AN ABSTRACT OF THE THESIS OF

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in PLANT PATHOLOGY (Major) presented on June 28, 1967 (Date)

Title: AMINO ACIDS IN TISSUES FROM HEALTHY AND CROWN GALL INFECTED KALANCHOE PINNATA

Abstract approved: [redacted]
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Crown gall infected and healthy Kalanchoe pinnata (Lam.) Persoon plants were analyzed for soluble amino acids. Specifically the amino acids in young leaf, mature leaf, stem, root, and gall tissues were quantitatively measured. The plants were inoculated with the bacterium, Agrobacterium tumefaciens (Smith and Townsend) Conn., on one side of the stem to initiate unilaterial galls (UG) and on opposite sides of the stem to produce opposite galls (OG). Plants with opposite galls and unilateral galls were supplied with either low or high nitrogen levels in the root medium.

Diseased plants supplied with low nitrogen had larger amino acid totals in diseased young leaf and stem tissue, and a lower total in old leaf tissue than controls. There were no qualitative amino acid patterns common among the different tissues of diseased plants characteristic for the crown gall disease. When OG and UG plants

were compared, the OG plants had more alanine in young leaves, stems, and roots, but less alanine in old leaves. The major factor influencing the amino acid pools in the plant tissues appeared to be the low nitrogen level rather than the presence or position of the galls on the stem.

The high nitrogen OG plants had about half the amino acid total found in controls or UG plants. They had lower totals in young leaves, old leaves, and stems than control tissues; and lower totals in young leaves, old leaves, and galls than UG plants. The UG plants had lower totals in young leaves, stems and roots, but a higher total in old leaves than control tissues.

The OG plants had less aspartic acid, glutamic acid, glutamine, and arginine in young leaves, old leaves, and galls than UG plants. Stem tissue from OG plants had less aspartic acid and glutamic acid. Galls from OG plants had less aspartic acid, glutamic acid, alanine, glutamine, arginine, serine, and leucines than galls from UG plants.

No notable amino acid trends were common among tissues of diseased plants (OG and UG) supplied with high nitrogen. The amino acid pool levels in the different tissues of OG and UG plants appeared to be influenced as much by gall positions as by the physiological presence of the gall.

Since the gall positions on high nitrogen plants had such an

effect on amino acid levels in plant tissues, workers who study crown gall diseased plants in the future should be cognizant of this fact when inoculating plants. Biochemical and physiological differences which may be attributed to differences between diseased and healthy plants could be due to gall positions on the plant.

AMINO ACIDS IN TISSUES FROM HEALTHY AND CROWN GALL INFECTED KALANCHOE PINNATA

by

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A THESIS

submitted to

OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

MASTER OF ARTS

June 1968

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[redacted]

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Date thesis is presented June 28, 1967

Typed by Joanne Wenstrom for Raymond Gene Harris

ACKNOWLEDGMENT

The author wishes to thank Dr. Ira W. Deep for his valuable guidance during the research investigations and initial preparations of the manuscript. Appreciation is given to Dr. Thomas C. Allen for his helpful suggestions and assistance during the preparation of the manuscript.

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INTRODUCTION

The crown gall disease caused by the bacterium Agrobacterium tumefaciens is an abnormal growth disease. The bacteria invade a wound in the host plant and cause healthy cells to be converted to tumorous cells. The tumor cells are characterized initially by rapid cell division, followed by cell hyperplasia and hypertrophy, and undifferentiated cell proliferation which results in a crown gall tumor.

In the study of the crown gall disease, most workers have been interested in elucidating the biochemical and physiological changes which are correlated with the conversion of healthy host cells to actively dividing tumor cells. One experimental approach has been to isolate tumor and normal tissue for tissue culture studies. Habituated and incompletely transformed tumor tissues and possible intermediate steps in the conversion of healthy cells to completely tumorous cells have been studied. Only a few workers have investigated the physiological changes which occur within the intact diseased plant as a result of crown gall development.

In this study, the objectives were to attempt to detect some biochemical differences between healthy and crown gall diseased plants. Specifically the young leaves, mature leaves, stems, roots

and galls of diseased and healthy Kalanchoe pinnata (Lam.) Persoon were analyzed qualitatively and quantitatively for their soluble amino acid contents. Inoculation procedures were varied to determine the effect of gall positions upon the soluble amino acid concentrations and distributions in the different organs of the host plant. Plants with identical inoculation treatments were grown in the presence of low and high nitrogen levels in the root medium to determine the possible effect of different levels of nitrogen nutrition on amino acid concentrations in healthy and diseased plant tissues.

LITERATURE REVIEW

Normal, habituated, and crown gall tumor tissues of grape were examined in tissue culture by Lee (1952) for total nitrogen, soluble nitrogen, and total crude protein. He found crown gall tissue contained the highest percentage of total nitrogen, soluble nitrogen, and estimated crude protein. Habituated tissues had the next highest amounts and normal tissues had the lowest concentrations. Lee hydrolyzed the three tissues with acid and found that, qualitatively, their amino acid contents were the same.

Robson, Budd, and Yost (1959) compared normal and crown gall tissues in tissue culture and reported that tumor tissue had twice as much nitrogen and protein on a per cell basis, and 2.5 times as much DNA as normal cells. Neisch and Hibber (1940) measured the water soluble protein content in cultures of tumor tissue and found it to be six times greater than in normal tissues. They found that tumor tissue retained 64% of its Kjeldahl nitrogen in the form of protein while normal tissue retained only 39%.

Simonson and Roberts (1962) cultured normal and tumor tissue from Boston ivy, cactus, tobacco, periwinkle, Virginia creeper and carrot. All tissues were analyzed for soluble amino compounds. They found that tissue from each plant species had its own characteristic pattern of amino compounds when grown in tissue culture on the

same synthetic medium. The amino compound contents of normal and tumor tissues from a particular plant species were the same qualitatively but differed quantitatively. They also found more similarities with the amino compounds of normal and tumor tissues from the one plant species than between tumor tissues from different species. Amino acid comparisons between tumor tissues of the different plant species yielded no similarities which were specific for, or characteristic of, the tumorous tissue.

Tissue culture methods have been examined critically by some workers. Generally, such investigations have shown that the physiology, cytology, and composition of plant tissues cultured in tissue culture are subject to change when grown on different synthetic media. In addition, the isolated tissue in tissue culture may be quite different biochemically when compared with the plant parts from which they were isolated. Tulecke et al. (1962) reported differences in the physiology and biochemistry of tissue cultures and the respective plant parts of Ginkgo biloba L. This work was continued by Weinstein and Tulecke et al. (1959) with tissue cultures and normal plant parts of Agave toumevana Trel. Later Weinstein et al. (1962) examined physiological and biochemical differences between rose tissue culture and rose stem tissue. The general conclusions from these investigations were that tissue grown in culture resembles the original tissue. The isolated tissues were shown to retain some of their inherent

capabilities. They still may have the capacity to produce specific compounds and have the ability to regenerate a whole plant.

Spurr, Hildebrandt, and Riker (1964) studied tumor and normal tissues in culture and found that the growth and oxidase activity of the tissues in culture were controlled by the culture medium composition and bore no obvious relationships to the growth and oxidase activity of the corresponding intact plants. Steward, Thompson, and Pollard (1958) performed similar investigations and reported that the proportion of alcohol-soluble to alcohol-insoluble nitrogen was greater in resting tissues than in proliferating tissues in culture. They showed that differences in nitrogen composition do exist between actively growing and non-growing tissues.

