed reduction of pH value from pink stage to young fruit stage may be due to its rapid reduction in the fruit proper. It has been widely known that the fungus-growth is controlled by the hydrogen-ion concentration of the culture media. DUNN (33) experimenting the effect of various acids and salts upon the growth of *Sclerotinia cinerea* concluded that hydrogen-ion is not always the main cause of the toxicity of various acids which have effect on spore germination and mycelial growth, having found that though the addition of NaOH changes pH from 3.8 to 4.0 or 5.2 or a little more without real injury, and in the case of phosphoric acid and sulphuric acid the optimum fungus growth was attained between pH 2.8 and 3.9. YAMAMOTO (156) found in his cultural experiment with *Sclerotinia Mali* that the range of pH for the development of this fungus is from 2.9 to 7.3, and there are two optima at 3.0 and 5.0. It has been proved in culture media that the difference in resistance is based on the difference in reaction, but in spite of the endeavors of a number of workers it is not conclusively proven in living host tissues. There are only E. F. SMITH and QUIRK (1926) who proved that such relation exists in *Bacterium tumefaciens*

(KÖHLER). It may be hardly possible to regard a greater pH value in the axis tissue (Table XXXVI) as the main cause of the disease resistance in the cluster. That the fruit proper in each portion of the cluster is far more suitable for the development of the fungus than the axis is conceivable. So far as pH-relation is concerned it is very interesting that the fruit is the most suitable substratum for fungi at all stages following the blossom stage. It should be noticed that the pH value of the fruit proper decreases rapidly with its development, while in the stalk and the axis any marked change of pH value dose not occur. The writer does not think to interpret the essence of the disease resistance only as a result of change of water content and H-ion concentration.

From the morphological stand point two kinds of the protecting tissue served for the disease resistance may be observed in the axis. One of them is the development of cortical and wood fibers and the other is the formation of wound callus tissue at the boundary of the lesion. NAMIKAWA (88) found that the mechanical elements in the blossom stalk begin to develop at about the end of the blooming period and the cell wall thickening of the sclerenchym fiber in the normal fruit appears to be a little stronger than that of the abscissed one. He further recognized that there is a development of the stone cells which stand compactly at the outer half of the sclerenchym-fiber and that the group of the cortical fiber is arranged discontinuously in ring form, the stone cells filling the space between them. Thus the cell wall thickening and its lignification do not begin only in these stone cells but also in the pith cells. The development of these cortical fibers and stone cells is completed at the end of June or at the beginning of July. The development of these mechanical elements in the axis is more pronounced showing high differentiation gradually from the anthesis to the petal fall. It is necessary for the hyphae to pass along the vascular tissue or through a point where the fibers are not well developed, in order to enter the inner fundamental tissue or the pith, breaking through these barriers. It is evident that the fungus invasion becomes difficult according as the tissues of axis differentiate more highly, consequently the hyphae attack the cortical fundamental tissue only vertically or tangentially. The wound callus tissue develops regardless of the direction of the hyphal invasion enveloping the lesion until at last there appears a distinct demarcation separating the lesion and the healthy tissue. In the axis-blight, however, it can not be proven a priori whether the callus development or the check of disease was the cause or the result as far at least as the anatomical observations were concerned. The development

of the disease resistant character in the axis seems to be simply understood if considered from morphological aspects, but since the causal fungus directly attacks the adjacent stalk of the comrade fruit proceeding along the sub-epidermal layer as soon as it enters the axis, the development of such mechanical elements will be of no use to prevent the fungal invasion. Accordingly the problem can not but look again for a physiological solution.

In brief, since it is a determined fact that the disease resistance in the axis develops in its growing process and so prevents the occurrence of axis-blight, whatever the nature of it may be, there is found no other practical control measure than depending on this aquired disease resistance. According to practical observation, however, all the rational cultural methods which prevent the abscission and so increase the percentage set have been proved to be at the same time effective for the control of the young fruit-rot and the axis-blight. It has been already mentioned that the rare climatic conditions in 1931 throughout Aomori Prefecture and Hokkaido gave the valuable data for the study on the *Monilia* disease. From these experiences and his own researches the writer should like to conclude that both the diseases can be effectively controlled.

VII CONCLUSION AND SUMMARY

1. Studies on the mode of infection of *Sclerotinia Mali* TAK. which inflicts the greatest economical damage upon the apple grower in Japan has been undertaken with special reference to the young fruit-rot and its prevention. The present thesis deals with the data obtained during the past nine years from 1924 to 1932 in both the Aomori Agricultural Experiment Station and the Hokkaido Imperial University.

2. The names of blossom-blight or young fruit-rot have been applied for the *Monilia* disease which attacks leaves, blossoms, young fruits, cluster axis and shoots. But inconsequence of having worked on the symptom and the pathogenesis in detail, it was found that the *Monilia* disease in general must be divided into four phases, viz., leaf-blight, blossom-blight, young fruit-rot and axis-blight, and further it was shown that the former two and the latter two are diseases which must come under different categories respectively considering from the mode of infection.

3. Inoculation experiments were executed on the basis of the suggestion that the young fruit-rot may perhaps be caused by stigma-infection for the reason that chemical control measures have been effective only for the blossom-blight but not for the young fruit-rot.

4. From the results of inoculation experiments on the different parts of blossom organs it was proved that the stigma is the main entrance of the causal fungus in the case of the young fruit-rot while it can not penetrate directly into the epidermis of the style, the receptacle and the stalk.

5. Having considered that a close intervention may occur between the pollen-tube and the mycelium, some inoculation experiments accompanied by pollination were undertaken. There is a tendency in pre- and simultaneous inoculation with pollination to result in a higher percentage of diseased fruits than in pre-pollination, and inoculation accompanied by self-pollination tends to give a higher percentage of the disease occurrence than that with cross pollination. In the case of open pollination, too, pre-inoculation tends to be more infective than post-inoculation. The disease percentage is decreased markedly when the infection occurs after the time elapsed from cross pollination.

6. The macroconidia obtained in cultures cause the young fruit-rot as well as the natural ones invading via stigma. The ascospores also cause the disease.

