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No. 5]

[1916

XXII.—A PHOMA DISEASE OF LAVENDER.

STUDIES FROM THE PATHOLOGICAL LABORATORY: II.

WILLIAM B. BRIERLEY.

(With Plates.)

In the early summer of 1915 my attention was drawn to two large beds of *Lavandula officinalis* in which practically every plant was dying. Affected shoots presented a dry, dirty brownish-grey colour, and the epidermis tended to split away in minute silvery flakes. The leaves on a diseased stem preserved their normal appearance for some time and then somewhat rapidly wilted and became brown and shrivelled. All portions of the plant above the dry discoloured areas died. This is a not uncommon disease of lavender, and at times is the cause of serious loss to growers. At first individual shoots only are affected but finally the complete plant is involved, and in a bed the disease rapidly spreads until all the plants are in a weak or dying condition.

A careful examination of a diseased shoot showed that the shrivelled portion of the stem under the flaking epidermis was studded with very minute blackish-brown points which proved to be the pycnidia of a fungus ramifying in the tissues. This fungus was identified as *Phoma lavandulae*, Gabotto, a species hitherto unrecorded in England.

The only other determination of this fungus is that of its original discoverer, who gives the following description of it.

Phoma lavandulae, nov. sp.

“Pycnidiis corticalibus, solitaris, lenticularibus, prominulis atris. Basidiis hyalinis, acicularibus 12–14 μ long. Sporulis fusoides, 2-guttulatis, hyalinis, 4–2 μ .”

“In ramis Siccis Lavandulae officinalis Chaix. Mirabello in Pedem.”*

As this description represented our knowledge of the fungus, no figures having been published, and as the fungus was sus-

* Dottore L. Gabotto. Contribuzione alla Flora Micologica Pedemontana. Nuovo Giorn. Bot. Ital., vol. xii., p. 69. 1905.

pected of pathogenic qualities, it was considered advisable to make a more detailed investigation of its morphology and biological relationships.

LIFE HISTORY OF THE FUNGUS IN PURE CULTURE.

The pycnospores usually germinate in a period of from one to three or four hours on nutrient media at room temperature (approximately 14–16° C.), and a moderate mycelial growth occurs. At first this is pure white but with increasing age becomes slightly grey. The medium directly beneath the colony is coloured brownish-red or variously from this to a dirty grey, the coloration not being constant for any particular nutrient medium or strength of medium.

1. Undulate terminal cells from mycelium growing on potato gelatine. (Swift $\frac{1}{4}$ obj. $\times$ III. eyepiece.)

The superficial hyphae are hyaline and septate; and smooth and regular with the exception of the terminal cell which is frequently undulate. (*Text Fig. 1.*)

The cells are uninucleate and the transverse walls are formed by the ingrowth of a ring-like diaphragm of cell wall substance. (*Pl. V. Fig. 1.*) The cell nucleus contains one or very rarely two nucleoli, and occasional granules which stain with nuclear stains. Fat is present in the vacuoles of the cytoplasm and not infrequently there is distinct evidence of glycogen. The cell walls stain dull yellow with Schultze's chlor-zinc-iodine solution.

The mycelium penetrates the nutrient medium to a depth of from five to eight millimetres, and the submersed hyphae are often very irregular with nodulose, barrel-shaped or globular cells. (*Text Fig. 2.*)

2. Irregular forms of submersed hyphae from various media. (Swift $\frac{1}{8}$ obj. $\times$ III. eyepiece.)

When growing on a nutrient liquid such as aqueous extract of fresh lavender shoots, the character of the submersed hyphae is maintained although not in so exaggerated a form.

After a few days' growth the superficial hyphae begin to abstrict conidia, which at first are hyaline and thin-walled, but later become thick-walled and dark brown in colour.

In about 14 to 17 days pycnidial formation occurs.

Under unusual conditions or with age the cells of the hyphae enlarge, become thick-walled and brown and very easily separate forming chlamydospores.

The fungus grows best at a temperature of about 18–20° C.

Pycnospores.—The pycnospores are hyaline, fusoid in shape, and frequently contain one or two vacuoles. (*Pl. VI. Fig. 1.*) Their average measurement is $4 \times 2 \mu$, but very often spores of 5μ and occasionally of 6μ in length may be found. Their walls stain bluish with Schultze's chlor-zinc-iodine solution and the contents appear to consist largely of glycogen.

The germ tube may be protruded from the side or from the end in which case the spore appears merely to elongate into a hypha. (*Pl. VI. Fig. 1. Text Fig. 3a.*).

3a. Pycnospore fifteen hours after germination. Spore more highly magnified at the left side. (Swift $\frac{1}{4}$ obj. $\times$ I. eyepiece.)

3b. The same colony after three days' growth. Note the main lines of hyphae, which perhaps correspond to the distributive hyphae when the fungus is growing on its host. Spores in a hanging drop of lavender extract in a Van Tieghem cell at room temperature. (Swift $\frac{3}{8}$ obj. $\times$ I. eyepiece.)

