MYCOLOGICAL STUDIES. I
ON THE "SPOTTING" OF APPLES IN GREAT BRITAIN

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(With 6 Text-figures.)

CONTENTS

1. Introduction.

This investigation was undertaken with a view to enquiring into the nature and origin of the spots which occur on the surface of a large number of varieties of apple, including many much prized for culinary and dessert purposes in this country; these spots, which begin to appear towards the end of the summer, spoil the appearance of the apple and are often the cause of premature decay.

This "spotting" of apples is prevalent in the United States and investigations have been undertaken or are in progress at several of the Agricultural Experimental Stations there. The earlier workers, notably L. R. Jones (Vermont)[1], Longyear (Michigan)[2] and Lamson (New Hampshire)[3], attributed the "spotting" to various fungi, for example,

[1] Jones, L. R. *Vt. Agr. Exp. Sta. Rept.* 5 (1891), p. 133.

[2] Longyear, B. O. *Spec. Bull. Mich. Agr. Expt. Sta.* 25 (March, 1904).

[3] Lamson, W. H. *N. H. Coll. Bulls.* Nos. 27 (Apr. 1895), 45 (May, 1897), 65 (May, 1899), 101 (Apr. 1903).

Dothidea pomigena (L. R. Jones), *Phyllachora pomigena* (Schw.) Sacc. (Longyear), but lacked the opportunity to verify their suppositions by cultural experiments.

In 1908 Charles E. Brooks[1] proved by cultural methods that *Cylindrosporium pomi*, in a later paper[2] identified as *Phoma pomi*, was capable of causing "spotting" in the Baldwin variety.

In 1911 W. M. Scott[3] isolated *Cylindrosporium pomi* and species of *Alternaria* from the Jonathan variety, but concludes that the cause of the disease is unknown. *Alternaria* has been obtained also by M. T. Cook and G. W. Martin (1914)[4] from large light brown spots on Jonathan apples.

Finally E. C. Stakman and R. C. Rose[5] have announced an investigation into a fruit spot of the Wealthy apple.

Besides the fungi found during enquiries more directly concerned with the "spotting" of apples, others which generally cause rotting or twig canker have been recorded as causing "spotting," for example, *Phoma mali* Schultz. et Sacc. (Charles E. Lewis[6]), *Physalospora cydonieae* (Clinton)[7], *Phyllosticta solitaria* (John W. Roberts[8]), and *Glomerella cingulata* (Dastur[9]).

In Britain, on the other hand, little work has been done. Towards the end of 1914, some correspondence was published in the *Gardeners' Chronicle*[10] with reference to a disease of apples which was puzzling fruit growers. The apples were covered with sunken spots which often developed after the fruit was stored. The varieties affected at the time were Ecklinville Seedling, Warner's King, Cox's Pomona, James Grieve, and Rival. The disease was also developing in Peasgood's Nonsuch, Gascoigne's Scarlet Seedling, Newton Wonder, and Blenheim Pippin. Fruits of Gascoigne's Seedling remarkable for size and colour, and apparently sound early in November (1914), were at the end of the month unrecognisable through the disease. This disease was thought to be due to *Cylindrosporium pomi*; but it was pointed out that no

[1] Brooks, Charles E. *Bull. Torr. Bot. Club*, 35 (1908), p. 423.

[2] Brooks, Charles E. and Black, Caroline. *Phyt.* II (1912), p. 63.

[3] Scott, W. M. *Phyt.* I (1911), p. 32.

[4] Cook, M. T. and Martin, G. W. *Phyt.* III (1913), p. 119.

[5] Stakman, E. C. and Rose, R. C. *Phyt.* IV (1914), p. 333.

[6] Lewis, Charles E. *Maine Agr. Exp. Sta. Bull.* No. 170 (1909).

[7] Clinton, G. P. *Connec. Agr. Exp. Sta. Rept.* Part V (1905), p. 264.

[8] Roberts, John W. *U.S.A. Dept. Agr. Bur. Pl. Ind. Bull.* 534 (1917), p. 1.

[9] Dastur, J. F. *Ann. App. Biol.* VI (1920), p. 262.

[10] "A Southern Grower." *Gard. Chron.* No. 1457 (Nov. 28, 1914), p. 357; Cornish, P. E., *l.c.* p. 357.

English mycologist appeared to have given any considerable attention to the subject, and that it was very important that mycologists in this country should be able to inform growers to which malady, whether bitter pit or Cylindrosporium spot, any attack was due.

These considerations furnished the incentive to study the "spotting" problem. Early in the following year we received reports from Kent, Surrey and Berkshire of "spotting" in other varieties, notably in Bramley's Seedling, Cox's Orange Pippin and Allington Pippin, and specimens were forwarded for examination by several fruit growers. It was reported from Kent that very many of the finest and best ripened apples had become covered with very small red spots: this had not prevented the apples from keeping, but it had greatly injured their sale. The trouble developed entirely in apples in store. Barker[1] reported "spotting" in Allington Pippin and Bramley's Seedling from Worcestershire, Cambridgeshire, Oxfordshire and Sussex, and concluded that the trouble was general throughout apple growing districts.

2. SYMPTOMS.

The spots show considerable variety in form and colour. They are sometimes green, of a darker shade than the normal skin colour, as for example in Lord Derby, Newton Wonder, and Reinette du Canada, where dark green blotches show up conspicuously on a yellow-green skin. In the case of a pigmented apple, the spots are usually of a darker shade of red or purple than the normal (Scarlet Nonpareil). In varieties with partial pigmentation, the spots are frequently coloured rose, red or purple (Mrs Philimore, purple spots on a green ground); coloured spots also appear in normally unpigmented varieties such as Old Nonpareil, Reinette du Canada (blotches with purple edging), and Lane's Prince Albert (dark brown blotches bordered pinkish red).

