A BACTERIAL LEAFSPOT OF TOBACCO

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INTRODUCTION

During the past five years a bacterial leafspot of tobacco occurring in Wisconsin has been the subject of investigation together with observations on tobacco leafspots in general in various tobacco districts of the United States. The tobacco leaf is subject to an exceptionally large number of diseases, originating from a variety of causes, in some cases with distinctive and constant symptoms, but more often confusing when any determination or classification based on symptoms is attempted. This confusion is in some respects magnified by the fragmentary early literature and is due largely to common names having been applied without adequate consideration as to the causal agents of the diseases. Recently two or three leaf diseases have been described, however, in sufficient detail to permit of definite reference. It is the purpose of this paper to contribute to the knowledge of one other such disease.

CAUSE

This disease, which is not believed to be new to tobacco growers in Wisconsin, has been found to be due to a bacterial organism apparently previously undescribed. The causal organism has been named Bacterium melleum, n. sp., and a description of the organism and the symptoms of the disease it causes is given in this paper.

COMMON NAMES

The leafspot to be described here is ordinarily called "rust" by Wisconsin tobacco growers. This name, which is not a good one from a phytopathological standpoint, is in fact in use throughout most of the tobacco districts of the world, for a variety of leaf diseases of tobacco, and is, therefore, a very unreliable term. The term "rust" is frequently limited by the use of such combinations as "red," "brown," "white," and "black rust;" but these again are of little significance when applied by different individuals and have little definite relation to the causal agent concerned. Other terms such as "firing," "black fire," "field fire," "wildfire," "speck," and "frogeye" are terms which have been commonly used as synonymous with "rust," though the term "frogeye" is now generally limited to the disease caused by Cercospora nicotianae.

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El. and Ev. and more recently "wildfire" has become the accepted common name for the disease caused by *Bacterium tabacum* Wolf and Foster.

The leafspot diseases of tobacco naturally fall into three disease categories, as regards cause, namely, (1) those due to nonparasitic agents, (2) those due to fungi, and (3) those due to bacteria. It is probable that additional terms should not be added to the list of common names until a satisfactory basis of classification based on these categories is established. However, we have come to refer in the laboratory to the disease herein described as the "Wisconsin bacterial leafspot" disease, and this term may tentatively be preferable to the commonly used term "rust."

**OTHER BACTERIAL LEAFSPOTS**

Although it is only within recent years that certain leafspots of tobacco have been definitely shown to be of bacterial origin, it is fairly certain that one or more have existed from the earliest days of tobacco culture in this country. The earliest treatises on tobacco culture refer to "rust" and "firing," although in most cases it would be difficult to judge the nature of the causative agent from the descriptions of these diseases. Killebrew and Myrick, for instance, wrote as follows—

another field fire, called "black fire," which is totally different from the red field fire, is caused by excessive humidity and occurs only after continued rains of several days' duration with hot weather. This black fire is much more to be dreaded than the brown rust or red field fire, for it attacks the plants while immature, involving all the leaves. Sometimes the disease will spread over a field in two or three days and ruin the crop, making black deadened spots as large as a silver dollar, but this rarely happens.

This disease was undoubtedly parasitic in nature, especially in view of the fact that these experienced observers separated it from other symptoms probably of nonparasitic origin. It is also quite likely that this disease was one of the two tobacco leafspot diseases recently shown to be of bacterial origin in this country.

Similarly the disease described in this paper as "Wisconsin leafspot" has probably existed in Wisconsin for 50 years or more along with other leafspots under the name of "rust," but now referred to by some of the older growers as "old-fashioned rust," on account of the fact that it has not been as prevalent in recent years as in the earlier days of the industry in this State.

Apparently the first leafspot disease of tobacco attributed to bacteria was that of "la rouille blanche" (white rust) of France, ascribed by Delacroix in 1905 to *Bacillus maculicola*. The description of this disease is not sufficient to afford adequate comparison with our American leafspots, but in any case the description given indicates that it is different from the Wisconsin bacterial leafspot. Honing in 1914 described a rust occurring in Deli (Sumatra) which he showed to be due to a bacterial organism which he named *Bacterium pseudozoogloeae*. This disease was also known as "black rust," although it evidently was not the "wildfire" disease of America, since neither the description of the causal organism nor that of the symptoms of the disease correspond with that of wildfire. Honing's disease corresponds more closely to that of the Wisconsin leafspot, although, as will be shown by later comparison, they differ in several respects.