The direction of the germ tubes is quite indiscriminate and does not appear to be conditioned either by the incidence of light rays or the local proximity of nutrient matter.

In distilled water very slender unbranched germ tubes were produced which never developed to a greater length than 35μ . In tap water slender feebly branching germ tubes were protruded, growing to about 150μ in length. In bouillon few spores germinated and the feeble germ tubes produced did not exceed 95μ

in length. The spores very frequently became swollen and irregular. In the majority of aqueous plant extracts, or these with agar and gelatine, germination freely occurred, and a moderate but never luxuriant growth resulted. (*Text Fig. 3b.*)

In pure nitrogen-free media such as glucose or levulose, germination was delayed, and was then only very feeble, the germ tube rarely exceeding 20–25 μ . Normal germination was brought about by the addition of nitrogen containing compounds such as ammonium tartrate, peptone or asparagine, but not potassium nitrate nor ammonium chloride.

Centrifugalisation for ten minutes had no visible effect on the spores. After fifteen minutes a slightly decreased power of germination was noticeable and after twenty-five to thirty-five minutes' treatment not more than one half the spores were capable of producing germ tubes. The inhibited spores stained an unusually dark brown colour with Schultze's chlor-zinc-iodine solution, but otherwise no difference could be detected.

A few observations were made on the effect of various chemical fumes on the germinative capacity of spores air dried from a suspension in water on a cover slip. In each case the latter was inverted over the mouth of the bottle containing the liquid for three minutes, exposed to the air for five minutes and then a drop of aqueous extract of lavender shoots placed over the spores and the cover slip inverted over a Van Tieghem cell and kept in the dark at room temperature. After exposure to formalin, toluene, benzole, ammonia and ether, no germination occurred. Chloroform and creosote killed a large number of the spores and the remainder only produced feeble germ-tubes. Acetone and turpentine appeared to retard and enfeeble the germination of only a small proportion of the spores.

The pycnosporos when in the pycnidium are very much more resistant to all adverse conditions than when in suspension in water or air dried upon a glass slide, and pycnidia on a shoot left exposed throughout the winter still contained a very few germinable pycnosporos on March 3rd.

Conidia.—After a few days' growth in pure culture on a nutrient medium conidial formation occurs. Any of the aerial hyphae may give origin to conidia but their production is chiefly from erect rather short hyphae four to eight cells in length, which are rather more stout than the remaining aerial mycelium. (*Pl. VI. Fig. 2.*) The first conidium is usually budded off from the end of the terminal cell, but very soon other conidia are abstricted indiscriminately from any portion of any cell towards the tip of the filament. Very frequently a conidium while still attached to the conidiophore gives origin, often from its side, to other conidia. The conidia may be somewhat irregular in shape, but are chiefly fusoid and a distinct neck is to be found at the point of abstriction. (*Pl. VI. Figs. 2, 3.*) They are commonly unicellular and uninuclear, but occasionally a spore consisting of two or three cells may be seen. (*Text Fig. 4c.*) When first formed they are hyaline and thin-walled and remain in this condition for some time, especially if they become free from the conidiophore.

Finally their walls thicken and become dark brown or brownish-grey. Ultimately the cells of the conidiophore itself not infrequently become thick-walled and brown, and when separated are

4a. Thick-walled brown conidium twenty-four hours after germination (Swift $\frac{1}{8}$ obj. $\times$ I. eyepiece.)

4b. Spore of same. (Swift $\frac{1}{8}$ obj. $\times$ I. eyepiece.)

4c. Thick-walled brown conidia five hours after germination. Note the multi-cellular conidia. (Swift $\frac{1}{8}$ obj. $\times$ I. eyepiece.)

The spores had all been subjected to a temperature below zero and then placed in a hanging drop of lavender extract in a Van Tieghem cell and left at room temperature. The conidia were from a culture on potato gelatine.

hardly to be distinguished from the brown conidia in appearance and germinative capacity.

Usually one, and rarely more than two, germ tubes are protruded from any portion of the spore and the growth is stouter and more frequently branched than that described for the pycnospores. (*Text Figs. 4a, b, c.*) The conidia measure 12–20 μ by 3–6 μ .

Hyaline Conidia.—The hyaline conidia approximate very closely to the pycnospores in their germinative capacity, but are perhaps slightly more resistant to adverse conditions.

Their walls stain yellowish-brown with Schultze's chlor-zinc-iodine solution and their contents consist largely of glycogen.

Thick-walled brown Conidia.—In water and nutrient media these spores do not germinate under a period of at least twenty-five days. This resting period may be curtailed or even eliminated by subjecting the spores to a temperature below freezing point or to the action of artificial gastric or pancreatic juice. The spores are equally susceptible with the pycnospores

to the influence of centrifugalisation, but much more resistant to desiccation and the action of chemical fumes. When the resting period of the spores has been eliminated their resistant quality is very greatly reduced.

