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A METHOD FOR THE CONTROL OF CROWN GALL IN THE APPLE NURSERY

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INTRODUCTION

The problem of crown gall in the root-grafted apple nursery is a very serious one. The nurseryman who has to discard only 5 to 10 per cent of his trees on careful inspection at digging time is usually considered fortunate. Losses of 25 to 50 per cent are not uncommon, and certain lots of trees in extreme cases have even shown a loss of 95 per cent. These serious losses to nurserymen and also the general interest of orchardists in obtaining disease-free stock for planting have made the writers feel the importance of this phase of the crown-gall problem. Present-day nursery stock, especially when grown by the root-grafting method, appears to contain a higher percentage of infection than formerly, and there also appears to be more trouble in recently planted apple orchards than in those planted a generation ago. Whether this supposition is true or not may be open to question. It may be that nursery and orchard trees are examined more critically as increased attention is given to orchard diseases.

Before the bacterial cause of this disease was known George G. Hedgcock, in his extensive studies of crown gall, tried many experiments in the propagation of root-grafted apple trees and made many observations which resulted in progress toward the control of this disease. The discovery of the bacterial nature of this previously obscure disease by Erwin F. Smith and his associates in 1907 marked

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a real epoch in the history of crown gall and appeared to open possibilities of finding satisfactory and practical remedies for the trouble. Nevertheless, in so far as the control of crown gall in the apple nursery is concerned, no very extensive practical applications of these important discoveries have as yet materialized.

This circular is published for the purpose of making immediately available to nurserymen and others interested in the propagation and growing of root-grafted apple trees in nurseries a new method of greatly reducing the attacks of crown gall. This preliminary report, to be followed later by a technical paper containing full details, also discusses briefly certain problems concerning the diagnosis of crown gall. It is hoped that the results of these experiments will tend to answer some of the questions concerning the identification of this disease.

FIG. 1.—Typical trees classified as clean

EXPERIMENTS AND RESULTS

As early as 1909 the senior writer used a formaldehyde solution as a dip for apple stocks and scions before grafting, as a means of controlling crown gall. Observations always indicated the practicability of this method, and nurserymen accordingly have been advised in correspondence as to this treatment for at least a decade. The need of more exact knowledge was so apparent that in 1921 experiments were initiated in an attempt to obtain more definite information on this phase as well as on related phases of the subject. This investigation, covering a period of five years, appears to confirm conclusively the observations of the writers extending over a much longer period. In these experiments this formaldehyde-solution treatment with modifications has consistently proved of value in controlling the crown-gall disease on apple grafts. For the past three years one of the organic-mercury compounds has also been tested as a dip and has proved distinctly more efficacious than the formaldehyde-solution treatment. Mercury compounds are among the most powerful of germicides, but the inorganic compounds, such as corrosive sublimate, are often very injurious to plant life, even in extremely dilute solutions. A number of new organic-mercury compounds have recently been put on the market and have proved of value in the control of other plant diseases. This particular one was tried in the hope that it would be effective in controlling crown gall and would be noninjurious to the apple

grafts. This proved to be the case. In a number of experiments performed in 1925 complete confirmation of results of experiments initiated in 1921 with the formaldehyde solution and in 1923 with the organic-mercury preparation has been obtained. In practically all of these experiments the grafts were wrapped either with raffia or with a cheap grade of muslin in such a manner as to cover completely the graft unions. The trees were dug as 1-year grafts. Neither the stand nor the amount of growth was reduced by the use of the organic-mercury compound as compared with untreated grafts grown as checks. The trees were classified into three grades: (1) Galled trees, with further subdivision into those with large and those with small galls; (2) doubtful trees, that is, trees exhibiting symptoms so slight as to be open to question or difference of opinion; and (3) clean trees, that is, those trees which showed no symptoms of gall or hairy-root. It should be stated that practically all of the so-called doubtful trees would have been classified as clean trees in an ordinary inspection. The accompanying illustrations were made from photographs of trees grown in the experiments of 1925. Figure 1 illustrates examples of clean trees. Figure 2 illustrates the type of tree which has been classified as possessing a small gall, whereas Figure 3 illustrates two types of trees classified as having large galls, which have invariably encircled more than 40 per cent of the circumference of the tree. Figure 4 represents one of the types of trees classified as doubtful.