Reference is made by number (italic) to "Literature cited," p. 492-493.
Wolf and Foster (7) in 1917 described the wildfire disease as it occurred in North Carolina and Virginia, following an unusually severe outbreak and proved the causal organism to be a bacterium, which they named *Bacterium tabacum*. This disease apparently has since spread to most of the other tobacco districts in the United States, seemingly from the North Carolina epidemic of 1917 as a center of infection. This disease is not readily distinguished from the Wisconsin leafspot in general symptoms, although the chlorotic area around the point of infection is usually larger and more common than in the Wisconsin leafspot. The wildfire organism is also a much more vigorous parasite than the Wisconsin leafspot organism, and the disease may consequently be much more prevalent and serious where it occurs. Fromme and Murray (3) investigated a leafspot disease in Virginia, which had apparently also existed for a considerable time in that State, and found it to be due to a bacterium which they named *Bacterium angulatum*. This disease has been named by them "angular leafspot" on account of the angular shape of the lesions. It cannot be readily confused with other bacterial leafspots but may be difficult to distinguish at times from certain nonparasitic spots when judged by symptoms alone.

**DESCRIPTION OF THE DISEASE**

The Wisconsin bacterial leafspot has been found ordinarily on the lower leaves of the plants in the field. Usually it is most marked on the lowest leaves but has been observed during this investigation up to the middle leaves. In severe outbreaks, not seen since the beginning of this study but earlier noted and referred to by others as "old-fashioned rust," the leaves on the entire plant may be involved. That the disease may occur on young leaves is evidenced, however, by the observation of several infections in seed beds from which the causal organism has been isolated. The older leaves are seemingly more predisposed to a rapid collapse and death, and finally, browning of the tissue when once infected, but when artificially inoculated by needle punctures the top leaves show equal predisposition to infection, and the development of the chlorotic area surrounding the point of infection is even more marked than on lower leaves. The common occurrence of the disease on the lower leaves in the field is due quite likely to the more favorable environmental conditions offered there for infection and progress of the disease.

The disease in the seed beds ordinarily is inconspicuous and not as typical as in the field. The spots are usually small and more angular than in the field and the chlorotic area less distinct (Pl. 2, A). The old lesions are usually small and light-colored, but when they run together the young leaves present the appearance of being blighted.

In the field the young spots are usually round, frequently with a small central fleck surrounded by a distinct chlorotic area or halo, identical with that of the wildfire disease. Under other conditions this halo may not appear at all or may disappear rapidly, the tissue surrounding the point of infection collapsing and soon turning brown in color, in some cases possibly white. Enlarging spots may or may not be limited by the veins of the leaf. At times the infected area seems to pass over the vein without injury to it. At other times, however, the parasite may enter the vein and follow it, producing an elongated lesion. The diameter of the spots may vary from 1 mm. to 1 cm. or more, frequently coalescing, and hence involving large areas of the leaf. The old lesions
are usually distinctly brown in color, sometimes brownish white, frequently with a dark center giving a "birds-eye" appearance. Concentric rings, usually are not present, though apparently they may occur (Pl. 1, A, B).

An interesting symptom frequently evident in the greenhouse after artificial infection is the formation of a secondary ring of small lesions 2 or 3 mm. beyond the circumference of the primary lesion. This ring, often perfect in shape, seems to follow as a result of renewed activity by the parasite about the primary lesion following a checking of the disease. The chlorotic area surrounding the center of infection has been found to be relatively free of organisms, as was found by Miss Elliott (2) in the halo-blight of oats.

