Thesis For M. S.

Apple Tree Anthracnose

By A. B. Cordley.

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Some Observations

on

Apple Tree Anthracnose.

For several years past the apple orchards of the Pacific Northwest, including western Oregon, Washington and British Columbia, have suffered seriously from the attacks of a fungous disease which has been known locally as "canker," "dead spot," or "black spot."

Although of considerable economic importance,, the disease seems to have been entirely overlooked by mycologists and nothing, of importance, concerning its nature has been recorded. When it was announced by Mr. Paddock, of the New York Experiment Station at Geneva, that Sphaeropsis malorum, the cause of the well known "black rot" of the apple and quince, is also the cause of a bark disease of apple trees, it was hoped that his discoveries would explain the cause of the similar western disease, but only a cursory examination was needed to show that this is not the case. Recently I have had, with Mr. Paddock, the privilege of comparing the two

diseases with the result that we were both convinced that they are entirely distinct.

In deciding to ignore the term canker--the most commonly used of the local names which have been applied to the disease, and in proposing for it the name of Apple Tree Anthracnose, I hope to avoid confusion in the designation of the disease in the future. The term, canker, is most commonly used in European works on plant diseases to designate injuries to the bark caused by the various species of Nectria; and in the eastern United States it has been applied by Mr. Paddock to a somewhat similar disease of apple bark caused by Sphaeropsis malorum Peck. The term Anthracnose, while it has perhaps no definite botanical significance, seems appropriate from the fact that the fungus which causes it and for which we here propose the name Gloesporium malicorticis is closely related to numerous other fungi of economic importance which have quite generally been designated as Anthracnoses.

Apple Tree Anthracnose attacks principally the smaller branches--those under two or three inches in diameter--although it also occurs upon the larger ones and upon the trunks of young trees. It ap-

pears first in fall, soon after the fall rains begin, as small, irregular, brown, sometimes slightly depressed areas of the bark. During the fall and winter months, it spreads but slowly, but with the advent of warmer weather in spring, growth takes place rapidly until under favorable conditions the disease may invade an area several inches in diameter. Such areas under observation at Corvallis, Or. the past season ceased to enlarge late in May, and early in June the first evidence of spore formation was seen. At that time the diseased areas were dark brown in color, markedly depressed, and in most instances limited by ragged, irregular fissures which separated the dead from the surrounding living tissues. (see fig. 1.) These dead spots vary in size from those not more than one-half inch in diameter to extensive areas two or three inches wide by six or eight inches long. Occasionally a single area completely girdles a branch thus killing at once its distal portion; but more commonly only a dead spot occurs, from which in the course of a few months the bark sloughs off leaving an ugly wound which requires several years to heal.

When these wounds are at all numerous the branches are exceedingly rough and disfigured and are moreover greatly weakened.

Early in June the first acervuli were observed. They appeared as small conical elevations of the epidermis and were scattered irregularly over the diseased area. By the end of June these elevations had increased considerably in size and in a few instances the overlying epidermis had been ruptured so as to expose the cream colored conidial mass. Material collected at that time and taken by me to Cornell University, where it was examined about the middle of July, revealed the presence of a few conidia none of which, however, could be induced to germinate. In material which was collected in July but which was not examined until early in October the conidia were more abundant but in dilution cultures in potato agar only two spores were observed to germinate. However, material which was collected at Corvalles, October 4, and which reached me a week later, had developed numerous conidia which germinated readily both in water

and in nutrient agar cultures. It would appear, therefore, that although evidences of the formation of acervuli may be noted early in June, mature conidia are not present in quantity before August or September.

Sections through a mature acervulus (see fig. 2.) show a subepidermal stroma from which arise comparatively long, closely compacted basidia on which the elliptical curved conidia are borne. As growth proceeds the overlying epidermis is ruptured and the mature conidia are set free. A true pycnidium is not developed. When first exposed the conidial mass is a delicate creamy tint but with age the outer surface becomes dark colored or even black. The conidia (see fig. 3.) are continuous, hyaline or with a greenish tinge, elliptical, curved, coarsely granular and measure 5-7 x 16-28μ. Average about 6-x 27μ.

