

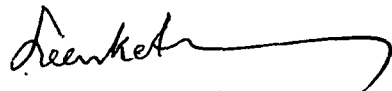
FINAL REPORT

ON

N.A.S.A. CONTRACT NAS 9-10947

entitled

DEVELOPMENT OF GERM-FREE PLANTS AND TISSUE CULTURES



S. Venkateswaran
Associate Professor

February, 1973

THOMAS, JAMES

NO:

U.S.A. COMPT WAS 2-1-1977

delivered

REPRODUCTION OF CHINESE-AMERICAN CULTURE

Associate Professor
S. J. Richardson

1973, January 1

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FINAL REPORT

ON

CONTRACT NAS 9-10947

This is the final report of the performances on the N.A.S.A. Contract NAS 9-10947 entitled:

DEVELOPMENT OF GERM-FREE PLANTS AND TISSUE CULTURES

This contract was awarded to the University of Houston with Dr. S. Venketeswaran as the Principal Investigator. This contract was a continuing effort of the botanical program for experiments at the Lunar Receiving Laboratory, M.S.C. It started on June, 1970 until September 1, 1972. The title of the contract remained unchanged.

This Contractor has fulfilled all the obligations as required by the Technical Monitor for fulfilling the experiments on the Apollo program. Four scientific publications and 6 presentations at National meetings have resulted from this contract.

The final report consists of the Progress Reports and comments.

FINAL COMMENTS ON CONTRACT NAS 9-10947

At the end of September 30, 1972, this contractor requested an extension of this contract until December 31, 1972 without any additional funding. The reason for this request was based on:

- (1) fulfilling supply of plant material/tissue culture for performing experiments on the Apollo 17 lunar samples- with no additional funding
- (2) maintaining the liaison between the University of Houston and the Botany Program at the Lunar Receiving Laboratory, Manned Spacecraft Center.

The contract was terminated as of December 31, 1972. All the reports requested in the contract have been submitted.

This is the final Report on this Contract, NAS 9-10947.

Respectfully submitted,

S. Venketeswaran
Associate Professor
Principal Investigator

Capital Equipments

No major capital equipments were bought under this N.A.S.A. Contract NAS 9-10947. Capital equipments bought or provided by N.A.S.A. under earlier Contracts to the Principal Investigator were used.

Papers Published under this N.A.S.A. Contract NAS 9-10947

1. Walkinshaw, C. H., S., Venketeswaran, et al. (1972). Effect of Lunar materials on plant tissue culture. Space Life Science 11, 119-131.
2. Walkinshaw, C. H., P. H. Johnson and S. Venketeswaran. 1972. Elemental abundances in callus tissues of carrots, rice, soybean and tobacco. In vitro 6: 391-396.
3. Weete, J. D., S. Venketeswaran and J. L. Laseter. 1971. two populations of aliphatic hydrocarbons in teratoma and habituated tissue cultures of tobacco. Phytochem. 10: 939-943.
4. Walkinshaw, C. H., H. C. Sweet, S. Venketeswaran and W. H. Horne. 1970. Results of Apollo 11 and 12 Quarantine studies on plants. Bioscience 20: 1297-1302.

Papers Presented at the Annual Meetings during this Contract
NAS 9-10947.

- 1971 - Walkinshaw, C. H. and S. Venketeswaran. Usefulness of pine tissue cultures in forestry and space research. In vitro 6: p. 379.
- 1970 - Venketeswaran, S. et al. Development of the Botany Protocol for the Apollo Mission. Am. J. Bot. 57, p. 736.
- 1970 - Venketeswaran, S. et al. Studies on Tissue Cultures of Higher Plants after Exposure to Lunar Soil after Apollo 11 and 12. Am. J. Bot. 57, p. 736.
- 1970 - Sweet, H., Walkinshaw, C. H. and S. Venketeswaran - Reactions of Several Species of Lower Plants to Lunar Materials, Am. J. Bot., 57, pp. 736.
- 1970 - Mahlberg, P., et al. and S. Venketeswaran. Ultrastructural observations on plasma membrane related invaginations in cultured cells employed in the Lunar test systems for Apollo 12. Am. J. Bot. 57, pp. 637.
- 1969 - Venketeswaran, S. et al. Plant tissue cultures for the Lunar Botany Program. XI Internat'l. Bot. Congress. p. 228.

Report on Work Performed

on

N.A.S.A. Contract NAS 9-10947

entitled

DEVELOPMENT OF GERM-FREE PLANTS AND TISSUE CULTURES

S. Venketeswaran
Associate Professor

June 1, 1970 - August 30, 1970

Abbreviations

I. Media = protocol are included in report.

Mod muk = modified Murashige and Skoog

SIK = SU + IAA + Kinetin

K = Knudson

10XW = 10 X White

1xW = 1 X White

Hoag = Haogland's

H = Heller's

V = Voth

MIK = Modified MUK + IAA + Kinetin

II. Cultures:

PTA = Pteridium aqualiumHOB = Todea barbaraL Cer = Lycopodium cernumMar. = Marchantia polymorphaPine L = Pinus lambertianaPine P = Pinus palustrisPine E = Pinus elliottiiDC = Daucus carotaSun = Sunflower = Helianthus annusSoy = Soybean = Glycine sojaHap = Haplopappus gracilis

SHIPMENTS TO N.A.S.A.

<u>Date</u>	<u>Cultures</u>	<u>Media</u>	<u>Number Taken</u>	<u>Date Last Transfer</u>
June 11, 1970 Thursday	PTA	Knudson	48	6/ 9
	Soy	SV	12	5/21 6/ 2
	Sun	SV	12	5/11 6/ 1
	Carrot	SV	48	6/ 4 5/27
	Haplopappus	SV	24	6/ 2 5/21
Total = 144 cultures				
June 16, 1970 Tuesday	Rice	Hellers	85	6/15 6/ 8
	PTA	Knudson	52	6/15
	tDB	Knudson	29	6/15
	L Cer	Knudson	26	6/15
Total = 192 cultures				
June 24, 1970 Wednesday	Soy	SV	23	6/12
	Sun	SV	20	6/ 1 6/ 2
	Pine	Mod. Muk.	30	6/ 4
		SV	200	
		Mod. Muk.	50	
		10 X W	48	
		1 X W	50	
		Hellers	50	
Total = 73 cultures; 398 bottles of media				
June 30, 1970 Tuesday	TDB	Knudson	24	6/11 6/18
	Soy	SV	24	6/17
	D.C	SIX	32	6/ 4
	Carrot	SV	16	5/27
Total = 96 cultures				

SHIPMENTS TO N.A.S.A. (continued)

<u>Date</u>	<u>Cultures</u>	<u>Media</u>	<u>Number Taken</u>	<u>Date Last Transfer</u>
July 8, 1970 Wednesday	Soy	SV	3	6/17 6/25 6/26
	Sun	SV	37	6/23 6/ 2
	D.C	SIK	1	5/21
	Carrot	SV	1	6/ 4
	Rice	Heller's	12	6/22
	PTA	Knudson's	1	6/ 9 6/10
Total = 261 cultures				
July 13, 1970 Monday	Marchantia	Hoagland's	12	7/ 8 7/10
	PtA	Knudson's	100	7/ 9 7/10 7/13
	TDB	Knudson's	9	7/ 9 7/10
	L Cer	Knudson's	1	7/ 9 7/10
Total = 302 cultures				
July 14, 1970 Tuesday		Knudson's	100	
		SIK	100	
		SU	200	
Total = 450				
July 15, 1970 Wednesday	PTA	Knudson's	45	7/15
		SV	200	
		Knudson's	100	
Total = 345				
July 16, 1970 Thursday	PTA	Knudson's	78	7/15 7/16
	PTA	Knudson's	21	7/14 7/15
	PTA	Knudson's	7	6/ 9
	TDB	Knudson's	23	6/18
	L Cer	Knudson's	11	6/10
	Marchantia	Hoagland's and Voth's	32	6/ 6
Total = 172				

SHIPMENTS TO N.A.S.A. (continued)

10

<u>Date</u>	<u>Cultures</u>	<u>Media</u>	<u>Number Taken</u>	<u>Date Last Transfer</u>
July 21, 1970 Tuesday	Note: Media was made in Mason jars, 100 mls per jar, in order to run a pilot test on new procedures of decontamination.			
	Soy	SV	1	7/21
	Sun	SV	1	7/21
	Carrot	SV	1	7/21
	Haplopappas	SV	1	7/21
	Rice	Heller's	1	7/21
	Pine	Mod. Muk.	1	7/21
	Corn	W 1 X	1	7/21
	To b	W 10 X	1	7/21
	D.C	SIK	1	7/21
	PTA	Knudson's	1	7/21
	L. Cer	knudson's	1	7/21
	TDB	Knudson's	1	7/21
	Marchantia	Hoagland's	1	7/21
Total = 13 cultures (in Mason jars)				

July 28, 1970 Tuesday	Soy	SV	100	7/26
	To b	10 X W	120	7/28

Note: Special transfers for Dr. Weete's experiments; portions of each mother culture were frozen as transfers were being made, in order to have zero time for the experiment.

Total = 220 cultures

SHIPMENTS TO N.A.S.A. (continued)

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<u>Date</u>	<u>Cultures</u>	<u>Media</u>	<u>Number taken</u>	<u>Date Last transfer</u>
Jly. 30, 1970	Okra	SV	184	6/11 7/15
Thursday	Soy	SV	48	7/26
	Sun	SV	36	7/28

Total = 268 cultures

Aug. 3, 1970-Monday

Note: Media preparations began for mission ready cultures and contamination checks for these cultures.

SV	300
Heller's	100
Mod. Muk.	100

Total = 500 bottles of media

Aug. 4, 1970	SIK	100
Tuesday	Knudson's	200

Total = 300 bottles of media

Aug. 7, 1970-Friday

Note: Contamination checks were completed on all required cultures for the mission.

Soy	SV	80	8/5	8/7
Sun	SV	80	8/5	8/7
Carrot	SV	80	8/5	8/7
Rice	Heller's	80	8/5	8/7
Tob Hab	10 X W	80	8/5	8/7
D.C.	SIK	50	8/5	8/7
Okra	SV	80	8/5	8/7
Hap.	SV	50	8/5	8/7
Tomato	SV	50	8/5	8/7
Pine L.	Mod. Muk.	50	8/5	8/7
Pine P.		80	8/5	8/7
Pine E.		50	8/5	8/7
PTA	Knudson's	80	8/5	8/7
TDB	Knudson's	80	8/5	8/7
L Cer	Knudson's	80	8/5	8/7
Marchantia	Voth	80	8/5	8/7

SUBCULTURING AT N.A.S.A.**SUBCULTURING AT N.A.S.A.**

<u>Date</u>	<u>Culture</u>	<u>Media</u>	<u>12 Number</u>
July 13, 1970	Soy	SV	143
Monday	Sun	SV	26
July 14, 1970	Carrot	SV	45
Tuesday	Sun	SV	88
July 15, 1970	D.C.	SIK	112
Wednesday	Carrot		85
July 16, 1970	Rice	Heller's	122
Thursday			

Note: All necessary poly-propalyne was prepared at
University of Houston and carried to N.A.S.A.

