

ON DISEASES OF PLUM TREES CAUSED BY SOME SPECIES OF *CYTOSPORA*.

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INTRODUCTION.

WITHIN the last few years, an increasing number of plum trees in the fruit plantations of Cambridgeshire have exhibited symptoms of a "die-back" disease, followed by the formation of pycnidia, from which red semi-gelatinous tendrils are exuded, these spore-masses belonging to the genus *Cytospora*, the members of which are recognised as conidial stages of various species of *Valsa*.

This investigation was commenced at Cambridge, at the suggestion of Mr F. T. Brooks, to whom I am much indebted for assistance. The work was continued at Nottingham, and I desire to express my thanks to Prof. J. W. Carr for permission to work in his laboratory.

No serious damage to mature plum trees, caused by members of the genus *Valsa*, has hitherto been recorded in England, though Massee (4) has described *Eutypella prunastri* (Sacc.) as attacking young plum stocks. Aderhold (1) in Germany, and Wormald (5) in this country, have described a strikingly similar disease of cherry trees caused by *Valsa* (*Cytospora*) *leucostoma*. As all efforts to obtain mature asci have failed, it is impossible to give the species of the fungus or fungi investigated with any approach to certainty.

FIELD OBSERVATIONS AND DESCRIPTION OF FRUCTIFICATIONS.

The diseased trees examined belonged to three varieties, Victoria, Prince of Wales, and Pond's Seedling. The trees attacked range from those 4-5 years old, up to the very largest, and the attack usually proves fatal. The first sign of attack is a withering of the leaves, usually progressing from the top of the tree downwards. Next, areas of bark, which may be on the main stem or a side branch, collapse and turn

brown; on these, after a considerable interval, numerous crater-like or lenticular fructifications develop, which almost invariably prove to be pycnidia. A certain amount of gum appears at the junction areas, but considering the proneness of plum trees to gum production its presence cannot be taken as a characteristic of these diseases.

This general description holds for all the trees examined, but there are differences in detail considerable enough to warrant separate treatment. The following record indicates the nature of these.

A. Victoria.

All the specimens, with one exception, were from trees on plantations at Willingham, Cambridgeshire.

(1) A small shoot from an old tree, of which large lateral branches, six years of age, were affected. The shoot was dead. Numerous small crater-like swellings, 1 mm. in diameter, of the bark were in evidence, due to hard pycnidia, seated in the deeper cortical layers. These pycnidia were seated on a poorly developed, dirty-white stroma, sharply limited from the surrounding tissues by a dark tough "skin." Each pycnidium had a single central pore, and was many-chambered; the walls were lined with sparingly-branched hyaline conidiophores, so closely packed as to form a palisade-like tissue.

The spores, borne singly at the tips of conidiophore branches, were continuous, sickle-shaped, hyaline bodies, averaging $7\ \mu$ long, and $1.5\ \mu$ broad, with two oil bodies. In moist weather, they are extruded in enormous numbers as pink tendrils, semi-gelatinous at first, becoming horny on drying. On wetting, the tendrils disintegrate into their component spores.

(2) A tree about five years old, from the University Farm, Cambridge, was cut down in August, 1913, and kept exposed on the laboratory roof till the following October. It had then developed numerous pycnidia borne several on each erumpent oval dark stroma. As no traces of this fungus were present in August it must here have developed as a saprophyte. Each pycnidium had several pores. Pink tendrils were extruded, made up of spores similar to those described above, but $5\ \mu$ long by $1\ \mu$ broad.

(3) A tree nine years old, dead. This specimen was taken 18 ins. from the ground line region, which was also the point of attack. The upper part of the tree was still green. The stromata were black, erumpent, and lenticular; each bearing several pycnidia (Figs. 1 and 2). Over the exterior of each fissure was a thin white covering, specially

noticeable on wetting the bark. Dark red tendrils were extruded, spores as above, 5μ by 1μ .

(4) A piece of bark only. The stromata were large and pulverulent,



Fig. 2.



Fig. 1.

Fig. 3.