PREVALENCE OF THE DISEASE

The causal organism of the Wisconsin leafspot disease was first isolated in the spring of 1917. On account of the similarity of other leafspots, nothing conclusive can be said as to the prevalence of the disease prior to that time, although the writer feels confident that within 20 years of casual observation previous to 1917 he has seen a number of more serious cases of the disease than have been noted since. This belief is strengthened by the testimony of a number of the older tobacco growers in the State, who recall complete losses of portions of crops from "rust," which, from our subsequent observations on nonparasitic leafspots, are not believed to develop to such an extent on the type of tobacco grown in this State, with the possible exception of the "rust" following mosaic. "Rust" following as a result of mosaic is not, however, ordinarily limited to such an extent by the topography of the land and the opportunity for infection as is the bacterial leafspot. Since 1917 a number of mild occurrences of the disease have been seen within a 25-mile radius of Madison, and in most cases have been identified by isolation of the causal organism and inoculation experiments. Search has been made for this disease in a number of other tobacco districts, mostly in Kentucky, Maryland, Pennsylvania, and in the Connecticut Valley. Only one specimen has been collected which can with certainty be said to be the same disease, and this was from Kentucky in 1919. One collection from Connecticut in July, 1919, proved to be the "wildfire" disease, and was the first record of that disease in the Connecticut Valley. Similarly, collections made in 1920 from Maryland, Kentucky, and Ohio proved to be wildfire.

ISOLATIONS

The first isolation of the Wisconsin leafspot organism was made in June, 1917, from the seed beds at the experiment station at Madison. At about the same time specimens of a leafspot on tobacco seedlings (wildfire) were received from Mr. E. G. Moss, in charge of the branch tobacco station, Oxford, N. C. This disease was at first thought to be due to a fungus, and preliminary isolation and infection experiments were conducted from this standpoint with negative results. Bacteria were soon after isolated and infection secured. Word was then received that Dr. Frederick A. Wolf, of the North Carolina Station who was working on the same disease, had established the bacterial relationship, no doubt a few days before our own conclusion had been reached. A few weeks later the writer visited the North Carolina section and had the opportunity of noting a second serious outbreak of the disease in that section.
On his return to Wisconsin similar leaf spots were noted, among them certain spots on a row of a southern type of tobacco in the experimental plots at Madison, though not occurring nearly as seriously as in the fields seen in North Carolina. The records show that two of the isolations from this row gave white organisms, one of which was infectious. Unfortunately this culture died before a detailed study could be made of it, so that we are not at all certain that it was the wildfire organism. The writer's earlier isolation from the seed bed leafspots and later isolations from the field yielded, however, only yellow infectious organisms. No white organism has since been isolated except following known cases of inoculation with the wildfire organism. It is felt that this explanation should be made here in view of a statement made by Wolf and Foster (7) as a result of correspondence, to the effect that wildfire occurred in Wisconsin in 1917. While the "similar spot" referred to has developed to be what we now call the "Wisconsin leafspot," there is still some probability that we did have one case of wildfire on a row of southern tobacco in 1917, and if so, it seems likely that it was the result of seed-borne infection. In any case wildfire can not be said to have occurred in Wisconsin in 1917 in the sense that it has since been reported from other States, nor was it introduced in that manner until 1922.

During the last five years a large number of isolation tests have been made from various sorts of leafspots of tobacco. Wherever fairly fresh and young spots of the wildfire disease or the Wisconsin leafspot have been plated out, no difficulty has been encountered in getting pure cultures at once, so that the distinction between these two diseases has been readily established. The method employed has been simply to select a young lesion, cut it out with a scalpel, and rinse it through 8 to 10 sterile water blanks with vigorous shaking. It was then transferred to a tube of bouillon, mashed with a sterile scalpel or rod on the side of the tube, rinsed into the bouillon and allowed to stand 15 to 30 minutes. One to six loopfuls of the bouillon were then transferred to melted potato agar at about 45° C. and plates poured. Cultures were usually kept in stock on potato-dextrose agar in the ice box. Twelve different isolations of the Wisconsin leafspot organisms have been made over a period of five years. Practically all the cultural character studies were made with one organism (culture No. 141), and where this culture was not used we have made sure that we have used a pathogenic organism corresponding in ordinary cultural characteristics.

INOCULATION EXPERIMENT

A very considerable number of inoculation experiments have been made in connection with demonstrating the pathogenicity of the organism isolated, testing the strains following growth in culture for different periods, and for comparison with other bacterial leafspots of tobacco, particularly wildfire. Repeated trials have also been made comparing inoculation by spraying and by needle puncture, under very variable environmental conditions. It has been found that, while good infection is always secured on wounded leaves with a virulent strain of the Wisconsin leafspot organism, practically no infection at all has ever been secured by simply spraying the plants with a suspension of the organism in water.

