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# XXXIV.—ON A TREE OF *AESCULUS PAVIA* KILLED BY *BOTRYTIS CINEREA*.

STUDIES FROM THE PATHOLOGICAL LABORATORY: V.

WILLIAM B. BRIERLEY.

(With Plate.)

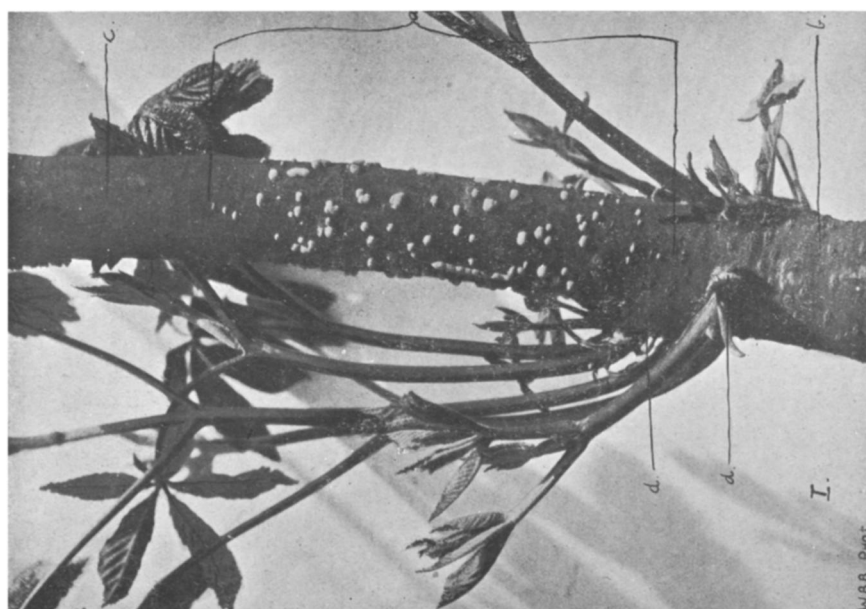
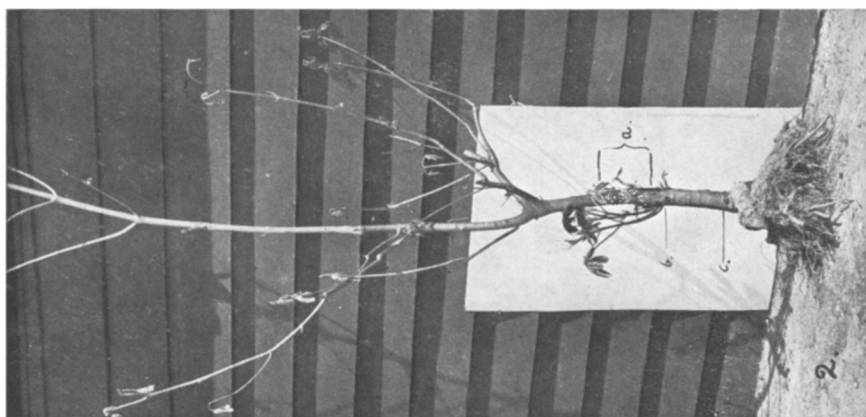
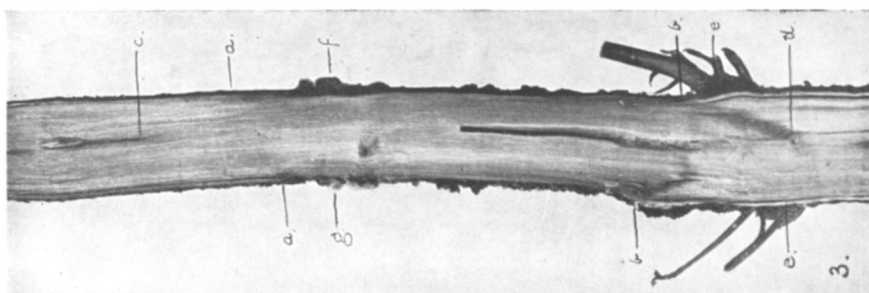
On May 28th a young specimen of *Aesculus Pavia* growing in the Royal Botanic Gardens, Kew, was observed to be in an unhealthy condition. The leaves were thoroughly wilted and the tree was apparently suffering from the effects of extreme drought. Several specimens of *Aesculus* sp. were standing in the immediate vicinity and as these seemed to be perfectly healthy with turgid extended leaves, a closer examination of the original tree was made. Around the base of the main stem was a circle of bare earth about one metre in diameter, and this soil shewed no appreciable difference in humidity or general appearance from that surrounding the neighbouring trees. About twenty centimetres above the soil surface a circle of new shoots, averaging three to five centimetres in length, sprouted vigorously from the stem. Commencing immediately above these shoots a zone of about ten or twelve centimetres of the bark was slightly shrunken, and sodden or discoloured in appearance; and bore a few immature pustules of *Botrytis* conidiophores. The leaves of the tree were green but hanging in a wilted and limp condition.

Two days later the leaves were no longer flaccid but dry and brittle, and the entire upper portion of the tree was in a desiccated state. The basal shoots were of a dark maroon-purple colour\* and growing with extraordinary rapidity and vigour, the longer ones being twenty-three to twenty-six centimetres in length (Pl. vii. Figs. 1 and 2). The diseased region immediately above these showed an abundance of the pale-smoke-grey pustules of *Botrytis*, which obviously coincided with the position of the lenticels in the bark (Pl. vii. Fig. 1, a). Above this region the lenticels were inconspicuous and small, whilst below they were large and tumescent (Pl. vii. Fig. 1, b, c). The diseased tree was about four and a half metres in height and of a moderately strong and vigorous growth. Its age was six years, and the affected region of the stem had a diameter of 4.5 cms. On May 31st the specimen was taken from the ground and photographed (Pl. vii. Figs. 1 and 2).

The diagnosis appeared perfectly straightforward and simple, and the death of the tree was attributed to an invasion of the main stem by *Botrytis*, which, plugging the conducting elements, effectively cut off the water supply to the upper portion of the tree, giving rise to the symptoms of extreme drought. This barrier would cause an excess of food material to accumulate immediately below the diseased region and in consequence the latent or adventitious buds situated here would be stimulated into active development, and a circle of new and vigorous shoots

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\* All the colour terms used are in accordance with Ridgway, R., "Color Standards and Color Nomenclature," Washington, 1912.



