

# Further Studies of the 'Brown Rot' Fungi.

## I. A Shoot-Wilt and Canker of Plum Trees caused by *Sclerotinia cinerea*.

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With Plates XIII and XIV.

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### I. GENERAL OBSERVATIONS.

ABOUT the middle of May 1919 a row of Victoria plum trees in the fruit plantation at Wye College was examined for the early symptoms of 'Wither-Tip', a disease from which some of the trees had suffered in previous years. This disease, described fully elsewhere,<sup>1</sup> is characterized, as the name implies, by a withering of the tips of the young green terminal shoots of the branches. In the present case the terminal shoots had made but little growth at the time the examination was made and typical instances of Wither-Tip were not seen. It was found, however, that a number of short leafy shoots had been recently killed; these shoots were borne laterally on the twigs produced the previous year, and they were such as would have remained short and become 'fruiting spurs' in the following season.<sup>2</sup> The largest leaves on these wilted shoots were only from 2 to

<sup>1</sup> Wormald, H. : A 'Wither-Tip' of Plum Trees. *Ann. Appl. Biol.*, v, 1918, pp. 28-59.

<sup>2</sup> Each of these lateral shoots consists of a number of leaves borne on a very short axis; the buds which develop in the axils of these leaves give rise to leaves and flowers the following spring (Plate XIII, Fig. 6).

3 cm. in length, and the wilt, therefore, must have occurred soon after the buds expanded (Plate XIII, Fig. 1).

Usually only one wilted shoot was to be found on a twig, but sometimes two and occasionally three were found on one twig. They were most numerous on trees which had been infected with the 'Wither-Tip' disease during the previous year; the withered tips had persisted through the winter, and during May bore numerous powdery tufts of *Monilia cinerea*, the conidial stage of *Sclerotinia cinerea*, (Bon.) Schröter. The wilted shoots were, as a rule, in close proximity to the dead tips, those shoots immediately below such tips being particularly subject to attack. It was suspected, therefore, that the wilt was caused by *S. cinerea*, the withered tips of the previous year's shoots serving, in all probability, as the principal source of infection, at any rate in this particular instance, since mummied fruit was absent from most of the trees (a result of the almost total failure of the crop in 1918), while a few of the trees bore one or two mummies only. The weather had been wet and rather cold while the buds were expanding, and the damp atmosphere probably favoured infection and also induced free development of conidia on the pustules of the withered shoots.

During the first week of June it was noticed that cankers had developed round those nodes where the wilted shoots were inserted. In a few cases the canker had girdled the twig and so caused a withering of that portion distal to the canker (Plate XIII, Fig. 5). Some of the cankers which had only partly girdled the twigs were labelled, so that they could be kept under observation to see what rate of progress the cankers made, but from that time onward no further increase in the size of the cankers could be detected, and on cutting cankers across during the first week of July it was found that already callus was developing from the edges of the lesions and tending to cut off the dead bark.

No external signs of a fungus were found on the dead leaves or on the cankers at this stage, except in one case where on one shoot pustules of *Monilia cinerea* were found on the petioles. The almost complete absence of *Monilia* pustules from the leaves was undoubtedly due to the dry weather which generally prevailed about this time.<sup>1</sup> When, however, infected shoots were placed in a moist atmosphere at room temperature (about 18° C.) *Monilia* fructifications appeared on the dead leaves within twenty-four hours.

The cankers were seen as slightly sunken areas, approximately elliptical in surface view, being broadest at the node, but, as a rule, they extended downwards to a greater distance than upwards. Thus of seventeen cankers which were measured two showed an upward extension equal to the downward, while in the rest the downward extension was the greater. The

<sup>1</sup> The pustules seen on the petioles were discovered on a day following a showery evening.

cankers examined were on twigs 3 to 7 mm. in diameter, and in most cases had extended laterally about half-way round the twigs; one of the largest of these was 4.4 cm. in length, extending from the node 1.8 cm. upwards and 2.6 cm. downwards.

The infected tissues undergo a disintegration resulting in gummosis, the gum frequently being so copious as to exude in drops (Plate XIII, Fig. 2). A characteristic feature of these lesions is a necrosis of the young xylem elements far in advance of the fungal hyphae. Mycelium is to be found only in the tissues of infected shoots and in the cankers, while disintegration of the xylem elements can be traced for several centimetres (as far as 10 cm. in one case) from the cankers. On cutting a twig transversely at a short distance above or below a canker the disintegrated xylem is seen as a row of dark dots, just visible to the naked eye and easily seen with a pocket lens (see Plate XIV, Fig. 10), forming an arc parallel with, and about 0.5 mm. from, the cambium layer, and situated on the same side of the twig as the canker. Microscopic examination resolves the dots as gum 'pockets' in the xylem. In longitudinal radial section through a cankered node the necrosis is seen as a dark line extending upwards and downwards from the canker and again parallel with the line that indicates the position of the cambium layer (Figs. 8 and 9). It was present even when there was no visible canker at the node apart from the base of the withered shoot itself. As with the cankers themselves, this necrosis of the xylem extends, as a rule, farther below the node than above it.