7. From the results of stigma-sterilization experiments it is shown that Bordeaux-mixture, copper carbonate and lime sulfur smeared on the stigma reduces neither the percentage set nor the disease.

8. Ringing and defoliation experiments do not show any recognizable evidence of positive effects on the development of the disease.

9. From the results of stigma-inoculation experiments and observations in field neither resistant variety of apple nor resistant species of *Malus* has been found. The stigma-infection of *Sclerotinia Mali* to pears, quinces and medlars are positive while negative in cherries and plums.

10. Anatomical studies on the behaviour of hyphae and pollen-tubes were carried out to prove accurately the fact of the stigma-infection of the *Monilia* fungus on the apple. The results indicate that the fungus invades into the ovule passing along just the same course of the pollen-tube and after that it spreads over every tissue. The hyphae germinated from the spores attached to the stigmatic surface enter the style between the papillate cells of the stigma, proceeding straight down the conducting tissue without branching and building particular mycelial passage, and invade the seed cavity through the suture of carpel margins. The hyphae, thereafter, grow down along the epidermal surface of the carpel or the placenta toward the funicle and at last penetrate into the nucellus passing through the micropyle. The hyphae in the embryo sac or the nucellus become thickened

and are stained deeply. The hyphae attack not only the ovule but also the ovarial wall, invading from the funicle into the mesocarp and the torus reaching at last the toral surface. Less amount of hyphae than pollen-tubes are seen in the seed cavity in the case of pre-pollination, while in the cases of pre- or simultaneous inoculation, on the contrary, pollen-tubes are less than hyphae in quantity. There are withered ovules in which well stained mycelia are present, and consequently it is evident that the stigma-infection of the causal fungus is the essential cause of the abscission phenomena of the young fruits.

11. The rate of elongation of the pollen-tube is not affected only by whether it is self pollination or cross one, but also by the climatic or nutritional conditions. The pollen-tube reached the embryo sac 72 hours after pollination in the blooming time of 1930 when the weather was warm, while it required over 120 hours in 1931 when it was cool. The growth of the pollen-tubes were slow and most ovules shrinked in the material obtained from cut branches preserved in the greenhouse or in the incubator. The growth of the hyphae is also influenced by the temperature, i.e., they invaded into the embryo sac 48 hours after inoculation in 1930, while it required 120 hours in 1931. In the incubator (20° C.) the hyphae invaded the embryo sac within 90 hours after inoculation, but it required 120 hours in the greenhouse (about 25° C.).

12. In the fruit stalk or the cluster axis the hyphae, proceeding straight, attack mainly the subepidermal layer or the pith. They destruct the parenchymatous cells, but not the epidermis, fibers and spiral vessels though they like to proceed along the vascular bundles. The hyphae in the cortical fundamental tissue of the stalk or the axis some time invade into the pith easily if the mechanical elements are not well developed.

13. Although it is evident from the symptoms and the results of inoculation experiments that the blossom-blight shift from the leaf-blight, but not by independent stigma-infection, this is especially verified furthermore by a leaf-inoculation experiment with the ascospore. The infection, however, does not occur on the leaves which passed even a little their very young stage no matter what kind of spores either ascospores or macroconidia may be used. The apothecium appears more in wet condition, and in Sapporo it is formed during 2 or 3 weeks from late April to early May corresponding to the leafing period of the *Malus* plants.

14. The macroconidia formation on the parts diseased in the previous year is not yet found is spite of careful observations in orchards for several years, and attempts also failed to get them formed in the laboratory. From

these facts it is suggested that the ascospores must be responsible for the leaf-blight.

15. According to the results of inoculation experiments and anatomical studies it is certain that the young fruit-rot would be resulted by the stigma-infection of the macroconidia or the ascospores. Since it is macroconidia that are produced in much greater numbers in the blooming time, the young fruit-rot must be caused mainly by them.

16. It is evident that the young fruit-rot and the blossom-blight belong to different categories in the mode of infection. That is, it is recognized that protecting spray will be effective to the blossom-blight, but not to the young fruit-rot.

17. The axis-blight is evidently converted from the young fruit-rot as proven from the symptoms and the inoculation experiments. There is no direct measure for the prevention of the axis-blight except to destroy the young fruit-rot, but its indirect prevention is possible by utilizing the abscission phenomena of the diseased fruits and disease resistnace in the axis tissue itself. That is, by employing all the cultural means such as to strengthen the axis and to increase the percentage set by forcing the development of fruits the damages will be surely reduced.

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Explanation of plates

Plate III

Young fruits of inoculation and pollination experiments in 1931.

Fig. 1. Simultaneously inoculated.

Figs. 2, 3, 4 and 5 show the diseased fruits pollinated 2, 3, 4 and 6 days after inoculation respectively.

Fig. 6. The diseased and healthy young fruits inoculated one day after pollination.

Plate IV

Figs. 7, 8 and 9. Show the diseased and healthy young fruits inoculated respectively 2, 3 and 5 days after pollination.

Fig. 10. The young fruit-rot in advanced stage : A- the disease has reached the stalk base ; B- the disease has proceeded to the white point ; C- depressed browing of the lesion is shown on the toral surface ; D- a healthy fruit.

Fig. 11. The young fruit-rot, the lesion at the core and a drop of secreted juice are shown.

Fig. 12. Apothecia collected on the 27th of April, 1931.

Plate V

Fig. 13. The blossom-blight on *Malus prunifolia* Borkh. (a) and the leaf-blight on the same species (b).

Fig. 14. The blossome-blight on the Jonathan apple and macroconidia are already formed on the affected leaf.

Fig. 15. The axis-blight on *Malus prunifolia* Borkh.

Fig. 16. Papillate cells on the stigma showing the conducting tissue with deep staining, TOJ (1). × 147.

Fig. 17. A hypha proceeding down the conducting tissue of the upper portion of the ovary; Rawls apple inoculated 24 hours after anthesis. × 147.

Fig. 18. Cross sectien of upper end of ovary; c– central cavity, cc– seed cavity, two cross sections of the hyphae are indicated by an arrow, GJC (5). × 147.

