AN EXPERIMENT ON CONTROLLING THE CROWN GALL OF THE APPLE TREE

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Introduction

Each year the nurserymen in Virginia suffer heavy losses due to t the crown gall disease, especially in apple trees. As high as 35 per cent of the apple trees are sometimes infected and since the diseased trees cannot be sold many thousands of them have to be discarded and destroyed. If this disease can, in a mesaure, be controlled it can be readily seen that a tremendous saving will be made in the nursery industry. With this in mind the experiment on the control of the crown gall of the apple tree was begun.

History of Work Done on The Crown Gall Disease

(1)

According to Mr. Wm. B. Alwood, we had practically no acquaintance with crown gall of the apple tree previous to 1896. Early European writers however, reported the loss of many grape vines due to galled roots, and considerable work had been done on root galls of some other plants. (2) Smith who did the first work on the disease in the United States Departmant of Agriculture, in 1892, studied the disease as it occurred on peaches, but "did not then have bacteria in mind". Later, however, in 1904, according to him (Smith) the Bureau of Plant Industry received a number of Marguerite or Paris daisy plants all of which were affected with gall-like growths on various parts of the stems and leaves. He, with the assistance of Brown and Tyson, began examination and experiment with these. Bacteria in the interior of the undecayed galls were first detected by Mr. Smith in fresh unstained thin sections which had been made by Dr. Tyson.

For two years experiments in inoculation were carried on and in 1906 the bacteria was isolated in agar cultures. "Inoculations from these cultures gave rise to gall growths on the inoculated plants." Many plants were used in Mr. Smiths experiments and among them was the apple. The following is a synopsis of his conclusions respecting crown gall.

"The galls are often invaded by saprophytic bacteria, especially the softer galls. They also invite various parasites, nematodes, fungus root rot, fire blight of apple pear etc; and some of these are able to cause great a damage."

"Even the hardest crown galls are due to the bacteria which closely resemble those found in the softest."

"Overfed plants are more subject to the disease than those making moderate growth."

"The apple hairy root, hitherto a disease of unknown origin and supposed to be non-infectuous has been shown to be due to bacteria which actually and morphologically differ, if at all, only slightly from the crown gall organism. This causal organism is not located in the roots themselves but in the flattened tumor from which they arise."

"Typical hairynroot has been produced on sound apple seedlings by pure culture inoculations, and in the same way on sugar beet both galls and hairy root have been obtained."

"On the apple when the tumors are very fleshy decay sets in earlier than when they wre woody. The decaying or cracking galls affords opportunity for the entrance of rain water, and many sorts of insects, bacteria, and fungi

which bring about more or less destruction of supporting tissue not involved in the original tumor. Up to this time (1911) the best method of dealing with this disaase remains the old one of strict inspection of nursery stock and the condemnation of all trees and shrubs found diseased, and until we know to the contrary, excassive callous should be regarded as incipient gall. The nurserymans remedy lies in careful methods and the abandonment of infected soils".

(1)

In 1902, Alwood, carried on experiments at the Virginia Agricultural Experiment Station. In these experiments various schemes were tried out and the following paragraphs are conclusions from the results obtained.

"The organism which produces the abnormal growth known as crown gall on tha apple appears to gain entrance to the apple seedling in the nursery".

"The diseased seedlings can be detected by inspection. The unusual amount of fibrous roots at and below the crown being the characteristic depended upon for recognition of the trouble".

"Nurserymen can select the seedlings used so as to largely control this trouble. No one should expect to entirely prevent its occurrence in the nursery now that it has become so wide spread".

"Persons planting fruit trees should reject with the greatest care all trees which show the cancerous growth about the crown or a sufficient abnormal development of fibrous roots about the crown to warrant belief that the plants are diseased".

"Apparently, crown gall can be readily inoculated from a diseased plant into healthy ones, hence diseased plants should not be allowed to remain among healthy ones in the orchard".

