EFFECTS OF MINERAL NUTRITION ON DEVELOPMENT OF CROWN GALL CAUSED BY *AGROBACTERIUM TUMEFACIENS*

by

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INTRODUCTION

Crown gall caused by *Agrobacterium tumefaciens* affects a considerable number of host plants in nature, and a much wider range of plants has been shown to be susceptible by experimental inoculation. It is an important disease of apple, cherry, peach and plum. In the Pacific Northwest crown gall causes considerable losses in the nursery because quarantine regulations prevent the sale of infected plants.

This disease has been studied intensively particularly from the standpoint of the physiology of tumor tissue and the mechanisms involved in conversion of normal tissue to tumor tissue. Considerable work has been done on the effect of mineral nutrition on growth of tumor tissue in vitro, but very little work has been done on the effect of various ions on gall development in vivo.

In the present investigation the effects of levels of nitrogen, phosphorus and potassium and the interaction among these elements on the growth of crown galls were determined. Since boron has been reported to have a direct influence on meristamatic activity, the effect of this element on gall growth has been studied.

It has been suggested that crown galls reduce growth of the host because of nutritional competition between host and gall tissue. Most of these observations have not been supported by data on the nutrient content of healthy and infected plants. In this investigation the nitrogen, phosphorus, potassium, calcium and boron content of healthy and gall infected plants was determined. The amounts of these elements in normal tissue and gall tissue were also compared.

All previous experiments on the influence of mineral nutrients on gall development were carried out with herbaceous plants. In this investigation both herbaceous plants (Bonny Best tomato) and woody plants (Mazzard cherry) were used.

REVIEW OF LITERATURE

The relationship between nutrition, soil fertility and the severity of disease has been studied intensively by many workers. Wingard (56) reviewed the literature in this field up to 1941. Since that time some excellent research has been done especially on the effect of nutrients on wilt diseases and damping off (6, 25, 47, 51, 52, 48 and 5).

No broad generalization can be made at present regarding the relation between nutrient level and disease development. Some diseases are severe on weak, under-nournished plants while others are most destructive when plants are vigorous (McNew 26).

Although some information is available on the nutrient requirements of tumor tissue in vitro (40, 16, 15, 41, 38 and 17), very little work has been done on the effect of host mineral nutrition on gall development. Hope *et al*. (17) pointed out that host tumor competition and interaction can be avoided by in vitro culture and that determination of gall tissue metabolism in vivo is open to criticism. But in spite of the complex nature of the problem, it is important to study the physiology of the host-tumor interactions as well as physiology of the isolated tumor tissue.

The early work on the effect of mineral nutrition on gall development was done by European workers. Rives (42) found that plants without potassium developed larger galls. Verona (50) reported that the fresh weight of the tumors on Ricinus was reduced by potassium application. Sempio (44) determined the effect of metals on the growth of the tumors on Ricinus. He showed that potassium, lead, barium, zinc and strontium stimulated growth of the galls while uranium, thorium and aluminum had no effect. He explained the effects of these minerals on the basis of stimulation or depression of host resistance. Kent (18) found that the size of crown galls on tomato was significantly reduced by adding lithium nitrate to the soil. He concluded that lithium either affects the bacterium directly in the host or inhibits the manufacture of a growth substance required for the gall development. Link et al. (25) in comparing gall size to the diameter of the stem found that tumors on tomato plants with minus nitrogen were relatively larger than tumors on plus nitrogen plants. Earlier Link et al. (24) reported that gall production in tomato plants was favored by low nitrogen and high carbohydrate. Klein (20) explained the stunted and nitrogen deficient appearance of the tumor bearing plants on the basis of mobilization of the nitrogen of the whole plant to the tumor. Levine's (23) results suggested that a well nourished, vigorously growing host develops large galls compared to a poorly growing host.

A number of investigators have reported that mineral nutrients tend to be higher in tumor tissue than in normal tissue. Nagy et al. (34) found that gall tissue contains more total nitrogen than normal tissue. Neish et al. (35) analyzed normal tissue and gall tissue of sugar beets and found that the tumor tissue has a higher content of crude protein, starch, pectins, cellulose, lignin and total ash than healthy tissue. Sugar content was lower in the gall tissue. He suggested that sucrose is utilized by the tumor tissue for the specific synthesis of proteins. Klein (19) reported that both soluble nitrogen and protein nitrogen is higher in the gall tissue. Klein (19) and Taso et al. (49) found that phosphorus accumulated in the gall tissue of tomato and Bryophyllum. Hope (17) showed that on a per-cell basis, the tumor tissue has about twice as much nitrogen and phosphorus as the normal tissue and about twice as much protein and nucleic acid. Wood and Braun (57) recently reported that tumor cells have a very effective ion-transport system. They found that normal cells require significantly higher levels of nitrate, phosphate and potassium for optimum growth.

It has been suggested that galls reduce the growth of the host. Deep and Young (8) reported that healthy cherry trees had an approximately 15 per cent greater increase in stem diameter over a three year period than gall bearing trees. Position of galls on the roots correlated with the degree of reduction in growth. Link et al. (25) found that galls reduced the growth of tomato plants especially in minus nitrogen plants. Riker et al. (39) found no differences in growth of healthy and gall infected apple trees after 25 years.

Link et al. (25) suggested that growth of galled plants may be reduced by mobilization of nutrients from the whole plant to the galls. Sempio (44) concluded that the effect of a tumor on the host is not direct but that the neoplastic tissue is the real parasite of normal tissue and grows completely at the expense of normal tissue. Melhus et al. (28) and Muncie et al. (31) found that stems of apple and peach trees infected by crown gall conducted considerably less water than normal stems. This suggests that the reduced growth of diseased plants may result from a reduction in the supply of water and mineral nutrients.

It is clear that growth of galls is influenced by the host mineral nutrition, but the nature of this influence is not known. It is beyond the scope of this review to discuss in detail the role of nitrogen, phosphorus and potassium in plants. There are excellent reviews dealing with this subject (9, 53, 30, 13, 22).

Although considerable work has been done on the role of boron in plants, there is still some controversy. A number of investigators have suggested that boron may be required for normal cell division, mitosis, cell enlargement and maturation (54, 55). Neals reported that the growth of roots of flax, field bean, broad bean and pea stopped in media lacking boron (33, 34).

It has been reported that boron is essential or beneficial for the normal germination of pollen grains and the growth of pollen tubes, Gouch et al. (10) and Okelly (37).

Boron may have a very important role in carbohydrate translocation. Gouch et al. (10, 11) suggested the formation of a sugar-borate complex which diffuses more readily through cell membranes. Skok (46) suggested that the effect of boron deficiency on sugar translocation is through an effect on cellular activity rather than the formation of a sugar-borate complex. Melbrath et al. (29) found that the phloem of tomato plants was killed by lack of boron thereby causing a reduction in carbohydrate translocation. Scott (43) found through in vitro studies using starch phosphorylase that boron competitively inhibits the formation of starch. This could lead to less sugar available for translocation.

Boron may be involved in protein synthesis or in the synthesis of some wall component such as pectin (37).

Whittington (55) supported the conclusion that boron is essential for pectin synthesis.

GENERAL PROCEDURES

I. Preparation of Inoculum and Methods of Inoculation

The inoculum used in all experiments was prepared from a mixed culture of two virulent strains of *Agrobacterium tumefaciens* supplied by the Department of Botany and Plant Pathology at Oregon State University. The organism was cultured on potato dextrose agar for three to six days. Inoculations were made by the following methods:

A. The File Method

This is a slight modification of a method reported by Ark *et al.* (1). The stems were wounded by filing to the cambium layer, and a disk of inoculum which was removed from the agar plate by a cork borer was attached to each wound with Scotch tape. This prevented rapid drying of the inoculated area.

B. Inoculation with a Needle

Inoculations were made by dipping the needle in a suspension of the organism and pushing it into the stem. In one experiment the tip of the needle was bent to form a 90 degree angle. This facilitated a uniform depth of wounding when the plants were inoculated. In another experiment the needle was pushed completely through the stem.

II. Planting Medium

When tomato plants were used the seeds were germinated in washed white sand. The seedlings were transferred to the nutrient solution or planting medium when they were about three to four inches in height. The planting medium used in most experiments contained a mixture of 75 per cent sand and 25 per cent peat moss. This is one of the media reported in the University of California Manual (2). It is reported to be very low in major elements and to supply the necessary minor elements. The amount of fertilizer used is shown under each experiment.

III. Nutrient Solutions

Hoagland's solution and minus boron Hoagland's solution were used in all boron deficiency experiments. The formulas for Hoagland's solution No. 1 and No. 2 are reported in California Circular 347 (14). The details of each treatment are given in each experiment.

IV. Sampling for Tissue Analysis

Leaf samples were taken either by harvesting the entire plant tops in the case of small plants or by collecting leaves at random from the middle shoots when the plants were large. Gall tissue was removed from the stems with a razor blade. The plant materials were dried in a 120° F. oven. The dried material was ground in a Wiley mill to pass a 20 mesh screen.

V. Tissue Analysis

The analyses were made by the following methods:

Mineral Elements	Methods of Analysis Reported
Nitrogen	Murneek, A. F. and P. H. Heinze. Speed and accuracy in determination of total nitrogen. University of Missouri. Agricultural Experiment Station Research Bulletin 261. 1937.
Phosphorus	Fiske, C. H. and Subba Row Y. The colorimetric determination of phosphorus. Journal of Biological Chemistry 66:375-400. 1945.
Boron	Dible, Wit, Emil Truog and K. C. Berger. Boron determination in soil and plants. Analytical Chemistry 26:418-421. 1954.
Potassium and Calcium	Brown, J. E., Omund Lilleland and R. K. Jackson. Further notes on the use of flame methods for analysis of plant material for potassium, calcium, magnesium and sodium. Proceedings, American Society of Horticultural Science 56:12-22. 1950.

THE EFFECT OF BORON DEFICIENCY ON THE DEVELOPMENT OF CROWN GALL ON MAZZARD CHERRY

Design of Experiment and Results

This experiment was carried out in aerated solution cultures in the greenhouse using three gallon capacity crocks. One year old Mazzard cherry trees which had been selected for uniformity of stem diameter were used. Two cherry trees were placed in each crock, and each crock was considered as a plot. A randomized block design was selected with two treatments and six replications. Number one Hoagland's solution was applied to one treatment and Hoagland's solution minus boron to the other. The soltuions were changed every 15 to 20 days.

The cherry trees were placed in the crocks and tap water was used until new roots developed. The crocks were then washed with distilled water, and the tap water was replaced with Hoagland's solution or Hoagland's solution minus boron.

Each cherry tree was inoculated with Agrobacterium tumefaciens in five places using the file method described in the General Procedure section.

Galls started to develop normally in both treatments. Two weeks after inoculation a white mass of tumor tissue was evident at most of the points of inoculation. Galls were well advanced by the fifth week after inoculation.

There was no indication of boron deficiency at this time. Leaf samples were collected and analyzed for boron. The results for both boron analysis and gall development are shown in Table 1. At this time galls were present on both minus and plus boron plants. From 60 points of inoculation 55 galls developed on plus boron plants and 58 developed on minus boron plants.

Table 1. THE EFFECT OF BORON LEVEL IN MAZZARD CHERRY LEAVES ON GALL DEVELOPMENT FIVE WEEKS AFTER INOCULATION.

	Plus Boron		Minus Boron	
Reps.	No. of Galls	ppm Boron of the Leaves	No. of Galls	ppm Boron of the Leaves
1	10	105.0	10	30.0
2	9	86.0	10	48.0
3	10	105.0	10	51.0
4	8	105.0	8	42.0
5	10	108.0	10	33.0
6	8	105.0	10	33.0

ppm Boron on dry weight basis.

Leaf analysis indicated that boron content of plus boron plants was about three times higher than minus boron plants. The average boron content of the minus boron plants was 39.1 ppm on a dry weight basis. This value is above the deficiency level of most stone fruit trees.

By the eighth week after inoculation galls which were developing on minus boron plants ceased to grow and began to dry up. At that time four replications, 1, 2, 5 and 6, were selected, and one new shoot of one tree in each replication was inoculated in five places.

The experiment was terminated after thirteen weeks. At that time all galls on the minus boron plants were completely inactive and the tissue was dry and brown while galls of the plus boron plants were large and typical of normal galls (figs. 1A and 1B).

Leaf samples were collected again at the end of the experiment for analysis. Boron levels and number of galls are shown in Table 2. Galls of the minus boron plants which failed to develop normally are designated as zero galls in Table 2. Thirty-nine galls developed on the plus boron plants. This means that 16 galls failed to develop after the first reading. On the other hand, all 58 galls of the minus boron plants failed to develop.

At this time boron level in the minus boron plants had dropped to near the deficiency level. Boron level in the plus boron plants had dropped slightly, but this is normal behavior of boron levels toward the end of the growing season.

Table 3 shows the gall development and leaf analysis of the new shoots which had been inoculated at the eighth week. In this case there was never any indication of gall development on the minus boron plants. This differs from the first inoculation where galls started to develop but

Figure 1A. THE EFFECT OF BORON ON GALL DEVELOPMENT ON MAZZARD CHERRY. GALLS HAVE DEVELOPED ONLY ON PLANTS WHICH HAVE BEEN SUPPLIED WITH BORON.

-B +B

Figure 1B. ENLARGED SECTION OF FIG. 1A.

Table 2. THE EFFECT OF BORON LEVEL IN MAZZARD CHERRY LEAVES ON GALL DEVELOPMENT 13 WEEKS AFTER INOCULATION.

Reps.	Plus Boron		Minus Boron	
	No. of Galls	ppm Boron of the Leaves	No. of Galls	ppm Boron of the Leaves
1	8	78.0	0	17.0
2	9	84.0	0	22.0
3	10	94.0	0	22.0
4	6	91.0	0	22.0
5	6	91.0	0	13.0
6	6	86.0	0	16.0

ppm boron on dry weight basis

Table 3. THE EFFECT OF BORON LEVEL IN MAZZARD CHERRY LEAVES ON GALL DEVELOPMENT ON THE NEW SHOOTS WHICH WERE INOCULATED EIGHT WEEKS AFTER THE ORIGINAL INOCULATIONS.

Reps.	Plus Boron		Minus Boron	
	No. of Galls	ppm Boron of the Leaves	No. of Galls	ppm Boron of the Leaves
1	5	78.0	0	17.0
2	5	84.0	0	22.0
5	5	91.0	0	13.0
6	5	86.0	0	16.0

ppm boron on dry weight basis.