Other workers have examined the tumor and normal tissues in plants. The most extensive work was carried out by Klein (1952). He grew numerous tomato plants and followed changes in the nitrogen metabolism of the tumors from inoculation to the development of crown gall tumors. Throughout the experiments he found that the amounts of soluble and protein nitrogen were greater in tumor tissue than in normal tissue. The major increase of protein nitrogen in the tumor tissue occurred late in tumor development and was initiated when the tumor switched from numerical cell increase to cell volume increase. The soluble nitrogen followed the same trend and increased, but the accumulation rate was slower. Since soluble

nitrogen and protein both increased in tumorous tissue during the maturation phase, the increased protein synthesis did not happen entirely at the expense of the amino acids in the soluble amino acid pool.

Steward, Thompson, and Pollard (1958) reported on nitrogen metabolism in crown gall infected leaves of Kalanchoe daigremontiana Hamet and Perrier. They found that tumors had considerably larger amounts of protein than normal leaves or normal parts of tumor bearing leaves. Neisch and Hibbert (1940) working with healthy and tumor tissues of beets found that crude protein content was higher in tumor tissue than in normal tissue of tumorous beets or in healthy beet tissue. Hussin's (1962) data on total nitrogen in tumor and normal tomato tissues followed the trends noted by Steward and Neisch. He found that the level of total nitrogen was higher in gall than in normal tomato tissues. Link and Goddard (1951) showed that tumors developing on nitrogen deficient plants contained less than half the total nitrogen found in tumors grown on plants supplied with an adequate supply of nitrogen. They also noted that the level of total nitrogen increased in older tumors. Further evidence along this line has been presented by James and Barbara Lippincott (1964). They worked with crown gall infected carrot phloem slices and found that tumor tissue contained twice as much protein as normal tissue on a wet or dry weight basis.

Seitz and Hochster (1964) determined the soluble amino compounds from normal plant parts and plant tumors of tomato and tobacco. They found the amino acid, lysopine, in tumor and normal tissues of both plants. Again the amino compounds of tumor and normal tissues were qualitatively the same, but there appeared to be quantitative differences between the two tissues.

Some general trends concerning the nitrogen metabolism of tumor and healthy tissues can be noted in previous research. In both tissue culture and plant tissue studies, the tumor tissue has more soluble protein, insoluble protein, total nitrogen, crude protein, and soluble amino compounds when compared with normal or habituated tissue. Tumor tissues from different plant species have shown no similarities in amino acids which are specific for or characteristic of the tumorous state. The soluble amino contents of tumor and normal tissues of the same plant species have been shown to differ quantitatively and not qualitatively. Additionally, caution must be used in relating biochemical and physiological data from tissue culture studies to the possible physiological processes of the intact plant. The physiological and biochemical compositions of tissues in tissue culture have been shown to be quite different from that found in plant tissues from the intact plant.

MATERIALS AND METHODS

Design of Experiments 1 and 2

Two experiments were set up using Kalanchoe pinnata plants. The plants were propagated vegetatively and were selected for uniform size prior to the experiments. The plants were grown in no. 10 cans containing soil very low in nitrogen content. The experiments were conducted in a randomized block design in the greenhouse. Plants were harvested 100 days after inoculations.

Experiment 1 was set up in the Fall of 1964 and consisted of 6 treatments with 15 replications;

1. Low nitrogen control.
2. Low nitrogen: plants wounded 6 places, 3 wounds on opposite sides of stem.
3. Low nitrogen: plants inoculated with crown gall bacteria in 6 places, 3 inoculations on opposite sides of the stem.
4. High nitrogen control.
5. High nitrogen: plants wounded 6 places, 3 wounds on each side of stem.
6. High nitrogen: plants inoculated with crown gall bacteria in 6 places, 3 inoculations on opposite sides of the stem.

Experiment 2 was set up in the Fall of 1965 and consisted of 6

treatments with 10 replications;

1. Low nitrogen control.
2. Low nitrogen: plants inoculated 4 times with crown gall bacteria, 2 inoculations on each side of the stem.
3. Low nitrogen: plants inoculated 4 times with crown gall bacteria, all 4 inoculations on the same side of the stem.
4. High nitrogen control.
5. High nitrogen: plants inoculated 4 times with crown gall bacteria, 2 inoculations on each side of the stem.
6. High nitrogen: plants inoculated 4 times with crown gall bacteria, all 4 inoculations on the same side of the stem.

The wounded control treatments were not repeated in Experiment 2 because the controls and the wounded controls gave identical results in Experiment 1.

Inoculation Procedures

The plants were inoculated by dipping a needle in a bacterial suspension and pushing the needle 1/4 inch into the base of the stem. A constant inoculation depth was maintained by bending the inoculation needle at right angles 1/4 inch from the tip. The inoculation loci were 1/2 inch apart.

Fertilization Schedules

Identical nutrient solutions were used in both experiments. The high nitrogen solution contained 20 ml 1M NH_4NO_3, 20 ml 1/12 M $Ca(H_2PO_4)_2$, 10 ml 1/2 M KCL, and tap water to give a liter volume. Fifty ml of the solution were added every 9 days to each plant in Experiment 1, while Experiment 2, 100 ml were added to each plant at the same time intervals. In Experiment 2, 1100 ml of the high nitrogen solution were added to each plant while only 550 ml were added to each plant in Experiment 1.

The low nitrogen nutrient solution contained the same amounts of $Ca(H_2PO_4)_2$ and KCL as the high nitrogen solution. In Experiment 1, the low nitrogen solution contained 1 ml of 1M NH_4NO_3 per liter. In Experiment 2, NH_4NO_3 was omitted to increase the magnitude of difference between high and low nitrogen treatments. The volumes and schedule in applying the low nitrogen solution to the individual plants were identical to those used with high nitrogen plants.

Harvesting Procedures

In both experiments, the young leaves, mature leaves, stem pieces, and galls were harvested separately. The roots were collected only from plants in Experiment 2. Fully expanded mature leaves were taken from the third or fourth internodes, numbered down from

the apical meristem. Partially expanded young leaves were taken from the first or second internodes. Galls, near the base of the stem, were cut off with a razor blade, and the stem pieces were collected from the top three internodes. In every case, 15 g lots were obtained, placed in plastic bags, and stored in the deep freeze at -20°C until extraction.

Extraction Procedure and Column Preparation

Each 15 g fresh weight sample was placed in 150 ml of 95% ethanol and homogenized in an omnimixer for 3 minutes. The homogenate was filtered through 2 thicknesses of cheese cloth and concentrated to approximately 3 ml in a flash evaporator. Fifty ml of distilled water then were added to the flask to resuspend all amino compounds and the solution was filtered through Whatman 3 filter paper in a Buchner funnel. The filtrate was passed through a Dower-50-X-4 cation exchange resin column which retained the amino acids. The column was prepared by placing 4 g of the cation exchange resin in a 1/2 cm diameter glass tube, 50 cm in length. The glass tube was stoppered with a glass wool plug held in place with cheese cloth and a rubber band. The column was rinsed with 50 ml of distilled water, and the amino compounds were eluted from the column with 40 ml 2N NH_4OH. Then the eluted solution was evaporated to dryness in a flash evaporator. To resuspend the amino compounds,

4 ml of a 10% solution of isopropyl alcohol in distilled water were added to the dry flask and rotated around the surface. Finally the 4 ml extract was stored in a deep freeze.

Two Dimensional Paper Chromatography

Two hundred μl of each extract were spotted on a 18 x 22 inch sheet of Whatman 1 Chromatography paper. The papers were placed in a chamber for descending chromatography and equilibrated for 12-15 hours with 0.3 M NH_4OH solution. A phenol-water solvent was used for the first directional run. This solvent was prepared by adding 120 ml of deionized water and 1.2 g of 8-equinolinol to a pound of Mallinckrodt Gilt Label phenol and adjusting to pH 4.5. After a 24 hour phenol solvent run, the papers were removed and dried. The second solvent system was a butanol-acetic acid-deionized water solution, 4:1:5, v/v/v. The papers were run for 30 to 33 hours, using the aqueous lower phase of the butanol-acetic acid-deionized water solution for equilibration and the upper phase as the solvent. The chromatograms were developed by dipping in a 0.3% ninhydrin acetone solution and by placing them in an oven at 60°C for 1 hour. After development, the ninhydrin-positive spots were identified and numbered.