Plate VI

Fig. 19. Cross section of style-base; sc–stylar cavity which comunicates to the central cavity of ovary, m– cross section of mycelial passage, RPBI (3). × 147.

Fig. 20. Cross section of upper end of ovary; c– central cavity, s– a part of seed cavity showing some pieces of a hypha, m– mycelial passage, RPBI (4). × 147.

Fig. 21. A pollen-tube approaching the seed cavity and the upper portion of central cavity (p) are shown; e– entrance of seed cavity, IIIF (3). × 147.

Fig. 22. The hyphae accompanied by the empty pollen-tube are passing down the funicle surface, BB (2). × 147.

Fig. 23. Germination and penetration of macroconidia on the stigma, GJC (4). × 80.

Fig. 24. The tip of the pollen-tube in the conducting tissue; Porter apple, open-pollinated and collected 24 hours after anthesis. × 147.

Plate VII

Fig. 25. A mycelial passage in the conducting tissue, rampant growth of pubescence is shown in the stylar cavity, BB (2). × 147.

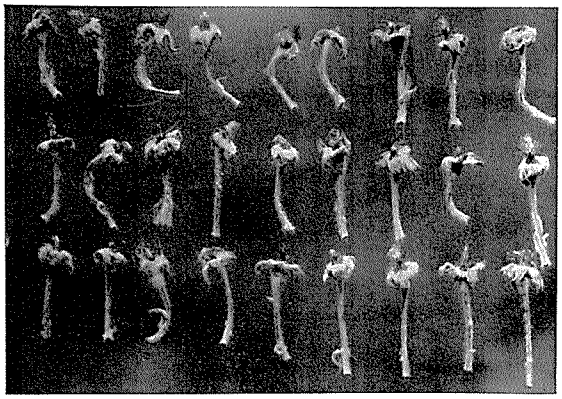
Fig. 26. Cross section of style-base showing mycelial passage (m), IB (5). × 147.
Fig. 27. Cross section of style-base showing the well stained conducting tissue before the hypha and the pollen-tube pass, TJO (1). × 147.
FiS. 28. Hyphae passing through the micropyle, some hyphae have already entered the bottom of the nucellus and the embryo sac. CC (2). × 147.
Fig. 29. The mycelium in the nucellus, GJC (4). × 147.
Fig. 30. Branching of the hypha in the seed cavity, TOJ (5). × 147.

Plate VIII

Fig. 31. The mycelium spreading over the funicle tissue, GJC (5). × 147.
Fig. 32. Disorganization of the internal integuments and vigorous hyphae in the nucellus, IB (7). × 147.
Fig. 33. A bundle of hyphae entering the seed cavity; one hypha has reached the ovule, TJO (4). × 147.
Fig. 34. The infected tissue of core; e–carpel epidermis, g– subsided cavity, p– primary vascular bundle, sc– seed cavity, EE (1). × 147.
Fig. 35. The mycelium passing from the pith region to the toral part. The cavity (x) communicates to the toral surface, EE (1). × 147.
Fig. 36. Longitudinal section of the margin of seed cavity showing the hyphae broken out of the carpel epidermis, EE (1). × 147.

Plate IX

Fig. 37. Hypha running through the vascular bundle, EE (1). × 147
Fig. 38. Cross section of young stalk; the hyphae which have attacked cortical layer (c) are about to invade into the vascular tissue (v) passing through the cambium. × 147.
Fig. 39. Cross section of style-base; abnormal tip of shrunken pollen-tube is shown, TOJ (6). × 147.
Fig. 40. Cross section of withered ovule showing well stained hyphae, TJO (6). × 147.
Fig. 41. Hypha and pollen-tube proceeding down in parallel at the style-base, the latter is stained well, BB (1). × 147.
Fig. 42. Longitudinal section of midrib of diseased leaf; vigorous hyphae are shown running along the vascular bundle. × 147.



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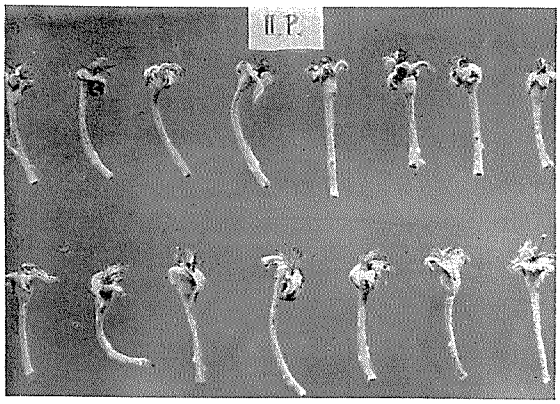

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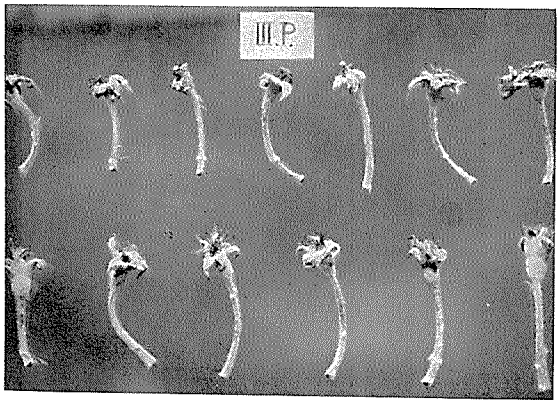

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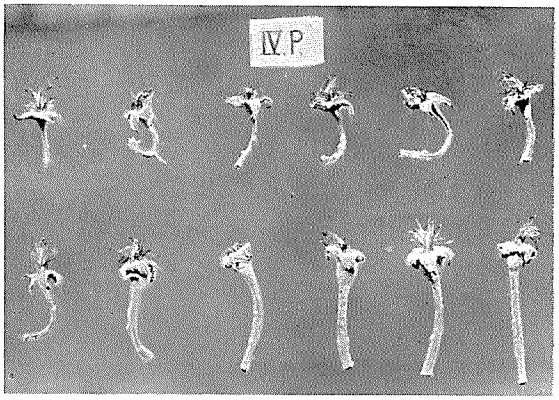