New Culture Initiation

Routine surface sterilization and seed germination procedures were followed.

I. Okra -

<u>Seedling Section</u>		<u>Media</u>	<u>Number</u>
Okra stem	on	SV media	25
Okra root	on	SV media	25
Okra stem	on	MIK media	10
Okra root	on	MIK media	10

II. Lettuce -

Lettuce stem	on	SIX media	20
Lettuce root	on	SIX media	16
Lettuce stem	on	SV media	25
Lettuce root	on	SV media ²	25
Lettuce stem	on	1 X W	37
Lettuce root	on	1 X W	21

III. Tomato

Tomato stem	on	SV	50
Tomato root	on	SV	50

IV. Popcorn

Popcorn stem	on	1 X W	50
Popcorn roots	on	1 X W	50

TISSUES CULTURED ON MEDIUM: SUNFLOWER; CARROT;

SOYBEAN, HAPLOPAPPUS

<u>Macronutrients</u>	<u>Stock Solution, gm/liter</u>	<u>Stock Solution, ml/liter of medium</u>	<u>Final concentration, mg/liter</u>
(1) NH_4NO_3	82.5	20.0	1650.0
KNO_3	95.0		1900.00
(2) $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	88.0	5.0	440.0
(3) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	74.0	5.0	370.0
KH_2PO_4	34.0		170.0
(4) Na_2EDTA	7.45	5.0	37.3
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	5.57		27.8
<u>Micronutrients</u>			
(5) $\text{MnSO}_4 \cdot \text{H}_2\text{O}$	4.5		4.5
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	1.5		1.5
H_3BO_3	1.5		1.5
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.04	1.0	0.04
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.25		0.25
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.005		0.005
AlCl_3	0.003		0.003
$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	0.003		0.003
KI	0.001		0.001
<u>Vitamins and Amino acids</u>			
(6) Nicotinic acid	0.5		0.5
Thiamine HCl	0.1	1.0	0.1
Pyridoxine HCl	0.1		0.1
Glycine	3.0		3.0
(7) NAA	0.175	1.0	0.175
(8) 2,4-D	0.221	1.0	0.221
(9) Yeast Extract	1		0.18
(10) Coconut Milk		100.0	100
(11) Ascorbic Acid	50.0		50.0
(12) Sucrose	30		30
(13) Agar	10		10

S.V. MEDIUM AND IAA AND KINETIN

TISSUE CULTURED ON MEDIUM: DANCUS CAROTA
pH 5.5-6.0

	<u>Major Solution</u>	<u>Stock Solution, gm/liter</u>	<u>Stock Solution ml/liter of medium</u>	<u>Final Concentration mg/liter</u>
(1)	NH_4NO_3	82.5	20.0	1650.0
	KNO_3	95.0		1900.0
(2)	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	88.0	5.0	440.0
(3)	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	74.0	5.0	370.0
	KH_2PO_4	34.0		170.0
(4)	Na_2EDTA	7.45	5.0	37.3
	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	5.57		27.8

Micro Nutrients

(5)	$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	4.5		4.5
	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	1.5		1.5
	H_3BO_3	1.5		1.5
	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.04	1.0	0.04
	$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.25		0.25
	$\text{CoCl}_3 \cdot 6\text{H}_2\text{O}$	0.005		0.005
	AlCl_3	0.003		0.003
	$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	0.003		0.003
	KI	0.001		0.001

S.V. MEDIUM AND IAA AND KINETIN

TISSUE CULTURED ON MEDIUM DANCUS CAROTA (continued)

pH 5.5-6.0

<u>Major Solution</u>	<u>Stock Solution, gm/liter</u>	<u>Stock Solution ml/liter of medium</u>	<u>Final Concentration mg/liter</u>
(6) <u>Vitamins and Amino Acids</u>			
Nicotinic acid	0.5		0.5
Thiamine HCl	0.1	1.0	0.1
Pyridoxine HCl	0.1		0.1
Glycine	3.0		3.0
(7) NAA	0.175	1.0	0.175
(8) 2,4-D	0.221	1.0	0.221
(9) Yeast Extract	0.1		0.18
(10) Coconut Milk		100.0	10%
(11) Ascorbic Acid	50.0	1.0	50.0
(12) Kinetin	0.04	1.0	0.04
(13) IAA	2.0	1.0	2.0
Sucrose	30		
Agar	10		

TISSUES CULTURED ON MEDIUM: PINUS PALUSTRISPINUS LAMBERTIANAPINUS ELLIOTTII

pH 5.7-5.8

	<u>Major Solution</u>	<u>Stock Solution, gm/liter</u>	<u>Stock solution, m/l liter of medium</u>	<u>Final concentration, mg/liter</u>
(1)	NH ₄ NO ₃	16.5		1650.0
	KNO ₃	19.0	100.0	1900.0
	CaCl ₂ ·2H ₂ O	4.4		440.0
	MgSO ₄ ·7H ₂ O	3.7		370.0
	KH ₂ PO ₄	1.7		170.0
(2)	Na ₂ -EDTA	7.45	5	37.3
	FeSO ₄ ·7H ₂ O	5.57		27.8
	<u>Minor Solution</u>			
(3)	HBO ₃	6.2		6.2
	MnSO ₄ ·4H ₂ O	22.3		22.3
	ZnSO ₄ ·4H ₂ O	8.6	1.0	8.6
	KI	0.83		0.83
	Na ₂ MoO ₄ ·2H ₂ O	0.25		0.25
	CuSO ₄ ·5H ₂ O	0.025		0.025
	CoCl ₂ ·6H ₂ O	0.025		0.025
	<u>Vitamins and Amino acids</u>			
(4)	Nicotinic acid	0.5		0.5
	Thiamine HCl	0.1	0.1	0.1
	Pyridoxine HCl	0.1		0.1
	Kinetin	0.5		0.5
(5)	2,4-D	5	1.0	5.0
(6)	Inositol	100	1.0	100.0
(7)	Asparagine	100	1.0	100.0
(8)	Ascorbic acid	50	1.0	50.0
(9)	Sucrose	30		30
(10)	Agar	10		1.00

HELLER'S MEDIUM
TISSUES CULTURED ON MEDIUM: RICE

pH: 5.5-6.0

(1) <u>Major Solution</u>	<u>Stock Solution, gm/liter</u>	<u>Stock Solution ml/liter of medium</u>	<u>Final concentration, mg/liter</u>
KCl	100.0	7.5	750.0
NaNO ₃	100.00	6.0	600.0
MgSO ₄ ·7H ₂ O	100.0	2.5	250.0
NaH ₂ PO ₄ ·H ₂ O	10.0	12.5	125.0
CaCl ₂ ·2H ₂ O	10.0	7.5	75.0
FeCl ₃ ·6H ₂ O	1.0	1.0	1.0
 (2) <u>Minor Solution</u>			
ZnSO ₄ ·7H ₂ O	1.0		1.0
H ₃ BO ₃	1.0	1.0	1.0
MnSO ₄ ·4H ₂ O	0.01		0.01
CuSO ₄ ·4H ₂ O	0.03		0.03
AlCl ₃	0.03		0.03
NiCl ₂ ·6H ₂ O	0.03		0.03
KI	0.01		0.01

HELLER'S MEDIUM

TISSUES CULTURED ON MEDIUM: RICE (continued)

pH: 5.5-6.0

(3)	<u>Vitamins and Amino acids</u>	<u>Stock Solution, gm/liter</u>	<u>Stock Solution ml/liter of medium</u>	<u>Final con- centration, mg/liter</u>
	Glycine	3.0		3.0
	Thiamine HCl	0.5	1.0	0.5
	Nicotinic acid	0.5		0.5
	Pyridoxine	0.5		0.5
(4)	D,L-Tryptophane	60.0	1.0	60.0
(5)	2,4-D	2.0	1.0	2.0
(6)	Coconut milk		100.00	100
(7)	Yeast extract			0.50
(8)	Sucrose		30.0 g/l	30
(9)	Agar		8.0-10.0 g/l	0.80-10

"The macroelements (KCl , NaNO_3 , $\text{Na}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$) are very soluble (100 g/l but these stock solutions are relatively concentrated. The others ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) are less soluble and so should be kept in more dilute solutions (10 g/l).

The minor elements with exception of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ are grouped together into one solution and one ml of the stock solution is used to make a liter of media. The ferric chloride is kept in a separate solution because it causes precipitation when grouped with other minor elements."
(R. J. Gautheret, 1959).

TISSUE CULTURED ON MEDIUM: Euphorbia

pH: 5.5-6.0

<u>Major Solution</u>		<u>Stock Solution, gm/liter</u>	<u>Stock Solution ml/liter of medium</u>	<u>Final concentration mg/liter</u>
Solution	Component			
(1)	NH_4NO_3	16.5		1650.0
	KNO_3	19.0	100.0	1900.0
	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	4.4		440.0
	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	3.7		370.0
	KH_2PO_4	1.7		170.0
(2)	$\text{Na}_2\text{-EDTA}$	7.45	5.0	37.3
	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	5.57		27.8
<u>(3) Minor Solution</u>		<u>Stock Solution gm/liter</u>	<u>Stock Solution ml/liter of medium</u>	<u>Final concentration mg/liter</u>
Solution	Component			
	HNO_3	6.2		6.2
	$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	22.3		22.3
	$\text{ZnSO}_4 \cdot 4\text{H}_2\text{O}$	8.6	1.0	8.6
	KI	0.83		0.83
	$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.25		0.25
	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.025		0.025
	$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.025		0.025

(4)	<u>Vitamins and Amino acids</u>	<u>Stock Solution gm/liter</u>	<u>Stock Solution ml/liter of medium</u>	<u>Final con- centration mg/liter</u>
Solution Component				
	Nicotinic acid	0.5		0.5
	Thiamine HCl	0.1	1.0	0.1
	Pyridoxine HCl	0.1		0.1
	Kinetin	0.5		0.5
(5)	IAA	2.0	1.0	2.0
(6)	Kinetin	.04	1.0	0.04
(7)	Ascorbic acid	50.0	1.0	50.0
(8)	Myoinosital	100.0	1.0	100.0
(9)	Sucrose	30.0		30
(10)	Agar	10.0		10

KNUDSON'S MEDIA

TISSUE CULTURES ON MEDIUM:

LYCOPODIUM CORNUUM (CLUB MOSS)

PTERIDIUM AQUALIMUM (FERN)

TODEA BARBARA (FERN)

pH 5.5-6.0

	<u>Major Solution</u>	<u>Stock Solution, gm/liter</u>	<u>Stock Solution ml/liter of medium</u>	<u>Final Concentration mg/liter</u>
(1)	$\text{Ca}(\text{NO}_3)_2$	100	10	1000
(2)	K_2HPO_4	25	10	250
(3)	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	25	10	250
(4)	$(\text{NH}_4)_2\text{SO}_4$	5	10	50
(5)	FePO_4	5	10	50
	<u>Minor Solution</u> (SV Minor)			
(6)	$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	4.5		4.5
	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	1.5		1.5
	H_3BO_3	1.5		1.5
	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.04	1.0	0.04
	$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.25		0.25
	$\text{CoCl}_3 \cdot 6\text{H}_2\text{O}$	0.005		0.005
	AlCl_3	0.003		0.003
	$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	0.003		0.003
	KI	0.001		0.001
(7)	Sucrose	20		200
(8)	Agar	10		100
	H_2O up to 1 liter			

Note: FePO_4 dissolves in conc. HCl 1g/10 mls.