Elution Techniques and Standard Curves of Amino Compounds

Each ninhydrin-positive spot on the chromatogram was cut out and cut into pieces approximately 0.25 cm^2. The pieces were placed in a test tube containing 5 ml of 50% ethanol. The test tubes were shaken, covered, and placed in the refrigerator. After 23 to 26 hours, the tubes were removed, shaken again, and set aside for 30 minutes. The paper fragments settled to the bottom and the optical density readings were taken with a Spectronic 20 colorimeter. The elution period was chosen because Porter, Margolis, and Sharp (1957) showed that color intensity remains constant between 24 and 30 hours. Their technique was adapted for this study. They reported that all amino acids except asparagine and proline can be read accurately at 570 mμ. Proline was read at 430 mμ and asparagine at 360 mμ. Blanks for calibration of the Spectronic 20 were taken from each chromatogram. A blank spot was taken near the phenol front and used as a control for determinations of proline, tyrosine, valine, tryptophane, histidine, lysine, arginine, and the leucines. The other blank was taken near serine and used for determinations of the remaining amino compounds. See Figure 1 for distribution of amino compounds and control blanks on the chromatograms.

Standard curves were run for the known amino compounds by preparing standard solutions containing 10, 20, 30, 40, 50, 60 or 80 μg

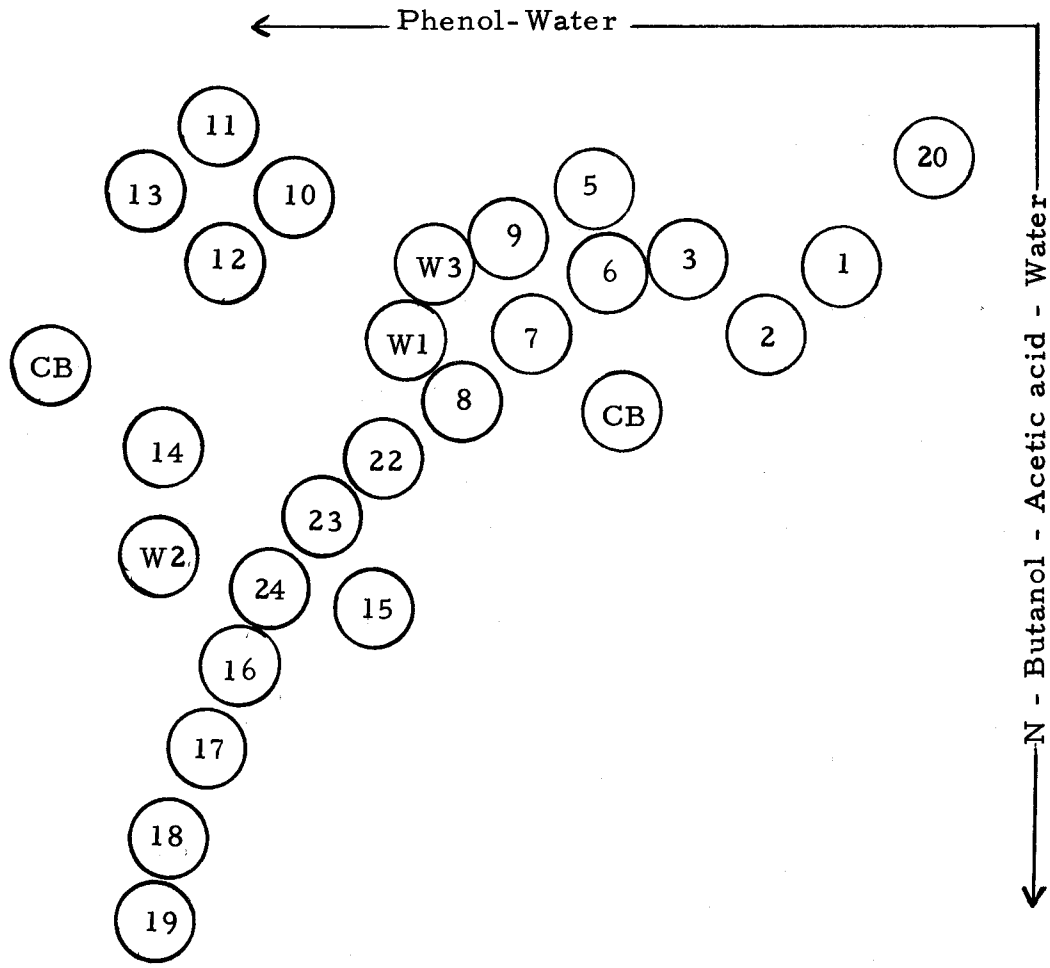

Figure 1. Amino acid map.

The amino acid spots are designated by the following numbers: 1, asparatic acid; 2, glutamic acid; 3, serine; 5, asparagine; 6, glycine; 7, threonine; 8, alanine; 9, glutamine; 10, histidine; 11, lysine; 12, methionine sulfoxide; 13, arginine; 14, proline; 15, tyrosine; 16, methionine and valine; 17, tryptophane; 18, phenylalanine; 19, leucines; 20, cysteic acid; 22, β-alanine; 23, α-aminobutyric acid; 24, γ-aminobutyric acid; unknowns, W-1, W-2, W-3; and control blanks, CB.

of each amino acid per 200 μl of solution. For each standard, 200 μl were spotted on a chromatography paper, which was processed as indicated earlier. Standard curves were prepared by determining the optical density for each amino acid in the standard solutions. At least 3 replications were analyzed and measured for each optical density reading listed in Table 1. By comparing optical densities of amino compounds in the extracts with the standard curves, the amounts of each amino acid could be determined quantitatively. The amino acid tolerances necessary to compensate for experiment error are listed in the far right column of Table 1.

Table 1. Optical density values for amino acid standard curves. Two hundred micro liters of each standard solution were spotted on chromatograms to give the optical density value listed below.

	Amino Acids	10	20	30	40	50	60	80	Tolerance
1.	Asparatic acid	.16	.29	.48	.54	.66	.70	.92	.03 - .04
2.	Glutamic acid	.18	.40	.62	.78	.93	1.25	1.50	.02 - .03
3.	Serine	.33	.60	.87	1.14	1.33	1.40	1.78	.02
5.	Asparagine	.08	.16	.26	.32	.39	.46	.61	.06 - .07
6.	Glycine	.21	.46	.64	.80	.93	1.30	1.30	.02 - .03
7.	Threonine	.16	.37	.61	.80	1.05	1.27	1.75	.03 - .04
8.	Alanine	.28	.62	.91	1.30	1.50	2.00	2.00	.02
9.	Glutamine	.05	.14	.21	.33	.44	.55	.74	.06
10.	Histidine	.04	.07	.12	.20	.31	.41	.56	.06 - .07
11.	Lysine	.05	.12	.23	.32	.43	.64	.84	.05
12.	Methionine sulfoxide	.10	.19	.35	.51	.67	.80	1.20	.05
12.[1]	Methionine sulfoxide	.08	.13	.19	.26	.27	.38		.05
13.	Arginine	.06	.11	.18	.27	.38	.53	.84	.05
14.	Proline	.05	.04	.07	.09	.10	.13	.16	.20
16.	Valine	.19	.42	.75	1.15	1.35	1.73	2.00	.02
16.[2]	Methionine	.05	.15	.28	.34	.50	.70	.93	.04 - .05
17.	Tryptophane	.02	.09	.16	.28	.32	.37	.54	.08 - .09
18.	Phenylalanine	.08	.14	.25	.34	.43	.57	.74	.05
19.	Isoleucine	.12	.24	.45	.68	1.00	1.20	1.80	.03
20.	Cysteic acid	.10	.22	.35	.44	.57	.62	.75	.06
22.	β-alanine	.07	.23		.60			1.25	.04 - .05
24.	γ-aminobutyric acid	.14	.39		.88			1.78	.03

[1] From degradation of methionine during chromatography.

