

# BACTERIAL STRIPE DISEASES OF SUGARCANE IN LOUISIANA<sup>1</sup>

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## INTRODUCTION

In the spring of 1927 leaf-stripe diseases, with symptoms similar to those of certain tropical bacterial diseases, occurred commonly on sugarcane throughout the entire sugar belt of Louisiana (2).<sup>2</sup> As no bacterial diseases of cane had been known to occur in Louisiana, investigations to determine the identity and importance of these troubles were started. As a result of these investigations, three distinct leaf-stripe diseases have been recognized in Louisiana. These are known as (1) the red stripe and top rot, (2) the mottled stripe, and (3) the white stripe. The first two are known to be caused by bacteria. The third one, the white stripe, is of considerable interest, as the stripe symptoms resemble very closely those of the leaf-scald disease of Java (8) and Australia (6). However, the disease seems to have but little economic importance in Louisiana, and as yet no organism has been isolated from the stripes. For these reasons it is not discussed in the present paper.

## THE RED STRIPE AND TOP ROT DISEASE

Of the leaf-stripe diseases occurring in Louisiana, the one known as red stripe and top rot is the most important. This disease occurs on the leaves and leaf sheaths, producing red to maroon stripes (fig. 1, A), and in the stems producing a definite rot which ordinarily begins at the top of the plant.

On the leaves the first symptom of the disease is the appearance of water-soaked stripes which, especially on the margins, gradually become tinted with maroon. These stripes under favorable conditions develop rapidly, and by the end of four to five days reach a length of 15 to 40 cm. and a width of 1 to 4 mm. By that time they have taken on a distinct dark red or maroon color. Usually each stripe is bordered by a yellowish or chlorotic zone of about the same width as the stripe. The stripes are not confined to the leaves, but extend down on the sheath, where they usually become wider, sometimes reaching a width of 1 to 2 cm. Often in later stages many of the stripes coalesce, forming bands with alternate maroon stripes and chlorotic areas.

In the stem the first symptom of the disease is a slight reddening of the fibrovascular bundles in the growing point. Later the red color gradually extends down the stem, forming a red ring in cross section about one-quarter to one-half the distance from the rind to the center. The central portion of the stem rapidly takes on a water-soaked appearance, while the portion outside of the red ring changes very

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slowly. As the disease progresses this condition gradually extends to the extreme base of the stem. Finally deterioration of the water-soaked area occurs and the tissues break apart, leaving a hollow central cylinder. By this time, the leaves at the top of the plant are dead