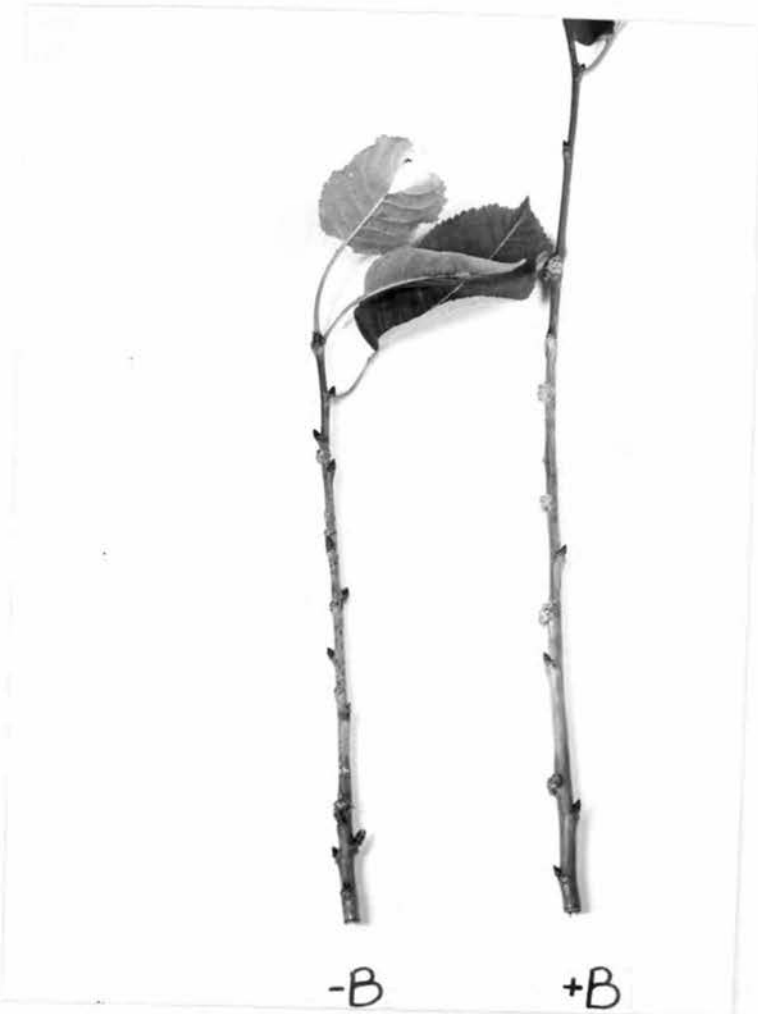


Figure 2. THE EFFECT OF BORON ON GALL DEVELOPMENT ON THE NEW GROWTH OF MAZZARD CHERRY TREES.

degenerated. While no galls developed on the minus boron plants all 20 galls developed on the plus boron plants (fig. 2). The boron level was about at the deficiency range for stone fruit trees in the minus boron plants and was about five times higher in the plus boron plants.

THE EFFECT OF BORON DEFICIENCY ON THE DEVELOPMENT OF CROWN GALL ON BONNY BEST TOMATO

Design of Experiment and Results

The experiment was carried out in the laboratory using fluorescent lights. Bonny Best tomato plants were grown in aerated solution cultures in one liter capacity brown jars. One tomato plant grown in each jar was considered a plot. A randomized block design was used with four treatments and four replications. Hoagland's solution number one and Hoagland's solution minus boron were used in the experiment as follows:

1. Plants were grown in minus boron Hoagland's solution throughout the experiment.
2. Plants were grown in plus boron Hoagland's solution throughout the experiment.
3. Plants were grown in complete Hoagland's solution until the plants were inoculated. The roots were then rinsed off with distilled water, and minus boron Hoagland's solution was added.
4. Plants were grown in minus boron Hoagland's solution until the plants were inoculated. A plus boron Hoagland's solution was then added.

Three weeks after the plants were placed in the solution culture the stem of each plant was inoculated with *Agrobacterium tumefaciens* in three places using the bent needle technique described in the General Procedure section.

The experiment was terminated five weeks after the plants were inoculated. Observations were made on gall development, and leaf samples were taken for boron analysis.

All the plants of treatment 2 (plus boron) grew normally, and galls started to develop. Plants of the first treatment (minus boron) were small in size and slightly yellowish. Galls developed very slowly on these plants. On the other hand, plants of the third treatments which did not receive boron after inoculation started to show deficiency symptoms. The active terminal growing points turned yellowish, followed by die-back and browning of the small leaves. The older leaves kept their green color, but they were rolled up and had occasional yellow spots. Galls did not develop on these plants. The plants of the fourth treatment grew normally after adding boron to the solution and typical galls developed (fig. 3A, 3B).

The results of gall development and boron levels in the corresponding plants are shown in Table 4. The galls of treatment number one are designated as zero gall. Although there was a slight development of gall tissue, they cannot be considered comparable to the galls of

Figure 3A. THE EFFECT OF BORON ON DEVELOPMENT OF GALLS ON BONNY BEST TOMATO.

1. Minus Boron
2. Plus Boron
3. Minus Boron after Inoculation
4. Plus Boron after Inoculation

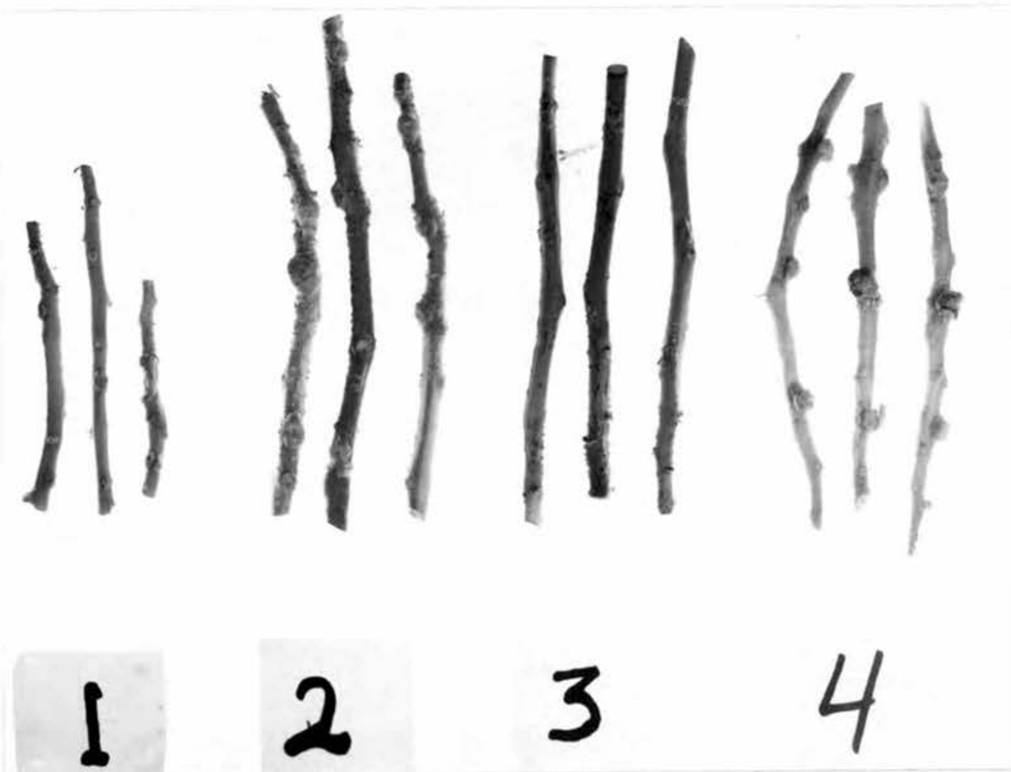


Figure 3B. THE EFFECT OF BORON ON DEVELOPMENT OF GALLS ON BONNY BEST TOMATO.

1. Minus Boron
2. Plus Boron
3. Minus Boron after Inoculation
4. Plus Boron after Inoculation

Table 4. THE EFFECT OF LEVELS OF BORON IN THE LEAVES OF BONNY BEST TOMATO PLANTS ON THE DEVELOPMENT OF CROWN GALL.

	Treat. No. 1		Treat. No. 2		Treat. No. 3			Treat. No. 4	
Reps.	No. of Galls	ppm B	No. of Galls	ppm B	No. of Galls	ppm B of old Leaves	ppm B of Yellow Leaves	No. of Galls	ppm B
1	0	25.0	3	82	0	26	13.0	3	84
2	0	19.0	3	84	0	43	--	3	100
3	0	23.0	3	100	0	51	18.0	3	110
4	0	24.0	3	140	0	48	36.0	3	125

treatments two and four. The galls of treatments two and four developed rapidly and were large by the end of the fifth week. No galls developed on any plants of treatment number three although some of the inoculated areas were swollen to a slight degree.

The results of leaf analysis are shown in Table 4. Boron content of the leaves of treatment one was very low. Both treatments two and four had a high boron content. The plants of treatment three were sampled differently. Each plant was separated into the yellow part and the old green part. The old green leaves were higher in boron while the yellowish leaves which developed after the plants were inoculated and supplied with a minus boron solution were very low in boron.

THE EFFECT OF BORON DEFICIENCY ON INITIATION OF CROWN GALL ON BONNY BEST TOMATO

The four to six day period following inoculation with *Agrobacterium tumefaciens* is very critical because during this time conversion of normal cells to tumor cells occurs. This is the period of tumor initiation as opposed to tumor development which occurs subsequently. This experiment was designed to determine whether boron deficiency has an effect on tumor initiation as well as tumor development.

Design of Experiment and Results

Bonny Best tomato seeds were germinated in sand, and the seedlings were transferred to nutrient solutions when they were four inches in height. The experiment was carried out under fluorescent lights in the laboratory using aerated solution cultures in brown liter bottles. A randomized block design was used with six treatments and three replications. The plants grew normally in a complete Hoagland's number two nutrient solution for three weeks, then the following treatments were made:

Treatment	1/25	1/26	1/27*	Date 1/28	1/29	1/30	1/31
1 Plus boron	+B	+B	+B	+B	+B	+B	+B
2 Minus boron for 5 days	+B	-B	-B	-B	-B	-B	+B
3 Minus boron for 4 days	+B	+B	-B	-B	-B	-B	+B
4 Minus boron for 3 days	+B	+B	+B	-B	-B	-B	+B
5 Minus boron for 2 days	+B	+B	+B	+B	-B	-B	+B
6 Minus boron for 1 day	+B	+B	+B	+B	+B	-B	+B

*Plants were inoculated in four places that day by the bent needle method described in the General Procedures section.

Treatment number one was supplied continuously with boron. The roots and jars of treatment number two were washed with distilled water and fresh Hoagland's solution minus boron was added one day before inoculation. Hoagland's solution minus boron was added to treatment number three immediately after inoculation. Treatments four, five, and six had the complete Hoagland's solution replaced with a minus boron Hoagland's solution one, two, and three days, respectively, after inoculation. Boron was added to all treatments four days after inoculation.

The nutrient solution for all treatments was changed every week, and the contents of each bottle was brought to volume when necessary with distilled water. The plants grew normally and no deficiency symptoms were noticed in any treatment. The experiment was terminated five weeks after inoculation. Leaf samples were taken for analysis and final observations on gall development were made.

Table 5. EFFECT OF BORON ON INITIATION OF CROWN GALL ON BONNY BEST TOMATO PLANTS.

Treatments	Number of Galls of Four Possible in Replication		
	1	2	3
1 Plus boron	4	2	4
2 Minus boron for 5 days	4	4	4
3 Minus boron for 4 days	4	4	4
4 Minus boron for 3 days	4	4	4
5 Minus boron for 2 days	4	4	4
6 Minus boron for 1 day	4	4	4

As indicated by Table 5 galls developed in all treatments. Boron was not added to treatment two for five days, but a gall developed at each point of inoculation. These results indicate that boron is not necessary during the initiation period.

THE EFFECT OF NITROGEN, PHOSPHORUS AND POTASSIUM ON GALL DEVELOPMENT ON BONNY BEST TOMATO PLANTS.

Introduction

The findings of Link *et al.* (25) indicated that tomato plants grown in a low nitrogen medium develop relatively smaller galls. The purpose of this experiment was to determine the effect of levels of nitrogen, potassium and phosphorus on development of crown gall on Bonny Best tomato plants and to determine whether the interaction among these three elements affects gall development.

Materials and Methods

Six weeks old Bonny Best tomato seedlings were planted in No. 10 cans containing a mixture made up of 75 per cent sand and 25 per cent peat moss. A randomized block factorial design was used with 12 treatments and five replications. The experiment was carried out in the greenhouse where the temperature ranged from 75° to 85° F. Fertilizers were added in liquid form starting one week after planting. The following treatments were applied:

	Treatments N	P	K	(A) Nitrogen	(B) Phosphorus	(C) Potassium
1	1	1	1	1 cc/liter	2 cc/liter	1 cc/liter
2	1	1	2	1 cc/liter	2 cc/liter	10 cc/liter
3	1	2	1	1 cc/liter	20 cc/liter	1 cc/liter
4	1	2	2	1 cc/liter	20 cc/liter	10 cc/liter
5	2	1	1	5 cc/liter	2 cc/liter	1 cc/liter
6	2	1	2	5 cc/liter	2 cc/liter	10 cc/liter
7	2	2	1	5 cc/liter	20 cc/liter	1 cc/liter
8	2	2	2	5 cc/liter	20 cc/liter	10 cc/liter
9	3	1	1	20 cc/liter	2 cc/liter	1 cc/liter
10	3	1	2	20 cc/liter	2 cc/liter	10 cc/liter
11	3	2	1	20 cc/liter	20 cc/liter	1 cc/liter
12	3	2	2	20 cc/liter	20 cc/liter	10 cc/liter

A 1 Molar NH_4No_3

B 1/12 Molar $Ca(HPo_4)_2$

C 1/2 Molar K Cl

The amount of each nutrient for a given treatment was pipetted into a one liter measuring cup and brought to volume with tap water. Two hundred milliliters of the diluted fertilizers were added to each plant every two weeks.

After 23 days all plants were inoculated in three places on the stem using the bent needle method described in the General Procedures section.

The experiment was terminated and data were collected approximately six weeks after making inoculations. The stem diameter above and below each gall was measured, and the average of both values was considered as the stem diameter. The diameter of the galls was measured and the gall stem ratio was calculated by dividing the gall diameter by stem diameter. This will be referred to as the relative gall size. Three observations were made per plant and the average values for the three observations are shown in Table 6 for each plant in the experiment.