HOAGLAND'S MEDIUM ($\frac{1}{4}$ STRENGTH)TISSUES CULTURED ON MEDIUM: MARCHANTIA

pH 6.5-7.5

	<u>Major Solution</u>	<u>Stock Solution, gm/liter</u>	<u>Stock Solution ml/liter of medium</u>	<u>Final Concentration mg/liter</u>
(1)	$\text{Ca}(\text{NO}_3)_2$	164	1.25	205
(2)	KNO_3	101	1.25	126.25
(3)	KH_2PO_4	136	0.5	68
(4)	MgSO_4	120	0.25	40
(5)	$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	1.0	1.0	1.0
	<u>Minor Solution</u>			
(6)	H_3BO_3	2.86		2.8
	$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	1.81	1.0	1.81
	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.22		0.22
	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.08		0.08
	$\text{H}_2\text{MnO}_4 \cdot \text{H}_2\text{O}$	0.02		0.02
(7)	Sucrose	20		20
(8)	Agar	10		10
	H_2O			to make 1 liter

VOTH MEDIUM

TISSUES CULTURED ON MEDIUM: MARCHANTIA

pH 6.5 - 7.5

	<u>Major Solution</u>	<u>Stock Solution, gm/liter</u>	<u>Stock Solution ml/liter of medium</u>
(1)	KNO ₃	50.56	1.66
(2)	Ca (NO ₃) ₂	164.16	1.44
(3)	Mg (NO ₃) ₂	148.41	1.2
(4)	KH ₂ PO ₄	68.04	0.8
(5)	Mg SO ₄	120.50	1.6
(6)	Sucrose	20	
(7)	Agar	10	
	H ₂ O	to make 1 liter	

Reference:

Voth, Paul D. Effects of Nutrient-Solution Concentration on the Growth of Marchantia Polymorpha. The Botanical Gazette. 104, No. 4, 591 - 601.

WHITE'S IX MEDIUM

TISSUES CULTURED ON MEDIUM: CORN

pH 6.1-7.0

	<u>Macroelements</u>	<u>Stock Solution, gm/liter</u>	<u>Stock Solution, ml/liter of medium</u>	<u>Final concentration, mg/liter</u>
(1)	Ca(NO ₃) ₂ ·4H ₂ O	6.0		300.0
	Na ₂ SO ₄	4.0	50.0	200.0
	KNO ₃	1.6		80.0
	KCl	1.3		65.0
	NaH ₂ PO ₄ ·H ₂ O	0.33		16.5
	MgSO ₄ ·7H ₂ O	14.4		720.0
	<u>Microelements</u>			
(2)	ZnSO ₄ ·7H ₂ O	3.0		3.0
	MnSO ₄ ·4H ₂ O	7.0	1.0	7.0
	H ₃ BO ₃	1.5		1.5
	KI	0.75		0.75
	CuSO ₄ ·5H ₂ O	0.001		0.001
	Na ₂ MoO ₄ ·2H ₂ O	0.25		0.25
(3)	FeCl ₃	1.5	1.0	1.5
(4)	IAA	1.0	1.0	1.0
(5)	<u>Vitamins and Amino acids</u>			
	Glycine	3.0		3.0
	Thiamine HCl	0.5	1.0	0.5
	Nicotinic acid	0.5		0.5
	Pyridoxine	0.5		0.5
(6)	Coconut milk		100.0	10%
(7)	Yeast Extract	5		0.5%
(8)	Sucrose	20		2%
(9)	FOR INITIATION OF CALLAS:			
	2,4-D	1.0		1.0
	NAA	1.0		1.0
(10)	Agar	10		1.0%

Report on Work Performed

on

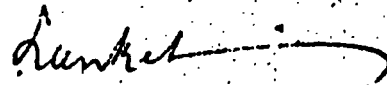
N.A.S.A. Contract NAS 9-10947

entitled

DEVELOPMENT OF GERM-FREE PLANTS AND TISSUE CULTURES

for the period of

September 1, 1970 - November 30, 1970



S. Venkateswaran

Abbreviations

I. Media: (protocols are included in report)

MM	=	Modified Murashige and Skoog (MOD MUK)
SIK	=	SV + IAA + Kinetin
SV	=	Modified Bonner
K	=	Knudson
10XW	=	10 X WHITE
1XW	=	1 X WHITE
Hoag	=	HOAGLAND'S
H	=	HELLER'S
V	=	VOTH
MIK	=	Modified MUK + IAA + Kinetin
SAB	=	Sabouraud broth
thio	=	Thioglycolate broth

II. Cultures:

PTA	=	<u>Pteridium aqualium</u>
TOB	=	<u>Todea barbara</u>
LCer	=	<u>Lycopodium cernum</u>
March	=	<u>Marchantia polymorpha</u>
Pine L.	=	<u>Pinus lambertiana</u>
Pine P.	=	<u>Pinus polustris</u>
Pine E.	=	<u>Pinus elliottii</u>
D.C.	=	<u>Daucus carota</u> (wild carrot)
Sun.	=	Sunflower = <u>Helianthus annus</u>
Soy	=	Soybean = <u>Glycine soja</u>
Hap	=	<u>Haplopappus gracilis</u>
Hab. tob	=	Habituated tobacco
tera tob	=	teratoma tobacco

Last
Transf.
Date

<u>Date</u>	<u>Cultures</u>	<u>Media</u>	<u>Number Taken</u>	<u>Purpose</u>	<u>Last Transf. Date</u>
September 2, 1970 Wednesday	Sun	SV	93	Back-up	8/31
	Soy	SV	33	Back-up	8/28
	Hab	10XW	64	Back-up	8/14
	Lettuce	SV	3	for research	8/ 5
Total - 193 Cultures					
Other Items:					
Ether - for research 6 1/4 jars					
PTA for research 9.0 g (lypholyzed) dry wt.					
PTA for research- 5.24 g fresh wt.					
September 4, 1970 Friday	Marchantia for research		3.2 g dry wt.		
	tobacco for research		5.21 g dry wt.		
September 7, 1970 Monday	Soy	SV	86	Back-up	9/ 4
	Sun	SV	74	Back-up	9/ 2 9/ 3
	Pine	mm	50	Back-up	9/ 3
September 10, 1970 Thursday	Okra	SV	65	Back-up	9/ 8
	Pine L.	mm	55	Back-up	9/ 9
September 21, 1970 Monday	Hab tob	10XW	35	Research	9/14
	Okra Stem	SV	105	Back-up	9/16
	D. Carrot	SIK	99	Back-up	9/15, 9/14

<u>Date</u>	<u>Cultures</u>	<u>Media</u>	<u>Number taken</u>	<u>Purpose</u>	<u>Last Transf. Date</u>
September 21, 1970 Monday (contd.)					
	Marchantia	Hoagland- (minus) sucrose	32	Nutritional study	9/19
	Marchantia	Voth- (minus) sucrose	38	Nutritional study	9/20
	Hab. tob	10XW	28	Back-up	8/14
Total = 337 cultures					
September 22, 1970 Tuesday					
	Soybean	SV	3	Mission	9/ 9
	Sunflower	SV	3	Mission	9/10
	Carrot	SV	3	Mission	9/10
	Okra	SV	3	Mission	9/ 9
	Lettuce	SV	2	Mission	9/11
	Haplopappus	SV	2	Mission	9/11
	D. Carrot	SIK	2	Mission	9/12
	Hab. Tobacco	10XW	3	Mission	9/ 8
	Rice	HELLER'S	3	Mission	9/12
	Pine E.	MM	2	Mission	9/16
	Pine L.	MM	2	Mission	9/16
	Pine P.	MM	2	Mission	9/16
	TDB	K	3	Mission	9/17
	PTA	K	3	Mission	9/18
	L Cer	K	2	Mission	9/18
	Marchantia	Voth	3	Mission	9/21

NOTE:

These cultures had been through two contamination checks using sab and thio media and had shown to be free from contaminants. They were placed in pint sized Mason jars, and sealed tightly with Mason jar lids to make possible entry into and decontamination of the cabinet lines. The best cultures of all species were used in preparing the mission cultures. Refer to mission culture report.

Total = 41 cultures

<u>Date</u>	<u>Cultures</u>	<u>Media</u>	<u>Number taken</u>	<u>Purpose</u>	<u>Last Transf. Date</u>
September 23, 1970 Wednesday					
	Marchantia	V#1	42	Nutritional study	9/22
	Marchantia	V	13	Nutritional study	9/22
	Hab. tob	10XW	35	Research	9/21
Total = 90 cultures					
October 1, 1970 Thursday					
		SV	20	pint size Mason jars 100 mls/jar	Mission lettuce
	Hab. tob	10XW	35	Research	9/28
	PTA	K	35	Research	9/28
	Marchantia	Voth #2	10	Nutritional study	9/24
	Rice	Heller's	35	Back-up	9/30
Total = 115 cultures 20 jars of media					
October 14, 1970 Wednesday					
	Hab. tob	10XW	25	Research	10/ 6
	PTA	K	19	Research	10/ 6
	Carrot	SV	97	Back-up	10/ 8
	Hab. tob	10XW	23	Research	10/12
	PTA	K	10	Research	10/12
	Lettuce	SV	39	Back-up	10/13
		HELLER'S	40	pint sized Mason jars 100 mls/jar	Mission
		SV	47	"	Mission
		MM	15	"	Mission
(Mason jar caps were delivered wrapped in foil; caps and media were ready for auto-claving into cabinet line)					
Total = 213 cultures 102 jars of media					

<u>Date</u>	<u>Cultures</u>	<u>Media</u>	<u>Number Taken</u>	<u>Purpose</u>	<u>Last Trans. Date</u>
October 21, 1970 Wednesday					
	Hab. tob	10XW	25	Research	10/20
	PTA	K	24	Research	10/19
	Pine E.	MM	42	Back-up	10/15
	Pine L.	MM	18	Back-up	10/15
	Pine P.	MM	24	Back-up	10/15
		10XW	36	pint-sized Mason jars 100 mls per jar - Mission	
	Hab. tob - fresh wt. = 56.7 g dry wt. = 3.2 g			Research	
	Total = 133 cultures 36 jars of media				
November 4, 1970 Wednesday					
		MM	36	pint-sized Mason jars 100 mls/jar- Mission	
		SV	40	pint-sized Mason jars 100 mls/jar- Mission	
	Hab. tob 42.0 g fresh wt. 2.93 g dry wt.			for Research	
	PTA	K	15	cultures given from back-up	
	Total = 76 jars of media for research 15 cultures				
November 9, 1970 Monday					
	Pine L.	MM	12	Back-up	10/21
	Pine E.	MM	38	Back-up	10/21, 10/23
	Rice	HELLER'S	29	Back-up	10/23
	Hab. tob	10XW	42	Research	10/26, 11/3
	PTA	K	33	Research	10/26, 11/2
		SV	30	pint-size Mason jars 100 mls/jar Mission	

Date	Cultures	Media	Number taken	Purpose	Last Transf. Date
November 9, 1970 (contd.)					
Monday		MM	2 pint-sized Mason jars 100 mls/jar	Mission	

Other Items:

1. Protocols for:

- a. White's media #1 - 4
- b. Voth media
- c. Miller's/Fowler's work on Marchantia

2. Evaluation sheets for mission plant and tissue cultures - 500

Total = 174 cultures
32 jars of media

November 17, 1970

Tuesday

Soybean	SV	138	Back-up	11/15-11/16
Sunflower	SV	35	Back-up	11/16
Tera. tob.	10XW	55	Back-up	11/15
Haplopappus	SV	49	Back-up	11/ 9
Euphorbia	SV	50	Back-up	11/14,11/26

NOTE: A contamination check was done on 25 of the 50 Euphorbia cultures in preparation for mission.