[2] Methionine and valine are located in the same area of a developed chromatogram (Figure 1).

RESULTS

The amounts of individual amino acids in leaf, stem, and root tissues of healthy and crown gall diseased Kalanchoe plants were determined by paper chromatography. Two experiments were performed, one in the Fall of 1964 and another in the Fall of 1965. These were not identical but certain treatments were similar. In Experiment 1, the diseased plants were inoculated 3 times with crown gall bacteria on opposite sides of the stem (OG). In Experiment 2, the plants were either inoculated 2 times on opposite sides of the stem (OG) in one treatment or were inoculated 4 times on one side of the stem (UG) in another treatment. (Henceforth the opposite gall and unilateral gall positions will be noted as OG and UG, respectively). Wounded controls were not included in Experiment 2, since the wounded controls of Experiment 1 yielded amino acid amounts identical to those obtained from the non-punctured controls.

Quantitative amino acid differences were observed between diseased and healthy plants. Interestingly, the position of the galls on the stems also influenced the amounts of amino acids in the different tissues. In both experiments, the high nitrogen OG plants had lower amino acid totals than UG plants.

Kalanchoe Plant Heights

In Experiment 2 the low nitrogen controls averaged 9.6 cm, the OG plants averaged 9.0 cm, and the low nitrogen UG plants averaged 9.2 cm in height (Table 2). The high nitrogen diseased plants were 4 cm shorter than the control plants. At the high nitrogen level, the controls averaged 20.6 cm, the OG plants averaged 16.3 cm, and the UG plants averaged 16.5 cm.

Amino Acid Contrasts Between Low Nitrogen Diseased and Healthy Plants

The amino acid concentration of the young leaves, old leaves, stems, and roots of plants in each group were totaled and are listed in Table 3. At the low nitrogen level, the diseased plant totals of both experiments were greater than the healthy plants. In both experiments, the diseased young leaf and stem tissues had larger amino acid totals than found in the controls (Table 4). But the diseased old leaves had lower amino acid totals than the controls. No measurable differences were evident between the totals of diseased and control root tissues.

Some of the individual amino acids comprising the tissue totals (Table 4) are listed in Tables 5 and 6. Tables containing all data from Experiment 1 and 2 are located in the appendix (Appendix Tables 1-10). Data on the amino acids, showing major differences

Table 2. The effects of gall positions on heights of Kalanchoe pinnata in Experiment 2.

Treatments	Replications										
	1	2	3	4	5	6	7	8	9	10	Averages
1. Low nitrogen control	11.2	8.3	10.3	8.5	9.8	10.2	10.3	7.7	9.2	10.1	9.6 cm
2. Low nitrogen opposite galls	9.9	8.7	7.8	8.3	8.2	10.8	8.9	9.2	9.8	8.8	9.0 cm
3. Low nitrogen unilateral galls	9.0	9.0	8.7	7.3	8.9	9.7	10.7	9.1	10.5	9.5	9.2 cm
4. High nitrogen control	22.5	23.4	21.3	16.7	18.5	22.7	22.1	21.2	19.9	17.2	20.6 cm
5. High nitrogen opposite galls	19.3	19.5	16.0	15.8	14.3	19.2	15.5	16.1	12.8	14.5	16.3 cm
6. High nitrogen unilateral galls	17.5	16.1	19.2	18.7	14.3	18.2	16.8	14.6	15.1	14.5	16.5 cm

Table 3. Plant amino acid totals in μmoles/grams of wet weight tissues. One gram of each tissue listed was used in the totals.

Treatments	Exp.	YL OL[1] S (3g)	YL OL[2] S R(4g)	YL OL[2] S G(4g)	YL OL S[3] R G(5g)
1. Low nitrogen control	1	4.76			
3. Low nitrogen opposite galls	1	5.43		8.97	
1. Low nitrogen control	2	5.61	8.16		
2. Low nitrogen opposite galls	2	6.21	8.97	10.31	13.07
3. Low nitrogen unilateral galls	2	6.16	8.38	10.26	12.48
4. High nitrogen control	1	15.97			
6. High nitrogen opposite galls	1	9.74		12.49	
4. High nitrogen control	2	19.78	24.56		
5. High nitrogen opposite galls	2	12.62	17.16	18.00	22.54
6. High nitrogen unilateral galls	2	20.82	25.63	30.20	35.01

[1]Micromoles/3g of wet weight tissue.

[2]Micromoles/4g of wet weight tissue.

[3]Micromoles/5g of wet weight tissue.

[4]The following abbreviations were used: YL for young leaves, OL for old leaves, S for stems, R for roots, and G for galls.

Table 4. Tissue amino acid totals in Experiments 1 and 2. Numbers indicate total amounts of soluble amino acids in μmoles/gram of fresh weight tissue.

Treatments	Exp.	Young leaves	Old leaves	Stems	Roots	Galls
1. Low nitrogen control	1	1.88	1.41	1.47		
3. Low nitrogen opposite galls	1	1.98	1.32	2.13		3.84
1. Low nitrogen control	2	2.01	1.74	1.86	2.55	
2. Low nitrogen opposite galls	2	2.53	1.45	2.23	2.76	4.10
3. Low nitrogen unilateral galls	2	2.63	1.40	2.13	2.22	4.10
4. High nitrogen control	1	8.36	2.77	4.84		
6. High nitrogen opposite galls	1	5.63	1.94	2.17		2.75
4. High nitrogen control	2	9.80	6.45	3.53	4.78	
5. High nitrogen opposite galls	2	5.04	4.35	3.23	4.54	5.38
6. High nitrogen unilateral galls	2	8.36	9.55	2.91	4.52	9.38

in amounts between diseased and healthy tissues, were excerpted from the appendix tables and incorporated into Table 5 for low nitrogen and Table 6 for high nitrogen tissues.

When the amino acid amounts from diseased tissues were contrasted with control tissues, measurable differences were evident (Table 5). Young leaves from OG plants had less asparatic acid and glutamic acid and more alanine and glutamine than the controls. The young leaves from UG plants followed the same trend with more alanine and glutamine than the control young leaves (Table 5).

The control old leaves had more asparatic acid and glutamic acid than diseased old leaves from OG plants or UG plants. Old leaves from UG plants had more alanine than the control old leaves or old leaves from OG plants. Stem pieces from OG plants had more asparatic acid and alanine than control stem pieces. Alanine was the only amino acid which showed a measurable difference between healthy and diseased root tissues. Those from UG plants had less alanine than the control roots (Table 5).

If the tissues or plants with opposite galls and unilateral galls were combined under the title of "diseased tissues and plants", several definite trends were evident when the "diseased tissues" were contrasted with the healthy tissues. The young leaves from "diseased plants" had more alanine and glutamine than the controls (Table 5). Old leaves from "diseased plants" had less asparatic

Table 5. Low nitrogen tissues. Numbers indicate μmoles of amino acid/gram of fresh wet tissue.

