A Botrytis Form Causing Storage Rot in Sugar Beets

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In the spring of 1941 a disease, previously not recorded by the Great Western Sugar Company and possibly new to the beet-sugar industry as a whole, was found on roots of the sugar beet *{Beta vulgaris* L.) kept in a storage cellar at the Experiment Station at Longmont, Colorado, It was a root rot with which was associated a fungus apparently of the form genus *fiolrytis,* and which readily produced sclerotia in culture.

Botrytis forms and more or less related *Sclerotinia* forms are known to cause rots of many vegetables and fruits. Rarely, however, are beets mentioned us host plants to these organisms, liamsey (3), in "*Sclerotina* species causing decay of vegetables under transit and market conditions,'" mentioned *Botrytis cinerea,* Pers. very briefly and in no way to indicate a connect ion with disease of beets.[3] Beet roots were found to be highly r-esistanl Jo inoculation with */Sclerotinia libertiana. S. intermedia, S. ricini,* and S. *minor,* with which forms the *Botrytis* form under discussion does not seem to be connected. In a later publication (4) the same author discussed *Botrytis cinerea* among potato pathogens but did not give the beet as a host. Beet roots were mentioned only in a reference to his paper previously given (3). Hodges (2) in 1936 in his study of ''Fungi of Sugar Beets" did not report *Botrytis* or *Sclerotinia* forms among fungi recovered.

Conditions Under Which the Fungus Occurred and Was Discovered

Most of the beets in the root cellar had been tested for sugar content and checked for presence of rot during the latter two-thirds of the month of December 3 910. In some of the material a considerable number of diseased roots was found. This was believed to be caused by difficulties in lowering the temperature in the newly constructed root cellar to a, satisfactory level during the first part of the storage period. If the individual beets, when examined, were found to be attacked only moderately by rot, the affected tissue was carved out,

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[2]This paper was presented by Dr. TT. K. Brewbaker.

[3]Numbers in parentheses refer to literature Cited.

and the roots were put back in storage. The testing process also resulted in a certain injury to the beets by leaving a drill hole of approximately 7/8-inch diameter. The routine procedure employed in this work no doubt permitted many roots to go back in storage before proper drying and healing of the wound surfaces were accomplished, and this fact probably helped to develop the trouble that followed later.

The amount of rot encountered at the time of the testing was, of course, a matter of some concern, but the situation was not considered in any sense alarming. The types of rot noted exhibited nothing different from what had been observed in numerous instances in the past. As usual, *Phoma* seemed to be the organism most frequently found, with *Fusarium, Pythium,* and *Rhizoctonia* forms occurring in sporadic cases. Saprophytes and facultative or weak parasites, such as species of *Penicillium, Mucor,* etc., naturally complicated the picture by their presence.

During the latter part of March and early in April, however, root rots were again found active. At this time some individual roots were in a rather advanced state of decay, often exhibiting an external fungus flora with a variety of types and colors. No attempt will be made to give the exact amount of damage caused by any one of the specific offenders among these organisms. It may be mentioned, however, that *Phoma* was again obtained from a large number of root-tissue plantings. At the same time the *Botrytis* form, connected with the new form of rot already mentioned, was frequently recovered from similar tissue plantings.

The first tissue, mycelium, and spore-plantings from the diseased beets were made during the last week of March, consisting of a limited number of test-tube slants with beet-juice agar, representing five different roots, two of which later proved to be infected with the *Botrytis* form now under discussion. A considerable number of similar cultures was put up during the first few days of April, mostly on potato-dextrose agar, and while both of these types of cultures produced attachment organs (see under "The Organism") as well as the typical *Botrytis* spore stage of the newly found organism, the potato-dextrose cultures also produced sclerotia very readily. Thus, while there had been at first an inclination to consider the unusual gray growth as merely representing another chance pickup, likely to disappear as suddenly as it had appeared, it now became clear that it had to be taken more seriously. The unusual mycelium and spore growth continued to appear on many of the beets, together with corresponding internal decay, and the agar cultures continued to yield this fungus with its *Botrytis* spore stage and its sclerotia in a great number of cases.