The writer can not state with certainty the relation of normal field infection to wounded tissue. He has not been able to find from observa-
tion that wounding by insects or other means has played any part in infection. It seemed rather that infection in the field was dependent upon the occurrence of favorable environmental conditions. Every attempt to duplicate such conditions experimentally has thus far given negative results. The writer has as yet, however, done nothing as regards the intrinsic predisposition of the plant itself to infection, and it is not improbable that the host grown under different conditions as regards chemical and physical relationships may be considerably altered thereby as regards predisposition. This belief is strengthened by the fact that similar experience has been had with frequent attempts at securing good infection with the wildfire organism by spraying under greenhouse conditions, while with this organism we know that under other conditions good infection may be secured in this manner.

The method of inoculation by wounding has been essentially that of puncturing the leaf with a needle point which has been dipped in a suspension of the organism in water and permitting a small droplet of the watery suspension to cover the wound. In this manner, when a virulent strain of Wisconsin leafspot organism is used, infection is secured in two to five days, and symptoms develop which compare favorably with those from *Bacterium tabacum* in size of chlorotic area or lesions obtained (Pl. 2, B). On the other hand, it is certain that under field conditions the Wisconsin leafspot organism is not as virulent as the wildfire organism, and that the former can be pathogenic only under more limited conditions than is the latter. Under field conditions the chlorotic area or halo formed by the Wisconsin leafspot organism is not normally as marked as that of wildfire (Pl. 1, A) and frequently may not occur at all (Pl. 3, A) on older leaves, where conditions are seemingly more favorable for a rapid collapse of the leaf tissue than is the case on the younger leaves.

Considerable uncertainty has been experienced throughout the progress of this investigation as to the continued pathogenicity of the organisms carried in culture on potato-dextrose agar, and as a result frequent inoculations have been made to test this point with various cultures. In large measure, the same has been true of the wildfire organisms carried along simultaneously.

A large number of subcultures have died, lost their pathogenicity completely, or in considerable part (Pl. 2, B).

This has been due apparently in most cases to an unfavorable cultural medium, although in many cases this occurred on potato-dextrose agar made according to the same formula as other batches in which organisms have been kept alive and virulent through transfers kept in the refrigerator for three years or more.

A limited number of inoculations have been made upon other host plants aside from ordinary tobacco (*Nicotiana tabacum*). Infection has been secured upon various other species of Nicotiana, especially *N. glauca* and *N. rustica*, and also upon the tomato, together with some indication of infection upon certain cereals.

**CULTURAL CHARACTERS**

**MORPHOLOGY.**—The organism is a short motile rod with rounded ends, occurring singly, in pairs, or occasionally in short chains. Measurements under various conditions have ranged from 0.5 to 0.8 microns in width by 1 to 2.4 microns in length, averaging about 0.6 by 1.8.
Stained by the Caesar-Gil and modified Pittfield methods for flagella from 24-hour-old cultures, the organism shows from one to several polar flagella, usually two or three, but as many as seven have been counted. The flagella are ordinarily from three to five times as long as the organism itself. The organism has been stained with carbol fuchsin, methylene blue, and anilin gentian violet. No spores or involu-

POTATO-DEXTROSE AGAR.—Most of the cultural work has been done on potato-dextrose agar, as this had been found to be most useful for rapid comparative purposes on account of the color imparted to the agar. Colonies in agar plates are first visible in about 36 hours at about 22° to 26° C., increasing to 3 to 5 mm. in diameter in six days. Colonies are at first grayish white, changing on most media on about the third day to a distinct yellow, after which a transparent light yellow tinge develops in the potato agar. The colonies are round, shining, convex, and yellow with opaque centers. The submerged colonies are lenticular. On agar slants a distinct growth may appear along the line of inoculation in 24 hours, and this broadens gradually at the base, becoming echinulate (Pl. 4 A). The growth becomes fairly heavy in 3 to 5 days but rarely covers the slant, usually developing a fairly deep orange color. Certain potato-dextrose agar media will color up in a few days, but ordinarily more gradual coloration occurs, the media becoming usually a bright honey yellow, which may extend to a depth of 2 inches or more from the growth down into the tube. Where the pigment is rapidly absorbed by the agar, the growth does not take on a deep color. Most of the yellow bacterial plant pathogens known to science have been cultivated simultaneously, but with none has this characteristic of yellowing potato-dextrose agar been nearly as distinct, and with the majority seemingly it does not occur. The pigment on potato-dextrose agar is soluble in water, sulphurous acid, ammonia, methyl and ethyl alcohols, and hydrogen peroxid. It is apparently destroyed by hydrochloric acid, toluene, xylol, benzoI, and carbon disulphid. Its production is slowed up mark-

GELATIN.—Gelatin is rapidly liquefied in thickly sown plates, usually within 48 hours. In gelatin stabs liquefaction usually begins in 48 hours (assumes a stratiform shape), and may require two to three weeks for completion.