Fig. 4.

nearly 5 mm. long, dark olive-green in colour, and sunken in the bark (Fig. 3). There was a sharp line of demarcation from the surrounding tissue. Each stroma bore several immature perithecia.

B. Prince of Wales.

(5) One mature tree was dead, two or three others were dying back from the top. All diseased trees were found at Histon, Cambridgeshire. Small, blue-green, sunken pycnidia were borne singly on poorly-developed stromata, erumpent on keeping (Fig. 4). Pink tendrils of the usual type were developed in a moist chamber.

C. Pond's Seedling.

(6) A mature tree at Long Sutton near Wisbech, one side of which was dying. The disease had spread from above downwards. Pycnidia were borne in groups on sunken, whitish, poorly-developed stromata. There were pink tendrils of spores, the dimensions of which were 5μ by 1μ . At the time Mr Brooks obtained this material the foliage of the parts of the tree affected was wilted and brown, presenting a scorched appearance.

(7) Piece of branch about 4 ins. in diameter from a tree at Long Sutton. Pycnidia and spores as in (6) above, but stromata erumpent, and dark-coloured.

It is possible broadly to divide the above into:

(a) Those with stromata well developed, dark coloured, and erumpent—Victoria (2) and (3) and Pond's Seedling (7).

(b) Those with stromata poorly developed, light coloured, and sunken—Victoria (1) and Pond's Seedling (6).

Too much stress, however, cannot be placed on this classification, in view of the great similarity of the spores and tendrils (except in the case of Victoria (3) where the dark-red tendrils and white crust point to a wider divergence) and also, as will be seen later, on account of the marked influence of media on pycnidial development.

The presence of a stroma more or less deeply seated in the bark, and sharply delimited from the surrounding tissues, indicates that all the fungi belong to the sub-genus *Leucostoma* of the genus *Valsa*, assuming, as is highly probable, that the stroma of the perithecial stage is similar to that bearing pycnidia. Beyond this it is not possible to go. The chief distinction from *Eutypella prunastri* appears to be in the production of well-marked tendrils of spores (cf. Massee, *l.c.*).

EFFECTS OF THE FUNGI ON THE TISSUES OF THE HOST.

The action of the fungi on the tissue of the host has been examined. The stains found most effective in this examination were Delafield's Haematoxylin, and the double stain Picric-Aniline Blue (picric acid being added to saturation).

It is found that the hyphae travel in the soft tissues of the bark, thence spreading laterally into the wood (cf. Fig. 5).

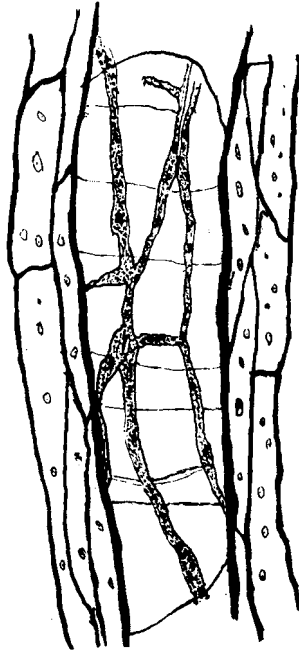


Fig. 5.

In all the tissues gum makes its appearance, in the bark and medullary rays as droplets; in the wood large masses are of frequent occurrence. As pointed out above, the diseased areas are easily identified by their collapsed bark. This collapse is caused by the death and decay of all living cells, only the fibrous cells remaining. It is usual to find above the junction areas of collapsed and healthy areas of bark, considerable lengths of red or brown wood, in which no hyphae can be traced, but gum is abundant (cf. Fig. 6). This phenomenon, however caused, is of common occurrence.

Lateral penetration of the hyphae takes place through the pits which abound in the tissues of the wood (cf. Fig. 7). This penetration is very slow, and often limited to the young wood, *e.g.* in a dead branch 8 ins. in diameter, from a Prince of Wales tree, hyphae could be found in the outermost $\frac{3}{4}$ ins., and in the inner part of this zone only in the medullary rays. In the vessels themselves the hyphae were limited to the outermost $\frac{1}{2}$ inch.