Chlamydo-spores.—If the fungus be grown on a very stiff agar medium (*e.g.*, 5 per cent. potato agar), the colonies seldom attain a diameter of more than two centimetres, and after ten or twelve days' growth the mycelium breaks up into chlamydo-spores. (*Text Figs. 5, 6.*)

5. Chlamydo-spores from a culture on 4 per cent. lavender agar.

6a. Chlamydo-spores five hours after germination.

6b. Chlamydo-spore fifteen hours after germination.

The chlamydo-spores were from an old culture on prune gelatine. They were subjected to the action of trypsin in a faintly alkaline medium for three hours, washed and placed in a hanging drop of lavender extract in a Van Tieghem cell, and left at room temperature.

(All figs. Swift $\frac{1}{8}$ obj. $\times$ III. eyepiece.)

The cells become irregularly swollen, spindle-shaped, barrel-shaped, or even globular. Their walls become thickened, often very irregularly, and their colour changes to brown. The mycelium becomes brittle and easily disintegrates. This form of sporulation tends to occur on all media when the culture is old or growing under unfavourable conditions. The chlamydo-spores germinate in water and the majority of nutrient media, but only after a minimum period of twenty-one days and a usual period of twenty-five to thirty days. As in the case of the thick-walled brown conidia, this resting period may be eliminated by freezing or the action of gastric or pancreatic juice. In resistant quality to adverse conditions the chlamydo-spores approximate to the thick-walled brown conidia.

Formation of Pycnidia.—Pycnidial formation, although abundant on diseased plants, is only sparingly and inconstantly

found when the fungus is grown in pure culture on a nutrient medium. No attempt was made to elucidate the cytology of the process of development. The pycnidia originate as small knots or tangles of hyphae.—(*Pl. V., Figs. 2, 3, 4*)—the separate strands interweaving very intimately to form a compact mass. This increases in size, the hyphae become abundantly septate, and those on the periphery become slightly thick-walled and assume a brownish-black coloration. (*Text Fig. 7c.*)

The inner tissues are very delicate and show a radiate arrangement which is demarcated from a wall tissue. (*Text Figs. 7b, c.*)

The central portion of this delicate tissue is very finely divided. For some considerable time the pycnidium is without orientation and there is no indication of the point at which the ostiole will be formed. When about one-half to two-thirds grown, a portion of the wall tissue of the pycnidium develops rather more rapidly than the remainder and bulges internally and externally. (*Text Fig. 7d. Pl. V., Fig. 5.*) The pycnidia grow immediately below the surface of the medium, only the merest film of the nutrient matter covering them, and the swollen portion of the wall is found immediately below this film—*i.e.*, remote from the mass of substratum. This swelling is covered externally by the carbonaceous layer of peripheral tissue which encloses the pycnidium, but the tissue of the swollen portion of the wall is delicate, this being most marked in its central portion. The formation of this swelling is the first visible sign of orientation in the pycnidium, and marks the position of the future ostiole. About this stage of development the basal portion of the wall of the pycnidium begins to increase in thickness and hyphae grow out from the wall into the surrounding medium, so that not infrequently the pycnidium appears to be seated on a small stroma or basal pad of tissue. (*Text Fig. 7c, d, e, f.*) At the same time folds of the wall grow into the central tissues. The wall is clearly divided into a peripheral irregular carbonaceous layer, one to three or four cells thick, a middle pseudoparenchymatous tissue four to six cells in thickness, and an inner very delicate hymenial lining from which spring the basidia. (*Pl. V., Fig. 5. Pl. VI., Figs. 4, 5.*) These have exceedingly delicate walls and are turgid, with a dense very finely granular protoplasm. The centre is filled with a dense mass of turgid hyaline very minute cells, forming the extremities of the basidia and being the first pycnospores. The actual formation of the ostiole—(*Text Fig. 7f*)—is brought about partially by the disintegration or possible autolysis of the very delicate central cells of the “ostiole tissue” and partly by the pressure exerted by the internal elements, which on the rupture of the ostiole pour forth in a diffuse mass. The peripheral layer of thick-walled brown cells originally covering the ostiole is either torn completely free—(*Text Fig. 7h*)—ruptured—(*Text Fig. 7f., Pl. II., Fig. 4*)—or pushed over to one side—(*Text Fig. 7g*). Pycnospore formation continues for some considerable time after the first rupture of the ostiole. Not infrequently two or three pycnidia in immediate contiguity fuse

together during their early stages of development and the walls separating them are absorbed into the central sporogenous tissues. When mature the irregular fruit may dehisce by one central ostiole or each loculus may possess its own opening. The development of the pycnidium is diagrammatically represented in Text figure 7.

