Crown gall of plants.

(15)

CROWN GALL OF PLANTS

ERWIN F. SMITH

[From PHYTOPATHOLOGY, Vol. I, No. 1, February, 1911]

CROWN GALL OF PLANTS

ERWIN F. SMITH

(WITH PLATES II AND III)

This disease is characterized by marked overgrowth of the affected tissues. It occurs on plants of many families and in various regions of the world. In the United States it has been designated "crown gall" because it occurs very frequently on the crown of the plant, but it is found also on roots and on stems above the earth surface. In Europe where it occurs on grape, rose, raspberry, beet, etc., it is known by a variety of names: canker, krebs, wurzelkropf, broussins, rogna.

For the past six years team work has been done on crown gall in the Bureau of Plant Industry by the writer and three colleagues, Dr. C. O. Townsend (first five years), Miss Alice C. Haskins (first two years), and Miss Nellie A Brown (last four years). Other co-workers have also contributed a little here and there,—cultures set, sections cut, flagella stained, chemical analyses made.

A bulletin on this subject is in preparation and to this publication those readers are referred who desire further proofs in confirmation of the statements here affirmed.

CAUSE OF THE DISEASE

This disease is due to *bacteria*: not to fungi, not to myxomycetes, not to mites, not to frosts, not to disturbances in nutrition. All these alleged causes have had their champions but they are not the true cause. They are all *post hoc* inferences based for the most part only on microscopic examinations made, in so far as organisms have been incriminated, in the later stages of the disease, when various saprophytes are certain to be present. Of clear cut and convincing experimental evidence in their favor there is not a scrap.

Bacteria were seen by the writer in fresh unstained sections of immature and non-necrosed crown galls in February, 1904, and two years later we obtained convincing proof of the gall-producing power of a particular schizomycete, obtained from galls by means of agar poured-plates, sub-cultured on agar from the colonies, and inoculated into sound plants. This organism was then named *Bacterium tumefaciens* S. & T. Since that date our experiments have been continuous, and we have obtained successful inoculations from pure cultures and sub-cultures on more than a thousand plants. Sometimes we have failed but gradually we have come to know many of the sources of our failures, and nothing in our experiments has led us to wish to modify in any material way our first published statement (Science, April 26, 1907, and Centralbl. f. Bakt., Abt. 2, Bd. XX, No. 1/3, 1907), or

subsequent statements (Science, Feb. 12, 1909 and August 13, 1909). As evidence in point the reader may consult the two plates accompanying this text, remembering that these results were obtained by pure culture inoculations, not by graftings (we have made no grafting experiments), and that we have obtained hundreds of others equally convincing ; *e. g.* of 43 hops inoculated with pure cultures from the daisy (two experiments, one by Miss Brown and one by myself) 42 developed galls where punctured ; of 85 sugar beets inoculated with pure cultures from the daisy (5 experiments) 83 contracted the disease, and 82 only at the point of inoculation ; of 12 carnations inoculated by Townsend every one developed tumors. On the other hand with rare exceptions our numerous controls have remained free. We may then admit that these overgrowths are due to bacteria. Are they all due to the same organism ?

CROSS INOCULATIONS

Early in our experiments we began to make inoculations on a variety of plants. The first work was done on the gall of the Paris daisy, but as opportunity came we made platings from tumors on other plants. Up to the present time we have obtained tumor-producing schizomycetes from overgrowths on plants belonging to many widely separated families (Compositae to Salicaceae). These organisms are closely alike on various culture media, and many of them are readily cross-inoculable, *e. g. daisy* to peach, radish, grape, sugar-beet, hop ; *peach* to daisy, apple, Pelargonium, sugar-beet, poplar ; *hop* to daisy, tomato, sugar-beet ; *grape* to almond, sugar-beet ; *poplar* to cactus, oleander, sugar-beet ; *willow* to daisy. Some cross-inoculate more readily than others, and there are also slight cultural differences. The morphological differences are trifling. In general it may be said that all plants susceptible to crown galls, *i. e.*, those on which they have been found in nature, are susceptible to artificial cross inoculation. The extent of natural cross-inoculation is less easily determined, but there is a good body of evidence going to show that it often takes place when one kind of plant follows another, *e. g.*, when peaches follow galled raspberries or apples.