Mr. George G. Hedgecock, of the United States Department of Agriculture, has done considerable work on crown gall and results of his work have been published in 1906,[3a-b] 1908,[4] and 1910.[5] The following paragraphs are taken from these publications.

In his publication in 1906,[3-a] -Crown Gall and Hairy Root Disease of the Apple, Mr. Hedgecock says that "hairy root is characterized both in seedlings and in grafted or budded trees by a stunted root system accompanied with excessive production of small fibrous roots, often originating in clusters from the main root or tap root. Galls often occur in connection with hairy root but these are a result of wounds rather than a form of this disease. Seedlings of the hairy root type, unless wounded remain free from galls".

"Apple crown gall is of two types. A hard callous form is common on grafted trees at the union of the root and scion, or at any other wounded place in the root system. The results of extensive inoculations with this type have failed to prove that this disease is of contagious nature".

"A second type is a soft form more common on seedlings, occurring more rarely on grafted trees. These soft galls often rot off. It is not certain, however, that they like the hard galls are replaced the following year by a new gall griwth from the ajacent live tissue of the host, nor is there proof yet that they are of contagious nature".

"Nurserymen are advised to be careful in the selectionof seedlings for grafting and budding. All rough, warty or galled seedlings should be thrown out for most of these will form rough rooted trees. Seedlings with tufted or hairy roots should also be rejected for these, as shown by experiments, develop into hairy rooted

trees with a very deficient root system. The hairy root disease, as it appears from the results of two years experiment is not contagious!"

(3b)

In another paper, in 1906, - The Wrapping of AppleGrafts and Its Relation to the Crown Gall Disease, in which work Mr. Hedgecock was assisted by Mr. Herman vonSchrenk, the results obtained by wrapping grafts with various materials are given. The kinds of wrapping were rubber, cloth, waxed paper, plain thread, waxed thread, and plain thread with the union waxed.

Conclusions from this experiment are- " In making grafts care should be taken to use root and scion pieces of as nearly the same size as possible, and that protection of the graft at the union will serve to induce better union and may also aid in keeping out disturbing factors".

"The use of either cloth or rubber as a material for wrapping apple grafts is recommended".

(4)

The work of Mr.Hedgedoock published in 1908 is the results of five years experimentation on the "Cross Inoculation of Fruit Trees and Shrubs with Crown gall". In these experiments a number og trees and shrubs were used but only the work pertaining to the apple tree will be included here.

"In the experiments with the apple, healthy seedlings were used. All these trees were wounded by making a downward slanting incision in the root. Some were used as checks and others were treated by inserting chips of apple crown gall, both hard and soft gall, in the incisions; all were wrapped as if grafted" The results of this experiment with the apple, Mr.Hedgecock says, "show quite conclusively that apple crown gall in its soft forms is contagious, but that in the hard form it is either slightly or not at all contagious".

The following paragraphs are the results of the five years experimentation as a whole, including work on all trees and shrubs.

"The soft galls from the almond, apricot, blackberry, cherry, peach, plum,

prune,and raspberry have been transferred easily to seedlings of the almond, apricot,peach and raspberry: less readily to those of blackberry,charry,plum prune,and pear;and with great difficulty to seedlings of the apple,chestnut, walnut,and rose".

In his"Field Studies of The Crown Gall and Hairy Root of The Apple" (5)
published in 1910,Mr. Hedgecock describes the various forms of crown gall and hairy root from observations and study in the field,and gives some recommendations suggested by the field study. The following are the recommendations given.

"In the nursery all diseased trees should be left in the field at the time of digging and burned as soon as dry".

"Get scions from healthy trees;never plant diseased trees in the scion orchard".

"Make close fitting root grafts,avoiding blunt ends of the root and scion in the union".

"Store root grafts preferably in sand".

"Plant the union of root grafts 3 to 4 inches below the surface of the ground".

In the résumé of,"The Structure and Development of Crown Gall : A (6)
Plant Cancer," by Smith,Brown,and McCulloch, a number of very interesting facts are given; some of them are included in the following paragraphs.