Treatments	Exp.	Asparatic acid 1	Glutamic acid 2	Alanine 8	Glutamine 9	Leucine 19
Young leaves	2					
Control		.34	.49	.33	.23	
Opposite galls		.16	.36	1.03	.38	
Unilateral galls		.30	.47	.78	.39	
Old leaves	2					
Control		.33	.43	.31		
Opposite galls		.20	.32	.36		
Unilateral galls		.11	.24	.54		
Stems	2					
Control		.23		.31		
Opposite galls		.33		.45		
Unilateral galls		.27		.36		
Roots	2					
Control				.45		
Opposite galls				.42		
Unilateral galls				.28		
Galls[1]	2					
Opposite galls & Unilateral galls		.22	.62	.76	.35	.38

[1]The galls from low nitrogen plants with opposite galls and plants with unilateral galls were combined for one extract.

Table 6. High nitrogen tissues. Numbers indicate μmoles of amino acid/gram of fresh weight tissue.

Treatments	Asparatic acid 1	Glutamic acid 2	Serine 3	Asparagine 5	Alanine 8	Glutamine 9	Arginine 13	Leucine 19	W2[1]
Young leaves									
Control	.41	.54		.23	.90	2.13	4.30		.26
Opposite galls	.23	.51		.16	1.19	1.22	1.05		.09
Unilateral galls	.40	.71		.35	1.07	1.70	2.78		.31
Old leaves									
Control	.41	.37			1.14	.43	3.10		
Opposite galls	.32	.43			.91	.36	1.65		
Unilateral galls	.45	.65			.94	2.62	3.32		
Stems									
Control	.44	.56				1.46	.08		
Opposite galls	.34	.40				1.26	.27		
Unilateral galls	.82	.68				.11	.20		
Roots									
Control						1.67	.49		
Opposite galls						1.50	.34		
Unilateral galls						1.45	.39		
Galls									
Opposite galls	.19	.56	.22		.82	1.08	.90	.40	.23
Unilateral galls	.54	.98	.43		1.36	1.89	1.58	.93	.77

[1]Data for W2 in the table are given in optical density units.

acid and glutamic acid than found in controls.

The stems and roots from "diseased plants" showed no measurable amino acid differences when compared with control stems and roots. There were no trends in common between the different tissues of the "diseased plant" when compared to control plants.

Contrasts Between Low Nitrogen Plants with Opposite Galls and Unilateral Galls

There were some differences in individual amino acid amounts between tissues from OG plants and UG plants (Table 5), but there were no differences in plant amino acid totals (Table 3). With tissue totals (Table 4), the root tissues were the only tissues showing a measurable difference between OG and UG plants. Roots from OG plants had a total of 2.76 μmole/g while roots from UG plants had 2.22 μmole/g.

Young leaves from OG plants had less asparatic acid, glutamic acid, and more alanine than young leaves from UG plants (Table 5). The old leaves from OG plants had less alanine and possibly more asparatic acid and glutamic acid than old leaves from UG plants. Stem pieces from OG plants had more alanine than stem pieces from UG plants. In the roots of OG plants alanine was in greater concentrations with a 0.45 μmole/g total while the total of UG plants was a 0.28 μmole/g.

No contrasts were possible between galls from OG plants and galls from UG plants in Experiment 2, since both were combined in one sample. However, a few differences were observed between Experiment 1 galls from OG plants and Experiment 2 combined galls from UG and OG plants (Table 7). The galls from Experiment 1 had less asparatic acid, glutamic acid, and more leucines than the combined gall extract of Experiment 2.

There was only one trend in common between tissues from OG plants when compared with tissues from UG plants. The OG plants had more alanine in the young leaves, stems, and roots and less alanine in the old leaves.

Amino Acid Contrasts Between High Nitrogen Healthy and Diseased Plants

At the high nitrogen levels, the diseased OG plants from both experiments showed general trends in amino acid totals. In Experiment 1 and 2, the plant total data of Table 3 demonstrated that the diseased OG plants had about half the amino acid total found in the control tissues, while the totals from UG plants were equivalent to those of the controls. This was further exemplified in the tissue totals listed in Table 4. In both experiments, the young leaf, old leaf, and stem tissue from OG plants had lower totals than found in the control tissues (Table 4). In Experiment 2, the root tissues

Table 7. Low and high nitrogen galls. Numbers indicate μmoles of amino acid/ gram of fresh weight tissue.

Treatment	Exp.	Asparatic acid 1	Glutamic acid 2	Serine 3	Asparagine 5	Glycine 6	Threonine 7	Alanine 8	Glutamine 9	Histidine 10	Lysine 11	Methionine sulfoxide 12	Arginine 13	Proline 14	Tyrosine 15	Valine, Methionine 16	Leucines 19	Cysteic acid 20	β-alanine 22	γ-aminobutyric acid 24	W1[1]	W2
3. Low nitrogen opposite galls	1	.09	.43	.16	<.10	<.09	.16	.66	.27		.16	<.10	.19	<.20	.18	.18	.94			<.06		.35
2. & 3 Low nitrogen galls[3]	2	.22	.62	.22	<.10	.11	.15	.76	.35	.23	.14	<.10	<..08	<.20	.13	.12	.38	T[2]	<.15	.14	T	.35
6. High nitrogen opposite galls	1	.18	.33	.20	<.10	<.06	.07	.51	.51				.32		<.11	<.06	<.10		<.15	<.06		.16
5. High nitrogen opposite galls	2	.19	.56	.22	<.10	<.09	.09	.82	1.08	<.09	<.14	<.10	.90	<.23	.11	.15	.40	T	.15	<.06	.01	.23
6. High nitrogen unilateral galls	2	.54	.98	.43	.11	.12	.20	1.36	1.89	<.09	.25	.11	1.58	<.20	.16	.22	.93		<.15	.17	.05	.77

[1] Date for W's in the table are given in optical density units.

[2] T represents trace amounts.

[3] The galls from plants with unilateral and opposite galls were combined for one extract in Experiment 2.

from the OG plants and UG plants had lower amino acid totals than corresponding control roots (Table 4). Also in Exp. 2, UG plants had lower amino acid totals in the young leaf, stem, and root tissues than the controls, but had a larger amino acid total in the old leaf tissue (Table 4).

In Exp. 2, the diseased young leaves from OG plants had lesser amounts of asparatic acid, glutamine, arginine, W-2, and greater amounts of alanine than found in the controls (Table 6). The young leaves from UG plants had smaller amounts of glutamine, arginine, and greater amounts of glutamic acid, alanine, and asparagine.

The old leaves from OG plants had less asparatic acid, alanine, and arginine than found in control old leaves. The old leaves from UG plants had less alanine, and more glutamic acid, arginine, and especially glutamine, than the controls. Control old leaves had only 0.43 μmoles/g of glutamine while UG plants had a 2.62 μmoles of glutamine (Table 6).

Stem pieces from OG plants had less asparatic acid, glutamic acid, and glutamine than the controls but more arginine (Table 6). The stem tissue from UG plants had more asparatic acid, glutamic acid, arginine, and much less glutamine than the controls, 0.11 μmoles of glutamine versus the 1.46 μmoles/g in the controls. Roots from UG plants or OG plants had less glutamine and arginine

than found in the control roots.

If the tissues of OG plants and UG plants were combined under the title of "diseased tissues or plants", there were some trends when the "diseased tissues" were contrasted with the healthy tissues. The young leaves from "diseased plants" had less glutamine and arginine; and more alanine than controls (Table 6). Old leaves from "diseased plants" had less alanine than controls. Stems from "diseased plants" showed more arginine than controls while the roots from "diseased plants" had less glutamine and arginine than controls. There were no notable trends common among the different tissues of the diseased plants when they were compared to control plants.

