

THE EFFECT OF VARIOUS METHODS OF WRAPPING
GRAFTS ON THE DEVELOPMENT OF CROWN GALL.

A Thesis Presented for the Degree of
Master of Science.

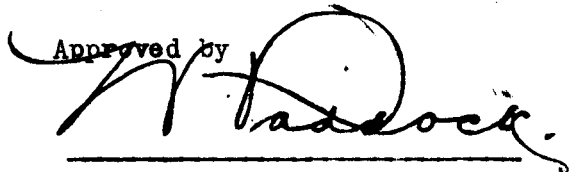
By

Edwin Warren Gordon, B. Sc.

THE OHIO STATE UNIVERSITY

1921

Approved by


W. H. Daddock

EFFECTS OF VARIOUS METHODS OF WRAPPING GRAFTS UPON
THE DEVELOPMENT OF CROWN GALL.

I N T R O D U C T I O N

Crown gall (*Bacterium Tumefaciens*) is a disease attacking such a large number of host plants that much work would be necessary to determine the extent of injury to each. It is evident from a review of the work that has been done in the past, that some kinds of plants are rarely if ever attacked while others are more so. When attacked, some are seriously damaged while others remain apparently perfectly normal except for the presence of the gall.

Until a comparatively recent date it was very difficult to distinguish Crown gall from some other malformations which may have been due to injury. The cause of Crown gall was unknown in spite of the fact that much work has been done on this subject.

Cavara of France was the first to lay this disease at the door of Bacteria but the true cause was explained in 1907 by Drs. Erwin F. Smith and C. O. Townsend who published a paper at this date demonstrating that the disease is produced by a bacterial organism (*Bacterium Tumefaciens*) and is not due to injury received in grafting, budding or otherwise; as was formerly supposed. Since this time all galls which are produced by this organism (*Bacterium Tumefaciens*) are classed as true Crown galls; while other galls which may be due to injury can only be called galls, or abnormal growths.

In general these abnormalities are of two kinds:

(1) True Crown galls on

a. Roots

b. Crown

c. Stem

(2) Hairy Roots.

In the latter case unusual tufts of fine roots are produced sometimes greatly damaging the main or anchor roots. Many orchardists and nursery men have been confused because the calluses that form during grafting and budding bear a marked resemblance to Crown gall in its early development and because of the growth of hairy like roots which may be due to the stimulation received by unfavorable conditions of moisture and temperature. The real final distinction should be made henceforth on the causative agent and not alone on the symptoms.

Galls caused by this organism may be said to be of three distinct classes:

1. Hard galls -
2. Soft galls -
3. Hairy root -

The soft galls are found to be more numerous on species of the stone fruits than on the pomaceous and it is that by some authorities that the nature of the gall depends to a large extent upon the tissue which is affected.

According to personal observation under the microscope, which is substantiated by many authorities, when the gall first begins its development there is a gradual pushing outward of a small area of the true cambium layer, which is transformed into large hypertrophied parenchyma cells. In its youngest stages the tissue of the gall is a mass of parenchyma with numerous minute areas of rapidly dividing meristems scattered thru it. The areas of meristematic tissue are centers of growth. As the galls become older these centers of growth increase in size and others originate in the newly formed parenchyma. The centers of these growths ultimately become most curiously twisted nodules of tracheides and woody fibers.

Many nursery men have claimed, and very consistently, that Crown gall is not infectious and that it is a blemish which is caused by an excessive development of callus tissue at the union, rather than a disease. The fallacy of this

supposition may easily be shown by microscopical examination or by a re-inoculation.

The following series of investigations were conducted to determine the effect of various wrappings upon the development of crown gall.

P R O C E D U R E

In order to determine the effect of the various wrappings upon the development of crown gall a number of grafts were made and various wrappings were used. Grafting and wrapping grafts is not a new idea, for it was done in the early days before Christ by Cato, Varro, Pliny and many other Agriculturists.