*To face page 315.]*

would arise. The deficiency of water in the upper portion of the stem would cause the lenticels to shrivel and become inconspicuous, whilst the excess of water below the diseased region would cause them to become tumescent.

The rapid killing of a large woody tree by the cutting off of food supplies owing to an invasion of the main stem some considerable distance above the soil surface is a sufficiently unusual mode of behaviour for *Botrytis* to make a further examination appear of some interest.

**Period of Attack.**—One of the striking features in this case is the extreme rapidity with which the upper portion of the tree was killed; and this is made more noteworthy when account is taken of the fact that the wood of *Aesculus Pavia* consists largely of fibres and tracheids, and that although the vessels are comparatively numerous they are of small diameter, and there is little wood-parenchyma tissue. In addition the medullary rays are usually one and rarely more than two cells in width, so that the wood is moderately compact and dense. The development of mycelium even in open woody tissues is usually very slow, and trees attacked by virulent and destructive fungus-parasites not infrequently remain alive for many years, only eventually succumbing to the cumulative effects of the invasion. Similarly in pure cultures of parasitic fungi on wood blocks, the penetrative power of the mycelium in a radial direction is slight, and after a period of several months the hyphae are not usually found at a greater depth than two or three centimetres. In the pure cultures of *Botrytis* upon sterilised blocks of willow and horse chestnut made by Brooks and Bartlett\* “the hyphae were seen to have penetrated but a short distance and were only found in those vessels and cells of the medullary rays which were near the surface of the blocks of wood”. The slight penetrative power of *Botrytis* in lignified tissues has recently been demonstrated from another point of view by Brown,† for he has shown that the active extract of this fungus, which, when injected into floral structures “produces rotting and death within half an hour” is totally without action on tissues of a hard woody nature. Again woody plants can, as a rule, withstand the cutting through of the greater part of their sap-wood by either artificial or mechanical means, or canker inducing and other fungi, without serious detriment to the immediate condition of their health. In consequence of the above factors the rapid wilting of a tree is only rarely due to fungal attack, and most usually must be attributed to fumes in the soil, extreme drought, or some other factor which affects in a general manner the whole root system. Where it appears that the wilting must be due to fungal invasion, these symptoms imply a remarkable rapidity of mycelial development, and a most unusual thoroughness and completeness of tissue permeation. If the fungus be *Botrytis* as in the present case this would seem even more noteworthy.

\* Brooks, F. T., and Bartlett, A. W.: Two Diseases of Gooseberry Bushes, *Ann. Mycol.*, vol. viii, No. 2, 1910.

† Brown, W.: “The Action of *Botrytis cinerea*,” *Ann. Bot.*, vol. xxix, 1915.

When the tree was first observed on May 28th. the leaves had completed their full expansion after the long winter, and were of normal size and growth, indicating that their development had not been interfered with in any way. They were thoroughly wilted, however, and hung in a limp and flaccid condition. Two days later the upper portion of the tree beginning from the *Botrytis* zone was in a desiccated state, and the leaves were brittle and easily detachable. These symptoms are significant of a very recent cause of drought due to a factor operating in so active and rapid a manner as to form almost immediately a complete barrier to all water conduction in the stem. If the *Botrytis* mycelium in the diseased region is this factor, as appears to be the only probable hypothesis, its behaviour is remarkable.

As the tree was only discovered during the late stages of the fungal invasion the exact length of time from the primary attack to the death of the host is unknown. A study of the weather conditions during the month of May is, however, instructive. From the 1st of May until May 16th no rain fell at Kew, and the weather was consistently bright and sunny. The temperature was comparatively high, the maximum during this period averaging  $20.2^{\circ}$  C. and the minimum  $6.7^{\circ}$  C. From May 16th until May 23rd the weather was dull and showery, 4.19 cms. of rain falling in seven days. The average maximum temperature fell by three degrees, whilst the average minimum increased by three. From May 24th until the end of the month there was a recurrence of the earlier weather conditions with a slightly increased temperature but occasional dull days. Rain only fell on the 29th May when 1 cm. was recorded.

It is well known that the active development of *Botrytis* is peculiarly dependent upon the presence of warm, humid, and equable conditions, and this is especially true of the infection stages. The brief wilting period indicating the unusual rapidity with which the fungus must have developed in the tissues has already been noted. When these factors are borne in mind, and the various phenomena presented by the host tree are correlated with the weather conditions, it appears extremely probable that the primary fungal attack must have occurred during the dull wet week of equable temperature extending from May 16th to May 23rd. This would give a maximum period of fifteen days from the initial penetration of the mycelium or germinating spore to a state of host permeation resulting in the complete desiccation of the tree.

**General Distribution of the Fungus in the Host.**—In order to determine the degree of penetration of the host tissues by the fungus, the main stem of the tree was carefully divided in a radial longitudinal plane. The diseased area was then visible by reason of its discoloration, and this was greatly accentuated by a few hours' immersion in water (Pl. vii. Fig. 3).

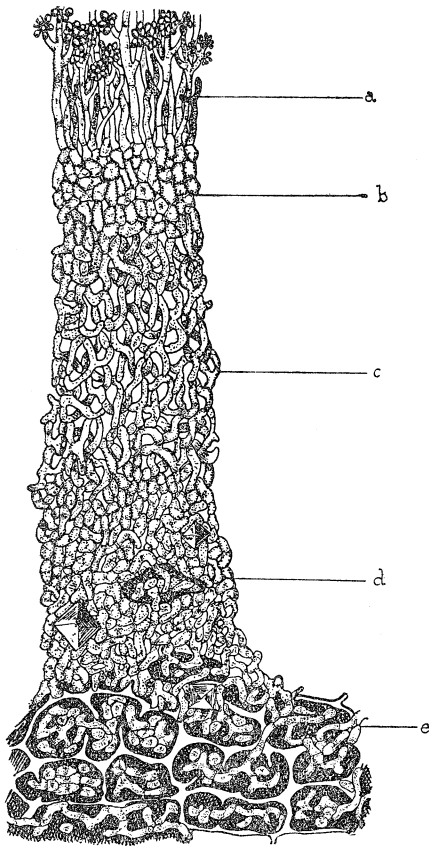
In section the diameter of the affected portion of the stem was appreciably less than that of the non-affected regions, but the most striking distinction lay in the general appearance and