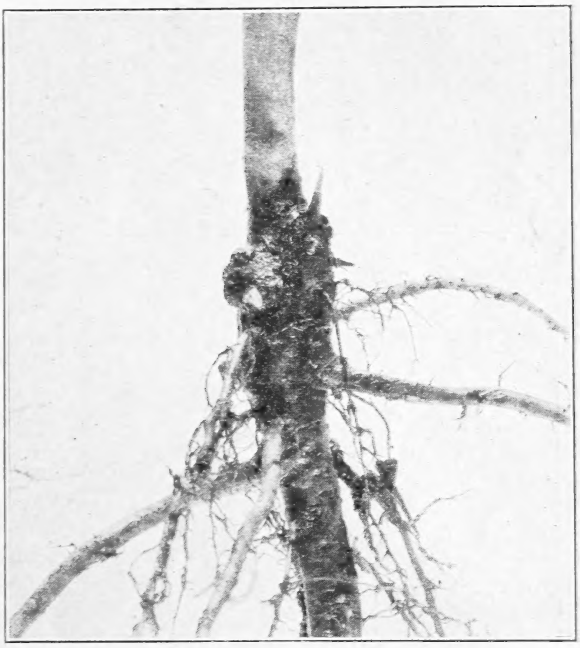

FIG. 2.—Type of tree classified as possessing a small gall

TABLE 1.—*Comparison of results obtained with all grafts treated with the organic-mercury compound and untreated grafts grown as checks*

Treated grafts			Untreated grafts		
Number	Percentage of galls		Number	Percentage of galls	
	Large	Total		Large	Total
2,619	2.0	6.1	2,619	28.7	32.6

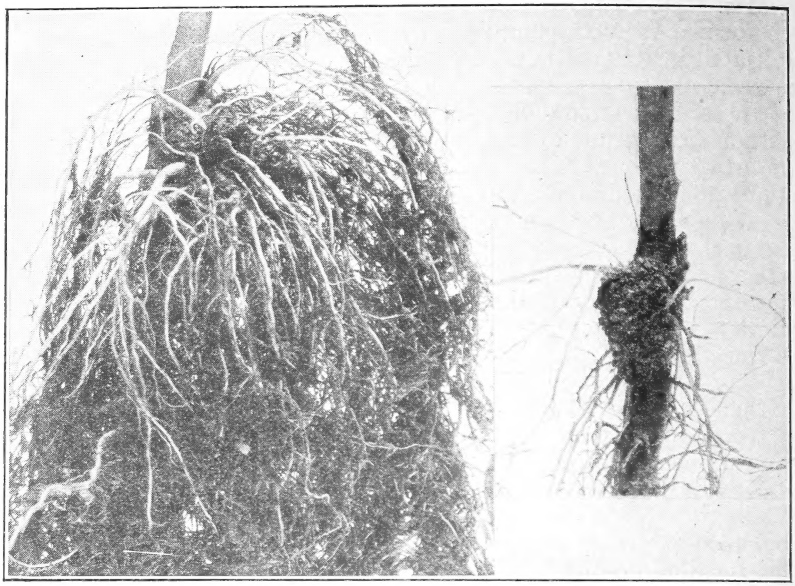

FIG. 3.—Typical trees classified as possessing large galls, one (left) showing the woolly-knot type and the other a simple form of crown gall