The materials used in this experiment for wrapping were:

- | | |
|------------------|------------------------|
| 1. Adhesive tape | 7. Wool yarn (unwaxed) |
| 2. Cloth | 8. Paraffin |
| 3. Cotton twine | 9. Plain paper |
| 4. Grafting Wax | 10. Pafia |
| 5. Waxed paper | 11. Rubber |
| 6. Waxed yarn | 12. Tree tanglefoot |

In addition to these, grafts were made by using no wrapping.

In the fall of 1919 grafts were made using seedlings of the Bartlett pear and the Damson plum. In the fall of 1920 grafts were made using wealthy apple seedlings. Since it must necessarily take time for the galls to develop on the woody plants and time was limited, the other tests were made using herbaceous plants. In January 1921 the first test with the herbaceous plants was made; followed by a second in March 1921 with a third in April 1921.

All grafts on the woody plants were of the type known as whip, or tongue, grafts and on the herbaceous plants the grafts were those known as splice grafts. In the March and April tests, in addition to the splice grafts, stabs or cuts were made in the stems just above the ground and these were inoculated from a gall which had developed on a tomato plant used in the January test. The

inoculation consisted in taking a small portion of the gall tissue and placing it in the slit and then wrapping with the various wrappings. As another test a few plants were inoculated with small portions taken from a gall which had developed on a Ben Davis seedling. In this test no definite results were obtained, for the plants did not show any conspicuous galls. According to this test better results are obtained when the inoculation is made from a gall which has developed on an herbaceous plant than when a gall grown on a woody plant is used.

Wrapping materials:

1. Adhesive tape was Bauer and Black's, which was cut into strips 1/4 inch wide by 8 inches long. This was wrapped around the grafts twice.
2. Cloth used was of the common cheapest grade of unbleached muslin which was cut in strips 1/4 inch wide and approximately 1 foot long. The wrapping was started in the middle and wrapped each way so that the union or graft was covered with two thicknesses of cloth and the ends were fastened by tying.
3. Cotton cord, or twine, was ordinary wrapping twine, and the graft was wrapped twice and fastened by tying.
4. Grafting Wax was 4 pounds of resin 2 pounds of Beeswax 1 Pound of tallow composition. This was applied just as cool as could easily be worked.
5. Waxed paper used was ordinary waxed paper such as is used for packing flowers. This was cut into strips 1/4 inch wide and 1 foot long. Grafts were doubly wrapped and fastened with grafting wax.
6. Waxed yarn, or cord, was wool yarn which had been dipped into wax until thoroughly saturated. Enough of this waxed yarn was used to give the union a double covering.
7. Wool yarn, or twine used was of the common red knitting variety. Grafts were carefully wrapped twice with this and the ends were tied.

8. Paraffin used was the ordinary high temperature kind, such as is used in making candles. This was melted and applied in a semi warm condition.

9. Plain paper used was ordinary brown wrapping paper cut 1/4 inch wide and 1 foot long. Grafts were wrapped with this and fastened with grafting wax.

10. Rafia was cut into strips about 1 foot long and was wrapped around the graft and fastened with grafting wax.

11. Rubber used was the thinnest obtainable, (thinner than balloon or dental rubber) and this was cut into strips 1/3 inch wide and 6 inches long. Enough of this was used to give two coverings to the graft and it was fastened with adhesive tape.

12. Tanglefoot used was ordinary tree tanglefoot which was placed around the graft and held in place by a strip of plain paper.

All grafts were inoculated from a pure culture grown on wheat extract Agar; the organism being isolated from a gall which had formed on a hop vine in the first three cases and from a gall which had formed on a raspberry vine in the other two.

R E S U L T S

The following table shows the percentage of galls which formed in the 1919 test when Bartlett pear and Damson plum seedlings were used.

Table No. 1

Results using Bartlett pear and Damson plum seedlings.