"Crown galls occur on a great variety of plants,but not always on the crown".

"They are injurious to the plant in varying degrees,depending on the

species,on the parts attacked,on the size and vigor of the individual etc. They are most injurioud to young and rapidly growing plants.

"Young,wellnourished,rapidly growing tissues take the disease more papidly than old or slow growing ones".

"The tissues of the gall multiply excessively and in opposition to the best interests of the plant".

"The gall tissue,which is often of a soft,fleshy nature is more subject to decay . It is not usually corked over,and the absence of a protective surface allows the ready entrance of water and other parasites".

"The tumor originates in the meristem,usually in the cambium region. It may perish within a few months or continue to grow (parts of it) for years".

"The relation between the host and the parasite may be regarded as a symbiosis in which the parasite has the advantage".

"The bacterium is a soil organism and planters should aim to keep their lands free from it by refusing to plant infected stock".

"Nurserymen should plant on uninfested land and carefully avoid heeling good stock into soil which has previously received infected plants. Nurserymen have been largely responsible for the dissemination of this disease".

"The organism is a wound parasite. Its entrance is favored by careless grafting,and by the presence of borers,nematodes etc."

Probably the most recent work on the infection and control of crown gall on the apple was done by Melhus and Maney (7) in Iowa. Their work consisted of two types of studies,viz. (1)infection and susceptibility of host tissues under diverse conditions,and (2) the influence of disinfectants and fungicides in preventing the development of crown gall.

A large number oftrees were studied in these experiments and various treatments,both in the infection experiments and in the disinfectant and fungicide experiments. Various methods of applying the treatments were also used in the experiments. Different methods of grafting,wrapping,and storing were also studied. The following are conclusions drawn from these experimants.

"The infection of apple grafts is readily accomplished by dipping the grafts just before planting in a viable bouillon culture of Bacterium tumefaciens. The majority of the galls occur at the union. The stock is less liable to become infected than the scion".

"Apple grafts were apparently equally susceptible to the crown gall organism where the callous was normal,excessive or slight".

"Well made and poorly made grafts showed little difference in the amount of crown gall that developed".

"Using an unusually large amount of string over the union of the grafs leads to girdling and excessive callousing of the trees,which seems to facilitate crown gall infection. Cloth applied over the union as a wrapper,either with or without string,decreases the amount of crown gall".

"Scion wood cut from trees infected with crown gall at the union did not show any increaseed amount of crown gall."

"Hairy root seedlings,when used as stock,did not transmit hairy root to the scion,but the stock portion of the graft remained infected in the majority of cases."

"Most of the crown gall infection takes place the first year on the grafts,during the formation of callous,at the union".

"Surface disinfection with formaldehyde (.16 percent), copper sulfate (.25 percent) and mercuric chloride (.1 percent), were seriously injurious to callousing of apple grafts. Fungicides which go into solution slowly, such as lead arsenate and bordeaux mixture, have a much less injurious effect on the callousing process".

"A strong bordeaux mixture (25-25-50) decreases the stand, tends to have a preserving action on the string and reduces the amount of crown gall".

"Resin sticker added to bordeaux mixture increases its toxic action and reduces the stand. The addition of lead arsenate or soaps to bordeaux mixture does not increase its toxic action on the grafts, but rather increases its adhesiveness and its fungicidal efficiency."

"More dilute bordeaux mixtures did not reduce the stand and proved nearly as beneficial in reducing crown gall as the stronger mixtures. The use of bordeaux mixture (8-8-50), with or without lead arsenate, reduced the percentage of crown gall 66 percent over the checks, and nearly 50 percent over the mean percent of crown gall in all the checks in the wealthy variety.

Experiment as Carried Out at W. T. Hoods Nursery
and at
The Virginia Agricultural Experiment Station .