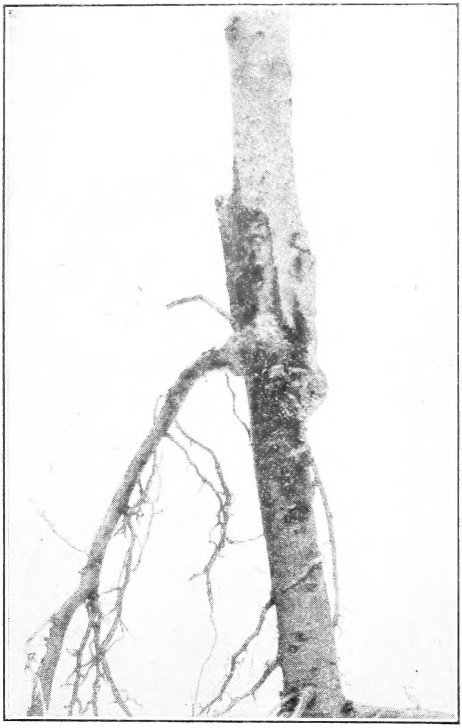

FIG. 4.—One of the types of trees classified as doubtful

In the experiments of 1925 the organic-mercury treatment was given to 2,619 grafts, representing a number of different varieties, with the result that the total number of trees obtained at digging time with both large and small galls amounted to 6.1 per cent, as compared with 32.6 per cent on 2,619 untreated grafts of the same varieties which were used as checks. The proportion of the total number of large galls in the treated grafts of all varieties was 2 per cent and in the untreated or check grafts 28.7 per cent, as shown in Table 1.

Figure 5 represents a typical lot of trees resulting from the use of the organic-mercury compound. This lot was selected because it illustrates about the average control obtained in the experiments in 1925, whereas

Figure 6 shows the corresponding lot of trees resulting from untreated grafts which were used as checks. The variety used in each case was Summer Rambo. In both illustrations the galled trees are on the left, the doubtful trees in the middle, and the clean trees on the right. Figure 7 shows a lot of Ben Davis trees selected as a sample of some of the best results obtained from grafts treated with the mercury compound, and Figure 8 shows the corresponding lot of Ben Davis trees resulting from untreated grafts used as checks. Reading from left to right, in both illustrations, it will be seen that there were no galled trees grown from the treated grafts, as compared with 48 galled trees in the check lot. This, of course, represents an example of absolute control that has not been met with very often in the experiments.

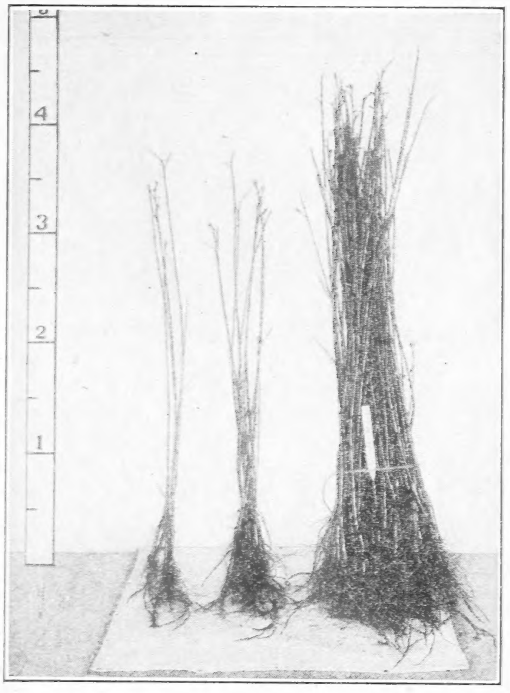

FIG. 5.—One of the lots of trees which after classifying, represented approximately the average of results obtained with the organic mercury-compound treatment. Variety, Summer Rambo. Reading from left to right: Galled trees (5), doubtful (11), clean (77)

DISCUSSION

Recently Riker and Keitt[1] have advanced the working hypothesis that "the malformations dealt with in the rejected nursery trees were not induced by the crown-gall organism." This hypothesis was formulated because of their negative results in attempts to isolate the organism from 175 trees which had been rejected on account of malformations supposedly due to crown gall. Since apparently similar malformations were not only controlled in size but also were largely prevented from forming by the action of germicides in the writers' experiments, their results are not in accord with the hypothesis of Riker and Keitt. It is difficult to assume that these malformations are due to any agency other than a pathogenic organism, since they are so definitely prevented by germicides.

Referring to Riker and Keitt's statement that malformations occur on grafts treated as aseptically as possible and "that gall-like formations may develop without the intervention of *Bacterium tumefaciens*," attention is called to the fact that Hedgcock[2] performed almost identical experiments more than 15 years ago and arrived at the following

[1] Riker, A. J., and Keitt, G. W. Crowngall in relation to nursery stock. *In* Science, vol. 62, p. 184–185. 1925.

[2] Hedgcock, G. G. Field studies of the crown-gall and hairy-root of the apple tree. U. S. Dept. Agr., Bur. Plant Indus. Bul. 186, 108 pp., illus. 1910.

significant conclusions: "The two regions where the formation of callus in root grafts was most abundant * * * coincide exactly with the position of more than 90 per cent of the galls, * * * indicating a close relation between the formation of callus and the development of the disease." All of the writers' observations and experiments furnish additional evidence to support these conclusions of Hedgcock.