colour of the diseased area. The upper portion of the stem was of a cartridge-buff colour, perfectly dry in appearance, and with a tendency for the tissue elements to tear up. Separating this from the diseased wood and marking the upper extent of mycelial development was a very diffuse olive-buff zone (Pl. vii. Fig. 3, a c a). This merged into the pale-olive-buff of the diseased tissues, which were dull and waterlogged in appearance. The lower limit of fungal growth was sharply defined by a narrow chamois coloured zone extending downward across the wood (Pl. vii. Fig. 3, b d b). The lower healthy portion of the stem contrasted sharply with the tissues above, being bright and richly sappy in appearance and of a marguerite-yellow colour. The diseased region of the cortex was even more sharply defined than the affected wood, for protruding through the collapsed sepia coloured cells were the pustules of *Botrytis* conidiophores. The dry deep-olive-buff coloured cortex above was sharply divided from the dead tissues (Pl. vii. Fig. 3, a) whilst the moist sappy cortex below was of a pale-glass-green colour and bordered abruptly on the diseased cortex (Pl. vii. Fig. 3, b). In addition the latter adhered closely to the wood, whilst the dry cortex above and more especially the healthy cortex below tended to split away from the wood in the plane of the cambium. The demarcation of the affected region has been described in some detail partly because of its distinctiveness, and partly because it shewed in a striking manner that the mycelium of the fungus advanced more rapidly in the woody cylinder of the host than in the cortical tissues (Pl. vii. Fig. 3, a-c, b-d), a result at variance with the experience of previous investigators.

There was no differential discoloration of the wood in transverse section as was found by Brooks and Bartlett in gooseberry bushes attacked by *Botrytis*.

**Distribution of the Fungus in the Cortex.**—The cortex, bast, and cambium are penetrated in all directions by the mycelium, and a careful examination of the tissues between the healthy and diseased regions, showed that the cells are killed in advance of the actual fungal invasion. In the affected zone the cells lose their content and the cellulose walls become swollen, yellowish in colour and highly refractive. The discoloration and death of the cells occurs before there is any visible effect on the cell-wall. The cell-wall substance is not infrequently finally disintegrated, the middle lamella being the last to disappear, and in the tissues, lacunae are formed which become filled with loosely formed pseudo-parenchymatous masses of mycelium. The relation of the mycelium to the cells of the host, and the nature of the parasitism of the fungus will be discussed in greater detail in a further communication. (Text Fig. 1, e.) It is interesting to compare this with the results obtained by Brown\* working with *Botrytis* extract. "It was shewn that the first demonstrable action consisted in the solution of the middle lamella uniting contiguous cells, with the result that coherence of the tissue was destroyed. The attack

\* Brown, W. On the Physiology of Parasitism, New Phyt. vol. xvi. 1917.



Text fig. 1.

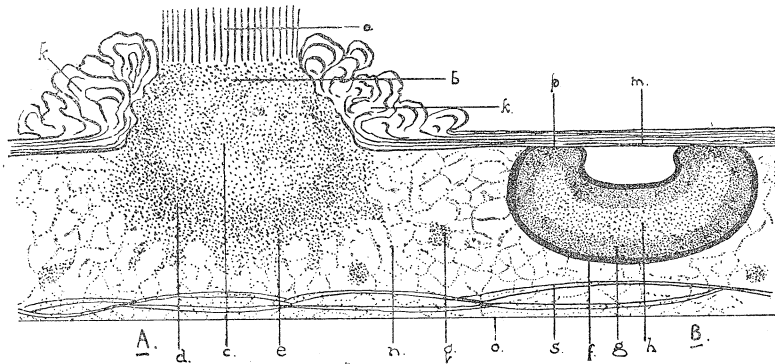
In the present case the hyphae were most usually found ramifying abundantly in a loose weft through the disorganised cortical cells, showing no discrimination for an intercellular as distinct from an intracellular course. Under each lenticel in the bark the hyphae mass together forming a pseudo-sclerotial structure which protrudes through the lenticel and resolves itself into a dense cluster of conidiophores (Text Fig. 1 & Pl. vii. Fig. 1, a, Fig. 3, f, g). These stromatic masses usually occupy about two-thirds of the depth of the cortex, are variable in size, and are plainly visible to the unaided vision. They are not sharply defined but are formed by the merging of the cortical-hyphae into a complex of disordered cortical cells and crystals embedded in a mycelial weft. (Text Figs. 1 and 2, e, d.) The centre of this structure is purely hyphal but never forms the solid dense tissue of a true sclerotium (Text Figs. 1 and 2 c). Passing through the lenticel to the outside of the stem this stroma gives rise directly to *Botrytis* conidiophores (Text Figs. 1 and 2, b, a).

\* Blackman, V. H. and Welsford, E. J. Infection by *Botrytis cinerea*, Ann. Bot. vol. xxx. 1916.

† Smith, R. E. The Parasitism of *Botrytis cinerea*, Bot. Gaz. xxxiii. 1902.

was, however, not confined to this, and the general body of the cell disintegrated, though complete solution of all constituents did not take place. At a comparatively late stage in the process the cells lost their power of becoming plasmolysed in hypertonic solutions. Thus of the two manifestations of the action of the extract, the toxic is subsequent to the enzymic". Blackman and Welsford,\* however, found that in the infection of the mesophyll of bean leaves by *Botrytis* "the first morbid change is seen as a slight disorganisation of the protoplast; the swelling of the wall is not noticeable till a later stage". The present results agree very well with those of Smith,† who states that "two different effects must be clearly distinguished, one following the other: first the death of the cells; and second the disintegration of their walls and contents."