The experiment was begun January 31, 1921, under the direction of Dr. F. D. Fromme, Plant Pathologist and Bacteriologist of the Virginia Agricultural Experiment Station. Suggestions offered by Dr. Fromme, and which were carried out, are as follows:

1.a.- Dip the cut surfaces of the stock and scion in a solution of copper sulfate, 1 pound to 10 gallons of water.

b.- Apply paste of copper sulfate and starch liberally on surface of the union with a brush then wrap in the usual way.

(Make the paste of ordinary clothes starch, to a pasty consistency and dissolve in it copper sulfate at the rate of 2 ounces to a quart of starch.)

2. Using 500 trees, divided into lots of 100 each and treat as follows:

Lot. 1. Make 100 grafts and store without treatment for checks.

Lot 2 . After scions has been cut for the graft, dip the cut end i in a suspension of crown gall organism. Maket the grafts and store without further treatment.

Lot 3. Treat scions as in Lot 2, make the grafts and tie them then paint the union liberally with paste of copper sulfate and starch.

Lot 4. Treat the scions as in Lot 2, allow dipped end to dry then dip in copper sulfate solution before union is made.

Lot 5. Treat scions as in Lot 4 and make the grafts, then apply the paste, giving both the dip and paste treatment.

3. Wrap some grafts with cloth, using strips about one inch wide and about four inches long. Dip about one half inch of one wnd of the cloth in hot grafting wax. Begin with the unwaxed end and wrap, press the waxed end down to hold the wrap.

4. Treat packing sand with formaldehyde, using one pint of 40% formaldehyde to fifty gallons of water, and apply one gallon of the solution to a cubic foot of sand.

That part of the experiment included in 1 a and b, 3 and 4 was carried out at Mr. W. T. Hood's Nursery, Richmond, Virginia, January 31 and

February 1, 1921. The following notes wontain the work done and results obtained. The varieties of which grafts were treated were Bonum and Stayman Winesap.

Bonum

Lot No. 1.

In this lot 475 grafts were used. After the unions were made and wrapped, the surfaces were painted with starch containing copper sulfate. The starch was in a pasty condition and was applied liberally with a brush.

Lot No.2.

In this lot 300 grafts were used. After the unions were made and wrapped the grafts were dipped in a copper sulfate solution so that the root and union were thoroughly covered.

Lot No. 3

In this lot 300 grafts were used. The cut end of the scions and the cut end of the roots were dipped in the copper sulfate solution before being united.

Lot No. 4.

In this lot 300 grafts were used. The unions were wrapped with strips of cloth about 1 inch wide and 4 inches long. One end of the cloth was dipped in hot grafting wax. Beginning with the unwaxed end the cloth was wound around the union and the waxed end pressed down.

Stayman Winesap

Lot No. 1.

In this lot 300 grafts were used. These were treated with the starch-

bluestone (copper sulfate) paste as was lot No. 1 of the bonums.

Lot No. 2.

In this lot 300 grafts were used. The cut end of the scions and the cut end of the roots (stocks) were dipped in the copper sulfate solution as was lot No. 3 of the bonums.

Lot No. 3.

In this lot 340 grafts were used. After the union was made the graft was dipped in a copper sulfate solution as was lot No. 2 of the bonums.

Lot No. 4.

In this lot 725 grafts were used. The unions were made and wrapped with cloth as was lot No. 4 of the bonums.

Soil Treatment

In this treatment 2 cubic feet of packing sahd was used. The sand was sprinkled with a solution of 1 pint of formaldehyde (40%) to 50 gallons of water. Applied 1 gallon of solution to a cubic foot of sand. The sand was covered and allowed to stand for 24 hours. It was the uncovered and allowed to "air"muntil it was dry enough for use. The grafts stored in this sand were not treated.

Results

In the spring at planting time when these grafts were examined it was found that all those treated with copper sulfate solution and copper sulfate starch paste were dead. No signs od callousing was evinced and the ends of the scions and stocks treated with the material were black and hard ,(dried-out-like.)

Two thousand and fifteen grafts were treated with the copper sulfate solutions and all were killed. This seems to prove conclusively that copper sulfate cannot be used as a preventative of crown gall, at least, at the strengths used in this experiment.