Late in July dilution cultures were made in neutral and in acid potato agar from material that had been collected the last of June. In these cultures not a single colony developed. October 4 similar cultures were made from material which was

collected about the middle of July. In this but two conidia could be found that had germinated. So few conidia had developed that it was difficult to obtain enough for satisfactory cultures without obtaining an extensive variety of contaminating growths. To obviate this difficulty, October 6 spores from a single acervulus were carefully removed with a flamed scalpel, teased out in a drop of sterilized water and transferred with a sterilized brush to marked places on plates of acid potato agar. These plates were examined daily and although numerous spores were seen, none were observed to germinate until October 10 when two, which had made a feeble growth, were transferred to tubes of sterilized bean stems. At the time the general failure of the spores to germinate was thought to be because they had lost their vitality through having been kept too long in the laboratory; but it now appears to have been because the conidia used were not mature, since spores from material collected October 4, have continued to germanate readily up to the middle of November.

In cell and in petrie dish cultures in potato agar, the conidia germinate readily in about twelve hours at a temperature of 22°c. At 29°, the germination is retarded indefinitely, although spores in cell cultures which had been kept at this temperature in the thermostat for 24 and 48 hours, germinated as usual when removed to the lower temperature. A germ tube is developed at one end of the conidium (see fig. 4) and the protoplasm begins to flow into it. Soon another tube pushes out, usually from the opposite end of the spore, and this is followed by others until from two to five tubes have been produced. In nearly all instances there is a slight bulbous enlargement at the origin of each tube much as is Gloesporium nervisequum #, but less marked.

Growth is comparatively slow, and at 24 hours from the time of sowing, the germ tubes are rarely more than twice the length of the spore. Even at this early stage, however, the production of second-

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Stoneman. A Comparative Study of the Development of Some Anthracnoses.

ary conidia has begun (see figs. 5 and 6). These are generally produced acrogenously from short lateral outgrowths of the germ-tubes or from the conidium much as described for Gloesporium fructigena [1] and for Colletotrichum gossypii.[2] Numerous instances have been observed, however, in which no such outgrowths could be seen, the secondary conidia having every appearance of being given off directly from the germ tube or even from the conidium itself. They are at first hyaline, later with a greenish tinge, granular, elliptical, rarely slightly curved, and always so far as observed decidedly smaller than the original conidium, although varying greatly with the character of the food supply. The most abundant production of these secondary spores is in the immediate vicinity of the conidium so that there is a tendency to produce an acervulus.

In three or four days the stellate colonies become visible to the unaided eye. They are cir-

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[1] Ibid. Bot. Gaz. xxvl, 12 pl. 1898.
[2] Atkinson, G. F. Anthracnose of Cotton. Jour. Myc. vol. 173-178, 1891.

cular in shape, with a slight greyish tinge, elevated and somewhat darker in the center where the production of secondary conidia is most abundant. In crowded cultures they rarely become more than 2-3 mm. in diameter but under more favorable conditions may attain a diameter of 4-8 mm. In its earlier stages, the mycelium which radiates quite uniformly in all directions from the center, is sparingly septate and without vacuoles. In the older colonies it becomes vacuolated and has been observed to break up into chains of irregular thick-walled dark colored cells.

In order to check the results, cultures on bean stems were obtained in various ways and at different times. October 10 two colonies which were supposed to have developed from the Gloesporium spores were transferred from petrie dish cultures to tubes of sterilized acid bean stems. October 12 the conidia from a single acervulus were teased out in sterilized water and with the tip of a sterilized needle a very few spores were transferred to each of several tubes of bean stems.