It should also be noted that the failure to find a parasitic organism or agency in diseased tissue at any given time does not necessarily justify the conclusion that the organism or agency is not present or that it has not been present at some previous time. Until conclusive evidence is brought forth proving that malformations "supposedly crown gall" are not due to the strongly pathogenic organism *Bacterium tumefaciens* Sm. and Tn., the present rigid inspection and rejection of crown-gall trees should be maintained. It is considered that this particular question is of vital importance to the orchardist.

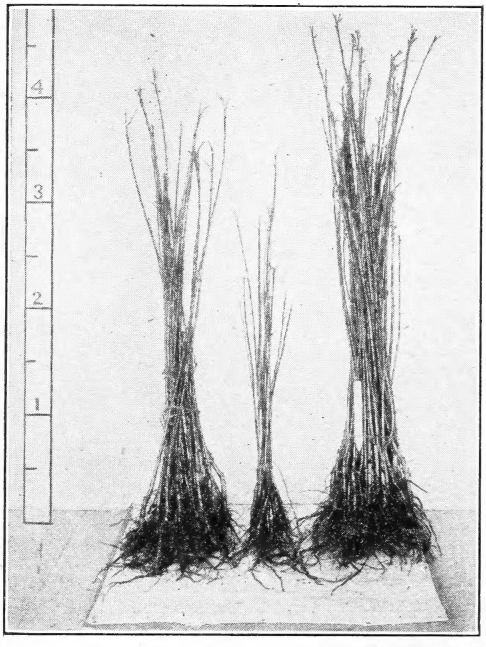

FIG. 6.—Lot of untreated Summer Rambo trees grown as checks for those shown in Figure 5. Reading from left to right: Galled trees (29), doubtful (14), clean (57)

RECOMMENDATIONS FOR CONTROL

Seedling apple trees of 1-year's growth ordinarily show a certain amount of hairy root and crown gall. These seedling trees with either type of the disease when lined out for budding or when used for grafting continue to carry the disease, in the great majority of cases at least. The experience of the writers is that the apple-seedling growers inspect the seedling stocks rather critically. Only an occasional gall and a few hairy-root trees may escape their scrutiny. However, when these diseased stocks are planted out or cut up into pieces for piece-root grafting, the nurseryman is simply propagating the disease. The best practice, therefore, is to inspect critically the seedling stocks before using them for grafting and to burn all the obviously infected ones. It has been generally accepted by both pathologists and nurserymen, at least since the work of Hedgcock, that the selection of roots free from crown-gall and hairy-root infection is an important factor in the control of this disease.

Since this disease is caused by a germ easily killed by germicides when it can be reached and since this germ may be present over the surface of the roots, the problem of killing the organisms present on the surface and of disinfecting the material resolves itself into

the question of finding a suitable germicide or fungicide and a suitable dosage which will kill the bacteria and not materially injure the roots of the trees. In this respect the organic-mercury compound, used as a dip in these experiments, represents a distinct improvement over the formaldehyde solution.

Well-made grafts properly fitted, especially those which have no overhang at the lower end, were shown by Hedgecock to be less susceptible to crown gall. They have, in fact, less exposed callus. The wrapping, of course, should be thoroughly done, whatever method is selected. Since the material has to be handled so much and since the freshly made grafts will stand this disinfectant, part of the writers' experiments also included dipping[3] the grafts the same day they were made, in order to disinfect and saturate the wrappers with the germicide and to give a second coating to the grafts themselves.