The grafts wrapped with cloth as described in notes were seemingly in good condition at planting time. These were examined on November 21, as well as could be done without taking them up, and they were found to be growing as well as other trees which were wrapped with twine in the regular way. Onet thousand and twenty-five trees were wrapped with the cloth. The data on crown gall infection will have to be taken later when the trees are taken up to be sold next fall (1922).

About 1500 or 2000 grafts were stored in sand treated with formaldehyde have not been located, consequently no notes have been taken. It is thought by men having charge of the planting that they were killed and were discarded with those killed by the copper sulfate. This is probably correct, since Melhus and Maney (7) state that a "Surface disinfection with formaldehyde (.16 per cent) was seriously injurious to callousing of apple trees.

That part of the experiment included in 2 also 3 and 4 was carried out at Blacksburg. The grafts being made and treatments applied between February 20th and March 1, 1921. Notes were taken three times; first, at the time the grafts were made; second, at the time the grafts were planted and third, when the trees were one year old.

Notes taken at time grafts were made.

Lot No. 1.

One hundred Staymand stored without treatment to be used as checks.

Lot No. 2.

Fifty Northern Spy and fifty Yorks were used. The scions after being cut were dipped in a suspension of the crown gall organism (Bacterium tumefaciens) before being united to the root. These were stored without further treatment.

Lot No. 3.

Fifty Northern Spy and fifty Yorks were used. The scions were treated as were those of lot No.2 and the unions made and tied. They were then painted with a starch-copper sulfate paste and stored. (The paste was the same as that used at Hoods nurseyy,and was applied to the union with a brush and allowed to "set" before the grafts were stored).

Lot No. 4.

Fifty Yorks and fifty Winesaps were used. The scions were treated as were those of lot No.2., but were allowed to dry and were then dipped in a solution of copper sulfate before being united to the root.(The copper sulfate solution was the same as that used at Hoods nursery).

Lot No. 5.

Seventy Stark and thirty Yorks were used. The scions were treated as were those of lot No.4,but after the union was made the starch paste was also used,thus giving a double treatment - the copper sulfate solution and the starch-copper sulfate paste treatments.

Another hundred grafts were wrapped with cloth. These were not inoculated or treated in any way. The object was to see if better or smoother unions would be made under this condition.

All the grafts ,except the hundred wrapped with cloth,were wrapped with waxed string (ordinary wrapping twine dipped in grafting wax).

Notes Taken at Planting Time - April 7,1921

Lot No. 1.

These were Staymans with no treatment, to be used as checks. They wrer calloused and showed signs of sprouting,but were not vigorous looking.

Lot No. 2.

This lot were Northern Spy and Yorks,treated with the crown gall organism,to be used as checks, These were calloused and in better condition than lot No. 1. Had sprouted more and were more vigorous looking.

Lot No. 3.

Northern Spy and Yorks were treated with the crown gall organism and then painted with starch bluestone paste. These were all dead;there was no callous formed and the root and scion were dead where the paste had been applied. Some roots put out shoots below the dead part,but the scions showed no signs of life.

Lot No. 4.

This lot,Yorks and Winesaps,treated with the crown gall organism and dipped in copper sulfate solution,were all dead and had much the same appearance as those of lot No. 3.

Lot No. 5.

This lot,Starks and Yorks,treated with the crown gall organism and dipped in copper sulfate solution also painted with starch paste were dead as were those of lots 3 and 4.

In the lot of trees wrapped with cloth the callousing was somewhat slowed than of those wrapped with twine.

Results

As is shown in notes taken at planting time, all grafts treated with the copper sulfate solutions were killed; the material in the proportions used being too strong. The remaining grafts, 100 Staymans, 50 Spy, 50 York, and 100 Grimes, however, were planted and the following notes taken when the trees were one year old.