October 17 six more colonies were transferred from plate cultures to tubes of bean stems and four days later four more tubes were inoculated by transferring colonies which had been grown in cell cultures. On the 17th a dilution culture was made in acid potato agar. On the 18th after the spores had germinated a number of them were carefully marked and on the 21st when the colonies had become visible to the unaided eye six of them were carefully transferred to tubes of acid bean stems and three to tubes which had not been made acid. Oct.25th a similar dilution culture was made in which the following day a number of colonies were carefully marked. These plates were then allowed to stand until Nov. 14th in order that any foreign growths which might be present should have an opportunity to develope, when eight of the marked colonies which still remained entirely distinct from all other growths were transferred to acid bean stems.

The first growth to appear on bean stems is invariably the production of a few scattered comparatively thick, more or less branched stromal growths which arise above the substratum at the

point of infection. These shortly become covered with a growth of floculent white mycelium and from this center the entire surface of the stem and of the liquid becomes covered with a dense gelatinous looking stroma which on the surface of the stem is covered with the floculent white mycelium. (See Fig. VII

In the course of ten days or two weeks there is usually an abundant production of sori, which, seen by transmitted light, are of an olive green color but which give to the surface of the stroma a glistening black appearance when viewed by reflected light. In the older cultures the stroma has invariably become of a deep salmon color, the cause of which has as yet not been determined. We have also observed slight elevations of the stroma which when sectioned suggested the development of an acervulus although in no such instances have conidia been observed. In a few instances we have also observed on the surface of the stroma small spherical, olive green, perethecia like bodies, covered with a scant mycelial growth, which possibly presage the development of an ascigerous

form. In no instance, however, have asci as yet been found in these bodies.

To determine whether the fungus studied is the cause of the disease under consideration, on Oct. 30th, thirty-six inoculations were made on sections of apple limbs. Twelve sections each from 3/4 to 1 1/2 inches in diameter and 4-6 inches long were selected and divided into groups of four sections each. One of these groups was thoroughly washed in a solution of mercuric chloride, 1-10000, after which it was thoroughly rinsed in water. The other two groups were left untreated. The sections in each group were numbered 1, 2, 3, 4, and upon each section three inoculations were made. On sections 1 and 3 in each group the inoculations were made by scraping up the epidermis with a flamed scalpel and applying directly to the exposed cortex and cambium small portions of bean stem cultures bearing an abundant mycelial growth and conidia. In inoculating sections 2-4 care was taken to select portions on which the bark was uninjured and to make the applications without in the slight-

est abraiding the epidermis. The sections when prepared were placed in fruit jars containing moist sand, and which had been thoroughly sterilized by steam heat. In about a week after the inoculations were made slightly discolored areas were observed about several of the points of infection and by Nov. 20th, three weeks from the time they were made, these areas had developed all the characteristics of the disease as seen in nature; being brown distinctly depressed and separated from the surrounding healthy portions by a zone of hypertrophied tissues which are marked by numerous rugged fissures in the epidermis through which the underlying chlorophyll bearing tissues may be seen. (Sm. Fig. VIII) The peculiar appearance of this zone attracted the attention of Professor Atkinson who, on examination found it to be an oedematous condition of the tissues produced no doubt by the excessive supply of moisture in the jars and by the stimulating effect of the fungus. Portions of these tissues were selected for a more careful histological study but

they were unfortunately lost in an accident and we can only refer for a consideration of oedematous tissues to an excellent article by Professor Atkinson on the Oedema of the Tomato.#

It was hoped that, by applying some of the spores to wounded tissues while care was taken to apply others to uninjured areas of the cuticle, some light might be thrown upon the manner in which the fungus first gains entrance to the cortical tissues; but owing to the fact that no results were obtained from any of the sections treated with mercuric chloride, the test was unsatisfactory.

