THE ANTHRACNOSE DISEASE OF DAPHNE MEZEREUM

caused by Marssonina daphnes (Desm. et Rob.) Mag.

by

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The hardy ornamental, Daphne mezereum, is a familiar border plant in the southern coast regions of British Columbia. Well known for its pink or white spikes of perfumed flowers, which appear in January or February when few other plants are in bloom, and for its brilliant red berries and waxy foliage during the summer, this shrub is becoming increasingly popular in parks and private gardens. However, within the last three years the propagation of this shrub has been seriously threatened by the sudden occurrence of an anthracnose or leaf-spotting disease, which prior to this time had been unknown in North America. This disease repeatedly defoliates the plants, stunts or completely kills the bloom and usually kills young seedlings. The work reported herein was undertaken to study this disease under local conditions and, if possible, to find practical control measures.

THE SUSCEPT

Plants affected. The disease has been found only on Daphne mezereum. Other species of Daphne are apparently immune. Attempts to infect artificially D. cneorum and D. laureola have been unsuccessful, and beds of the former,

growing underneath severely infected bushes of D. mezereum, have remained completely free of the disease during three years of observation. The numerous other species of daphne have not been tested since they are grown only rarely in British Columbia. There is no reference in the scanty literature on this disease to its occurrence on other species.

Parts affected. The most apparent injury caused by daphne anthracnose is to the leaves. The lesions are small but usually very numerous and characteristically located along the mid-rib, resulting in defoliation of the plant. As many as three crops of leaves have been destroyed in one season, leaving the plant very weak in the fall and subject to winter injury. Since the daphne is used as an ornamental shrub the unsightly appearance of the spotted leaves, even without defoliation, seriously reduces its attractiveness. Flower infection occurs in the spring, causing reduced and deformed bloom. Stem infections have been observed on the current season's new growth but they rarely kill the shoot. Spindly suckers, starting late in the fall, have been killed back by lesions of the anthracnose. Maturing buds become infected in the fall but are not killed. Lesions are formed on the bud scales but the fungus remains dormant until spring before sporulating.

Varietal susceptibility. Although there are several varieties of Daphne mezereum recorded by Kew Gardens, only

the pink flowered form and a white flowered variety, D. mezereum var. alba, are known in British Columbia. Of these two the pink form is much more commonly grown. Both these varieties are very susceptible to the disease. Occasionally a dark pink form is found, which, although by no means immune, is considerably more resistant than the common pink. Nurserymen report that when daphnes are raised from seed a small percentage of the plants will be of the dark form. This strain is characterized by larger flowers which are almost red in colour. The dormant buds are of a reddish tinge and the petioles and mid-ribs of the leaves are also redder than those of the common pink form.

Another peculiar daphne is found on the campus and as yet has not been identified. It is similar to the mezereum except that the bloom is paler, the branches thicker and more spreading and the leaves in more definite whorls. This daphne is completely immune to anthracnose, according to field observations and laboratory tests and, although not as showy as the common pink mezereum, it may be valuable as a substitute. Efforts are being made to determine the variety name of this type.

Because of the severity of the anthracnose on the pink form of daphne, nurserymen who propagate this species from seed would be well advised to select the dark pink form which is equally beautiful but more disease resistant.

THE DISEASE

Names. Although the causal fungus of daphne anthracnose was first described in 1843, there is still very little literature on the disease itself. Laubert (7) described the disease from Germany in 1931 but did not give it a common name. The first published work in English was by Green (4) in 1935. He used the name "Daphne Leaf Spot" to describe the disease. Local names used by gardeners and nurserymen include "Daphne Blight" and "Scab." The name "Daphne Anthracnose" is suggested as being the most accurate. The lesions are necrotic and hypoplastic and therefore, according to Jenkins' (6) classification, the disease should be called an anthracnose. This name will be used throughout this paper.

History and Range. The disease is of European origin. The pathogene was first described by Desmaziere in 1843, when it was found on native European D. mezereum (Sacc. Syll. Fung. III: 498). In 1915 the anthracnose was reported by Bubak and Kabat on wild daphnes in the Tyrol (1). Laubert (7) in 1931 in Germany described the disease as very injurious in nurseries and gave a short description of symptoms and etiology. Green (4) found the fungus on the cultivated daphne in England but reported that it was very rare and certainly not of economic importance.

The most recent reports indicate that the trouble is known in France, Switzerland, Italy, Denmark, Austria,

Latvia, Holland, Great Britain and Australia, as well as in British Columbia, Canada, (4), (3) and (11). Apparently the disease in these European countries is of minor importance since there is practically no literature available on the subject.

Daphne anthracnose was first reported in British Columbia in June, 1936, when specimens were sent to Mr. J. W. Eastham, Provincial Plant Pathologist, from Layritz Nurseries, Limited, Vancouver. Mr. Eastham found that the disease was severe on the daphnes at this nursery and also at their nursery in Victoria. About the same time the disease was noticed by Dr. F. Dickson, Professor of Botany at the University of British Columbia, on the D. mezereum plants on the campus of the University. Further investigations by the author during the same summer showed that a few plants in private gardens were diseased but general infection had not occurred. The studies were continued the next summer when it was found that the disease had spread to gardens in widely separated parts of the city. In the summer of 1938 diseased plants were found in all the suburban nurseries visited. Several nurserymen reported that their entire plantings of seedling stock had been killed by this disease in spite of attempted control by spraying. A number of plantings in public parks throughout the city have already been taken out because of the ravages of the anthracnose. At the present time the disease has

not been reported anywhere else in North America.

It has not been possible to determine when and how the disease was introduced into British Columbia. A check through the files of the Plant Inspection Office in Vancouver showed that, although the Layritz Nurseries had not imported daphnes from any country where the disease is known for at least two years prior to the outbreak, this plant has in the past been imported from Holland. It is quite possible that some of this stock was diseased, or at least carried the spores of the pathogene.

Economic importance. The economic importance of the daphne in Canada is not very great. The plant is confined to mild, temperate regions where the winters are open and the summers relatively cool and humid, such as in southern British Columbia. There are probably more daphnes grown here than in any other part of Canada. In this area the daphne is of definite importance to nurserymen and gardeners and unless methods are found to check the damages of anthracnose, the use of this shrub as an important ornamental will have to be discontinued.

SYMPTOMATOLOGY.

Symptoms of the disease are found on almost all parts of the plant. The buds, blossoms, leaves, berries and young shoots are all attacked by the anthracnose.

The most destructive phase of the disease occurs on the

leaves (Plate I). The first symptoms of infection are minute dark green or black dots. These usually appear on areas of the leaves where free water can accumulate. The mid-rib of a daphne leaf is sunken and it is in this trough that most of the lesions appear. The fact that the lesions are generally clustered on the mid-rib at the base of the leaf blade and on the short petiole accounts for the defoliating action of this disease. Within a few days the spots enlarge, finally to a diameter of 1 to 2 mm., coalesce and turn dark grey or pale brown in colour. The margins of these lesions are very definite and the surface is slightly raised. The grey, erumpent nature of the lesions at this stage is due to the formation of a glistening, waxy mass of spores over the surface. The infections remain in this stage until a rain or heavy dew washes away the spore masses, leaving shallow, brown depressions which do not enlarge but generally produce a new crop of spores. Lesions have been found on both sides of the leaves but the greatest number occurs on the upper surface.

Blossom infection is characterized by malformed or completely killed flowers (Plate II). Usually the lesions occur on the calyx lobes causing them to curl and die. If the buds become infected before they open they become mummified and remain to spread the disease to the young leaves which appear as soon as the blooming period is over. The lesions on the berries are similar to those on the

leaves but are smaller in size, seldom becoming larger than 1 mm. in diameter. A normal berry is bright red in colour and about 1 cm. in diameter. Severely infected berries do not colour as completely and are smaller in size. Premature berry drop is a phase of the disease due more to a weakened condition of the plant than to pedicle infections.