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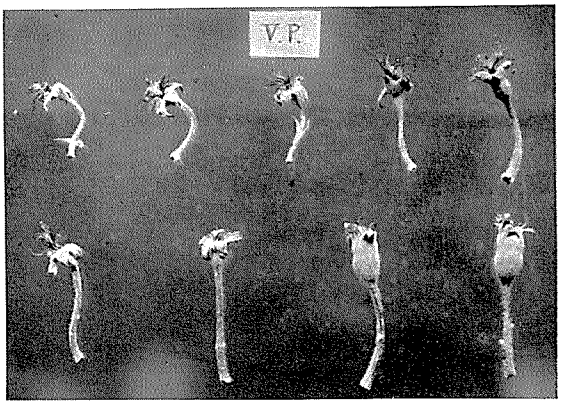
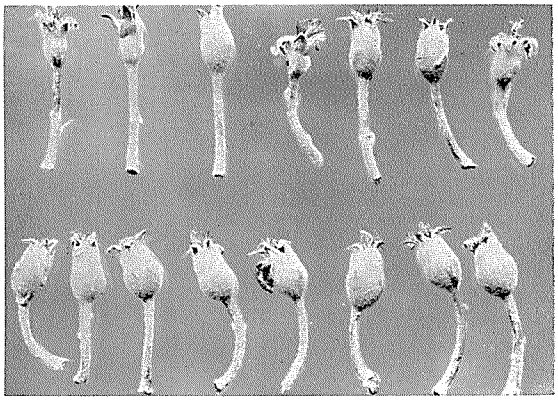


Fig. 5



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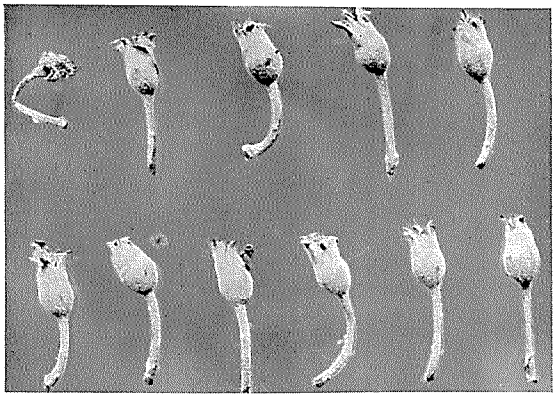

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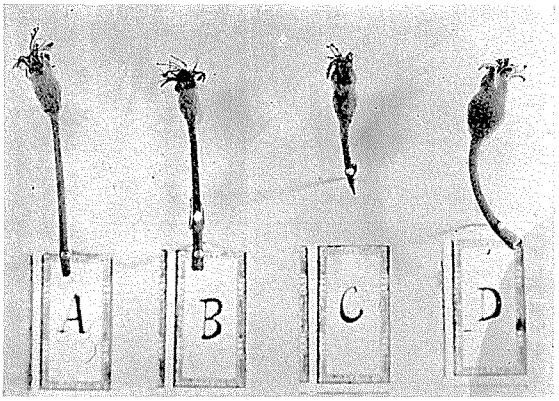

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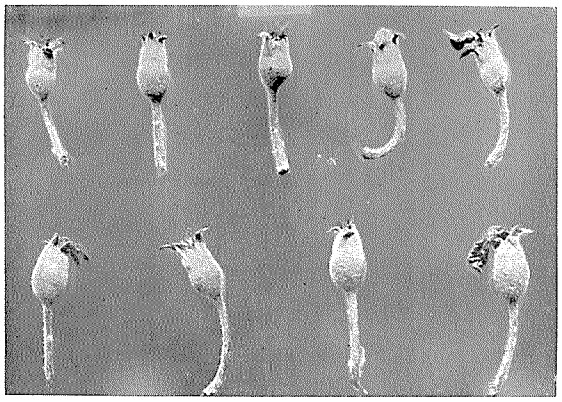

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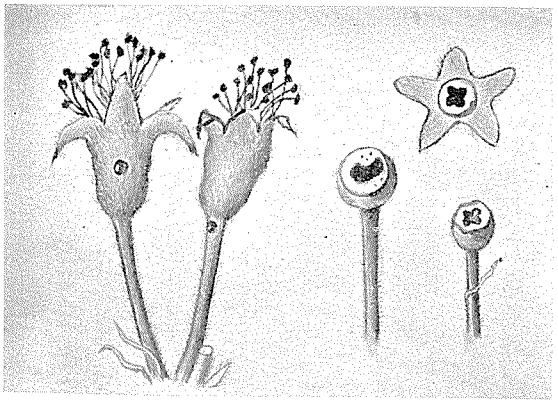

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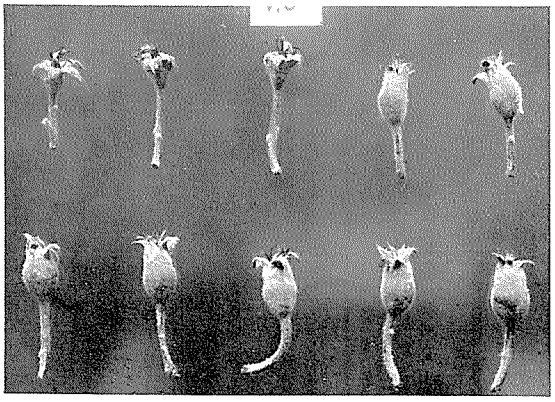
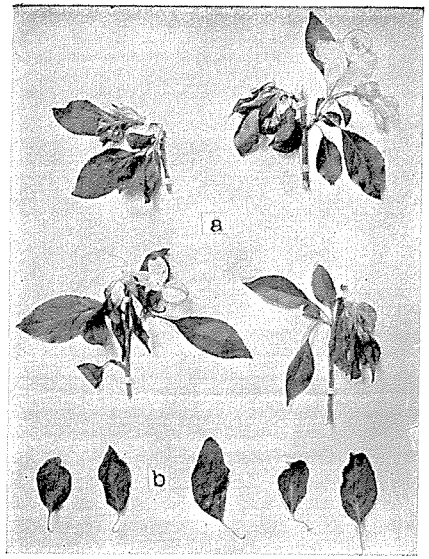