The Disease

The disease caused by *the Botrytis* form under discussion, as generally observed at the experiment station, was a root rot in which the tissues under attack were at first quite uniformly invaded and discolored on a broad and rather regular front (fig. 1), with no appreciable change in the apparent moisture content. A general collapse of tissues followed in a much later stage. The discoloration varied over a considerable range from a very dark brown, which in some cases bordered on black, to a pale brown with a tinge of pink. Any and all locations on the beet root seemed to be suitable for the initial attack, if the conditions were favorable, and it was frequently observed that several points on the same rout had been attacked independently. However, it was evident in the beets studied that the wounds caused by the test drill or by carving out diseased tissue, as mentioned earlier, and also the broken root tip, served as by far the most preferred and effective ports of entry for the fungus. Thus, this organism may be a wound parasite in a general sense but able to attack the beets through rather small and inconspicuous blemishes in the epidermis, by the lateral roots, or around buds on the crown after perhaps having gained a foothold in collapsed remnants of petioles. This point could not be investigated conclusively because of insufficient material for study purposes at the time of the discovery of the disease. A few surface applications of inoculum gave negative results.

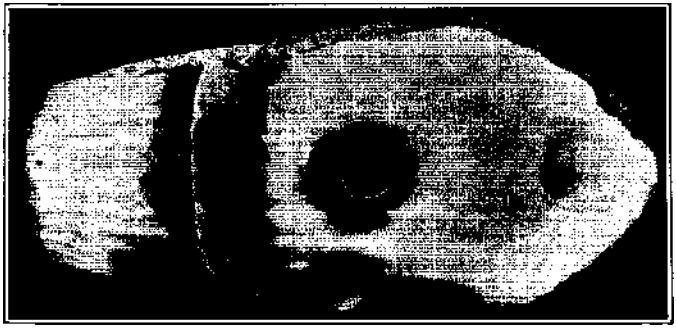

Figure 1.—Spread of the Botrytis rot from two holes made at right angles to each other. Note advance in tissue over rather even front, with relatively dark discoloring, and conidia formation on mycelium in holes. Specimen inoculated and kept ar 4° to 0° C. for 42 days. Advance much slower than in some cases. Decay on upper right side should be disregarded in this connection. Gray spots noticeable on areas of decay do not represent mycelium and conidia, as in the holes, but small fibrous particles of beet tissue which rapidly lost moisture when beet was cut.

Inoculation of roots under pyramid-shaped plugs (approximately 12 x 12 mm.) was usually successful, provided a fairly large amount of mycelium, with or without the conidial spore stage, was introduced. Inoculation of holes (fig. 1) made with a ½-inch tube borer was tried in a small number of cases, all of which were successful. These holes were permitted to dry and heal over for 1½ to 2½ hours before *the* inoculum was applied. They were not plugged. The beets were kept in a refrigerator at a temperature of 4° to 9° C.

Most of the *inoculated specimens* were kept at 4° to 9° C. for the duration of the test. This was for the purpose of simulating the somewhat too high storage temperature that the *beets had* been subjected to before the fungus was discovered. The rapidity with which the rot established itself and spread through the tissue at this temperature was considerably less than at room temperature, judging from a comparison with a few of the first inoculations made. However, the penetration of the discoloration at the lower temperature in a number of cases amounted to as much as 10 to 25 mm. in 25 days and, in a few cases, exceeded this figure appreciably. The *Botrytis* spore stage often began to appear on the surface mycelium after 14 days. From the 25 last inoculations, the organism was recovered in 100 percent of the cultures even in spite of notable differences in penetration and color of the different loci.

The Organism.

The mycelium of this *Botrytis* form as found on the beets usually exhibited a gray color of varying shade which seemed to become decidedly darker with age (fig. 1 and 2). Young and luxuriantly growing mycelium in rare cases was found to be practically white,

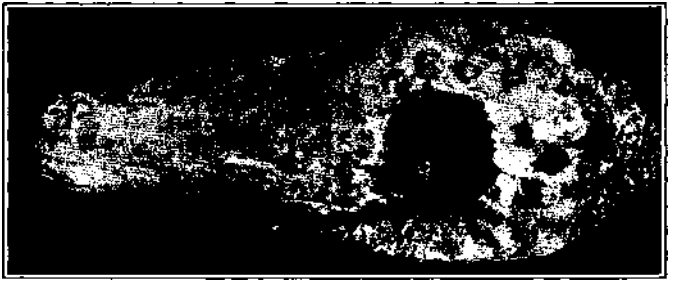

Figure 2.—Myeelium on conidial growth very dark as compared with growth shown in holes on figure 1. Specimen old and noticeably shrunken when photographed, June 14. Sclerotia formed in identification number not surrounded by surface mycelium. Some penicillium present at right..