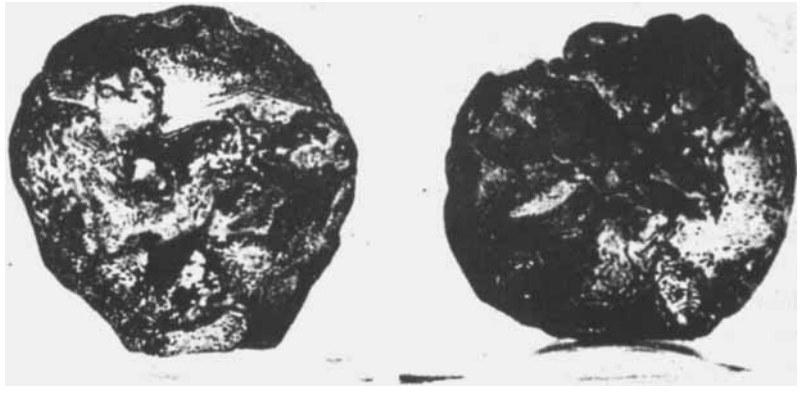
Curiously mottled blotches are sometimes formed with the mottlings in purple, purplish brown and green; for example, Ribston Pippin, Old Nonpareil, Cox's Orange Pippin, Charles Ross (brown, and purplish brown).

Dark brown spots occur in Ecklinville Seedling, Yorkshire Greening, etc.; pale brown spots in Cox's Orange Pippin, Charles Ross, Emperor Alexander, Peasgood's Nonsuch (spots with irregular contour), and Duke of Devonshire. In Hollandbury the spots are dark, of irregular outline, angular and so numerous as to give the apple a fantastic appearance. The

[1] Barker, B. T. P. *Ann. Rept. Agr. and Hort. Res. Sta., Long Ashton* (1914), pp. 97–99.

dark brown, slightly sunken spots in Wellington resemble a scurf; in Tamplin they are often round, sunken, numerous and very small.

Minute black markings of various kinds are frequently present in the brown spots which add a great variety of minute detail. The spot may be almost uniformly black (September Beauty, Rev. W. Wilks, Early River, Wolf River), or black dendritic markings may appear (Newton Wonder). These appearances are due to the presence of fungi with dusky mycelium, for example, *Alternaria*, *Dematium pullulans*, etc. Again, the spot may be dotted in various patterns with the dark sclerotial or other reproductive bodies of various fungi, and the pattern will vary with the kind and degree of development each has attained. The following fungi have been found to produce this "dotted" effect:



(*a*) (*b*)

Fig. 1. Photographic reproduction showing mummification following "spotting" of apples.

(1) *Pleospora pomorum*—black sterile perithecia.
(2) *Valsa* sp.—necks of the perithecia.
(3) *Polyopeus purpureus*—dark brown pycnidia (Early River, Stirling Castle).
(4) *Myxosporium mali*—black sclerotial bodies.
(5) An unidentified fungus with thick glistening walls—black sclerotia.

Perithecia often escape recognition since only a portion of the perithecium (*Pleospora*), or only the extremity of the neck (*Valsa*) protrudes above the surface of the apple.

It is not meant to be understood that the spots named are necessarily of different origin; a purple spot and a brown may differ in aspect merely

through varietal characteristics of the apples on which they occur: conversely, spots of a somewhat similar category—brown spots, for example—should not be held to indicate a likeness of origin, since a brown colour is one of the commonest symptoms of a pathological condition.

Spots of different kinds may occur on the same variety and even on the same apple. There were on Lane's Prince Albert, received from Berkshire at the end of April, 1915, pale brown spots with a dark brown centre; chocolate brown spots with a pale centre, and blackish spots mottled with green; some spots were variously dotted, others not. On other apples of this variety received at the same time, dendritic markings were present on a pale brown ground, and in the same variety greenish and purplish sunken blotches and dark brown spots with a pinkish red border also occur.

"Spotting" has been observed in about one hundred varieties of apple, and occurs in a widely representative series of varieties—early, mid-season and late, and whether culinary, dessert or exhibition—including a number of sorts most prized in commerce. The varieties which escape include hard-fleshed apples, notably the russets (Christmas Pearmain, Worcester Pearmain, etc., with the exception of Hubbard's Pearmain): the late pippins (Allen's Everlasting—a seedling from Sturmer Pippin, Fearn's Pippin, etc.); also, as far as observations of 1917–18 show, certain other varieties with crisp sub-acid flesh, such as Barnack Beauty, Gloria Mundi, Belle Dubois.

"Spotting" in relation to the lenticels.

The surface of the apple is studded with numerous minute pale, more or less stellate, apertures which are more conspicuous on some varieties than others. These are usually referred to as lenticels[1], although they do not possess the typical structure of such organs. McAlpine[2] states that they are formed through rupture of the stomata as a result of expansion as the apple increases in girth; he gives a photographic reproduction of a pore showing the remains of a stoma (McAlpine, Plate XIV, fig. 102). They take their origin therefore in the same way as lenticels.

In a number of cases the spots originate at the lenticels. A careful examination will show a complete series of stages from discoloured lenticels to spots. In Wellington the smallest spots just encircle the

[1] The stomata are not all ruptured by the time the apple reaches maturity: stomata were observed quite late in the season in a large specimen of an unknown variety.

[2] McAlpine. "Bitter Pit Investigation," *First Progress Report* (1911–12), p. 40.

lenticel, and there are others of every intermediate stage between these and the largest in the apple. In Ecklinville Seedling nearly every lenticel in the apple is brown and in a large number of them brownness has spread round to form a spot varying from the size of the lenticel itself to one-eighth of an inch in diameter. On Stirling Castle with dark spots the lenticels show up conspicuously brown. On Golden Spire there are dark brown spots on one apple and pale brown on another, the size of a pin's head. On Lane's Prince Albert received in December 1915 from Berkshire, the smallest spots were of the same dimension as the lenticels.

These observations confirm those of Charles Brooks[1], Barker[2], Cook and Martin[3] and others as to the importance of the lenticel in relation to incipient "spotting"; but the lenticels are not the only centres of origin, since the opportunity for infection occurs wherever stomata exist or where the skin is injured by cracks or wounds of any kind.