These, and to a certain extent the basal tissues are partially extruded pushing back in all directions the corky tissues of the lenticel. (Text Fig. 2, A, a, b, k.) The conidiophorous-stromata or pseudo-sclerotia are only found in relation to the lenticels and as far as could be determined every lenticel in the diseased region was occupied in this manner (Pl. vii. Fig. 1, a), this probably being the cause of the sodden, water-logged appearance of the affected tissues. Somewhat similar structures have been described on the vine by Istvánffi\* and on lime trees by Smith.† In the former case the stromata were considerably more definite in structure, and were very small, being formed under the



Text fig. 2.

cuticle; whilst in the latter, they commenced as true sclerotia, but as they passed to the exterior of the host became diffuse, and finally resolved into conidiophores. In neither case is any relation to the lenticels mentioned, nor do the figures indicate that this is present.

In addition to these structures in *Aesculus Pavia* true sclerotia are formed in equal abundance. These average from 1.5 mm. to 2 mm. in diameter, and from 0.5 to 1 mm. in thickness (Text Fig. 2, B). They are circular or irregularly oval in outline and possess one convex and one concave side, the latter being closely adpressed to the bark of the host. (Text Fig. 2, B.) The central portion consists of a mass of elongate ramifying hyphae which toward the periphery form a dense and solid tissue enclosed in a brownish-black skin one or two cells in thickness. This skin is absent from the sclerotial ring in contact with the bark (Text Fig. 2, B, p), and this is paralleled by tube cultures of *Botrytis*. Here the sclerotia are strongly concave and most usually adhere to the glass, the narrow, irregular, contact surface of the fungus body being free from the black skin enclosing all other surfaces of the sclerotium. The sclerotia are perfectly discrete bodies with little mycelial attachment at any stage

\* Istvánffi, Gy. de: Etudes microbiologiques et mycologiques sur le rot gris de la vigne—*Botrytis cinerea* ou *Sclerotinia Fuckeliana*, Annales de l'Institut Central Ampélogique Royal Hongroise, tome iii, 1905.

† Smith, R. E. *Botrytis* and *Sclerotinia*: their relation to certain plant diseases and to each other, Bot. Gaz. xxix. 1900.



of their development, and are of a firm cheese-like consistency. Their distribution in the cortex contrasts markedly with that of the pseudo-sclerotia for they are never found correlated with lenticels, but are always adpressed to the unbroken bark (Text Fig. 2, A, B). The sclerotia and conidiophorous-stromata are not successive stages in the development of single bodies; but are discrete entities without intermediate structures, and may be formed simultaneously, for examples of each in various stages of growth are to be found side by side. Whatever the factors may be which determine whether the cortical hyphae at a given spot form a sclerotium, or a stromatic pustule resolving into conidiophores, they evidently bear a direct relation to the lenticels. It is improbable that this stimulus is one of aeration for it is difficult to conceive that this could have so local and so constant an application throughout the diseased cortex. A more probable hypothesis is that the stimulus is one of pressure or contact, and this would derive support from the fact that in tube cultures of *Botrytis*, the conidiophores are formed over the free surface of the medium, whilst the sclerotia are most usually formed in contact with the glass at the periphery.

**Distribution of Fungus in the Wood.**—The symptoms of extreme drought presented by the specimen had aroused the expectation that the water conducting tissues in the stem would be completely plugged by hyphae. So far from this being the case, however, transverse sections through any portion of the diseased wood showed practically an absence of fungus. The explanation of this was given by radial longitudinal sections for in these it was seen that the mycelium was almost totally confined to the *living* tissues of the central cylinder. From the cortex the fungus passed into the medullary rays which it traversed in a radial direction, and in which it was so abundant as to form the most prominent feature in stained sections, often completely filling the cells of this tissue with masses of fungal hyphae. The mycelium also invaded freely the cells of the wood-parenchyma, but only very rarely could a filament be discovered in any of the water-conducting elements of the stem or in the dead mechanical fibres. The fungus was absent from the pith although ray cells bordering on this tissue contained the fungus in quantity. Sections were taken from all portions of the affected zone, and the constancy and exactitude of this differential distribution confirmed.

**Cause of Death of Host.**—The absence of the mycelium from the vessels and tracheids immediately proved the original diagnosis to be erroneous, for this was based on the assumption that the water-conducting elements of the stem were choked by masses of hyphae, as in the *Verticillium* disease of potatoes,\* or the Wilt disease of cotton.† The extraordinary vigour and turgidity of

\* Pethybridge, G. H. The *Verticillium* Disease of the Potato, Sci. Proc. Roy. Dub. Soc., vol. xv. 1916.

† Smith, E. F. Wilt Disease of Cotton, etc., Div. Veg. Phys. and Path. U.S. Dept. Agr. Bull. 17. 1889.

the new shoots, and the tumescence of the lenticels below the diseased zone, indicated that here there was no lack of water, and that root pressure was operating in a perfectly normal way. On the other hand the rapid wilting and finally the complete desiccation of the tree commencing from immediately above the diseased zone, showed equally conclusively that the passage of this water up the stem was totally inhibited by some factor in the affected region. Yet in this zone itself the water-conducting elements were free from the presence of the fungus, whilst on the other hand the medullary rays and wood-parenchyma tissues were killed and occluded by the hyphae.

These results seemed to lend strong support to the view that the living parenchymatous cells of the wood and medullary rays are fundamentally important and integral parts of the tissues concerned in the raising of water in the plant, a theory early formulated by Westermaier\* and Godlewski,† and more recently upheld by Ursprung‡ and in a slightly modified form by Ewart.§

It is well known that if a localised region of a branch still attached to a tree be killed by artificial means, the leaves above this region eventually wilt and shrivel, the rapidity of the fading being roughly proportionate to the length of the affected portion of the branch.¶ According to the above hypothesis the symptoms of drought shown in this case are directly due to the suppression of the vital activity of the cells of the medullary rays and wood parenchyma, which so reduces the supply of water to the foliage above that the leaves quickly fade. Such a condition appears to be exactly paralleled in the present specimen of *Aesculus Pavia*, the artificial lethal agency being replaced by the action of the *Botrytis* mycelium. The general application and truth of this explanation has, however, been subjected to very severe criticism. Strasburger|| for example in 1891 demonstrated that stems more than ten and a half metres long continued to conduct water after they had been completely killed by steam, and similar experiments have been repeated by many observers since, which seem to prove indubitably that even when the vital activity of the cells of the wood has been eliminated, water under the action of purely physical forces rises in the stems of high trees. It is evident therefore that the inhibition, by death, of the vital functions of the living cells of the central cylinder in a zone ten or twelve centimetres in extent at the base of the specimen of *Aesculus Pavia*, could not be the primary cause of the wilting of the foliage and the desiccation of the tissues.