The cell walls are practically unaffected by these fungi, sections from a large dead branch failing to show any discoloration after prolonged treatment with Schultz's reagent; in consequence dead wood, although brittle, shows no signs of crumbling.

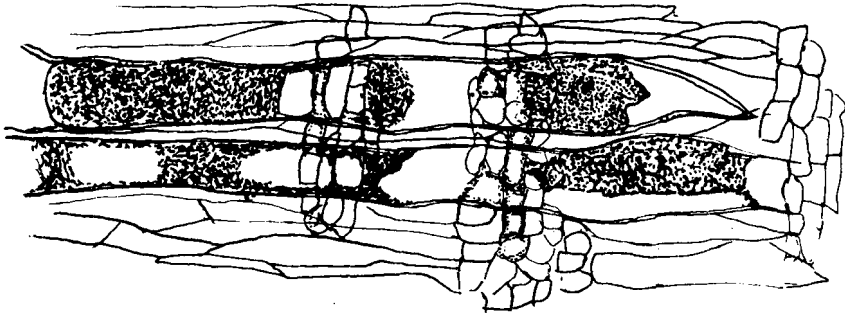


Fig. 6.

The formation of pycnidia is preceded by an aggregation of hyphae in the tissues of the bark to a small hard pustule, which gradually enlarges, splitting the bark in the process. Owing to the friability of diseased bark, this process could not be followed in detail.

In "Victoria (3)," the hyphae are dark coloured, large, fairly thick walled, and stain with difficulty; in the remainder, they are thin walled, narrow, hyaline, and stain readily. With this single exception, the above description applies to all the specimens examined.

CULTURE EXPERIMENTS AND CHARACTERS OF THE FUNGI IN PURE CULTURE.

All attempts to bring about maturation of the asci in the only perithecius found having failed, the description which follows applies entirely to cultures obtained from conidia.

Separate experiments in regard to the germination of spores were made with all the "strains" mentioned above, and there was complete agreement in the results attained.

Germination readily took place in twenty-four hours at room temperature (summer) in all natural nutriment media, *e.g.* grape juice, fruit extracts stiffened with agar or gelatine, and plum wood extract. Moistened strips of plum wood and solid media, such as carrots or potatoes, also gave good results.

No germination took place in distilled, rain, or tap water, or in water collected after slowly trickling down a healthy plum shoot.

The limits of vitality of the spores are not known, although (a) spores readily germinated after five days' soaking in water, when some nutrient material was added, (b) spores from tendrils which had been kept for four months in the dry laboratory air readily germinated, (c) spores

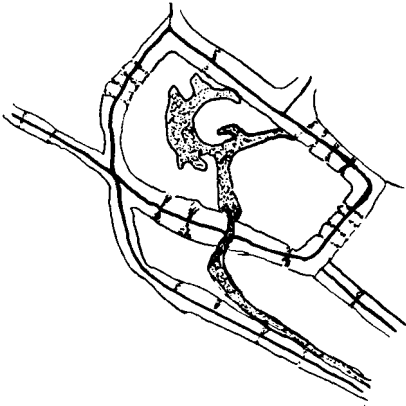


Fig. 7.

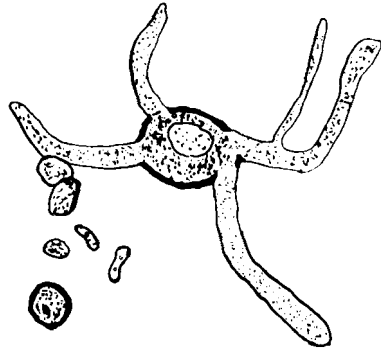


Fig. 8.

from tendrils which had been kept three months in culture vessels in a saturated atmosphere, likewise germinated. These facts indicate considerable vitality within wide limits.

Germination tests were also carried out with artificial media. In those containing no nitrogen, such as solutions of glucose, saccharose, or either of these with the addition of phosphates, no germination took place. On the addition of nitrogen in simple combinations, such as ammonium salts, only a slight swelling resulted. Ammonium tartrate and non-poisonous nitrates give better results, and small germ-tubes were produced. The addition of organic nitrogen, *e.g.* peptone or albumen, brought about normal germination.