Low nitrogen plants were yellowish and grew poorly while high and intermediate nitrogen plants were more vigorous. The plants responded primarily to nitrogen level as indicated by the stem diameter measurements.

A composite sample of the five replications was obtained for each treatment. Analyses were made for nitrogen, phosphorus, potassium and boron using techniques indicated in the General Procedures section. An individual sampling by replication was not possible due to the small size of the low nutrient plants.

Results

The data in Table 6 were analyzed using the individual degrees of freedom technique. Results of the analysis of variance are shown in Table 7. The relative size of the galls was significantly larger in low nitrogen plants (fig. 4). Adding potassium to the plants also caused a reduction in the relative size of the galls. Phosphorus had no effect on relative size of the galls in this experiment, but the interaction between nitrogen and phosphorus was effective. In other words the rate of reduction of the relative size of the galls by the nitrogen increased when phosphorus was added (fig. 5).

Table 6. THE EFFECT OF LEVELS OF NITROGEN, PHOSPHORUS AND POTASSIUM ON RELATIVE SIZE OF CROWN GALLS ON BONNY BEST TOMATO.

	Treatments			Relative Gall Size in Replications:						
	N	P	K	1	2	3	4	5	Total	Mean
1	1	1	1	2.14*	2.25	2.32	2.06	2.85	11.62	2.32
2	1	1	2	2.06	2.28	1.77	2.23	2.66	11.00	2.20
3	1	2	1	1.86	2.32	2.57	2.63	3.00	12.38	2.47
4	1	2	2	2.00	2.66	2.57	2.08	2.38	11.69	2.33
5	2	1	1	1.96	2.40	2.06	2.03	1.93	10.43	2.08
6	2	1	2	2.01	2.29	1.53	1.74	1.88	9.45	1.89
7	2	2	1	1.50	2.18	2.24	2.52	2.18	10.62	2.10
8	2	2	2	1.47	2.03	1.86	2.06	1.94	9.36	1.87
9	3	1	1	1.84	2.64	1.96	1.71	1.86	10.01	2.00
10	3	1	2	1.71	1.94	1.45	1.87	1.92	8.80	1.76
11	3	2	1	1.89	1.96	1.37	1.85	1.64	8.71	1.74
12	3	2	2	1.33	1.63	1.40	1.47	1.47	7.30	1.43

* Each number presents the average of three observations.

Table 7. ANALYSIS OF VARIANCE OF THE DATA OF TABLE 6.

Variation Due to:	Sum of Squares	Degrees of Freedom	Mean Square	F	Remarks
Nitrogen L	3.4692	1	3.4692	54.206	Significant*
Nitrogen Q	.0294	1	.0294	.459	
Phosphorus	.0299	1	.0299	.467	
Potassium	.6161	1	.6161	9.629	Significant*
Nitrogen L x Phosphorus	.4708	1	.4708	7.356	Significant*
Nitrogen Q x Phosphorus	.0224	1	.0224	.350	
Nitrogen L x Potassium	.0372	1	.0372	.581	
Nitrogen Q x Potassium	.0034	1	.0034	.053	
Potassium x Phosphorus	.0068	1	.0068	.0106	
Nitrogen L x Potassium x Phosphorus	.0012	1	.0012	.0018	
Nitrogen Q x Potassium x Phosphorus	.00033	1	.00033	.0051	
Error		44	0.064		

*Significant to the 1 per cent level.

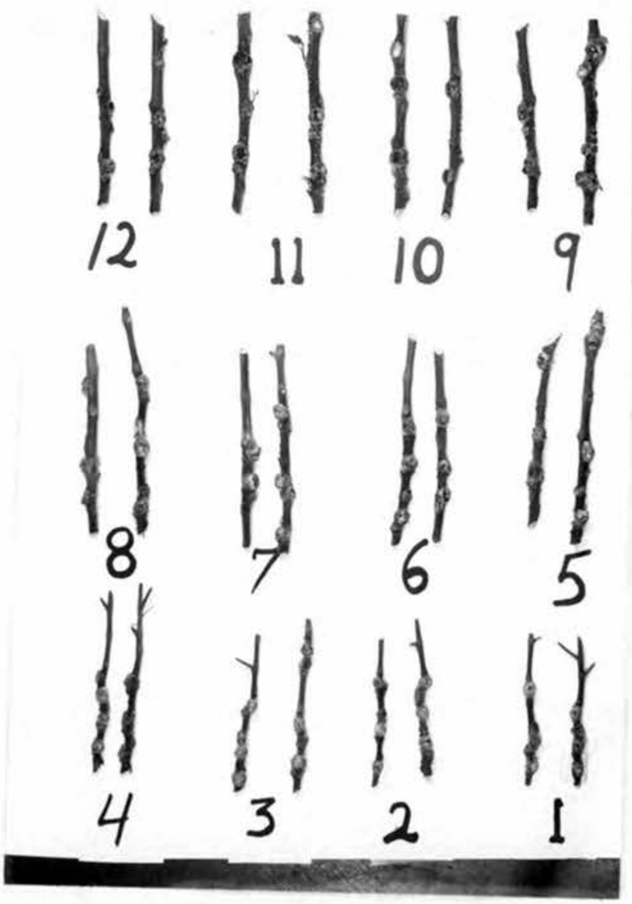


Figure 4. THE EFFECT OF NITROGEN, PHOSPHORUS AND POTASSIUM ON THE RELATIVE SIZE OF THE GALLS ON BONNY BEST TOMATO.

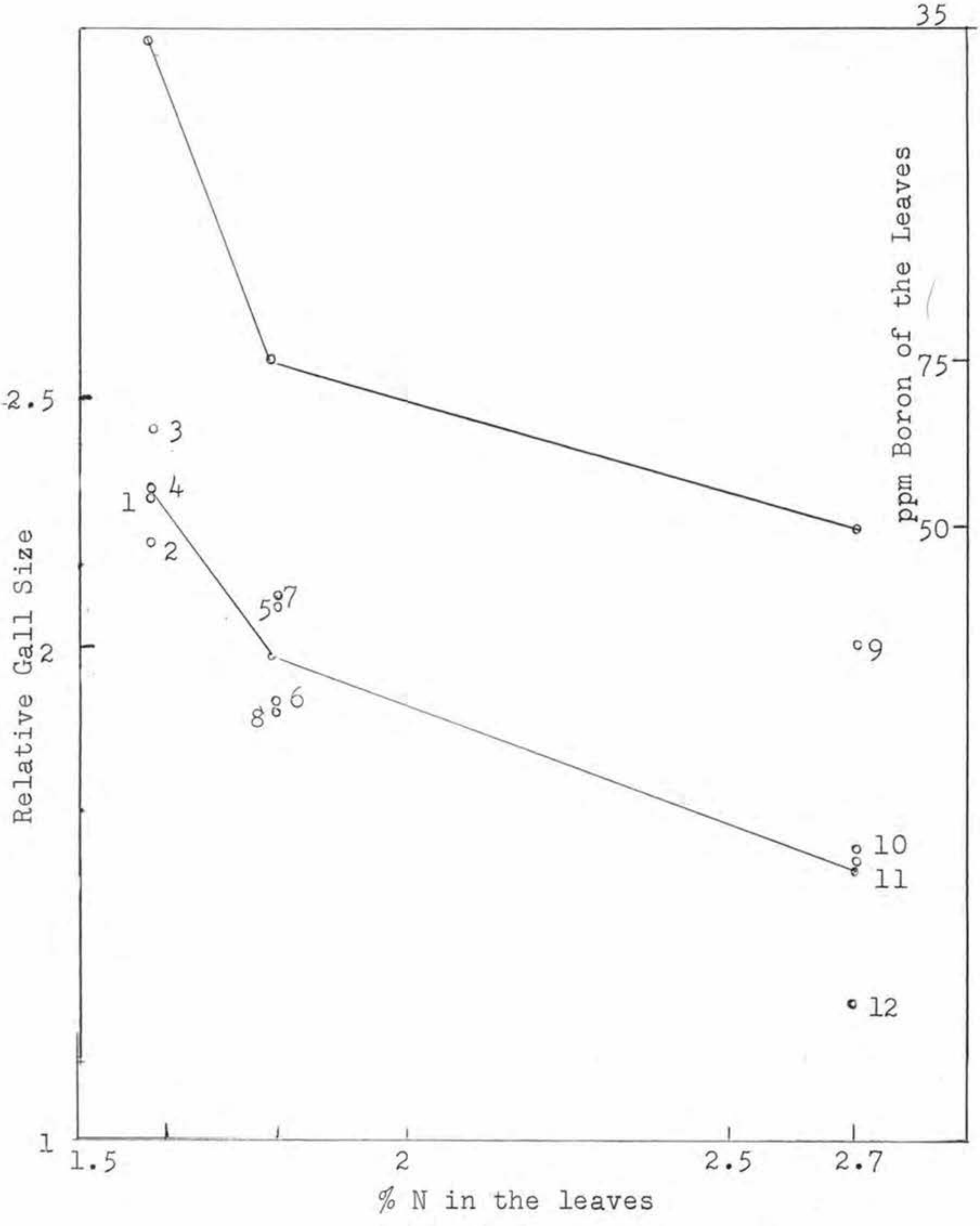


Figure 5. EFFECTS OF NITROGEN LEVEL ON THE RELATIVE SIZE AND BORON CONTENT OF BONNY BEST TOMATO. (The numbers on the curve represent the number of each treatment)

The relation between the relative gall size and nitrogen levels was significantly linear (fig. 4). The average relative gall size of five replications for each treatment is represented by each circle on Figure 5. The reduction in relative gall size as affected by nitrogen levels is shown by the line which passes through the mean of the four treatments at each nitrogen level. The deviation of the four treatment means from the line shows the effect of adding phosphorus or potassium on relative size of the galls. For example, at the highest nitrogen level treatment 12 gave the lowest relative gall size. This can be explained by the additive effect of nitrogen and potassium and the first order interaction between nitrogen and phosphorus. Treatment nine had the highest average relative gall size at the high nitrogen level because the level of potassium and phosphorus was low. Adding potassium alone or phosphorus alone reduced the relative gall size from 2.00 to 1.76 and 1.74, respectively. By adding both potassium and phosphorus the relative gall size dropped to 1.43.

The results of analysis of the leaf tissue are shown in Table 8. Nitrogen level in the leaf increased on the average from 1.6 per cent to 1.8 per cent for the second level and to 2.8 per cent for the high level. Potassium level was higher in all the treatments which received the second potassium level. The difference between

Table 8. NITROGEN, PHOSPHORUS, POTASSIUM, AND BORON CONTENT OF BONNY BEST TOMATO LEAVES.

Treatments N	P	K	% * Nitrogen	% * Phosphorus	% * Potassium	Boron ppm
1	1	1	1.60	.22	3.2	132.0
1	1	2	1.60	.14	4.3	134.0
1	2	1	1.60	.25	3.4	128.0
1	2	2	1.50	.20	3.9	124.0
2	1	1	1.80	.13	2.9	77.0
2	1	2	2.18	.15	4.5	73.0
2	2	1	1.60	.25	3.0	82.0
2	2	2	1.75	.20	3.7	70.0
3	1	1	2.75	.13	2.5	49.0
3	1	2	2.86	.12	3.6	51.0
3	2	1	2.55	.19	2.1	49.0
3	2	2	2.88	.20	3.5	45.0

* On dry weight basis.

first level and second level phosphorus application was not great. This might explain why phosphorus alone did not affect the relative size of galls in this experiment. There were no boron treatments in this experiment, but because of the previous results the level of boron in the leaves was determined. Boron level on the average dropped from about 130 ppm in plants from the lowest level of nitrogen to 75 ppm and 48.5 ppm in plants from the intermediate and highest levels of nitrogen, respectively. The effect of the reduction of boron level with increasing nitrogen is of great interest since reduction in gall size with increasing nitrogen could be due to a change in boron level rather than a direct result of changing nitrogen levels. This indicates the importance of determining the interaction between different mineral elements in a study of this nature.

THE EFFECT OF NITROGEN AND POTASSIUM ON GALL DEVELOPMENT ON MAZZARD CHERRY

This experiment was designed to test the effect of levels of nitrogen and potassium on development of galls on Mazzard cherry. There was a decrease in gall size relative to stem size in tomato plants when nitrogen and potassium were increased from a low level to an optimum level. This relationship has been tested with a woody plant, Mazzard cherry.

Materials and Methods

One year old Mazzard cherry trees were selected for uniformity. The stems were cut back, leaving 5-8 buds. The trees were planted in a mixture of 75 per cent sand and 25 per cent peat moss. The experiment was carried out in the greenhouse when the temperature was between 75-85° F.

A randomized block factorial design was selected for the experiment with 12 treatments and five replications. The treatments are shown below. Nutrient solutions were first applied four weeks after planting. In addition to nitrogen and potassium, 150 ml. of the following nutrient solution was added to each plant three weeks after inoculation: (1) $CaCO_3$ 50 grams in three liters; (2) 50 ml. of 0.05 molar Ca $(H_2PO_4)_2$ in three liters;

(3) 6 ml. of 0.5 molar $MgSO_4$ in three liter.

The stems of the trees were inoculated in four places by wounding with a file then attaching an agar disk of inoculum to the wounded areas with Scotch tape.

The following treatments were applied:

Treatment	N[1]	K[2]	Galls
1	0	0	0
2	0	0	Gall
3	0	20 ml	0
4	0	20 ml	Gall
5	5 ml	0	0
6	5 ml	0	Gall
7	5 ml	20 ml	0
8	5 ml	20 ml	Gall
9	30 ml	0	0
10	30 ml	0	Gall
11	30 ml	20 ml	0
12	30 ml	20 ml	Gall

[1] Nitrogen added as one molar NH_4NO_3 5 cc/liter or 30 cc/liter.

[2] Potassium added as 0.5 molar K_2SO_4 20 cc/liter

The experiment was terminated three months following inoculation. Leaf samples were collected from each tree, and all samples were analyzed for percentage nitrogen. Since the individual samples were small, a composite sample of five replications was used for potassium, calcium, and phosphorus determinations. Samples of gall tissue were collected from the plants in treatments 4, 6, 8, 10 and 12, and analyzed for the same elements. The methods of analysis for each of these elements are listed in the General Procedures section.

The ratio of gall diameter to stem diameter was calculated for each tree (gall diameter divided by stem diameter). The weight of the new growth was also determined.