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\* Westermaier, W. Zur Kenntniss der osmotischen Leistungen des lebenden Parenchym's Ber. der deut. bot. Gesell., Bd. i, 1883.

† Godlewski, E. Zur Theorie der Wasserbewegung in den Pflanzen, Pringsheim's Jahrb. f. Wiss. Bot. Bd. xv, 1884.

‡ Ursprung, A. Zur Frage nach der Beteiligung lebender Zellen am Saftsteigen, Beihefte z. Bot. Centralb. Bd. 28, 1912.

§ Ewart, A. J. The Ascent of Water in Trees, Phil. Trans. Roy. Soc. Lond. vol. 108B, 1905; and vol. 199B, 1908.

¶ See Janse, J. M. Der Aufsteigende Strom in der Pflanze, Jahrb. f. Wiss. Bot., i, Bd. 45, 1908; ii, Bd. 52, 1913, and Ursprung, A., loc. cit.

|| Strasburger, E. Ueber den Bau und Verrichtungen der Leitungsbahnen in den Pflanzen, Jena, 1891.

Furthermore it has been abundantly demonstrated by Weber,\* Janse,† Vesque,‡ and more recently by Dixon,§ that the cessation of the transpiration stream in a branch which has been killed in a local region, is not due to the removal of the vital activities of the living cells, but to the clogging of the conducting vessels above the diseased region, by the products of the morbid changes in the dead tissues, which contaminate the ascending water-flow. This brown gum-like clogging material is deposited in the walls and lumina of the conducting elements immediately bordering the killed region. The adjoining living cells are stimulated to the development of tyloses and so ultimately the passage of water up the stem is completely blocked. Dixon has also shown that the "physical and chemical nature of the sap is profoundly altered by steaming the branch through which it passes" and that the consequent accumulation of poisonous substances in the leaves ultimately causes their wilting and death, even if these branches are given a double supply of water. Attention may be drawn to the fact that it is not the particular method adopted to kill the localised region, which produces the changes, but the morbid products emanating from the dead tissues. In the case of branches attached to trees the period elapsing before wilting of the leaves becomes evident, is rarely less than four or five days and is usually considerably longer; but shoots experimentally poisoned by the degenerative products may show wilting of the leaves in as short a period as two and a half days,¶ a time corresponding approximately with the wilting period in the present specimen of *Aesculus Pavia*.

It appeared not improbable therefore that in the latter case the death of the tree might have resulted from the combined effect of the poisoning of the leaves, and the clogging of the tracheae, by morbid products arising from the cells of the medullary rays and wood-parenchyma which had been killed by the *Botrytis*. This hypothesis would reasonably correlate the presence of the diseased zone of the tree with the symptoms of rapid and extreme drought shown by the foliage.

There were at once evident, however, a number of facts which would not coincide with this theory. If, for example, the leaves were poisoned by the accumulation in their tissues of necrotic matter, their transpiratory functions would cease before the tracheae in the stem were clogged by morbid products, and the immediate result of this would be a slight excess of water in the tissues of the upper portion of the tree. It is difficult therefore to conceive how such a process could in any way give rise to the state of extreme desiccation exhibited by the wood and cortical tissue of the diseased *Aesculus Pavia*. Furthermore Dixon has

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\* Weber, C. A. Ueber den Einfluss höherer Temperaturen zu leiten, Ber. d. Deutsch Bot. Gesell. Bd. 3, 1885.

† Janse, loc. cit.

‡ Vesque, J. Sur le prétendu rôle des tissus vivants du bois dans l'ascension de la sève, Compt. rend. Tome 101, 1885.

§ Dixon, H. H. Transpiration and the Ascent of Sap in Plants, London, 1914.

¶ Dixon, H. H.: loc. cit.

pointed out that "the leaves which fade after their supporting branch has been killed by heat, fade in a different manner from those which wilt owing to a lack of water. In the former case the margin of the leaf first becomes darkened and this darkened region gradually invaded the leaf between the veins. It then dries and shrivels whilst the green parts immediately round the veins remain comparatively fresh. As this change is taking place these veins usually become pink and finally brown. This coloration is particularly noticeable when the leaves are viewed with transmitted light. Shrivelling and withering of the leaf, except at the edges, does not occur until after these changes are complete". "On the other hand, when leaves fade simply from an insufficient water supply, *e.g.*, on a branch severed from a tree, shrivelling comes on while they are still green. Blackening appears only after shrivelling and occurs in irregular patches. The veins do not change colour and the walls of the tracheae do not appear coloured in transverse section. The first colour change is when the cell-contents of the mesophyll and parenchyma of the veins colour brown after death". It may again be emphasised that the manner of localised killing, whether by steam, chemicals, fungus mycelium or other lethal agency is without importance; the distinction is one between a process of poisoning by the products of morbid and degenerative changes, and the effects merely of a shortage of water.

Now it has already been noted that in the present specimen of *Aesculus Pavia* the leaves at first were limp and flaccid but of a normal green colour. When the tree was taken from the ground three days later the foliage was shrivelled, dry and brittle, and during the subsequent week the leaves merely withered in the manner characteristic of drought, and without any of the symptoms of poisoning.

The desiccated condition of the tissues of the stem, and the manner in which the leaves faded, were strong presumptive evidence that poisoning of the foliage due to contamination of the sap-flow by morbid products, had not occurred. This, however, did not negative the probability that the conducting elements in the stem immediately above the diseased region were choked by the degenerative matter emanating from the dead tissues; and that this clogging, supplemented by tylose formation, was the factor inhibiting the flow of the transpiration stream in the tree.

To determine the soundness of this hypothesis, a careful examination, using microchemical methods, was made of the tissues immediately above the diseased zone and these were compared minutely with corresponding tissues from other parts of the stem. No trace could be detected of any deposition of morbid products either in the walls or in the lumina of the tissues in question, and although, as will be noted later, tyloses were not infrequently present they were not more abundant in this region than in any other portion of the tree. Thus although at first it had appeared probable that the death of the *Aesculus Pavia* under consideration was either a direct result of the suppression of the vital activities of the cells of the medullary rays and wood-parenchyma

in the affected region; or an indirect result of their destruction, by reason of the clogging of the tracheae in the contiguous zone immediately above, by the morbid products of their degeneration; no evidence in support of either of these views could finally be discovered.