Observations on the extension of the gum 'pockets' from the original lesion were recorded in four cases, as follows:

<i>Description of the original lesion.</i>	<i>Extension of gummosis (xylem necrosis) from the original lesion.</i>	
	<i>Upwards.</i>	<i>Downwards.</i>
1. No canker visible on outside. Shoot dead and tissues brown as far as and including xylem formed in the current year. Hyphae found in the shoot only.	2.1 cm.	1.5 cm.
2. No canker visible on outside. The inner basal tissues of the shoot not brown; the younger tissues of the shoot brown and containing hyphae.	0.3 cm.	1.5 cm.
3. Canker present, extending two-thirds round the twig and upwards from the node for 1.6 cm., downwards for 2.4 cm.	5.5 cm.	8.5 cm.
4. Canker present, two-thirds round the twig, extending upwards from the node for 1.3 cm., downwards for 1.8 cm.	5.0 cm.	10.0 cm.

No organism has been found in these disintegrated tissues beyond the cankers, and the necrosis appears to be brought about by an enzyme (secreted by the fungus) which diffuses along the young vessels and causes their hydrolysis. Whatever may be the cause, the action ceases early in the growing season, for if, in the winter following infection, a twig is cut across at 2 or 3 cm. above or below a canker, the disintegrated vessels are found to be embedded in the wood and confined to the inner edge of the ring of

xylem produced during the season in which infection occurred (Plate XIV, Fig. 11).

Although pustules of *Monilia cinerea* may develop on the dead leaves during wet weather in the summer, they are not found on the bark of the shoots or on the cankers until winter approaches. Early in December *Monilia* fructifications were seen on the bark of a number of the dead shoots and on the petioles of the withered leaves. Conidial fructifications continue to develop during the winter months, and in February nearly all the cankers labelled in June of the previous year bore grey conidial tufts, usually at the base of the short shoots, but also frequently on the bark of the cankers. The cankers by this time were, in many cases, nearly covered with callus, which, developing from the two sides, had almost reached the middle line and had caused the bark to become ruptured (Plate XIII, Figs. 3 and 4).

The conidia produced on the *Monilia* pustules of the cankers during winter and spring have dimensions similar to those of *M. cinerea* found on the mummied fruit and on withered tips at that time of the year. Conidia taken from a pustule growing from a canker in March showed variation in size from  $7.5 \times 5.5 \mu$  to  $16 \times 11.5 \mu$ ; they were mostly, however, within the range  $10-12.5 \times 7-9.5 \mu$ , and the average of 100 conidia was  $11.3 \times 8.4 \mu$ .

The conidia produced on the young leaves in summer are larger than those of the cankers, their size being of the same order as that of the 'summer conidia' of the fruit.<sup>1</sup>

Although the wilting of the short shoots of the plum trees had not been observed previous to the spring of 1919, or if noticed had not been recognized as a 'Brown Rot' disease, there was evidence that it had occurred during the seasons 1918 and 1917 on some of the trees examined, for certain cankers were found (in 1919) which from their condition and their position on the branches indicated that infection had taken place through the short shoots. Thus on two-year-old wood there were cankers at nodes where there must have been short leafy shoots during the previous year. These cankers were partly covered by callus and almost invariably bore pustules on the short dead shoots or on the cankers; they correspond to, and were similar to, those cankers which were kept under observation and found to produce pustules during the season subsequent to infection. Somewhat similar cankers, but with callus further developed and bearing no pustules (most of the dead bark having peeled away), were found on the three-year-old wood; these were evidently a further stage in the formation and the healing over of the Shoot-Wilt cankers (Plate XIV, Fig. 7).

## II. ISOLATION AND CULTURAL EXPERIMENTS.

Pure cultures of *Sclerotinia cinerea* could easily be obtained from the mycelium found in the tissues of the young cankers. The surface of

<sup>1</sup> See p. 315.

a canker was first cleansed by wiping it with cotton-wool moistened with 95 per cent. alcohol; transverse sections were then made through the cankered portion and the sections placed in sterile water in a flamed watch-glass.<sup>1</sup> As a further precaution against contamination the outer layers of the bark were teased away with flamed needles, and particles of the internal brown tissues (bark and wood) were removed to a second watch-glass of water, from which they were transferred to carrot agar or prune agar in Petri dishes. In such plates, kept at room temperature (about 18° C.), the hyphae grew out readily and within six days had given rise to discs of mycelium 2.1 to 2.5 cm. in diameter; at this stage further primary growth was checked, but fan-shaped lobes appeared at certain points on the margin, and these gave rise to a zone of mycelium around the primary growth, and later another zone developed in a similar way (Plate XIV, Fig. 13). This mode of growth in agar plates is also shown by cultures of *Sclerotinia cinerea* when obtained directly from ascospores, and, as pointed out in previous papers,<sup>2</sup> is a character which distinguishes this fungus, as found in Britain, not only from *S. fructigena*, but also from the Brown Rot fungus which is common in America.

Cultures obtained in this way in June and July were almost invariably pure, so far as could be seen. For further experimental work it was considered desirable, however, to obtain cultures derived from single conidia. The agar-plate cultures were quite barren, but on transferring a little of the mycelium to sterilized potato in tubes, grey tufts of conidiophores with their chains of conidia appeared within a week. Conidia taken from the cultures on potato were isolated<sup>3</sup> on agar plates and the resulting 'sporelings' gave pure line cultures.

In winter pure line cultures were obtained direct from conidia taken from a canker; in general habit such cultures resembled those obtained from the barren mycelium of the young cankers.