Results

The effects of nitrogen and potassium on the average relative size of the galls for each tree are shown in Table 9. The data of Table 9 were analyzed and the analysis of variance is presented in Table 10. Nitrogen level significantly affected the relative size of the galls (figs. 6 and 7). Plants with highest nitrogen level had the lowest relative gall size. Plants with intermediate nitrogen had the highest index for gall size while plants with the lowest nitrogen were intermediate with regard to relative size of galls. The average relative size of the galls in low, intermediate, and high nitrogen plants was 2.31, 2.69, and 1.60 respectively. Potassium had no effect on gall size under the conditions of this experiment. Although the interaction between potassium and nitrogen was not significant when the analysis was based on the amount of potassium added, there was a consistent reduction in relative gall size when the actual amount of potassium in the tissue was higher (Table 11).

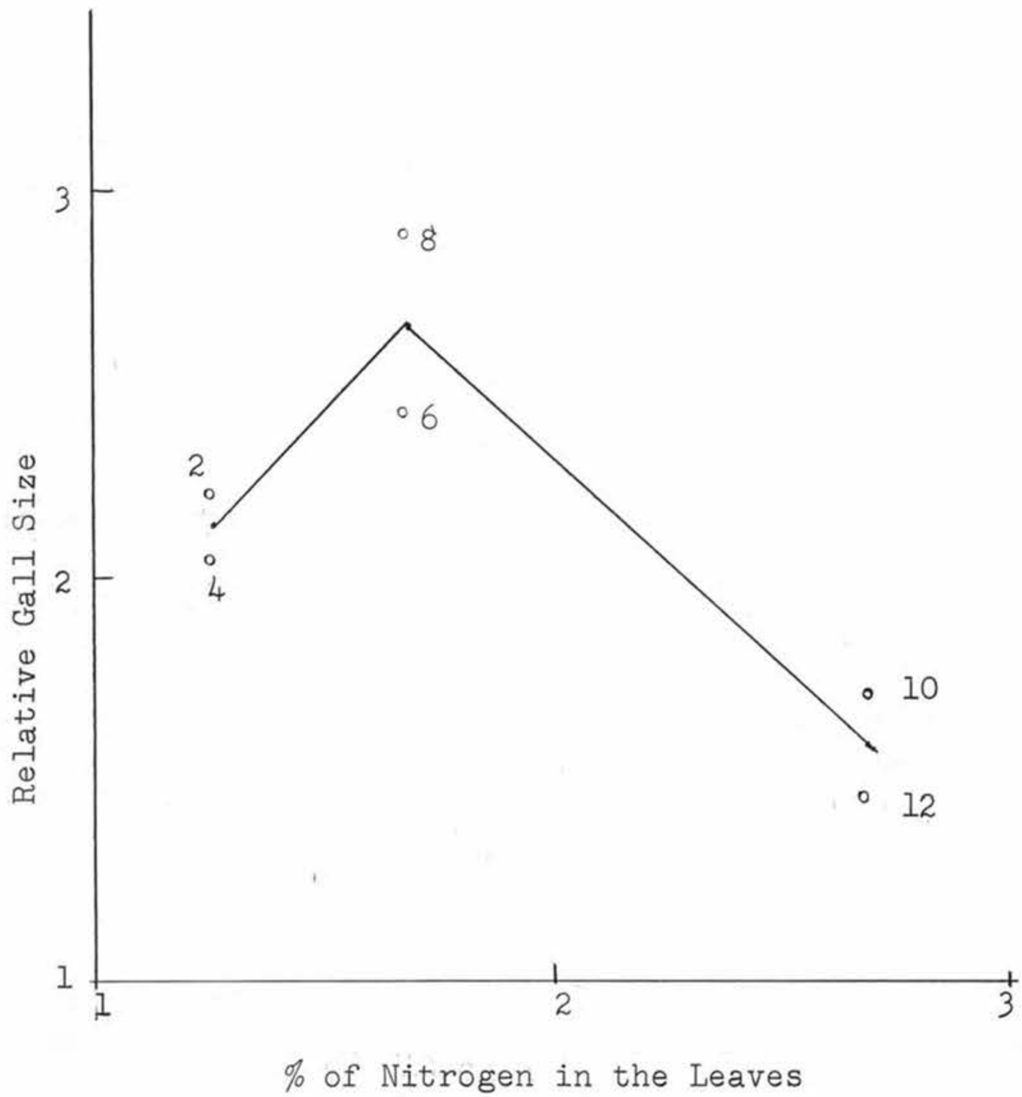


Figure 6. THE EFFECT OF NITROGEN AND POTASSIUM TREATMENTS ON THE RELATIVE SIZE OF THE GALLS ON MAZZARD CHERRY. (The numbers on the curve represent the number of each treatment.

Figure 7. THE EFFECT OF NITROGEN AND POTASSIUM ON THE RELATIVE SIZE OF THE GALLS ON MAZZARD CHERRY.

Table 9. THE EFFECT OF NITROGEN AND POTASSIUM ON THE RELATIVE SIZE OF THE GALLS ON MAZZARD CHERRY.

Number	Treatments N	Treatments K	Relative Size of Galls in Replications: 1	2	3	4	5	Total	Mean
2	0	0	2.40	2.10	2.25	2.16	2.53	11.44	2.28
4	0	1	2.25	2.70	2.60	1.60	2.56	11.71	2.34
6	1	0	2.84	2.88	1.94	2.41	2.25	12.33	2.46
8	1	1	2.47	3.88	3.30	2.56	2.40	14.61	2.92
10	2	0	1.63	2.33	1.77	1.58	1.45	8.76	1.73
12	2	1	1.40	1.45	1.46	1.58	1.39	7.28	1.45
	Totals		12.99	15.34	13.33	11.89	12.58	66.13	13.22

Table 10. ANALYSIS OF VARIANCE OF THE DATA OF TABLE 11.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	Remarks
Treatments	6.870	5			
Nitrogen L	2.527	1	2.527	19.498	Significant*
Nitrogen Q	3.596	1	3.596	27.75	Significant*
Potassium	0.0381	1	0.038	.029	
Nitrogen L x Potassium	0.153	1	0.153	1.181	
Nitrogen Q x Potassium	0.554	1	0.554	4.280	
Error	2.592	20	0.1296		

* Significant to the 1 per cent level.

Table 11. THE EFFECT OF POTASSIUM AND NITROGEN CONTENT OF THE LEAVES ON THE RELATIVE SIZE OF THE GALLS ON MAZZARD CHERRY.

Treatment Number	% Nitrogen of the Leaves	% Potassium of the Leaves	Relative Gall Size
2	1.22	0.65	2.28
4	1.23	1.08	2.34
6	1.74	1.66	2.46
8	1.59	.93	2.92
10	2.68	1.28	1.75
12	2.52	1.70	1.45

The effects of the various treatments on content of nitrogen, phosphorus, potassium, and calcium in the leaves are reported in Tables 12 and 13. The percentage of nitrogen in the leaves of plants with galls was found to be similar to that of healthy plants (Table 14). There was also no constant variation between the healthy and the gall bearing plants with regard to potassium, phosphorus and calcium content.

Table 12. THE EFFECT OF NITROGEN, POTASSIUM AND GALLS ON MINERAL CONTENT OF MAZZARD CHERRY LEAVES AND NITROGEN CONTENT OF THE GALLS.

Treatments			Per Cent Nitrogen		Per Cent Potassium	Per Cent Phosphorus	Per Cent Calcium
N	K	Gall	Leaves	Gall	Leaves	Leaves	Leaves
0	0	0	1.18		.65	.190	2.27
0	0	Gall	1.22		.65	.163	2.10
0	1	0	1.15		.85	.220	2.05
0	1	Gall	1.23	1.10	1.08	.290	2.30
1	0	0	1.71		1.24	.290	1.88
1	0	Gall	1.74	1.16	1.66	.143	2.10
1	1	0	1.52		1.16	.150	1.88
1	1	Gall	1.59	1.24	.93	.135	2.10
2	0	0	2.59		1.00	.118	1.88
2	0	Gall	2.68	1.80	1.24	.125	1.88
2	1	0	2.57		1.70	.125	1.78
2	1	Gall	2.52	1.80	1.70	.110	1.78

Table 13. THE EFFECT OF NITROGEN, POTASSIUM, AND GALLS ON THE LEVEL OF NITROGEN IN LEAVES OF MAZZARD CHERRY.

Treatments			Percentage Nitrogen in the Leaves Replications					
N	K	D	1	2	3	4	5	Total
0	0	0	1.40	1.04	.90	1.16	1.40	5.90
0	0	Gall	1.60	1.33	.85	1.13	1.22	6.13
0	1	0	1.27	1.00	1.22	1.10	1.17	5.76
0	1	Gall	1.01	1.24	1.15	1.27	1.50	6.17
1	0	0	1.64	1.60	1.82	1.88	1.64	8.58
1	0	Gall	1.68	2.10	1.56	1.72	1.68	8.74
1	1	0	1.76	1.40	1.12	1.60	1.76	7.64
1	1	Gall	1.84	1.34	1.44	1.58	1.76	7.96
2	0	0	2.52	2.76	2.44	2.58	2.66	12.96
2	0	Gall	2.74	2.72	2.40	2.74	2.80	13.40
2	1	0	2.84	2.60	2.38	2.36	2.68	12.86
2	1	Gall	2.70	2.70	2.32	2.36	2.56	12.64
Totals			23.00	21.83	19.60	21.48	22.83	108.74

NOTE: See the table of analysis of variance (Table 16).

Table 14. ANALYSIS OF VARIANCE OF THE DATA OF TABLE 13.

Variation Due to:	Sum of Squares	Degrees of Freedom	Mean Square	F	Remarks
Replication	0.619				
Treatment	20.534				
Nitrogen Linear	19.460	1	19.460	705.070	Significant*
Nitrogen Quadratic	0.922	1	0.922	33.400	Significant*
Potassium	0.145	1	0.145	5.253	Significant**
Galls	0.019	1	0.019	0.688	Not Significant
Error	1.217	44	0.0276		

* Significant to the 1 per cent level.

**Significant to the 5 per cent level.

Nitrogen levels in the tissue of the galls from treatments 4, 6, 8, 10 and 12 are shown in Table 12. Nitrogen content of the galls of high nitrogen plants was higher than that of low and intermediate nitrogen plants. Nitrogen content of the galls of treatment 4 was 1.10 per cent while the galls of treatments 6 and 8 contained 1.16 and 1.24 per cent nitrogen. The galls of treatments 10 and 12 each contained 1.80 per cent nitrogen.

The effect of the various treatments on the new terminal growth of the trees is shown in Table 15. The results of the analysis of variance, Table 16, indicates that nitrogen was the main factor responsible for the increase of shoot weight or growth of the trees (fig. 8). Potassium level had no effect on the new growth of the shoots in this experiment. The presence of galls on the shoots did not affect their vigor significantly, but there was considerable difference in the amount of new growth in treatments 7 and 8 which differed only in presence of galls. The average weight of the new shoots of treatment 8 was 2.12 grams while treatment 7, which received the same nutrient solution as treatment 8 but had no galls, had new shoots which averaged 3.3 grams in weight (fig. 9).

Figure 8. THE EFFECT OF LEVEL OF NITROGEN ON THE GROWTH OF MAZZARD CHERRY TREES.

Figure 9. THE EFFECT OF GALLS ON THE GROWTH OF MAZZARD CHERRY. TREATMENT 8 AND TREATMENT 7 RECEIVED THE SAME NUTRIENT.

Table 15. THE EFFECT OF NITROGEN, POTASSIUM, AND GALLS ON THE GROWTH OF THE MAZZARD CHERRY PLANTS.

	Treatments			Weight of the New Shoots in Grams						
				Replication						
	N	K	Galls	1	2	3	4	5	Total	Mean
1	0	0	0	.60	.90	.20	1.10	.50	3.30	.66
2	0	0	Gall	.50	.65	.90	.80	.20	3.05	.62
3	0	1	0	.25	.90	.45	.50	.40	2.50	.50
4	0	1	Gall	.40	.45	.80	.20	.25	2.10	.42
5	1	0	0	1.80	1.65	1.30	1.10	3.40	9.25	1.98
6	1	0	Gall	1.40	.55	5.15	2.00	2.75	11.85	2.37
7	1	1	0	4.55	2.65	2.75	2.35	3.30	15.60	3.12
8	1	1	Gall	2.00	1.85	1.85	3.35	1.20	10.25	2.05
9	2	0	0	14.40	6.40	9.00	12.50	2.40	44.70	8.94
10	2	0	Gall	4.20	5.20	9.50	11.50	11.70	42.10	8.42
11	2	1	0	9.10	8.30	13.20	13.00	12.00	55.60	11.12
12	2	1	Gall	7.00	5.60	7.90	9.20	8.40	38.10	7.62
	Total			46.20	35.10	53.00	57.60	46.50	238.40	

NOTE: See the table of analysis of variance in the text.

Table 16. ANALYSIS OF VARIANCE

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	Remarks
Nitrogen Linear	28747.2025/40	1	718.680	188.778	Significant*
Nitrogen Quadratic	9516.0025/120	1	79.300	20.830	Significant*
Potassium	98.0100/60	1	1.633		
Disease	552.2500/60	1	9.201	2.416	Not Significant
NLK	74.8225/40	1	1.870		
NQK	18.9225/120	1	.157		
NLD	378.3025/40	1	9.457		
NQD	232.5625/120	1	1.438		
KD	529.0000/60	1	8.816		
NLKD	217.5625/40	1	5.439		
NQKD	.7225/120	1	.006		
Error	167.532/44	44	3.807		

* Significant to the 1 per cent level.

THE EFFECT OF CROWN GALL ON GROWTH AND MINERAL CONTENT OF BONNY BEST TOMATO

Introduction

The effect of crown gall on the host has been reviewed. It is generally observed that galls reduce the growth of the host, but in some investigations galls have caused no noticeable effect on growth. The data obtained by Link *et al*. (15) showed that tumors on minus-nitrogen plants affect the host severely causing a depression of the rate of axis elongation, reduction in leaf formation, and at times death of the growing points.

Two experiments were carried out to determine the effect of galls on growth of the host and on content of mineral nutrients in host tissue.