7. Diagrammatic representation of the development of the pycnidium. The line x—y represents the surface of the culture medium or epidermis of the host.
- Undifferentiated pseudoparenchymatous mass of mycelium.
 - The central tissues demarcated from the wall tissue.
 - The wall tissues differentiate into three layers and the central tissues show a radial arrangement.
 - The pycnidium orientated by the development of a delicate mass of tissue immediately below the outer film of substratum, and the growth of hyphae from the pycnidium to form a basal "stroma." The central portion of the inner tissues becomes very finely divided, and projections or tongues of wall tissue grow into the delicate inner tissues.
 - Differentiation is more marked and the ostiole begins to form.
 - On the final rupture of the ostiole the pycnosporos issue forth in a diffuse mass.
 - Rupture of the ostiole by the pushing over to one side of the outer wall layer.
 - Rupture of the ostiole by the tearing free of the outer wall layer.

The diameter varies from 80-150 μ , and the pycnidium may be pear-shaped, globular, or oblately spheroidal. The basidia are 12-14 μ long and more or less acicular, with a swollen base, or phial shaped. (*Pl. II., Fig. 5.*)

A few observations were made with a view to ascertaining what factors condition the orientation of the pycnidium, for the latter in early stages of its development is isodiametrical.

Two plate cultures were supported on their edges so that the immature pycnidia on the one plate received the light through the medium on their potential basal sides, whilst those on the other plate received it from above on their potential ostiolar sides. The face of each plate remote from the light was rendered opaque. The development of the pycnidia in both cases was perfectly normal, the ostiole being away from the nutrient substratum.

A portion of a plate culture containing developing pycnidia was cut out, placed on the bottom of a small sterile bottle, and this exhausted as far as possible of air. The pycnidia matured in a perfectly normal manner.

A plate culture with young pycnidia was flooded with its own nutrient medium (potato gelatine) to a depth of about six millimetres, so that the developing structures were in a middle plane. The pycnidia matured and were rather larger than usual, and hyphae from the wall grew out into the overlying food layer. The orientation of the pycnidia was completely disturbed, no definite ostiole was formed, and in many cases the wall was quite irregularly burst to allow of the exit of the spores. In other cases no opening of any kind was formed.

When a very thin film of nutrient material was poured over a plate containing developing pycnidia or the added layer was very frothy from violent shaking the disturbing effect, although still apparent, was not nearly so well marked.

These observations, though somewhat primitive and inconclusive, would appear to indicate that relation to food supply, and not light or aeration, is the primary factor conditioning the orientation of the pycnidium.

Infection Experiments.—Infection experiments were carried out on *Lavandula officinalis*, using pure cultures of the fungus grown on lavender gelatine. The results of this work are summarised below.

Experimental Infections on Lavandula officinalis.

Treatment.	Spore.	First Distinct Signs of Infection.	Result.
1. Suspension of spores in sterile water brushed on the surface of 12 shoots.	Pycnospore	On 11 shoots in 9-10 days. On 1 shoot in 15 days	Pycnidial formation in about 31 days.
	Hyaline Conidium.	On 10 shoots in 10-11 days. On 2 shoots in 17 days	Pycnidial formation in about 37 days.
	Brown Conidium.	None after 30 days ...	None.
	Chlamydo-spore.	None after 30 days ...	None.

Treatment.	Spore.	First Distinct Signs of Infection.	Result.
2. Spores digested in artificial gastric juice for 12 hours; then washed in sterile water and brushed on the surface of 12 shoots.	Pycnospore	On 1 shoot after 14 days.	Slight infection of 1 shoot. No pycnidial formation. Feeble mycelium in shoot determined as <i>Phoma lavandulae</i> .
	Hyaline Conidium.	None after 30 days ...	None.
	Brown Conidium.	On 5 shoots in 12 days On 1 shoot in 18 days	Pycnidial formation on 4 shoots after about 40 days. Mycelium in 2 remaining shoots identified as <i>Phoma lavandulae</i> .
	Chlamydo-spore.	On 7 shoots in 11-12 days.	Pycnidial formation on 6 shoots after about 40 days. Mycelium in remaining shoot identified as <i>Phoma lavandulae</i> .
3. Suspension of spores in sterile water frozen for about 14 minutes, and subsequently brushed on the surface of 12 shoots.	Pycnospore	None after 30 days ...	None.
	Hyaline Conidium.	None after 30 days ...	None.
	Brown Conidium.	On 7 shoots in 14 days	Pycnidial formation on 3 shoots after about 40 days. On 1 shoot after about 60 days. Mycelium in 3 remaining shoots identified as <i>Phoma lavandulae</i> .
	Chlamydo-spore.	On 8 shoots in 12-14 days. On 1 shoot in 17 days	Very sparing pycnidial formation on 5 shoots after about 40-43 days. Mycelium in 3 remaining shoots identified as <i>Phoma lavandulae</i> .