Kind of wrapping	Large gall	Medium gall	Small gall	No gall
(1) Rubber	20%	30%	40%	10%
(2) Cloth	35%	40%	17%	8%
(4) Grafting wax	50%	20%	27%	3%
No wrapping	50%	30%	18%	2%

Approximately 8% of the grafts did not successfully unite, so the percentages given in Table No. 1 were derived from only those grafts which had successfully united. In this test which was made in the fall of 1919, cions were stored in white river sand (medium texture) until April 1920 when they were set in specially prepared soil and were dug for examination August 1920. When dug the rubber was as yet intact as far as weathering was concerned, but it was badly stretched, and often perforated by portions of the galls which had developed. The cloth was almost wholly rotted, but had remained intact long enough to hold the cion in place until the union was completed. The wax had almost been destroyed when the seedlings were set out in the spring and had been destroyed when examined in the fall of the year. Due to the continued moist condition of the soil. The wax proved to be very little better than no wrapping at all.

In a good many instances the time required for the development of galls on hard woods varies from 6 weeks to almost an indefinite period.. In the test commenced in the fall of 1920, the results obtained were not definite since time was limited and the grafts were so disturbed that approximately 95% of them were lost; and of the remainder only two galls developed. These were on the grafts which had been wrapped with waxed wool twine for practically all others had been destroyed. In this case, seedling apple trees were used and consequently the galls were very small, almost unnoticable at the time of the examination.

In the January 1921 test, herbaceous plants were used and many of these were lost during the process of grafting due to the damping off fungus (*Pythium de Baryanum*). Results are given only of those plants which were successfully grafted, allowance being made for those that were lost by this fungus which was much more prevalent due to the favorable conditions which were found in the greenhouse.

In this test plants from 3 - 6 weeks old were used. The cuts were made clean and under as near sterile conditions as possible. In each case the top was

grafted onto its original root. Care was taken in the inoculation so that the conditions were the best possible. After the grafts were made the plants were placed in the propagating room, where they were allowed to remain until united, a matter of about 10 days, and they were then placed in the green house proper. Here almost daily observations were made.

The following table is the result of the January 1921 test using an herbaceous plant, tomatos:

Table No. II.

Effect of various cion wrappings on Crown gall in tomato plant grafts (splice).

Kind of wrapping	Large gall	Medium gall	Small gall	No gall
Waxed cord	40%	30%	30%	- - -
Cotton cord	50%	38%	12%	- - -
Rafia	55%	35%	10%	- - -
Paper plain	60%	25%	15%	- - -
Grafting wax	65%	30%	5%	- - -
No wrapper	65%	32%	3%	- - -

In this case all the plants were affected to a greater or lesser extent and the effect of the wrappers is shown only by the size of the galls produced. In this case the waxed wool twine or yarn seemed to give the best results while grafting wax and plain paper gave but very little better results than those secured with no wrapping at all. In the case of no wrapping the plants were repotted and the union was packed tightly with earth.

Table No. III give the results of the experiment tried on Tomatos in March 1921.

Table No. III.

Effect of various cion wrappings on crown gall in tomato plant grafts (splice).

Kinds of wrapping	Large gall	Medium gall	Small gall	No gall
Waxed yarn	40%	32%	28%	- - -
Paper waxed	48%	40%	12%	- - -
Paper plain	60%	24%	16%	- - -
Rubber	49%	40%	11%	- - -
Cotton twine	50%	35%	15%	- - -
Rafia	50%	30%	20%	- - -

In this test waxed yarn proved to be the best while plain paper was the poorest. Results from the rubber wrappings may be due to the fact that rubber strips were much narrower than formerly used. In the case of these they were repotted after being grafted and a half inch of white river sand, fine texture, was scattered over the top of the top. A smaller percentage of these plants were lost because of the damping off fungus.

Table No. IV.