Total = 327 cultures

November 23, 1970

Sunflower	SV	93	Back-up	11/17
Haplopappus	SV	18	Back-up	11/17
Pine E.	HELLER'S MM	7	Nutritional study	11/20
Pine E.	SV - MM	7	Nutritional study	11/20
Pine E.	WHITE - MM	7	Nutritional study	11/20
Pine E.	MM #1	7	Nutritional study	11/20
Pine E.	MM	7	Nutritional study	11/20
Pine L.	HELLER'S - MM	3	Nutritional study	11/20
Pine L.	SV - MM	3	Nutritional study	11/20

<u>Date</u>	<u>Cultures</u>	<u>Media</u>	<u>Number taken</u>	<u>Purpose</u>	<u>Last Transf. Date</u>
November 23, 1970 (contd.)					
Pine L.	WHITE - MM	3	Nutritional study	11/20	
Pine L.	MM #1	3	Nutritional study	11/20	
Pine L.	MM	3	Nutritional study	11/20	
Pine E.	MM #1	30	Back-up	11/20	
Pine L.	MM #1	4	Back-up	11/20	
Total = 196 cultures					

MILLER'S VOTH #5 (from Miller's Bottles)

<u>Major Solutions</u>	<u>Stock Solution</u> <u>g/l</u>	<u>Stock Solution</u> <u>mls/l of medium</u>	<u>Final</u> <u>Concentration</u> <u>mg/l</u>
(1) KNO_3	0.5 molar	1.6	
(2) $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	0.5 molar	1.4	
(3) $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$	0.5 molar	1.2	
(4) KH_2PO_4	0.5 molar	0.8	
(5) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.5 molar	1.6	
<u>Minor Solution</u>			
(6) $\text{MnSO}_4 \cdot \text{H}_2\text{O}$	0.2	1.0	0.2
(7) ZnCl_2	0.2	1.0	0.2
(8) $\text{Na}_2\text{B}_4\text{O}_7$	0.2	1.0	0.2
(9) FeSO_4	0.02	1.0	0.02
(10) Agar	10 g/l		1%

pH not adjusted

 H_2O to make 1 liter

Report on Work Performed

on

N.A.S.A. Contract NAS 9-10947

entitled

Development of Germ-Free Plants and Tissue Cultures
for the Period of

December 1, 1970 - February 28, 1971

S. Venketeswaran
Associate Professor

Following is a list of the shipments made from the University of Houston to N.A.S.A. during the given three month period. Abbreviations and media protocols are identical to those in previous reports which can be used for referral.

<u>Shipments</u>					
<u>Date</u>	<u>Cultures</u>	<u>Media</u>	<u>Number taken</u>	<u>Purpose</u>	<u>Last Trans. Date</u>
December 1, 1970	Pine e.	MOD. MUK-1	50	Back-up	11/24
	Pine l.	MOD. MUK-1	5	Back-up	11/24
	Okra S	SV	72	Back-up	11/24
	Okra R	SV	8	Back-up	11/24

Total = 135 cultures

MOD. MUK-#1 100 1/2 pint size Pine

Mason jars

50 mls/jar

Knudson's 100 50 mls/jar Lower plants
(PTA, TDB, L. Cer.)

Other items:

Caps wrapped in foil were delivered at the same time as the jars.

December 2, 1970

SV	60	1/2 pint Mason jars (50 mls/jar)	Soybean
SV	60		Carrot
SV	60		Sunflower
SV	60		Okra
SV	30		Lettuce

Total = 270 jars of media

Note

The jars were properly labelled with the name of the media and the species which it was for. The caps were wrapped in foil and delivered with the jars.

Shipments (continued)

<u>Date</u>	<u>Cultures</u>	<u>Media</u>	<u>Number taken</u>	<u>Purpose</u>	<u>Last Trans. Date</u>
December 7, 1970	PTA	K	51 (regular bottles)	Back-up	12/3
	TDB	K	60 (1/2 pt. Mason jars)	Mission	12/4-12/6
	Harvey's		12 (regular bottles)	for Pine	
Total = 111 cultures 12 bottles of media					

Note

A contamination check using sab. and thio. was used on TDB cultures.

December 28, 1970	SIK		60 (1/2 pt. Mason jars-50 mls/jar)	Mission-in cabinet lines
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Total = 60 jars of media

December 29, 1970	HELLER'S		60 (1/2 pt. Mason jars-50 mls/jar)	Rice
	10XW		60 (1/2 pt. Mason jars-50 mls/jar)	Hab. tob.

Total = 120 jars of media

January 25, 1971	KNUDSON'S		40 (1/2 pt. Mason jars-50 mls/jar)	TDB
	KNUDSON'S		20 (1/2 pt. Mason jars-50 mls/jar)	PTA
	SV		50 (1/2 pt. Mason jars-50 mls/jar)	Soybean
	SV		20 (1/2 pt. Mason jars-50 mls/jar)	Lettuce

Total = 130 jars of media

Shipments (continued)

<u>Date</u>	<u>Cultures</u>	<u>Media</u>	<u>Number taken</u>	<u>Purpose</u>	<u>Last Trans. Date</u>
January 26, 1971	Hab. tob.	10XW	100 (1/2 pt. Mason jars)	Research	
Total = 100 cultures					
Other items:					
160 gms. of fresh weight Hab. tob.					
February 8, 1971	Marchantia	V-5	77	Back-up	2/3, 2/4, 1/23
	Pine e.	MOD.MUK	117	Research	2/7
		10XW	102	Mission	2/7
		SV	72	Mission	2/7
(1/2 pt. Mason jars)					
Total = 194 jars of cultures					
174 jars of media					
February 25, 1971	Pine e.	MM1	40	Mission	2/24
	Lettuce	SV	30	Mission	2/22
	Okra	SV	24	Mission	2/22, 1/8, 1/11
	PTA	KNUDSON'S	16	Mission	2/16
	Sphar. d.	MACKER'S	35	Mission	2/20, 2/23
	Marchan- tia	V-5	52	Mission	12/19, 1/18
	Corn	W1X	17	Mission	2/22
	Eurphorbia	SV	10	Mission	2/23
	TDB	KNUDSON'S	36	Mission	1/21, 2/18
Total = 260 cultures					

Note

The cultures were checked in sabouraud and thio-glycolate media for contamination and showed negative results. They were prepared, sealed and labeled properly in 1/2 pint size Mason jars.

This third quarterly report does not include the details of the work performed for the Apollo 14 Mission. The work began in August, 1970 and was completed in February, 1971- most of the work being done during the fall of 1970. Therefore, such performance has been described and submitted in the second quarterly report along with the work done during the fall period.

Nutritional work has been continued with Marchantia, corn, and slash pine. There has been success with Marchantia and corn. A report of these experiments will be submitted at a later date. Slash pine is presently being tested on 39 media types which vary in salt concentrations and hormonal concentrations. A full report of this also will be submitted at a later date.

Report on Work Performed

on

N.A.S.A. Contract NAS 9-1094

entitled

Development of Germ-Free Plant and Tissue Cultures

for the Period of

March 1, 1971 - May 31, 1971

S. Venketeswaran
Associate Professor

Included in this report is a list of shipments taken from the University of Houston to N.A.S.A. during the given period. All mission work had been completed by March 1, 1970 and is therefore included in previous reports. Nutritional research has been continued with slash pine with the initiation and maintenance of pine cell suspension cultures. Light and phase-contrast microscopy has been used for studying the cell cycle of pine. Suspension cultures have been plated out to test for viability, clonal growth, and contamination. Growth studies on inoculum size, media variation and basalt and earth treatment have been completed using fresh-weight and dry-weight measurements. Details of pine studies will be submitted at a later date.

Shipments

<u>Date</u>	<u>Cultures</u>	<u>Media</u>	<u>Number</u>	<u>taken</u>	<u>Purpose</u>	<u>Last Trans. Date</u>
March 12, 1971	Marchantia	V-5	39		Research	2/3
	Marchantia	KNUDSON'S	13		Research	3/9
	Pine e.	MM-1	78		Research	3/10, 3/11
	Hab tob.	10X	37		Research	3/2

Total = 167 cultures

Note: All are in 1/2 pint size Mason jars.

March 19, 1971	L. cer.	KNUDSON'S	18		Back-up	3/18
	PTA	KNUDSON'S	18		Back-up	3/17
	TDB	KNUDSON'S	18		Back-up	3/1
	Pine e.	MM-1	100		Back-up	2/12, 2/16, 3/17, 3/16

Total = 154 cultures.

April 2, 1971	Marchantia	V5-A	18		Nutritional study	3/29
		V5-B	18		Nutritional study	3/29
		V5-C	18		Nutritional study	3/29
		V5-D	18		Nutritional study	3/29
		V5-E	18		Nutritional study	3/29
		V5-F	18		Nutritional study	3/29
		V5-G	18		Nutritional study	3/29
	Hab. tob.	10XW	18		Back-up	3/2, 1/15
	Rice	HELLER'S	25		Back-up	3/18
	Soybean	SV	25		Back-up	3/9, 2/4
	Marchantia	V5	30		Back-up	3/18
	Pine e.	MM1	179		Back-up	3/30, 3/16, 3/17, 2/15, 2/10

Total = 428 cultures

Shipments (continued)

43

<u>Date</u>	<u>Cultures</u>	<u>Media</u>	<u>Number taken</u>	<u>Purpose</u>	<u>Last Trans. Date</u>
May 19, 1971	Hab. tob.	10XW	25	Research	5/5
	Pine e.	MM-4	32	Research	5/5
	Pine e.	MM	27	Research	5/5
	Total = 84 cultures.				
May 27, 1971	Pine e.	MM1 (modifi- cations)	15	Research	4/29, 5/4
	Pine e.	MM1	40	Research	4/29, 5/4
	Hab. tob.	W10X	25	Research	5/24

Report of Work Performed

on

N.A.S.A. Contract NAS 9-10947

entitled

DEVELOPMENT OF GERM-FREE PLANTS AND TISSUE CULTURES

for the period of

June 1, 1971 - August 31, 1971

S. Venkateswaran

During the month of June through August, efforts were concentrated primarily in preparation work for Apollo 15. In June, contamination checks were done on all cultures to be used for mission. Two small tissue samples from each newly transferred culture were taken--one was placed in thioglycolate medium, the other in sabouraud broth. Contamination check data is submitted with this report. Only those cultures which showed negative contamination tests were shipped to N.A.S.A. A list of these shipments is included in the report. This list also includes all media and supplies needed at N.A.S.A. for mission and research purposes.