The cultures of the Shoot-Wilt fungus on sterilized potato were typical of *Sclerotinia cinerea* f. *pruni*, conidia being produced more freely than is the case in potato cultures of *S. cinerea* f. *mali*. These conidia were larger than the 'winter conidia' produced on the cankers, and were of the same order as those produced on the leaves and fruit in summer, the average size being approximately  $17 \times 12 \mu$ .

<sup>1</sup> Two watch-glasses were sterilized simultaneously, by passing them several times through a Bunsen flame, and left to cool with one inverted over the other to eliminate as far as possible contamination by the entrance of spores floating in the air.

<sup>2</sup> Ann. Bot., xxxiv, p. 164; xxxv, 1921, p. 129.

<sup>3</sup> For details of the method adopted by the author when isolating *Monilia* conidia on agar plates see Ann. Bot., xxxiii, pp. 371-2.

## III. INOCULATION EXPERIMENTS.

Using pure cultures of the fungus, inoculation experiments were carried out as follows:

(a) Inoculation of plum leaves on short shoots to confirm the evidence supplied by observations in the open that the conidia of *Sclerotinia cinerea* are able to cause infection of the leaves and produce cankers on the twigs by invasion from the infected leaves.

(b) Inoculation of plums (fruit) to ascertain whether the fungus, when raised from the small 'winter conidia', produces typical 'summer conidia' when grown on the fruit, as was shown to be the case in the 'Wither-Tip' disease.

(c) Inoculation of apple flowers to determine whether the form causing this Shoot-Wilt and canker of plum trees is identical with the form causing the 'Blossom-Wilt and Canker' disease of apple trees (forma *mali*) or with the form commonly occurring on plum and cherry trees (forma *pruni*), the latter in a considerable number of inoculations having invariably failed to induce Blossom-Wilt of apple trees.

(a) *Inoculation Experiment on Plum Leaves.**Experiment 1.*

Young shoots of Victoria plum trees growing in pots in the greenhouse were used in this experiment. Two series of inoculations were made on April 1, 1920, viz.:

(a) Five of the shoots were sprayed with distilled water; one leaf on each shoot was then punctured (four punctures in a group between the midrib and the margin of the leaf) with a sterile needle and the punctured parts inoculated with conidia of the fungus. The conidia had been produced in cultures on steamed potato, and the inoculation was made by taking a particle of the potato on a needle and bringing the conidia-bearing surface in contact with the wounded leaf.

(b) In the second series seven shoots were also sprayed with distilled water, and one leaf on each was inoculated with conidia but without the preliminary puncturing.

One leaf only in each series became infected; notes taken on the rate of progress of the disease in these two cases are as follows:

<i>April 1.</i>	<i>April 14.</i>	<i>April 17.</i>	<i>April 22.</i>
(a) One leaf on each of five shoots inoculated at punctures.	One leaf only infected; a brown discoloration extends to a distance of 8 mm. from the punctures.	The whole leaf is flaccid; a brown area, about 1 cm. wide, extends from near the apex of the leaf into the petiole; the rest of the leaf is green.	Infected leaf withered; the other leaves of the shoot are also wilting.
(b) One leaf on each of seven shoots inoculated without punctures.	One leaf only infected; a brown discoloration extends from the middle of one edge to the base of the lamina.	Petiole and lower parts of lamina brown; apical portion of leaf still green, but flaccid; the other leaves on the shoot are also wilting.	A canker has girdled the twig; the leaves of the terminal portion of the twig are wilting.

Later the infected twigs were cut off and it was found that in both of them the mycelium had invaded the twig from the shoot, since hyphae were seen in the cortex. A drop of gum had oozed out to the surface of one canker. Plate cultures obtained by placing particles of the cortex (three particles taken from each canker) on agar, in the way already described for isolating the fungus from naturally infected cankers, gave rise in every case to typical cultures of *Sclerotinia cinerea*.

In the infected leaf of the first series of inoculations it was seen that the browning began at the punctures. In the leaf of the second series infection started at the edge of the leaf, and there was some doubt as to whether the germ tubes had actually penetrated the uninjured epidermis; the leaf was quite young, its edges being still recurved, at the time of inoculation, and it is possible that, in the act of placing conidia on the lower epidermis, the margin of the leaf may have been slightly injured. This result therefore, particularly as it was the only positive one of that series, cannot be taken as proof that infection can take place on an uninjured leaf. The experiment, however, affords evidence that conidia of *Sclerotinia cinerea* are able to produce infection of plum leaves through small wounds, if not through the uninjured surface, and also that the mycelium in an infected leaf may extend into the shoot and also into the twig bearing the shoot.

Experiment 1, being carried out under greenhouse conditions, eliminated the action of frost as a possible primary cause of Shoot-Wilt.

#### *Experiment 2.*

This experiment was carried out on Victoria plum trees in the College plantation in the spring of 1921. Short lateral shoots were labelled and the twigs bearing them were sprayed with sterile distilled water; one leaf on each shoot was inoculated, a leaf which had attained to about half its full size being selected and marked with a loop of cotton tied loosely round the petiole. As in the previous experiment, two series of inoculations were made, (a) in which the leaves were punctured before inoculation, the punctures on each leaf being four in number, about 2 mm. apart, and situated midway between the middle of the midrib and the margin on one side, and (b) in which the leaves were not punctured. Leaves used as controls and for comparison were sprayed, some being punctured, but not inoculated. Six leaves in each series were inoculated on May 3.