It was evident therefore that some other factor existed in the diseased zone; a factor which operated so effectively and rapidly that within a period of a few days a barrier was formed completely checking the upward flow of water in the tree. Below the limit of fungal extension (Pl. vii. Fig. 3, b d b), the tissues were exuberantly healthy and turgid; above the limit of mycelial growth (Pl. vii. Fig. 3, a c a), the tissues were dry and shrivelled from want of water. In the diseased zone all the living tissues of the stem had been killed and occupied by the fungus, whilst the water-conducting channels were free from hyphae. The death of the cortical, ray and wood-parenchyma tissues, was apparently without importance in the conduction of water up the tree; whilst the conducting elements immediately above and below the diseased region were perfectly free from occlusion by morbid products, and did not contain an abnormal number of tyloses.

The only feasible hypothesis remaining seemed to be that although the water conducting tissues of the diseased region of the stem were free from fungus, they must yet be mechanically occluded in some other way. To determine the nature of this occlusion, if present, a thorough visual and microchemical examination of the diseased tissues was carried out, and these were minutely compared with corresponding healthy tissues from below the affected region, and dry tissues from above. The only ascertainable difference was found to be in the relative abundance of tylose formation in the several regions of the stem. In the duramen xylem of all portions of the woody cylinder tyloses were abundant, and in the peripheral actively-conducting tissues of the healthy wood they were not infrequently present. They are thin walled and in almost all cases extend in a single series along the narrow vessels. The tyloses were difficult of accurate observation in the diseased sap wood, for they were collapsed and shrivelled and usually matted closely together. A careful comparative estimation, however, revealed that there were approximately ten times as many tyloses in a given diseased area as in a corresponding area of healthy wood. It was evident therefore that one immediate effect of the fungal invasion had been to stimulate the wood-parenchyma cells to the active formation of tyloses.

Marshall Ward\* demonstrated that in the invasion of tissues by *Botrytis* the host cells are killed in advance of the hyphae by an enzymic body secreted by the young fungus cells. Brown† has recently investigated this active principle and has shown that it is possibly a protoplasmic toxin of an enzymic nature and that it possesses a relatively high coefficient of diffusion. Although it has not yet been proved of *Botrytis* extract it is a

\* Marshall Ward, H. A Lily Disease, Ann. Bot. vol. ii, 1888.

† Brown, loc. cit.

well known property of many toxic bodies, that in dilute solution they act as growth stimulants, and proliferation of plant cells as a result of exposure to various poisonous substances has recently been abundantly demonstrated by Smith.\* When the tissues of the present host were invaded the active principle of the fungus would diffuse rapidly through the cells, and at the extreme limit of its diffusion range it would appear probable that the highly dilute toxic body stimulated the living wood-parenchyma cells to the formation of intrusive vesicles. The period of time during which the tyloses must have been formed could only be very brief, for as has been noted the active principle of *Botrytis* diffuses rapidly, and the toxic substance would very shortly be present in a lethal concentration. Immediately this acted upon the cells of the wood-parenchyma, the tyloses would collapse and shrivel together choking the lumina of the tracheae with dead matter; the rapidity of this process being manifested in the brief wilting period of the foliage of the tree. These plugs of dead tyloses were sufficiently abundant for it to be not improbable that they were primarily responsible for the complete interruption of the water stream in the stem of the *Aesculus Pavia* under consideration.

**Longitudinal Extension of Fungus in Host.**—As the roots of the tree were in a normal state of activity the blocking of the transpiration current would immediately result in an excess of raw food material in the region below the barrier. Here there had formerly been a few branches the cut ends of which were occluded by wound cork (Pl. vii. Figs. 1, d, and 3, e). The excess of food material stimulated the growth of adventitious buds from this callus, resulting in a whorl of vigorously growing shoots (Pl. vii. Figs. 2 and 1). In addition to utilising the food excess from below, these shoots would, within a certain range draw upon the tissues above them, so that these cells would be depleted of food content, and a "starvation zone" would thus be established between the whorl of shoots, and the downward trend of fungal extension. A careful examination of the tissues shewed that mycelial penetration in a longitudinal direction had apparently ceased, the limits being marked by the discoloured zones already described. Downward this cessation was sudden, corresponding with the sharply defined line which extended across and through the tissues, but which in the sap-wood region always lay above the insertion of the shoots (Pl. vii. Fig. 3, b d b). It was found that in the narrow intervening region, the tissues, although beyond the range of diffusion of the active principle of the fungus, were practically devoid of content, and it would appear probable that the limitation of downward mycelial extension was due to the inability of the *Botrytis* to cross this "starvation zone". In an upward direction the cessation of growth was much more gradual, corresponding with the comparatively broad, diffuse, discoloured zone marking the boundary of the diseased region (Pl. vii. Fig. 3, a c a); and the inability of the fungus to develop further was probably due to the increasing desiccation of the wood.

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\* Smith, E. F. Mechanism of Tumour Growth in Crown-gall, Jour. Agr. Res. vol. viii. 1917.