Also during this time period initial growth studies were attempted. Data is included in the report. In order to determine optimal inoculum size for culture initiation, fresh weight for a period of six weeks was measured using three different inoculum sizes: 1 gram, 2 grams, and 3 grams. These were done on both mm and mm-1 media to distinguish between the normal media and the normal with coconut milk and yeast extract.

A series of nutritional studies that were initiated six months ago were terminated. A report was written on these experiments and is included as supplementary information to the report.

Abbreviations

I. Media: (protocols are included in report)

MM	=	Modified Murashige and Skoog (MOD MUX)
SIK	=	SV + IAA + Kinetin
SV	=	Modified Bonner
K	=	Knudson
10XW	=	10 X WHITE
1XW	=	1 X WHITE
Hoag	=	HOAGLAND'S
H	=	HELLER'S
V	=	VOTH
MIK	=	Modified MUX + IAA + Kinetin
SAB	=	Sabouraud broth
thio	=	Thioglycolate broth

II. Cultures:

PTA	=	<u>Pteridium aquilium</u>
TOS	=	<u>Todea barbara</u>
LCer	=	<u>Lycopodium cernuum</u>
March	=	<u>Marchantia polymorpha</u>
Pine L.	=	<u>Pinus lambertiana</u>
Pine P.	=	<u>Pinus palustris</u>
Pine E.	=	<u>Pinus elliotii</u>
D.C.	=	<u>Daucus carota</u> (wild carrot)
Sun.	=	Sunflower = <u>Helianthus annuus</u>
Soy	=	Soybean = <u>Glycine soja</u>
Hap	=	<u>Haplopappus gracilis</u>
Hab. tob	=	Habituated tobacco
tera tob	=	Teratoma tobacco

Shipments

<u>Date</u>	<u>Cultures</u>	<u>Media</u>	<u>Number taken</u>	<u>Purpose</u>	<u>Last Trans. Date</u>
June 15, 1971	Hab. tob.	10XW	20	Research	5/3
	Hab. tob	10XW	18	Research	6/8
	Hab. tob	10XW	16	Research	6/15
	Pine e.	MM-vari- ations	15	Research	4/29, 5/5, 6/2, 5/24
June 29, 1971	Marchantia	K	40	Mission	
	PTA	K	40	Mission	
	Pine e.	MM-1	20	Mission	
	Soy	SV	20	Mission	
	Hab. tob.	10XW	20	Mission	
	Rice	HELLER'S	11	Mission	
	Corn	MM-1	7	Mission	
	D. carrot	SV	12	Mission	
July 1, 1971	Hab. tob.	10XW	25	Research	6/29
	Hab. tob.	10XW	30	Research	6/22
	Pine e.	MM-1	30	Research	6/28
July 8, 1971	L. Cer	K	40	Mission	6/9
	Pine e.	MM-1	30	Mission	6/8
	Soybean	SV	15	Mission	6/4
	Hab. tob.	10XW	30	Mission	6/8, 6/22
July 12, 1971		W10X	60	Mission	
		MM-1	60	Mission	
		SV	140	Mission	
		HELLER'S	60	Mission	

July 19,
1971

Polypropylene blocks for transfers
500 Mason jar seals for decontamination procedures
at N.A.S.A.

<u>Date</u>	<u>Cultures</u>	<u>Media</u>	<u>Number taken</u>	<u>Purpose</u>	<u>Last Trans. Date</u>
July 23, 1971		KNUDSON's	180	Mission	
August 4, 1971	Soybean	SV	20	Mission	8/2
August 12, 1971	Soybean	SV-liquid	4	Research	6/29, 8/4
	Pine e.	MM-solid	30	Research	8/3, 8/12
	Soybean	SV-solid	30	Research	8/10, 8/11
	Hab. tob.	W10X	110	Research	7/13, 7/20 7/27, 8/5
	Pine e.	Liquid MM-1 on solid MM-1	8	Research	8/4, 8/11
	Pine e.	MM-liquid (small inoculum)	3	Research	7/30
	Pine e.	Buffered MM liquid	3	Research	7/12
	Pine e.	Conditioned MM liquid	3	Research	7/12
	Pine e.	Conditioned MM 1st transfer	8	Research	8/10
	Pine e.	MM-liquid (large inoculum)	8	Research	8/5
August 16, 1971		SV	100	Mission	
		HELLER'S	40	Mission	
		W1X	20	Research	
		MM-1	100	Mission	

CONTAMINATION CHECK - APOLLO 15
(June, 1971)

Each subculture was tested with thioglycolate and trypticase soy at the time of subculturing. Test tubes of the contamination check media were prepared at the University of Houston. Two samples from each new subculture were taken, one of which was placed in thioglycolate and the other of which was placed in trypticase soy. Ten days were allowed as the incubation period at room temperature. If any contamination appeared in the test tubes, the respective culture was discarded.

Data

<u>Name of Culture</u>	<u>Soy</u>	<u>Sun</u>	<u>D.carrot</u>	<u>Rice</u>	<u>Corn</u>	<u>Med. tob.</u>	<u>Pine o.</u>	<u>FTA</u>	<u>L.Car</u>
Number of new sub-cultures	60	60	38	24	12	60	60	60	60
Number of cultures contaminated in try	0	1	16	7	4	5	4	14	14
% contaminated in try	0	1.6	42	29.1	33.3	8.3	6.6	23.3	23.3
Number contaminated in Thio	0	0	15	8	3	5	3	15	16
% contaminated in Thio	0	0	39.4	33.3	25	8.3	5	25	26.6
TOTAL % contaminated	0	1.6	42	29.1	25	8.3	6.6	25	26.6

Growth Studies Data

MM	Date	Days	Culture	Wt - T ₁	Wt - T ₂	Δwt.
	5/ 3/71	07	1a	3.0	3.2	+0.2
	5/10/71	14	2a	3.0	4.8	+1.8
	5/18/71	22	3a	2.9	8.6	+5.7
	5/26/71	30	4a	3.2	13.6	+10.4
	6/ 2/71	37	5a	2.9	11.2	+8.3
	6/ 9/71	44	6a	3.1	12.4	+9.3
	5/ 3/71	07	1b	2.0	3.2	+1.2
	5/10/71	14	2b	2.2	5.4	+3.2
	5/18/71	22	3b	2.2	2.0	+0.8
	5/26/71	30	4b	2.1	7.0	+4.9
	6/ 2/71	37	5b	1.9	9.7	+7.6
	6/ 9/71	44	6b	1.8	10.4	+8.6
	5/ 3/71	06	1c	0.9	1.6	+0.7
	5/10/71	13	2c	0.9	2.3	+1.4
	5/18/71	21	3c	0.9	3.8	+2.0
	5/26/71	29	4c	0.9	6.6	+5.7
	6/ 2/71	36	5c	0.9	11.0	+10.1
	6/ 9/71	43	6c	1.1	13.0	+11.9

wt = weight

T₁ = time of initiation of cultureT₂ = time of harvest

Δwt = change of weight

MM-1	Harvest Date	Age in Days	No. Culture	Wt - T ₁	Wt - T ₂	Δwt.
	5/ 3/71	10	1a	3.4	4.5	+1.1
	5/10/71	17	2a	3.1	6.9	+3.8
	5/18/71	25	3a	3.3	10.2	+7.0
	5/26/71	33	4a	2.8	11.6	+8.8
	6/ 2/71	40	5a	2.9	15.8	+12.9
	6/ 9/71	47	6a	3.0	14.0	+11.0
	5/ 3/71	10	1b	2.1	1.8	+0.3
	5/ 3/71	10	10b	2.0	2.3	+0.3
	5/10/71	17	2b	1.9	4.1	+2.2
	5/18/71	25	3b	1.9	5.6	+3.7
	5/26/71	33	4b	2.0	4.7	+2.7
	6/ 2/71	40	5b	2.1	5.2	+3.1
	6/ 9/71	47	6b	2.1	7.7	+5.6
	5/ 3/71	07	1c	1.1	1.4	-0.3
	5/10/71	14	2c	1.2	1.7	+0.5
	5/18/71	22	3c	1.1	1.5	+0.4
	5/26/71	30	4c	1.1	3.7	+2.6
	6/ 2/71	37	5c	1.2	6.7	+5.5
	6/ 9/71	44	6c	1.2	1.5	+0.3

Dry wts. in grams

<u>1 wk.</u>	<u>MM</u>	<u>MM-1</u>
1a	.1765	.32
1b	.1945	.18
1c	.1046	.21
<u>4 wk.</u>		
4b	.3535	.26
4c	.3125	.29
4a	.4353	.39
<u>6 wk.</u>		
6a	.50	.52
6b	.41	.40
6a	.58	.11

Data Terms:

Wt - t_1 = weight in grams at time of initiation of culture

Wt - T_2 = weight at time of harvest of culture

Δ wt = change in weight

a = 3.0 gram inoculum size

b = 2.0 gram inoculum size

c = 1.0 gram inoculum size

MM-1 = Media with coconut milk and yeast extract

MM = Control media without coconut milk and yeast extract

1a = 3.0 gram inoculum, culture #1

2a = 3.0 gram inoculum, culture #2

etc.

Nutritional Studies on Pinus elliotii

The following is a review of the results obtained on the nutritional studies on Pinus elliotii tissue culture. Requirements of established major salt solutions such as Harvey's, Heller's, White's and SV's were tested. Individual elements, additives, and extracts were tested in varying concentrations. The results were recorded according to the following numerical point system:

- 1 = no signs of growth since subculture
- 2 = signs of growth but small amount, little or no greening
- 3 = large growth, but no greening, healthy
- 4 = large growth, with greening, healthy

This system allows a fast coverage of the type or visual description of the cultures based on growth rate (size) and pigmentation without any judgement as to which is considered the best culture. At least ten cultures on each media type were used. The average evaluation is tabulated in the report.

Maintenance of pine callus on Harvey's medium was attempted three times. The first attempt resulted in death after just one generation. The next two attempts survived the first generation but not the subcultures (generations) thereafter. After two months the cultures were totally brown and remained the size of the original subcultures. After four months there was still no sign of growth.

Mod. Muk. medium was the control medium for most of the test components. It is completely defined, containing no coconut milk or yeast extract as does MM-1 medium. Initially, adding coconut milk and yeast extract to the medium gave increase in growth rate and yellow to green pigmentation. But once these cultures were

obtained, maintenance on Mod. Muk. was possible without any visual decline in the condition of the cultures. After two to three generations, Mod. Muk. continued to improve the condition of the cultures, while MM-1 now showed signs of decline.

Heller's, White's, and SV's major salts were tested in media identical to MM-1 (with coconut milk and yeast extract) except for the major salts. After one generation, both Heller's and White's cultures showed rapid decline in growth and were dead within three months. SV's salts gave a continual improvement through six months maintenance.

Manganese and magnesium ion concentrations were individually tested both in the presence and absence of coconut milk and yeast extract. In the manganese test, there was little variation in the size of the cultures. All seemed to grow at one of the fastest rates obtained thus far. Differences were noted in pigmentation. MM-4 having 10 mg/l of Mn^{++} showed the least browning with many areas pale green. This concentration was the highest tested for this element. With Mg^{++} opposite results were obtained. The least browning was with the lowest Mg^{++} concentration tested, 185 mg/l. Higher concentrations seemed to be toxic to growth, since very little growth with much browning was common to cultures at 740 mg/l. Coconut milk and yeast extract did not noticeably affect the results of these tests. Thus the cultures are now maintained on the defined media.