CULTURAL AND OTHER DIFFICULTIES

Bacterium tumefaciens dies readily in some of the common culture media, and also gradually loses its virulence. It often occurs in the tumors in a cultivable condition only in extremely small numbers so as to be missed very readily. It also comes up on agar plates poured from the galls more slowly than the saprophytes and the latter were the first colonies we considered, and also undoubtedly the ones which have

attracted the attention of other workers. Both yellow and white non-pathogenic schizomycetes occur in crown galls and colonies of some of the latter on agar closely resemble the tumor-producing organism. Furthermore the organism does not make pockets or abscess cavities in the tumors such as are common in many bacterial diseases of animals and plants. Moreover, it does not stain well in the tissues and consequently is certain to be overlooked in any ordinary examination of stained slides. The microscope alone cannot be depended upon. We should never have gotton anywhere had we depended solely or principally upon that instrument. The best proof of the existence of the organism in the tissues is our ability to obtain it (sometimes in nearly or quite pure culture) by the poured-plate method from young non-necrosed galls after thorough surface sterilization. Old galls are unsuited for preparation of cultures.

HARD VS. SOFT GALLS

Various writers have maintained that there are two kinds of galls, especially on the apple tree, viz. hard galls and soft galls, the former being non-infectious and comprising most of the specimens met with. This has been a favorite argument of certain dealers in trees and Dr. Hedgcock at one time maintained this view. Our experiments, however, do not warrant any such distinction. The writer produced slow-growing hard galls on apple trees by inoculation with an organism taken from a soft gall of the peach ; and the hardest of hard galls of apple, selected for us by Dr. Hedgcock as typical, yielded colonies closely resembling those plated from soft galls, and with some of these colonies galls were produced on other plants. Whatever may be the factors leading in the apple to hard gall in some cases and soft gall in others both are due to infectious bacteria, and both kinds of gall are to be avoided in making plantings. Owing to the slow growth of these galls on many apple trees it has been maintained with some show of reason that they are harmless, and in some cases this may be true, although in the photograph of two rows of apple trees one galled and the other not, submitted by Dr. Hedgcock in support of this contention, the writer was able immediately to pick out the galled row before he had seen the legend. But even if this contention were generally true, which it is not, there are other reasons why all galled trees should be rejected in planting, namely because the gall is not covered by a smooth, sound, *protective* bark layer, and consequently often affords a ready entrance to other parasites. Some convincing details are given in the bulletin.

APPLE HAIRY ROOT

This disease supposed to be distinct from crown gall and non-infectious we have also proved to be due to bacteria scarcely distinguishable from those occurring in ordinary crown gall. These bacteria are found in the nodular base from which the tufted clusters of roots arise. We have induced the disease by pure culture inoculation not only in the apple but also in other plants and the disease would seem to belong with crown gall as only a special form of the latter. As a tentative hypothesis until further experiments are made we may assume either: (1) That the hairy root organism while resembling the crown gall organism is not identical with it; or (2) that they are the same, and if infection takes place in a certain group of cells an ordinary gall will develop, while if other special groups of cells are first invaded, *i. e.* root anlage, then a cluster of the fleshy roots will develop. Some of our inoculation experiments point to the latter conclusion.

NATURE OF THE GALL

Superficially these growths are like those due to various fungi, to gall insects, to *Plasmodiophora brassicae*, or to the olive-tubercle organism, but structurally they are different. Their manner of growth and their histology strongly suggest certain malignant animal tumors. The writer has twice called attention to this publicly at meetings of the American Society for Cancer Research (first in 1909) and has summarized the evidence somewhat carefully in the bulletin referred to. More recently (October, 1910) Jensen of Denmark has expressed similar views respecting the crown gall of the sugar-beet, but without knowing of our results.

One of the principal biological problems of today, and one of the most interesting in the whole range of pathology, is to determine if possible the chemical stimulus responsible for overgrowths. This problem is of immense and wide-reaching importance not only to the pathologist but also to the physiologist and all others interested in cell mechanics. In conclusion I may be permitted to express the hope that in these plant tumors, now so easily producible by a definite microorganism, we possess means of determining the cause of cell division and possibly of shedding some light on the origin of certain malignant animal tumors.

NOTE.—Massee has recently claimed to confirm Toumey's finding of *Dendrophagus* in crown gall of rose and chrysanthemum (Kew Bulletin No. 9, 1910). He also notes that he could find no trace of *B. tumefaciens*. The last statement is not surprising, and the first one appears to rest on no better evidence than a somewhat hasty microscopic examination, without attempt at pure cultures or inoculations. Such a microscopic examination, even if it were a very long and careful