NUTRIENT BROTH.—Decided clouding occurs in 24 hours in beef-

POTATO CYLINDERS.—Good growth in 48 hours, of brownish yellow color. Growth is profuse in 5 days, with increasing yellowing of organism along line of streak and darkening of medium. Tests with iodin indicate marked conversion of starch to amylodextrin, but diastatic action on the whole is feeble as compared with Bacterium campestre.
LITMUS MILK.—The color becomes more intense on the second day, with the formation of a thin blue layer in the upper portion of the medium which disappears about the third day. After 4 days the casein is precipitated, and in 6 more days the clearing has proceeded two-thirds of the way down the tube. The liquid is dark green on top and shades down to a tan just above the casein. The medium on long standing finally becomes a deep blue-green.

COHN’S SOLUTION.—Marked clouding occurs in 2 days or less, followed in about 4 days by the appearance of a heavy white crystalline membrane on the surface and a faint greenish tinge below it. On long standing the medium becomes light yellow in color and contains a flocculent precipitate.

FERMI’S SOLUTION.—Clouding becomes very marked after 2 days. After about 8 days the medium takes on a light greenish tinge, but this is not as marked as with Bacterium tabacum. After about 10 days or more a fairly heavy membrane is formed and the sediment increases. On longer standing the medium turns to an intense honey-yellow color.

BEEF AGAR STROKE.—On beef agar slants growth is distinct in 24 hours, grayish white in color, turning finally to a deeper yellow. Growth less profuse than on potato agar and no coloring of medium evident.

LOEFFLER’S BLOOD SERUM.—Growth grayish yellow, spreading, resulting in gradual liquefaction of medium.

NITRATE IN NITRATE BROTH.—There was no reduction of nitrates in nitrate peptone broth.

INDOL, AMMONIA, AND HYDROGEN SULPHID.—Negative tests for all by usual methods.

FERMENTATION TESTS.—From a 2 per cent Difco peptone solution five different carbon media were made by adding 1 per cent of the following: Saccharose, dextrose, lactose, glycerine, and dextrin. In fermentation tubes no gas was formed with any of these compounds. Distinct clouding appeared in the open arms in 48 hours. In the case of saccharose and dextrose slow clouding also appeared in the closed arms. No acid was produced in any of the tubes.

LITMUS SUGAR AGARS.—Tests with lactose, glycerine, saccharose, dextrose, dextrin, agars showed no formation of acid in any case.

TOLERATION OF ACIDS AND SODIUM CHLORID.—No growth was obtained in tubes of neutral beef-peptone broth to which 0.3 per cent of malic, citric, or tartaric acid had been added (pH values 3.6 to 4.0). A concentration of 0.2 per cent of malic and tartaric limited growth but 0.2 citric did not. Two to 3 per cent sodium chlorid limited growth markedly and 4 per cent inhibited growth entirely.

OPTIMUM REACTION AND TOLERATION LIMITS.—The best growth in beef-peptone broth was secured at + 10 to + 15 Fuller’s scale. The maximum for growth lies apparently close to + 20, + 22 giving no growth. While good growth was secured in some instances as low as —20 it is not believed that these results are significant, since the broth after adjustment and standing for some time usually rose to —4 or higher.

TEMPERATURE RELATIONS.—The optimum for growth in culture lies close to 26° to 28° C. No growth was secured at 35° to 36° or below 7° to 9°. The thermal death point found by subjecting freshly inoculated tubes of bouillon to different temperatures for 10 minutes was found to be about 57°.
RESISTANCE TO DESICCATION.—The organism dried on cover slips in sterile Petri dishes did not lose its power of growth until after 14 days.

RELATION TO OXYGEN.—No growth in atmosphere freed from oxygen by pyrogallol-KOH method. Some growth in closed arm of fermentation tubes with saccarose and dextrose.