No definite controls were kept, but all the inoculated plants were either amongst others in a bed or had other plants in their immediate vicinity. The disease only appeared on those shoots experimentally infected. Where no pycnidial formation occurred the infected shoots were carefully sectioned and the mycelium present compared minutely with mycelium definitely known to be that of *Phoma lavandulae*.

Similar infection experiments were carried out on *Lavandula spica* with approximately equal results. Infection experiments were made with pycnospores only on *Lavandula vera* and *Lavandula lanata*, and pycnidial formation obtained in both cases.

Attempts were made to infect the following plants: *Chrysanthemum* sp., *Salvia* sp., *Vinca* sp., *Artemisia* sp., *Santolina* sp., *Aristolochia* sp., *Aster* sp., *Malva* sp., *Helianthus* sp., *Lindera* sp., *Crataegus* sp., *Calluna* sp., and *Syringa* sp.

The first trials were made with pycnospores directly transferred from lavender shoots, and these were placed both on the normal surface or in wounded tissues. No infections occurred.

Subsequently the fungus was transferred to a mush made by grinding the externally sterilised and washed fresh tissues of the individual plants with fine sterile sand in a mortar. No growth occurred on mush of *Artemisia*, *Santolina*, *Helianthus*,

and *Calluna*, and on each of the others but a slight growth was obtained. This appeared most promising on *Salvia*, *Vinca*, *Aster*, and *Malva*, and subcultures were made to freshly prepared mush of these plants. Portions of the most vigorously growing mycelium with a little mush were then placed on wounded and unwounded surfaces of the suitable plants. No infection occurred, and subsequent examination showed that the mycelium in every case had died after growing for a little time in the mush.

It would appear, therefore, that the fungus is pathogenic to the genus *Lavandula*, and probably confined to this genus.

8. Shoot of *Lavandula officinalis* infected at K_1 with pycnosporos and at K with thick-walled brown conidia treated with gastric juice. Shoot drawn eighteen days later. Pycnidia were subsequently formed on the stem portion but not on the leaves. ($\frac{2}{3}$ normal size.)

Relation of Fungus to Tissues of Host.—The fungus is primarily found in the stem tissues of its host. Rarely the leaf shows infection distinct from its supporting stem, and this

may occasionally be produced by artificial inoculation. (*Text Fig. 8.*) Pycnidia have never been found on the leaves.

At first the fungus mycelium spreads in the cortex of the host, radiating in fairly well defined "strands" or "tracks" from the point of infection. Much of this early mycelium may be regarded as distributive in that it consists of elongate little branched hyphae which penetrate the tissues principally in a direction parallel to the axis of the host. Later the mycelium is freely branched and forms a complex ramifying system in the host tissues. From the cortex the hyphae pass to the phloem groups and thence along the medullary rays to the pith, in which the fine mycelium is very abundant. (*Text Fig. 9.*)

9a. Hyphae of fungus ramifying in pith cells of host.
 9b. Hyphae passing through pits in the walls of pith cells of host.
 The hyphae are very slender and freely branched.
 (Swift $\frac{1}{8}$ obj. $\times$ III. eyepiece, reduced by one half.)

From the medullary rays and phloem groups the hyphae penetrate the xylem elements, branching little, but either running longitudinally through them or crossing at right angles to their length.

The hyphae enter the cells through the pits which are abundantly present in the walls—(*Text Fig. 9b*)—and where a hypha passes through a deep pit as in the walls of sclerenchymatous or tracheidal elements a definite constriction is visible. (*Pl. V., Figs. 6, 7, 8.*)

At point of entry and exit to such a pit an appressorium like dilation is present in the hypha.

The cells of the cortex, phloem, and cambium are rapidly killed and collapse—(*Pl. V., Fig. 9; Pl. VI. Fig. 4*)—and there appears to be no response of any kind on the part of the host. The cell walls do not seem to be affected by the fungus, and no trace of enzyme action could be discerned.

The pycnidia of the fungus are formed immediately below the epidermis, or at a depth of a few cells, and usually cause the latter to split away from the collapsed cortex. (*Pl. V., Fig. 9; Pl. VI., Fig. 4.*)

No conidial formation occurs on the host plant, but in old dried diseased shoots occasional chlamydospores are found. (*Pl. V., Fig. 6.*) These are frequently in organic connection with the *Phoma* mycelium. By teasing the tissues apart individual spores may be picked up on a glass hair and thus isolated. In size and appearance they resemble the chlamydospores found in artificial culture media. Of six spores tested in lavender extract three germinated and these only in ten, thirty and thirty-three days respectively. Of six spores digested with gastric juice, washed and placed in lavender extract, two germinated, both in about twelve hours. Unfortunately no growth of mycelium could be obtained from these spores, but there can be little doubt that they are homologous with the chlamydospores found in pure cultures of the fungus.