Fig. 9

Fig. 12



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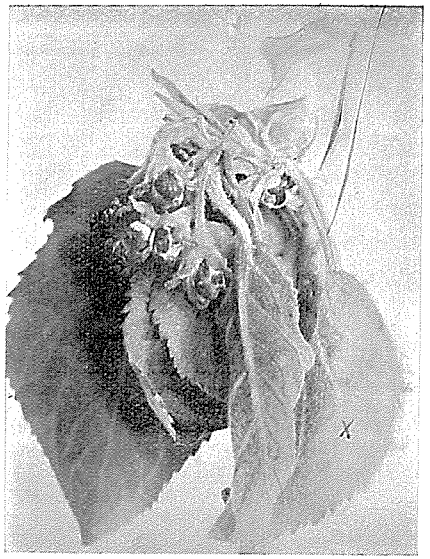

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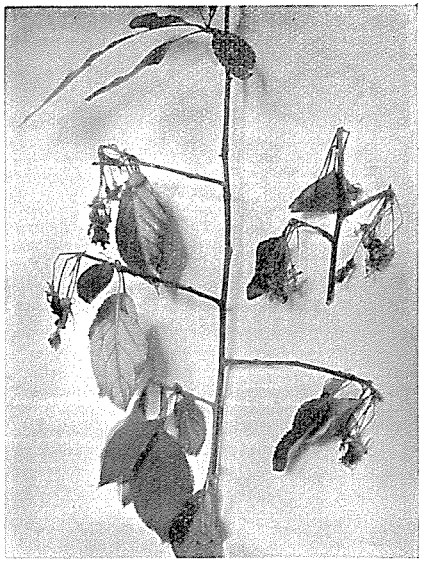

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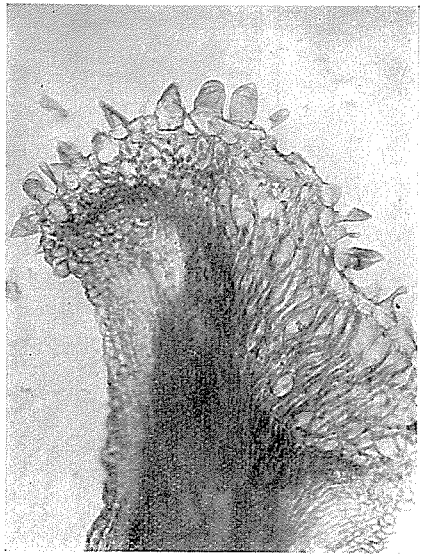

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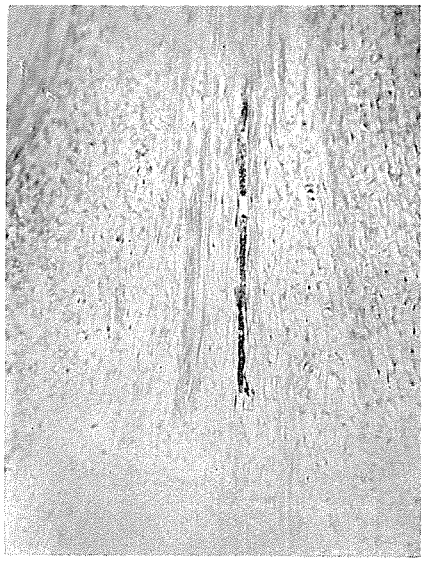

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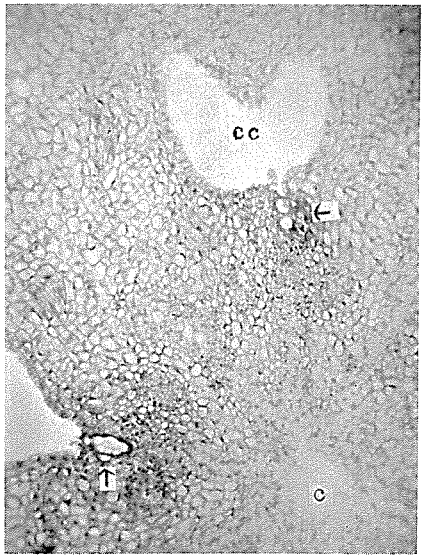

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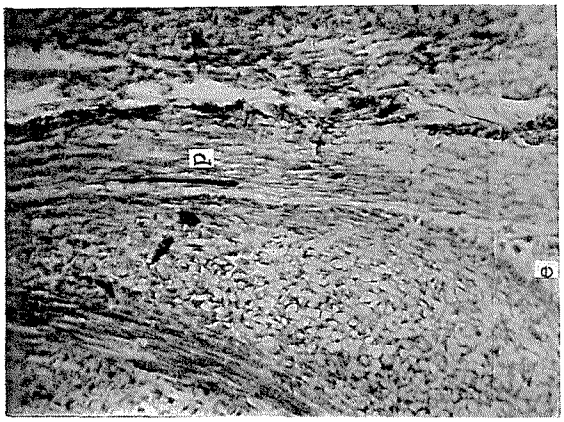

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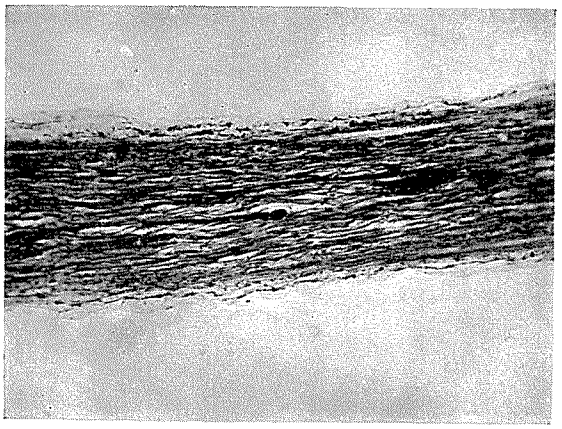