Experiment I. Inoculation Made by Pushing the Needle Half Way Through the Stem

Material and Methods

Bonny Best tomato seeds were germinated in sand. The seedlings were transferred to No. 10 cans containing a mixture of 75 per cent sand and 25 per cent peat moss. The plants were selected for uniformity in height and number of leaves prior to setting up the experiment. A randomized block design was used for the experiment with four treatments and five replications. The four treatments were:

1. Control.
2. Stems inoculated in three places.
3. Stems inoculated in six places.
4. Stems inoculated in nine places.

The plants were inoculated four weeks after the seedlings had been transferred. Inoculations were made by pushing the needle half way through the stem after it had been dipped in a culture of Agrobacterium tumefaciens. The needle was bent to a 90° angle to facilitate a uniform depth of inoculation.

Host mineral nutrition was maintained at a low level throughout the experiment. One hundred milliliters of liquid fertilizer containing 30 ml of 1 molar NH_4No_3, 20 ml of .05 molar Ca $(H_2Po_4)_2$ and 15 ml of 0.5 molar K_2So_4 per liter were added to each plant two weeks after planting. One hundred milliliters of Hoagland's solution were supplied to each plant three weeks after the first addition, and a final nutrient additive of 50 milliliters of Hoagland's solution per plant was supplied after two weeks. The plants grew but were not vigorous.

The experiment was terminated seven weeks after inoculation. The height of each plant was measured. Plants were removed from the cans carefully in order to prevent damage to the roots. The plants were dried in a

75° C. oven, and the dry weight of both tops (stems and leaves) and roots were taken. The tops were ground and analyses made for nitrogen, potassium, phosphorus, and calcium. Gall tissue from all plants was collected, and analyses were made for the same elements. Analyses were performed by the methods cited in the General Procedures section.

Results

The effect of the galls on the height of the tomato plants is shown in Table 17.

Table 17. THE EFFECT OF GALLS ON HEIGHT OF BONNY BEST TOMATO PLANTS SIX WEEKS AFTER INOCULATION.

	Height in Inches of Plants with:			
Replication	No Galls	3 Galls	6 Galls	9 Galls
1	19.2	18.0	19.2	--
2	18.8	22.0	17.5	20.1
3	18.8	20.0	19.5	21.5
4	18.2	17.3	18.2	18.2
5	20.0	21.0	21.5	21.5
Mean	19.0	19.6	19.1	20.1

Galls did not affect the height of the tomato plants. The mean height of the control plants and the 3, 6, and 9 gall plants was 19.0, 19.6, 19.1, and 20.1 inches, respectively (fig. 10).

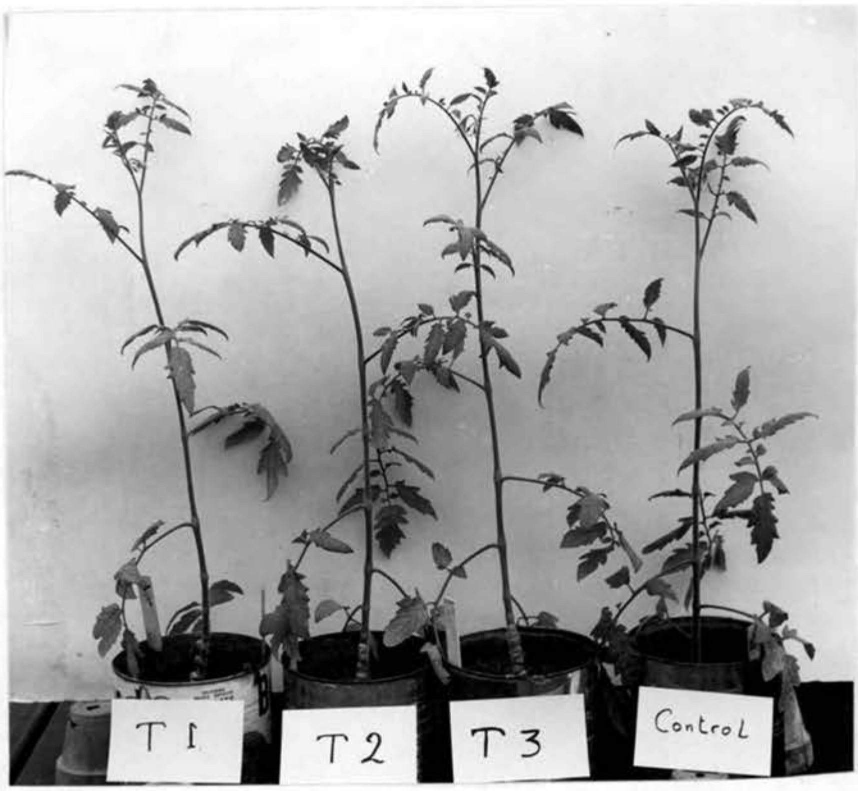
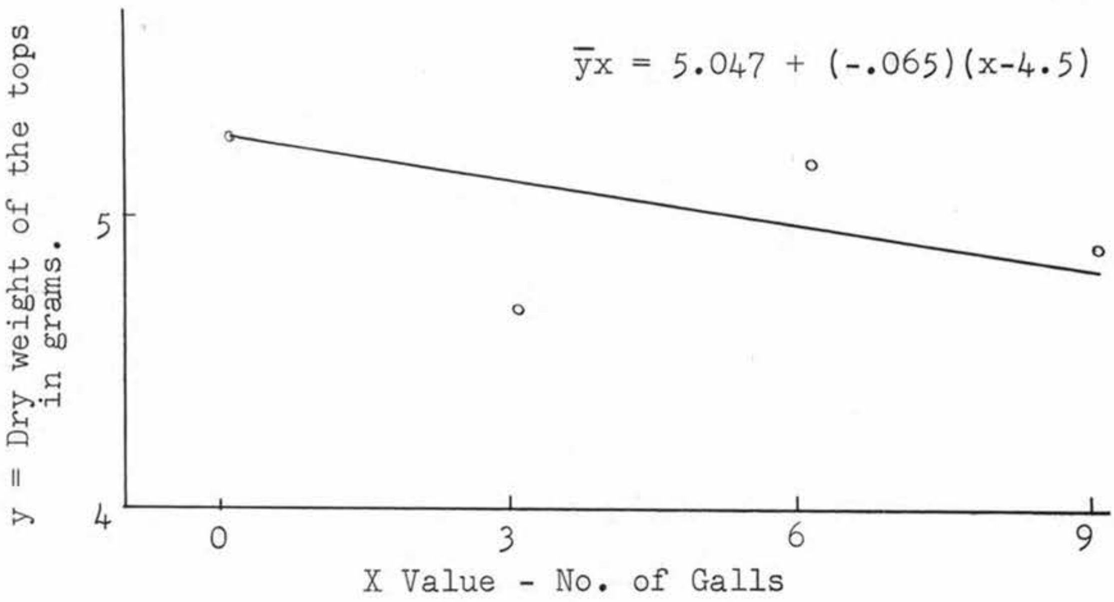


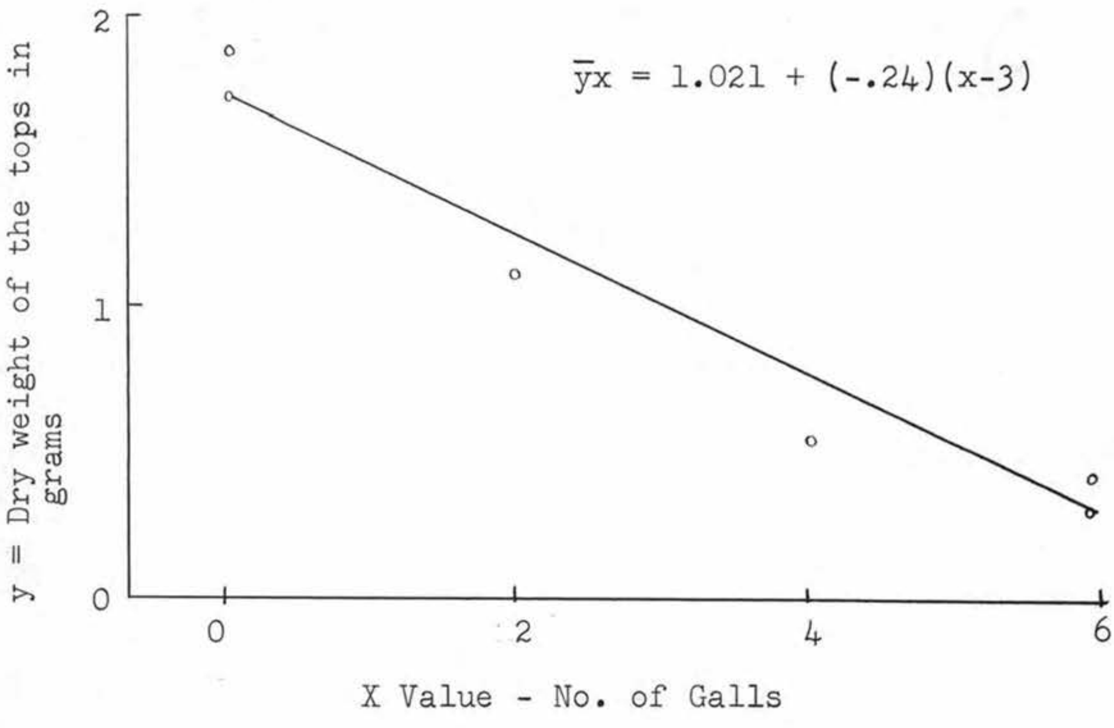
Figure 10. THE EFFECT OF GALLS ON THE GROWTH OF BONNY BEST TOMATO PLANTS.

Dry weights of the tops are shown in Table 18. In many ways dry weight is a better indication of amount of growth than height measurements. Since the test of linearity (Table 19) indicated that the relation between the treatment (x) and the top dry weight (y) is linear, the data of Table 18 were analyzed statistically by the method of regression analysis. The results of the analysis, Table 19, and the slope of the regression line (fig. 11, Experiment I) demonstrate that there is no difference in dry weight of the tops. The mean dry weight of the tops of the control plants was 5.63 grams, while the mean dry weight of the tops of the plants with 3, 6 and 9 galls was 4.68 grams, 5.02 grams and 4.86 grams, respectively.

The effect of the galls on the dry weights of the roots is shown in Table 20. The mean dry weight of the roots was .78 grams for the control, and 0.62, 0.70 and 0.68 for the 3, 6 and 9 gall plants respectively. The slope of the regression line (fig. 12, Experiment I) indicates that the rate of change in weight of the roots among the different treatments is not significant.

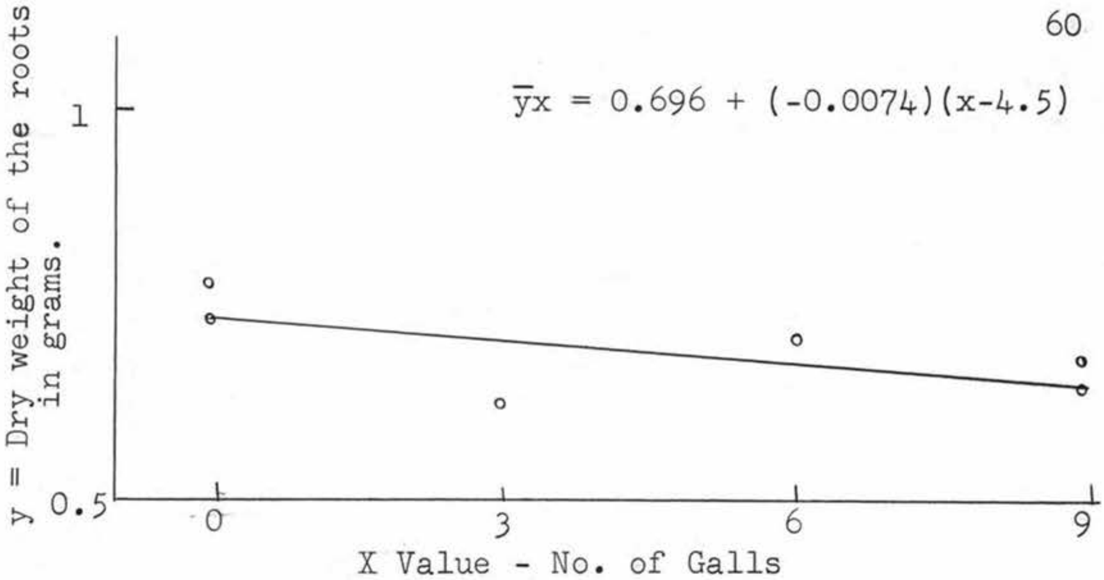


Experiment I. Galls on the side of the stem.

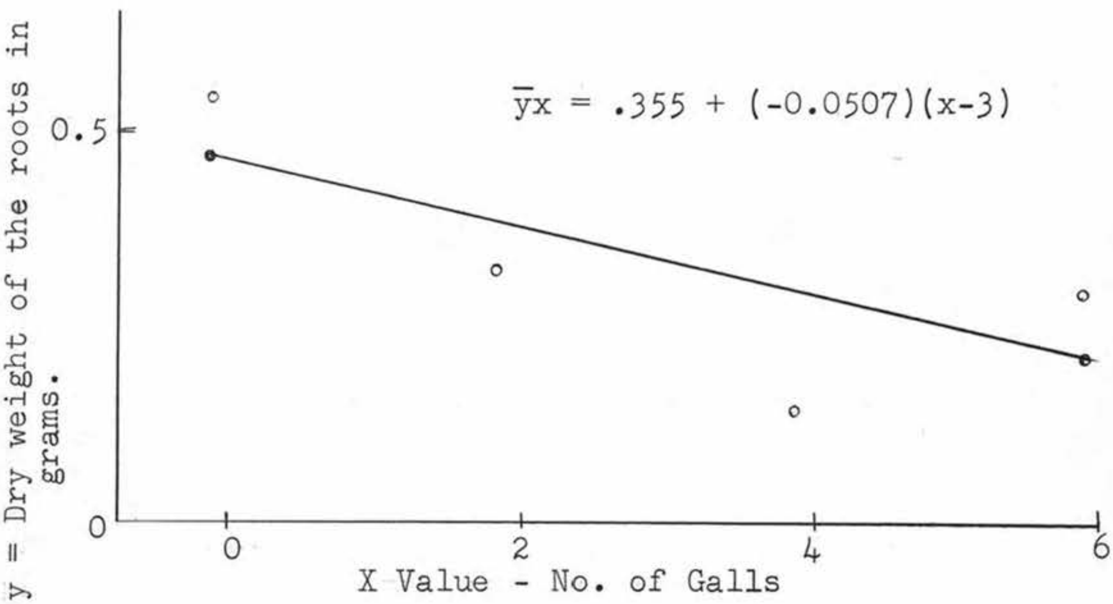


Experiment II. Galls through the stem.

Figure 11. THE EFFECT OF GALL POSITION ON DRY WEIGHT OF THE TOPS.



