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FIELD EXPERIMENTS WITH CROWN GALL, 1913-1917

B. YOUNGBLOOD, Director.
COLLEGE STATION, BRAZOS COUNTY, TEXAS

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FIELD EXPERIMENTS WITH CROWN GALL, 1913-1917

BY

H. NESS, M. S., HORTICULTURIST IN CHARGE.

DESCRIPTION OF DISEASE

Crown gall is a cancerous growth occurring on a large number of both cultivated and wild plants. It is more common on woody or shrubby perennials than on plants more succulent and of shorter life, although several of that class are subject to its attack.

Among the worst sufferers are the following: peaches and other stone fruits, apples and other core fruits, grapes, blackberries, and raspberries. Besides these, which constitute the bulk of our orchard and garden fruits, many forest trees and shrubs have been found to be more or less ready subjects to it.

SYMPTOMS.

The symptoms of crown gall vary somewhat for the different species of host plants. In some the attack is on the root, immediately under the surface of the soil, as in the peach and the apple. In others, both the root and the stem may become the seat of the disease, as in the grape. In still other species it prefers the stem and branches, as in the olive, whence it has been known as the "olive knot" in Southern Europe. The young and growing parts, whether roots or stems, are the places of the attack: especially is a wound or bruise on such tissue an easy starting point. The disease can be communicated to any part of the host plant through wounds. For instance, in pruning peach trees preparatory to planting, it is easy to infect the sound tops from the diseased roots with the pruning shears. Galls from that cause have arisen in my experiments with the peach.

The first appearance of the disease is signified by a small swelling of soft, fleshy tissue, not very different from a wart or similar swelling not due to pathogenic causes. Soon the swelling outgrows the surrounding tissues and becomes a rough-surfaced tumor, globular, or elongated and irregular in outline, as its growth takes place faster in one direction than in another. While the edges of the tumor are constantly extending into new and sound tissues, the central or older portions of it decay, producing a deep cavity of dead tissue on the host plant. This cavity obstructs the flow of the sap and gradually reduces the vitality of the affected plant.

Dr. George Hedgecock, who has given this disease a most thorough investigation in the apple, recognizes two forms of tumors on that host, namely, a soft form and a hard form. The soft form is similar to the soft gall of the peach, and, according to Dr. Hedgecock (National Nurseryman, August, 1910, Vol. XIX) either rots away and disappears or becomes a hard gall. He also states that in his orchard experiments a large per cent. of the apple trees diseased with the crown gall (soft gall) recovered entirely from the disease; many others developed hairy roots of the woolly knot from the surface of the hard crown gall.

In the peach the recovery from the soft gall, or crown gall, must be extremely seldom. A case of it has never come to my notice, in spite of the large number of affected peach trees handled in my investigations. I have heard of such spontaneous cures in the peach, but I must conclude that they are so rare that crown gall in the peach may be looked upon as incurable. The surface of the hard gall in the apple becomes densely covered with threadlike roots, called whiskers. According to Dr. Hedgecock this form is less contagious than the soft gall of the apple—but surely fatal. Besides the whiskered roots in the apple, there are other deformities, such as the loss of the tap-root, and in its place the production of many weak, shallow-growing lateral roots. The same changes take place even more decidedly in the root system of an affected peach; its tap-root is early dwarfed, while weak and deformed laterals are multiplied.

The progress in the growth of the tumors varies not only with the species of the host, but also with the individuals of the same varieties; some trees living for several seasons without showing any marked distress, while others show unthriftiness from the start. The usual appearance of the affected tree will, however, soon show decrease in vitality and loss in the density and luster of the foliage, feebleness in growth of the shoots, and reduction in the size and quality of the fruit. Thus no abatement in this condition of the tree, or cure, need be looked for, since the very soil in which it stands has become a source of infection.

TRANSMISSION.

Crown gall is very contagious, so that a single infected tree in an orchard is a menace to every sound tree in that orchard. Infection takes place with great ease and rapidity. In the cultivation of the orchard or nursery, the plow or cultivator moves the dirt from an infected tree to the sound trees. Heavy showers and burrowing rodents are powerful distributers of the infection. A sick tree heeled in with well trees will, in a short time, start the galls on the wounded roots of the healthy trees, provided the temperature is sufficient to allow healing of wounds. Infection may also take place in a shipment in which unsound trees are packed with sound trees.

The absence of galls is no certain sign of non-infection. In 1913 I needed a number of young peach trees infected with crown gall for use in my experiments. They were obtained by heeling in for about a month's time the needed number of sound trees, mixed with a similar number of affected trees. On the first of February, when the sound trees were treated with the others, they showed no sign of infection except for the rather marked callous of the wounds at the ends of the amputated roots. Ten trees of the healthy group planted without treatment or disinfection developed severe galls during the season's growth in the test-orchard. The land upon which these trees grew while developing galls had for several seasons been planted in sorghum and other grass-like crops, hence was not liable to previous crown gall infection.

DISTRIBUTION.