Stem infections on the current season's growth are occasionally found. They are erumpent, olive green in colour and somewhat elongated vertically. Such lesions are never more than 2 mm. in diameter. These infections do not kill the twig and are considered unimportant compared to other phases of the disease.

The lesions on the dormant bud scales are quite characteristic (Plate III). They vary in size from less than 1 mm. in diameter to elongated lesions 1 cm. in length. The margins of these spots are clearly defined and ringed by a white, papery fringe of the broken cuticle and epidermis. The center of these lesions is coal-black, stroma-like and distinctly erumpent. Bud scale infection occurs in the late summer as the buds are maturing and the black lesions can be found at any time during the winter before the buds open and the scales fall.

Gross symptoms on a severely infected plant resemble severe frost injury. The tender young leaves wilt and turn yellow before dropping, while the older leaves show dead or flaccid portions where the lesions have been most

numerous. Later on in the season, defoliation is the most common general symptom. Severely infected plants, after losing one crop of leaves, usually do not produce a full new set before the leaves are killed off again. As a result such plants have only a whorl of new leaves at the tips of the stems as contrasted to the usual heavy foliation of a healthy plant. The plants thus present a general ragged, straggly appearance.

ETIOLOGY

Name, History and Classification of the Pathogene. The causal organism of the daphne anthracnose is Marssonina daphnes (Desm. et Rob.) Mag., an imperfect fungus of the family Melanconiaceae. The earliest record of this fungus dates from 1843 when Desmaziere described the fungus by the name of Septoria daphnes Desm. et Rob. (Sacc. Syll. Fung. III: 498). Later Oudeman (Mat. Myc. Neerl. II, p. 28) described a fungus which he named Gleosporium Daphne Oudem. The pathogene was transferred by Saccardo to the genus Marssonia (spelled Marsonia by Saccardo) which had been erected by Fischer (Raben. F. Eur. n.1857) in 1957 (Sacc. III: 769). In 1923 Magnus (Sec. V. Höhn, 1923) changed the title of the genus to Marssonina because the previous name had been preoccupied by a phanerogamic genus. Therefore at the present time the accepted name of the fungus is Marssonina daphnes (Desm. et Rob.) Mag. An apparently different fungus, Gleosporium mezerei Cke. was described

from England by Cooke in 1890 (Grevillea 19: p.8, 1890), differing from M. daphnes in having non-septate spores. Correspondence between the author and Dr. Bisby of Kew Gardens resulted in a re-examination of Cooke's original material, in 1937, which showed that, to quote Dr. Bisby, "Gleosporium mezerei Cooke is identical with Marssonina daphnes (Desm. et Rob.) Sacc. .... M. daphnes is the older name and it would be a good thing to relegate the imperfectly described G. mezerei to synonymy." Apparently the confusion resulted from the fact that immature spores of the fungus are non-septate and approximately 15 X 6 u in size, which is a Gleosporium character, but mature spores are mostly 1-septate and more usually 20 u long, which characters place the fungus in the genus Marssonina. Connors in the Canadian Plant Disease Survey, 1937, reported the outbreak of the disease in British Columbia and identified the fungus as G. mezerei Cke. Later, in private correspondence the identification was changed to M. daphnes. Grove (5) in 1937, in his monograph of British Stem and Leaf Fungi, listed G. mezerei as "probably a young state of M. daphnes," although he did not examine Cooke's material. Many species of Marssonina have been found to be the imperfect stages of both Discomycetes and Pyrenomycetes, but there is still no perfect stage of M. daphnes known.

Attempts to locate a perfect stage were made during the study. Fallen leaves were examined periodically during the

winter, as were the berries. Bud scale lesions were examined at all stages and twig lesions studied whenever they were found. On none of these structures was there any indication of a perfect stage. The fallen parts of the plants always decayed in the field long before blooming time, precluding the possibility of a perfect stage forming on them in the spring. It is possible that in less humid climates the leaves, berries and bloom would persist on the ground until spring.

Morphology of Pathogene. The fruiting body of M.daphnes covers the whole of the surface of the individual lesion. It is of the typical acervulus type and is formed in the epidermal layer of the plant tissue (Plate IV , Fig. 1). The spores are produced in a gelatinous matrix on very short, simple conidiophores. They are obovate, slightly bent to one side, hyaline and with numerous guttulations (Plate VI , Fig. 3). A basal septum is formed in mature spores, making two unequal cells, the larger apical cell usually being the one to produce a germ-tube. Measurements on 100 spores from natural lesions on daphne leaves showed that the average size of the spores is 8 X 20 u, with a range in width from 7 to 9 u and in length from 18 to 21 u. The mycelium is completely intra-cellular and very characteristic in shape, being alternately swollen and constricted, branching and tortuous (Plate IV , Fig. 2). This peculiar type of mycelium is found in all the tissues attacked and in culture.

Pathogenicity. Two attempts to infect healthy daphne plants in the green-house with M. daphnes from single spore cultures were unsuccessful. This may have been due to incorrect humidity relations, but since time and facilities were not available to correct this error, the green-house tests were abandoned in favour of excised leaf cultures.

The method of culturing the daphne leaves was a modification of that used by Clinton and McCormick (2). The leaves were suspended over a culture solution in Petri dishes or trays, on floating waxed-paper. The leaf petioles projected into the solution through small slips in the paper (Plate XI). This method was found very satisfactory since it held the leaves free from the solution and yet the petioles were always immersed. The leaves were held securely in the paper and could be transferred, atomized or photographed with ease. Although a two percent sucrose solution was used in early trials, it was found that pure water kept the leaves in good health for at least six weeks. Since contaminations were eliminated by using water and since two weeks were sufficient for the pathogenicity studies, this medium was used in all subsequent cultures. It was found necessary to keep the covers on the Petri plates in order to maintain a sufficiently high humidity. Satisfactory lighting was obtained from north windows and all cultures were kept in the laboratory at room temperature.

Inoculations on excised leaves readily gave infections

after an incubation period of ten days. Pathogenicity was demonstrated in one instance where spores from a mono-sporic culture of M. daphnes gave typical infections on the excised leaves. Subsequent re-isolations from these anthracnose lesions yielded cultures indistinguishable from the original isolations. No difficulty was experienced in causing infection by atomizing spores from diseased leaves on to the leaves in culture.

Life History of the Pathogene. To understand the life cycles of the daphne anthracnose pathogene it is necessary to know the seasonal development of the daphne plant. Under local conditions the daphne has a very short period of dormancy. Usually the flowers appear in January or February, before the opening of the leaves. The bloom is clustered along the previous season's growth, forming a dense spike of flowers. The blooming period is relatively prolonged, lasting from a month to six weeks. Generally in late March the new leaves appear, mostly from leaf buds among the dead or dying flowers. By the end of April the plant is in full leaf and remains so until late in the winter. The bright scarlet berries ripen in the late summer and, on healthy plants, persist well into the winter. The buds on the current season's growth enter a short dormant period in the fall prior to the next season's development.

The primary cycles. The life history of M. daphnes consists of a series of cycles, none of which contains a

perfect stage of the fungus (Plate IX). The primary cycle takes place on the blossoms. The inoculum is supplied by the sporulation of overwintered lesions on the bud scales. Because the flowers are clustered close together on the stem, the transfer of inoculum is easily effected by splashing rain or even a heavy dew, the dense spike also maintaining a favourable high humidity for spore germination. Because the fruiting bodies are typical acervuli water is essential for spore dissemination as a solvent for the gelatinous matrix in which the spores are embedded. Infections occur both on the petal-like calyx lobes and on the calyx tube. The resulting lesions produce spores before the blossoms drop. This is the end of the primary cycle; there is no saprogenesis as far as is known, the fallen bloom playing no further part in the life history.