Contrasts Between High Nitrogen Plants With Opposite Galls and Unilateral Galls

In Experiment 2, OG plants had smaller amino acid totals in the young leaf and old leaf and gall tissues when compared to UG plants (Table 4). Young leaves from OG plants had a 5.04 μmole total, while young leaves from UG plants had a 8.36 total. The old leaves from OG plants had only a 4.35 μmole total and the old leaves from UG plants had a 9.55 total. The stems from diseased OG plants had a greater amino acid total than the stems from UG plants, while the root totals of both OG and UG plants were equivalent.

Young leaves from OG plants had less asparatic acid, glutamic

acid, asparagine, glutamine, arginine, and W-2 than young leaves from UG plants (Table 6). The old leaves from OG plants had less asparatic acid, glutamic acid, glutamine, and arginine, than found in old leaves from UG plants.

The stem pieces from OG plants had less asparatic acid and glutamic acid than stem pieces from UG plants. They had more glutamine than stem pieces from UG plants (Table 6). The roots from OG plants had individual amino acid amounts comparable to the amounts in the roots from UG plants. It appeared that the amino acid differences between OG plants and UG plants were due to the different gall positions on the stem.

In Experiment 2, the galls from OG plants had a total of 5. 38 μmole, which was less than the 9. 38 total from galls of UG plants (Table 4). The galls from OG plants had less asparatic acid, glutamic acid, alanine, glutamine, arginine, W-2, serine, and leucines than found in galls from UG plants (Table 6).

In general, the UG plants had more asparatic acid and glutamic acid in young leaves, old leaves, stems, and galls than OG plants (Table 6). Also glutamine and arginine were found in higher concentrations in the young leaves, old leaves, and galls of UG plants than in OG plants.

In both Experiments 1 and 2, the galls from OG plants had a similar amino acid pattern (Table 7). Qualitatively they had the

same amino acids. But in Experiment 2, galls from OG plants had more glutamic acid, alanine, glutamine, arginine, leucines, and W-2 than galls from Experiment 1 OG plants. The higher concentrations of these amino acids in the Experiment 2 galls wasn't unusual since Experiment 2 plants did receive twice as much nitrogen as Experiment 1 plants.

DISCUSSION

The presence of crown gall tumors on Kalanchoe stems altered the soluble amino acid pools in the different plant tissues. Whether the amino acid metabolism was directly affected by the tumors or whether the changes were indirect consequences of other metabolic reactions was not evident.

When low and high nitrogen plants were compared, no trends or correlations were found with individual amino acids or amino acid totals which were characteristic for the crown gall diseased plants. The diseased young leaf and stem tissues from low nitrogen plants had larger amino acid totals, while the old leaves had lower totals than the controls (Table 4). The opposite was true for high nitrogen plants, with the young leaves, stems, and roots showing lower amino acid totals than controls. The old leaves from UG plants had a greater total while those from OG plants had a smaller total than the controls (Table 4).

At the individual amino acid levels, alanine was the only amino acid showing even a possible trend in both low and high nitrogen diseased plants (Tables 5 and 6). The young leaves had more alanine than their respective controls. The high nitrogen galls from OG plants had amino acid concentrations equal to half those found in galls from UG plants (Table 6). Perhaps this trend would have been

noted with low nitrogen galls from OG and UG plants if they had not been combined in one extract.

The major influencing factor on amino acid pool levels in low nitrogen plants seemed to be the nitrogen level rather than the galls or the gall positions on the stem. The low nitrogen plants were stunted, yellow in color, and showed other nitrogen deficiency symptoms, conversely the high nitrogen plants were dark green and showed no nitrogen deficiency symptoms. Further analysis between low and high nitrogen plants, diseased or healthy, yielded no meaningful trends or correlations.

In both Experiments 1 and 2, the OG plants with high nitrogen levels had about half the amino acid total found in the control and the UG plants (Table 3). The plant tissues from OG plants had lower amino acid totals in young leaves, old leaves, stems than control plants and lower totals in young leaves, old leaves, and galls than UG plants (Table 4). When UG plants were compared with controls, they had lower amino acid totals in young leaves, stems, and root tissues. If OG plants and UG plants were grouped together as "diseased plants", there were few amino acids which were characteristic of the crown gall disease. Young leaves from diseased plants had less glutamine and arginine and more alanine. Old leaves had less alanine, stems had more arginine, and roots had less glutamine and arginine than controls. There was no amino acid

trend common among the different tissues of UG and OG plants.

There were just as many amino acid differences between tissues of OG plants and UG plants as there were between tissues of OG plants and the control plants (Table 6 and p. 25). In general, OG plants had less asparatic acid and glutamic acid in young leaves, old leaves, stems, and galls than UG plants. Glutamine and arginine were at lower levels in young leaves, old leaves, and galls of OG plants than in UG plants. In stem tissue from OG plants, there were 1.26 μmoles of glutamine and only 0.11 μmoles in stem tissue from UG plants. The data suggested that levels in amino acid pools were affected as much by the positioning of the galls as by the physiological presence of the gall.

Several intriguing questions materialized as the data above were collected. It was evident that gall position on the stem influenced, directly or indirectly, the amino acid pools of the different tissues. But which plants were more characteristic of crown gall diseased plants; the OG plants, UG plants, or neither? Young leaf, old leaf, stem, and root amino acid analyses from crown gall infected Mazzard cherry seedlings would suggest neither, if the data were applicable to Kalanchoe plants. The analyses showed no qualitative or quantitative amino acid differences between the control and the diseased plants parts from seedlings with crown gall tumors on the roots (Deep and Harris, 1964). If the galls are located on the

roots, one could say that a crown gall tumor on a plant does not influence the amino acid pools of the other plant tissues. Since most of the galls are found on the roots in nature, this approach suggests that the plant with galled roots would be more characteristic of the gall diseased plant.

Hussin (1962) in one of his crown gall experiments used Bonny Best tomatoes and inoculated them as the Kalanchoe plants were inoculated in this study. In one treatment he inoculated the plants 6 times on one side of the stem (UG) and in another treatment inoculated the stems 3 times on opposite sides of the stem (OG). He found that the growth of UG plants was not significantly reduced while the top and root growth of OG plants was significantly reduced. In our study, the tops of both UG and OG Kalanchoe plants were reduced (Table 2). His tentative hypothesis to explain this phenomenon was an initial malformation of phloem elements by tumor formation with consequent reduction in translocation of organic compounds to the roots. The reduction would affect the size of the root system and ultimately affect the leaf and stem growth. Most interesting were his tissue analyses of OG and UG tomato plants. Total nitrogen, phosphorous, and potassium levels were the same in OG plants, UG plants, and control plants. Projecting Hussin's data on tomatoes to identical treatments on Kalanchoe plants, raised an interesting question. If the OG, UG, and control plants had the same nitrogen

levels, how could one account for total nitrogen in OG plants especially when the amino acid levels of OG plants were one-half those of the controls and UG plants? Did the OG plants have more protein, nucleoprotein, or other nitrogenous substances than found in the controls or UG plants?

Research data of others were supported in this investigation. Tumor tissue of Kalanchoe plants had larger amino acid totals than the stem tissues from healthy and crown gall diseased plants. Workers using different plants and plant tissues have shown consistently that gall tissue had more soluble protein, insoluble protein, amino acids, total nitrogen, and crude protein when compared with normal or habituated tissues (Literature Review). Additionally, this study showed that the different plant tissues of diseased plants had no amino acid patterns or trends which were specific for the crown gall disease complex. Seitz and Hochster (1964) determined soluble amino acids from normal plant parts and tumors of tomato and tobacco and found that amino acids from tumor and normal tissues were qualitatively the same but quantitatively there were more in the tumor tissue. Simonson and Roberts (1962) analyzed the amino acids in normal and tumor tissues of Boston ivy, periwinkle, cactus, tobacco, carrot, and Virginia creeper. They found more amino acids in gall tissues than in normal tissues. Tumor tissues from the different plant species showed no similarities in

amino acids which were specific for or characteristic of the tumorous state.