Development of "spotting."

"Spotting" usually appears towards the end of July, and there is a considerable development in August. On September 29th, 1915, "spotting" was observed on 31 varieties at Wisley. The production of new spots goes on continuously throughout the storage period until the coming of spring: over 200 spots were counted on a specimen received from Sussex in 1915.

The progress of "spotting" was specially studied in a collection of over ninety varieties of apples at the Royal Horticultural Society's Gardens in 1917–18, from two to four specimens of each variety being examined. Of these, 30 varieties remained free during the season. In some kinds spots developed early in the season and remained without further development; for example, in King of Tomkins County (seasonal period September–April), depressed blackish brown spots up to $\frac{1}{4}$ in. diameter were present early in November, but remained with little increase in size until May. In others, there was a steady increase in size; thus in Ribston Pippin (November–January) numerous minute brown spots were present early in November: these increased to $\frac{1}{8}$ in. (November 22nd) and $\frac{1}{4}$ in. in diameter (November 28th); soon afterwards the apples became rotten. In Yorkshire Greening (October–January), there were few depressed brown spots and brown spots with

[1] Brooks, Charles E. *Bull. Torr. Bot. Club.* 35 (1908), p. 423.

[2] Barker, B. T. P. *Ann. Rept. Agr. and Hort. Res. Sta., Long Ashton* (1914), p. 98.

[3] Cooke, M. T. and Martin, G. W. *Phyt. l.c.*

a black centre up to $\frac{1}{4}$ in. in diameter; these remained arrested until January 31st, when the largest spot was 1 in. in diameter; the apple then rotted. In over 20 varieties, rotting which could be traced to "spotting" had set in before the varieties were out of season.

3. THE FUNGI CONCERNED.

Owing to the fact that so few of the black bodies found in "spot" areas proved fertile, cultural methods were adopted in the hope that reproductive bodies which would facilitate the work of identification, would be formed in the media employed.

The apple was first washed in ·1 per cent. mercuric chloride solution, and then rinsed thoroughly in sterile water: small cubes from 1–5 mm. long were cut out rapidly with a sterile knife and dropped into apple extract or on the surface of apple agar—in some cases the cubes were immersed for a few seconds in mercuric chloride followed by washing in sterile water before they were transferred to the medium. Control cubes were taken from unspotted parts of the apple and invariably gave negative results. Cubes taken from bitter pit areas also yielded negative results.

The first fungi were isolated in the autumn of 1915, but owing to circumstances a number of cultures were abandoned and only those considered to be of importance at the time were carried on. Further work was started in the autumn and winter 1917–18 and another series of cultures obtained: these included all the fungi carried on from 1915, with the exception of species of *Alternaria* and *Sclerotium*, and in addition a number of others which were not obtained in the first period.

The total number of isolations in both periods exceeds 400: of these, 140 were in the first period and 260 in the second. The actual number of failures to isolate fungi in the first period exceeded 50 per cent., due in some cases to the use of liquid apple extract, and in others to over-sterilising in mercuric chloride the inoculation cubes. In the second period out of 38 placed in extract, 19 failed—exactly 50 per cent., whereas of those placed on apple agar only seven failed, being cubes obtained from four varieties of apple.

The fungi present in the original cultures were separated by plating out or by transference to slant tubes; in this way several fungi whose identity could be determined were obtained in pure culture; and notably *Leptosphaeria vagabunda* Sacc., *Coryneum foliicolum* Fuck, *Fusarium*

mali[1] Allerch., *Myxosporium mali*[2] Bresadola and *Alternaria grossularieae* Jacz[3].

Others whose identity could not be determined were regarded as separate fungi from the fact that the general characteristics remained fairly constant in successive sub-cultures. They were transferred to potato mush agar in the autumn of 1918 and in the majority of cases reproductive bodies were formed. These fungi[4] include:

(*a*) Species of a phomoid genus differing from *Phoma* in possessing compound, unilocular pycnidia with multiple necks, to which the name *Polyopeus*[5] is given—*P. purpureus*, *P. pomi*, *P. recurvatus* and *P. aureus*.

(*b*) An aggregate species of *Fuckelia*—*F. botryoidea*.

(*c*) A species of *Coniothyrium* with lobed pycnidia—*C. convolutum*, and a form of *C. cydoniae*.

(*d*) A species of *Alternaria* forming in media "pockets" of conidia —*A. pomicola*.

(*e*) A species of *Pleospora*—*P. pomorum*, with a dematiaceous stage of the *Stemphylium pyriforme* type. The identity of the conidial and ascigerous stages has been proved by reciprocal single spore cultures.

(*f*) A species of *Sclerotium*—*S. stellatum*, somewhat resembling *S. bataticolum*[6].

Three other fungi have not yet formed reproductive bodies rendering identification impossible; they are:

(*a*) The thick-walled fungus to which reference has been made.

(*b*) A slow-growing fungus presenting a dingy white and somewhat powdery appearance at the surface of the medium when grown on apple agar. This fungus was obtained 21 times from spots, with a centrally situated lenticel, varying in size from $\frac{1}{16}$ to $\frac{1}{8}$ in. in diameter.

(*c*) A sterile fungus, isolated from Ben's Red and King of Tomkins County, producing brilliant colours when grown on different media. On apple agar the colours vary from dull pink to bright orange-yellow; on potato slants they vary from pink to yellow, yellow-green with a

[1] See Allescher. *Ber. Bot. Ver. Landshut.* XII, p. 130 (1892); and Oudemans, *Cat. Champ. Pays Bas*, p. 531.

[2] See Bresadola. *Hedwigia*, p. 382 (1897).

[3] This apple Alternaria proved morphologically identical with *Alternaria grossularieae* (Jaczewsky, *Bull. Soc. Myc. Fr.* XXII, 1906, p. 122), isolated by one of us from gooseberries.

[4] For descriptions of the new species see the *Journal of Botany*, vol. LVIII, p. 239. (Oct. 1920).

[5] The morphological and physiological characteristics of this genus will be described elsewhere.

