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FINAL REPORT

CONTRACT NAS 9-9211

STUDY OF EFFECTS OF LUNAR MATERIALS ON
BOTANICAL SYSTEMS (APOLLO XI).

by

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FINAL REPORT

Agency: National Aeronautics and Space Administration

Contract: NAS 9-9211

Program: Lunar Receiving Laboratory, Houston, Texas

Area: Botanical area

Title: Study of Effects of Lunar Materials on Botanical Systems (Apollo XI).

Period: February 3, 1969 - September 30, 1969

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I. Histological Examination and Evaluation of Botanical Tissues.

A. Introduction.

The broad purpose of this facet of the quarantine program is to analyze the Botanical Test Systems for their degree of normality following their growth under the four test conditions involving the lunar soil and the control conditions which are outlined in the Botanical Protocol.

The plant materials analyzed in this study were cultured and subsequently collected according to the procedures outlined in the Botanical Protocol. Each collection vial, containing one to three pieces of material, was recognizable only by an identification number which will serve for specimen identification throughout this report. The evaluation of the materials as presented in this report, therefore, represents an objective study of the tissues since I did not know the experimental treatment of the test specimens. These materials were taken to my laboratory at Indiana University for detailed study following their collection and certification by the Health and Safety officer at the Lunar Receiving Laboratory. The processed materials were embedded in paraffin or Epon resin, sectioned, stained and mounted under a coverglass on 1 x 3 inch glass slides or on grids in preparation for examination by light or electron microscopy

Four broad categories of tissues or organs were collected for examination:

- a. Callus tissues
- b. Cotyledon segments
- c. Leaf disks
- d. Root segments

Two pieces of each collected sample, with the exception of some root tips, were processed and prepared for histological examination. Two slides or 3 grids from each sample, with the exception of the above mentioned root tips, were prepared in anticipation of any possible loss or inadvertant destruction of a collection number during one of the many stages involved in the preparation of histological specimens. The specimens that were fixed in Bouins fixative for 24 hours, were microtomed at 8 microns and stained with Heidenhain's haematoxylin and erythrosin B; those prepared for electron microscopy were stained with uranyl acetate and lead citrate.

B. Materials Prepared for Analysis.

Specimen recognition during the analysis of the tissues was by code number and all recorded data will be presented with these numerical designations. Two sets of slide specimens which represent specimens from each sample, are included with this report. These sets represent a total of 390 permanent microscopic slides. Several grids were prepared from each specimen, and several negatives were prepared from one or more grids of each specimen. The materials that were photographed represent structural features which were considered to be particularly interesting in the thin sections. A total of 70 photographs accompany this final report.

C. Numerical Bases Utilized in Analysis.

A numerical basis was established for the evaluation of the tissues whereby a selected number of characters was graded from 1 (abnormal) to 5 (normal). The characters chosen represented histological features which respond rapidly to known earthly infectious agents and thereby represent indicators of various pathological conditions. Changes from the normal for

these various histological features were to be considered as symptomatic of the presence of a foreign substance in the test plants.

Each slide specimen was evaluated and tabulated on an individual data report sheet. Examples of the several data collection sheets are included for reference. Callus tissues were evaluated on the bases of the general cell histology of the cultured cells. The evaluation of the leaf, cotyledon and root segments included the histological appearance of the gross tissue histology as well as, a more detailed analysis of the cellular histology. The numerical evaluation of the several tissues can be outlined as follows:

<u>Tissue</u>	<u>Cellular Histology</u>	<u>Gross Histology</u>	<u>Total Points Possible</u>
Leaf or cotyledon	85	45	130
Root Tip	140	60	200
Callus tissues (light)	35		35
Callus tissue (electron)	60		60

D. Results of the Light Microscopic Analyses of the Experimental Tissues.

Presentation of the results from these analyses are provided in the following tables (TABLES I - IV):

TABLE I

Evaluation of the cotyledonary tissues (cross sectional views).

<u>Cotyledon</u>	<u>Cellular Histology</u>	<u>Gross Histology</u>	<u>Total Points</u>
10169 T	85	45	130
10178 T	59	40	99
10187 T	85	45	130
10209 T	83	44	127
10211 T	85	45	130
10226 T	60	37	97
10235 T	62	41	103
10257 T	62	41	103
10267 T	85	45	130
10274 T	82	44	126

<u>Cotyledon</u>	<u>Cellular Histology</u>	<u>Gross Histology</u>	<u>Total Points</u>
10290 T	83	44	127
10297 T	17	9	26
10299 T	85	45	130
10306 T	85	45	130
10315 T	83	45	128
10322 T	52	36	88
10330 T	51	34	85
10331 T	85	45	130
10337 T	69	39	108
10339 T	83	42	125
10362 T	66	37	103
10369 T	85	45	130
10385 T	85	45	130
10386 T	45	29	74
10410 T	60	37	97
10419 T	85	45	130
10441 T	77	43	120
10449 T	-	-	-
10467 T	85	45	130
10473 T	85	45	130

The above data represent a summary of the tabulated data which were accumulated on the data report sheets. These sheets, one Gross Histology sheet and one Cellular Histology sheet for each specimen examined, are included in this report and represent pages 17-76. Only two copies of these pages were prepared. One was sent to the Technical Monitor, Dr. Charles Walkinshaw, as an integral part of this report, while the other was retained for my file.