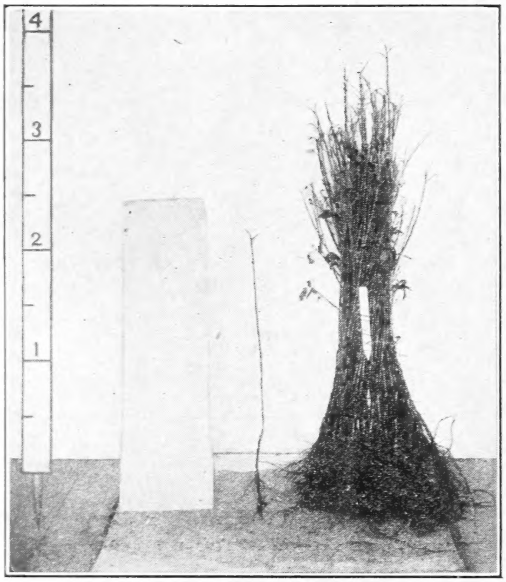

FIG. 7.—Lot of Ben Davis trees selected as showing some of the best results obtained in controlling crown gall by the use of the organic-mercury compound. Reading from left to right: Galled trees (none in this case), doubtful (1), clean (99)

Since there is still a possibility, after the grafts have been stored and the young tender callus developed, that this may carry incipient infections, and as it is desirable to give another dose of the germicide just before planting, a third dipping[3] was tried and found to be noninjurious to the graft and helpful in the control. Melhus and Maney[4] also report the use of Bordeaux mixture as a dip for apple grafts.

There is a possibility that the grafting knives or other tools and the benches and tables may become infected. It is not probable that all obscure cases will be eliminated in the inspection, and dormant infection would, of course, escape notice. Furthermore, the knives may cut through bacterial infection beneath the thin outer bark which was not reached by the disinfectant. Although there is no definite proof that stock can be contaminated with crown gall by means of grafting knives, there is proof that the pear-blight organism can be carried on grafting and pruning knives. In view of the fact that it is rather easy occasionally to disinfect the tools and grafting benches, it is thought good practice to utilize this method of control, even though its value is not fully proved.

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[3] Later experiments in a repetition of these treatments have tended to minimize the importance of the second and third dipping and to increase the importance of the first dipping.

[4] Melhus, I. E., and Maney, T. J. A study of the control of crown-gall on apple grafts in the nursery. Iowa Agr. Exp. Sta. Research Bul. 69, pp. 159–172. 1921.

The following treatment of apple grafts for the control of crown gall is recommended as a result of a large number of experiments over a number of years:

(1) Select seedlings free from hairy-root and crown-gall infection.

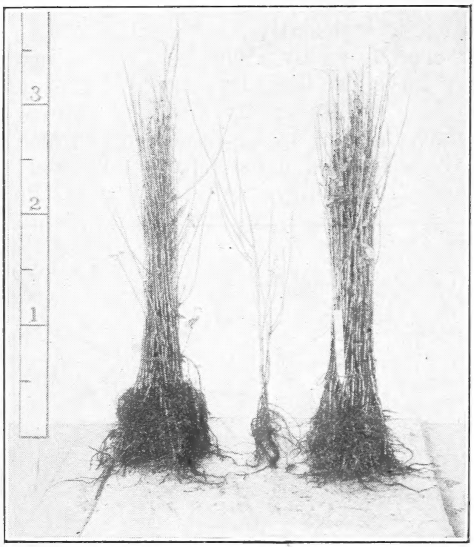

FIG. 8.—Trees from untreated Ben Davis grafts grown as checks for the lot illustrated in Figure 7. Reading from left to right: Galled trees (48), doubtful (4), clean (61)

(2) After washing, dip for 10 minutes uncut seedlings and scions in a solution consisting of 1 part of hydroxymercurichlorophenol[5] to 400 parts of water (approximately at the rate of 1 ounce to 3 gallons). In this as well as in subsequent dips do not rinse with water, and keep the solution in either a wooden or a nonmetallic container. The uncut seedlings and scions should be allowed to become surface dried before cutting for the grafting operation.

(3) Dip the grafts, which should be well fitted and carefully wrapped, in this same freshly made solution for about 5 seconds.[6]

(4) Store grafts under cool conditions and dip them for about 5 seconds in a freshly made solution of the mercury compound immediately before planting.[6]

(5) Keep the bench on which the grafting is done, as well as all grafting tools, disinfected by frequent washings with a germicide.

[5] Obtainable on the market in powdered form under the trade name "Semesan."

[6] Later experiments have shown excellent control with the first dipping alone and very little additional benefit from the second and third dippings. Since there has been found to be some danger of injury, under unfavorable conditions of storing and growing the grafts, by the second and third dippings here suggested, it is possible to eliminate these additional dippings without appreciable loss of control.

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