Though crown gall is peculiarly a nursery disease, it also occurs on wild vegetation. Land freshly cleared from the forest is, therefore, likely to be infected, especially where patches of blackberries and their relatives have existed. Freshly cleared land should not be planted to orchards. But after several seasons in the ordinary farm crops, the danger of the original infection will pass. This disease has been known for a long time in Europe, principally in the olive and grape-growing regions. Its destructiveness has, however, been much more marked in this country, particularly in the peach-growing regions of the South, where the larger number of nurseries and peach orchards are more or less infected.

PROBABLE CAUSE OF RAPID SPREAD.

The rapid spread of crown gall in Texas seems to date back to the time when the planting of commercial peach orchards had its great boom; that is, twenty-five to thirty years ago. The old family orchards consisted mainly of selected seedlings of the Indian Cling type —a peach celebrated for longevity and hardiness. In the orchards and nurseries of that time crown gall was unknown or very little known. The great freedom from this disease was evidenced by the uniformly long life of the peach tree. The grafted trees of thirty years ago were also more regular bearers, and had on the average a much longer life than our present peach trees. The reasons for these facts can be easily explained, when we remember that the modern peach is an abnormity selected for its fruit only; that its advent brought contempt and destruction to the old seedling orchards; and that the greatly increased demand for peach seed, for stock to bud on, had also to be satisfied from the same abnormity.

From an observation made in growing two rows of peach seedlings a few years ago, I am strongly of the opinion that peach stock obtained from seed now current in the market (practically the only kind available to the nurseryman) is much more susceptible to the crown gall than stock from the old-fashioned Indian Cling seed. One of these nursery rows was planted with seed picked from Indian Cling trees which were more than twenty-five years old. The seedlings were left without budding the first year. In the spring of the next year, the second row, adjacent to the first, was planted with peach seed bought on the market. The next fall the trees in both rows were dug up. Those from the Indian Cling peach showed no signs of crown gall, while those of the second row, from seed current in the market, contained many galls. This occurrence, although not the result of an experiment nor reduced to experimental data, is indicative, nevertheless, of what facts experiments of this kind might bring out.

CLIMATE AND SOIL.

The climate of the South, with its frost-free and moist winters, is very favorable to the activity of this disease. Heeled in or newly transplanted trees show after a few weeks, even during the coldest part of the winter, evident signs of callous growth in the wounds and bruises upon the roots. Where crown gall infection is present, the superabundance of this callous growth is indicative of the beginning of the crown gall disease itself.

The behavior of crown gall, as regards soils, seems to be about the same as for the sandy, porous soils of East Texas and the stiff, impervious, gray clayey soils of Brazos county. For the black waxy prairie soils, it is declared to be less general, though not absent.

CROWN GALL DUE TO BACTERIA.

The bacterial origin of crown gall was proved only a very few years ago by Erwin Smith and his assistants, pathologists of the Bureau of Plant Industry, United States Department of Agriculture. Their researches are embodied in Bulletin No. 213, Bureau of Plant Industry, issued February 28, 1911, and prove beyond all doubt that the organism, which they have named *Bacterium turmifaciens,* is capable of inducing cancerous tumors on a multitude of widely different species scattered throughout the vegetable kingdom, from the sunflower family to the willow family. In regard to soft gall and hard gall, they found no difference as to causes and final effects. The effect of several germicides on the *Bacterium turmifaciens* in pure cultures was noted. Accounts of attempts to cure gally trees established in the orchard are mentioned (p. 184) as giving negative results. In some of these attempts solutions of bluestone were applied to the wounds left by the removal of the galls, but the galls returned.

FAILURE TO CURE TREES IN THE ORCHARD.

The futility of attempts to cure crown gall on trees established in the orchard is at once evident, when we remember that the extent of the disease cannot easily be determined, nor can remedies be applied without severe injury to the trees from the operation itself. The return of the galls is certain, because the protection due to antiseptics is only temporary and local, since the soil will remain infected beyond the reach of the disinfectants.

DISINFECTION BEFORE PLANTING.

So long as the absence of visible galls is taken as evidence of sound trees, crown gall will continue to spread in spite of all nursery inspection and care on the part of the nurseryman. No cure, or even mitigation of the disease, after the infected tree has been planted, can be hoped for. On the other hand, if the galls are removed in their initial stage and equal disinfection is given to all the trees, whether infected or apparently sound, immediately before the planting, absolute cure and future prevention of the disease would be the logical result.

EXPERIMENT TO DISCOVER PROPER REMEDIES

In February of the year 1911 an experiment was started by the writer for the purpose of discovering the proper germicides, their strengths of solution, and the time of exposure necessary to kill the crown gall germ, without killing or injuring the trees.

A large number of both peach and apple trees of transplanting age and size were secured, but as many of them proved to be so severely affected that they had to be discarded, only 150 peach trees and 50 apple trees could be included in the experiment. Even these were so severely wounded by the amputation of oversized galls that it was evident from the start that the experiments would not be conclusive. The trees most ideal for these experiments would be such as had evident galls, but so small that their removal would leave no serious wounds on the tree.

PLAN OF EXPERIMENT.

The trees were divided into series, according to the germicide used, and each series into lots (designated by letters), according to the strength of the solution and the length of exposure. Each lot of peach trees included five trees, while in the case of apple trees there were ten trees in each series with no lots.