TABLE II

Evaluation of the leaf tissues (cross sectional views):

<u>Leaf</u>	<u>Cellular Histology</u>	<u>Gross Histology</u>	<u>Total Points</u>
10489	85	45	130
10504	85	45	130

<u>Leaf</u>	<u>Cellular Histology</u>	<u>Gross Histology</u>	<u>Total Points</u>
10528	85	45	130
10531	85	45	130
10537	61	29	90
10545	85	45	130
10556	85	45	130
10560	85	45	130
10568	85	45	130
10570	74	44	118
10571	85	45	130
10581	83	44	127
10592	85	45	130
10609	68	34	102
10615	85	45	130
10625	85	45	130
10639	85	45	130
10645	85	45	130
10651	85	45	130
10659	65	36	101
10663	85	45	130
10669	85	45	130
10680	85	45	130
10689	84	44	128
10694	85	45	130
10701	85	45	130
10703	85	45	130
10709	85	45	130
10737	76	41	117
10749	85	45	130
10751	84	44	128
10754	77	44	121
10778	85	45	130
10781	85	45	130
10820	85	45	130
10825	70	41	111
10831	85	45	130
10836	85	45	130
10837	83	44	127

<u>Leaf</u>	<u>Cellular Histology</u>	<u>Gross Histology</u>	<u>Total Points</u>
10840	85	45	130
10844	79	44	123
10845	85	45	130
10860	85	45	130
10870	85	45	130
10887	85	45	130
10907	85	45	130
10931	85	45	130
10947	85	45	130
10951	85	45	130
10953	85	45	130
10970	81	43	124
11000	85	45	130

The above data represent a summary of the tabulated data which were accumulated on the data report sheets. These sheets, one Gross Histology sheet and one Cellular Histology sheet for each specimen examined, are included in this report and represent pages 77-190. Only two copies of these pages were prepared. One was sent to the Technical Monitor, Dr. Charles Walkinshaw, as an integral part of this report, while the other was retained for my file.

TABLE III

Evaluation of the root tips (cross and longisectonal views);

<u>Root</u>	<u>Cellular Histology</u>	<u>Gross Histology</u>	<u>Total Points</u>
10169 R	59	40	99
10178 R	140	60	200
10187 R	140	60	200
10200 R	140	60	200
10209 R	140	60	200
10226 R	140	60	200
10235 R	132	46	178
10257 R	135	59	194
10267 R	136	58	194
10274 R	140	60	200
10290 R	-	-	-

<u>Root</u>	<u>Cellular Histology</u>	<u>Gross Histology</u>	<u>Total Points</u>
10297 R	140	60	200
10299 R	-	-	--
10306 R	112	49	161
10315 R	136	57	193
10322 R	138	58	196
10330 R	134	60	194
10331 R	140	60	200
10337 R	140	60	200
10339 R	140	60	200
10362 R	138	60	198
10369 R	140	60	200
10385 R	100	32	132
10386 R	140	60	200
10401 R	-	-	-
10419 R	140	60	200
10441 R	-	-	-
10449 R	140	60	200
10467 R	140	60	200
10437 R	140	60	200

The above data represent a summary of the tabulated data which were accumulated on the data report sheets. These sheets, one Gross Histology sheet and one Cellular Histology sheet for each specimen examined, are included in this report and represent pages 191-250. Only two copies of these pages were prepared. One was sent to the Technical Monitor, Dr. Charles Walkinshaw, as an integral part of this report, while the other was retained for my file.

TABLE IV

Evaluation of callus tissues:

<u>Callus</u>	<u>Cellular Histology</u>	<u>Total Points</u>
11003	32	32
11006	35	35
11007	35	35
11012	29	29
11017	35	35

<u>Callus</u>	<u>Cellular Histology</u>	<u>Total Points</u>
11020	35	35
11033	34	34
11036	33	33
11039	34	34
11046	23	23
11047	23	23
11055	35	35
11062	35	35
11069	34	34
11080	33	33
11084	34	34
11090	35	35
11091	33	33
11092	34	34
11097	35	35
11102	35	35
11105	35	35
11106	35	35
11121	35	35
11130	33	33
11131	32	32
11134	32	32
11144	32	32
11145	35	35
11156	29	29
11157	33	33
11183	31	31
11184	35	35
11185	35	35
11186	34	34
11189	32	32
11197	33	33
11198	32	32
11200	32	32
11202	32	32
11210	35	35
11218	35	35

<u>Callus</u>	<u>Cellular Histology</u>	<u>Total Points</u>
11219	32	32
11225	34	34
11227	35	35
11229	35	35
11240	35	35
11259	35	35
11264	35	35
11268	35	35
11271	34	34
11272	35	35
11276	35	35
11277	35	35
11279	35	35
11285	35	35
11288	34	34
11292	35	35
11303	35	35
11309	35	35
11312	35	35
11316	35	35
11317	35	35
11318	35	35

The above data represent a summary of the tabulated data which were accumulated on the data report sheets. These sheets, one Tissue Culture sheet for each specimen examined, are included in this report and represent pages 251-330. Only one copy of these pages was prepared and sent to the Technical Monitor, Dr. Charles Walkinshaw, as an integral part of this report.; a second copy was kept for my files.