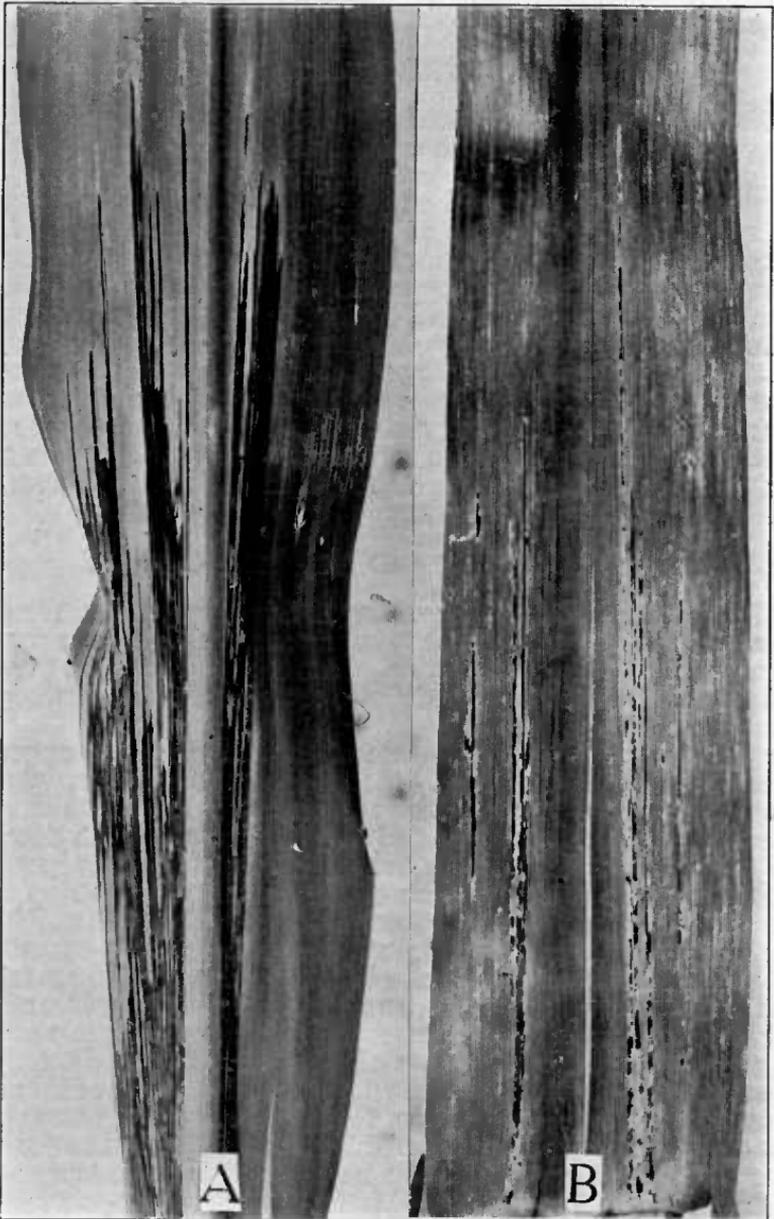


FIGURE 1.—Bacterial stripe diseases on sugarcane leaves: A, Red stripe caused by *Phytomonas rubrilineans*. B, mottled stripe caused by *P. rubrisubalbicans*

and the upper part of the stem is badly rotted. The killing of the terminal bud also results in a sprouting and growth of the lateral buds. Like the main stem, these lateral shoots usually show a reddening of the central zone.

The red-stripe disease has been found widely distributed over the sugar belt of Louisiana on the following POJ<sup>3</sup> varieties: 2727, 2714, 826, 2725, 213, 234, 36, 36-M, 979, and 228. The most serious natural infection observed occurred at Youngsville in August, 1928, one plantation showing a 10 per cent infection of top rot on POJ-2727 and 5 per cent on POJ-2714.

The organism causing red stripe was first isolated from leaves of POJ-213 cane collected at Youngsville on August 25, 1927. Since then many cultures have been obtained from both leaves and stems.

#### INOCULATION EXPERIMENTS

Inoculation experiments with pure cultures of the organism isolated from both leaves and stems have been consistently successful. Most of the inoculations have been made by introducing the inoculum into the growing point with a syringe. In practically all cases deep red to maroon stripes have appeared on the leaves and a large percentage of the stalks have developed top rot.

An inoculation test carried on in the summer of 1929 is an example of a series of such tests made with various varieties. In this test a susceptible variety, POJ-2725, was inoculated with five different cultures. Fifty plants were inoculated with each culture, and 50 plants were used for controls. After 12 days every plant of the 250 inoculated had red stripes, and 131 were developing top rot. On the controls no top rot or stripe symptoms developed around the needle punctures.

Inoculation experiments on different varieties have shown that POJ-2727, POJ-2725, and POJ-826 are very susceptible to the disease, while D-74 and the POJ varieties 36, 36-M, 234, and 213 are moderately susceptible. It is doubtful whether the disease would ever become serious in the latter varieties, but it could become quite serious in the former ones.

#### RELATION TO TROPICAL DISEASES

The red stripe and top rot disease as it occurs in Louisiana bears a close resemblance to diseases described from several tropical countries. Wakker and Went (?) described a top rot from Java and included a colored illustration which presents exactly the same symptoms as have been observed in Louisiana material. The evidence is very strong that the troubles are identical.

In Australia Cottrell-Dormer (1) and Wood (9) described a top rot and associated it with red stripes on the leaves. The disease was also produced by inoculating plants with mixed bacterial cultures. According to these authors, the Australian disease is confined to the upper joints of a stalk and does not spread through the stalk to the roots as is the case with the Louisiana disease. However, as the other symptoms are very similar, there is a strong likelihood that the diseases are identical.

In Hawaii Lee and his associates (4, 5) have described a red-stripe disease of Tip varieties and have named the bacterial pathogene *Phytomonas rubrilineans*. It is stated that the disease also attacks the stalks occasionally, but this point is not stressed. The stripes as described and illustrated are very similar to those produced by the

<sup>3</sup> Proefstation Oost Java.

red-stripe disease in Louisiana. The cultural characters of the two organisms are also very similar. A few slight differences have been observed, but it is very doubtful whether these differences are important. As the varieties of cane used in the Hawaiian investigations are entirely different from those grown in Louisiana, it has not been possible to compare the susceptibility of different varieties to the two organisms.