At the beginning it was realized that such inoculations would not offer absolute proof of the parasitic nature of the fungus since it is of course possible that sections of limbs may offer less resistance to the fungus than they would had they not been removed from the tree. But the fact that these sections were taken from the tree in fall and were inoculated immediately, before any

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Atkinson, G. F. Oedema of the Tomato. Bull. Cornell Univ. Expt. Sta. p.75-108. Pl.8. 1893.

great change in the tissues could have taken place, together with the fact that the inoculations were followed by such a virulent attack of the disease, seem to offer at least a very strong probability of its parasitic nature.

Before any experiments in controlling the disease could be intelligently undertaken, it was necessary to know something of its nature. Having shown that it is caused by a certain fungus, the question of most interest is, can it be controlled? And, if so, how? My absence from the state, while studying the fungus itself, necessarily prevented me from conducting any experiments in controlling it, but from what I now know of the disease I believe that I may safely assert that it can be controlled. We have seen that the spores are developed and probably distributed during the late summer and fall months and that they undoubtedly germinate after the fall rains begin. It is also known that bordeaux mixture and other copper compounds prevent the germination of the spores of most fungi. We therefore infer that if the trees be thoroughly

sprayed with bordeaux mixture or with the ammoniacal solution of copper carbonate, once soon after the fall rains begin and again as soon after the leaves fall as possible, the germination of the spores will be largely prevented and the spread of the disease be thereby checked. It is not expected that such a process will exterminate the disease, but it is believed that it will so reduce its ravages that it can no longer be considered a menace to the apple growing industry. For the latter of the two applications mentioned above bordeaux mixture, winter strength, should be used. For the former bordeaux, summer strength, may also be used, but if fruit is on the trees it would be better to use the ammoniacal solution of copper carbonate. Whichever spray is used should be thoroughly applied and applied as soon as possible after the fall rains begin. The fungus cannot be destroyed by sprays after it has once entered the tissues of its host.

In addition to the sprayings recommended, we

should advise owners of young orchards but little diseased, to carefully cut out and paint over with strong bordeaux all anthracnosed spots that may be observed. As stated in a preceding paragraph, it is possible that the mycelium of the fungus in the dead area of bark, after resting through the summer, may be stimulated to renewed activity by the fall rains and thus itself be an additional means of propagating the disease. Should this be the case, which we are at present inclined to doubt, spraying will not be entirely efficient in preventing the spread of the disease. For the present at least, or until the above supposition can be proved or disproved, it will be advisable to supplement the sprayings by using the knife wherever practicable. Old, badly diseased orchards can best be renovated by pruning severely and spraying thoroughly.

The fungus which appears never to have been described may be characterized as follows:

Gloesporium malicorticis n. sp.

Parasitic in the cortex of branches of Pyrus malus. Affected areas dark brown, limited by ragged irregular fissures, sometimes 2-3 x 6-8 inches, occasionally girdling the branch. Acervuli scattered, triangular, rupturing the epidermis, 300-800µ in diameter. No pycnidia. Conidial mass at first cream colored, later darker. Conidia borne on upright basidia which arise from a sub-epidermal stroma. Conidia continuous, coarsely granular, at first hyaline, later with greenish tinge, elliptical, curved, 5-7 x 16-28µ. Average 6x24µ. Those grown in cultures smaller and rarely curved.

Fig. I

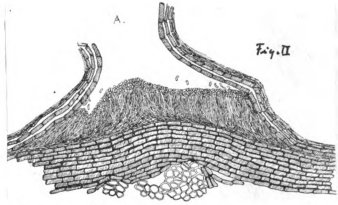

Fig. II

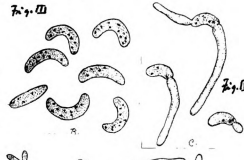

Fig. III

Fig. IV

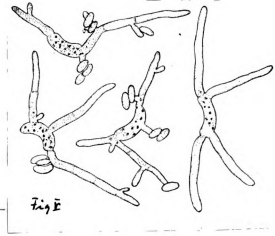

Fig. V