E. Results of the Electron Microscopic Analyses of the Experimental Tissues:

Presentation of the results from these analyses are provided in the following table (TABLE V).

TABLE V

<u>Callus</u>	<u>Cellular Histology</u>	<u>Total Points</u>
11089	57	57
11092	59	59
11104	58	58
11164	50	50
11174	41	41
11195	42	42
11204	50	50
11213	58	58
11232	59	59
11298	40	40
11319	53	53

The above data represent a summary of the tabulated data which were accumulated on the data report sheets. These sheets, one Cell Organization (Ultrastructure) sheet for each specimen examined, are included in this report and represent pages 330-342. Only one copy of these pages was prepared and sent to the Technical Monitor, Dr. Charles Walkinshaw, as an integral part of this report; a second copy was kept for my files.

F. Test Unit Termination Form.

The above data were recorded on the Test Unit Termination Forms, pages 1-9, as the information became available and these forms were subsequently forwarded to Dr. Walkinshaw to aid him in correlation and integration of the histological data with other data available to him.

II. Evaluation and Revision of the Histological Protocol of the Botanical Operation Procedure Manual OPV 452.2.2.2.

During the period of this contract, I have revised the above protocol and sent it to Dr. Walkinshaw. This work involved: the organization of Section 2.4.2.7, Histological Observations; Appendix A, Preparation of Botanical Specimens for Examination by Light Microscopy; and Appendix B, Preparation of Botanical Specimen's for Examination by Electron Microscopy. This work involved the preparation of a 27 page report, all of which has been received by you on previous dates.

III. Training of Personnel in Histological Procedures.

The successful accomplishment of this facet of the program was dependent on the training of personnel to perform the many tasks associated with the preparation of both light and electron microscopic specimens. This training was begun shortly after the initiation of the contract.

In addition to the training program, it was necessary to establish the proper procedures to be employed when handling the different tissues during the program. Different materials and staining procedures were employed during the early months of this contract which enabled me to determine the preferred preparative and staining procedures to be employed on the tissues. These studies resulted in the preparation of slides, grids and photographs which were sent to Dr. Walkinshaw along with the monthly reports.

In summary these materials have included:

Microscopic slides:	224
Electron microscopic grids:	31
Electron micrographs:	40

IV. Literature Documentation.

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V. Reports and Publications Accruing from the Initial Contract.

- a. Reports at the XI International Botanical Congress, Seattle, August 24-Sept. 2, 1969.
1. The vacuolar apparatus of plant cells: Origin of the vacuole.
 2. The vacuolar apparatus of plant cells: Ectoplasmasomes.
- b. Publications.
1. Ectoplasmasomes: Endocytic vesicles associated with the vacuolar apparatus in plant cells. P. Mahlberg, K. Olson and C. Walkinshaw. Submitted to *Planta*, September, 1969.

VI. Data Report Sheets.

These 306 report sheets are included as supplementary pages at the end of report.

VII. Test Unit Termination Forms.

Data derived from the analyses of the various plant tissues were collated and tabulated on the Test Unit Termination Form. This information was transmitted immediately to Dr. Walkinshaw who now possesses this information. This material consisted of 9 pages and represents supplementary pages for this report.

VIII. Summary of Materials Accompanying or Associated with this Final Report.

- A. Microscopic slides, 2 sets, totalling 390 slides.
- Set I Box 6 (several boxes)
- Set II Box 6 (several boxes)

This includes 2 slides of nearly all specimen identification numbers.

B. Electron microscopic grids, totalling grids.

Grid box:

This includes grids of nearly all specimen identification numbers.

C. Electron micrographs, totalling pictures.

This includes representative photographs of nearly all specimen identification numbers.

D. Data Report Sheets, including those related to Gross Histology, Cellular Histology, Tissue Culture, and Cell Organization (Ultrastructure) of the different tissues examined in this investigation, 326 pages. Report pages 17-342.

E. Test Unit Termination Form, pages. Report pages 343-352.

X. Supplies and Equipment Costing over \$100.

Enlarger	\$398.00
Interference-contrast micro-	
scopic equipment	2975.00
Diamond knives	852.00
Histocenter	710.00
Printer	410.00

XI. Listing of Electron Microscopic Grids Sent to Houston. Grid box 24969.

<u>Pinus lambertiana</u>	11164	A1 to A3
	11174	B1 to B3
	11195	C1 to C3
<u>Pinus palustris</u>	11204	E1 to E3
	11213	F1 to F2
	11225	G1 to G3
	11231	H1 to H3
	11232	J1 to J2
<u>Nicotiana tabacum</u>	11089	L1 to L3
	11092	M1 to M3
	11104	N1 to N3
<u>Zea mays</u>	11298	P1 to P3
	11319	Q1 to Q3