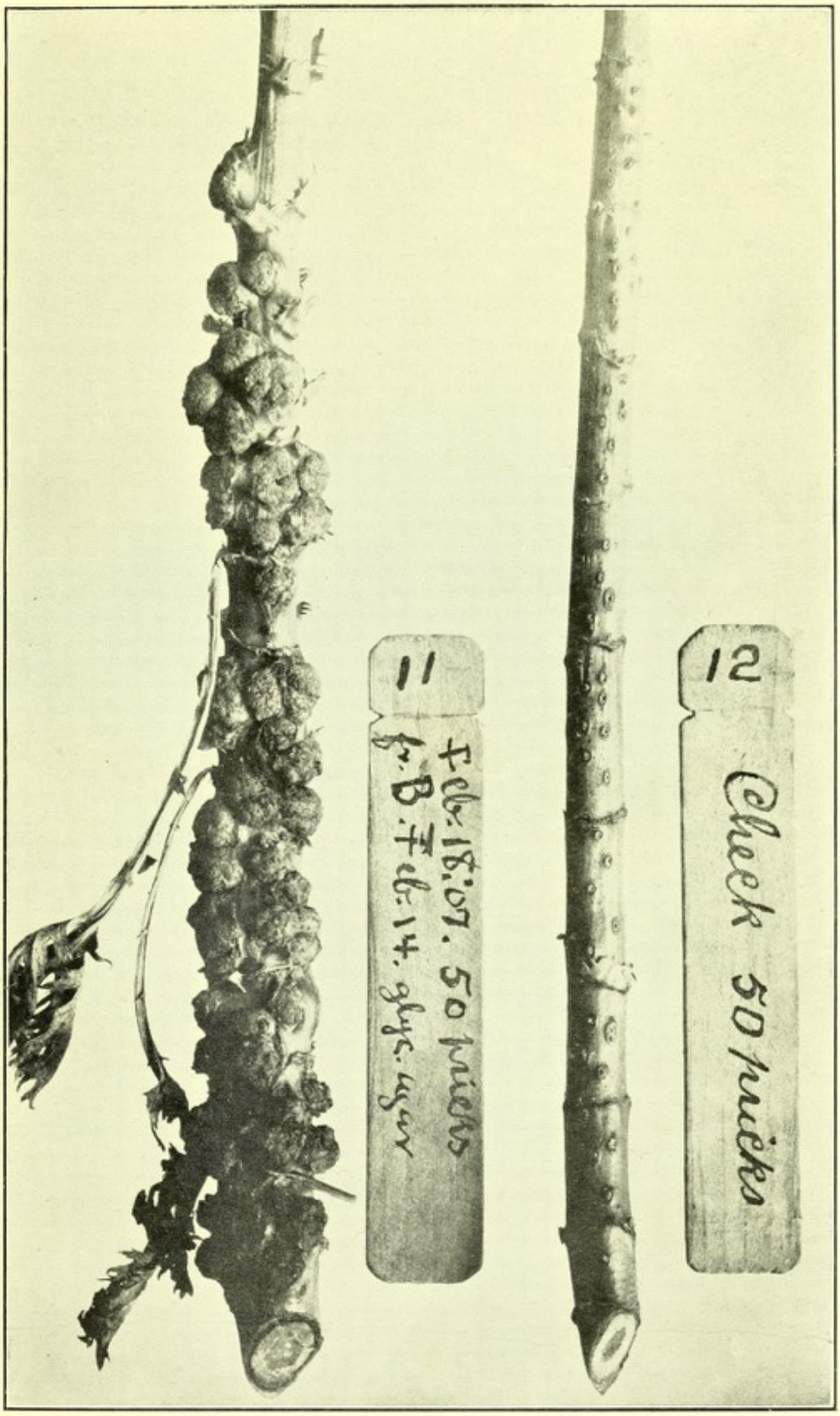

PLATE II. **Smith on Crown Gall.**

11. Fifty punctures with an infected needle; 12, fifty punctures with a sterile needle. The young inoculated plant forked into two twin branches of which 11 is one and 12 the other. Time two months and four days. Photo. April 22, 1907. Pure culture inoculation of daisy on daisy.