Since the gall positions on high nitrogen plants had such an effect on amino acid levels in Experiments 1 and 2, workers who study crown gall diseased plants in the future should be cognizant of this fact when inoculating the plants. Biochemical and physiological differences which may be attributed to differences between diseased and healthy plants, could be due to gall positions on the plant. Earlier workers have not mentioned this aspect in their research publications. In the literature a crown gall diseased plant is a plant with crown gall tumors. Klein (1952) inoculated tomatoes on the stems and followed changes in nitrogen metabolism of tumors from inoculation to development of crown gall tumors. Steward, Thompson, and Pollard (1958) inoculated leaves of Kalanchoe daigremontiana and found that tumors had considerably more protein than normal leaves or normal parts of tumor bearing leaves. Seitz and Hochster (1964) inoculated the stems of tomato and tobacco plants and determined the soluble amino compounds from normal plant parts and plant tumors. These are a few of the workers who have inoculated plants and analyzed the biochemical and physiological differences between diseased and healthy plants without considering the gall positions on the diseased plant.

SUMMARY

1. Only one general qualitative or quantitative difference was found between the amino acid composition of healthy Kalanchoe pinnata plants and those infected with crown gall. The young leaves of diseased plants had greater amounts of alanine than the controls.

2. The diseased plants supplied with low levels of nitrogen in the root medium had amino acid totals greater than controls. More specifically, the diseased young leaf and stem tissue had larger amino acid totals, while the diseased old leaf tissues had lower totals. There were no trends in amino acid compositions common among the different tissues of diseased plants supplied with low levels of nitrogen whether inoculated to obtain unilateral galls (UG) or to obtain opposite galls (OG) on the stem, when compared with controls.

3. Only one amino acid trend was evident between tissues of low nitrogen OG and UG plants. The OG plants had more alanine in young leaves, stems, and roots, but less alanine in old leaves. The major factor influencing the amino acid pool levels in low nitrogen plants appeared to be the low nitrogen levels rather than presence or position of the galls on the stem.

4. In both Experiments 1 and 2, the high nitrogen OG plants had about half the amino acid totals found in the control or UG plants.

OG plants had lower totals in young leaves, old leaves, and stems than control plants. They had lower totals in young leaves, old leaves, and galls when compared to UG plants. The UG plants had lower amino acid totals in young leaves, stems, and roots than controls, but a higher total in old leaves.

5. The high nitrogen OG plants had less asparatic acid and glutamic acid in young leaves, old leaves, stems and galls than UG plants. Glutamine and arginine were at lower levels in young leaves, old leaves, and galls of OG plants when compared to UG plants. The galls from OG plants had less asparatic acid, glutamic acid, alanine, glutamine, arginine, W-2, serine and leucines than found in galls from UG plants.

6. Young leaves from high nitrogen diseased plants, UG and OG plants, had less glutamine, arginine, and more alanine than controls. Old leaves had less alanine. Stems had more arginine while roots had less glutamine and arginine than controls. No notable amino acid trends were common among tissues of diseased high nitrogen plants when compared to control plants. The data suggested that amino acid pool levels in different plant tissues were influenced as much by gall positions as by the physiological presence of the gall.

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APPENDICES

Appendix table 1. Low nitrogen young leaves. Numbers indicate μmoles of amino acid/gram of fresh weight tissue.

Treatments	Exp.	Asparatic acid 1	Glutamic acid 2	Serine 3	Asparagine 5	Glycine 6	Threonine 7	Alanine 8	Glutamine 9	Arginine 13	Valine 16	Leucine 19	Cysteic acid 20	γ-aminobuytric acid 24	W1[1]	W2	W3
1. Control	1	.21	.46	.15	< .10		< .06	.51	< .09	< .08	< .06	< .10	T[2]	< .06		.08	
3. Opposite galls	1	.22	.47	.24			< .06	.60	.17		< .06	< .10	T	< .06		.11	
1. Control	2	.34	.49	.15	< .10	< .09	< .06	.33	.23		< .06	< .10	T	< .06	.04	T	T
2. Opposite galls	2	.16	.36	.13	< .10	< .09	< .06	1.03	.38		< .06	< .10	T	< .06	.04	T	.03
3. Unilateral galls	2	.30	.47	.14	< .10	< .09	< .06	.78	.39	< .08	< .06	< .10	T	< .06	.05	.09	

[1] Data for W's in above table are given in optical density units.

[2] T represents trace amounts.

Appendix table 2. Low nitrogen old leaves. Numbers indicate μmoles of amino acids/gram of fresh weight tissue.

Treatments	Exp.	Asparatic acid 1	Glutamic acid 2	Serine 3	Asparagine 5	Glycine 6	Threonine 7	Alanine 8	Glutamine 9	Valine 16	Leucines 19	Cysteic acid 20	γ-aminobutyric acid 24	W1[1]	W2	W3
1. Control	1	.32	.34	.06		<.09	.07	.28	<.09	<.06	<.10	T		T[2]		
3. Opposite galls	1	.27	.24	.05		<.09	.06	.28	.11	<.06	<.10	T	<.06	T	T	
1. Control	2	.33	.43	.09	<.10	<.09	.06	.31	.11	<.06	<.10	T	<.06	T	.04	.03
2. Opposite galls	2	.20	.32	.08		<.09	<.06	.36	.12	<.06	<.10	T	<.06	.02	.04	.03
3. Unilateral galls	2	.11	.24	.05		<.09	<.06	.54	.09	<.06	<.10	T	<.06	.02	T	

[1] Data for W's in above table are given in optical density units.

[2] T represents trace amounts.

Appendix table 3. Low nitrogen stems. Numbers indicate μmoles of amino acid/gram of fresh weight tissue.

Treatments	Exp.	Asparatic acid 1	Glutamic acid 2	Serine 3	Asparagine 5	Glycine 6	Threonine 7	Alanine 8	Glutamine 9	Tyrosine 15	Valine 16	Leucines 19	Cysteic acid 20	γ-aminobutyric acid 24	W1[1]	W2	W3
1. Control	1	.16	.27	.05		<.09	<.06	.22	.35	<.11	<.06	<.10	T[2]			T	
3. Opposite galls	1	.26	.19	.08	<.10	<.09	<.06	.21	.53	<.11	.07	<.10	T		T	T	T
1. Control	2	.23	.33	.06	<.10	<.09	<.06	.31	.46		<.06	<.10	T	<.06	T	.07	T
2. Opposite galls	2	.33	.40	.10	<.10	<.09	<.06	.45	.48		<.06	<.10	T	<.06	T	.06	.04
3. Unilateral galls	2	.27	.38	.08	<.10	<.09	<.06	.36	.46	<.11	<.06	<.10		<.06	T	T	

[1] Data for W's in above table are given in optical density units.

[2] T represents trace amounts.

Appendix table 4. Low nitrogen roots. Numbers indicate μmoles of amino acid/gram of fresh weight tissue.

Treatments	Exp.	Asparatic acid 1	Glutamic acid 2	Serine 3	Asparagine 5	Glycine 6	Threonine 7	Alanine 8	Glutamine 9	Arginine 13	Tyrosine 15	Valine 16	Leucines 19	γ-aminobutyric acid 24	W2[1]
1. Control	2	.22	.44	.11	<.10	<.09	.08	.45	.38	<.08	<.11	.14	.27	<.06	.19[2]
2. Opposite galls	2	.18	.42	.15	<.10	<.09	.12	.42	.43	<.08	<.11	.20	.35	.11	.22
3. Unilateral galls	2	.16	.34	.11	<.10	<.09	.11	.28	.34	<.08	<.11	.16	.28	.06	.18

[1] Data for W's in above table are given in optical density units.