The secondary cycles. The first leaf infection cycles commence with the transfer of inoculum from the diseased blossoms to the young leaves. The bud scales may supply some inoculum for these cycles but usually they have dropped before the leaves open. Because of the close association between the blossoms and the whorls of young leaves it is hardly possible for the leaves to escape inoculation from the diseased bloom. It has been noted that, as the leaves open, the flowers drop and are held in the whorls of leaves in the same manner that the loosened bud scales are held by the blossoms. Rain and dew are

necessary both for dissemination and germination. The points of severest infection on the leaves are invariably where free moisture can accumulate. Fallen debris and bloom on the leaf surfaces retain the moisture; the mid-rib trough, bent-down edges and leaf tips hold drops of water; a film of water will stay where two leaves come in contact. These areas are always the most severely affected. There is no relation between the stomata and the location of infections since most, but not all, of the lesions are on the upper surface of the leaf, while all of the stomata are on the lower surface. The relative infrequency of the under-leaf infections is probably due to the absence of free water on the lower surface and to the greater difficulty of inoculation. The incubation period has been found to be ten days with artificial inoculations on excised leaves. In the field new infections are quite evident within two weeks of a rainy period. At this time waxy, sporulating pustules are present, the spores from which constitute the inoculum for the repeated summer cycles.

The leaf cycles continue into late summer, as long as new leaves continue to appear. There is a striking relation between weather conditions and these cycles. During a dry spell, new, uninfected leaves are produced and remain healthy until after a rain. Then a new general infection appears, followed by another defoliation.

Other, less obvious, secondary cycles appear during the

summer on the shoots and on the ripening berries. As in the other cycles there is a close association between the source of inoculum and the susceptible tissue. The cycle on the shoots apparently carries the disease over winter, and, according to Green (4), these lesions sporulate in the spring. Because of the much greater prevalence of bud-scale infection, the role of twig lesions in initiating the primary flower infection cycles is relatively unimportant. The berries are infected in late summer from the diseased leaves. Some of these diseased berries spread the fungus to late-appearing leaves, and the cycle ends with the fall and decay of the fruit.

The final cycle of the season takes place on the buds formed in the axils of the leaves. In this case the proximity of inoculum to susceptible tissue is most strikingly illustrated. Any rain falling on the diseased leaves is drained down the mid-rib and bathes the axiliary bud with a suspension of spores. In this cycle the inoculation is so sure that there is practically one hundred percent bud-scale infection in the fall. The constant close proximity of the infection courts to the source of inoculum in all of the cycles largely explains the devastating nature of general infections following a period of rain. The habit of growth resulting in this condition is illustrated by Plate X. The bud-scale lesions develop into black, erumpent, stroma-like, over-wintering bodies which do not sporulate until blossoming

time in the spring (Plate III, Figs. 1 and 2). Spore production at that time supplies the inoculum for the start of the primary cycle again.

Pathological Histology. Histological studies on the relation between pathogene and suscept were made on naturally infected material except that some of the stages in penetration were obtained from artificial inoculations on excised leaves.

A modification of the lacto-phenol method (9) of clearing and staining leaves and other material was used with good results. Portions of leaves or petals, bark of young twigs and berry skins were treated by the following method: the fresh material was boiled in absolute alcohol for five minutes to remove the chlorophyll and other pigments and to fix the tissue. The tissue was then transferred to hot lacto-phenol for clearing. It was found that when the lacto-phenol was held at a slow boil for a few minutes and then rapidly cooled there was less danger of air bubbles in the tissue. A few drops of cotton-blue stain were added to the lacto-phenol, the concentration being determined by experimentation and varying with the tissue being treated. The cleared and stained material was mounted on microscope slides in lacto-phenol or glycerin jelly (8). In making slides of leaves and petals the epidermis was sometimes stripped off after clearing and staining. Since the early stages of infection are confined to the epidermal layer,

this technique gave a clearer slide. By this method the plant cells were left transparent and the fungus tissue was stained a dark blue, resulting in very clear differentiation. It was possible to follow the course of mycelium down into the spongy mesophyll and even to study infections in the lower epidermal cells through the rest of the leaf.

The spores apparently become firmly attached to the surface of the leaf by the secretion of some adhesive substance. Examination of spores by the India-ink method (8) showed that there is no thick gelatinous envelope around the spores. Stained spores on the surface of leaf material were always surrounded by a diffused stained area which would indicate a fungus secretion. Ungerminated spores on leaf material treated by the clearing method described above were not dislodged in the rather strenuous process.

Germination takes place from either the large or the small cell of the spore, but usually from the larger. The germ-tube always originates from the side of the spore which is in contact with the plant surface. A fine penetration tube is formed immediately under the spore and penetrates directly through the cuticle and epidermal cell wall. The fact that there is no horizontal growth of a superficial germ-tube is strikingly illustrated by the fact that spores have been seen lodged directly on a stomatal opening and yet the hyphae penetrate directly into the guard cell rather than through the stomatal opening. Soon after entering the

epidermal cell the hypha forms a small bulbous vesicle (Plate VI, Fig. 1). From this organ the typical swollen hyphal bodies radiate to the periphery of the cell (Plate VI, Fig.4). Adjacent cells are penetrated only when the invaded cell is practically filled with hyphae. The fungus spreads very slowly and mature single infections are seldom more than ten epidermal cells wide (Plate IV, Fig. 1). The palisade layer is generally penetrated and many of these cells have been found completely filled with fungous tissue. The spongy mesophyll is very rarely attacked from infections on the upper surface and no case has been seen where an infection has penetrated completely through the leaf. When the infections occur over the mid-rib the xylem elements become completely filled with hyphae which would explain the defoliating action of this disease. As the lesions mature the epidermal cells are ruptured by the pressure of the fungous growth and the sporulating layer is exposed (Plate V, IV). The immature spores are formed, before the cuticle is ruptured, on short conidiophores. When the cuticle breaks the gelatinous matrix extrudes, as shown in Plate I, Fig. 2. It has been noted that the cell walls of invaded tissues are considerably thicker than those of healthy tissues. This response on the part of the plant may be somewhat responsible for the restricted nature of the lesions.

Histological examination of dormant bud-scale lesions show that the black, erumpent surface is sclerotial in nature,

(Plate VII, Figs. 1 and 2). This structure is composed almost entirely of dark coloured closely knotted mycelium. In the spring the surface of the lesion becomes a sporulation layer.

Cultural Studies. M. daphnes was obtained in pure mono-sporic culture by the following method: a dilute suspension of spores from fresh leaf lesions was streaked on to thin, cleared agar in Petri plates. Isolated spores were located with a binocular microscope and marked by rings of India-ink on the under surface of the plate. After the spores had germinated they were picked out with a very fine-pointed platinum needle and transferred to test-tube slants of potato-dextrose agar. Although the fungus grew very slowly on this agar, sufficient single-spore cultures were obtained for the cultural studies.

A series of different media were used to determine the most satisfactory one for growth of the fungus. Table I shows the media used and the growth responses obtained after three weeks at approximately 22 degrees C. The "Difco" agars used are commercial preparations and were made according to the directions on the containers. Coon's agar was made from the formula given in Rawlin's "Phytopathological Methods" (9). The daphne twig agar was made by steeping 50 grams of dried daphne twigs in 500 cc's. of water by boiling for one-half hour. The extract was filtered and added to 500 cc's. of hot 4 percent agar, strained, tubed and autoclaved. The best growth was obtained on daphne twig agar

but even that medium gave very slow growth. Prune agar was almost as good, and, since the daphne material could not be easily obtained, most of the routine culturing was done on the prune agar. More detailed studies of cultural characteristics were abandoned because the slow growth of the organism would not allow for quantitative measurements of growth responses.

Table I. The reaction of M. daphnes to various media.