The details of germination are similar to those described by Aderhold (1). The sickle-shaped spores enlarge considerably, and in about ten

hours, by swelling along the short diameter, are converted into spheres, from which eight to ten hours later 1-4 germ tubes protrude (cf. Fig. 8).

The isolation in pure culture of members of this genus is rendered easy by the production of tendrils of spores. A small piece of tendril is placed in a drop of sterile water, and spores transferred from this to the required medium. When large numbers of cultures were required it was found most convenient to make stock cultures on grape-gelatine, from which mycelial inoculations were carried out.

In nearly all cases, mycelial growth followed by pycnidial formation takes place with great rapidity.

There is an entire absence of any definite stroma, or limiting layer; the pycnidia being developed on small cushions of hyphae (cf. Fig. 9)



Fig. 9.



Fig. 10.

and the hyphae of the general tissue passing gradually into conidiophores.

On cutting sections of a very young pustule (it is best to take one growing on agar material), it is seen to be a solid mass of interwoven hyphae. As growth continues, spaces appear, which later become chambers. These chambers are lined with sparingly-branched conidiophores, which are full of minute darkly staining granules, specially noticeable as the conidiophore walls stain only feebly. Later, conidia are abstricted from the tips of the conidiophores. One or more pores now appear in the pycnidia, through which drops of water are extruded, followed by the spores, either as pink tendrils, or more often, owing to the saturated atmosphere, as pink droplets.

The media employed included grape juice, raisin extract, plum wood extract, alone or stiffened with agar or gelatine; artificial nutrient solutions; and solids, *e.g.* potato, carrot, turnip, and plum wood.

Generally, it may be said that liquid media were not favourable to growth; gelatine cultures were marked by rapid and profuse mycelial growth at the expense of pycnidial formation, the pycnidia when formed being rudimentary; on agar, growth was slow, but pycnidia were abundantly formed.

Potato and turnip slices did not prove good media, growth was slow and pycnidial formation scanty. Carrot was highly satisfactory, large tendril-producing pycnidia being rapidly formed.

Growth on wood blocks, in the usual culture tubes, was very rapid on the surface, a dense mycelial felt being produced in five days. Penetration was however slow; in one case, no hyphae were at any depth greater than $\frac{1}{4}$ inch in a block inoculated two months before; after ten months penetration was complete. Pycnidia were formed in greater abundance on those blocks with bark attached; the bark is split by pressure from below, and through the fissures tufts of hyphae come to the surface; on these, pycnidia are developed in 3-4 weeks.

This formation of pycnidia on the surface of the medium, instead of being immersed, has been noted by Aderhold (1), and ascribed to the high moisture content of the air in culture vessels.

All spores produced in artificial culture were found to be of uniform size and shape, 5μ by 1μ , hyaline, continuous, and sickle-shaped, thus agreeing with those found in nature.

The following is a detailed description of certain peculiarities in some of the "strains"; for convenience each strain is described by the name and number of its host.

(a) *On raisin or grape-gelatine media.*

Victoria (1). Medium rapidly coloured black, hyphae remaining hyaline.

Victoria (2). Mycelium brown, no discoloration in medium.

Victoria (3). Hyphae dark, no discoloration.

Prince of Wales (5). Mycelium confined to the upper surface of medium, forming a light-brown skin. No discoloration of medium.

Pond's Seedling (6). No discoloration of medium. At the edge of the dish a peculiar "efflorescence" of the medium takes place, due to the production of snow-white, feathery aerial hyphae; these sometimes appear in a dish which is drying up.

(b) On raisin, grape juice, or plum agar media.

Victoria (1). Medium blackened. Pycnidia black, 1-2 mm. diameter covered with a light-grey mycelial felt (cf. Fig. 9).

Victoria (3). No discoloration of medium. Hyphae hyaline at first, dark later. Pycnidia as in (1).

Prince of Wales (5). Colourless mycelium. No discoloration. Pycnidia black, covered with grey felt.

Pond's Seedling (6). White mycelium. No discoloration. Formation of aerial hyphae similar to those produced on gelatine. Large white pycnidia, covered with a greenish felt (Fig. 10).