In series (b) brown spots or patches were observed on five of the inoculated leaves on May 8, mostly towards the base of the lamina; a few days later these brown portions had fallen out and no further change occurred. It is uncertain whether these were infection spots or not, but a comparison with the results obtained in series (a) and the fact that similar spots were present on comparatively few un-inoculated leaves suggest that they were a result of inoculation; assuming this to be the case, it would

appear that most of the conidia had been washed down towards the base of the lamina, for they had been placed near the middle of each leaf.

In series (a) the punctures showed brown margins in every case on May 8, and on some leaves the punctures were connected by brown cells; in three leaves the discoloration had extended from the punctures to the edge of the leaf on that side of the midrib. Later, further extension of the browning occurred, usually accompanied by a yellowing of the tissues in advance of the browning and by a distortion of the leaf as a result of a check in its development on the inoculated side. Finally, in four leaves the diseased portions dropped out and the rest of the leaf did not become affected; in each of the other two, however, the whole leaf was killed, and in one of them the disease extended into the axis of the shoot, causing the wilting of all the other leaves on the shoot, and then into the twig to form a canker (Plate XIV, Fig. 14). As these are features which have not previously been recorded, details of observation are here tabulated.

*Results on six plum leaves punctured and inoculated on May 3.*

<i>May 11.</i>	<i>May 16.</i>	<i>May 24.</i>
1. A browning of the tissues extends from the punctures to the margin of the leaf.	The browning extends from the midrib to the margin of the leaf on the punctured side and for a distance of 2 cm. along the edge; there is also a yellowing of the infected side accompanied by distortion.	Leaf withered and brown to base of petiole.
2. A browning extends from the punctures to the edge of the leaf.	The discoloration is now extending towards the midrib; there is a slight yellowing and distortion of the leaf between the punctures and the base of the leaf.	The infected portion has fallen away.
3. There is a brown margin to the punctures.	The browning extends for 2 mm. from the punctures; there is a slight yellowing and distortion on the infected side.	The diseased area, 5 × 6 mm., is now breaking away. [A few days later it had fallen out.]
4. Tissues brown between, and round the margins of, the punctures.	That portion of the leaf from the punctures to the margin is yellowish-green and there is some distortion.	Diseased portion fallen out.
5. The punctures have brown margins; two are connected by brown cells; leaf slightly distorted.	Punctures all connected by brown tissues, leaf distorted, and yellowish between the punctures and the base.	Infected portion fallen out.
6. Punctures connected by brown cells; browning extending to the margin of the leaf.	The browning extends as far as the margin of the leaf and to the midrib, and for 1.5 cm. along the edge; leaf distorted.	Infected leaf withered to base of petiole; other leaves of the shoot are wilting; the lower end of the petiole of one leaf is brown for 6 mm.

Later observations on leaves No. 1 and No. 6, the two which were killed outright, were as follows:

*No. 1.* On May 31 the tip of the shoot was dead but the lower leaves were alive; by June 10 no further development had occurred except that the infected leaf bore pustules of *Monilia cinerea*.



No. 6. On May 31 the whole shoot was dead and bore withered leaves; pustules of *M. cinerea* were present on the inoculated leaf. By June 4 infection had extended into the twig and there was a globule of gum at the node. On June 15 the twig was removed and photographed (Fig. 14); the canker on the twig was only about 1 cm. in length, but necrosis of the young xylem elements, extending beyond the canker for 0.5 cm. upwards and 4.5 cm. downwards, had taken place as in the case of natural infections. Mycelium was found in the brown tissues of the canker, and particles of transverse sections through the bark placed on agar plates gave rise to typical cultures of *Monilia cinerea*; a subculture, on steamed potato, from one of these produced grey *Monilia* fructifications within five days. Thus, as in Experiment 1, proof was obtained that the fungus used in inoculating the leaf had penetrated into the twig. No mycelium was found in the disintegrated xylem beyond the canker, and thick sections, taken at 1 cm., 2 cm., and 4 cm. below the canker, placed on agar, showed that the fungus was not present, since no growth resulted.

The browning of the tissues bordering the punctures in Experiment 2 was apparently the first external symptom of infection, for the punctures on the control leaves, on May 11 and later, had pale margins; on these uninoculated punctured leaves again there was no distortion, showing that merely puncturing the leaf does not check its growth on the injured side. A comparison with the control leaves therefore shows that infection occurred in all the inoculated punctured leaves of that experiment. The failure of the infection to extend to the base of the leaf even where definite brown areas appeared round the punctures may have been due to (1) the age of the leaves when inoculated, or (2) the very dry weather which prevailed almost throughout the whole period during which observations were made; in all probability both these factors influenced the result. With regard to the age of the leaves it would seem that very young leaves are more susceptible than older ones, since the leaves of shoots showing the wilt, when naturally infected, are almost invariably quite small when killed; this, together with the fact that, in Experiment 2, the one shoot which was eventually killed was almost fully developed when the infection reached the axis of the shoot, suggests that, in order to secure results more comparable with natural infections, the inoculations must be carried out on still younger leaves.