The minuteness of the pycnospores precluded the obtaining of definite information as to the manner in which they penetrate the host plant. It is to be noted, however, that under natural conditions the pycnospores are usually found in considerable number in or about the stomata, and that artificial infections very often appear to centre in a stoma.

Relation of Spores to Temperature and Desiccation.—A number of observations were made on the relation between the germinative capacity of the spores of the fungus, and various degrees of temperature and desiccation. The results are summarised in a tabulated form on pages 126-9.

The hyaline thin-walled spores are very considerably less resistant than the brown thick-walled spore forms. Both the former, and the majority of the latter, are killed by exposure to a temperature of 53° C. for 17 hours. The hyaline conidia are on the whole more resistant than the pycnospores, but both forms are unable to withstand a temperature below zero. When, however, the pycnospores remain in the pycnidia they are considerably more resistant to low temperatures, and may even survive over winter in a germinable condition.

The thick-walled brown conidia and chlamydospores are immune to the action of frost, and exposure to such a temperature merely results in the curtailment or elimination of their resting period.

Prolonged desiccation rapidly destroys the germinative capacity of the pycnospores and hyaline conidia, but is almost without effect on the thick-walled spore forms.

RELATION OF SPORES TO TEMPERATURE AND DESICCATION.

Room temperature approximately 14°—16° C.

Nutrient medium used for all experiments was aqueous extract of lavender shoots.

Treatment.	Spore.	Result.				Per cent. germination.
I. A suspension of spores in sterile water was smeared over six glass cover slips, and the films air dried. They were then placed in a paraffin oven at 53° C. for 17 hours. On removal a drop of nutrient medium was placed on the centre of each cover slip over the spores, and the cover slips inverted over Van Tieghem cells, and placed in a dark cupboard at room temperature.		Total number of spores.		Number of spores germinated.		
	Pycnospore ...	531		0		0
	Hyaline conidium.	327		0		0
	Brown conidium.	230		35		15
	Chlamydospore	123		27		22
				} After 27 days.		
II. Transfers were made from a suspension of spores in water to hanging drops of nutrient medium on six glass cover slips, and these inverted over Van Tieghem cells. They were placed in a paraffin oven at 53° C. for 17 hours, and then removed to a dark cupboard at room temperature.		Total number of spores.	No. of spores germinated during first 17 hours in paraffin oven.	No. of spores germinated later.	Total number of spores germinated.	
	Pycnospore ...	467	0	0	0	0
	Hyaline conidium.	218	0	0	0	0
	Brown conidium.	242	0	19	27	8
	Chlamydospore	153	0	23	23	15
				} After 27 days.		
III. Treatment as in I., but films placed for 17 hours in an incubator at 33-34° C.		Result.				
		Total number of spores.		Number of spores germinated.		
	Pycnospore ...	283		57		20
	Hyaline conidium.	320		74		23
	Brown conidium.	153		107		70
Chlamydospore	97		67		69	
				} In 24 hours.		
				} After 27 days.		
IV. Treatment as in II., but hanging drops placed for 17 hours in an incubator at 33-34° C.		Total number of spores.	No. of spores germinated during first 17 hours in incubator.	No. of spores germinated later.	Total number of spores germinated.	
	Pycnospore ...	193	0	104	104	54
	Hyaline conidium.	121	0	66	66	55
	Brown conidium.	350	0	213	213	61
	Chlamydospore	54	0	34	34	63
				} During next 24 hrs.		
				} After 27 days.		

Treatment.	Spore.	Result.				Per cent. germination.
V. Treatment as in I., but films placed for 17 hours in an incubator at 22-23° C.	Pycnospore ... Hyaline conidium. Brown conidium. Chlamydospore	Total number of spores.		Number of spores germinated.		60 63 73 64
		353		211		
		201		127		
		197		144		
		89		57		
VI. Treatment as in II., but hanging drops placed for 17 hours in an incubator at 22-23° C.	Pycnospore ... Hyaline conidium. Brown conidium. Chlamydospore	Total number of spores.	No. of spores germinated during first 17 hours in incubator.	No. of spores germinated later.	Total number of spores germinated.	79 75 84 86
		453	340	18	358	
		320	230	10	240	
		397	2*	301	303	
		156	0	135	135	
VII. Treatment as in I., but films placed for 17 hours in a dark cupboard at room temperature 14-16° C.	Pycnospore ... Hyaline conidium. Brown conidium. Chlamydospore	Result.				63 64 79 75
		Total number of spores.		Number of spores germinated.		
		327		206		
		404		258		
		233		184		
VIII. Treatment as in II., but hanging drops left for 17 hours in a dark cupboard at room temperature 14-16 C.	Pycnospore ... Hyaline conidium. Brown conidium. Chlamydospore	Total number of spores.	No. of spores germinated during first 17 hours in cupboard.	No. of spores germinated later.	Total number of spores germinated.	94 86 89 84
		125	117	0	117	
		300	255	3	258	
		99	4*	84	88	
		84	0	71	71	

* It is probable that these spores had not properly thickened their walls.