Medium.	pH	Average diameter growth in 3 weeks, on 4 colonies.				Final Average.	
Difco Prune Agar	5.1	9.75	9.37	8.25	8.37	8.93	mm.
" Corn Meal Agar	5.3	9.75	9.35	6.25	4.75	7.53	"
" Potato-dextrose Agar	5.4	7.00	8.00	6.22	-	7.21	"
" Malt Extract Agar	5.0	6.00	5.37	4.87	5.00	5.31	"
" Bean Pod Agar	5.0	5.00	6.37	5.00	5.00	5.34	"
" Lima Bean Agar	5.3	1.00	2.00	1.00	1.50	1.37	"
Coon's Synthetic Agar	5.1	nil	nil	nil	nil	nil	"
Daphne Twig Agar	5.3	9.00	9.50	9.50	9.37	9.34	"

A typical colony on daphne twig agar is compressed, erumpent, with a slightly convoluted surface and a clearly defined margin. It is almost black in colour with a narrow brown margin. The surface of mature colonies becomes covered with the waxy, white spore masses. A characteristic brown stain permeates the agar surrounding the colony, fading out about 1 cm. from the margin.

The spores germinate slowly in distilled water. Table II shows the results obtained from a germination series in Van Tiegham cells at various temperatures and using distilled water. Complete data on the effect of nutrient, pH and temperature were not obtained. The optimum temperature for

Table II. The effect of temperature on spore germination.

Temperature in degrees C.	% Germination after 24 hours.	% Germination after 48 hours.
5	0	2.3
10	3.0	7.6
15	24.2	32.5
20	52.4	75.0 (estim.)
25	0	0

spore germination lies between 20 and 25 degrees C. or approximately 70 degrees F. Very slight germination occurs if the temperature is below 15 degrees C. At 25 degrees C. and above the spores were killed before any sign of germination appeared. This information cannot be completely correlated with the temperature relations of the disease in the field but does help to explain why epiphytotics of the anthracnose are associated with hot summer weather.

EPIPHYTOLOGY

Weather conditions apparently play a very important part in influencing the severity of the attacks of daphne anthracnose. The unusually long period of apparent health

shown by the plants between blossom infection and general leaf infection seems to be due to unfavourable weather conditions for the disease during that time. There is certainly abundant inoculum present on the dead or dying bloom to infect the young leaves which are apparently very susceptible as shown by the severe attacks of disease on late-appearing leaves in the summer. Weather charts of the Vancouver district have been consulted in an effort to correlate temperature and humidity conditions with anthracnose epiphytotics. The date of first general leaf infection was noted during 1937 and 1938 and since the incubation period is approximately ten days, the weather conditions ten days prior to this date were studied.

In 1937 first general leaf infection was noticed on May 29. From the weather charts it was found that on May 17 and 18 the mean daily temperature was 55 degrees F., accompanied by light precipitation. Prior to that date the temperature had attained this height on two occasions, but without rain. Therefore these two days were the first during the year when humidity was high and temperature 55 degrees or higher.

In 1938 leaf infection first became general about May 15. That year the first two days during which rain accompanied a temperature over 55 degrees F. were May 7 and 8, which was one week prior to the first epiphytotic. As in 1937 such temperatures had been reached earlier in the

season, but not accompanied by rainfall.

Although two years' evidence cannot be considered conclusive, the facts are worth reporting in explaining the incidence of the disease in the summer. Whether or not it is true that temperatures above 55 degrees F., accompanied by rain, are necessary for infection will have to be shown by observations in future seasons. One substantiating fact is obtained from the spore germination tests. At 59 degrees F. there was 32 percent germination in 48 hours but only 7.5 percent at 50 degrees F., under optimum conditions of humidity and with a constant temperature.

CONTROL

Exclusion. Exclusionary measures would be of definite value in preventing the spread of daphne anthracnose to new areas. Long distance spread of the disease is almost entirely effected by movement of diseased stock and probably was the method of introducing the disease to southern British Columbia. At present the adjacent state of Washington imports considerable numbers of daphnes each year from B. C. Since there has been no report of daphne anthracnose in that state, and since it is hardly likely that infected plants will not be sent there eventually unless checked, it would be in the interests of daphne growers in Washington to ban imports of daphnes from infected areas. Although there is still the danger of natural spread of the disease down the coast, exclusionary measures

would greatly reduce the danger of the disease becoming established.

Eradication. Daphne anthracnose appears to be of such an infectious nature that there is little hope of being able to eradicate the disease in districts where it is now established. The area involved in British Columbia is still small but the suscept plants are so widely scattered that it would be practically impossible to attempt control by destroying the diseased bushes. Moreover, the disease is so easily spread that every infected bush in the whole area would have to be located and destroyed to effect complete eradication. In areas where the disease is not yet firmly established, roguing out infected plants is recommended.

Protection. The primary problem of controlling the disease where it is already widespread is one of protecting the plants from infection by spraying. Control by the use of fungicides is made difficult by the extreme and summer-long susceptibility of almost all parts of the plants. However, spraying experiments have been made during the last two summers in an effort to control or reduce the damages of the leaf infections.

1937 experiment. In 1937 the author experimented on fifty mature plants on the University campus. Five of the bushes were left unsprayed as checks and the remainder were divided into three equal groups. Every plant was classified as to size, vigour and location and the plants in

each test were as nearly as possible equal in all respects. The spray materials used were Lime Sulphur, in the form of a commercial preparation known as Microsul, produced by the Lunavale Products Limited, Lancaster, England; Burgundy Mixture in the form of Micronized Burgundy, produced by the same company; and home-made Bordeaux Mixture. The concentrations used were:

Microsul.....8 lbs. per 100 gals.

Micronized Burgundy.....4 lbs. per 100 gals.

Bordeaux Mixture.....4-4-40

A spreader and sticker known as Lethalate Wetting Fluid, made by the Lunavale Products Limited, was found to be satisfactory. Good coverage was obtained using 2 lbs. of Lethalate per 100 gallons of spray, a high concentration necessary because of the very waxy nature of the leaves. Only half this amount of spreader was used with Micronized Burgundy which is sold with the spreader added.

Three sprays of each material were applied in the most thorough manner possible. The dates of application were April 8, June 4 and July 13. At the end of this program the experiment had to be discontinued; therefore the results were taken on the amount of control effected up to that time. By the end of May the check plants showed general infection and were completely defoliated at the conclusion of the experiment.

The Lime Sulphur spray gave very poor control, the

defoliation being almost as severe as on the checks. The Micronized Burgundy gave fair control. Although there was moderate infection, the defoliation was much less severe than on the checks. The Bordeaux Mixture gave the best control. At the time of the last spray there were very few lesions on the leaves, there had been practically no defoliation and the plants were much more healthy than the rest of the plot. In spite of the good control with Bordeaux, these plants became infected later in the summer, and by the end of August all the plants had been defoliated.

An interesting after-effect of this spraying program was noted at blooming time the next spring. While classifying the plants for vigour it was observed that plants which had been sprayed with different sprays showed marked differences in earliness and amount of bloom. The results are shown in Table III.

Table III. The effect of spray treatments on following year's bloom.

Treatment used 1937	Time of Blooming			Amount of Bloom		
	Late	Medium	Early	Good	Medium	Slight
Bordeaux	100%	0	0	80	20	0
Burgundy	66	34	0	73	0	27
Lime Sulphur	13	40	47	26	20	54
Check	0	60	40	0	20	80

It will be seen that the better the spray control the later and more profuse was the bloom produced in the spring. A

logical explanation of this would be that the Bordeaux spray, and, to a lesser extent, the others, by delaying defoliation prevented the plants from becoming dormant as early as the checks, but at the same time gave them more vigour. On the other hand the badly defoliated checks became dormant earlier in the fall and bloomed earlier in the spring. The disease, however, left the plants in a weakened condition, reflected in poor bloom.

1938 experiment. The same plot of plants was sprayed in 1938 using only the Micronized Burgundy. Bordeaux was discontinued because, although it gave the best control in the previous year, the unsightly residue made it quite unsuitable as a spray for an ornamental plant. It seemed possible that the Burgundy might give satisfactory control if the applications were more frequent and the spraying continued later in the summer.