Experiment I. Galls on the side of the stem



Experiment II. Galls through the Stem.

Figure 12. THE EFFECT OF GALL POSITION ON DRY WEIGHT OF THE ROOTS.

Table 18. THE EFFECT OF GALLS ON THE DRY WEIGHT OF THE TOPS OF BONNY BEST TOMATO. EXPERIMENT I.

x / y	Dry Weight in Grams of Stems and Leaves of Plants with:			
	No Galls	3 Galls	6 Galls	9 Galls
Observations	6.70	4.95	5.70	5.50
	5.70	5.25	4.50	5.55
	5.55	4.00	5.30	4.60
	4.30	3.70	4.40	3.65
	5.90	5.50	5.20	5.00
Total	28.15	23.40	25.10	24.30
Mean	5.63	4.68	5.02	4.86

Regression Coefficient b = -.065

Regression equation = $\overline{y}x = 5.047 + (-.065)(x-4.5)$

Test of the hypothesis that B =0 is F = .163 with 12 and 18 d.f.

Table 19. ANALYSIS OF VARIANCE OF THE DATA OF TABLE 21. TEST OF LINEARITY.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Replications	6.8506	4		
Treatments	2.550	3		
Linear Regression	.9702	1		
Dev. from Linearity	.1579	2	.0789	.0411*
Error	2.3050	12	.1921	
Total	11.7063	19		

* Not significant.

Table 20. THE EFFECT OF GALLS ON THE DRY WEIGHT OF THE ROOTS OF BONNY BEST TOMATO. EXPERIMENT I.

x y	Dry Weight in Grams of Roots of Plants with:			
	No Galls	3 Galls	6 Galls	9 Galls
Observations	1.10	.65	.82	.86
	.71	.65	.65	.65
	.81	.60	.65	.55
	.60	.55	.70	.65
	.70	.65	.70	.75
Total	3.91	3.10	3.52	3.40
Mean	.78	.62	.70	.68

Regression Coefficient $b = -.0074$

Regression equation $= \bar{y}x = .696 + (-.0074)(x-4.5)$

Test of the hypothesis that $B = 0$ is $F = .0083$ with 1 and 18 d.f.

Content of Nitrogen, Phosphorus, Potassium, and Calcium in the Leaves of Healthy and Infected Plants

The average percentage nitrogen in the leaves (Table 21) was 1.09, 1.09, 1.13 and 1.28 in the control plants and the three, six and nine gall plants, respectively. There was no significant difference in leaf nitrogen as a result of galls on the stems. There was also no difference in level of phosphorus (Table 23), potassium (Table 22), and Calcium (Table 24) in leaf tissue of healthy and tumor bearing plants.

Table 21. NITROGEN CONTENT OF THE TOPS OF BONNY BEST TOMATO AT DIFFERENT LEVELS OF INOCULATION. EXPERIMENT I.

	Per Cent Nitrogen in the Stems and Leaves of Plant with:			
Replications	No Galls	3 Galls	6 Galls	9 Galls
1	1.20	1.10	1.16	1.12
2	1.10	1.18	1.06	1.10
3	1.16	1.00	1.16	1.80
4	.94	1.10	1.16	1.20
5	1.06	1.10	1.11	1.20
Total	5.46	5.48	5.65	6.42
Mean	1.09	1.09	1.13	1.28

Nitrogen content of the composite sample of the galls was 1.76 per cent.

Table 22. POTASSIUM CONTENT OF THE TOPS OF BONNY BEST TOMATO AT DIFFERENT LEVELS OF INOCULATION. EXPERIMENT I.

Replications	Per Cent Potassium in the Stems and Leaves of Plants with: No Galls	3 Galls	6 Galls	9 Galls
1	3.08	3.00	2.95	3.00
2	3.08	3.20	2.65	3.20
3	2.85	3.00	2.70	3.20
4	2.55	3.00	3.05	2.85
5	2.95	2.65	3.30	3.05
Total	14.51	14.85	14.65	15.30
Mean	2.90	2.97	2.93	3.06

Potassium content of the composite sample of the galls was 4.50 per cent.

Table 23. PHOSPHORUS CONTENT OF THE TOPS OF BONNY BEST TOMATO AT DIFFERENT LEVELS OF INOCULATION. EXPERIMENT I.

Replications	Per Cent Phosphorus in the Stems and Leaves of Plants with: No Galls	3 Galls	6 Galls	9 Galls
1	.170	.150	.163	.170
2	.163	.170	.150	.180
3	.150	.143	.170	.170
4	.125	.143	.170	.170
5	.170	.150	.170	.180
Total	.778	.756	.823	.870
Mean	.155	.151	.164	.174

Phosphorus content of the composite sample of the gall was 0.410 per cent.

Table 24. CALCIUM CONTENT OF THE TOPS OF BONNY BEST TOMATO AT DIFFERENT LEVELS OF INOCULATION. EXPERIMENT I.

Replication	Per Cent Calcium in the Stems and Leaves of Plants with: No Galls	3 Galls	6 Galls	9 Galls
1	1.70	1.65	1.62	1.78
2	1.62	1.50	1.55	1.70
3	1.62	1.55	1.78	1.65
4	----	1.65	1.83	1.65
5	1.60	1.50	1.83	1.60
Total	6.54	7.85	8.61	8.38
Mean	1.62	1.57	1.72	1.67

Calcium content of the composite sample of the galls was 1.20 per cent.

Content of Nitrogen, Phosphorus, Potassium, and Calcium in Gall Tissue and Leaf Tissue

The analysis of gall tissue showed that the level of nitrogen, phosphorus, and potassium was higher in gall tissue than in normal tissue. The mean percentage of nitrogen in the tops ranged from 1.09 to 1.28 while it was 1.76 in the galls. Leaf phosphorus content ranged from 0.155 to 0.174 per cent while it was 0.410 per cent in gall tissue. Potassium content of gall tissue was 4.50 per cent compared to 2.90 to 3.06 per cent in the tops. On the other hand, calcium content of the galls was 1.20 per cent, and the lowest value of calcium in the leaves was 1.57 per cent (Tables 21, 22, 23 and 24; fig. 13).

Figure 13. NITROGEN, PHOSPHORUS, POTASSIUM AND CALCIUM CONTENT OF THE LEAVES AND GALLS ON BONNY BEST TOMATO. EXPERIMENT I.

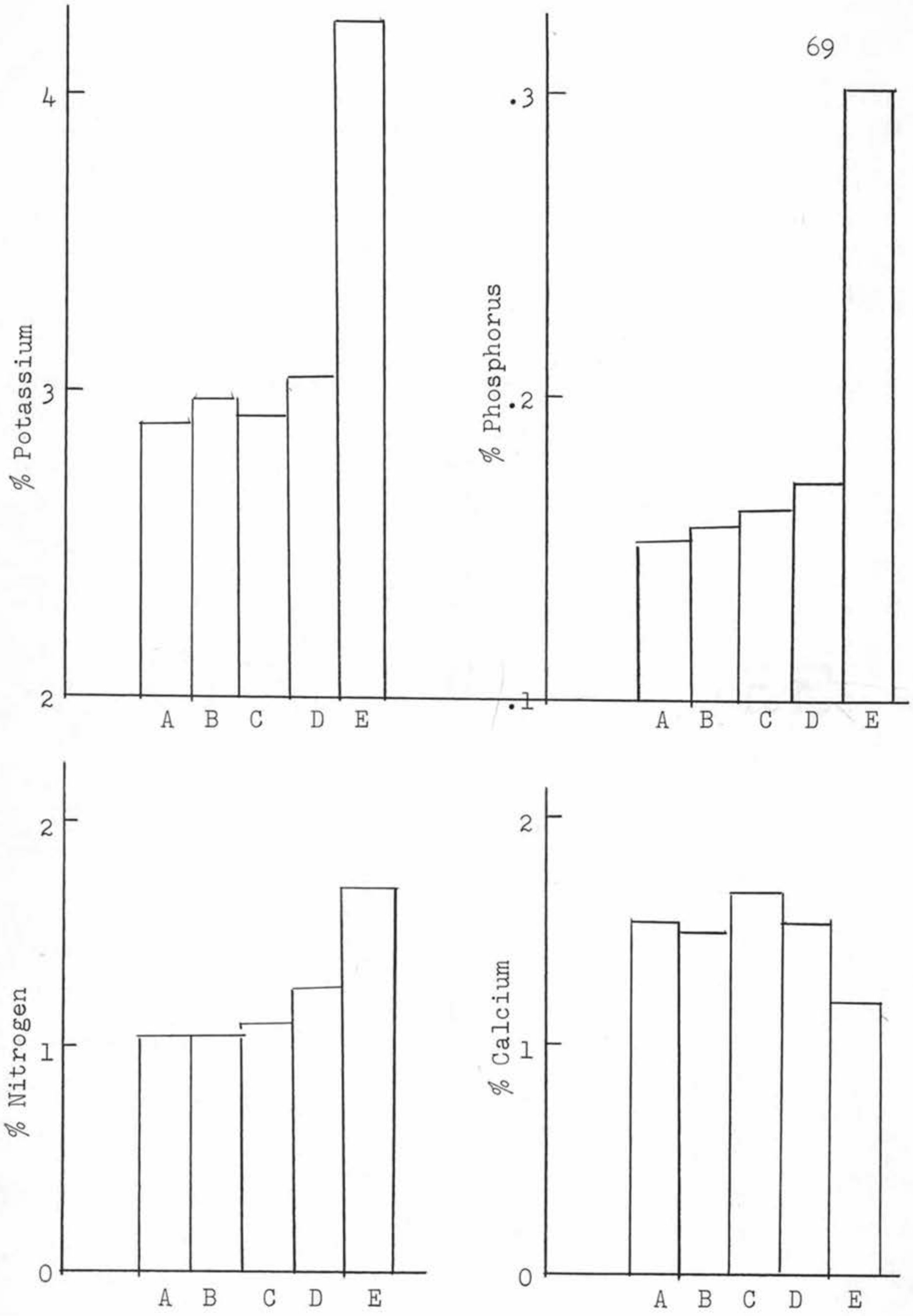
A Control

B Plants with three galls

C Plants with six galls

D Plants with nine galls

E Gall tissue



EXPERIMENT I

THE EFFECT OF CROWN GALL ON GROWTH AND MINERAL CONTENT OF BONNY BEST TOMATO.

Experiment II. Inoculation Made by Pushing the Needle Completely Through the Stem

Material and Methods

Bonny Best tomato seedlings were planted in No. 10 cans containing a mixture of 75 per cent sand and 25 per cent peat moss. They were selected for uniformity in height prior to setting up the experiment. A randomized block design was used for the experiment with four treatments and five replications. The treatments were:

1. Control
2. Stems inoculated in two places.
3. Stems inoculated in four places.
4. Stems inoculated in six places.

Plants were inoculated two weeks after the seedlings were transplanted. Each inoculation was made by dipping the needle in a dense suspension of *Agrobacterium tumefaciens* and pushing the needle completely through the center of the stem.

The plants were watered twice with Hoagland's solution. Twenty milliliters were supplied fifteen days after inoculation, and 100 milliliters were supplied three weeks after the first application.

The experiment was terminated seven weeks after inoculation. Data were collected on stem height, fresh weight of galls, and dry weight of tops and roots. Tops and gall tissue were analyzed for nitrogen, phosphorus, potassium, and calcium by the methods cited in the General Procedures section.

Results

The effect of galls on growth of tomato plants in height is shown in Table 25 and Figure 14.

Table 25. THE EFFECT OF GALLS ON THE HEIGHT OF BONNY BEST TOMATO PLANTS.

	Height of Plants in Inches						
	Replication						
Treatments	1	2	3	4	5	Total	Mean
Control	15.2	18.0	16.0	14.5	17.5	81.5	16.4
2 Galls	16.0	13.0	12.0	12.0	12.0	65.0	13.0
4 Galls	5.0	4.5	10.5	9.5	9.5	39.0	7.8
6 Galls	5.5	4.5	9.0	10.0	13.0	42.0	8.4

The height of the plants was reduced when galls were present. The average height of the control plants was 16.4 inches while plants with two galls averaged 13.0 inches in height. The height of the 4 gall and 6 gall plants was about half that of the control plants (fig. 14).

Figure 14. THE EFFECT OF CROWN GALL ON THE GROWTH OF BONNY BEST TOMATO.

The effect of galls on the dry weight of roots and tops and the relation between the fresh weight of gall tissue and the dry weight of normal tissue are shown in Figure 15 and Tables 26 and 27. The dry weight of both tops and roots decreased significantly as the total fresh weight of the galls increased. The average dry weight of the tops of control plants was 1.90 grams. This value decreased to 1.17 grams, 0.53 grams, and 0.47 grams as the total fresh weight of the gall tissue increased from 3.33 grams to 5.10 grams, and 5.70 grams, respectively. A similar effect on the root system was noted (Table 27).

The data of Tables 26 and 27 were analyzed by the method of regression analysis. The regression lines for the dry weights of the tops and roots are shown in Figures 11 and 12. The slope of the lines and the F tests indicate that galls affected the growth of the plants.

The results of the analysis of both leaf and gall tissue for nitrogen are presented in Table 28. Nitrogen content of leaf tissue was lower in the control and two gall plants than in the four gall and six gall plants. There was an accumulation of nitrogen in gall tissue. The mean percentage of nitrogen in the leaves of treatment two was 1.09 while the mean percentage of nitrogen in the galls of the same treatment was 1.76. The same relation was apparent in treatments three and four (Table 28).