The following germicides were used:

Series 1. Mercuric chloride (corrosive sublimate).
Series 2. Hydrogen-peroxide.
Series 3. Formalin (formaldehyde).
Series 4. Potassium permanganate.
Series 5. Copper sulphate (bluestone).
Series 6. Methyl violet.
Series 7. Salicylic acid.
Series 8. Mercuric chloride.
Series 9. Mercuric chloride.
Series 10. Murcuric chloride.
Series 11. Copper sulphate.
Series 12. No disinfectant used.

In order easily to bring weights and measures into accurate proportions, the metric system was used. The amount of water used in each case was 100 liters, which is nearly equivalent to 26.5 gallons. One hundred grams equal 3.527 ounces. For all ordinary purposes, a cubic centimeter of water equals one gram. After the galls had been removed and both the roots and the tops pruned, the roots and lower parts of the stem were submerged in the germicides for the lengths of time stated in the table.

The following table gives the scheme of disinfection and the results after a season's growth from the transplanting.

TABLE 1.

Peach Trees Disinfected February 13 and 14, 1911.

Series 1—Mercuric Chloride.

Lot.	Proportion.	Disinf.	Water.	Exposure.	At End of Season.	
					No. Alive.	No. Dead.
a	1:2000	50 gms.	100 l.	1-2 hr.	0	5
b	1:2000	50 gms.	100 l.	1 hr.	0	5
c	1:5000	20 gms.	100 l.	1 hr.	0	5
d	1:5000	20 gms.	100 l.	2 hrs.	0	5
e	1:10,000................	10 gms.	100 l.	2 hrs.	0	5
f	1:10,000................	10 gms.	100 l.	4 hrs.	0	5

All trees died before the end of the season, apparently due, at least in part, to the fatal nature of the corrosive sublimate.

Series 2—Hydrogen Peroxide.

Lot.	Proportion.	Disinf.	Water.	Exposure.	At End of Season.	
					No. Alive.	No. Dead.
a	1:2000	50 c.c.	100 l.	1 hr.	2	3
b	1:2000	50 c.c.	100 l.	2 hrs.	3	2
c	1:5000	20 c.c.	100 l.	2 hrs.	3	2
d	1:5000	20 c.c.	100 l.	3 hrs.	4	1
e	1:10,000................	10 c.c.	100 l.	3 hrs.	5	0
f	1:10,000................	10 c.c.	100 l.	5 hrs.	3	2

Galls returned on all trees that remained alive. Growth strong to very strong on the more sandy land of the lots "d," "e," and "f."

Series 3—Formalin.

Lot.	Proportion.	Disinf.	Water.	Exposure.	At End of Season.	
					No. Alive.	No. Dead.
a	1:2000	50 c.c.	100 l.	1 hr.	4	1
b	1:2000	50 c.c.	100 l.	2 hrs.	4	1
c	1:5000	20 c.c	100 l.	2 hrs.	3	2
d	1:5000	20 c.c.	100 l.	3 hrs.	4	1
e	1:10,000................	10 c.c.	100 l.	3 hrs.	4	1
f	1:10.000................	10 c.c	100 l.	5 hrs.	5	0

Galls returned in severe form on all trees alive. Some of the branches infected from the roots by the pruning shears. Growth of all trees, strong. Land, sandy.

Series 4—Potassium Permanganate.

Lot.	Proportion.	Disinf.	Water.	Exposure.	At End of Season.	
					No. Alive.	No. Dead.
a	1:500	200 gms.	100 l.	1 hr.	2	3
b	1:500	200 gms.	100 l.	2 hrs.	2	3
c	1:1000	100 gms.	100 l.	2 hrs.	3	2
d	1:1000	100 gms.	100 l.	3 hrs.	2	3
e	1:2000	50 gms.	100 l.	3 hrs.	3	2
f	1:2000	50 gms.	100 l.	5 hrs.	2	3

Galls returned in severe form on all trees alive. Growth, medium—weaker than in Series 3. Land, low, thin, compact.

TABLE 1—Continued.

Peach Trees Disinfected February 13 and 14, 1911.

Series 5—Copper Sulphate.

Lot.	Proportion.	Disinf.	Water.	Exposure.	At End of Season.	
					No. Alive.	No. Dead.
a	1:1000..................	100 gms.	100 l.	1 hr.	5	0
b	1:1000..................	100 gms.	100 l.	2 hrs.	1	4
c	1:2000..................	50 gms.	100 l.	2 hrs.	4	1
d	1:2000..................	50 gms.	100 l.	4 hrs.	2	3
e	1:4000..................	25 gms.	100 l.	4 hrs.	1	4
f	1:4000..................	25 gms.	100 l.	6 hrs.	2	4

In "a" galls present, some old scars clean. In "b" galls less severe, some old scars clean. In "c" galls less severe, some old scars clean. In "d," "e" and "f" galls light, fresh old scars clean.

Owing to a lack of suitable material, the investigation had, after the completion of Series 5, to be curtailed to five trees or one lot in each series.