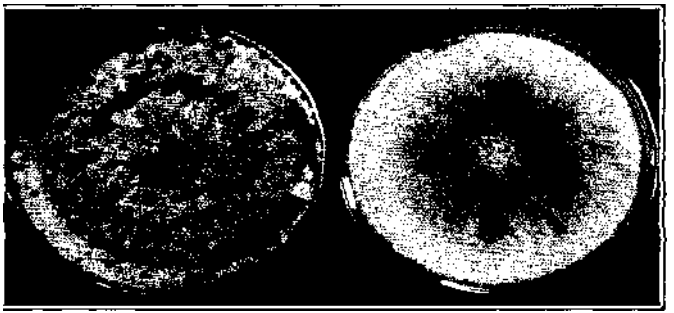

Figure 3 —Cultures of the Botrytis form under discussion grown on potato dextrose agar at room temperature. Older specimen 6 weeks old and showing numerous sclerotia. Conidial stage also present on some areas within 15 mm. from edge. Younger specimen 6 days old with growth reaching edae. (Growth thickening toward edge, of whitish color, and in this case with a faint touch of pink. Conidial development beginning.

for instance over a out surface of a rotting heel that had been kept in a warm and humid atmosphere, but it soon grew darker. In this brief sketch of the morphological nature of the fungus it may be pointed out that if corresponded closely, on most points comparable in spite of being on a different hosl, with the description of *Bolrytia,* and more specifically *Botrytis cinerca,* as given by Stevens (5). Conidiophoros and conidia corresponded in detail with the picture by Smith, used by Stevens. Attachment, organs were formed very early in culture ami corresponded with the description of those of *Sclerotinia fuckeliana* as given by Stevens (picture by Istvanffi). They were among the first features observed which indicated the presence of an unusual fungus on the beets. Sclerotia were not observed on the beets at the time of the discovery of the fungus but appeared in considerable numbers on some beets which were held through the first part of the summer (fig. 2). They seemed to form chiefly in cracks or depressions such as between buds on the crown or even in small injuries. Note the case of the badly affected beet (fig. 2), with rot spreading from the hole but sclerotia forming in the identification number independent of external mycelium. The picture was taken when the beet was noticeably shrunk and the *Botrytis* growth, much darker than in the earlier stages. As mentioned previously, sclerotia were also obtained readily on potato-dextrose agar (fig. 3), while they were small and few. if present at all, on the beet agar used in this work. The conidial stage developed readily on both media but generally less abundantly on potato-dextrose agar. Note the occurrence of conidial growth on some parts of older specimen (fig. 3).

Another interesting parallel was noted in one particular root that was inoculated on July *23* and kept in the refrigerator until September 3. This root was not fully turgid when inoculated, after this considerable period of storage, which fact may account at least in part for a pronounced shrinking of the two plugs, under which the inoculations were made, and of the beet tissue under the plugs. In one case, cavities were formed even independently of the shrunken space immediately under the plug. However, the interesting feature was the complete filling of the cavities under the plugs in both loci with a tough, rather rubber-like, cement-colored mass, which more than likely was of the same nature as a formation mentioned by Stevens (5) in his description of *Selerotinia fructiyena, cinerea,* and *laxa,* as follows :

"The mycelium within the fruit persists, turns olivaceous and forms large irregular sclerotioid masses which on the following- spring may produce fresh conidia."

As pointed out by Stevens (5), "The form genus *Botrytis* contains many parasites on various hosts. In some instances they are known to include ascigerous stages *(Sclerotinia)* in their life cycle ; in others no such relation is known, though it has often been assumed. Specific limitations are but poorly understood, and the relations between the various forms and between these forms and the ascigerous stages are in a state of much confusion."

This statement, made by Stevens about 20 years ago, applies to a very great extent today. The reviewer of "The perfect stage of *Botrytis cinerea'* by J. W. Graves and P. Lr. Drayton (1), makes the following statements:

"In this preliminary study, using about 70 isolates from various hosts and localities as a basis, the authors obtained mature apothecia belonging to the genus *Sclerotinia* from 9 isolates. The taxonomic significance of the development of these *sclerotinioid* apothecia by some of the common forms of *Botrytis cinerea* cannot be properly evaluated at present, and hence no change in nomenclature is proposed. However, it is believed that work now in progress with single-ascospore cultures will give some clue to the interpretation of the numerous variations observed and help to clarify the species concept in this perplexing group of fungi."