The chelating agent EDTA was tested in half the concentration of that in the control Mod. Muk. medium. After two generations some difference in growth was obtained. But very distinct was the extreme browning in the cultures with less EDTA.

At 220 mg/l and at 880 mg/l, calcium gave severe browning effects on the callus. The control is 440 mg/l which resulted in light tan coloration but no severity in browning.

Potassium phosphate, control at 170 mg/l, showed browning effects with decrease in concentration, and dark yellow to green effects with increase of concentrations. No visual effect on growth rate was noticed.

Depleting kinetin from the medium did not show any noticeable differences from the control. Growth and pigmentation were still considered satisfactory.

Varying asparagine concentrations affected growth and not pigmentation. A higher growth rate was very obvious at 25 mg/l asparagine than with complete depletion of asparagine from the medium.

Ascorbic acid seemed to be toxic to growth at increased concentrations. At 25 mg/l the cultures were approximately half the size of those grown without ascorbic acid although the latter showed more browning effects. All cultures did show sporadic greening.

Depleting all additives from the medium was compared to depleting major salts only and then minor salts only. After two generations the cultures grown without additives were dead. The other cultures were brown with very little growth.

Sucrose was tested at 5 g/l instead of 30 g/l. After two generations the cultures were almost dead.

Wood extract with Heiler's salts inhibited growth within one generation. The extract with Mod. Muk. media supported growth, but much browning continued.

Pine extract has resulted in decline of growth rate after two generations. The cultures were light tan, but no significant browning or greening was noticed.

Malt extract when added to Mod. Muk. medium seemed to inhibit growth and any yellow to green color. But when added with coconut milk, the cultures were much greener than any others throughout all the tests, but they were not large. The two additives combined enhanced pigmentation but not growth.

EVALUATIONS

Name of Media	Test Items	EVALUATIONS								
		1970 12/16	1971 1/12	1971 2/12	1971 3/10	1971 4/13	1971 5/2	1971 5/25	1971 6/25	1971 7/27
HARVEY'S major salts		1.0	-----	1.75	1.0	**	3.0	1.0	1.0	1.0
MOD.MUK.	w/o cm+ye	2.17	2.87	2.5	2.9		2.75	3.2	2.2	2.9
MM-1	w cm+ye	2.77	3.56	2.5	2.5		2.5	1.0	1.4	2.5
MM-2	HELLER'S major (w cm+ye)	2.43	1.5	1.2	1.0	*				----
MM-3	WHITE'S major (w cm+ye)	2.50	1.33	1.0	1.0	*				----
MM-4A	SV's major (w cm+ye)	2.0	2.62	2.86	3.1		3.28	*	*	----
MM-4	Mn ⁺⁺ =10mg/l (w cm+ye)			3.33	3.52		2.5	3.2	*	----
MM-5	Mn ⁺⁺ =4.5 mg/l (w cm+ye)			3.41	3.45		2.7	3.2	*	----
MM-6	Mn ⁺⁺ =1.0 mg/l (w m+ye)			3.91	3.74		2.38	3.2	*	----
MM-7	Mg ⁺⁺ =370 mg/l (w cm+ye)					***	1.0	2.8	*	----
MM-8	Mg ⁺⁺ =185 mg/l (w cm+ye)					***	3.0	2.8	*	----
MM-9	Mg ⁺⁺ =740 mg/l (w cm +ye)					***	2.9	2.0	*	----
MM-4	Mn ⁺⁺ =10 mg/l (w/o cm+ye)							3.6	2.5	3.3
MM-5	Mn ⁺⁺ =4.5 mg/l (w/o cm+ye)							2.8	2.27	3.0
MM-6	Mn ⁺⁺ =1.0 mg/l (w/o cm+ye)							2.2	2.8	3.0
MM-7	Mg ⁺⁺ =370 mg/l (w/o cm+ye)							2.8	2.1	3.0
MM-8	Mg ⁺⁺ =185 mg/l (w/o cm+ye)							2.2	2.4	3.5
MM-9	Mg ⁺⁺ =740 mg/l (w/o cm+ye)							2.8	2.3	2.5

* = Discontinued
 ** = Re-initiated
 *** = Initiated
 cm = coconut milk
 ye = yeast extract
 MM = MOD. MUK.

EVALUATIONS (continued)

Name of media	Test Items	1970										1971										1972	
		12/16	1/12	2/12	3/10	4/13	5/2	5/25	6/25	7/27	12/16	1/12	2/12	3/10	4/13	5/2	5/25	6/25	7/27				
MM-10	Na ₂ EDTA=37.3 mg/l (w/o cm+ye)	----	----	----	----	----	----	----	2.75	3.0													
MM-11	Na ₂ EDTA=18.65 mg/l (w/o cm+ye)	----	----	----	----	----	----	----	3.0	2.7													
MM-12	CaCl ₂ ·2H ₂ O=440 mg/l (w/o cm+ye)	----	----	----	----	----	----	----	2.75	2.5													
MM-13	CaCl ₂ ·2H ₂ O=220 mg/l (w/o cm +ye)	----	----	----	----	----	----	----	2.43	2.0													
MM-14	CaCl ₂ ·2H ₂ O=880 mg/l (w/o cm+ye)	----	----	----	----	----	----	----	2.6	3.0													
MM-15	KH ₂ PO ₄ =340 mg/l (w/o cm+ye)	----	----	----	----	----	----	----	2.5	2.7													
MM-16	KH ₂ PO ₄ =85 mg/l (w/o cm+ye)	----	----	----	----	----	----	----	1.25	1.6													
MM-17	KH ₂ PO ₄ =170 mg/l (w/o cm+ye)	----	----	----	----	----	----	----	2.4	2.7													
MM-18	FeSO ₄ - media would not make	----	----	----	----	----	----	----	----	----													
MM-19	MM w/o kinetin	----	----	----	----	----	----	----	2.33	3.0													
MM-20	MM w/asparagine=25 mg/l	----	----	----	----	----	----	----	2.6	3.0													
MM-21	MM w/o asparagine	----	----	----	----	----	----	----	3.0	2.0													
MM-22	MM w/sucrose=5g/l	----	----	----	----	----	----	----	2.0	1.3													
MM-23	MM-1 w/o minor elements	----	----	----	----	----	----	----	3.0	2.0													
MM-24	MM- w/o minor elements	----	----	----	----	----	----	----	3.0	2.2													
MM-25	MM major + minor salts only	----	----	----	----	----	----	----	2.0	1.0													

EVALUATIONS (continued)

Name of Media	Test Items	1970 12/16	1971 1/12	1971 2/12	1971 3/10	1971 4/13	1971 5/2	1971 5/25	1971 6/25	1971 7/27
MM-26 MM w/Ascorbic acid=100 mg/l		----	----	----	----	----	----	----	2.6	2.2
MM-27 MM w/Ascorbic acid= 25 mg/l		----	----	----	----	----	----	----	2.8	2.7
MM-28 MM w/o Ascorbic		----	----	----	----	----	----	----	3.0	3.2
MM/me malt extract		----	----	----	----	----	----	3.6	3.0	2.0
MM/me/cm malt extract + C.M.		----	----	----	----	----	----	3.8	3.0	3.2
MM/pine ex. pine extract		----	----	----	----	----	----	----	3.41	2.2
MM/wood ex. wood extract		----	----	----	----	----	----	----	3.5	2.8
HELLER'S/wood ex. wood extract		----	----	----	----	----	----	----	1.2	1.0

MODIFIED MURASHIGE AND SKOOG MEDIUM (MM-1)

TISSUES CULTURED ON MEDIUM: PINUS palustrisPINUS lambertianaPINUS elliottii

pH 5.7-5.8

		Stock Solution, gm/liter	Stock solution, ml/liter of medium	Final con- centration mg/liter
<u>Major Solution</u>				
Solution	Component			
(1)	NH ₄ NO ₃	16.5		1650.0
	KNO ₃	19.0	100.0	1900.0
	CaCl ₂ ·2H ₂ O	4.4		440.0
	MgSO ₄ ·7H ₂ O	3.7		370.0
	KH ₂ PO ₄	1.7		170.0
(2)	Na ₂ -EDTA	7.45	5	37.3
	FeSO ₄ ·7H ₂ O	5.57		27.8
<u>Minor Solution</u>		Stock Solution, gm/liter	Stock solution, ml/liter of medium	Final con- centration mg/liter
Solution	Component			
(3)	HBO ₃	6.2		6.2
	MnSO ₄ ·4H ₂ O	22.3		22.3
	ZnSO ₄ ·4H ₂ O	8.6	1.0	8.6
	KI	0.83		0.83
	Na ₂ MoO ₃ ·2H ₂ O	0.25		0.25
	CuSO ₄ ·5H ₂ O	0.025		0.025
	CoCl ₂ ·6H ₂ O	0.025		0.025

MODIFIED MURASHIGE AND SKOOG MEDIUM (MM-1) (continued)

Vitamins and Amino acids		Stock Solution, gm/liter	Stock Solution, ml/liter of medium	Final con- centration mg/liter
Solution Component				
(4)	Nicotinic acid	0.5		0.5
	Thiamine HCl	0.1	1.0	0.1
	Pyridoxine HCl	0.1		0.1
	Kinetin	0.5		0.5
(5)	2,4-D	5	1.0	5.0
(6)	Inositol	100	1.0	100.0
(7)	Asparagine	100	1.0	100.0
(8)	Ascorbic acid	50	1.0	50.0
(9)	Sucrose	30		30
(10)	Coconut milk	1	100	100
(11)	Yeast extract	1		10
(12)	Agar	10		1.00

Report of Work Performed

on

N.A.S.A. Contract NAS 9-10947

entitled

DEVELOPMENT OF GERM-FREE PLANTS AND TISSUE CULTURES

for the period of

September 1, 1971 - November 30, 1971

S. Venkateswaran

For Apollo 13, the University of Houston took full responsibility at N.A.S.A. of the number and condition of the plant tissue cultures required, as well as the entry of the cultures and necessary supplies into the cabinet lines and the maintenance of the germ-free cabinet lines during this period. This entailed utilization of paraformaldehyde gas for decontamination purposes, after which airwashes and wiping down of the cabinets and culture jars were necessary. For protective reasons, all cultures and media had to be sealed tightly with the Mason jar lids prior to running a decontamination of the cabinets.

The responsibilities also extended to the challenging of all mission cultures with the lunar, simulated lunar, and earth soils. Each culture was numerically labelled for future data purposes. A table with the information on plant species, treatments, and numbers used is included in this report.

During this report period, studies were started on inducing differentiation in Pinus elliotii callus tissue. This involved varying ratios of kinetin to indolactic acid and kinetin to naphthalene acetic acid - used modified Murashige and Skoog medium as a basal medium. These experiments are still in progress. A table of the variations is included in this report.