[6] Tubenhaus, J. J. *Phyt.* III (1913), p. 164; also Martin, William H. *Phyt.* VII (1917), p. 308.

metallic lustre and orange, and on potato agar plates bright shades of green and blue have appeared.

The effect produced by these various fungi in the tissues of the apple can only be outlined in very general terms. In sections through a small spot, where of course there may be one fungus or two or more associated fungi, the mycelium is present in the air spaces: the hyphae penetrate between the cells and envelop them in a web of filaments firmly adhering to the cell wall. The cells lose their hyaline character through changes in the protoplasm and plastids, and become brown—the tissue is darker when fungi with dusky mycelium are present. In some cases mycelium is present in a hyaline zone in advance of the discoloured region.

SPECIAL RELATIONS OF THE FUNGI CONCERNED IN "SPOTTING."

The fungi isolated from the smallest spots ($\frac{1}{16}$–$\frac{1}{8}$ in. diam.) include *Pleospora pomorum*[1], *Leptosphaeria vagabunda*, *Polyopeus purpureus*, *P. pomi*, *P. recurvatus*, *P. aureus*, *Fuckelia botryoidea*, *Coniothyrium convolutum*, *Alternaria grossularieae*, *Coryneum foliicolum*, *Myxosporium mali* and certain unidentified forms. Among these fungi are to be sought those responsible for the commencement of "spotting."

In the majority of cases small spots have yielded only one fungus, for example *Alternaria grossularieae* (Ben's Red); *Polyopeus purpureus* (Byford Wonder, Charles Ross, Bismarck). But spots of this category even when occurring on the same apple have not always yielded the same fungus, for example, the brown spots on Margil yielded *Polyopeus aureus*; the dark brown spots, *Fuckelia botryoidea*; again the pale brown spots on Frogmore Prolific yielded *Fuckelia botryoidea*; other spots, *Leptosphaeria vagabunda*. On September Beauty two spots yielded *Alternaria grossularieae* and two *Polyopeus purpureus*. Alternaria and Polyopeus spots occur also on Wolf River and Grenadier.

In some cases two or more fungi may be associated in small spots; for example, *Alternaria grossularieae*, *Polyopeus purpureus* and an unidentified fungus, in Byford Wonder. Here we have two fungi associated which in the same variety also occur singly in spots. In a spot on the variety Rev. Wilks, somewhat larger than those above mentioned, certain spots yield only *Alternaria grossularieae*, but other spots on the same apple yield the same fungus in association with *Myxosporium mali*.

[1] For the apple varieties from which *Pleospora pomorum* and other fungi have been isolated, see *Journal of Botany*, *l.c.* and *The Gardeners' Chronicle*, vol. LXVIII, October 30, 1920, p 216.

As regards the frequency with which a particular fungus occurs alone in spots, data can only be given for *Pleospora pomorum* and fungus *b*; the former was found unassociated in eight out of nine varieties studied (in the ninth case *Polyopeus purpureus* was present in addition); in the latter, in 12 out of 15.

Taking into account the total number of spots of all categories, *Pleospora pomorum*[1] has been isolated from 18, *Polyopeus purpureus* from 20 varieties. Only six of these varieties yielded both fungi. *Alternaria grossularieae* occurred in seven varieties of which two (Byford Wonder and Ben's Red) also yielded *Pleospora*, and four (Early River, Grenadier, Rev. W. Wilks, and September Beauty) *Polyopeus purpureus.*

The apples from which *Pleospora pomorum* has been isolated are by no means of a similar class; they include dessert (Cox's Orange, Ben's Red), culinary and dessert (Bramley's Seedling, Wealthy), and culinary (Byford Wonder) sorts; also varieties in season in late autumn (Loddington), and in winter (King of Tomkins County). *Polyopeus purpureus* occurs also in both culinary and dessert varieties, and notably in the culinary varieties Newton Wonder and Lane's Prince Albert, varieties with a wide seasonal range, but it also occurs in varieties with a restricted range as Grenadier.

Fungus *b* has been isolated from dessert and culinary sorts—Alfriston (Nov.–Apl.), Lord Hindlip (Jan.–May), Belle de Pontoise (Dec.–Feb.), Duke of Devonshire (Mar.–Apl.), etc.—chiefly from apples in season after November.

The surface spots present on apples badly affected with "bitter pit" frequently yielded fungi; for example, on Newton Wonder some spots yielded *Cladosporium epiphyllum*, others *Dematium pullulans* and fungus *b*, others slow-growing unidentified fungi (minute dark brown spots). Fungi were not obtained from "spotted" specimens of Calville Boisbunel, Crawley Beauty, Sanspareil and Yorkshire Greening.

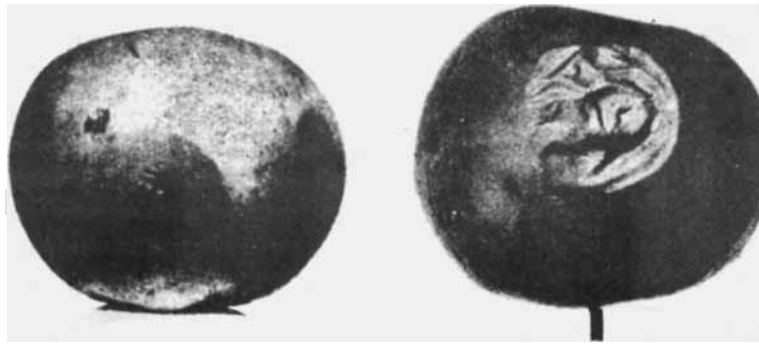
The largest spot-flora has been obtained from Cox's Orange Pippin —eight species excluding unnamed fungi: two fungi were obtained from Blenheim Orange, fungus *b* from small spots and later *Myxosporium mali*[2] from areas where rot had set in.

Of the important culinary varieties, Lane's Prince Albert yielded *Dematium pullulans* four times, *Polyopeus purpureus*, *recurvatus*, and

[1] The earliest date on which it has been obtained is Nov. 15th (Charles Ross), and the latest, early in March (Bismarck).