While the evidence is not absolutely conclusive that the Louisiana disease is identical with any of the tropical diseases, for the present, at least, the name given to the Hawaiian pathogene, *Phytomonas rubrilineans*, will also be used for the Louisiana organism. Eventually investigators in the Tropics will be able to test out such susceptible varieties as POJ-2727 and will be able to determine definitely whether the tropical and the Louisiana leaf stripe and top rot troubles are identical.

#### THE MOTTLED-STRIPE DISEASE

The second bacterial disease of cane which has been observed in Louisiana is of less economic importance than the red stripe. To this disease the name mottled stripe has been applied.

The mottled-stripe disease as it occurs in Louisiana is primarily a disease of the leaf blade. The stripes (fig. 1, B) which are characteristic of the disease are predominantly red in color, though frequently white areas or white margins occur. This difference in color often gives the appearance of red on a white background and has suggested the specific name *rubrisubalbicans* for the causal organism. The stripes run parallel to the leaf veins and range in length from very short up to a meter or more, while the width is usually from 1 to 4 mm. One to many stripes may occur on the same leaf. When many stripes occur they sometimes coalesce, forming mottled red and white bands. A microscopic examination of the diseased tissue shows the presence of motile bacteria in great numbers, though bacterial exudation on the surface of the leaves has not been observed.

Of the canes commonly planted in Louisiana, the disease occurs most commonly on D-74. Natural infection on the varieties POJ-213, 36, and 234 is not common.

The organism causing the mottled-stripe disease was first isolated from leaves of D-74 collected at Baton Rouge, La., on June 28, 1927. Small sections of stripe tissue were sterilized in 1-1,000 mercuric chloride solution for 30 seconds, washed three times in sterile water, and then macerated in a small amount of Bacto-dextrose broth. Dilution cultures were then made with Bacto-dextrose agar and pure-culture isolations obtained. Since then the organism has been isolated many times.

#### LEAF-INOCULATION TESTS

Numerous inoculations have been made to test the pathogenicity of the organism, to trace the development of the leaf stripe, and to determine the susceptibility of different cane varieties. Usually the inoculations were made by covering the upper surface of the leaf with a suspension of the bacteria in dextrose broth and then scratching the inoculum into the leaf with a sharp sterile needle. Some inoculations were also made by introducing a quantity of the bacterial suspension into the terminal growing region with a syringe.

The first inoculations were made on July 8, 1927. With the culture isolated on June 28 inoculations were made on the basal portion of leaves of D-74 cane. Ten leaves were inoculated and 10 controls were scratched with sterile dextrose broth. At the end of 5 days the inoculated leaves showed stripes 3 to 8 inches long, and at the end of 27 days from 14 to 38 inches. None of the controls developed stripes.

Many similar tests with different cultures made during the summers of 1928 and 1929 gave practically the same results. The original culture isolated in 1927 remained virulent throughout this period.

While inoculations carried on in the summer were invariably successful, those made in the fall, when the plants had practically ceased growth and the weather was cooler, were not satisfactory.

#### VARIETAL SUSCEPTIBILITY

The susceptibility of different varieties of cane to the mottled-stripe disease was determined by inoculation tests. In the first test, started on August 2, 1927, nine of the most important varieties were used. One hundred leaves of each were inoculated and 50 held as controls. In this test the disease developed most rapidly on D-74, but Louisiana Striped, SC-12/4, and Louisiana Purple were also quite susceptible. On the POJ varieties 36, 213, 228, 234, and 979, practically no stripes more than 4 inches long developed, which indicated that these varieties were resistant. The controls remained free of stripes.

In another test started on August 14, 1928, 54 varieties and seedlings were used. Ten plants of each were inoculated by introducing the inoculum into the terminal growing region with a syringe. The varieties that had been used the previous year showed the same relative resistance as in the first test. Other varieties found to be susceptible were D-95, Crystallina, POJ varieties 2364, 2222, 2714, 100, and U. S. seedlings 434, 1419, 726, and 672. The varieties found to be resistant included L-511, Cayana 10, Badilla, Co-281, Uba, Kavangire, POJ varieties 1228, 2379, 139, 36-M, U. S. seedlings 559, 593, 666, 547, 689, 541, 676, 766, 665, 1532, 600, 590, 576, 1444, 1389, 510, and CP seedlings 130, 177, and 807.