EFFECT OF SUNLIGHT.—Fifteen minutes' exposure of plates on ice to sunlight killed a few organisms, and practically all were killed on 30 to 60 minutes' exposure.

VITALITY AND VIRULENCE.—The organism can be kept in culture and maintain its virulence for at least three years. It may lose its virulence, however, upon certain media while still giving normal growth in culture or it may die out rapidly on presumably favorable cultural media for reasons not definitely understood.

GROUP NUMBER.—221.3333633. The name *Bacterium melleum*, n. sp., is suggested for this organism, basing the specific name on the honeylike color imparted to potato dextrose agar.

TECHNICAL DESCRIPTION

*Bacterium melleum*, n. sp.

A short rod with rounded ends, occurring singly, in pairs, or in chains. Average size 0.6 by 1.8 microns. Motile by a tuft of polar flagella usually two to three in number, but ranging from one to seven, and three to five times as long as the body of the organism. No spores or involution forms have been observed. Capsules are present. It is Gram-negative and not acid-fast. The organism is pale or orange yellow on most media, particularly on potato-dextrose agar, to which it imparts a honeylike pigment. Growth on potato agar stroke is abundant, echinulate, raised, glistening, smooth, and viscid; agar colonies grow rapidly, are circular, smooth, and convex. On nutrient broth the surface growth is slight or none, cloudy strong, and sediment somewhat flaky, more amorphous, and moderate in amount. In gelatin stabs growth is best at top with liquefaction, beginning in 3 days and complete in about 20 days. The coagulation of milk is prompt, coagulum slowly peptonized. Alkaline reaction with litmus milk, with prompt reduction. Good growth in Fermi's and Cohn's solution. No indol or ammonia produced. Nitrate in nitrate broth not reduced. Optimum temperature for growth about 26° to 28° C., maximum 35° to 36°. Thermal death point 57°. Optimum reaction for growth in broth +10 Fuller's scale. Pathogenic on *Nicotiana tabacum*, causing a leafspot with or without a chlorotic halo around the point of infection, usually followed by browning of affected tissue.

COMPARISON WITH OTHER BACTERIAL LEAFSPOTS OF TOBACCO

It is evident from the description of the causal organism that the Wisconsin leafspot differs from that of the previously described American tobacco leafspots, namely, wildfire and angular leafspot. The most striking difference is that of the color of the pathogenes, which is white in both of the latter diseases but yellow in the Wisconsin leafspot. As far as symptoms themselves are concerned, one could not with certainty distinguish between wildfire and Wisconsin leafspot, though the former is the more destructive and widespread and under field conditions usually possesses the most marked chlorotic halo.

The Wisconsin leafspot disease is not so readily distinguishable from the Sumatran disease described as black rust by Honing (4). Neither specimens of this disease nor the causal organism have been seen. Therefore Honing's description offers the only basis for comparison.

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2 According to the classification of the American Society of Bacteriologists the combination would be *Pseudomonas mellea* n. sp.
The illustration of black rust by Honing shows some resemblance to Wisconsin leafspot, but the disease does not seem to be identical with the latter. The chief points of difference between the two causal organisms may be summarized as follows:

**Bacterium pseudozoogloeae** (Honing)

1. Produces "black rust."
2. Apparently no chlorotic halo.
3. Produces lesions with concentric rings.
4. Size generally 1.5 microns by 0.7 to 1 micron.
5. 1 to 2 polar flagella.
6. Color usually yellowish gray.
7. Gelatin stab papillate; liquefaction napiform to saccate.
8. Milk coagulum not peptonized.
9. Litmus milk rendered acid.
10. Acid with dextrose, lactose, and saccharose broth.
11. Fluorescence yellowish green (in gelatin).

**Bacterium melleum, n. sp.**

1. Produces "brown rust."
2. Chlorotic halo frequently present.
3. Lesions usually not concentrically ringed.
4. Generally 1.8 microns by 0.6 micron.
5. 1 to 7 polar flagella.
6. Color usually orange-yellow.
7. Gelatin stab filiform; liquefaction stratiform.
8. Milk coagulum peptonized.
9. Litmus milk rendered alkaline.
10. No acid with dextrose, lactose, and saccharose broth.
11. Fluorescence honey-yellow on potato-dextrose agar.