[2] Also isolated from Byford Wonder, Domino, Duke of Devonshire, Lord Grosvenor, Lord Suffield, Rev. W. Wilks, Winter Hawthornden.

Cephalothecium roseum; Newton Wonder, *Polyopeus purpureus*, fungus *b* four times and *Cladosporium epiphyllum* twice; Potts's Seedling, *Polyopeus purpureus*, *Cladosporium epiphyllum*, *Fusarium mali*, and unidentified fungi; Ecklinville Seedling, *Dematium pullulans*[1] and fungus *b*; Bramley's Seedling, *Pleospora pomorum*, *Cladosporium epiphyllum*[2] and unidentified fungi; Peasgood's Nonsuch, unidentified fungi (reddish brown spots).



(*a*) (*b*)

Fig. 2 (*a*). Photographic reproduction of an apple inoculated with conidia of *Pleospora* at two points, after seven weeks, showing infertile perithecia (see Fig. 3 *a* for representation of the same apple after two weeks).

(*b*) Photographic representation of an apple inoculated with conidia of *Pleospora* at four points, after seven weeks (see Fig. 3 *d* for a diagrammatic representation of the same apple).

5. INOCULATIONS WITH *PLEOSPORA*[3] *POMORUM* HORNE.

On Jan. 17th, 1917, an apple of unknown variety was inoculated at four points by introducing, through punctures made with a sterile needle, fragments of mycelium bearing conidia (*Stemphylium*) of *Pleospora pomorum*. Five days afterwards, spots were observed at the points of inoculation. On Feb. 22nd, large dark brown areas had developed, and the apple was rotting. Since other spots had formed at points not inoculated another sound apple was inoculated on Jan. 17th, and a number of

[1] Also isolated from Allington Pippin, Christie Manson, Crawley Beauty, Lord Suffield, Tamplin, Scarlet Nonpariel, Stirling Castle.

[2] Also isolated from small spots in association with other fungi in Christie Manson, Landsburger Reinette, Lord Suffield, Tamplin and Winter Quarrenden.

[3] A full account of the reactions to various media is omitted for reasons of space.

control punctures were made on a third apple. On Feb. 22nd, brown spots ¼ in. diameter were observed, but again spots appeared at places not inoculated. However, on Feb. 28th, the four spots formed at the points of inoculation on this apple had coalesced to form an area 1 in. in diameter. The apple was then cut open and portions of diseased tissue were removed from the interior at a depth of ½ in. from the surface.

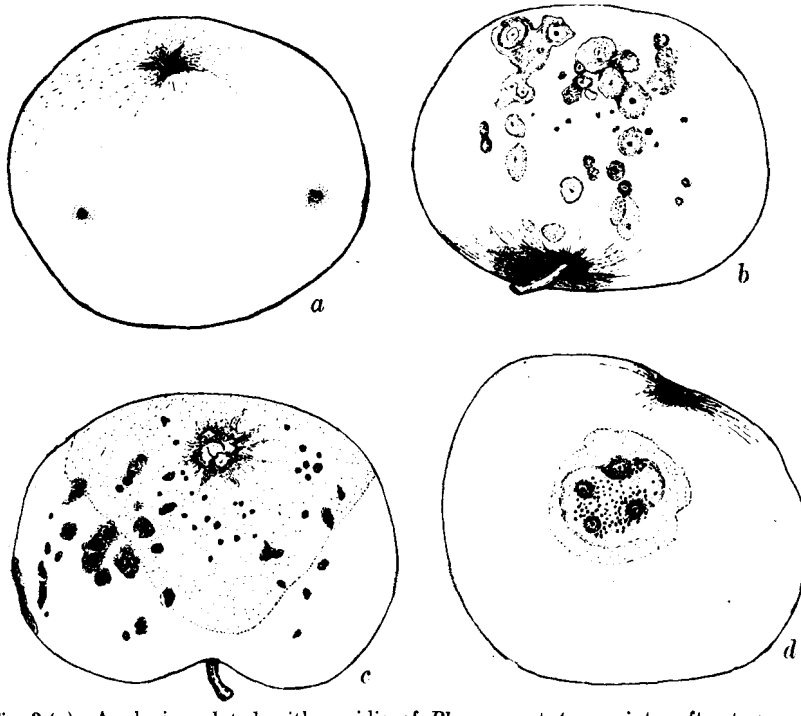


Fig. 3 (*a*). Apple inoculated with conidia of *Pleospora* at two points, after two weeks (see Fig. 2 *a* for photographic reproduction of the same apple after seven weeks).
(*b*) Apple affected with "spotting," March, 1915.
(*c*) Apple showing rot (shaded portion) following "spotting."
(*d*) Apple inoculated with conidia of *Pleospora* at four points, after seven weeks, showing infertile perithecia (see Fig. 2 *b* for a photographic reproduction of the same apple).

Some portions were placed in sterile petri dishes and others on the surface of apple agar in slant tubes. After a few days abundant conidia and later sterile perithecia of *Pleospora* were formed in both dishes and tubes.

Experiments were then made with known varieties. Specimens of Lane's Prince Albert and Newton Wonder were inoculated on March 14th. In the case of Lane's Prince Albert a spot about ⅜ in. in diameter was present on April 3rd, increasing to a pale brown area with dark centre

1½ ins. in diameter on April 11th and 2¼ ins. in diameter on April 23rd; in the case of Newton Wonder, small spots ⅛ in. in diameter were present on April 11th which increased to ½ in. on April 23rd. In both cases black, sterile perithecia of *Pleospora* developed in the diseased areas.

A number of apples were inoculated with *Pleospora pomorum* while still on the tree in August, 1917. A representative series of varieties was chosen including two varieties, viz. Bismarck and Charles Ross, from which this fungus had been isolated.