	Proportion.	Disinf.	Water.	Exposure.	At End of Season.	
					No. Alive.	No. Dead.
Series 6—Methyl Violet...........	1:4000	25 gms.	100 l.	3 hrs.	3	2
Series 7—Salicylic Acid............	1:500	200 gms.	100 l.	3 hrs.	1	4
Series 8—Mercuric Chloride.........	1:2000	50 gms.	100 l.	3 hrs.	0	5
Series 9—Mercuric Chloride.........	1:2000	50 gms.	100 l.	3 hrs.	0	5
Series 10—Mercuric Chloride........	1:2000	50 gms.	100 l.	3 hrs.	0	5
Series 11—Copper Sulphate........	1:1000	100 gms.	100 l.	3 hrs.	9	1
Series 12—No idsinfectant used.....	....	....	....	....	10	0

SUMMARY OF RESULTS.

In none of the series preceding Series 5 was there the slightest evidence that the remedy applied had in any way affected the progress of the disease. In Series 5, although every tree was affected, none of the galls arose from the edges of the old scars left by the removal of the gall previous to disinfection. The new galls had the appearances of being of recent origin. Their tissues were turgid and of a fresh, white color, showing rapid growth.

The galls in Series 2, 3 and 4 were mainly situated in scars after the old galls. Their tissues were matured and discolored. In many cases decay had started in their centers.

In Figure 2, No. 5, from Series 5, Lot "a," the profile of a young gall is visible to the right on the top of the upper cluster of root branches, while immediately underneath these is a large black patch showing the scar of an old amputated gall. By close inspection, it will be seen that the scar is clean and that sound healing has made good progress around its edges. Small fresh galls are seen in other places, especially at the collars of the lateral branches of the tap-root. The specimen, being one of the five trees of Lot "a," Series 5, was selected as a fair sample of its kind.

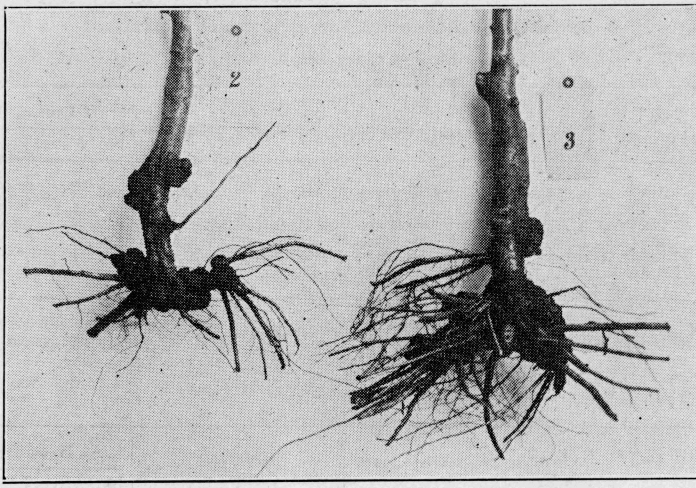

Figure 1.—Showing samples of trees of Series 2 and 3 at conclusion of experiment.

The trees of Series 8 were, upon examination, found to be affected with nematodes, and were therefore left out of serious consideration.

The treatment failed to remove the galls in Series 9.

Series 10 was composed of sound one-year-old seedling trees.

The results of Series 8, 9 and 10 were the same as those of Series 1—all trees died, unable to withstand the action of the mercury salt.

Series 11 consisted of ten sound trees heeled-in for a month with the infected material, so that callous growth was evident on the ends of

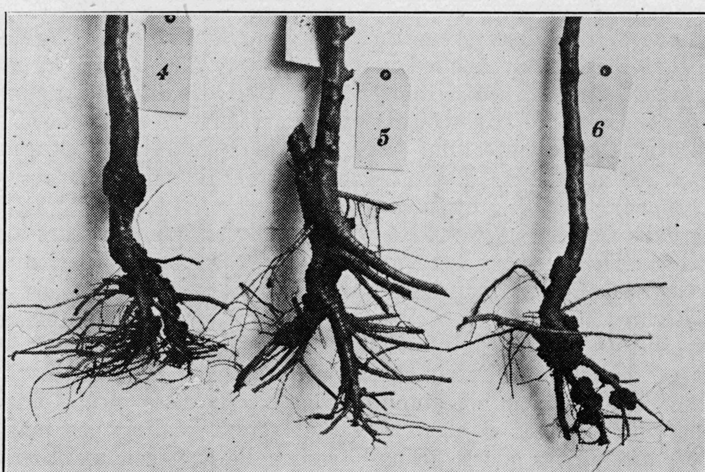

Figure 2.—No. 5 shows young gall and clean scar of amputated growth. Nos. 4 and 6 show similarly numbered series at end of experiment.

amputated roots. One of the nine trees which remained alive had acquired a small incipient gall.

Series 12 consisted of ten sound trees heeled-in with the infected trees, as in the case of those in Series 11, but not treated with any disinfectants before planting. All the trees lived, and made medium to strong growth; but all became strongly infected with galls.