#### STEM INOCULATIONS

In order to determine whether the mottled-stripe organism can attack parts of the plant other than the leaf, stem inoculations were made on 50 plants of D-74, and POJ-234 on August 4, 1927. A portion of an internode was covered with a 24-hour culture, and this inoculum was forced into the stem by puncturing with a sharp needle. For controls, portions of the stems of 40 plants of each variety were covered with sterile dextrose broth and punctured. On September 23, there was no noticeable difference between the inoculated plants and the controls. Aside from a slight reddening of a few fibrovascular bundles, no pathological symptoms were observed.

#### INOCULATIONS ON SEED CANE

A test started on August 9, 1929, was made to determine whether young cane plants can become infected readily from diseased seed cane. Thirty healthy single-eye seed pieces of the varieties D-74 and POJ-213 were sterilized for 10 minutes in a 1-1,000 mercuric chloride

solution. These were then washed twice in sterile tap water and immersed for 15 minutes in a 48-hour dextrose broth culture of the mottled-stripe organism. They were then planted separately in 6-inch pots which had been sterilized at 15 pounds pressure for 2½ hours. Thirty controls, not inoculated, were also planted. The pots were kept in the greenhouse and watered with sterile tap water.

Half of the plants were examined on September 7 and the remainder on November 11. On the latter date, the plants averaged 22 to 24 inches in height. The examination showed no differences between the plants from the inoculated and the control seed pieces. No mottled-stripe symptoms were observed on any of the plants.

As similar results were also obtained from field plantings made in the fall of 1927, it would seem that cane plants do not readily contract the disease from infected seed cane.

#### INOCULATIONS ON OTHER HOSTS

To determine whether the mottled-stripe organism is pathogenic to plants other than cane, 50 plants each of Johnson grass (*Holcus halepensis*), sorghum, corn, and cane were inoculated on August 4, 1928. After 18 days no mottled-stripe symptoms had developed on the corn plants. Of the sorghum inoculations all of the controls were negative, while 35 of the inoculated plants showed stripes ranging from one-half to 2 inches in length. The other 15 plants had been accidentally destroyed. Of the Johnson-grass inoculations the controls were negative, while 48 of the inoculated plants showed stripes one-fourth to one-half inch in length. The other two plants died from other causes. The sugarcane plants used as controls were negative, while the inoculated ones had stripes one-half to 4 inches in length. The test showed that the organism was parasitic to some extent on both Johnson grass and sorghum.

#### CULTURAL CHARACTERS

The mottled-stripe organism grows readily on most of the standard culture media.

On Bacto-dextrose agar plates (pH 7.0) at 30° C, the organism grows readily, the colonies reaching a diameter of 2 mm. in four days. By reflected light the colonies are circular, convex, entire, glistening, finely granular, viscid, milky-gray; by transmitted light, the margins are translucent and the centers are opaque and slightly buff tinted. In the medium surrounding the colonies, calcium oxalate crystals are abundant. Subsurface colonies after four days are small, lenticular, and opaque.

On Bacto-dextrose agar slants (pH 7.6) the growth after 24 hours is abundant, filiform, spreading at base, smooth, convex, glistening, gray-white, translucent to opaque, and slightly viscid. After seven days, growth is much more extensive and opaque. After the growth is loosened from the surface and agitated in distilled water, viscid strands remain suspended in the liquid for several minutes.

On Bacto-nutrient agar slants (pH 7.1) growth is at first scanty, filiform, glistening, smooth, translucent, gray-white, convex, and somewhat viscid. The growth is less abundant on this medium than on Bacto-dextrose agar.

On potato slants, after 24 hours, the growth is scanty, glistening, and light buff in color, and growth increases but slightly after further incubation.

On Bacto peptonized milk agar, after 24 hours, the growth is abundant, filiform, glistening, gray-white, raised, translucent, wet, and sticky. After 10 days the consistency is butyrous and the surface has a distinct iridescence, becoming more pronounced up to 24 days.

On Bacto-dextrose broth (pH 6.8), after 24 hours, a light lacy pellicle, which is easily precipitated, forms at the surface, and a heavy precipitate accumulates in the bottom of the tube. As the culture ages, new pellicles are formed and precipitated, increasing the amount of sediment. The latter becomes extremely viscid, and when agitated spirals upward in a ropy strand which slowly settles in a coil to the bottom of the tube when the agitation is stopped.