The method adopted was as follows: two spot-free apples were chosen of each variety and punctures made (using a sterile needle) in each; one apple was inoculated with a minute fragment of mycelium bearing conidia, and the other not inoculated. After inoculation the apples were enclosed in manilla bags similar to those used in pollination experiments. The apples, by design, were not sterilised before inoculation, as it was desired not to injure the surface of the apple in any way. The inoculant was therefore exposed to competition from fungi already present on the apple. Records of the appearance, etc., were taken at short intervals. As the apples ripened they were removed from the tree and stored under suitable conditions.

Of the 28 varieties inoculated, spots were not formed at the point of inoculation in five, viz. the firm-fleshed russet—Court Pender Platt, Norfolk Beaufin[1], Charles Ross[2], Ribston Pippin and Cellini. Small spots were formed which remained arrested for a period in seven varieties, of which five are culinary sorts—Alfriston, Allen's Everlasting, Beauty of Kent, Calville Boisbunel, Cockle's Pippin, King of the Pippins and Lord Derby. The remaining 16 varieties developed "spotting" more or less rapidly and rotting ensued—Allington Pippin, Belle de Pontoise, Bismarck, Bramley's Seedling, Cardinal, Crawley Beauty, Duchess Favourite, Early Victoria, Grenadier, Keswick Codling, Lane's Prince Albert, Potts's Seedling, Red Astrachan, Rival, Royal Jubilee and Wealthy.

In the case of five of the 16 varieties which eventually rotted—Allington Pippin (Jan 11th), Rival (Jan. 11th), Wealthy (Dec. 31st), Royal Jubilee and Cardinal—*Pleospora was re-isolated from the diseased tissue.* In the first four of these the development of spotting occurred between Sept. 12th and the end of November, that is approximately the time these varieties are in season. In the early variety, Cardinal,

[1] Large natural spots formed on the inoculated apples.

[2] The failure to infect Charles Ross is noteworthy, since *Pleospora* was isolated from this variety on Oct. 25th, 1915.

rotting took place earlier. A sound Cardinal apple placed in contact with the diseased specimen rotted within a month.

In the case of the varieties Rival, Wealthy, and Allington Pippin, *no fungus other than Pleospora was isolated from the rotting areas which developed in the apples inoculated with Pleospora.*

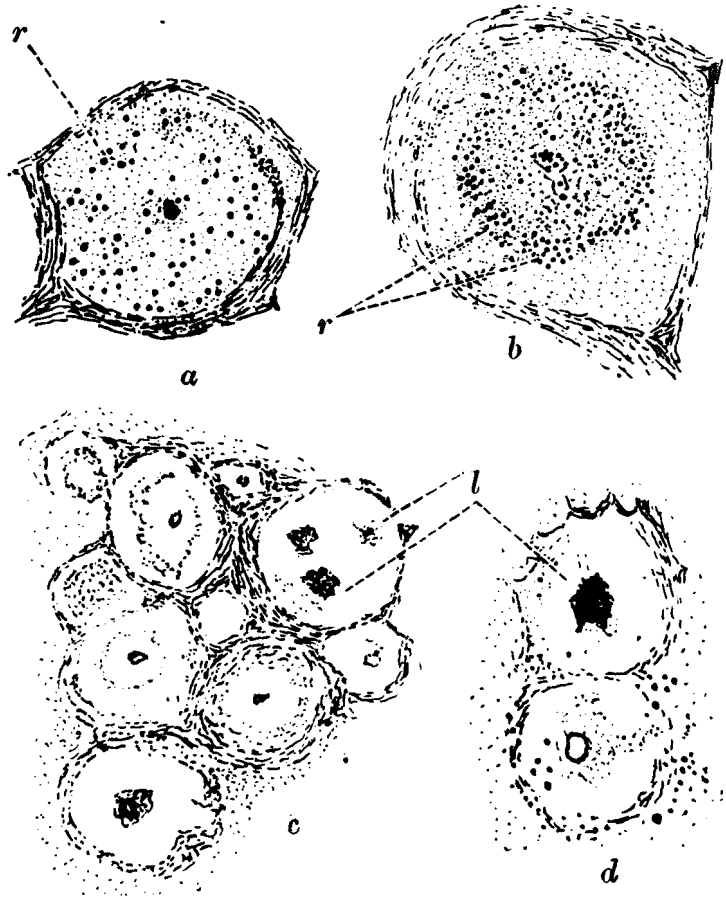


Fig. 4 (*a–b*). "Spots" on surface of apple enlarged showing black reproductive bodies of fungi (*r*). (*c–d*). Group of "spots" enlarged showing lenticels (*l*).

In the case of the Cardinal apple, however, the presence of a second fungus, *Polyopeus purpureus*, in the diseased tissue was revealed from re-isolation tests made after Sept. 13th. On the following day cubes from the diseased portions in which *Pleospora* mycelium was present were placed in petri dishes in a moist atmosphere. The mycelium which

developed was carried on in plate culture and eventually yielded either *Pleospora* or *Polyopeus purpureus*, or both. On Sept. 20th, some more