Also, studies on corn are continued from previous work. Media variations are listed in this report, and are modification of White's basal medium. In order to induce corn callus, fresh corn root explants were placed on all these modified White media and also on MM-1 media. For statistical reasons, these experiments are repeats of those done and reported at an earlier date, in which success was accomplished on MM-1 media only.

Shipments

<u>Date</u>	<u>Cultures</u>	<u>Media</u>	<u>Number taken</u>	<u>Purpose</u>	<u>Last Trans. Date</u>
September 1, 1971	Hab. tob.	10XW	25	Research	8/10
	Hab. tob.	10XW	25	Research	8/26
September 6, 1971		10XW	60	Mission	
		MM-1	60	Mission	
		SV	60	Mission	
September 8, 1971		SV	60	Mission	
September 20, 1971	Hab. tob.	10XW	25	Research	9/9
	Hab. tob.	10XW	25	Research	9/16
October 14, 1971		MM-1	25	Research	
		MM	25	Research	
October 28, 1971		SV	50	Mission	
October 29, 1971		SV	50	Mission	
November 3, 1971		4 books of polypropylene for mission			
November 29, 1971		MM-1	10	Research	
		SV	30	Research	

Mission Challenges

Arrangement in cabinet line	Species	Treatments and Numbers Treated							
		New Subcultures				Old Cultures			
		(0-2 wks old)				(2 wks or older)			
<u>Bio. Inc. #7</u>		<u>SLS</u>	<u>I</u>	<u>L</u>	<u>C</u>	<u>SLS</u>	<u>I</u>	<u>L</u>	<u>C</u>
Shelf #1-Soybean		5	5	5	5	5	5	5	5
	#2-Hab. tob.	5	5	5	5	5	5	5	5
	#3-Pine e.	5	5	5	5	5	5	5	5
	#4-Carrot	Not used for mission							
<u>Bio. Inc. #6</u>									
Shelf #1-Corn		5	5	5	5	5	5	5	5
	#2-Sun.	5	5	5	5	5	5	5	5
	#3-Carrot	--	--	--	--	10	10	10	10
	#4-Rice	--	--	--	--	5	5	5	5
<u>Bio. Inc. #5</u>									
Shelf #1-L.cer		5	5	5	5	5	5	5	5
	March.	5	5	5	5	5	5	5	5
	#2-PTA	--	--	--	--	10	10	10	10

Bio. Inc. = Biological Incubator

SLS = Simulated lunar soil

I = Iowa soil (earth)

L = Lunar soil

C = Control (no treatment)

WHITE'S 1X BASAL MEDIUM

pH 6.1-7.0

<u>MACROELEMENTS</u>	<u>STOCK SOLN.</u> <u>gm/liter</u>	<u>STOCK SOLN.</u> <u>ml/liter</u>	<u>FINAL CONC.</u> <u>mg/liter</u>
1) $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	6.0		300.0
Na_2SO_4	4.0		200.0
KNO_3	1.6	50.0	80.0
KCL	1.3		65.0
$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$	0.33		16.5
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	14.4		720.0
<u>MICROELEMENTS</u>			
2) $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	3.0		3.0
$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	7.0		7.0
H_3BO_3	1.5	1.0	1.5
KI	0.75		0.75
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.001		0.001
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.25		0.25
3) FeCl_3	1.5	1.0	1.5
<u>VITAMINS AND AMINO ACIDS</u>			
4) Glycine	3.0		3.0
Thiamine HCL	0.5	1.0	0.5
Nicotinic Acid	0.5		0.5
Pyridoxine	0.5		0.5
5) Sucrose		20 gm	20
6) Agar		10 gm	10

WHITE'S LX - MODIFICATIONS

<u>MODIFICATION</u>	<u>STOCK SOLN.</u> <u>gm/liter</u>	<u>STOCK SOLN.</u> <u>ml/liter</u>	<u>FINAL CONC.</u> <u>mg/liter</u>
<u>WLX - A</u>			
IAA	1.0	1.0	1.0
Coconut Milk		100.0	100
Yeast Extract		5 gm	0.50
2,4 D	1.0	1.0	1.0
HAA	1.0	1.0	1.0
<u>WLX - B</u>			
Basal Medium			
<u>WLX - C</u>			
Asparagine ($1.5 \times 10^{-2} M$)	1.98	1.0	1.98
<u>WLX - D</u>			
HAA	1.0	1.0	1.0
<u>WLX - E</u>			
Coconut Milk		100 ml	100
<u>WLX - F</u>			
HAA	1.0	1.0	1.0
Coconut Milk		100 ml	100
<u>WLX - G</u>			
DPU	1.0	1.0	1.0

WHITE'S 1X - MODIFICATIONS (continued)

<u>MODIFICATION</u>	<u>STOCK SOLN.</u> <u>gm/liter</u>	<u>STOCK SOLN.</u> <u>ml/liter</u>	<u>FINAL CONC.</u> <u>mg/liter</u>
<u>WLX - H</u>			
NAA	1.0	1.0	1.0
DPU	1.0	1.0	1.0
<u>WLX - I</u>			
Casein Hydro- lysate Broth	1.0	1.0	.10
<u>WLX - J</u>			
NAA	1.0	1.0	1.0
Casein Hydro- lysate Broth	1.0	1.0	1.0

MODIFIED MURASHIGE AND SKOOG MEDIUM (MM-1)

TISSUES CULTURED ON MEDIUM: PINUS palustris
PINUS lambertiana
PINUS elliottii

pH 5.7-5.8

Major Solution		Stock Solution, gm/liter	Stock solution, ml/liter of medium	Final concentration mg/liter
Solution	Component			
(1)	NH_4NO_3	16.5	100.0	1650.0
	KNO_3	19.0		1900.0
	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	4.4		440.0
	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	3.7		370.0
	KH_2PO_4	1.7		170.0
(2)	$\text{Na}_2\text{-EDTA}$	7.45	5	37.3
	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	5.57		27.8
Minor Solution		Stock Solution, gm/liter	Stock solution, ml/liter of medium	Final concentration mg/liter
Solution	Component			
(3)	HBO_3	6.2	1.0	6.2
	$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	22.3		22.3
	$\text{ZnSO}_4 \cdot 4\text{H}_2\text{O}$	8.6		8.6
	KI	0.83		0.83
	$\text{Na}_2\text{MoO}_3 \cdot 2\text{H}_2\text{O}$	0.25		0.25
	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.025		0.025
	$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.025		0.025

MODIFIED MURASHIGE AND SKOOG MEDIUM (M1-1) (continued)

<u>Vitamins and Amino acids</u>		<u>Stock Solution, gm/liter</u>	<u>Stock Solution, ml/liter of medium</u>	<u>Final con- centration mg/liter</u>
Solution Component				
(4)	Nicotinic acid	0.5		0.5
	Thiamine HCl	0.1	1.0	0.1
	Pyridoxine HCl	0.1		0.1
	Kinetin	0.5		0.5
(5)	2,4-D	5	1.0	5.0
(6)	Inositol	100	1.0	100.0
(7)	Asparagine	100	1.0	100.0
(8)	Ascorbic acid	50	1.0	50.0
(9)	Sucrose	30		30
(10)	Coconut milk	1	100	100
(11)	Yeast extract	1		10
(12)	Agar	10		1.00

MODIFIED MURASHIGE AND SKOOG MEDIUM (M-M medium)

Basal Medium

pH 5.7 - 5.8

	MAJOR SOLUTION	STOCK SOLN.	STOCK SOLN.	FINAL CONC.
		<u>gm/liter</u>	<u>ml/liter</u>	
1)	NH ₄ NO ₃	16.5		1650.0
	KNO ₃	19.0		1900.0
	CaCl ₂ ·2H ₂ O	4.4	100.0	440.0
	MgSO ₄ ·7H ₂ O	3.7		370.0
	KH ₂ PO ₄	1.7		170.0
2)	Na ₂ EDTA	3.725	10.0	37.3
	FeSO ₄ ·7H ₂ O	2.785		27.8
<u>MINOR SOLUTION</u>				
3)	HBO ₃	6.2		6.2
	MnSO ₄ ·7H ₂ O	22.3		22.3
	ZnSO ₄ ·4H ₂ O	8.6	1.0	8.6
	KI	0.83		0.83
	Na ₂ MoO ₄ ·2H ₂ O	0.25		0.25
	CuSO ₄ ·5H ₂ O	0.025		0.025
	CoCl ₂ ·6H ₂ O	0.025		0.025
<u>VITAMINS AND AMINO ACIDS</u>				
4)	Nicotinic acid	0.5		0.5
	Thiamine HCL	0.1	1.0	0.1
	Pyridoxine HCL	0.1		0.1
5)	2,4 D	5.0	1.0	5.0
6)	Inositol	10.0	10.0	100.0
7)	Asparagine	100.0	1.0	100.0
8)	Ascorbic acid	50.0	1.0	50.0
9)	Sucrose		30 gm	30
10)	Agar		10 gm	10

MODIFIED MURASHIGE AND SKOOG MEDIUM MODIFICATIONS

<u>MODIFICATION</u>	<u>STOCK SOLN.</u> <u>gm/liter</u>	<u>STOCK SOLN.</u> <u>ml/liter</u>	<u>FINAL CONC.</u> <u>mg/liter</u>
MM-A			
Basal Medium			
MM-B			
Kinetin	0.5	1.0	0.5
MM-C			
IAA	0.18	1.0	0.18
MM-D			
IAA	3.0	1.0	3.0
MM-E			
Kinetin	1.0	1.0	1.0
IAA	0.3	1.0	0.3
MM-F			
Kinetin	1.0	1.0	1.0
IAA	.005	1.0	0.005
MM-G			
Basal Medium			
MM-H			
Kinetin	0.5	1.0	0.5

MODIFIED MURASHIGE AND SKOOG MEDIUM MODIFICATIONS

<u>MODIFICATION</u>	<u>STOCK SOLN.</u> <u>gm/liter</u>	<u>STOCK SOLN.</u> <u>ml/liter</u>	<u>FINAL CONC.</u> <u>mg/liter</u>
MM-L			
NAA	3.0	1.0	310
MM-J			
NAA	3.0	1.0	3.0
MI-K			
Kinetin	1.0	1.0	1.0
NAA	0.03	1.0	0.03
MM-L			
Kinetin	1.0	1.0	1.0
NAA	0.005	1.0	0.005

Callus pine tissue cultures have been found to contain high concentrations of phenolic compounds. These end products constitute the results of the biosynthetic pathways utilizing aromatic amino acids and their precursors. The observance of these intracellularly stored compounds during electronmicroscopic studies has stimulated the curiosity of N.A.S.A. workers as to the controlling steps in their biosynthesis. The following bibliography is the result of a partially completed literature search for recent information in this area.

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Report on Work Performed
on
N.A.S.A. Contract NAS 9-10947
entitled

DEVELOPMENT OF GERM-FREE PLANTS AND TISSUE CULTURES
for the period of
December 1, 1971 - February 29, 1972

July 31, 1972

S. Venkateswaran

During the months of December, 1971 through February, 1972, efforts were concentrated on continued maintenance of the Apollo 15 plant tissue cultures in the cabinet-lines at N.A.S.A. Most of the lunar treated cultures were transferred to second generation growth in order to check for long term effects. Non-treated cultures also were transferred to act as controls and to eventually expand the numbers of cultures on hand for preparation of Apollo 16 mission requirements.