Sprays were applied on the following dates: April 18, May 4, May 20, June 9, July 15 and August 1. On the first spraying the concentration of Micronized Burgundy was 1 lb. in 8 gallons of water, which is much higher than the recommended strength, but a concentration which gave satisfactory control of rust on antirrhinums at Saanichton, without spray injury. This strength did, however, seriously injure the daphnes and the spray had to be diluted in subsequent applications to 1 lb. in 40 gallons before the injury was eliminated. The spray injury spots on the leaves

were very similar in appearance to the lesions of the anthracnose, making observations on control difficult. The plants so injured were greatly reduced in vitality, and although the subsequent sprays did not cause burning, the plants were in poor condition for the duration of the summer.

The plants were divided as before into three groups. The first group received all the sprays; the second group was not sprayed on April 18; and the third group was not sprayed on April 18 nor May 4. Although the intervals between sprays were not regular, an effort was made to apply the sprays before threatened rain in order to give the best protection possible.

Table IV. Results of spraying experiment - 1938.

Amount of Disease	Observed April 15				Observed May 12				Observed May 25				Observed July 5			
	#1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
No Disease	15	15	15	15	15	13	13	3	6	4						
Very Slight						2	2	3	5	3	1					
Slight								6	4	5			7	3	1	
Moderate								3		3	1		8	6		
Severe											13	15		6	14	15

Explanation:- the figures refer to the number of plants out of 15. The figures on the 5 check plants were multiplied by 3 to be comparable.

These numbers refer to the spray treatment, thus:

#1 - all 6 sprays applied.
2 - first spray omitted.
3 - second spray omitted.
4 - check - unsprayed.

From Table IV it will be seen that even the whole six sprays gave only partial control. This fact was obvious by the first of July and the two sprays following that date were of very little use. The probability of nurserymen or gardeners being willing to apply more than six sprays is remote; therefore the only conclusion is that summer spraying with Burgundy Mixture is not a practical control for the disease. In view of the results obtained during the previous year with Bordeaux, probably this spray would be more effective than Burgundy Mixture. Under certain conditions, such as in seedling plots and in nurseries, Bordeaux could be used to save the plants even if it destroyed the ornamental value.

There is a strong possibility that the disease can best be attacked in the fall at the time the bud-scale lesions form. A strong dormant or pre-dormant spray might control or reduce the over-wintering lesions. One private grower reported that he controlled the anthracnose by dipping young infected plants, during November, in a lye solution, 1 oz. in 10 gallons of solution. Although there were no check plants in his experiment, the report is authentic and may indicate the value of a dormant spray or dip. It is hoped that this work can be supplemented in the near future.

From these experiments the only recommendations that can be made are to use a strong dormant spray just after the normal leaf drop in the fall, followed if necessary by

summer sprays with Bordeaux Mixture. These summer sprays should commence early in May and continue during the summer, so spaced that the plant is completely covered with spray at the critical periods of rain.

SUMMARY

The anthracnose of the ornamental shrub, Daphne mezereum, a disease of European origin and previously unknown in North America, has appeared and reached serious proportions in southern British Columbia. The disease is a leaf spotting type, the most serious phase of which is defoliation, accompanied by blossom blight and premature berry drop. This trouble has become so severe that young nursery plantings have been completely killed and older plants greatly weakened, with a general loss in ornamental value. The causal agent is the Melanconiacious fungus Marssonina daphnes (Desm. et Rob.) Mag. The fungus Gleosporium mezerei Cke., previously believed to be responsible for another leaf disease of daphne, has been proved to be a synonym of M. daphnes. Pathological histology has been studied in detail. The fungus over-winters on the scales of the dormant buds in the form of stroma-like lesions, which sporulate during blossom time in the spring. Epiphytotics occur when high summer temperatures are associated with rain. Summer spraying has not proved to be an effective control, but hope is held for the use of a strong dormant spray followed by summer spraying with Bordeaux Mixture.

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BIBLIOGRAPHY

1. Bubak, F. Ein Beitrag zur Pilzflora vor Tirol und Istrien. Ann. Mycol. XII: 205-220, 1914.

2. Clinton, G. P. and Florence A. McCormick. Rust infection of leaves in Petri dishes. Bull. Conn. Agric. Exp. Sta. 260: 475-510, 1924.

3. Connors, I. L. Sixteenth annual report of the Canadian Plant Disease Survey. Can. Dept. Agric. 1936.

4. Green, D. E. A leaf spot of Daphne mezereum. Royal Hort. Soc. J. 60: 156-8, 1935.

5. Grove, W. B. British Stem and Leaf Fungi. Vol. 2. Cambridge University Press. 1937.

6. Jenkins, A. E. Application of the terms "Anthracnose" and "Scab" to plant diseases caused by Sphacelona and Gleosporium. Phytopath 23: 389-395, 1935.

7. Laubert, R. A very injurious disease of Daphne mezereum (German title). Der Blumen - und Pflanzenbau: 46: 138, 1931.

8. Lee, A. B. The Micrometrists Vade Mecum. Ninth edition. P. Blakiston's Son and Co., Philadelphia, 1924.

9. Rawlins, T. E. Phytopathological and Botanical Research Methods. John Wiley and Sons, Inc., London. 1933.

10. Saccardo, P.A. Sylloge Fungorum. Vol. 1-25 and supp. 1845 –

11. Stevenson, J. A. Foreign Plant Diseases. U.S.D.A., Wash. 1926.

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PLATE I. Fig. 1. Leaves of *Daphne mezereum* infected by *Marssonina daphnes*. From excised leaf culture. X 1.

Fig. 2. Infected daphne leaf showing waxy spore masses on lesions. X 8.

Fig. 3. The same, showing killing of mid-rib.

Photographs by Dr. Dickson.

PLATE I

Fig. 1.

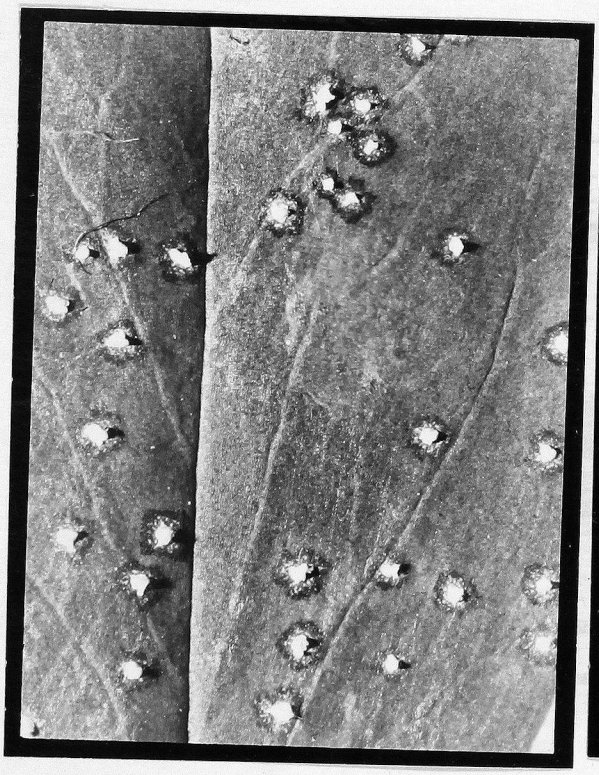

Fig. 2.

Fig. 3.

PLATE II. Blossom buds of *D. mezereum*. X 6.

The top three buds are healthy, the bottom three show the lesions of anthracnose.

PLATE II

PLATE III. Daphne anthracnose on the bud scales.

Fig. 1. Overwintering lesions.

Fig. 2. New lesions on folded leaves.

PLATE III

Fig. 1.

Fig. 2.

PLATE IV. Fig. 1. Semi-diagrammatic drawing of acervulus of M. daphnes, in cross section.