On Bacto-neutral red broth, clouding is moderate and the medium becomes eosin in color. The other characters are similar to those on Bacto-dextrose broth, though the sediment which develops is somewhat more viscid.

#### COMPARISON WITH RELATED BACTERIAL DISEASES

The mottled-stripe disease differs widely from the red-stripe disease as it occurs in Louisiana and as it has been described from Hawaii. The points of difference include the symptoms on the host, the susceptibility of various varieties, and the cultural characteristics of the causal organisms.

On the sugarcane plant, stripes of the mottled-stripe disease are red to mottled red and white, while those of the red-stripe disease are of a solid maroon color. The mottled-stripe disease does not attack the leaf sheaths or the stem, while the red stripe attacks both, causing a serious rot of the latter. Such varieties as POJ-36, 213, and 234 are very resistant to the mottled stripe but are susceptible to the red stripe.

The organisms causing the two diseases differ widely in certain important characteristics. The mottled-stripe organism produces capsules on dextrose media, does not liquefy gelatin, produces indol in 14 days, produces hydrogen sulphide in 3 days, exerts a rapid diastasic action on starch, and invariably produces an alkaline reaction in sucrose, dextrose, and lactose broth. On the other hand, the red-stripe organism, *Phytomonas rubrilineans*, does not produce capsules, liquefies gelatin, does not produce indol, produces hydrogen sulphide (does not according to Lee), exerts a slow diastasic action on starch (does not hydrolyze starch, according to Lee), and produces acid from dextrose (according to Lee).

The mottled-stripe organism has also been compared with the one which commonly causes a striping of Johnson-grass leaves in Louisiana. The latter organism is probably identical with the sorghum leaf-stripe organism, *Bacterium andropogoni* (3). The cultural characters of the two organisms are quite different, and the stripes on Johnson-grass leaves following inoculation with pure cultures are also different. The Johnson-grass organism invariably produces a broad stripe or blotch, as compared with the narrow stripe of the cane organism. (Fig. 2.)

## TECHNICAL DESCRIPTION

As the organism causing the mottled-stripe disease of sugarcane in Louisiana seems to be distinct from other named bacteria, it is here described as a new species.

***Phytomonas rubrisubalbicans* sp. nov.**

A motile, short rod, slightly curved, with rounded ends and polar flagella; single, in pairs or rarely in short chains of from 2 to 6 cells; no spores; capsules present on dextrose media; aerobic but grows moderately under anaerobic conditions; gray-white to a light buff on agar; gelatin not liquefied; indol produced; hydrogen sulphide produced; starch hydrolyzed, no acid or gas produced from dextrose, lactose, maltose, saccharose, mannite, or malt extract; optimum temperature 30° C., optimum reaction pH 6.8 to 8; Gram-negative; not acid-fast;

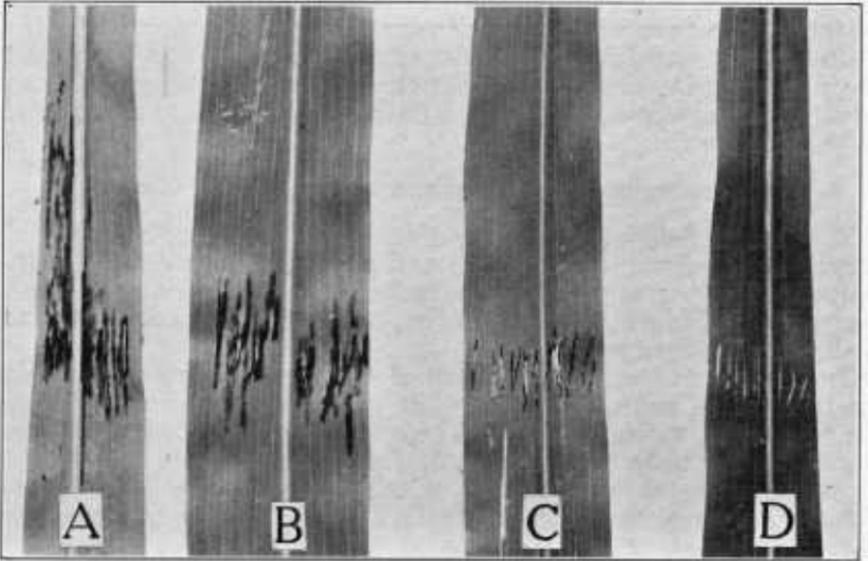


FIGURE 2.—Inoculation tests on leaves of Johnson grass: A, B, Leaves inoculated with *Bacterium andropogoni* isolated from Johnson grass; C, leaf inoculated with *Phytomonas rubrisubalbicans* from sugarcane; D, control, leaf scratched but not inoculated

pathogenic on leaves of sugarcane in Louisiana, causing the disease known as mottled stripe.