**Infection of the Host.**—The conidia of *Botrytis* show very great variability in their power of attacking plants, this depending upon the particular strain of the fungus, upon the host, and upon the incidental conditions in the environment at the time of infection. Marshall Ward found the spores capable of infecting lily leaves, whilst Kissling\* describing an epidemic of *Botrytis* among gentians found that the conidia could not attack the leaves. Infection occurred through the stigma and anthers, and this agrees with Nordhausen's results† in which non-cuticularised organs succumbed readily to the attacks of germinating conidia, whilst only under certain special conditions of humidity, lack of light or flaccidity could the leaves be infected. Potter‡ found no difficulty in directly infecting turnips and potato haulms with the conidia of *Botrytis*, whilst Brooks§ could only infect lettuce plants after they had been kept for some days in darkness or were yellowing. Brooks and Bartlett|| were unable to infect gooseberry bushes with conidia even when these were placed in wounds in the cortex. Tubeuf¶ and Behrens\*\* found that the spores germinated in water and immediately infected young developing shoots and needles of various conifers. Masee†† confirmed this, but stated that the spores cannot pierce the bark of a two year old seedling directly, but only as a wound parasite. I have found no difficulty‡‡ in directly infecting wounded surfaces of fig trees with conidia but have totally failed to infect uninjured surfaces. Blackman and Welsford (loc. cit.) found that spores very often failed to infect a bean leaf, and Brown (loc. cit.) from the same laboratory, has demonstrated that the cuticle of leaves offers a very great obstacle to the action of *Botrytis* extract, and further that with tissues of a "woody texture no definite action could be established in any case". As was pointed out by Marshall Ward (loc. cit.) conidia may in almost all cases be rendered capable of penetrating a cuticularised surface of a leaf or stem if their germ tubes be previously invigorated by saprophytic nourishment. Even this, however, does not appear to give them the power of infecting uninjured bark surfaces, and so far as I am aware all successful inoculations of woody plants by *Botrytis* have been achieved by

\* Kissling, E. Zur Biologie der *Botrytis cinerea*, Hedwigia vol. 28, 1889.

† Nordhausen, M. Beiträge zur Biologie parasitärer Pilze, Jahrb. Wiss. Bot. 1899.

‡ Potter, M. C. Rottenness of Turnips and Swedes in Store, Journ. Bd. Agric., vol. iii, 1896.

§ Brooks, F. T. Observations on the Biology of *Botrytis cinerea*, Ann. Bot., vol. xxii, 1908.

|| Brooks, F. T., and Bartlett, A. W. Two Diseases of Gooseberry Bushes, Ann. Mycol., vol. viii, 1910.

¶ Tubeuf, V. K. F. Beiträge zur Kenntnis der Baumkrankheiten, Berlin, 1888.

\*\* Behrens, J. Phytopathologische Notizen, Zeit. f. Pflanzenkrank Bd. v, 1895.

†† Masee, G. A Conifer Disease, Journ. Bd. Agric., vol. x, 1903.

‡‡ Brierley, W. B. Note on a *Botrytis* Disease of Fig Trees, Kew Bull. 1916, p. 225.

placing either spores or mycelium on previously wounded surfaces.

In the "die-back" diseases of woody plants caused by *Botrytis* such as those of fig trees\* ribes bushes† or roses‡ the germ tubes almost certainly enter through injured buds, while in the "die-back" of conifers, infection obtains through the tender young shoots and leaves. In the diseased sapling lime trees described by Smith and the seedling larches by Massee the attack commenced at the soil level, presumably being a direct invasion by saprophytic *Botrytis* in the soil, which entered through some injury caused probably in transplanting.

In the present specimen of *Aesculus Pavia* the distribution of the *Botrytis* pustules, the youngest being at the upper and lower growing regions and the mature ones being in the centre (Pl. vii. Fig. 1, a) indicates that the primary infection occurred approximately in the middle of the diseased region, and would therefore take place about twenty-six centimetres above the surface of the soil. This eliminates the possibility that the attack was a direct invasion of the tree by saprophytic mycelium from the soil as in the limes and larches noted above, and postulates a spore infection. The brief consideration of the biology of spore infection, however, has shown the great improbability of the penetration of an unwounded bark surface by a germinating conidium; but although a thorough inspection of the diseased area was made no wound could be detected. Nevertheless it is almost certain that a minute wound must have been present, and it would seem probable therefore that infection of the *Aesculus Pavia* occurred by a *Botrytis* spore, which chanced to be inserted under favourable weather conditions in this very minute wound in the bark of the tree—a combination of circumstances rarely to be repeated.

**Identity of Fungus.**—The conidiophores arising from the stromata in the cortex of the host were of the type characteristic of *Botrytis cinerea*, Pers., although the spores were somewhat larger than usual, measuring  $12\mu$ – $15\mu$  by  $8\mu$ – $12\mu$ . Cultures were made on potato agar of conidia, diseased bark and diseased wood, and in all cases a typical growth of *Botrytis cinerea*, Pers., was obtained. These were compared with cultures of *Botrytis* from the following sources:—(i) a strain growing saprophytically upon dead lilac leaves; (ii) a strain growing saprophytically upon woody mangolia shoots killed by frost; (iii) a strain causing a die-back of young cypress trees; (iv) a strain causing a "die-back" and fruit-rot of fig trees; (v) a strain obtained from the interior of woody galls on a bush of *Ribes alpinum*, Kew. No constant differences could be determined in the several cultures, although on their host plants these fungi vary greatly both in morphology and manner of behaviour. When cultures of *Botrytis* from *Aesculus Pavia* were inoculated into wounded lettuce plants the typical "lettuce drop" developed. Although therefore the

\* Brierley, W. B., loc. cit.

† Brooks, F. T., and Bartlett, A. W., loc. cit.

‡ Smith, R. E., loc. cit.



behaviour of the fungus in the present specimen is unusual, there is no evidence to prove that the fungus is a special physiological strain, or other than the common *Botrytis cinera*, Pers. reacting to the stimulus of the particular environment presented by *Aesculus Pavia*.

**Other Instances of Woody Trees Killed by Botrytis.**—The fungus *Botrytis* is not infrequently found upon large woody trees growing as a saprophyte, and in such cases is usually confined to young shoots and twigs which have been killed by frost, or other agency. Very rarely indeed does it develop in the woody tissues of the main stem. In a few cases the fungus is known as an active parasite upon trees, and then is usually the cause of a "die-back" of the shoots. In the common "die-back" of conifers due to *Botrytis* it cannot be said that the fungus attacks woody tissues for only the soft and tender green shoots are destroyed, and only very exceptionally does the mycelium extend into the older lignified tissues of the branches. In the "die-back" of fig trees, roses, and ribes bushes, it is again usually the young sappy shoots which are attacked. In these cases, however, the mycelium not infrequently develops into the hard lignified regions of the branches and occasionally even invades the main stem of the plants. The hyphae extend in a downward direction, growing most rapidly in the cortex, and gradually spreading inwards eventually penetrating all the tissues of the plant.