From this comparison it may be seen that the differences are decided in many instances and that the likelihood of the Sumatran and American leafspot being identical is very remote.

**PRACTICAL CONSIDERATIONS**

The Wisconsin bacterial leafspot or "rust" no doubt occurs annually in this State, or at least it has been found for the last five years without much difficulty, although not to such an extent as to cause much concern. As already stated, however, it is quite certain that in years past it has been the cause of considerable losses and the object of demand for control measures. This belief is strengthened by the writer's recent studies of nonparasitic spotting of tobacco, which might otherwise have been confused with the bacterial leafrust. The causal organism is not believed to be a vigorous parasite, and special conditions are necessary for infection without wounding. Aside from a period of rainy or humid weather, and possibly a fairly high temperature, we do not know the conditions which are necessary for infection, since these two requirements in themselves are apparently not sufficient. This conclusion is arrived at as the result of environmental studies in controlled temperature and humidity chambers.

There is some ground for the belief that plants may be predisposed to the disease from internal causes. This hypothesis may be illustrated by an observation of the behavior of this disease in field fertilizer plots at the Wisconsin Experiment Station. The plots concerned were in duplicate and were intended to compare the value of barnyard manure with commercial fertilizers. For some reason not clearly understood, the manured plots gave a yield considerably lower than the unfertilized plots. Counts were made of the number of infected plants in each plot and, as may be seen from Table I, a fairly close correlation existed between yield, or the fertilizer applied, and the amount of infection.
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### Table 1.—Percentage of “rust” on fertilizer test plots

<table>
<thead>
<tr>
<th>Plot No.</th>
<th>Fertilizer applied per acre.</th>
<th>Yield in pounds of cured leaf per acre.</th>
<th>Percentage of plants rusted.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Barnyard manure, 20 tons.</td>
<td>1,545</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>do</td>
<td>1,242</td>
<td>40</td>
</tr>
<tr>
<td>8</td>
<td>Average</td>
<td>1,393</td>
<td>44.5</td>
</tr>
<tr>
<td>1</td>
<td>No fertilizer</td>
<td>1,682</td>
<td>23</td>
</tr>
<tr>
<td>7</td>
<td>do</td>
<td>1,527</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>1,604</td>
<td>24.5</td>
</tr>
<tr>
<td>2</td>
<td>200 pounds nitrate of soda, 200 pounds sulphate of potash, 600 pounds acid phosphate.</td>
<td>1,695</td>
<td>11</td>
</tr>
<tr>
<td>6</td>
<td>do</td>
<td>1,735</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>1,715</td>
<td>10.5</td>
</tr>
</tbody>
</table>

It does not follow from this, however, that “rust” is more likely to occur in low-yielding crops, the manured plots in this test being in fact an average crop. The condition is rather one of “physiological or nutritional balance” in the plant, a condition, as yet very inadequately understood in relation to plant diseases. A practical suggestion for experimental work in reducing damage from this disease by proper fertilization is offered, however, by such observations.

It has usually been found that the first or primary infection starts in the seed bed and that the secondary infection in the field is a direct result of transplanting infected plants. The same seems to be especially true of the wildfire disease. In the springs of 1917 and 1918 infection with Wisconsin leafspot was first noted in the seed beds at the Wisconsin Agricultural Experiment Station, and subsequently secondary infection in the field was found in areas in the field planted from the infected areas in the seed beds, although a considerable time intervened between the two appearances of the disease, during which time it had apparently disappeared. This experience, together with a similar, and now common, experience with the wildfire disease, offers the suggestion that seedling plants from areas showing signs of infection should preferably not be transplanted into the field. It is not improbable that spraying with copper sprays in the seed beds as suggested for wildfire (5) may also help to control the Wisconsin bacterial leafspot should conditions warrant its use.

The manner in which the disease lives over winter is not definitely known. The causal organism may perhaps live over on the seed, on the cloth covers, possibly in the soil, or by other means. Until this is determined no satisfactory means of control from this standpoint can be offered.