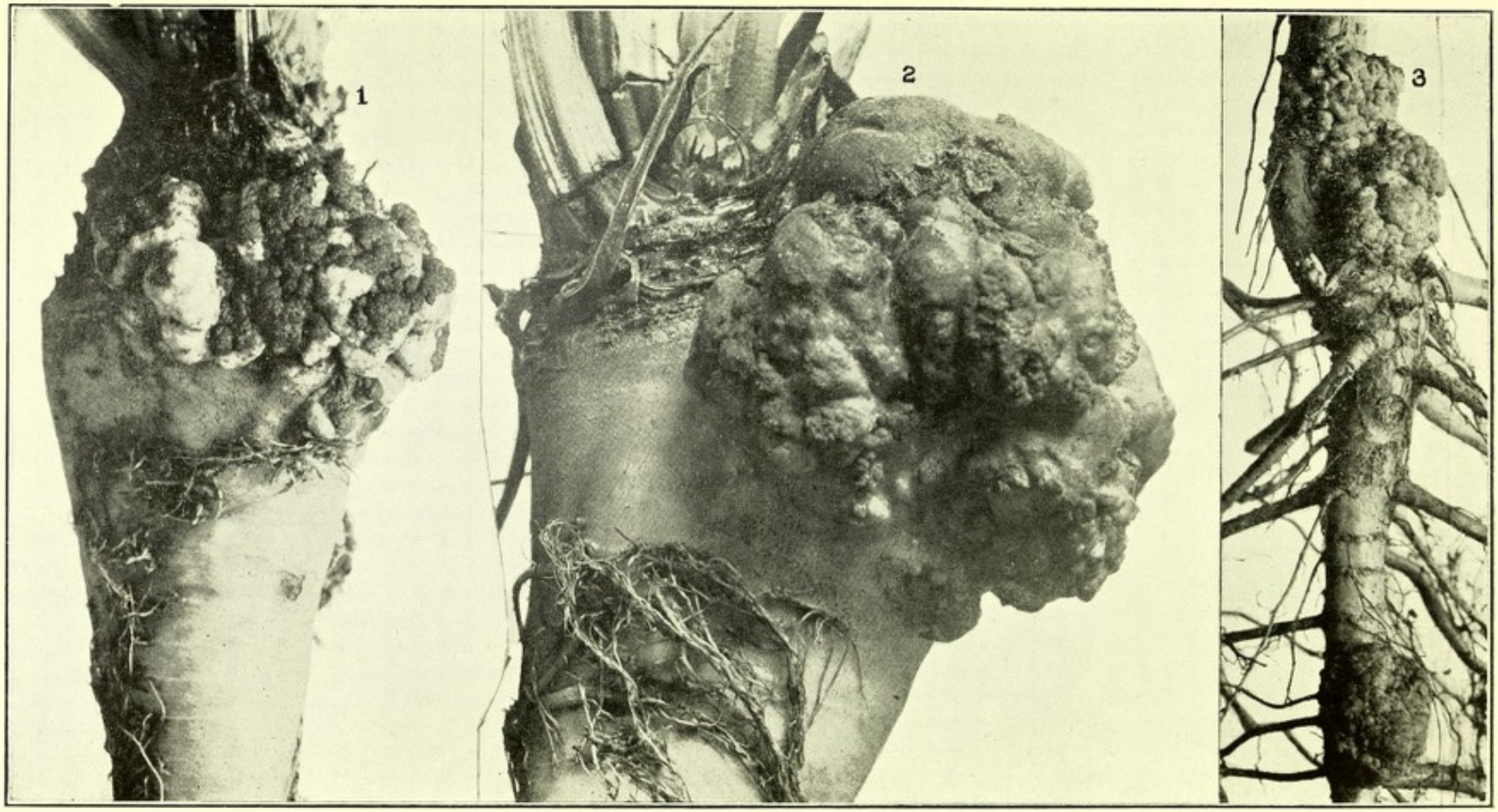

PLATE III. **Pure Culture Inoculations of Crown Gall.**

1. Grape on sugar beet, 47 days. Of 12 plants, 11 contracted the disease at place pricked. 1910.
2. Hop on sugar beet, 60 days. Inoc. in 1910 from about 26th sub-culture. Of 5 plants, all developed tumors.
3. Peach on peach, 50 days. Of 60 trees inoc., 55 developed tumors. The checks remained free. 1908.

one, would not enable any one to decide with certainty whether the granulations visible in certain cells should be regarded as the plasmodium of a myxomycete, or only as products of cell disorganization, which are often peculiarly liable to misinterpretation. The writer saw and studied Prof. Toumey's slides, but was never convinced that the organism described and figured by him (Arizona Bull. 33, page 54, figs. 28 and 29) had any connection whatsoever with the cell-changes in the gall also described and figured by him, *e. g.*, those on the plate facing page 49. But even granting Toumey's interpretation as correct, the etiological significance of the plasmodium has not been made out, since all sorts of non-parasitic soil organisms are likely to occur in galls when they are of some size. Toumey inoculated only ten plants altogether and obtained successful infections on *three* only of the ten. The inoculations were made in a country where the disease prevailed extensively and with spore material taken from the *cut surface of the gall*. The *Dendrophagus* spores, therefore, would be liable to contamination with anything occurring on the cut surface, especially the virulent schizomycete which can be demonstrated to be present in such galls and with which in pure sub-culture the disease can be reproduced, if the plants are in a young and actively growing condition, 100 times out of 100.

The writer has never seen anything on or in crown galls which has in any way led him to think that Myxomycetes have anything to do with their production. Phenomena similar to that described by Toumey were seen independently by the writer as long ago as 1893 in cells of the crown gall of the peach, and after six months' work upon the subject, the conclusion was reached that the appearances were artefacts and not living slime molds.

LABORATORY OF PLANT PATHOLOGY,
U. S. DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.