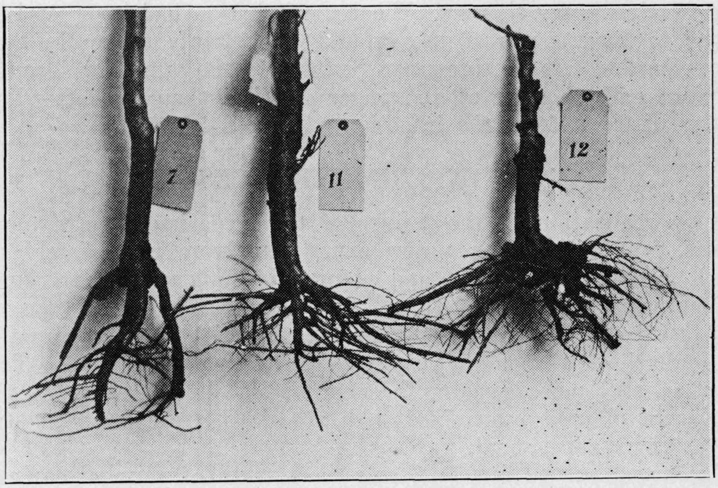

Figure 3.—No. 7 shows results of salicylic acid treatment; only living specimen. No. 11, sound tree after treatment with copper sulphate. No. 12, not treated with disinfectant.

APPLE RESULTS DIFFER SLIGHTLY.

In regard to apples, I wish to say that the tumors were so large that it was difficult to find fifty trees from which they could be removed without girdling the main root. They were, as above stated, divided into five series, and treated as in the case of the peaches, with the following solutions: first, mercuric chloride; second, copper sulphate; third, salicylic acid; fourth, methyl violet. The fifth was given no treatment beyond the amputation of the galls.

Many of the trees failed to revive, and those that did remained in a state of such low vitality that little conclusion could be drawn in regard to results of the treatment. It is, however, to be noted that *the ten trees dipped in mercuric chloride, the proportion being 1:2000 parts of water, and exposed to it for three hours, showed no greater mortality than the rest.*

COMMENTS ON RESULTS.

No positive results were obtained in these experiments, and indeed they could not have been expected when consideration is given to the severity of the galls with which the trees were affected. Yet one fact became patent after the season's growth, namely, that sulphate of copper, or bluestone solution, gave indication of ability to prevent the return of the removed galls, when the trees were treated with it as in these experiments.

The eventual return of the galls, or reinfection, in Series 5 and on one tree in Series 11, was to be expected, since both series were in rows contiguous to other series, where the disinfectants used proved ineffectual. The distance between the trees was only ten feet in each direction, with ground sloping sufficiently for a strong surface flow of water during heavy showers. That the infection had been carried from higher to lower parts of the ground was plainly shown in Series 5, where the first lots contiguous to Series 4 in the same row showed more incipient galls in spite of a stronger solution, than the latter lots at a greater distance from the infection of Series 4.

NEW EXPERIMENT NECESSARY

Upon realization of the failure of this experiment to give positive results, preparations for a new experiment were immediately started. Peach seeds were obtained and planted in sufficiently large quantities to insure ample choice of trees. The ground selected for the nursery had just been cleared of blackberries, of which a goodly number, on the lower half of the land, were affected with crown gall. To make infection still more certain, the peach seeds used on that part of the land were planted intermixed with the galls, fresh from the trees of the previous experiment.

The stand turned out fair, and the growth of young trees was also uniformly good. When dug during December, those on the lower ground were without exception affected with gall, while those on the higher ground showed no sign of galls. They were all heeled-in together and left until the first of February, when they were sorted out preparatory to disinfection.

The disinfection in this experiment was done on February 1, 1913. The planting could not be done on account of heavy rains until February 17. Meanwhile, the trees were heeled-in, each lot separated by a safe distance from the others. The land chosen for the planting had for several years been cultivated in corn or sorghum, hence not liable to contain any crown gall infection.

Each lot was in this case made up of ten trees, five of which had galls and five of which, to all appearances, were sound; but all of the same lot were disinfected similarly. Series 5 consisted of ten trees free from galls. They were planted without disinfection, as a check on the gall-free trees constituting half of each lot. The following table shows the treatment and results.

Peach Trees Disinfected February 1, 1913.

Series 1—Copper Sulphate, Bluestone.

Lot.	Proportion.	Disinf.	Water.	Exposure.	Results.	
					Originally Gally.	Originally Gall-free.
a	1:500	200 gms.	100 l.	1 hr.	5 gally, some sound scars.	3 trees alive 1 small gall 2 clean.
b	1:1000	100 gms.	100 l.	2 hrs.	1 gally, 4 clean sound scars	5 trees clean.
c	1:2000	50 gms.	100 l.	3 hrs.	4 gally, 1 clean	4 trees clean, 1 with gall.
d	1:2000	50 gms.	100 l.	6 hrs.	2 gally, 3 clean scars	4 trees clean, 1 with gall.

Series 2—Cromic Acid.