Due to the dry weather at the begining of the growing season and lack of cultivation throughout the summer, none of the trees made what could be called good growth, and many of them died. There was very little, if any, difference evidenced in the growth and callousing of any of the grafts (trees). The checks dipped in the bacterial suspension were as smooth as those receiving no treatment at all. Those wrapped with cloth were perhaps smoother and more evenly calloused than the others, except in cases where the stock (graft root) was larger than the scion. It seems that in all cases where the stock was larger than the scion, in an effort to heal over, an excessive amount of callous tissue was formed. This was evidenced in those wrapped with cloth as well as in those wrapped with the waxed twine. In most all cases where the stock and scion were of the same size and good fits made, smooth unions resulted, especielly those wrapped with cloth.

Some of the grafts were planted in rather rough gravelly soil and had a tendency to be rougher than those planted in better soil. More surplus callous tissue was evident, and it seemed to be softer - more watery- than that of the grafts grown in the better soil.

In cutting the tongue sometimes the end of the stock or scion, or

the ends of both, were left to long as shown in figure 1,D, and excessive callous formed on these ends,figure 1 E. This ball-like growth has a tendency to push the tongues apart,making it necessary for more callous tissue to be formed to heal over the union. Consequently a rough union resulted. In several cases where excessive callous was present some kind of maggots were found feeding in the soft tissue. Decay had set in and the tree would most assuredly have been destroyed. None of these maggots,however,were found in the grafts wrapped with cloth.

In almost every case where the union was smooth and it was evident that the healing was done readily,and without excessive callousing, theroot system was better than those showing excessive callous tissue.

Definite conclusions cannot be expected from a problem of this kind the first year and it will be carried further, but there are a few things worthy of note.

It seems that a great part of the battle in controlling crown gall is to keep down the growth of excessive callous,since the organism seems to take hold and thrive better in this tender rapid growing tissue,and to protect the union as far as possible against infection by careful wrapping. The first may be accomplished in making the grafts,by selecting stock and scion of as near the same size as possible and then making a good fit, and the second by the use of cloth or similar material for wrapping.

Since the type of soil seemed to enter in as a factor in this experiment,i.e.,regarding callous development, it might be well to select a more loamy soil in which to plant the grafts rather than a rough or gravely piece.

Fig. 1.