The falling out of the infected tissues in the majority of the leaves suggests that the cells are killed in advance of the hyphae; it is conceivable that such dead cells, rapidly becoming desiccated by the persistent dry condition of the atmosphere, would check further growth of the fungus and, as the uninfected tissues of the young leaf continued to grow, a line of rupture would appear between the dead and the living parts. On the other hand, the mere drying out of the infected tissues would tend to inhibit

the growth of the hyphae by rendering the food-stuffs in the leaf unavailable.

Two other series of inoculations similar to those described under Experiment 2 were carried out at the same time, using a culture derived originally from an ascospore of *Sclerotinia cinerea*. The early stages of infection again appeared, as shown by a brown discoloration round the punctures, together with a yellowing and distortion of the leaf in the neighbourhood of the infected areas, but in no case did infection extend to the base of the leaf, the brown parts eventually falling out.

It is evident from these experiments that the conidia of *Sclerotinia cinerea* are able to infect young Victoria plum leaves through punctures, but it has not yet been established that they can produce infection of uninjured leaves. In severe outbreaks of 'Wither-Tip' the disease is usually associated with aphid attacks; on the other hand, in the wilt of the short shoots there was no evidence of insect injury. The failure to obtain any instances of definite infection through unwounded leaves in these experiments, when observations in the open suggest that such cases occur in natural infections, may be understood when it is realized that the pustules of *Monilia cinerea* on mummied fruit and dead shoots produce conidia throughout the winter and spring, and that the leaves are therefore liable to infection, whenever favourable conditions supervene, from the time the buds open onwards.

An experiment carried out in the laboratory favours the idea that infection of uninjured leaves is possible. Young leaves, on long plum shoots placed with their cut ends in water and kept in a moist atmosphere, were readily infected by placing conidia of *Monilia cinerea*, taken direct from a dead shoot, in drops of water on the uninjured leaves. Brown areas appeared within two days at the inoculated spots, and on the sixth day after inoculation several of the leaves were brown throughout and grey *Monilia* pustules were present on them. This experiment is not conclusive evidence, as the shoots were under very abnormal conditions and the purity of the fungus was questionable; it suggests, however, a line for further research.

#### (b) *Inoculation of Plums (fruit).*

On July 2, 1919, four plums were inoculated by making a puncture through the skin of each with a sterile needle and inserting conidia taken from a culture of the Shoot-Wilt fungus growing on steamed potato. All became infected and produced grey *Monilia* pustules within a few days. Control plums, punctured but not inoculated, did not become infected.

By July 19 three of the infected plums had fallen, but the fourth was still on the tree and had communicated the rot to two others in contact with it. The dimensions of 100 conidia taken from this plum were found to

range from  $10 \times 8 \mu$  to  $26 \times 16 \mu$  and  $22 \times 18 \mu$ , the average being  $16.8 \times 12.5 \mu$ . These 'summer conidia' on the fruit were thus greater than those conidia produced on the dead shoots and cankers in winter, as will be seen by comparing the dimensions here given with those of the 'winter conidia'.<sup>1</sup>

These results confirm those of observations previously recorded,<sup>2</sup> which go to show that the conidia of *Sclerotinia cinerea* produced in winter, on shoots, cankers, and mummied fruit, are invariably distinctly smaller than those which develop on recently infected fruit, leaves, and flowers in summer.

(c) *Inoculation of Apple Flowers.*

This experiment was carried out on two trees (variety James Grieve) in the fruit plantation. Inflorescences were selected which bore flowers recently opened, and two flowers were inoculated on each inflorescence. On one tree five inflorescences were inoculated with the Shoot-Wilt *Monilia* and five with the Apple Blossom-Wilt fungus (*Monilia cinerea* f. *mali*); on the second tree three inflorescences were inoculated with the former and three with the latter. Thus each fungus was used to inoculate sixteen flowers on eight inflorescences. The inoculations were made by placing conidia from pure cultures on the stigmas.<sup>3</sup>

Of the flowers inoculated with the Apple Blossom-Wilt fungus all were killed. On one inflorescence the two inoculated flowers fell off without infection extending into the axis; in the rest, however, the mycelium grew into the spurs and killed all the flowers and leaves on those spurs, the wilting of the leaves being noticeable in from fourteen to seventeen days from the day the flowers were inoculated, a condition typical of the Apple Blossom-Wilt disease.<sup>4</sup>

Of the flowers inoculated with the Shoot-Wilt *Monilia* the preliminary symptoms of infection were observed in an early browning of the styles and a premature withering of the stamens and calyx lobes as seen by comparing the inoculated flowers with normal flowers of the same age. All the inoculated flowers, with the exception of two, fell before setting into fruit, and in no case did the fungus enter the axis of the inflorescence, the other flowers and the leaves showing no signs of infection. In this respect the Shoot-Wilt *Monilia* is biologically similar to isolations of *Sclerotinia cinerea* obtained from *Monilia* fructifications found on plums and cherries, and also to an isolation started from an ascospore when the ascigerous stage was found on mummied plums.<sup>5</sup>

<sup>1</sup> See p. 308.

<sup>2</sup> Ann. Bot., xxxiv, p. 161.

<sup>3</sup> For further details of the method adopted in inoculating flowers with *Monilia* conidia, see Ann. Bot., xxxiii, no. 131, pp. 388 and 390.

<sup>4</sup> Vide A Blossom-Wilt and Canker of Apple Trees. Ann. Appl. Biol., iii, 159, 1917.