Lot.	Proportion.	Disinf.	Water.	Exposure.	Results.	
					Originally Gally.	Originally Gall-free.
a	1:2000	50 gms.	100 l.	1 hr.	5 trees, galls very severe	5 trees, galls very severe.
b	1:5000	20 gms.	100 l.	1 hr.	5 trees, galls very severe	5 trees, galls very severe.
c	1:5000	20 gms.	100 l.	2 hrs.	5 trees, galls very severe	5 trees, galls very severe.
d	1:10,000	10 gms.	100 l.	4 hrs.	5 trees, galls very severe	5 trees, some galls on branches very severe.

Series 3—Carbolic Acid.

Lot.	Proportion.	Disinf.	Water.	Exposure.	Results.	
					Originally Gally.	Originally Gall-free.
a	1:300	333 gms.	100 l.	1 hr.	5 trees, galls very severe	5 trees, galls very severe.
b	1:500	200 gms.	100 l.	2 hrs.	5 trees, galls very severe	5 trees, galls very severe.

Series 4—Formalin.

Lot.	Proportion.	Disinf.	Water.	Exposure.	Results.	
					Originally Gally.	Originally Gall-free.
a	1:500	200 c.c.	100 l.	1 hr.	5 trees, galls severe	5 trees, galls severe.
b	1:500	200 c.c.	100 l.	2 hrs.	5 trees, galls severe	5 trees, galls severe.
c	1:1000	100 c.c.	100 l.	4 hrs.	5 trees, galls severe	5 trees, galls severe.

Series 5—No Disinfectant.

Ten trees, free from galls when planted. Result—All ten severely gally when taken up the next winter.

INFERENCE FROM SECOND EXPERIMENT.

The results of this experiment are:

(1) A corroboration of the efficacy of copper sulphate as a cure an antidote for the crown gall.

(2) Proof that freedom from galls is not freedom from infection.

(3) Proof that infection is certain from even slight contact wit the disease under growing conditions, as shown by Series 5, as well a by the five originally gall-free trees in each of the lots under Series 1 2, 3 and 4.

Cromic acid, carbolic acid, and formalin showed no power to kee the galls from returning on originally gally trees, or to prevent the development on trees merely exposed to infection. The same appeare to be true for potassium permanganate, methyl violet, and salicylic aci used in the first experiment.

Most of the chemicals used in these tests have a greater germicid

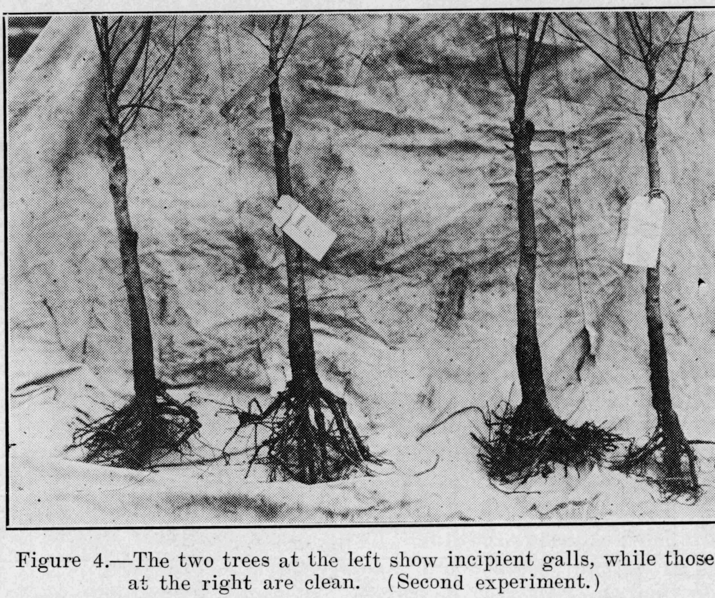

Figure 4.—The two trees at the left show incipient galls, while those at the right are clean. (Second experiment.)

power than copper sulphate. It seems strange, therefore, that th alone showed effectiveness. This fact must be due to the chemic affinity of the copper sulphate for the albuminoids of the living cel in both the host and the bacterium. This chemical action consists the union of the copper sulphate with the vital substance of the cel forming an insoluble precipitate within the cells penetrated by the sol tion. Thus both the disinfection of the wounds and the sealing them against further infection is accomplished.

Figure 4 shows four representatives of Series 1, Table 2. The tv trees to the left are from the gally half of lot "a"; the two to the rig are from the corresponding part of lot "b." On all four trees, t clean scars left from the removal of the galls are fairly visible. T peculiar formation of the root systems is due partly to the transplanting, partly to the galls during the first year's growth, or during the seedling year.

By referring to Table 2, Series 1, it will be seen that the treatment in lot "b" was apparently more effective than in lot "a," although the weaker solution of "b" was supposed to be equalized by the longer exposure. No inference can be drawn from this sole occurrence in regard to the comparative virtues of a stronger solution and a shorter exposure, or a weaker solution and a longer exposure. It is, however, a subject worthy of a special experiment with a larger number of trees.

In lots "c" and "d" the solution was undoubtedly too weak, although most of the old scars were clean and the new galls were most likely due to late infection.

Figure 5.—Representatives of Series 2, "a" and "b," one year after carbolic acid treatment. (Second experiment.)