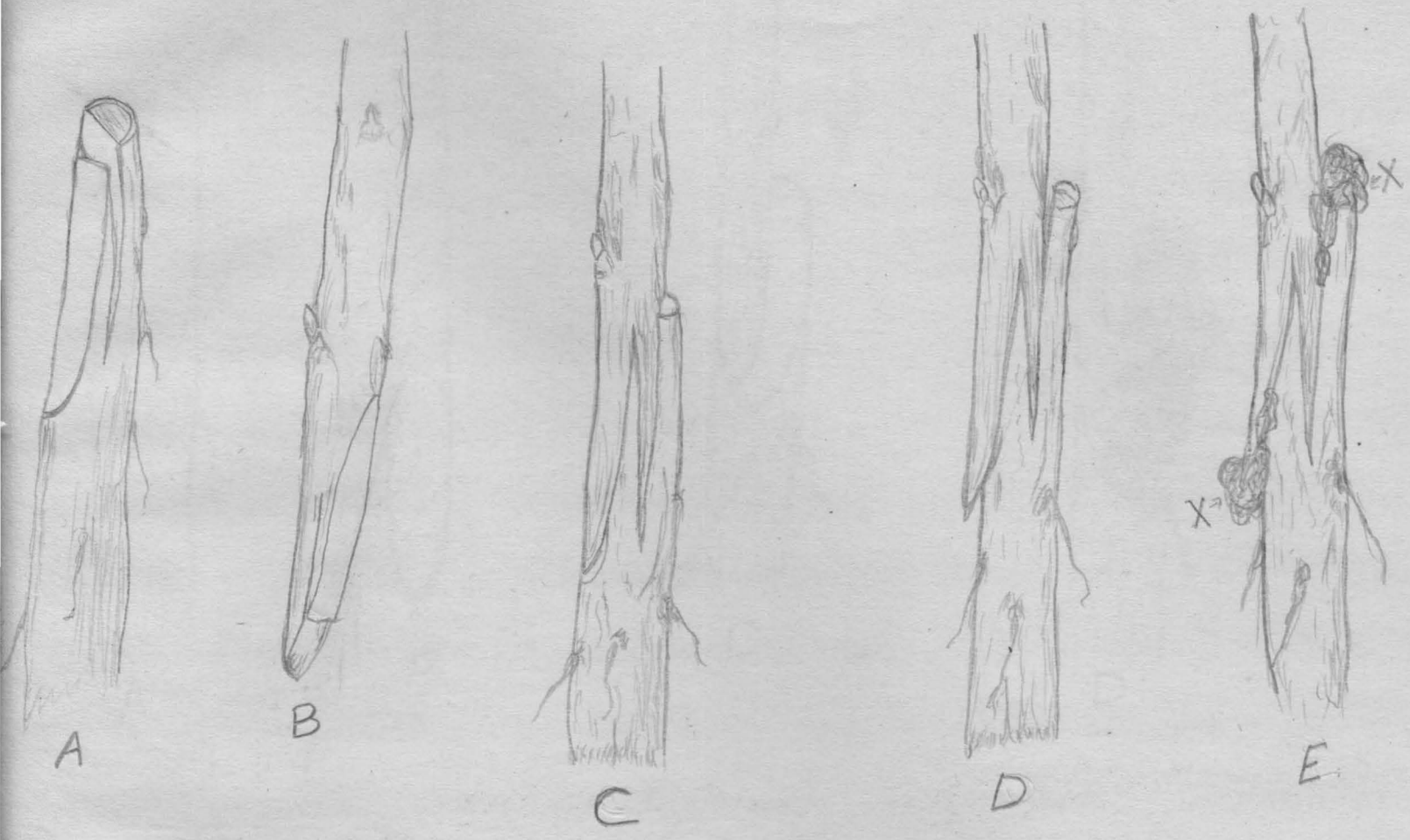

Figure 1 - A, the stock, B, the scion, C, the stock and scion united - a good fit. These will unite without forming an excess of callous tissue and the union will be smooth. D, the stock and scion united with the ends too long- a poor fit. Excess callous tissue will be formed on these ends (x - E) and a rough union will result. This excess tissue not only invites the crown gall organism but also invites fungus diseases and certain insects, especially those whose larvae feed upon decaying matter.

Fig. 2.