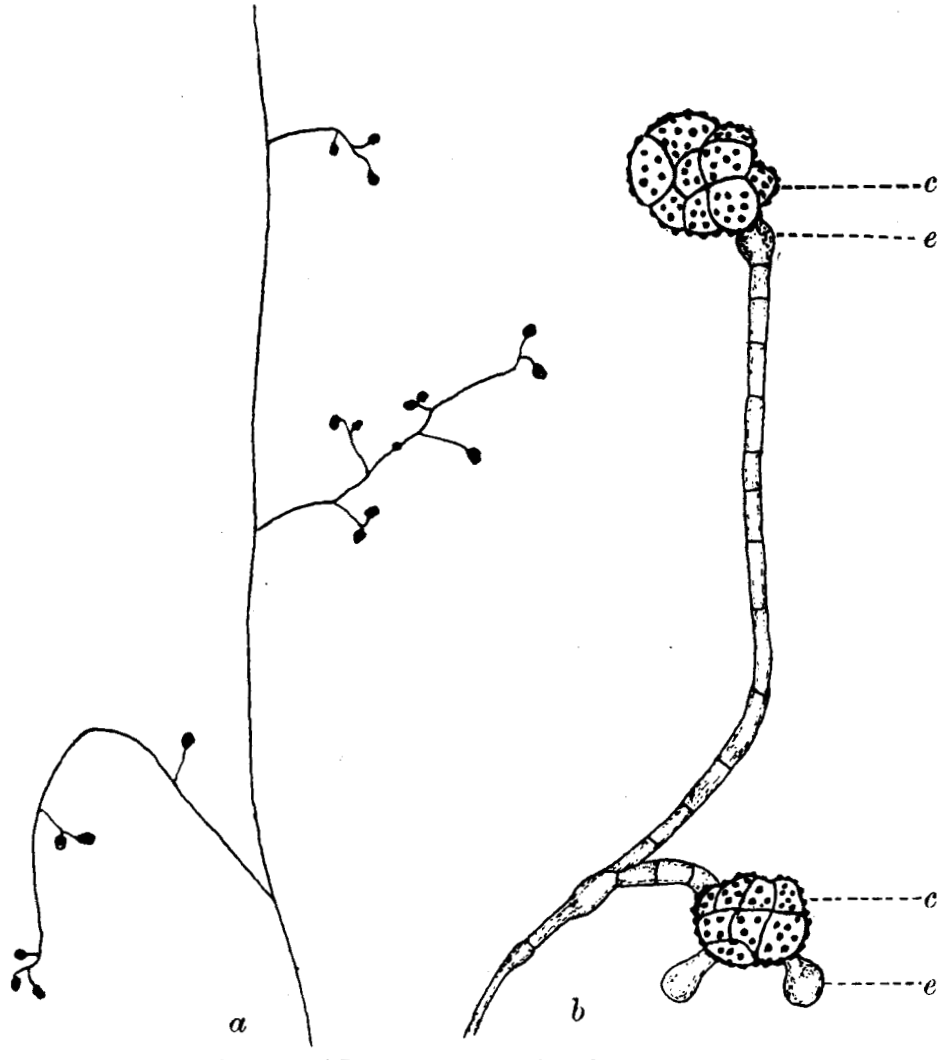


Fig. 5. Conidial stage of *Pleospora pomorum* (*Stemphylium*).
(*a*) Portion of conidiophore.
(*b*) Sporophore showing swollen end-cells (*e*) and mature conidia (*c*).

cubes were cut and dropped on the surface of apple agar in slant tubes. On this occasion abundant conidia (*Stemphylium*) were produced within four days, but no *Polyopeus*. On Sept. 26th masses of white mycelium

appeared at the surface of the apple: two tubes of agar were inoculated and yielded on Oct. 2nd, *Pleospora* and *Polyopeus purpureus* respectively.

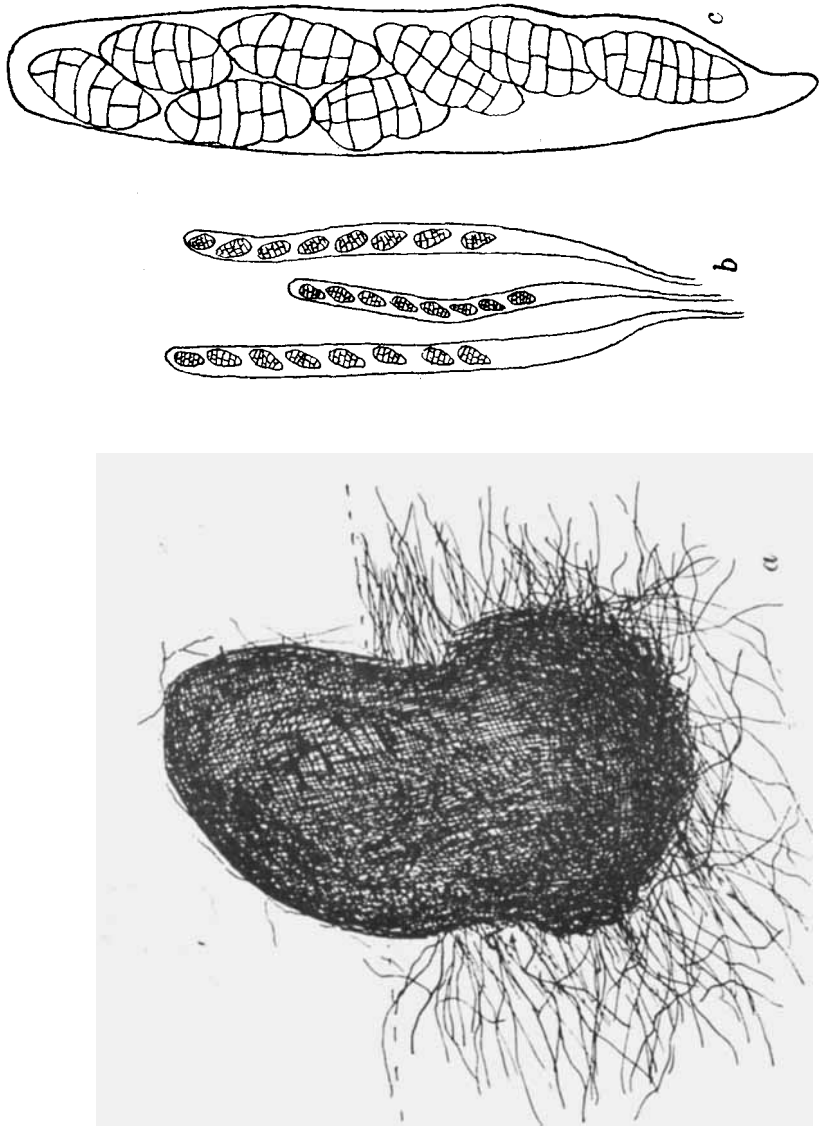


Fig. 6. Ascigerous stage of *Pleospora pomorum*.
(*a*) Perithecium. (*b*) Asci with spores in a single rank. (*c*) Ascus with spores in a double rank.