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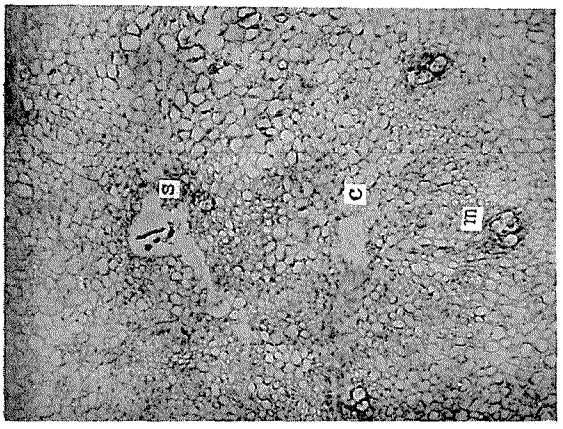

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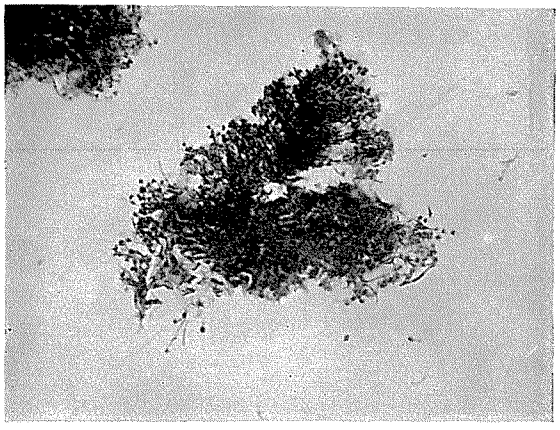

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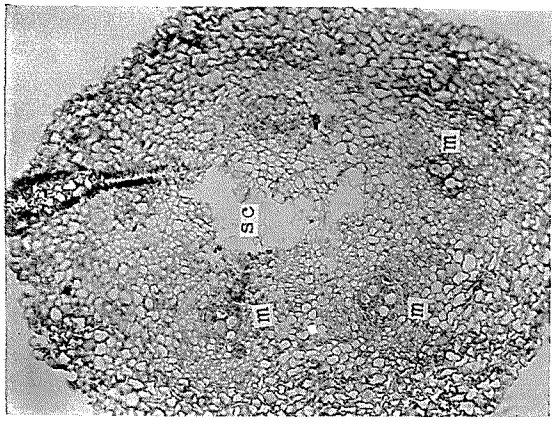