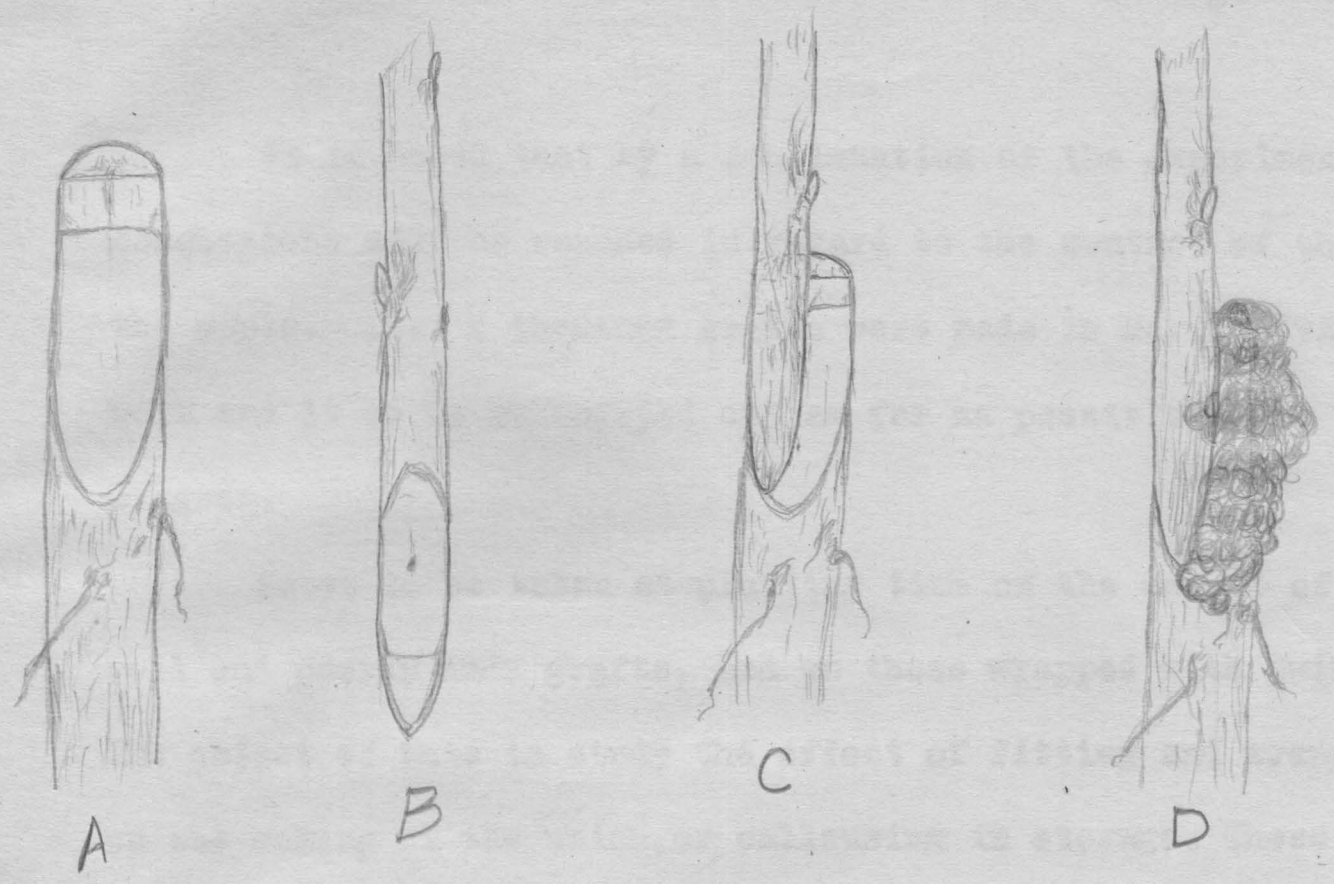

Figure 2 - A, the stock, B, the scion. Notice that the scion is much smaller than the stock. C, the stock and scion united - a poor fit, too much of cut surface of stock exposed, an excellent place for the crown gall organism to enter. An excessive amount of callousing will be necessary to heal this union, consequently a union as shown in D will be the result.

Plans for Continuation of the Experiment

It is hoped that by a continuation of the experiment, more definite conclusions will be reached in regard to the control of the crown gall of the apple. Over a thousand grafts were made in March (1922) for this work and it is to be carried out as far as possible under the following program.

Notes to be taken at planting time on the amount of callousing of well and poorly made grafts, and on those wrapped with twine and cloth. The object of this to study the effect of fitting and wrapping the grafts on the making of the union, or callousing in storage. These same studies to be made when the trees are about one year old- after a seasons growth.

After the trees start growing, infection experiments with the crown gall organism will be carried on. Various methods of infection and treatment will be studied; such as removing the dirt away from around the root and applying the organism with a brush, making wounds with a knife dipped in a culture of the organism, pouring a suspension of the organism around some of the roots, putting chips of old growing galls around the roots, and any other methods that may be thought of. If it is possible to apply some fungicides, such as bordeaux mixture, after infection treatments have been made, this will also be done.

Due to not having the organism in culture, the grafts this year had to be planted before being treated and the procedure as stated in the preceding paragraph will have to be followed, but it is hoped and in fact the plan is to follow somewhat the line of experiment that was carried out by Melhus and Maney (7) in Iowa.

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