<sup>5</sup> Vide On the Occurrence in Britain of the Ascigerous Stage of a Brown Rot Fungus. Ann. Bot., xxxv, No. 137, pp. 125-35, Jan. 1921.

IV. COMPARATIVE TESTS FOR PRESENCE OF OXIDASE  
IN CULTURES.

In a previous paper<sup>1</sup> it has been shown that two forms of *Monilia cinerea*, referred to as forma *mali* and forma *pruni*, are distinguishable not only by a difference in the degree of parasitism shown by the two forms when apple flowers are inoculated with their conidia, but also by a difference in the rate of secretion of an oxidase when the fungi are grown in liquid culture media. In that article the method adopted in applying the test for the presence of oxidase is given in detail;<sup>2</sup> the tests as applied to the Shoot-Wilt fungus were carried out as described there, except that another culture medium was used (viz. a 2 per cent. extract of prunes) and, the thermostat not being available, the cultures were grown, and the tests carried out, in a warm room at a temperature of about 20° C. instead of at 25° C. as in the previous experiment.

In the present instance two isolations of the Shoot-Wilt fungus were used, and, for comparison, a culture of *Sclerotinia cinerea* originally started from an ascospore, and two cultures of *Monilia cinerea* f. *mali* from apple trees, were tested simultaneously. For convenience these may be indicated by the letters *A*, *B*, *C*, *D*, and *E*, as follows:

- A*. Shoot-Wilt *Monilia*, Isol. I: from the mycelium of a Shoot-Wilt canker.
- B*. Shoot-Wilt *Monilia*, Isol. III: isolation from a conidium of a fructification on a Shoot-Wilt canker.
- C*. *Sclerotinia cinerea*, Isol. I: from an ascospore of an apothecium found on a mummied plum.
- D*. *Monilia cinerea* f. *mali*, Isol. XXIII: from a conidium of a Brown Rot canker on an apple tree (Kent).
- E*. *Monilia cinerea* f. *mali*, Isol. XXIV: from the mycelium in a dead spur of an apple tree (Ross-shire).

*B* and *E* were the fungi used in the experiments described under 'Inoculation of Apple Flowers'; *E* produced typical Apple Blossom-Wilt, while *B* failed to do so.

Two plate cultures of each were started, and when they were ten days old five of them (viz. one of each isolation) were tested for oxidase, guaiacum and pyrogallie acid (2 per cent. solution) being the reagents used. By the tenth day the mycelium of *D* and *E* was much darker than that of *A*, *B*, and *C*. The liquid was strained off from the mycelium, the former only being used in the tests.

*D* and *E* readily gave the oxidase reaction, a blue colour being evident in the guaiacum tubes within half an hour; the colour developed into

<sup>1</sup> Wormald, H.: The 'Brown Rot' Diseases of Fruit Trees. II. Ann. Bot., xxxiv, 1920, pp. 143-71.

<sup>2</sup> loc. cit., pp. 147-50.

a bright blue within the next half-hour, and later it was a still deeper blue. *C* gave a trace of colour at the end of two hours, *A* and *B* not until three hours. The colour gradually became a little deeper in tone, but it was still a pale blue in the tubes of *A*, *B*, and *C* at the end of twenty-four hours, the reaction being a little more pronounced with *C* than with *A* or *B*. A corresponding yellowing appeared in the tubes containing pyrogalllic acid, the colour again being more intense with *D* and *E* than with *A*, *B*, and *C*.

On the following day the other five cultures were similarly tested, with the same general result, *D* and *E* readily giving the oxidase reaction with guaiacum and with pyrogalllic acid, the rest giving a comparatively feeble reaction; *C*, however, again being a little more active than *A* or *B*.

None of the isolations used in this experiment had been previously tested for the oxidase reaction, so that the result is further evidence in support of the conclusion previously arrived at, that the forms *mali* and *pruni* can be distinguished in the laboratory by applying comparative tests for secretion of oxidase in liquid culture media. In this connexion the fact that the two isolations of forma *mali* used in the above experiment were obtained from specimens received from such widely separated counties as Kent and Ross-shire is not without interest.

#### V. 'SHOOT-WILT' AND 'WITHER-TIP' COMPARED.

The disease described in the present paper differs from 'Wither-Tip' primarily in the fact that in the former the short lateral shoots are affected, in the latter the long terminal shoots; both kinds of shoots bear leaves only and develop from buds produced on long shoots in the previous summer. The difference is not an absolute one, since under certain conditions, particularly if the terminal shoot is injured or checked in growth, the lateral shoots are induced to elongate and are then subject to 'Wither-Tip'.

Although the two have thus much in common, the disease at present under consideration shows certain features that do not appear in typical cases of 'Wither-Tip'. The wilt of the short shoots is noticeable early in the season, i.e. about the time the trees are in bloom, whereas 'Wither-Tip' is not conspicuous until later in the season, when the terminal shoots have reached a length of several inches. In 'Wither-Tip' the disease is confined to the current year's growth, since further extension of the fungus ceases before it reaches the older parts of the twig; the axis of a lateral shoot, on the other hand, is usually so short that the fungal hyphae pass almost directly from the infected leaves to the twig bearing the shoot and a canker of the bark and wood round the insertion of the shoot very frequently results, together with the gummosis of the young xylem elements as described above.