Treatment.	Spore.	Result.				Per cent. germination.
IX. Treatment as in I., but films placed in a capsule, which was then sealed, and left exposed to a temperature below freezing point for 17 hours.	Pycnospore ... Hyaline conidium. Brown conidium. Chlamydospore	Total number of spores.		No. of spores germinated after removal to room temperature.		2 3 74 80
		417	8	} During 24 hours.	}	
		372	11			
		197	146	} During 24 hours.	}	
		230	162			
X. Treatment as in II., but hanging drops placed in a capsule, and left exposed to a temperature below freezing point for 17 hours.	Pycnospore ... Hyaline conidium. Brown conidium. Chlamydospore	Total number of spores.	No. of spores germinated within 24 hours of removal from cold to room temperature.	No. of spores germinated during next 24 hours.	Total number of spores germinated.	0 2 63 75
		473	0	0	0	
		231	1*	4*	5	
		303	180	11	191	
		187	135	5	140	
XI. Treatment as in I., but films left in a dark cupboard at room temperature for 7 days.	Pycnospore ... Hyaline conidium Brown conidium Chlamydospore	Total number of spores.	Spores germinated during first 24 hours after removal.	Spores germinated during next 24 hours.	Total number of spores germinated.	63 62 4 0
		715	438	12	450	
		451	265	17	282	
		461	2	0	2	
		250	0	0	0	
XII. Treatment as in I., but films left in a dark cupboard at room temperature for 33 days.	Pycnospore ... Hyaline conidium Brown conidium Chlamydospore	583	23	10	33	6
		414	41	15	56	13
		195	150	14	164	84
		123	95	7	102	83

* It is probable that those spores were thick-walled brown conidia.

Treatment.	Spore.	Result.				Per cent. germination.
		Total number of spores.	Spores germinated in first 24 hours.	No. of spores germinated during next 24 hours.	Total number of spores germinated.	
XIII. Spores subjected to the action of artificial gastric juice for 12 hours, then washed, and placed in a hanging drop of lavender extract in a Van Tieghem cell at room temperature.	Pycnospore ...	265	1	0	1	4.4
	Hyaline conidium	123	3	0	3	3
	Brown conidium	97	64	16	80	82
	Chlamydospore	69	51	3	54	78
XIV. Suspension of spores in sterile water frozen for about 14 minutes, and subsequently covered by a hanging drop of nutrient medium in a Van Tieghem cell, and kept at room temperature.	Pycnospores ...	521	2	0	2	4.4
XV. Pycnidia teased from shoot, and suspended in water. Then frozen to slide for about 14 minutes. Picked out with glass hair, and placed in sterile water. Crushed, and loopfuls transferred to nutrient medium in a Van Tieghem cell.	Pycnospores ...	267	97	6	103	38

The Action of certain Enzymes on the Spores.—The pycnospores and hyaline conidia are killed by treatment with pepsin for twelve hours (artificial gastric juice, 100 c.c. of 0.4 per cent. hydrochloric acid added to 0.2 grams of pepsin dissolved in 100 c.c. of water). The effect of similar treatment on the thick-walled brown spore forms is to eliminate their resting period and render them capable of germination in a few hours. This result is apparently identical with that produced when the spores are subjected to a temperature below zero. One important point, however, is to be noted. Under normal conditions the spores are able to withstand repeated freezings, but after treatment with pepsin for twelve hours they are killed if exposed to frost for seventeen hours. Prolonged immersion in gastric juice or repeated alternate periods of immersion and desiccation reduce the germinative capacity of the spores. Spores which have passed through their resting period and are therefore immediately germinable are still resistant to repeated freezings, but not to repeated treatment with pepsin.

Treatment with trypsin (5 c.c. of Benger's liquor pancreaticus + 5 c.c. of 0.5 per cent. sodium carbonate) gave results much more variable but in effect substantially similar to those of pepsin.

Dilute solutions of diastase of malt, and invertase fairly rapidly reduced the germinative capacity of the thin-walled conidia and pycnosporos, and slowly that of the brown thick-walled spores. No effect on the duration of the resting period of the latter was observed.

Conclusion.—The life history of *Phoma lavandulae* on its host plant is simple. The overwintering of the fungus and spring infection of the host is by means of the occasional chlamydospores, and pycnosporos remaining in pycnidia, present in decaying fragments of old diseased shoots. The rapid spread of the disease during the summer months is due to the pycnosporos which are produced in immense numbers. By the removal of all affected shoots as soon as noted, and if possible before pycnidia are formed, the disease may be kept in check.

Summary.—A serious disease of the genus *Lavandula* is caused by *Phoma lavandulae*, Gabotto, a fungus which is here recorded for England.