The fungus under discussion has been pronounced by a reliable authority to be a *Botrytis* of the *cinerea* type. It is not identical with *Sclerotinia fuckeliana,* mentioned in the morphological comparisons, but is a closely related form.

Possible Origin of the Fungus in the. Case under Discussion

The origin of this fungus in the beet material at Longmont is largely a matter of conjecture. It is true that it appeared during the first season in which the newly constructed root cellar was in operation, but tliis fact in itself does not explain the matter. The most plausible explanation appears to be that the initial infection of the cellar occurred through the introduction of some used vegetable and fruit crates, and that the conditions happened to be favorable for growth and spread, particularly in view of the fact that the fungus develops well at a relatively low temperature.

It is regretted that this theory regarding the source of the inoculum could not be checked, since by the time the fungus had been recognized as a menace all the used crates obtained had been inside the cellar and thus had to be considered contaminated.

Upon the complete emptying of the cellar, it was disinfected with a solution of copper sulfate, and all the crates were treated by being immersed for 12 to 15 minutes in the same solution, the strength of which was approximately twice the strength used in treating grain.

Up to date the fungus has not reappeared, and it is hoped that by using great care in cleaning out the drill holes when testing the beets, by allowing considerable time for drying and healing of the wound surfaces before the roots are put back in storage, and by keeping a lower temperature in the root cellar the trouble will be prevented.

Summary

In the spring of 1941 a disease hitherto not recorded by the Great Western Sugar Company was found on roots of the sugar beet (*Beta vulgaris* L.) kept in a storage cellar at the Experiment Station at Longmont, Colorado. Isolations from attacked beets yielded a fungus of the form genus *Botrytis,* the morphology of which resembled closely the description of *Botrytis cinerea* Pers., and it has been designated as a *Botrytis* of the *cinerea* type. Wound-inoculation tests, with resulting rot and later recovery of the organism introduced, showed it decidedly pathogenic to sugar-beet roots under the conditions of the test.

It is considered probable that the disease was introduced with some used vegetable and fruit crates, which may have carried the original contamination.

After disinfection of the cellar and the crates, the disease did not reappear by the end of 1941.

Literature Cited

1. Graves, J. W., and Drayton, F. L. The Perfect Stage of *Botrytis cinerea.* Mycologia, 31:485-489. Illus. Review: Exp. Sta. Record 82:200-201. 1939.
2. Hodges, F. Allen. Fungi of Sugar Beets. Phytopathology 26: 550-563. Illus. 1936.
3. Ramsey, G. B. *Sclerotinia* Species Causing Decay of Vegetables under Transit and Market Conditions. Jour. Agri. Research 31:597-631. (630). Illus. 1925.
4. ——. *Botrytis* and *Sclerotinia* as Potato Tuber Pathogens. Phytopathology 31:439-448. (443). Illus. 1941.
5. Stevens, F. L. The Fungi which Cause Plant Diseases. The Macmillan Company, New York. pp. 138, 139, 141, 578, 579. 1919.

Dusting and Spraying Sugar Beets in Michigan for Control of Cercospora Leafspot

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During the years 1936, 1937, and 1938 the estimated loss to the sugar-beet crop in Michigan because of attacks of *Cercospora* leafspot averaged approximately 15 percent. This disease is one of long standing in the State, but only during recent years have there been succeeding serious losses to the sugar-beet industry.

In 1939, cooperative experiments and demonstrations were inaugurated with the various sugar companies, and dusting work was carried out on 26 blocks of beets in the Saginaw Valley. The dust material used was monohydrate copper, sulfate-lime 20-80, since previous work had shown this to be effective under moist conditions. The results of all these trials in 1939 showed conclusively that, in a year of moderate to heavy leafspot infection, the 20-80 copper-lime dust at 3 to 4 applications was an effective control, giving an average increase of approximately 2 tons of beets per acre, with an increase in sucrose of 1 percent, 1.2 percent increase in purity, and an increase in estimated recoverable sugar of 777 pounds per acre. These results

[1]Michigan Agricultural Experiment Station.