Fig. 2. Camera lucida drawing of young infection showing radiating hyphal bodies.

PLATE IV

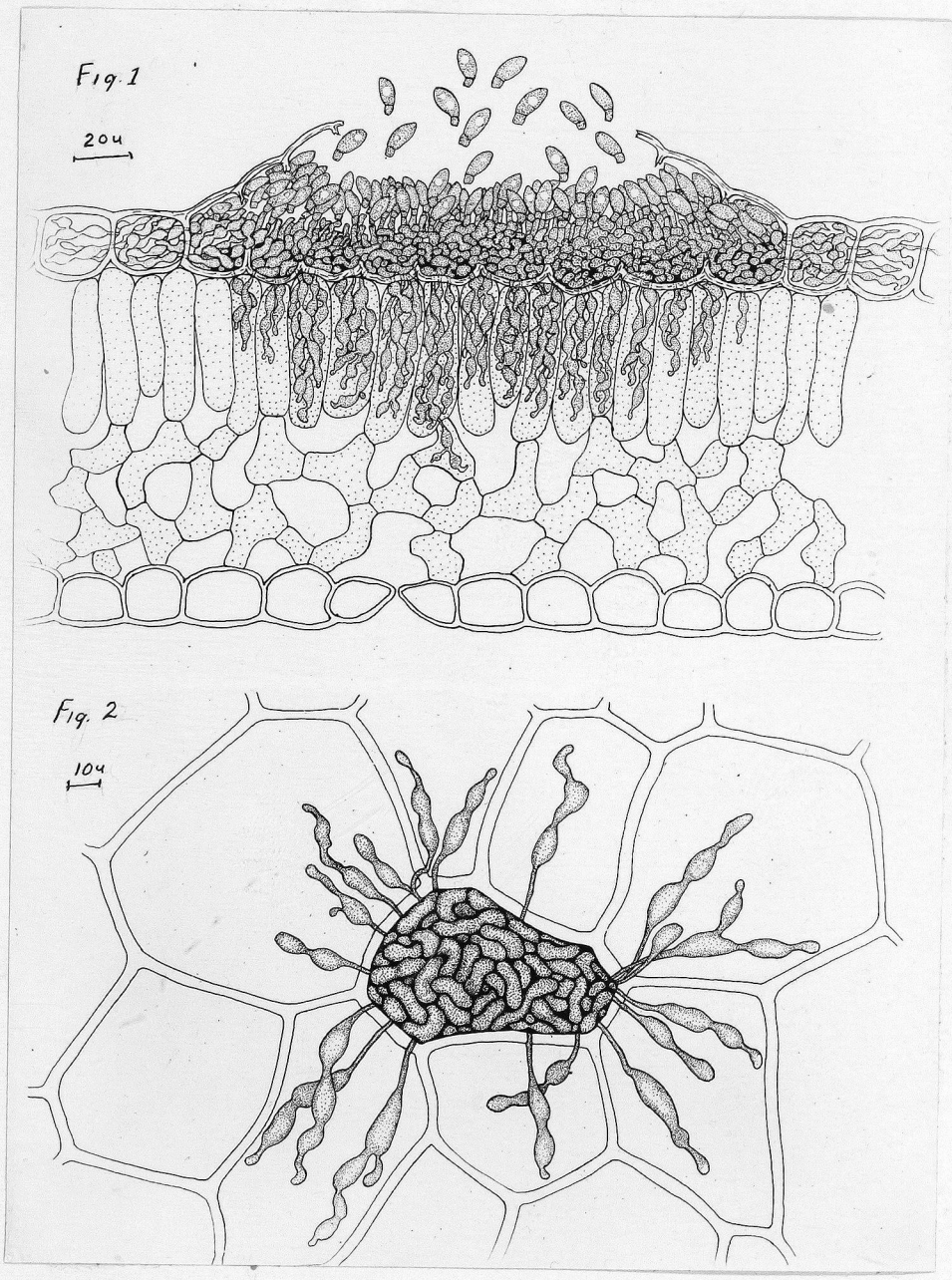

PLATE V. Fig. 1. Photomicrograph of cross section of acervulus of M. daphnes. X 500.

Fig. 2. Photomicrograph from cleared leaf preparation showing radiating hyphal bodies at edge of lesion. X 500.

PLATE V

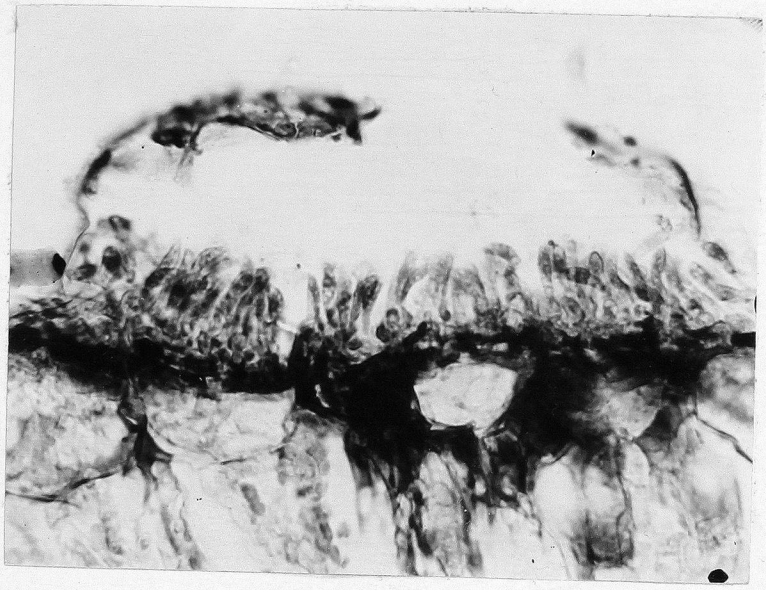

Fig. 1.

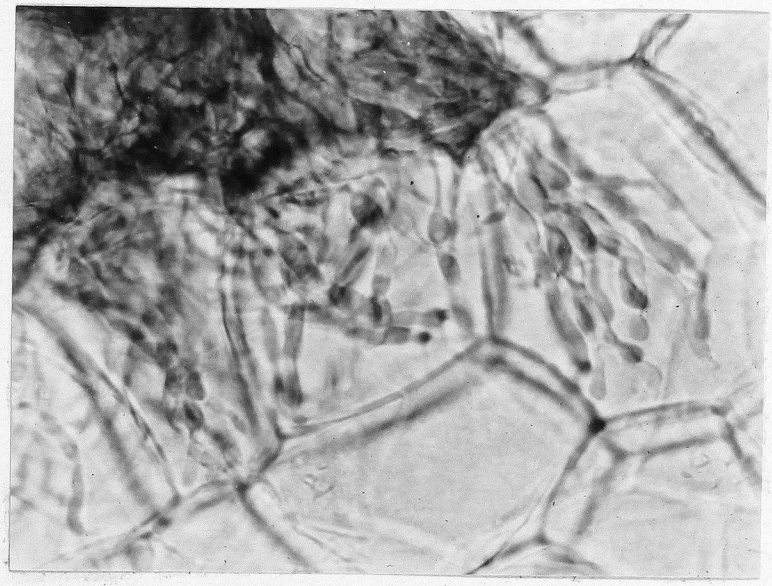

Fig. 2.

PLATE VI. Morphology of M. daphnes.

Figs. 1, 2, 4 and 5, camera lucida drawings of stages in penetration of the fungus into the epidermal cells of the daphne leaf.

Fig. 3. Spores of M. daphnes greatly enlarged. The actual size of spores is 8 x 20 u.