## SUMMARY

Investigations carried on in Louisiana during a period of three years have shown that three leaf-stripe diseases of sugarcane occur there. Two of them are discussed in this paper. One of these, known as red stripe and top rot, is closely related to or identical with stripe and top-rot diseases in Java, Australia, and Hawaii. The disease is characterized by long, deep-red or maroon colored stripes on the leaves and by a rapid decay of the central portion of the stem. This decay is followed by the dying of the apical portion of the plant.

The evidence indicates that the organism causing red stripe and top rot is *Phytomonas rubrilineans*. It was isolated many times from infected leaves and stems. Inoculation experiments on leaves and stems were invariably successful. Inoculation experiments with pure cultures of the red stripe and top rot organism have shown that the POJ varieties 2727, 2725, and 826 are very susceptible,

while the D-74 and POJ varieties 36, 36-M, 234, and 213 are moderately susceptible.

The other disease, named mottled stripe, is characterized by the presence of long stripes on the leaf blades. These are predominantly red in color, though a mottled red and white condition often occurs. An organism, described as *Phytomonas rubrisubulbicans*, sp. nov., was isolated many times from tissues showing mottled-stripe symptoms. Inoculation experiments on the leaves of cane with pure cultures were invariably successful. Inoculations in stems of cane were negative. As a result of the inoculation experiments, the following varieties of cane have been shown to be very susceptible to the mottled-stripe disease: D-74, POJ-2364, POJ-2222, POJ-2714, POJ-2727, Crystallina, D-95, SC-12/4, Louisiana Striped, Louisiana Purple, and POJ-100. Among the varieties showing considerable resistance to the disease, though not immune, were POJ varieties 36, 36-M, 234, 213, 228, 979, Co-281, and CP varieties 130, 177, and 807.

The mottled-stripe disease was transmitted to Johnson grass (*Holcus halepensis*) and sorghum, but not to corn.

#### LITERATURE CITED

- (1) COTTRELL-DORMER, W.  
1926. NOTES AND OBSERVATIONS ON THE RED STREAK ASSOCIATED WITH QUEENSLAND TOP ROT DISEASE. Queensland Agr. Jour. (2) 25: 406-414, illus.
- (2) EDGERTON, C. W., and CHRISTOPHER, W. N.  
1927. THE RED STRIPE DISEASE OF SUGARCANE. Planter and Sugar Manfr. 79: 63, illus.
- (3) ELLIOTT, C., and SMITH, E.  
1929. A BACTERIAL STRIPE DISEASE OF SORGHUM. Jour. Agr. Research 38: 1-22, illus.
- (4) LEE, H. A., and JENNINGS, W. C.  
1924. BACTERIAL RED STRIPE DISEASE OF TIP CANES. Hawaii. Sugar Planters' Assoc. Expt. Sta. Circ. 42, 4 p., illus.
- (5) ——— MARTIN, J. P., PURDY, H. A., BARNUM, C. C., JENNINGS, W. C., and WHEELER, D. M.  
1925. RED-STRIPE DISEASE STUDIES. Hawaii. Sugar Planters' Assoc. Expt. Sta., Dept. Path. 99 p., illus. Honolulu.
- (6) NORTH, D. S.  
1926. LEAF-SCALD, A BACTERIAL DISEASE OF SUGARCANE. Colon. Sugar Refining Co. [Sydney] Agr. Rpt. no. 8, 80 p., illus.
- (7) WAKKER, J. H., and WENT, F. A. F. C.  
1898. DE ZIEKTEN VAN HET SUIKERRIET OP JAVA DIE NIET DOOR DIEREN VEROORZAAKTE WORDEN. Deel 1, 217 p., illus. Leiden.
- (8) WILBRINK, G.  
1920. DE GOMZIEKTE VAN HET SUIKERRIET, HARE OORZAAK EN HARE BETSRIJDING. Arch. Suikerindus. Nederland. Indië 28: [1399]-1525.
- (9) WOOD, E. J. F.  
1927. TOP ROT IN SUGARCANE. Queensland Agr. Jour. 28: 208-211.