(c) On potato agar media.

Victoria (1). Greenish-black coloration of medium. Large pycnidia.

(d) On wheat flour agar media.

Victoria (1). No discoloration. Cream-coloured, almost invisible, mycelium. Grey-coloured large pycnidia.

(e) On acid and alkaline media.

By titration with normal acid or alkali, using phenol phthalein as an indicator, a series of raisin agar tubes was obtained, containing various concentrations of acid or alkali. It was found that the degrees of acidity or alkalinity limiting the growth of these fungi were 10 per cent. normal HCl and 5 per cent. normal NaOH.

Victoria (1). 10 per cent. normal HCl. Slow growth. No discoloration of medium.

5 per cent. normal HCl, neutral, and 5 per cent. normal NaOH. Normal growth, with discoloration of medium.

Pond's Seedling (6) and *Prince of Wales* (5). Normal growth at all concentrations, within above limits.

FORMATION OF PERITHECIA.

Prolonged but fruitless attempts were made to induce perithecial formation in culture. These included growing the fungi on pure agar, agar with a high concentration of nutrient material, acid and alkaline media, and upon wood. Cultures were also kept (a) at 28° C. for three months, (b) frozen, (c) exposed throughout the winter on the laboratory roof, (d) in the dark; but with negative results.

The recent work of Shear (3) and Harper (2) has indicated the existence of "strains" within the same species of fungus which behave differently in culture media. It seems probable that the fungi here investigated were conidial bearing strains only. The constant cultural differences described above point to the existence of different strains.

Inoculation experiments.

These have, so far, yielded negative results. A T-shaped cut was made in the bark of a healthy plum shoot, and a small piece of mycelium introduced under the edges of the cut.

Inoculations made in September, 1913, were examined a year later but no signs of infection could be found.

In view of the negative results yielded by the infection experiments up to the time of writing conclusive proof that the "die-back" disease under investigation is caused by the fungi in question is lacking. There is however strong presumptive evidence that they are responsible. Their presence in the tissues of the host in each case examined, the general character of the development of their fructifications on the diseased bark, the identity with or close relationship to *Cytospora leucostoma*, a parasitic fungus known to cause a strikingly similar disease on cherry trees, of the fungus isolated from the diseased areas of the affected plum trees in most cases examined, and the failure to find in the diseased parts any other organism to which the disease could be attributed, are points which, taken collectively, suggest that the fungi in question have caused the trouble. The lack of confirmative evidence from the infection experiments, although rendering absolute proof of the cause of the disease at this stage impossible, does not necessarily conflict with the view that these fungi are the cause, since it is always possible that the conditions necessary for successful infection did not obtain in the experiments already conducted.

SUMMARY.

- (1) A disease or diseases of plum trees believed to be caused by one or more species of *Cytospora* has been described.
- (2) The fungus isolated in most cases is closely related to or identical with *Cytospora leucostoma*.
- (3) Complete germination of the spores took place only in presence of organic nitrogen.
- (4) Pycnidia and spores were obtained in artificial culture, similar to those occurring in nature.
- (5) Attempts to induce perithecial formation failed.

LITERATURE.

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EXPLANATION OF FIGURES.

- Fig. 1. A piece of diseased bark of Victoria (3). Nat. size.
 Fig. 2. T.S. of pycnidia of Victoria (3). $\times 80$.
 Fig. 3. A piece of diseased bark of Victoria (4). Nat. size.
 Fig. 4. A piece of diseased bark of Prince of Wales (5). Nat. size.
 Fig. 5. L.S. of diseased wood of Prince of Wales. $\times 300$.
 Fig. 6. L.S. of wood of diseased Prince of Wales branch, above hyphae. $\times 220$.
 Fig. 7. T.S. of diseased wood of Prince of Wales. $\times 800$.
 Fig. 8. Germinating spores of fungus from Victoria (1). $\times 300$.
 Fig. 9. Petri dish culture of fungus from Victoria (1) on raisin agar.
 Fig. 10. Petri dish culture of fungus from Pond's Seedling (7) on raisin agar.