In Potts's Seedling (Sept.–Oct.), the diseased tissue obtained from artificially produced spots repeatedly yielded *Polyopeus purpureus* and not *Pleospora*. A spot which appeared naturally on the same apple also

yielded *Polyopeus purpureus*, but with *Fusarium mali* in addition. Another natural spot yielded an unidentified fungus. In this variety, *Pleospora* has made no progress, but a different fungus, *Polyopeus purpureus*, occurring in the natural spots on the same apple, has effected an entry[1] at the points where *Pleospora* was introduced. Again *Pleospora* was not re-isolated from Lane's Prince Albert, a variety with a wider seasonal range (Oct.–Mar.), but *Polyopeus purpureus* and another phomoid fungus were obtained instead from the artificially produced spots.

The results obtained in Grenadier (Sept.–Oct.) were somewhat different. Small brown spots appeared at the point of inoculation on Sept. 12th. These measured 3 mm. in diameter on Sept. 24th. On this date other spots were forming at the lenticels near the point of inoculation. A month later the original spots formed merely the nucleus of brown areas; in fact the apple was in a semi-rotten condition. On Nov. 8th fragments of tissue removed with sterile instruments from the diseased area near the original point of inoculation were placed on the surface of apple agar in slant tubes. After a few days *Polyopeus purpureus* and *Alternaria grossularieae* were present in the cultures. Cultures made in the same way but using tissue taken 2 ins. from the point of inoculation yielded *Alternaria grossularieae*. Finally, cultures made using eruptive mycelium taken from pustules appearing at the surface of the apple (Oct. 29th) yielded *Polyopeus purpureus*. *Pleospora pomorum* was not obtained at all, but instead two other fungi—*Polyopeus purpureus* and *Alternaria grossularieae*—which were not present in the inoculant.

6. CONTROL.

Susceptibility and Immunity.

This work has shown (Section 5) that *Pleospora pomorum* can parasitise at least three varieties of apple (Rival, Wealthy, Allington Pippin), whilst in certain other varieties (Cardinal, Grenadier, Potts's Seedling), originally inoculated with *Pleospora*, *Pleospora* was either not re-isolated (*Polyopeus purpureus* was obtained instead) or was re-isolated in association with other fungi (*Polyopeus purpureus*, *Alternaria grossularieae*, etc.). Considering together the observations noted in Section 4 and the facts recorded in Section 5, the evidence seems to suggest that both *Pleospora pomorum* and *Polyopeus purpureus* exhibit a preference for varieties,

[1] It is interesting to note in Norfolk Beaufin (Oct.–Dec.) artificially made punctures were not inoculated by the fungus or fungi causing the natural spots formed in October on the inoculated specimen.

but that the varieties preferred by the one are not necessarily those preferred by the other. But the varieties Rival, Wealthy and Allington Pippin, in season from October to December inclusive, were inoculated when unripe whereas Cardinal, Grenadier and Potts's Seedling, in season from August to October inclusive, were inoculated when ripe. Hence the inoculations in the two cases are not strictly comparable since the varieties were not inoculated at the same phase of apple development. The supposition that *Pleospora pomorum* and *Polyopeus purpureus* each exhibit a preference for varieties, for example Rival and Cardinal respectively, is therefore open to the objection that a given variety may be susceptible to one fungus at an early stage of its development and to another at a later stage; in fact, there may be a definite fungal succession. Questions of this kind can only be settled conclusively by carrying out, throughout the season, continuous comparative series of inoculations on specially selected varieties. Certain subsidiary phenomena for example, "arrested spotting" and recrudescence of "spotting" after a period of rest, would then be better understood. Considerable help would be afforded by a parallel study of the seasonal chemical changes taking place in the varieties selected for experimental purposes.

Outdoor measures.

The risks of summer infection could be considerably reduced by spraying with Burgundy mixture when the fruit is young. Very probably a weak solution thoroughly applied, similar to that successfully used at Wisley by the authors[1] to prevent the infection of gooseberries by the American gooseberry mildew, would serve the purpose. The treatment should be repeated each year, for until the life-histories of the fungi concerned in "spotting" and the various sources of summer infection are completely known, it is impossible to take comprehensive measures against seasonal recurrence. The success of spraying as a means of control has been repeatedly demonstrated in America by Lamson[2] and others.

Indoor measures.

Summer spraying should practically prevent the appearance of "spotting" in store unless the fungi exist in the fruit-room itself. Where no spraying has been practised, the development of "spotting" could

[1] Horne, Arthur S., in *The Gardeners' Chronicle*, LIX, p. 310 (June 10. 1916); and Horne, Eleanor V., in *The Garden*, LXXXI, No. 2374, 174 (May 19, 1917).

[2] Lamson, W. H. *N. H. Coll. Bulls.* Nos. 27 (Apr. 1895), 45 (May, 1897), 65 (May, 1899), 101 (Apr. 1903).

be retarded by storing at lower temperatures than those frequently adopted. It is however eminently desirable to systematically disinfect the fruit-room since some of the fungi enumerated in this paper can grow slowly and even sporulate at a comparatively low temperature (0°–5° C.).

7. SUMMARY.

This paper presents the results so far obtained in an investigation, which has been carried on since 1915, into "spotting" in apples. The symptoms of "spotting" as they are found and develop in numerous varieties of apple are described. Several fungi have been isolated from spots and cultured in various artificial media with the production of spores.

They include a new genus of Phomatales (*Polyopeus*) and nine new species, of which at least one, *Pleospora pomorum*, as the result of experimental inoculations, has been proved capable of parasitising apples. The fungi do not include any of the species hitherto reported as causal organisms in the United States, the only centre where investigations into the "spotting" of apples, as distinct from the "bitter pit" problem, has been undertaken.

The work was commenced at the Wisley Gardens of the Royal Horticultural Society. During the building of the Society's laboratory it was continued, by the kindness of Professor V. H. Blackman, in the Department of Plant Physiology and Pathology at the Imperial College of Science and Technology. In was later carried on again at Wisley; the work however has been completed at the Imperial College.

The authors' thanks are due to Professor Blackman for his kind help and criticism during the investigation.