Only three cases have been described in which in nature *Botrytis* has invaded a woody host at the base of the plant. The first was by Smith in 1900 (loc. cit.) the host plants being young saplings, three to five feet high, of *Tilia parviflora* and *Tilia grandiflora*. The attack commenced at the ground level, and the hyphae spread rapidly upwards in the tissues discolouring the bark in advance of the wood. The invasion occurred probably during the winter months and its progress was sufficiently slow to permit of the neighbouring saplings becoming fully leaved whilst the diseased trees remained in bud, the buds, however, being green and apparently sound. The cortical tissues were gradually destroyed, but the bast was practically unaffected, and a few fungus filaments only could be found in the outermost portion of the wood. Bursting through the bark were "a sort of half formed sclerotia having the normal cellular structure at the base, but lacking a definite surface layer, and resolving above into a dense mass of conidiophores". Under artificial conditions ordinary black-skinned sclerotia developed later in the cortex. The second instance is given in a brief account by Massee (loc. cit.) of a number of larch seedlings attacked at the soil level by *Botrytis*. The cortex was thoroughly permeated by the fungus which formed sclerotia embedded in the tissues. Later these burst through the bark and gave rise to conidiophores.

In 1903 Miss Lorrain Smith\* described a disease of the gooseberry caused by *Botrytis*. The host was attacked at the ground level and the bark destroyed; the mycelium of the fungus per-

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\* Smith, A. L. A Disease of the Gooseberry. Journ. Bot. vol. xli, 1903.

meating the inner cortex and bast almost to the first branches and downwards into the roots. Sclerotia were found on the outside of the bark.

These cases present a number of interesting comparisons with the diseased *Aesculus Pavia*. In the former the infection occurred at the soil level presumably by *Botrytis* growing saprophytically in the soil and the mycelium spread slowly upwards destroying the cortex. In the diseased *Aesculus* the infection took place probably by a spore some twenty-six centimetres above the soil level and the mycelium spread rapidly in all directions, penetrating the medullary rays as far as the pith. In the gooseberry, lime trees and the larch seedlings there was no direct effect on the transpiration stream and the eventual death of the upper portion of the hosts was merely part of a general necrosis. In the *Aesculus* the cutting of the water supply to the upper part of the tree was rapid and complete, and although not a direct result of mycelial thrombosis, was due to a choking of the conducting elements by tyloses formed as a direct reaction to fungal stimulation. The diseased lime saplings normally showed only a form of conidiophorous stromata, whilst in the gooseberry and larch seedlings true sclerotia only were present; these, however, in the latter apparently very shortly giving rise to conidiophores. In the specimen of *Aesculus* both types of structure were present normally and bore a very definite and constant relation to the lenticels.

Thus the disease of lime trees, gooseberry bushes and larches have much in common, but apart from their similar etiology show little resemblance to the diseased *Aesculus Pavia* under consideration.

#### CONCLUSION.

The points of interest in this specimen to which attention may be drawn are as follow:—

The position on the tree at which infection occurred.

The symptoms of the disease and the rapidity with which the host, a comparatively large woody tree, was killed.

The distribution of the fungus in the tissues and especially its absence from the water-conducting channels.

The induction of the formation of tyloses which created an effective barrier to the transpiration stream.

The development by the fungus of conidiophorous stromata and of true sclerotia, and the constant relation of these to the lenticels.

A number of very interesting features were presented by the morphology of the fungus in the tissues, and these will be described in a further communication.

I am glad to record my indebtedness to Miss M. N. Owen, Temporary Technical Assistant in the Laboratory, for the preparation of many slides upon which much of the information in the present paper is based.

## EXPLANATION OF TEXT FIGURES AND PLATE.

Text Figure 1.—Semidiagrammatic vertical section through a small conidiophorous stroma, *a*—conidiophores; *b*—slightly more solid cushion of tissue which resolves into conidiophores; *c*—loose mycelial structure free from host elements; *d*—complex of hyphae, crystals and cortical cells; *e*—mycelium ramifying in the cortical tissue.

Text Figure 2.—Diagrammatic representation of cortex with A—conidiophorous stroma; and B—true sclerotium. A: *a*—conidiophores; *b*—conidiophore cushion; *c*—loose hyphal tissue; *d*—complex of fungus and host elements; *e*—cortical hyphae massing together to form the stroma; *k*—lenticular tissue pushed outwards; *n*—hyphae in cortex; *o*—cambium; *s*—bast fibres; *q*—lacuna in cortex filled by pseudoparenchymatous mass of mycelium. B: *f*—sclerotial skin; *g*—dense tissue of sclerotium; *h*—loose internal tissue; *p*—sclerotial ring from which the dark skin is absent; *m*—unbroken surface of bark.

## EXPLANATION OF PLATE VII.

Fig. 1.—*a*—diseased zone showing *Botrytis* pustules. The young pustules are situated at the margin of the zone, and the mature ones in the middle. Their distribution corresponds with that of the lenticels; *b*—healthy stem shewing tumescent lenticels; *c*—dry stem in which the lenticels may only be seen with difficulty; *d*—wound-callus occluding old branches and giving rise to vigorous adventitious shoots.

Fig. 2.—General appearance of tree immediately after removal from ground. *a*—diseased zone; *b*—whorl of developing shoots; *c*—soil level. The upper portion of the tree is in a thoroughly desiccated condition.

Fig. 3.—Radial longitudinal section through diseased zone; *aca*—upper limit of fungal growth; *bdb*—lower limit of fungal growth; *e*—wound callus from which the new shoots spring; *f*—pustule of *Botrytis*; *g*—fungus pustule showing conidiophorous stroma. Note the dryness of the upper region of the section and the cortex splitting away from the wood. The hollow in the centre of the section represents the position of the pith.

XXXV.—THE GENUS *COCOS*.

In 1886 Dr. O. Beccari published in *Malpighia* vol. i. p. 343, a preliminary study of the palms included in the genus *Cocos*, Linn.; he has now supplemented this by a revision, published in the *L'Agriculture Coloniale*, x. p. 435 (Florence, 1916), in which nine distinct genera are recognised and distinguished as in the key reproduced below. *Barbosa*, *Arecastrum*, *Butia* and *Glaziova*, regarded as subgenera in the earlier publication, are now raised to generic rank, but the name *Glaziova*, Mart. (1871), has been replaced by that of *Syagrus*, Mart. (1824), in order to