Figure 7 shows representative specimens of Series 5 (Table 2) after a year's growth in the plat. When planted they were free from visible blemish, but were infected and served as a check on the gall-free but similarly infected trees in the lots. These trees prove very conclusively that infection is certain, even from short contact, and that absence of galls is no proof of non-infection. The galls of these trees showed evidence of a whole season's growth, and the older parts of the galls were in many cases dead and decaying, most likely due to infection before planting.

In these experiments positive and absolute results are lacking, but after the completion of the above attempts, strong circumstantial evidences have been gained, showing that copper sulphate alone is worthy of further trial—a fact that will simplify future experiments.

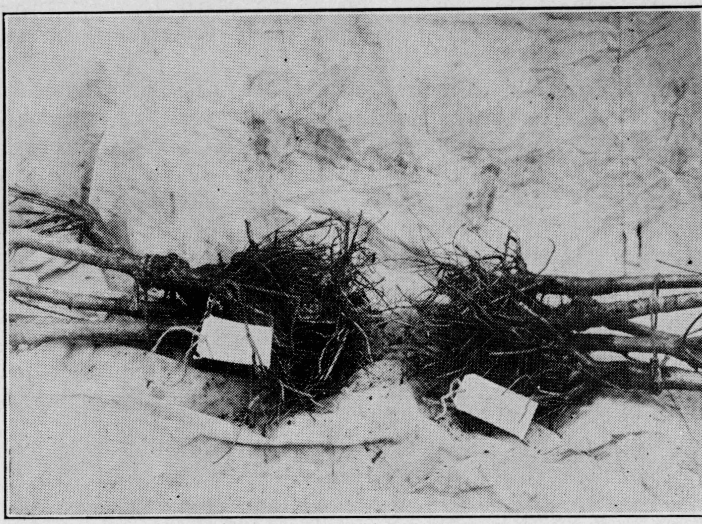

Figure 6.—Representatives of Series 3, "a" and "b," one year after carbolic acid treatment. (Second experiment.)

The following propositions also arise:

(1) In both of these experiments, trees treated with substances capable and incapable of disinfection have been planted in proximity to each other, and the consequent reinfection has, to a considerable degree, obscured the results.

(2) The data on the relative germicidal powers of the disinfect-

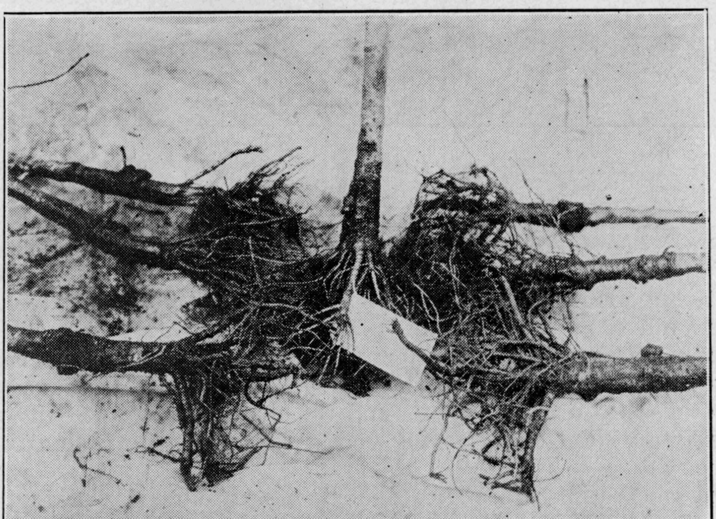

Figure 7.—Representatives of Series 5 one year after planting in experiment plat. (Second treatment.)

ants used were those obtained from pure cultures on artificial substrata, and may, therefore, be quite different from what they would be when obtained directly from the parasite on its natural host plant. Hence, the recorded figures of the above original may mislead, instead of guide, in experiments of this kind.

(3) The approximate degree of concentration of the copper sulphate, which will disinfect the host without endangering its life, is yet to be determined. This can best be done by experiments wherein all other substances are excluded, and copper sulphate alone used in solutions of various strength and times of exposure.

FURTHER EXPERIMENTATION

To clear up these problems, a new lot of peach seed was sown in infected ground during February, 1914. A year later the seedlings were taken up preparatory to disinfection and replanting. The galls on them had become very large, so that their amputation left rather severe wounds. A hundred of these trees, together with twenty out of fifty very gally apple trees, were selected for the experiment.

The mortality of the peach trees and their unthriftiness were such that no results were obtained. The twenty apple trees suffered much from the amputation necessary to remove the overgrown galls, but were, nevertheless, made use of and divided into two series. The solution, which was the same for both lots, was copper sulphate in proportion of 1 part to 250 parts of water, or 400 grams to 100 l. water. Series 1 was given two hours of exposure: Series 2, three hours.

Results for Series 1: Seven trees living; growth 3 to 4 feet long; two trees with young galls; one without visible galls but with suspicious roots, yet not the typical whiskers; four cured, showing clean scars.

Results for Series 2: Eight trees alive; growth 3 to 5 feet; five trees cured, showing large clean scars in process of healing and normal roots; three trees reinfected, one of them with gall showing initial whiskers.