In pure culture on nutrient media the fungus produces hyaline thin-walled pycnosporos, hyaline thin-walled conidia—which later become thick-walled and brown—and thick-walled brown chlamydospores.

The thin-walled spores germinate almost immediately, are little resistant to desiccation and are killed by frost.

The thick-walled spores are fairly resistant to prolonged desiccation, and only germinate after a resting period which may be eliminated by freezing or the action of pepsin or trypsin.

All spore forms germinate freely in vegetable nutrient media and the optimum temperature for the growth of the mycelium is about 18–20° C.

The development of the pycnidium is described and observations indicating that its orientation is conditioned by its relation to the food supply.

Infection experiments were carried out and demonstrated the pathogenicity of the fungus to the genus *Lavandula* and the fact that the fungus is probably confined to this genus.

The fungus mycelium ramifies throughout the host tissues and causes the cortex and phloem groups to collapse. The hyphae pass from cell to cell through the abundant pits in the walls.

The pycnidia are formed immediately below the epidermis which is lifted away from the cortex. Conidial formation is absent from the normal life cycle but chlamydospores are occasionally formed.

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Phoma lavandulae.

EXPLANATION OF PLATES.

PLATE V.

Fig. 1.—Formation of transverse wall in hypha by the ingrowth of a ring-like diaphragm of cell wall substance. (Leitz $\frac{1}{2}$ oil immersion $\times 8$ oc.)

Figs. 2, 3 and 4.—Young stages in the formation of pycnidia. (Swift $\frac{1}{2}$ obj. $\times$ III. eyepiece.)

Fig. 5.—Swelling in wall tissues at the position of the future ostiole. This drawing was made from a specimen immediately prior to dehiscence; the pycnosporos are ready to emerge. (Swift $\frac{1}{2}$ obj. $\times$ III. eyepiece.)

Fig. 6.—Chlamydospores of fungus in host tissue. (Swift $\frac{1}{2}$ obj. $\times$ III. eyepiece.)

Figs. 6, 7 and 8.—Passage of hyphae through cell walls of host. In the pit the hypha is constricted but dilates at points of entry and exit. (Swift $\frac{1}{2}$ obj. $\times$ III. eyepiece.)

Fig. 9.—Longitudinal section of a lavender stem attacked by the fungus. The developing pycnidia have lifted the epidermis away from the collapsed cortical tissues. (Swift $\frac{2}{3}$ obj. $\times$ I. eyepiece.)

PLATE VI.

Fig. 1.—Pycnosporos germinating on lavender extract at room temperature. (Swift $\frac{1}{2}$ obj. $\times$ III. eyepiece.)

Fig. 2.—Formation of thin-walled conidia on prune gelatine at room temperature. Note the very characteristic necks formed at point of abstriction. (Swift $\frac{1}{2}$ obj. $\times$ III. eyepiece.)

Fig. 3.—Thick-walled brown conidia from same culture as 2 but eight days later. (Swift $\frac{1}{2}$ obj. $\times$ III. eyepiece.)

Fig. 4.—Vertical section of a mature dehiscent pycnidium growing on its host. Note the collapsed cortex and lifted epidermis, the basal stroma of thick-walled hyphae and the ingrowths from the wall tissue into the receptacle. (Swift $\frac{1}{2}$ obj. $\times$ III. eyepiece.)

Fig. 5.—Basidia abstricting spores. (Swift $\frac{1}{2}$ obj. $\times$ III. eyepiece.)

XXIII.—DECADES KEWENSES

PLANTARUM NOVARUM IN HERBARIO HORTII REGII
CONSERVATARUM.

DECAS LXXXVIII.

871. *Glyptopetalum Lawsonii*, Gamble [Celastraceae-Celastraeae]; species *G. zeylanico*, Thwaites, affinis, foliis coriaceis apicem versus tantum grosse serratis, cymis 7-floris axillaribus vel supra-axillaribus differt.

Frutex erectus, glaber, ramulis crassis glabris supremis paullo complanatis. *Folia* opposita vel subalterna, elliptico-obovata vel elliptica, apice acuta, obtusa vel interdum emarginata, basi cuneata, 5–9 cm. longa, 3–6 cm. lata, coriacea, siccitate pallide viridia, integra vel apicem versus dentibus paucis (3–4) grosse serrata; costa crassa, nervis lateralibus utrinsecus 7–9 elevatis inter se reticulatione conspicua junctis; petioli crassi, 5 mm. longi. *Cymae* axillares vel supra-axillares, dichotomae, 6–7-florae; pedunculus 2–3 cm. longus, complanatus, ad apicem bracteolis 2 oblongis 4 mm. longis munitus; rami primarii 8–10 mm. longi, bracteolis parvis muniti, secundarii circiter 4–5 mm. longi, etiam bracteolis parvis ad mediam partem instructi. *Calyx* 4-lobus, lobis brevibus ad apices nigro-scariosis.