PLATE VI

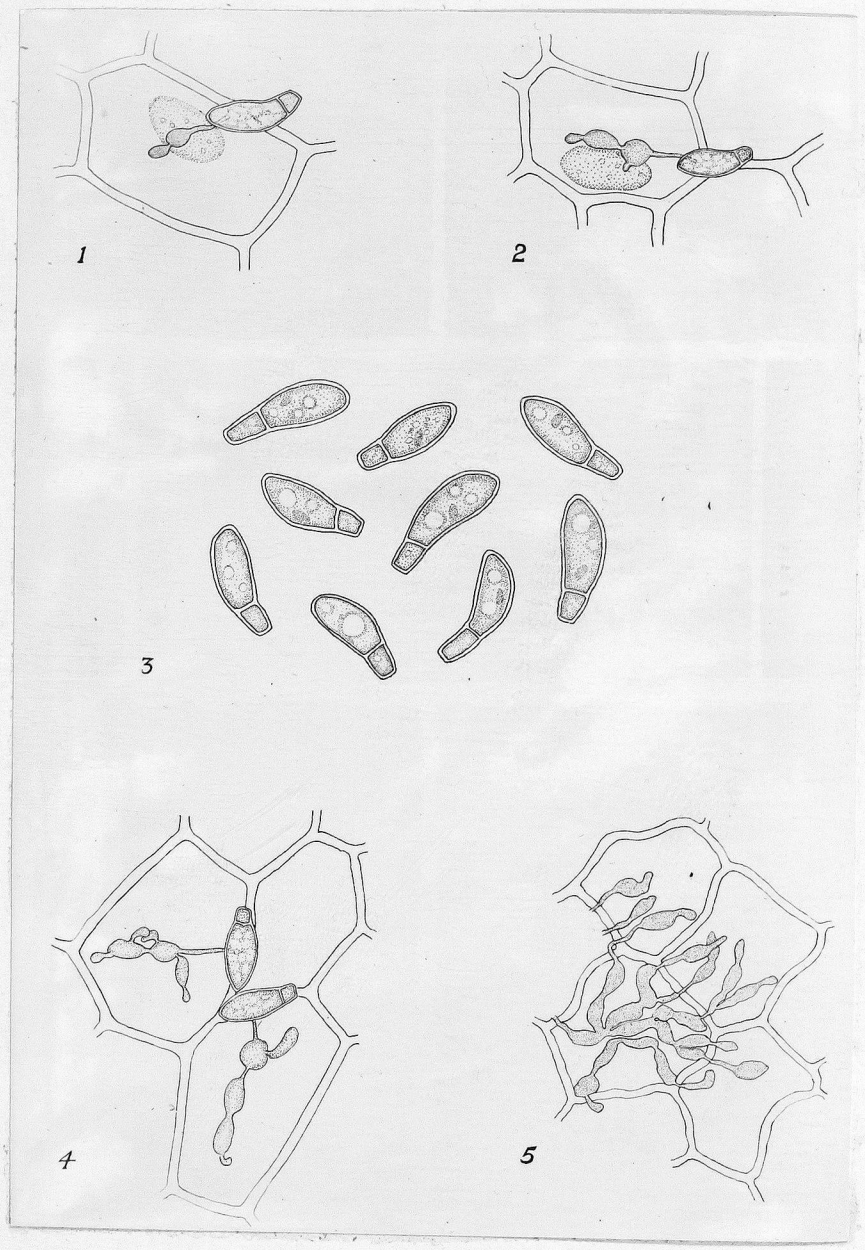

PLATE VII. Photomicrographs of bud-scale lesions.

Fig. 1. Free-hand section of a single lesion, showing cells packed with mycelium and illustrating the stromatic nature of the lesion. X 100.

Fig. 2. Portion of same lesion X 500.

PLATE VII

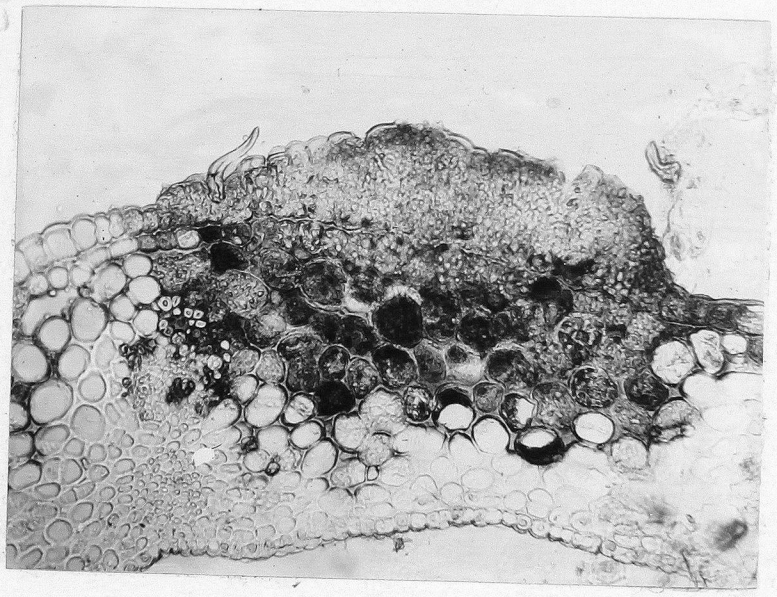

Fig. 1.

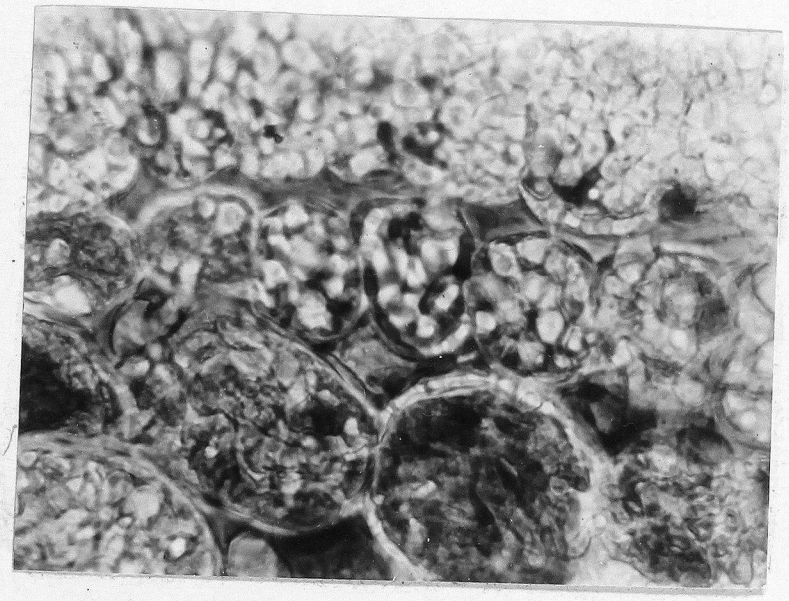

Fig. 2.

PLATE VIII. Figs. 1 and 2. Photomicrographs of young petal infections showing hyphal bodies in epidermal cells. X 500.

PLATE VIII

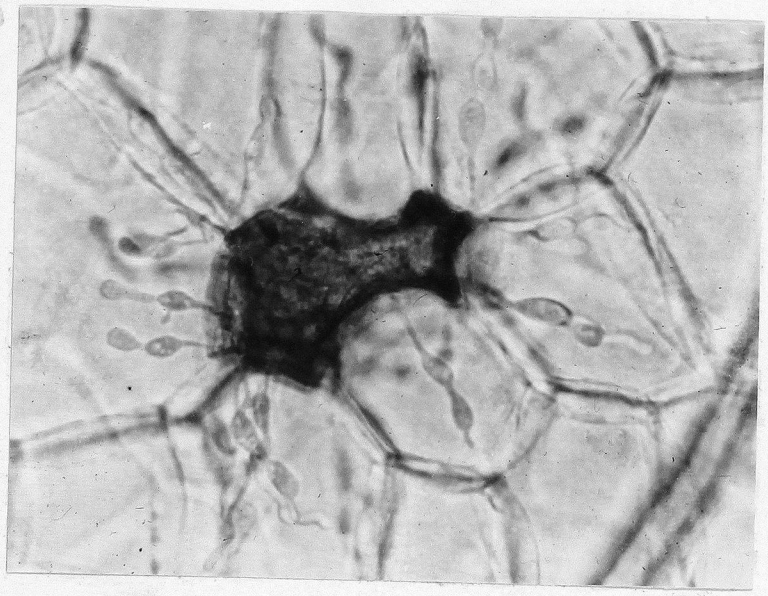

Fig. 1.