[2] T represents trace amounts.

Appendix table 5. High nitrogen young leaves. Numbers indicate μmoles of amino acid/gram of fresh weight tissue.

Treatments	Exp.	Asparatic acid 1	Glutamic acid 2	Serine 3	Asparagine 5	Glycine 6	Threonine 7	Alanine 8	Glutamine 9	Histidine 10	Lysine 11	Methionine 12	Arginine 13	Proline 14	Tyrosine 15	Valine 16	Leucines 19	Cysteic acid 20	γ-aminobutyric acid 24	W1[1]	W2	W3
4. Control	1	.45	.73	.33	<.10	<.09	.11	.96	2.30				3.06			<.06	<.10	T[2]	<.06		.24	
6. Opposite gall	1	.05	.38	.18	<.10		<.04	.85	1.15				2.56		<.11	<.06	<.10		<.06		.07	
4. Control	2	.41	.54	.30	.23	<.09	.13	.90	2.13	<.09	.16		4.30	<.20		<.06	.18	T	<.06	.14	.26	T
5. Opposite galls	2	.23	.51	.24	.16	<.09	.09	1.19	1.22				1.05			<.06	.14	T	<.06	.02	.09	
6. Unilateral galls	2	.40	.71	.27	.35	<.09	.10	1.07	1.70	<.09	.14	T	2.78	<.20	<.11	<.06	.23	T	<.06	.09	.31	

[1] Data for W's in the table are given in optical density units.

[2] T represents trace amounts.

Appendix table 6. High nitrogen old leaves. Numbers indicate μmoles of amino acid/gram of fresh weight tissue.

Treatments	Exp.	Asparatic acid 1	Glutamic acid 2	Serine 3	Asparagine 5	Glycine 6	Threonine 7	Alamine 8	Glutamine 9	Histidine 10	Lysine 11	Arginine 13	Proline 14	Tyrosine 15	Valine 16	Leucines 19	Cysteic acid 20	γ-aminobutyric acid 24	W1[1]	W2	W3
4. Control	1	.52	.62	.08	< .10	< .09	.08	.66	.32			< .08			< .06	< .10	T[2]	< .06	T	T	T
6. Opposite galls	1	.13	.33	.04	< .09	< .09	.07	.69	.23			< .08			< .06	< .10	T	< .06	T		
4. Control	2	.41	.37	.06	< .10	< .09	.08	1.14	.43	.15	.15	3.10		< .11	< .06	.14	T	< .06	.04	.10	T
5. Opposite galls	2	.32	.43	.06	< .10	< .09	.07	.91	.36			1.65		< .11	< .06	.13	T	< .06	.07	T	
6. Unilateral galls	2	.45	.65	.10	< .10	.28	.08	.94	2.64	.22	< .14	3.32	< .20	< .11	< .06	.16	T	.12	T	.10	

[1] Data for W's in the table are given in optical density units.

[2] T represents trace amounts.

Appendix table 7. High nitrogen stems. Numbers indicate μmoles of amino acid/gram of fresh weight tissue.

Treatments	Exp.	Asparatic acid 1	Glutamic acid 2	Serine 3	Asparagine 5	Glycine 6	Threonine 7	Alanine 8	Glutamine 9	Arginine 13	Tyrosine 15	Valine 16	Leucines 19	Cysteic acid 20	γ-aminobutyric acid 24	W1[1]	W2	W3
4. Control	1	.40	.54	.08	<.10	<.09	<.07	.39	2.76	.14	<.11	<.06	<.10	T[2]			T	T
6. Opposite galls	1	.18	.25	.04	<.10	<.09	<.04	.19	.97	<.08	<.11	<.06	<.10	T				
4. Control	2	.44	.56	.10	<.10	<.09	<.06	.31	1.46	<.08	<.11	<.06	<.10	T	<.06	T	T	T
5. Opposite galls	2	.34	.40	.08	<.10	<.09	<.06	.30	1.26	.27	<.11	<.06	<.10	T	<.06	T	.05	
6. Unilateral galls	2	.82	.68	.15	<.10	<.09	<.06	.39	.11	.20	<.11	<.06	.14	T		T	.13	

[1] Data for W's in table are given in optical density units.

[2] T represents trace amounts.

Appendix table 8. High nitrogen roots. Numbers indicate μmoles of amino acid/gram of fresh weight tissue.

Treatment	Exp.	Asparatic acid 1	Glutamic acid 2	Serine 3	Asparagine 5	Glycine 6	Threonine 7	Alanine 8	Glutamine 9	Lysine 11	Arginine 13	Tyrosine 15	Valine 16	Leucines 19	β-alanine 22	γ-aminobutyric acid 24	W2
4. Control	2	.24	.58	.18	< .10	< .09	.12	.51	1.67		.49	< .11	.19	.36		.14	.33
5. Opposite galls	2	.26	.63	.15	< .10	< .09	.15	.57	1.50		.34	< .11	.19	.34		.12	.24
6. Unilateral galls	2	.29	.65	.10	< .10	< .09	.15	.51	1.45	< .14	.39	< .11	.20	.35	< .15	.13	.25

[1]Data for W's in the table are given in optical density units.

Appendix table 9. Low nitrogen galls. Numbers indicate μmoles of amino acid/gram of fresh weight tissue.

Treatment	Exp.	Asparatic acid 1	Glutamic acid 2	Serine 3	Asparagine 5	Glycine 6	Threonine 7	Alanine 8	Glutamine 9	Histidine 10	Lysine 11	Methionine sulfoxide 12	Arginine 13	Proline 14	Tyrosine 15	Valine, Methionine 16	Leucines 19	Cysteic acid 20	β-alanine 22	γ-aminobutyric acid 24	W1[2]	W2
3. Opposite galls	1	.09	.43	.16	<.10	<.09	.16	.66	.27		.16	<.10	.19	<.20	.18	.18	.94			<.06		.35
2. & 3. galls[1]	2	.22	.62	.22	<.10	.11	.15	.76	.35	.23	.14	<.10	<.08	<.20	.13	.12	.38	T[3]	<.15	.14	T	.35

[1]The galls from plants with unilateral and opposite galls were combined for one extract in Experiment 2.

[2]Data for W's in the table are given in optical density units.

[3]T represents trace amounts.

Appendix table 10. High nitrogen galls. Numbers indicate μmoles of amino acid/gram of fresh weight tissue.

Treatment	Exp.	Asparatic acid 1	Glutamic acid 2	Serine 3	Asparagine 5	Glycine 6	Threonine 7	Alanine 8	Glutamine 9	Histidine 10	Lysine 11	Methionine sulfoxide 12	Arginine 13	Proline 14	Tyrosine 15	Valine, Methionine 16	Leucines 19	Cysteic acid 20	β-alanine 22	γ-aminobutyric acid 24	W1[1]	W2
6. Opposite galls	1	.18	.33	.20	<.10	<.06	.07	.51	.51				.32		<.11	<.06	<.10		<.15	<.06		.16
5. Opposite galls	2	.19	.56	.22	<.10	<.09	.09	.82	1.08	<.09	<.14	<.10	.90	<.23	.11	.15	.40	T[2]	<.15	<.06	.01	.23
6. Unilateral galls	2	.54	.98	.43	.11	.12	.20	1.36	1.89	<.09	.25	.11	1.58	<.20	.16	.22	.93		<.15	.17	.05	.77

[1] Data for W's in the table are given in optical density units.

[2] T represents trace amounts.