Fig. 19

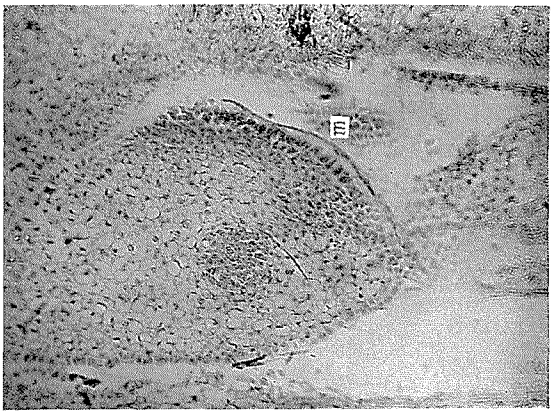

Fig. 22

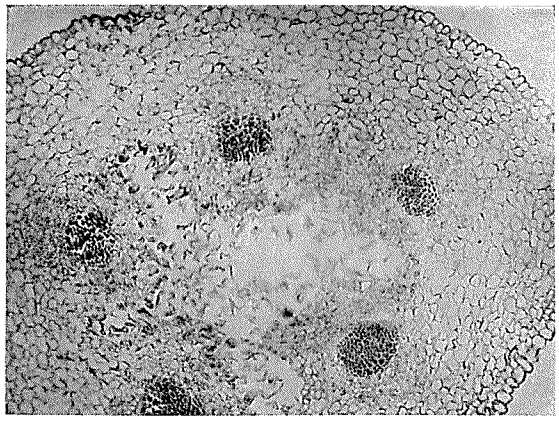

Fig. 27

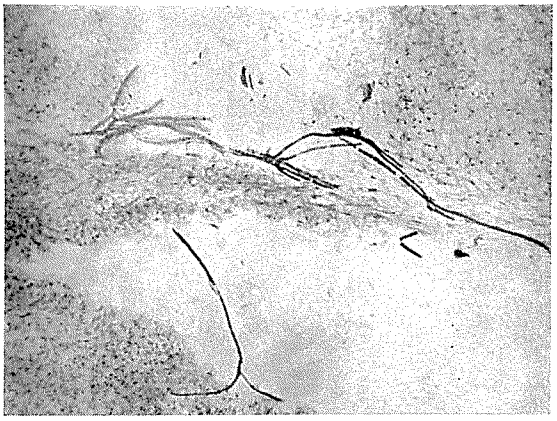

Fig. 30

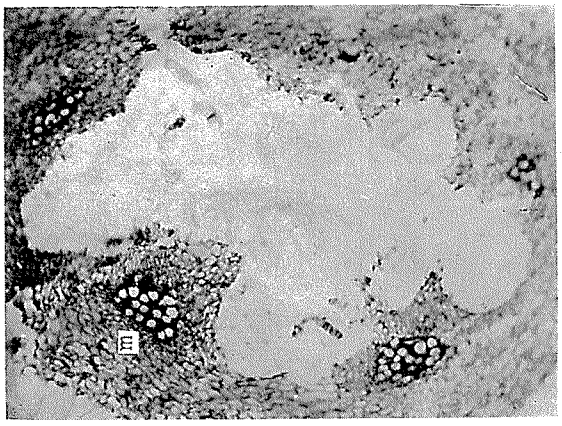

Fig. 26

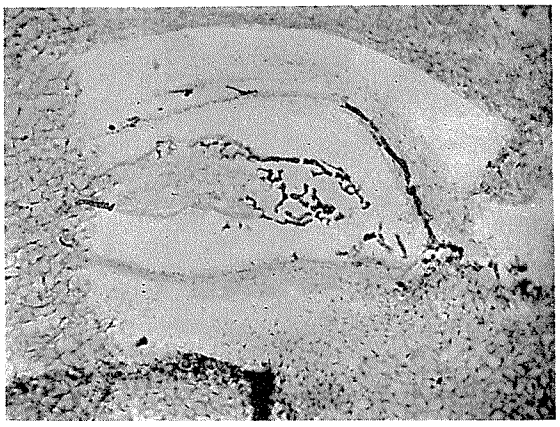

Fig. 29

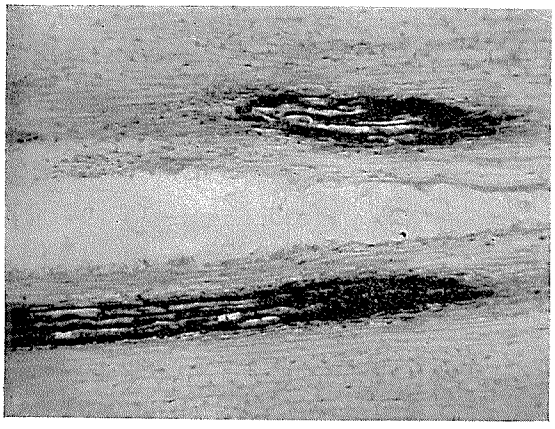

Fig. 25

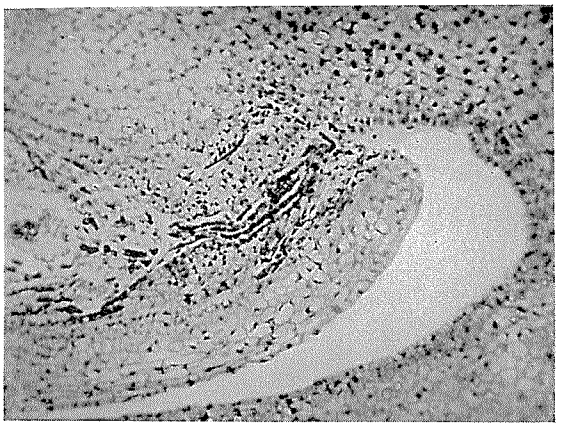

Fig. 28

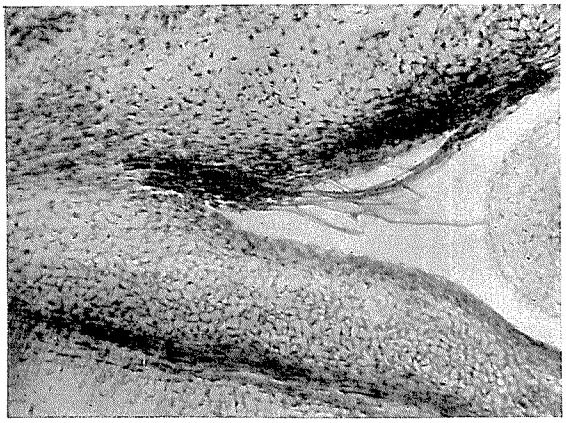

Fig. 33

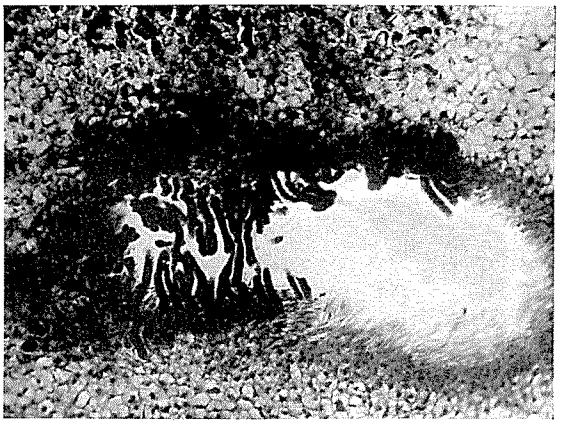

Fig. 36