In these series the soil undoubtedly became infected during April, when heavy freshets broke over the nursery ground, on which the peach seedlings for this experiment had been grown.

Again, as the experiment failed to furnish absolute data, new peach trees were prepared, according to the same scheme and with the same purpose in view. These were planted in 1916 in a new place, far from orchard or nursery ground. But, owing to the prolonged rains, the planting became late, and the soil in less good texture. A prolonged drouth followed, in which nearly all the trees were lost, and the experiment consequently failed.

The apple trees in this, the third experiment, gave strong additional evidences of the disinfecting power of copper sulphate solution against the crown gall disease.

A new experiment will be made as soon as a proper number of trees suitably infected can be grown for the purpose. The object of the work will be to give answers to the questions remaining unsolved by the failure of the two last experiments and as stated in the three preceding propositions.

PRACTICAL INFERENCE FROM THESE EXPERIMENTS

Although these investigations remain incomplete and unfinished, this much is proved:

(1) That no nursery inspection is any guaranty or protection against the spread of the crown gall disease.

(2) That solutions of copper sulphate of proper strength and time of exposure will disinfect nursery stock infested with the crown gall disease preparatory to transplanting into the orchard.

(3) That infection of sound trees can very readily take place in the heeling-in trench, if they happen to be mixed with infected trees; and that the same thing consequently can take place in a shipment of trees so mixed.

RECOMMENDATIONS TO PLANTERS.

Bulletin No. 213, "Crown Gall of Plants: Its Cause and Remedy" (p. 196), issued by the Bureau of Plant Industry, Washington, D. C., charges the nurseryman with the duty of restricting and retrenching upon this disease, and recommends a very close nursery inspection.

It becomes very plain, even from these preliminary experiments, that neither the nurseryman nor the nursery inspector can effectively combat this disease. No active nurseryman can carry on his business isolated from other nurseries; that is, without exchange or introduction of new stock. He will, therefore, sooner or later have his premises infected.

The nursery inspector, even if present in the nursery to inspect the shipments, would be able only to reject those trees that showed the positive presence of galls, while as great or a greater number of invisibly infected trees would necessarily have to be passed up by him as sound stock.

Hence, it becomes self-evident that the planter can accept no guaranty, either from the nurseryman or from the nursery inspector; nor would it be reasonable for the planter to demand satisfaction from the nurseryman or inspector, if crown gall should develop in his plantation, provided the trees were, to all appearances, clean and sound at the time of delivery.

COUNTERACT SPREAD BY DIPPING.

What remedy is then left to counteract the spread of this terrible orchard and nursery pest? *Dipping in copper sulphate solution.*

When and where should this dipping be done? The dipping should be done immediately before planting, and the proper place is where the trees are to be planted, or on the planter's own premises.

It is evident that the disinfection could not safely be done in the nursery because, according to the nature of the disease, the infection would everywhere be lurking there while, on the contrary, the ground of the orchardist ought to be safe from probable reinfection.

What strength of solution, and what length of exposure to it, are proper? A positive answer on these points has not yet been obtained from my experiments, and must, therefore, be referred to the best indications observed.

In Table 2, Series 1, lots "a" and "b," give fair indications. Of these two, lot "b" is especially worthy of consideration; first, because there was no loss of trees; second, the disinfection was perfect, except in one case, where reinfection came from the soil, as evidenced by the clean scars upon the affected tree. In this case the proportion, as indicated in the table, was 1:1000, or 100 grams copper sulphate to 100 l. water, which, according to avoirdupois measure, is about 3.5 ounces of copper sulphate to 26 gallons of water, the time of exposure being two hours.

From my observations throughout these experiments, I am of the opinion that peach trees, uninjured by crown gall or other blemish, can stand the double of that dose. I recommend, therefore, that it be made: *7 ounces of copper sulphate to 26 gallons of water, and the exposure two hours.*

In the case of apple trees, which seem to demand and can stand a stronger dose, I recommend the proportion: *1 part of copper sulphate to 250 parts of water, or about 1 pound of copper sulphate to 26 gallons of water, and the exposure two hours.*

Washing off of the adhering dirt and pruning preparatory to planting should be done immediately before dipping. That is, the branches should be cut back to the proper number and proportion and the lacerated ends of the roots trimmed, leaving smooth, clean cuts. Too long and slender roots should be cut back proportionately to their thickness. The whole root system should be examined carefully for any suspicious swellings or protuberances which may be initial galls not yet broken through the epidermis. These, if discovered, should be cut out, in order to give the solution a chance to come into direct contact with the tissues of their interiors. The trees may be tied into bundles of convenient size for dipping, and each bundle given a label, indicating the time, so that the dipping may be done as uniformly as safety requires.

The bundle of trees should be plunged deep enough into the solution so that the trees are covered to a distance of several inches above the collars of the roots. The pruned tops should also be thoroughly wetted with the solution to prevent any infection being carried from the roots by the pruning shears.

This simple method of disinfecting is not only to be recommended for the orchard, but for the nursery as well. By taking advantage of this in all new planting on uninfected land, and in the various forms of nursery rotation, the infected area can gradually be restricted.