Although a considerable number of varieties of tobacco have been grown at the Wisconsin station annually, these have not all been equally exposed to infection. From limited observations and experiments, however, the writer feels safe in concluding that differences in varietal susceptibility or resistance are small, if in fact they exist at all.
SUMMARY

A bacterial leafspot disease of tobacco has been under observation and study in Wisconsin for five years. This disease is one of three or more different leafspots of tobacco commonly grouped under the common name "rust" by the growers in Wisconsin. The other "rust" spots appear to be nonparasitic in nature.

This disease has not been especially serious in recent years, but it is believed that it is the "rust" which has been most serious in past years and may become so again at any time that the required favorable conditions for its occurrence appear.

The Wisconsin leafspot of tobacco differs from both the wildfire leafspot and the angular leafspot occurring in other sections of the United States, and from the black rust occurring in Sumatra, all of which are bacterial in nature. The symptoms of the wildfire disease and of the Wisconsin leafspot organism, however, are much the same.

The disease usually manifests itself by round, brown, or rust-colored spots, usually less than \( \frac{1}{2} \) inch in diameter, but frequently running together to form larger irregular lesions. Frequently the young lesions are marked by a distinct chlorotic area or halo surrounding the point of infection. Under field conditions infection usually starts on or is confined to the lower leaves. Lesions may also occur on young leaves in the seed beds.

The disease is caused by a yellow bacterial organism apparently previously undescribed. The name *Bacterium melleum*, n. sp., is suggested, and the more common morphological and cultural characteristics are given.

Artificial infection has been secured only through wounding by needle pricks. Under the conditions of the inoculation experiments in the greenhouse this has also been more or less true with the wildfire organism, which has been studied comparatively in practically all of the work done with the Wisconsin leafspot organism. Under field conditions it is not believed that wounding is necessary for infection. Temperature and humidity conditions in themselves do not apparently govern the occurrence of infection. Some data secured indicate that predisposition may be influenced by the fertilizing materials available.

It is believed that the disease ordinarily starts in the seed beds, from which it is transferred to the field. Growers are advised, therefore, not to use plants from infected seed bed areas for transplanting.

LITERATURE CITED


(5) Jenkins, E. H., and Chapman, G. H.  
1922. Condensed Recommendations for the Control of Wildfire. Conn.  

(6) Killebrew, J. B., and Myrick, Herbert.  
1897. Tobacco Leaf. Its Culture and Cure, Marketing and Manufacture.  
xiv, 520 p., 137 fig. New York. Chronological list of works on  
tobacco, p. 495–496.

(7) Wolf, Frederick A., and Foster, A. C.  
pl. 15–16. Literature cited, p. 458.
PLATE i

A.—Leaf of Havana Seed tobacco, showing fairly typical symptoms of the Wisconsin leafspot ("rust") disease. Natural infection.

B.—More detailed view of portion of leaf similar to that illustrated in A, showing the central points of infection, the coalescence of the spots, and their relation to the veins.

C.—A leaf showing a fairly typical case of "wildfire" on Havana Seed tobacco. Compare with Wisconsin leafspot. This leaf was collected in Connecticut in July, 1919, and was the first authentic case of the wildfire disease in New England.

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PLATE 2

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A.—Leaves from tobacco seedlings in seed beds, showing typical lesions. Such leaves readily carry the infestation to the fields. Note the halo surrounding the points of infection (B, C, and D). Artificial inoculation with leafspot organisms by means of pin pricks on tobacco leaves, illustrating their similarity under certain conditions (B, C, D, and E).

B.—Leaf inoculated with Wisconsin leafspot organism.
C.—Leaf inoculated with wildfire organisms.
D.—Leaf inoculated with Wisconsin leafspot organism with lowered virulence.
E.—Control, pricked, but not inoculated.
PLATE 3

A.—A leaf of tobacco inoculated with the Wisconsin leafspot organism by means of pin pricks. Tissue collapsed rapidly around point of inoculation and turned brown, no halo developing. Similar spotting without halo may also develop in the wildfire disease.

B.—Typical colonies of the Wisconsin leafspot organism on potato agar plate.
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PLATE 3

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A.—Streak of Wisconsin leafspot organism on potato-dextrose agar after four days' growth at 20° to 22° C. in comparison with control tube. Note color difference of culture medium, as a result of development of yellow pigment on potato-dextrose agar.

B.—Photomicrograph of Wisconsin leafspot organism. Carbolfuchsin stain. X3,000.

C.—Line drawing of Wisconsin leafspot organism, showing flagella.