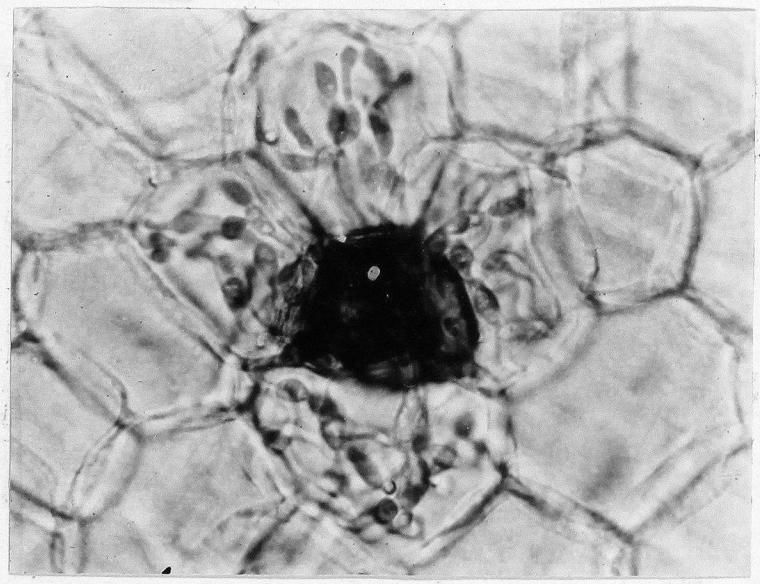

Fig. 2.

PLATE IX. Diagram showing the various cycles of *M. daphnes* on *D. mezereum.*

PLATE IX.

DIAGRAMMATIC YEARLY CYCLE
of
Marssonina daphnes on Daphne mezereum.

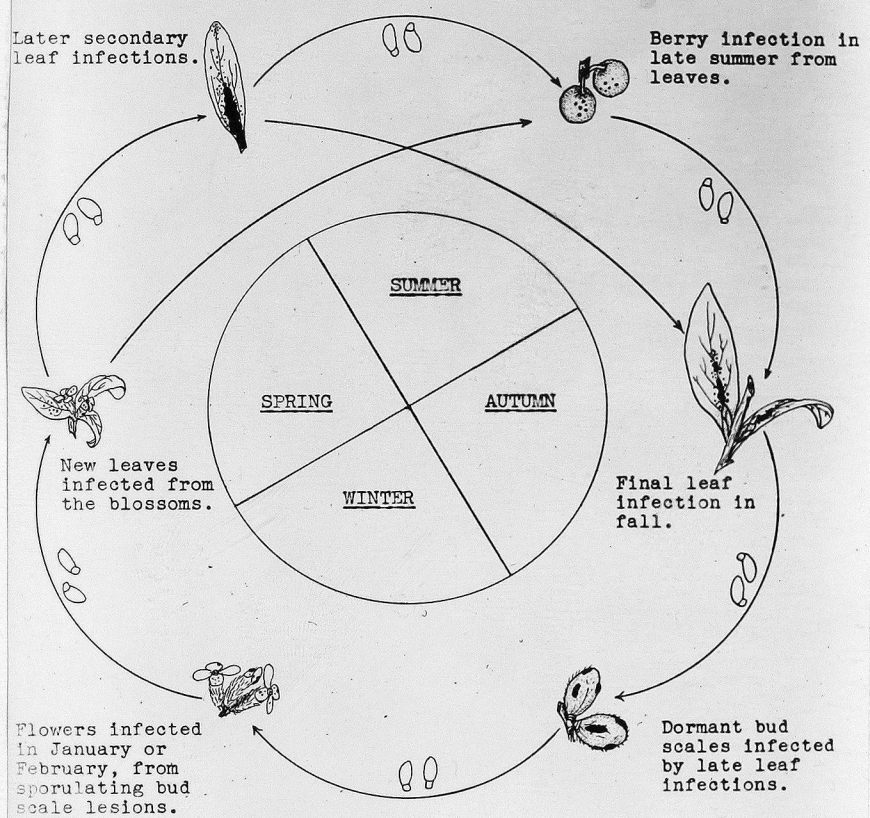

PLATE X. A spray of daphne bloom, illustrating the dense clusters of flowers in close proximity to the new leaf whorls.

Copies from Nutthall's "Beautiful Flowering Shrubs."

PLATE X

PLATE XI. Photographs of excised leaf cultures, showing the method used to support the leaves.

Fig. 1. A Petri dish culture.

Fig. 2. A tray culture, showing minute lesions of M. daphnes resulting from artificial inoculation.

Photographed by Dr. Dickson.

PLATE XI

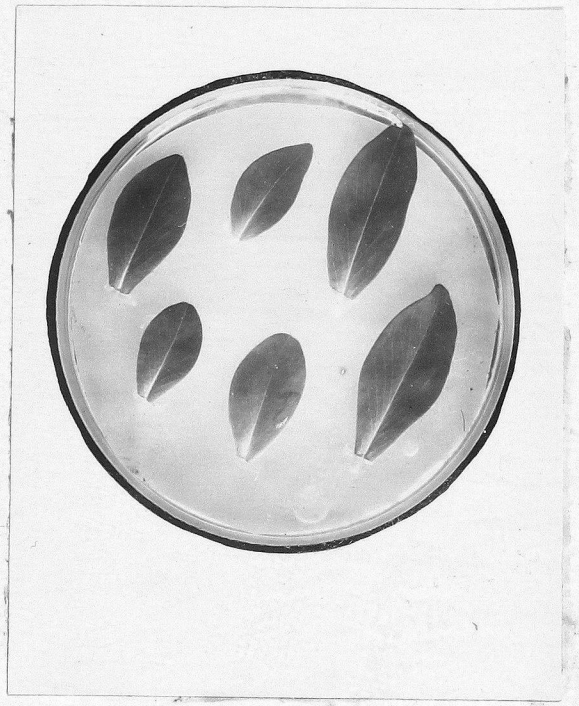

Fig. 1.

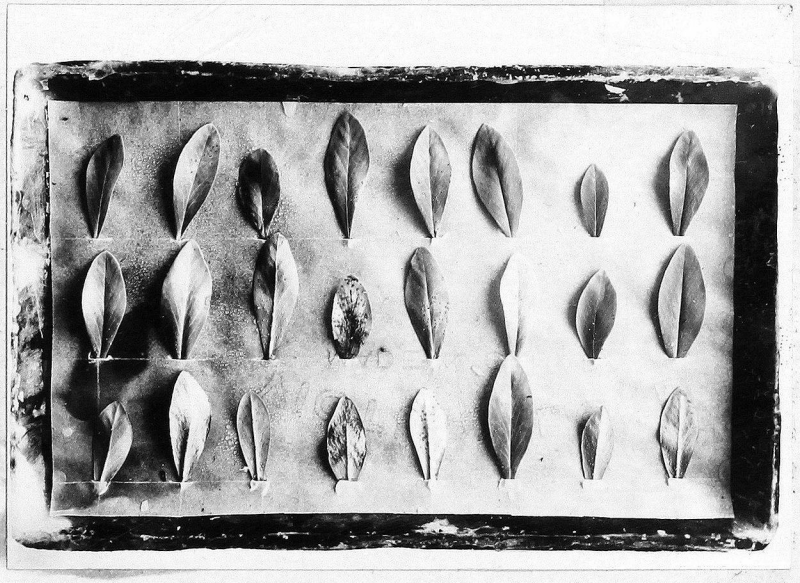

Fig. 2.