In both forms of disease *Monilia* fructifications may appear on the leaves, under favourable conditions, during spring and summer, but as a rule

A a

they are not found on the bark of the shoots (or on the cankers) until the following winter.<sup>1</sup>

## VI. THE ECONOMIC IMPORTANCE AND THE CONTROL OF THE DISEASE.

No estimates have yet been obtained as to the losses due to Shoot-Wilt, but the direct damage caused by the disease is probably inappreciable except in certain seasons when there is mild damp weather as the leaf-buds are unfolding. The short shoots are, as already explained, incipient fruit spurs, and the killing of a number of such shoots one year means a corresponding reduction in the number of inflorescences the following year (Fig. 6). The fact that further extension of the mycelium often ceases before the cankers girdle the twigs is of some significance, since such lesions tend to become healed over, so that little direct harm is done in those cases unless many shoots become infected.

Perhaps the chief economic importance of the disease is the fact that not only may the mycelium in the withered leaves give rise to the *Monilia* fructifications during the season in which infection occurs and so cause further dissemination of conidia that year, but the dead shoots and cankers become, in the following season, sources of infection which are easily overlooked. When it is remembered that *Sclerotinia cinerea* infects not only the leaves (as shown in this article) but also the flowers (often causing serious outbreaks of Blossom-Wilt) and the fruit, all possible sources of infection must be taken into consideration if attempts to keep the Brown Rot diseases under control are to be successful.

The cankers on the one-year-old twigs are too small for their excision to be a practical operation, and to cut back behind them would often mean removing a number of incipient fruit spurs. It cannot be over-emphasized that mummied fruit and twigs killed by the Brown Rot fungi should be removed and destroyed by fire whenever this is at all practicable. To supplement this treatment the writer recommends, in cases where the 'Shoot-Wilt' disease is known to be present, the application, in winter, of a caustic alkali wash to which soap has been added, the soap being necessary to ensure a complete wetting of the powdery *Monilia* fructifications. Such a wash has not yet been thoroughly tested as a means of controlling Brown Rot diseases, but it has been found, in experiments on a small scale, that a spray-fluid containing 1 per cent. caustic soda and 1 per cent. soft soap, used as a winter wash shortly before the buds open, will either destroy the fructifications or render them sterile for some weeks.

<sup>1</sup> The writer has found *Monilia* fructifications on the bark of a recently killed plum shoot on one occasion only—on a shoot affected with 'Wither-Tip' in May 1921.

## VII. SUMMARY.

1. A wilt of the short shoots of Victoria plum trees is described.
2. The shoots are killed soon after the leaves unfold and mycelium extends from the dead shoots into the twigs bearing them, causing cankers.
3. The disintegration of the infected parts results in gummosis of the tissues, and gum often exudes in drops.
4. A necrosis of the young xylem elements can be traced for several centimetres above and below a canker, but the mycelium extends no farther than the actual canker.
5. The fructifications of *Monilia cinerea* are sometimes to be found on the infected leaves during the summer, but they do not appear on the cankers until the following winter and spring.
6. Conidia taken from a canker in winter had an average size of  $11.3 \times 8.4 \mu$ , but when the fungus was grown on plums (fruit) in summer the average size of the conidia produced under these conditions was  $16.8 \times 12.5 \mu$ .
7. Shoot-Wilt has been induced on plum trees by inoculating punctured leaves with conidia of the fungus grown in pure cultures.
8. The fungus causing the disease is *Sclerotinia cinerea*, (Bon.) Schröter, f. *pruni*, as shown by its—
  - (a) morphology,
  - (b) mode of growth in pure cultures,
  - (c) inability to invade the axes of apple inflorescences when flowers are inoculated with conidia,
  - (d) comparatively slow rate of secretion of an oxidase when growing in liquid culture media.

## EXPLANATION OF PLATES XIII AND XIV.

### PLATE XIII.

- Fig. 1. Typical example of 'Shoot-Wilt'.
- Fig. 2. Two cankers, the result of infection through short shoots, showing a copious flow of gum.
- Figs. 3 and 4. Cankers as seen in the winter following infection of the shoots; at this stage the cankers bear conidial fructification of *Sclerotinia cinerea*.
- Fig. 5. A twig showing the terminal portion killed by infection through a short shoot.
- Fig. 6. Portion of a plum twig at the time of blossoming, showing the remains of a short shoot killed during the previous season; the uninfected shoots have developed into flowering spurs.

### PLATE XIV.

- Fig. 7. Shoot-Wilt cankers showing condition during the second winter after infection; the lesions are almost covered with callus.

Fig. 8. A twig cut longitudinally to pass through the base of the wilted shoot on the right; there is no definite canker on the twig, but necrosis of the xylem is seen as a dark line on the inner side of the cambium line (natural size).

Fig. 9. Portion of the twig shown in Fig. 8 as seen with a lens ( $\times 4$ ). *c.*, the cambium; *n.*, necrosis of xylem elements shown by an almost continuous line of gum-pockets.

Fig. 10. Section of twig, as seen with a lens ( $\times 5$ ), at 2 cm. above a Shoot-Wilt canker; necrosis of the xylem is seen as an arc of dark spots ('gum-pockets') on the right; condition in June of the same year in which infection occurred.