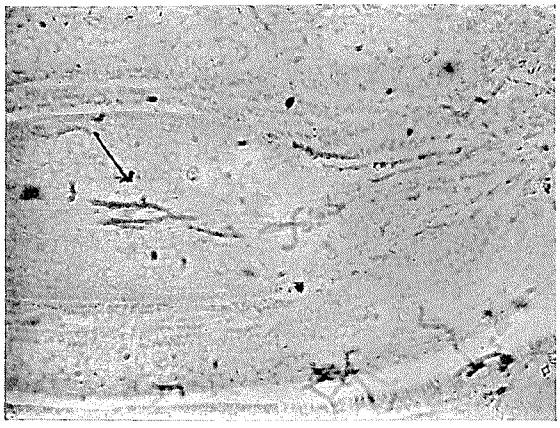

Fig. 32

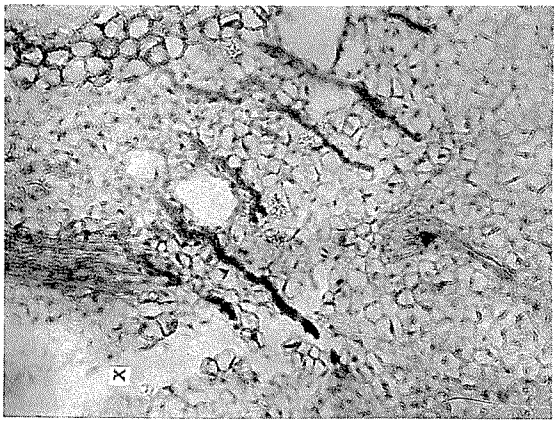

Fig. 35

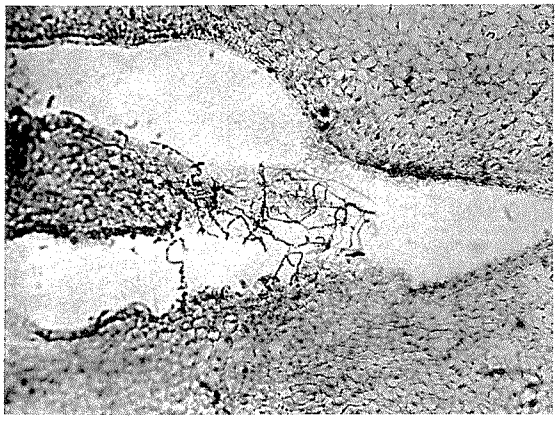

Fig. 31

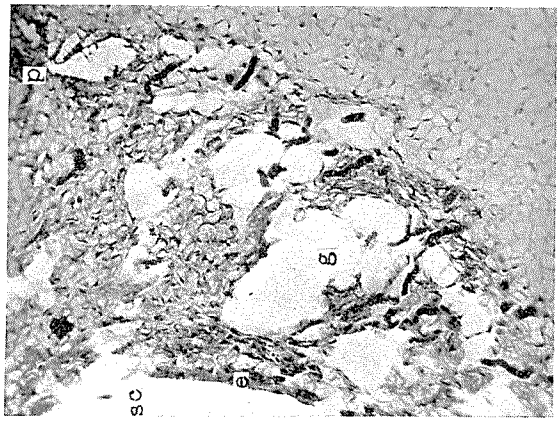

Fig. 34

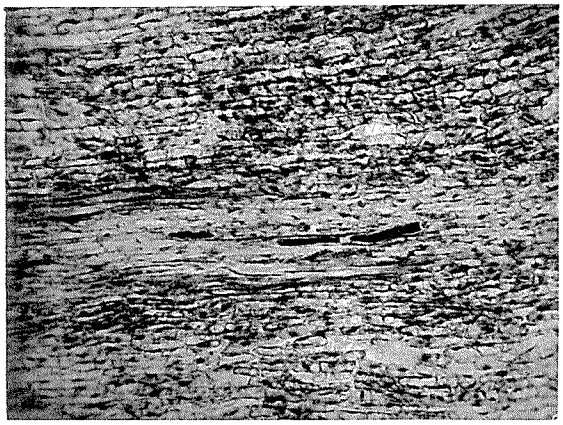

Fig. 39

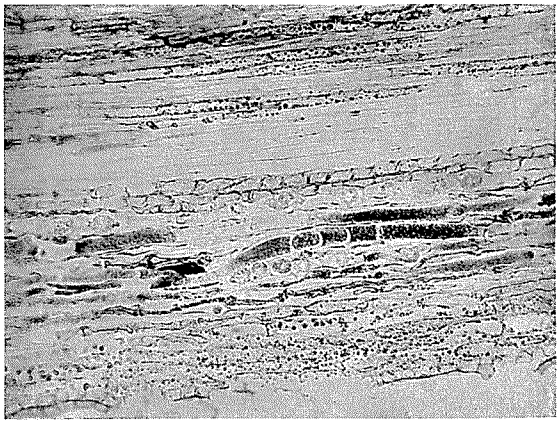

Fig. 42

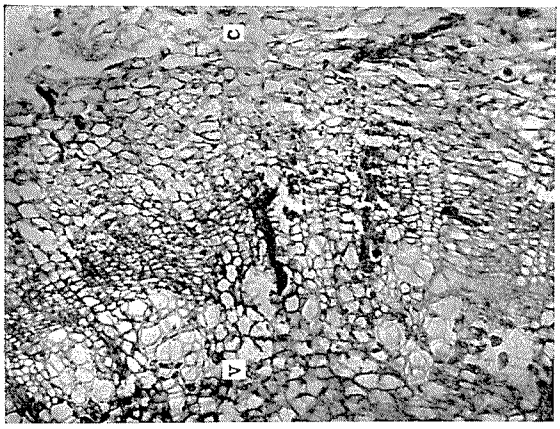

Fig. 38

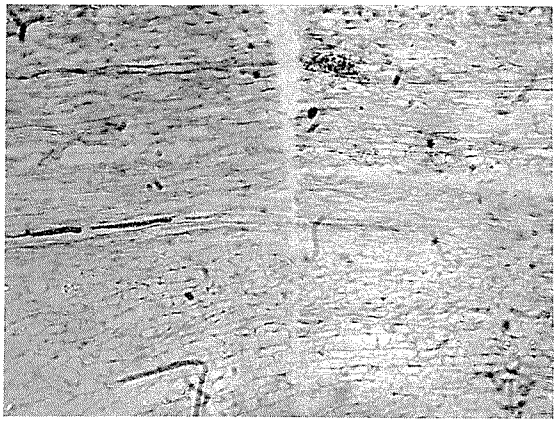

Fig. 41

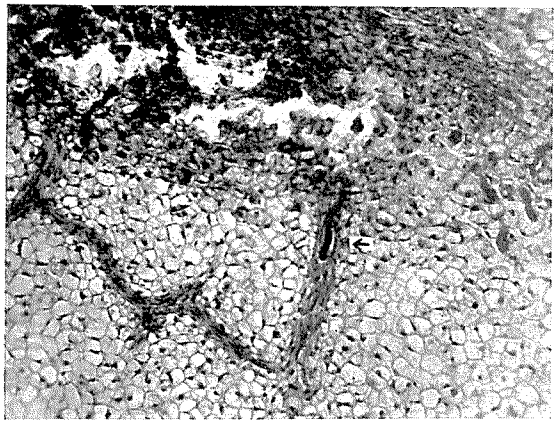

Fig. 37

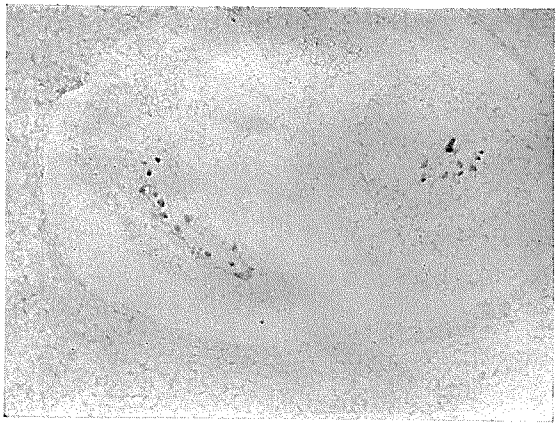

Fig. 40