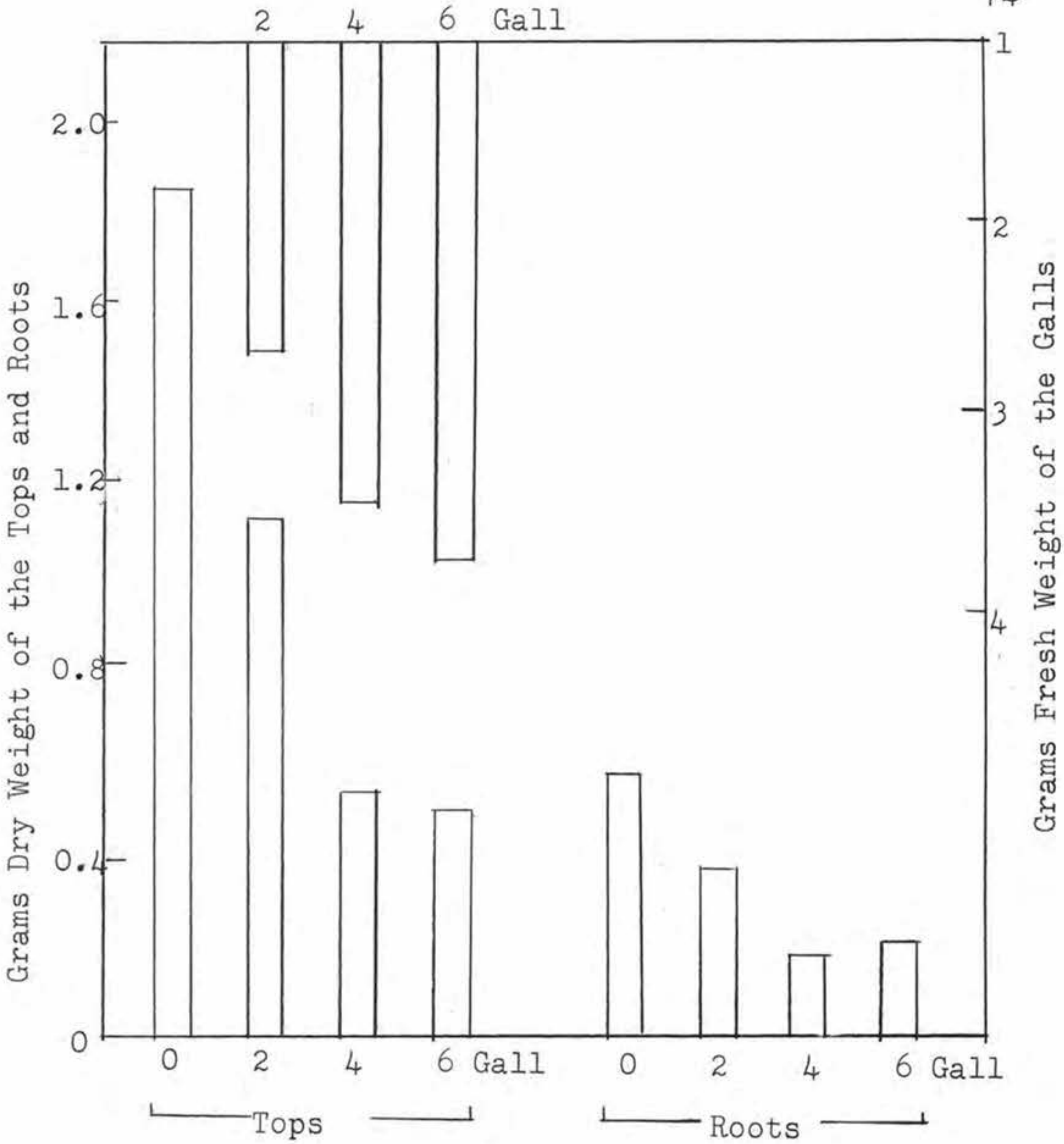


Figure 15. CORRELATION BETWEEN FRESH WEIGHT OF GALL TISSUE AND DRY WEIGHT OF TOPS AND ROOTS OF BONNY BEST TOMATO AT DIFFERENT LEVELS OF INOCULATION.

Table 26. THE EFFECT OF THE GALLS ON DRY WEIGHT OF THE TOPS OF BONNY BEST TOMATO. EXPERIMENT II.

x	Dry Weight in Grams of Stems and Leaves of Plants with:			
y	No Galls	2 Galls	4 Galls	6 Galls
	1.75	1.80	.55	.20
	2.15	1.16	.12	.15
Oobservations	1.82	.80	.80	.30
	1.85	1.02	.70	.75
	2.35	1.11	.50	.95
Total	9.52	5.88	2.67	2.35
Mean	1.90	1.17	.530	.470

Regression coefficient $b = -0.24$

Regression equation $\bar{y}x = 1.021 + (-0.24)(x-3)$

Test of the hypothesis that $B = o = F = 28.76$ with 1 and 18 d.f.

Table 27. THE EFFECT OF GALLS ON THE DRY WEIGHT OF THE ROOTS OF BONNY BEST TOMATO. EXPERIMENT II.

x y	Dry Weight in Grams of Roots of Plants with:			
	No Galls	2 Galls	4 Galls	6 Galls
	.55	.50	.13	.25
	.80	.34	.10	.10
Observations	.60	.40	.30	.30
	.40	.30	.20	.40
	.60	.20	.14	.50
Total	2.95	1.75	.87	1.55
Mean	.570	.340	.150	.300

Regression coefficient $b = -.0507$

Regression equation $\bar{y}x = .355 + (-.0507)(x-3)$

Test of the hypothesis that $B = 0$ $F = 18.00$ with 1 and 18 d.f.

Table 28. NITROGEN CONTENT OF THE TOPS AND GALLS OF BONNY BEST TOMATO AT DIFFERENT LEVELS OF INOCULATION. EXPERIMENT II.

	Per Cent Nitrogen in the Stems and Leaves of Plants with:				Per Cent Nitrogen in the Galls of Plants with:		
Replications	No Galls	2 Galls	4 Galls	6 Galls	2 Galls	4 Galls	6 Galls
1	1.00	1.12	1.40	2.30	1.50	2.10	2.40
2	1.00	1.00	2.20	2.23	1.55	2.30	2.20
3	1.00	1.08	1.18	1.62	2.00	2.00	2.10
4	1.16	1.08	1.24	1.80	1.90	1.80	2.05
5	1.00	1.18	1.28	1.30	1.85	1.76	1.64
Total	5.16	5.46	7.30	9.15	8.80	9.96	10.39
Mean	1.03	1.09	1.46	1.83	1.76	1.99	2.07

Potassium content of the leaf and gall tissue is shown in Table 29. The percentage of potassium in the leaves of the control and treatment two plants was lower than the percentage in the leaves of treatment three and four plants. Potassium content of the gall tissue was higher than potassium content of the leaves (Table 29).

The data on content of phosphorus in the leaves and galls are shown in Table 30. There was a slight increase in percentage of phosphorus in the leaves of gall bearing plants as opposed to the control, and in all treatments gall tissue was higher in phosphorus than the leaf tissue on the same plants (Table 30).

The calcium content of leaf and gall tissue is shown in Table 31. Calcium content of the leaf tissue did not change much in treatments one, two and three, but it was higher in treatment four. Gall tissue, on the other hand, was lower in calcium compared to the leaves of the same plants (Table 31).

The data on mineral content of gall tissue and the stem and leaf tissue for each treatment in Experiment II are illustrated in Figure 16. Each bar indicates the average value of the corresponding treatments. The nutrient element content of the plants with galls usually showed an increase rather than a decrease when compared with healthy plants. There was a higher percentage of nitrogen, phosphorus, and potassium in leaves of gall bearing as opposed to healthy plants, but the percentage of calcium was lower in gall bearing plants.

Table 29. POTASSIUM CONTENT OF THE TOPS AND GALLS OF BONNY BEST TOMATO AT DIFFERENT LEVELS OF INOCULATION. EXPERIMENT II.

	Per Cent Potassium in Stems and Leaves of Plants with:				Per Cent Potassium in Galls of Plants with:		
Replications	No Galls	2 Galls	4 Galls	6 Galls	2 Galls	4 Galls	6 Galls
1	2.35	2.00	3.50	2.60	2.60	3.30	4.3
2	2.25	2.00		3.15	2.90	4.50	4.1
3	2.35	2.50	3.10	2.50	3.30	4.00	4.2
4	2.50	3.00	3.55	2.60	3.40	3.40	3.7
5	2.60	2.90	3.50	4.60	3.30	4.30	3.2
Total	12.05	12.40	13.65	15.45	15.50	18.60	19.5
Mean	2.41	2.48	3.41	3.10	3.10	3.70	3.9

Table 30. PHOSPHORUS CONTENT OF THE TOPS AND GALLS OF BONNY BEST TOMATO AT DIFFERENT LEVELS OF INOCULATION. EXPERIMENT II.

	Percentage Phosphorus in the Stems and Leaves of Plants with:				Percentage Phosphorus in the Galls of Plants with:		
Replications	No Galls	2 Galls	4 Galls	6 Galls	2 Galls	4 Galls	6 Galls
1	.300	.280	.270	.450	.400	.480	.450
2	.270	.300		.300	.480	.450	.400
3	.380	.360	.250	.304	.500	.480	.560
4	.360	.380	.400	.300	.560	.540	.500
5	.240	.380	.440	.300	.540	.560	.400
Total	1.55	1.76	1.36	1.65	2.52	2.51	2.31
Mean	.310	.352	.340	.330	.504	.502	.462

Table 31. CALCIUM CONTENT OF THE TOPS AND GALLS OF BONNY BEST TOMATO AT DIFFERENT LEVELS OF INOCULATION. EXPERIMENT II.

	Percentage Calcium in the Stems and Leaves of Plants with:				Percentage Calcium in Galls of Plants with:		
Replications	No Galls	2 Galls	4 Galls	6 Galls	2 Galls	4 Galls	6 Galls
1	1.65	1.40	1.45	3.00	1.15	1.20	1.00
2	1.88	1.45		2.80	1.20	1.40	1.20
3	1.45	1.60	1.78	3.20	1.15	1.20	1.30
4	1.32	1.40	1.65	2.05	1.25	1.25	1.25
5	2.00	1.32	1.45	1.65	1.25	1.20	1.20
Total	8.30	7.17	6.33	12.65	6.00	6.25	5.95
Mean	1.66	1.44	1.58	2.54	1.20	1.25	1.19

Figure 16. NITROGEN, PHOSPHORUS, POTASSIUM, AND CALCIUM CONTENT OF THE LEAVES AND GALLS ON BONNY BEST TOMATO. EXPERIMENT II.

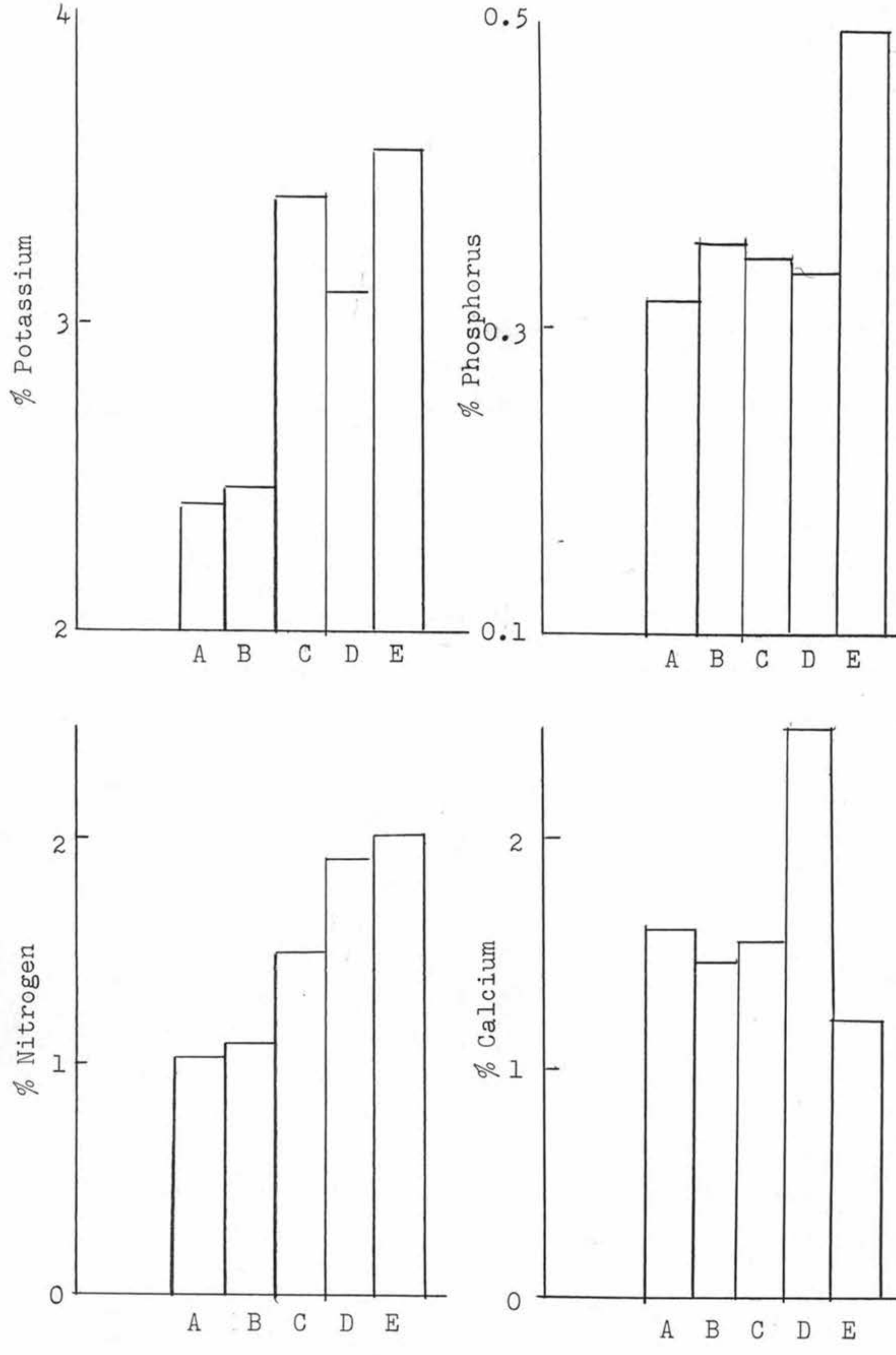
A Control

B Plants with three galls

C Plants with six galls

D Plants with nine galls

E Gall tissue



EXPERIMENT II

DISCUSSION

Effects of Boron on Development of Crown Gall

During the four week period following inoculation, galls began to develop on Mazzard cherry trees from both minus and plus boron treatments. By the end of the fourth week boron level in both treatments was above the deficiency level for stone fruit trees. There was no morphological difference in size, shape, or appearance between the galls of the two treatments because there was sufficient boron during this period for gall development.

The effect of boron deficiency became apparent about the eighth week after inoculation. Galls of the minus boron plants began to dry up leaving necrotic areas, while the galls of plus boron plants continued to develop normally (Table 2). The results of leaf analysis at that time indicated that the amount of boron in minus boron plants was at the deficiency level, while plus boron plants contained sufficient boron for continued growth of trees and development of galls (Table 2 and Figures 1A and 1B). No galls developed on the new shoots of minus boron plants which were inoculated during the eighth week, but galls did develop on the corresponding shoots of plus boron plants (Table 3 and Figure 2). There were no severe boron deficiency symptoms on minus boron plants and no striking differences of any kind in the growth of plus and minus boron plants at the end of eight weeks when galls had begun to dry up.