Fig. 11. Section across a twig at 2 cm. below a Shoot-Wilt canker, showing the 'gum-pockets' embedded in the xylem; condition in March of the year following infection.  $\times 5$ .

Fig. 12. Section through a canker on a two-year-old twig (about 12 months after infection), showing the callus covering the lesion.  $\times 5$ .

Fig. 13. Pure culture of *Sclerotinia cinerea*, obtained by placing a particle of infected tissues from a canker on carrot agar, 20 days old, growing at room temperature (slightly reduced). [Compare culture of *S. cinerea* derived from an ascospore, as shown in Ann. Bot., vol. xxxv, Plate VI, Fig. 4.]

Fig. 14. Portion of a twig bearing short shoots, one of which has been killed as a result of inoculating a single leaf (punctured) with conidia of *S. cinerea*.

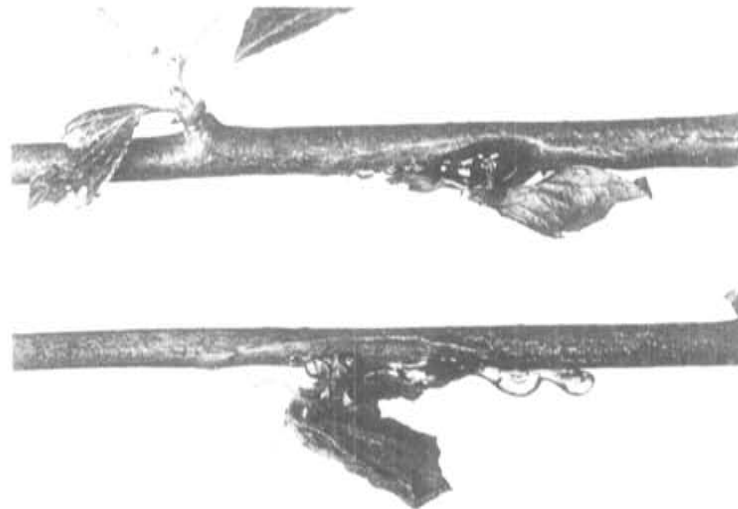




1.



3.



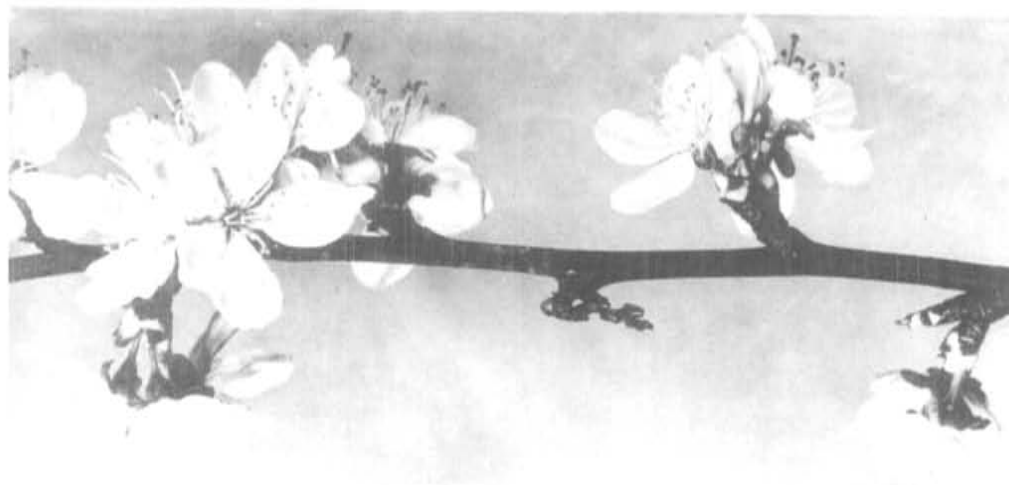
2.



4.



5.



6.

WORMALD - SHOOT-WILT.

Hutch. coll.





7.



8.



9.



10.



11.



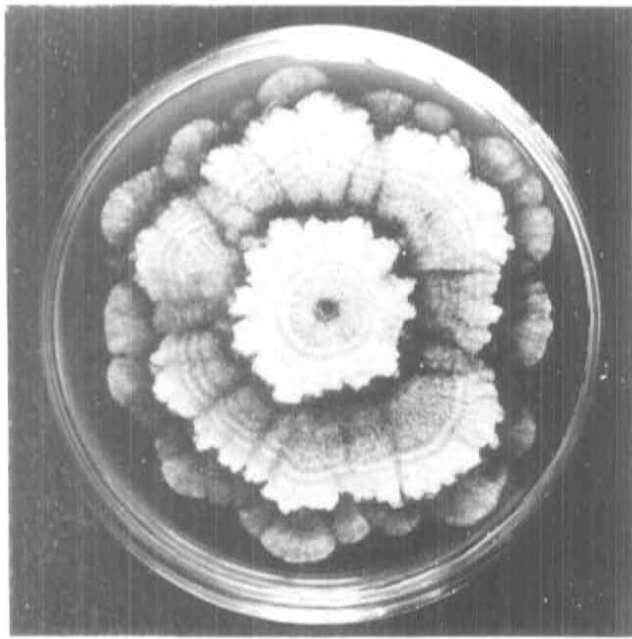
12.

WORMALD - SHOOT-WILT.



14.

Witch coil



13.