Effect of various cion wrappings on crown gall in tomato plant grafts.(stab)

Kind of wrapper	Large gall	Medium gall	Small gall	No gall
Rubber	35%	45%	20%	- - -
Adhesive tape	35%	40%	24%	1%
Waxed yarn	40%	35%	25%	- - -
Cotton twine	50%	30%	20%	- - -
Waxed paper	50%	30%	20%	- - -
Plain paper	60%	30%	10%	- - -
No wrapping	60%	40%	- -	- - -

From this test it is shown that adhesive tape is slightly better than the

other wrappers used and rubber is a close second; while plain paper is very little better than no wrapping. In only one case was there a plant which continued growth without being affected with gall.

The following is a test which was made in April 1921 and much better results were secured, very few of the plants were lost because of damping off fungus. Several of them were lost due to other causes.

Table No. V.

Effect of various cion wrappings on crown gall in tomato plant grafts (splice).

Kind of wrapping	Large gall	Medium gall	Small gall	No gall
Tree tanglefoot	- -	- -	10%	90%
Rubber	10%	50%	40%	- -
Adhesive tape	25%	30%	45%	- -
Waxed yarn	25%	35%	40%	- -
Wool cord	30%	40%	30%	- -
Cotton twine	35%	40%	25%	- -
Paraffin	35%	40%	25%	- -
Rafia	35%	45%	20%	- -
Waxed paper	35%	50%	15%	- -
Plain paper	50%	40%	10%	- -

In this test rubber gave the best results, closely followed by adhesive tape. With the exception of tree tanglefoot which did not favor the development of the gall but left the plants in such a sickly condition that they were below normal in growth. The tissues of these plants seemed to be partially destroyed, which may be due to some toxic agent which is a constituent of tanglefoot for it gives acid test with litmus paper. Again plain paper shows

the largest both in size and quantity development of galls almost as was shown by the check test.

In March 1921 a test was made using St. Regis raspberries but no definite results were obtained. Not being able to secure new wood growth the experiment was tried on old wood, and the plants were badly injured during the early stages and dies before showing any symptoms of crown gall.

C O N C L U S I O N

The limited scope of this series of investigation conducted by the writer, while in his estimation conclusively demonstrating certain fundamental facts, does not justify the establishing of definite rules in regard to the effect of various wrappings upon the development of crown gall. In these experiments the conclusions drawn are almost wholly from work done on herbaceous plants.

1. Crown gall develops chiefly on young and rapidly growing tissue.
2. That galls may be controlled to a limited extent by wrappings.
3. That rubber and adhesive tape gives a greater percentage of no galls.
4. That cloth and waxed yarn gives nearly as good results as either rubber or adhesive tape.
5. That plain paper and grafting wax are very little better than no wrapping.
6. That due to the expense connected with the use of rubber or adhesive tape it will be found to be more economical in the end to use cloth or waxed yarn.

B I B L I O G R A P H Y

Duggar, B.M. Fungous disease of plants.

Green, L. and Melhus, I.E. The effect of crown gall upon the young
apple orchard.

Iowa Research Bulletin #50.

Hedgcock, Geo. C. Crown gall, Hairy Root disease of the Apple.

Bureau Plant Industry U.S. Dept. Agl. Bulletin #90.

Hedgcock, Geo. C. The Cross Inoculation of Fruit Trees, and shrubs with
Crown gall. Bureau Plant Industry U.S. Dept. Agl. Bull. #31.

Orchard Studies XIII Virginia Agricultural Experiment Station.

Selby, A. D. Diseases of the Peach Ohio Agl. Experiment Station Bulletin #92.

Smith, Erwin F. and Townsend, C.O. A Plant Tumor of Bacterial Origin.

Report April 1907.

Smith, E. F., Brown, Nellie A. and Townsend, C.O. Crown Gall of Plants, its
cause and remedy. Bureau plant Industry U.S. Dept. Agl. Bull. #213.

Toumey, J.W. An inquiry into the cause and nature of Crown gall. Arizona
Agl. Experiment Station Bull. #33.