It has been shown in this investigation that galls can grow to an optimum value even under limited nitrogen supply and that large galls develop on plants under conditions of severe nitrogen deficiency. Galls degenerated, however, on Mazzard cherry even before boron deficiency symptoms became evident. A possible explanation for this is the immobility of boron as opposed to nitrogen; that is, boron does not move readily from mature tissue to growing areas while nitrogen does.

A similar relation between gall development and boron levels was obtained with Bonny Best tomato. Very small galls started to form on tomato plants which were maintained continuously in a minus boron solution, but they did not develop to normal size. The average boron content of the leaves of these plants was 22.7 ppm. This boron, which was present as contamination, was apparently sufficient to permit slow growth of the plants and development of a small amount of gall tissue.

The reaction of plants which received no boron after inoculation was more severe than the reaction of plants which were grown continuously in a boron deficient medium (fig. 3A). After one week the new leaves turned yellow, and there was severe terminal necrosis. Galls did not even begin to develop on these plants. Apparently the boron present as contamination was sufficient to maintain growth of a weak plant but not enough to permit continued growth of a vigorous plant. Boron content of the leaves of these plants was as low as 13 ppm in the necrotic terminal leaves and as high as 51 ppm in the older leaves.

Typical galls developed on plants which received boron throughout the experiment and on plants which received no boron until inoculation and then were transferred to a plus boron solution. Furthermore, the plants which received no boron prior to inoculation contained as much of this element at the end of the experiment as plants which had been supplied with boron continuously (Table 4, fig. 3A and 3B).

Boron deficiency seems to affect the growth and development rather than initiation of crown gall. Boron was withheld from tomato plants one day prior to inoculation and up to four days after inoculation then supplied again (Table 5). The four day period following inoculation is reported to be the time when normal tissue is converted to gall tissue. Lack of boron during this period had no affect on gall initiation since galls developed normally on all plants.

Boron is required in normal plant growth, but the specific role of boron in growth is not known. The two mechanisms which are supported by the greatest amount of experimental evidence are that boron is needed in cell division or cell enlargement, Whittington (54, 55), Neals (33, 34), and that boron deficiency has an effect on sugar translocation (10, 43, 46, 29).

It is of interest that available sugar has been reported to be important in gall development through its effect on protein synthesis (35, 36, 7). It is possible that sugar in boron deficient plants is less available for the formation of galls, but boron deficiency would be equally effective in preventing gall growth if boron were required for cell division or cell enlargement.

Effect of Nitrogen, Phosphorus, and Potassium on Crown Gall Development.

The relationship between relative size of crown galls and nitrogen content of the leaves of Bonny Best tomato plants was linear (fig. 5 and Table 6); that is, when nitrogen content of the leaves was high the relative gall size was significantly small. The relative size of the crown galls on Mazzard cherry increased to an optimum value in the second level of nitrogen, then dropped to a minimum value in the highest level of nitrogen. The rise in the growth curve to an optimum indicated that some nitrogen is needed for maximum gall development in Mazzard cherry. Since some nitrogen was added to the tomato plants (1 ml/liter of one molar NH_4NO_3), perhaps this amount was sufficient to permit development of galls to optimum size. It is also possible that woody plants might behave differently from herbaceous plants in this respect.

Link et al. (25) noted the decrease in relative gall size with increase in level of nitrogen. However, these investigators could not have obtained information on the optimum level of nitrogen for gall development because they worked with only two levels of nitrogen. The present results contradict Levine's statement (23) that a vigorously growing host develops large galls.

Link et al. (25) and Klein (20) explained the increased growth of tumors on a low nitrogen plant by the capacity of tumors to mobilize nutrients from the host. Their observations are not supported by this investigation since the results of leaf analysis indicate that the mineral nutrient content of the stems and leaves was not reduced by the presence of galls; in fact mineral nutrient level was usually higher than in the non-tumor bearing plants (figs. 13 and 16). The fact that some nitrogen is needed for optimum growth of the galls also seems to contradict this hypothesis.

Link et al. (25) further suggested that inadequate vascularization between tumor and host of the high nitrogen plants prevents the tumors on these plants from mobilizing nitrogenous materials of the host. This contention is contradicted by the finding that nitrogen content of the gall tissue varied directly with the amount of nitrogen supplied to the plants. Nitrogen content of the galls on Mazzard cherry was 1.10, 1.20, and 1.80 per cent in low, intermediate, and high nitrogen plants, respectively.

An explanation of the decrease in relative gall size on the basis of tumor activity alone is misleading because two other factors, host plant activity and its independent growth and the interaction between host tissue and gall tissue, should also be considered.

Although galls possess a great capacity for growth and development independent of the host perhaps because of a high auxin concentration (12), high enzyme activity (3, 20), and a more effective ion-transport system (57), their ability to grow is limited to an optimum value in the same manner that host growth is limited. Because of the more efficient metabolic systems in gall tissue it seems reasonable that tumors might reach their optimum growth with a lower nutrient supply than would normal tissue. Once the galls reach their optimum growth, however, their size in relation to the stem diameter of the host is determined by growth of the host. If the host is growing under a limited nutrient supply, its growth is very poor and galls on such a host are relatively large. Galls borne on high nutrient plants reach an optimum rate of growth and grow no faster with added nutrients while the normal host tissue continues to respond by further growth. Although galls on such plants would tend to be large, the ratio between gall size and stem size would be relatively small. The results of the present investigation support this hypothesis. The average diameter of the galls on low, intermediate, and high nitrogen plants was 9.77, 11.0, and 11.5 millimeters. The last two values are very similar, suggesting that the optimum value for the growth of the galls has been reached. On the other hand, the rate of the growth of the host continued to increase as nitrogen level increased. The average diameter of the host plants was 4.2, 5.5, and 6.7 mm. in the low, intermediate, and high nitrogen plants, respectively.

The decrease in relative gall size with increased nitrogen may also be explained by an interaction between nutrient elements. It has been shown that boron is required for gall development. An increase in nitrogen level drastically reduced the boron content of the foliage of Bonny Best tomatoes even though all plants were growing in uniform media with regard to boron supply.

Reduction in level of boron from 125 ppm in the low nitrogen plants to 75 and 50 ppm in the intermediate and high nitrogen plants may be the factor influencing relative gall size. The normal tissue responded to added nitrogen by increased growth, but growth of the gall tissue may have been affected by the reduced boron level.

The effect of potassium on relative size of galls on Bonny Best tomato plants was similar to that of nitrogen; that is, adding potassium caused a reduction in the relative gall size (Table 7). The interaction between potassium and nitrogen was not significant. Although each of these elements acted independently to reduce gall size, the rate of reduction in relative gall size was not increased by adding both potassium and nitrogen simultaneously.

No significant effect on relative size of galls on Mazzard cherry resulted when potassium was added. Perhaps the amount of potassium was not great enough to be effective. There was very little difference in the amount of potassium present in plus and minus potassium plants in this experiment. Statistically, the interaction between nitrogen and potassium was not significant. In one treatment, however, the potassium content of the leaves of the minus potassium plants was greater than that of the plus potassium plants. If the actual potassium content is considered rather than the supplied potassium, the interaction between potassium and nitrogen is quite evident. Relative gall size was not affected by potassium content when the nitrogen supply was very low, but the relative gall size was even smaller on intermediate and high nitrogen plants when the tissue had a high potassium content. It appears that at a low level of nitrogen there is no response to the addition of potassium because growth of both gall and normal tissue is limited by the amount of available nitrogen. This also suggests that there may have been no interaction between potassium and nitrogen in affecting gall size on Bonny Best tomato because potassium levels were not sufficiently different or the level of nitrogen was never low enough.

It is suggested that potassium influences relative size of galls in the same manner as nitrogen, that is, through its effect on host vigor.

Phosphorus alone had no effect on the relative size of the galls on Bonny Best tomato, but the interaction between nitrogen and phosphorus was significant. When phosphorus was added to the plants which received the high level of nitrogen, a greater reduction in relative size of the galls resulted. Addition of phosphorus to plants which received the lowest level of nitrogen did not influence relative gall size because the rate of growth of both the gall and host tissue was again limited by nitrogen level.

The independent effects of nitrogen, potassium, and phosphorus, and the interactions of these three elements support the previously stated hypothesis that the optimum growth rate of gall tissue is reached with a relatively low nutrient supply; therefore, the rate of growth of the host plant is the principal factor which determines the relative size of galls. With both Bonny Best tomato and Mazzard cherry, treatments in which the highest level of nutrient was supplied resulted in the most vigorous plants and the smallest relative gall size.

The Effect of Crown Gall on the Growth of the Host

When Bonny Best tomato was inoculated so that galls occurred only on one side of the stems, there was no significant reduction in growth as measured by height, dry weight of stems and leaves or dry weight of the roots (Tables 17, 18, and 20 and Figures 10, 11, and 12). When plants were inoculated by pushing the needle completely through the stem so that galls developed on both sides, plant height, dry weight of stems and leaves, and dry weight of roots were all reduced (Tables 25, 26 and 27, Figures 11, 12, and 14).

There was no significant difference in dry weight of the new growth of inoculated and uninoculated Mazzard cherry plants. There was, however, considerable difference between the inoculated and uninoculated plants at the mineral nutrient level which most favored gall growth over normal stem growth. New shoots of the inoculated plants had an average 2.05 grams weight while new shoots of the uninoculated plants had an average weight of 3.2 grams.

The data which demonstrate the effect of crown gall on growth agree with the work reported by Link et al. (25) and Deep and Young (8), while the data demonstrating no effect on growth agree with the results reported by Riker et al. (39).

Link et al. (25) and Sempio (45) interpreted the reduction in size of gall bearing plants to an interference with host nutrition by the mobilization of nutrients from the host to the gall. Although gall tissue analysis showed an accumulation of mineral nutrients in the galls, analysis of leaf and stem tissue from the same plants did not show a reduction in such elements. On the contrary, there was a higher percentage of nitrogen, potassium, and phosphorus in diseased plants than in the comparable healthy plants (Figures 13 and 16).

Although the manner in which galls affect growth is still controversial, data from this investigation suggest that more is involved than prevention of translocation of mineral nutrients, and these data do not support the theory that there is a mobilization of nutrients from the host to the galls. The results of this investigation suggest that the position of galls and the degree of malformation of the stem determines the degree of effect on growth. Nine galls on the side of the host did not reduce the growth while four galls on both sides of the stem significantly reduced plant size.

These data suggest that galls prevent normal functioning of the xylem or phloem. Melhus et al. (28) and Muncie (31) reported that young apple and peach trees translocated less water when galls were present on the stems. However, several lines of evidence suggest that this may not be the significant factor affecting plant growth. Severely galled plants seldom wilt, and histological examination of galled stems reveals the presence of a large amount of recognizable xylem tissue. It is also questionable whether the degree of inhibition of translocated water which was usually obtained by Melhus and Muncie would affect plant growth.

The effect of galls on growth of the roots has been ignored by previous workers. Such an effect was demonstrated in this investigation. It is possible that galls influence growth of the entire plant through an initial effect on the roots. This effect could be brought about by crushing of phloem elements by the galls with subsequent interference with the translocation of carbohydrates and other organic nutrients to the roots. It has been reported that galls on tomato plants caused a crushing of the phloem elements (21).

Accumulation of Mineral Nutrients in Gall Tissue

Nitrogen, phosphorus and potassium levels were higher in gall tissue of Bonny Best tomato than in normal healthy tissue while calcium content was lower in gall tissue than in normal tissue (Figures 13 and 16). The accumulation of nitrogen and phosphorus has been reported previously by Nagg *et al*. (32), Klein (19), Taso *et al*. (49) and Hope (17).

The accumulation of potassium, which is very striking, has not been reported previously. The amount of potassium in gall tissue was 4.5 per cent while the average potassium content of the healthy control plants was 2.9 per cent. This is of interest because of the possible relationship between the high enzymatic activity of gall tissue and high potassium content of the tissue. The low calcium content of gall tissue might be expected since calcium is found in older tissue, especially in crystal form, and it is an immobile element.

SUMMARY AND CONCLUSIONS

1. Crown galls did not develop on inoculated Mazzard cherry and Bonny Best tomato plants when they were maintained in minus boron Hoagland's solution. This was found to be an effect on gall development rather than gall initiation.
2. The effects of nitrogen, potassium, and phosphorus on relative size of galls on Bonny Best tomato were investigated, and the effects of nitrogen and potassium on relative size of galls on Mazzard cherry were determined.
3. An increase in nitrogen or potassium caused a reduction in relative size of the galls on Bonny Best tomato. There was an interaction between nitrogen and phosphorus; that is, adding phosphorus to the plants with the high level of nitrogen caused an even greater reduction in relative gall size.
4. Nitrogen alone and the interaction between nitrogen and potassium caused a reduction in the relative size of galls on Mazzard cherry.
5. It has been postulated that the relative size of crown galls is determined by the independent growth of the host and the galls. The optimum growth rate of gall tissue is reached when nutrient supply is relatively low. This may be because of the active

metabolic systems present in galls. The optimum growth rate of normal host tissue is not reached until nutrient supply is much higher. For this reason when nutrient supply is low, gall diameter becomes much larger than stem diameter. When nutrient supply is high, both gall tissue and stem tissue grow at the optimum rate, and there is much less difference between gall and stem diameter.

6. The presence of galls had no influence on mineral nutrient content of the leaves of Mazzard cherry.
7. When Bonny Best tomato plants were inoculated so galls developed on one side of the stems, there was no effect on the growth of the plants. When tomato plants were inoculated so galls developed on both sides of the stems, plant height, dry weight of stems and leaves, and dry weight of roots were reduced.
8. The data suggest that the degree of malformation of the vascular tissue of the stem is the factor which reduces growth of the host. The hypothesis has been made that galls reduce root growth by causing malformation of the phloem. Reduction in size of the root system could affect growth of stems and leaves.

9. Mineral nutrient content of the stems and leaves of gall bearing Bonny Best tomato plants was similar to that of healthy plants, and in some cases mineral content was even higher in diseased plants.
10. Nitrogen, phosphorus, and potassium accumulated in gall tissue on Bonny Best tomato, while calcium content of gall tissue was lower than that of healthy tissue. The accumulation of potassium has not been reported previously, and the possible correlation between high potassium content and high enzymatic activity of gall tissue should be explored.

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