The cabinet-lines were maintained sterile throughout the entire time span. Therefore, it was unnecessary to run para-formaldehyde decontaminations as prior to Apollo 14 in the summer of 1971. Swab tests were taken weekly to determine and confirm the sterility of the cabinet-lines.

The air-conditioning fan in the lower plant cabinet would periodically fail to work. Whenever this occurred, the temperature would soon rise forcing the cabinets to go on high-temperature, self-cut-off which included shut-off of the light system in that cabinet. In order to repair the fan, the sterility of the cabinet lines would have to have been sacrificed to allow someone to enter the cabinet in question. It was decided not to repair the fan, but to risk its continued performance. To relieve the pressure on the fan, the doors connecting each cabinet in the cabinet-line are now left open enabling more air-flow and some temperature control from adjacent cabinets. This would help keep the cabinet in question from going on high-temperature cut-off. There has been no reported trouble with the fan and/or cabinet since this time.

A list of the work performed at N.A.S.A. is included in the report along with a list of the shipments from the University to

N.A.S.A. Because the University of Houston was held responsible for photography of mission work, there is a separate list which gives the labelled numbers for all mission cultures categorized by the treatment and age of culture. Those marked with "p" are the cultures used for documentary photographing. Soybean, sunflower, pine, and tobacco were photographed in December 1, 1971 (p 1). The lower plants were photographed on December 20, 1971 (p 3). All other photographs were taken on December 14, 1971 (p 2).

Efforts were also directed toward the continuance of pine nutritional studies. Several experiments were started during the time period of this report. Only one was started and terminated by February. It is included in this report. All other experiments will be reported in the next quarterly report.

The "Pine Bud-Formation Experiment" was a preliminary effort to check the response of Pinus elliotti to attempts at inducing differentiated tissue. This was done by varying ratios of kinetin, IAA, and NAA. The media variations are listed in this report. Visual observation of the cultures showed differences only in growth and pigmentation. No indications of bud or root formations were detected. The results generally were as follows: (refer to media protocols)

<u>Media</u>	<u>Observation</u>
MM-G	Large growth; not as large as control; very little browning.
MM-H	Control: large growth; very little browning.
MM-I	Less growth than control; yellow to green callus; no browning.
MM-J	Similar to control with more browning.
MM-K	Large growth; more browning than MM-J.
MM-L	Little growth; very much browning.

All cultures, except controls, placed on MM-A media through MM-F died within three weeks. Attempts to salvage them on second generation transfers were in vain.

PINE BUD FORMATIVE EXPERIMENT

<u>MODIFICATION</u>	<u>STOCK SOLN.</u> <u>gm/liter</u>	<u>STOCK SOLN.</u> <u>ml/liter</u>	<u>FINAL CONC.</u> <u>mg/liter</u>
MM-A			
Basal Medium			
MM-B			
Kinetin	0.5	1.0	0.5
MM-C			
IAA	0.18	1.0	0.18
MM-D			
IAA	3.0	1.0	3.0
MM-E			
Kinetin	1.0	1.0	1.0
IAA	0.3	1.0	0.3
MM-F			
Kinetin	1.0	1.0	1.0
IAA	.005	1.0	0.005
MM-G			
Basal Medium			
MM-H			
Kinetin	0.5	1.0	0.5

PINE BUD FORMATIVE EXPERIMENT (continued)

<u>MODIFICATION</u>	<u>STOCK SOLN.</u> <u>gm/liter</u>	<u>STOCK SOLN.</u> <u>ml/liter</u>	<u>FINAL CONC.</u> <u>mg/liter</u>
MM-L			
NAA	3.0	1.0	310
MM-J			
NAA	3.0	1.0	3.0
MM-K			
Kinetin	1.0	1.0	1.0
NAA	0.03	1.0	0.03
MM-L			
Kinetin	1.0	1.0	1.0
NAA	0.005	1.0	0.005

MODIFIED MURASHIGE AND SKOOG MEDIUM (M-M medium)

Basal Medium

pH 5.7 - 5.8

MAJOR SOLUTION	STOCK SOLN. <u>gm/liter</u>	STOCK SOLN. <u>ml/liter</u>	FINAL CONC. <u>mg/liter</u>
1) NH_4NO_3	16.5		1650.0
KNO_3	19.0		1900.0
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	4.4	100.0	440.0
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	3.7		370.0
KH_2PO_4	1.7		170.0
2) Na_2EDTA	3.725	10.0	37.3
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	2.785		27.8
<u>MINOR SOLUTION</u>			
3) HBO_3	6.2		6.2
$\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$	22.3		22.3
$\text{ZnSO}_4 \cdot 4\text{H}_2\text{O}$	8.6	1.0	8.6
KI	0.83		0.83
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.25		0.25
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.025		0.025
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.025		0.025
<u>VITAMINS AND AMINO ACIDS</u>			
4) Nicotinic acid	0.5		0.5
Thiamine HCL	0.1	1.0	0.1
Pyridoxine HCL	0.1		0.1
5) 2,4 D	5.0	1.0	5.0
6) Inositol	10.0	10.0	100.0
7) Asparagine	100.0	1.0	100.0
8) Ascorbic acid	50.0	1.0	50.0
9) Sucrose		30 gm	3%
10) Agar		10 gm	1%

Bud Formation Experimental Media
(initiation of experiment on December 7, 1971)

MM-A	w/o Kinetin, w/o IAA (basal media)
MM-B	w/ Kinetin, w/o IAA control
MM-C	w/o Kinetin, w/ IAA (.18 mg/l)
MM-D	w/o Kinetin, w/ IAA (3.0 mg/l)
MM-E	w/ Kinetin, (1.0 mg/l), w/ IAA (.03 mg/l)
MM-F	w/ Kinetin, (1.0 mg/l), w/ IAA (.005 mg/l)
MM-G	w/o Kinetin, w/o NAA
MM-H	w/ Kinetin, w/o NAA control
MM-I	w/o Kinetin, w/ NAA (.18 mg/l)
MM-J	w/o Kinetin, w/NAA (3.0 mg/l)
MM-K	w/ Kinetin, (1.0 mg/l), w/ NAA (.03 mg/l)
MM-L	w/ Kinetin, (1.0 mg/l), w/ NAA (.005 mg/l)

Shipments

<u>Date</u>	<u>Cultures</u>	<u>Media</u>	<u>Number taken</u>	<u>Purpose</u>	<u>Last Trans. Date</u>
December 7, 1971	Hab. tob.	10X	100	Mission	12/9
	Soy	SV	100	Mission	
	Carrot	SV	50	Mission	
January 18, 1972	Marchantia	K	50	Mission	12/20
	L. Cer.	K	50	Mission	
	D. Carrot	SV	50	Mission	
	Soy	SV	50	Mission	12/21
	Hab. tob.	10X	50	Mission	
	Corn	MM-1	50	Mission	
	D. Carrot	SV	50	Mission	
February 9, 1972	Hab. tob	10X	100	Research	2/27
February 25 1972	Corn	MM_ 1	50	Mission	2/27

Work Performed at N.A.S.A.

<u>Date</u>	<u>Culture</u>	<u>Media</u>	<u>Number of Transfers</u>	<u>Type of Treatment</u>
12/ 9/71	Hab. tob.	10XW	40	Lunar, 2nd. generation
12/ 9/71	Hab. tob.	10XW	40	Controls
12/ 9/71	Soybean	SV	40	Lunar, 2nd. generation
12/ 9/71	Soybean	SV	40	Controls
12/ 9/71	D. Carrot	SV	40	Lunar, 2nd. generation
1/20/72	Marchantia	K	40	Control
1/20/72	L. Cer.	K	27	Control
1/20/72	D. Carrot	SV	15	*SLS
1/20/72	D. Carrot	SV	15	Iowa
1/20/72	D. Carrot	SV	20	Controls
1/21/72	Soybean	SV	40	Controls
1/21/72	Hab. tob.	10XW	40	Control
1/21/72	Corn	MM-1	40	Control
1/21/72	D. Carrot	SV	40	Control
2/27/72	Corn	MM-1	15	Lunar, 2nd. generation

*SLS = Simulated lunar soil.

Apollo 15

Plant Tissue Culture

NOTE: top = challenge material placed on top bottom = challenge material placed beneath of two week old culture. newly transferred culture.

Culture	LUNAR		SIMULATED LUNAR		IOWA		CONTROL	
	top	bottom	top	bottom	top	bottom	top	bottom
Soybean	51001	51006	51011	51016	51021 p-1-2	51026	51031 p-2	51036
	51002 p-2	51007	51012p-1-2	51017	51022	51027 p-1	51032	51037
	51003	51008 p-1	51013	51018	51023	51028 p-2	51033 p-1	51038
	51004	51009	51014	51019 p-1-2	51024	51029	51034	51039 p-1
	51005 p-1	51010 p-2	51015	51020	51025	51030	51035	51040
Sunflower	51041	51046 p-1	51051	51056	51061	51066	51071 p-1	51076
	51042	51047	51052 p-1	51057	51062 p-1	51067	51072	51077 p-1
	51043 p-1	51048	51053	51058	51063	51068	51073	51078
	51044	51049	51054	51059 p-1	51064	51069 p-1	51074	51079
	51045	51050	51054	51060	51065	51070	51075	51080
Pine	51081 p-1	51086	51091	51096	51101 p-1	51106	51111	51116
	51082	51087	51092	51097 p-1	51102	51107 p-1	51112 p-1	51117
	51083	51088	51093 p-1	51098	51103	51108	51113	51118 p-1
	51084	51089 p-1	51094	51099	51104	51109	51114	51119
	51085	51090	51095	51100	51105	51110	51115	51120

Apollo 15 (continued)

<u>Culture</u>	<u>LUNAR</u>		<u>SIMULATED LUNAR</u>		<u>IOWA</u>		<u>CONTROL</u>	
	<u>top</u>	<u>bottom</u>	<u>top</u>	<u>bottom</u>	<u>top</u>	<u>bottom</u>	<u>top</u>	<u>bottom</u>
Tobacco	51121	51126	51131	51136 p-1	51146	51141 p-1	51151	51156 p-1
	51122	51127	51132 p-1	51137	51147	51142	51152 p-1	51157
	51123 p-1	51128	51133	51138	51148	51143	51153	51158
	51124	51129	51134	51139	51149 p-1	51144	51154	51159
	51125	51130	51135	51140	51150	51145	51155	51160

D. carrot

51161	51171	51181	51191
51162	51172	51182	51192
51163	51173	51183	51193
51164	51174	51184	51194
51165	51175	51185	51195
51166	51176	51186	51196
51167	51177	51187	51197
51168	51178	51188	
51169	51179		

Apollo 15 (continued)

<u>Culture</u>	<u>LUNAR</u>		<u>SIMULATED LUNAR</u>		<u>IOWA</u>		<u>CONTROL</u>	
	<u>top</u>	<u>bottom</u>	<u>top</u>	<u>bottom</u>	<u>top</u>	<u>bottom</u>	<u>top</u>	<u>bottom</u>
<u>Rice</u>	51201		51206		51211		51216	
	51202		51207		51212		51217	
	51203		51208		51213		51218	
	51204		51209		51214		51219	
	51205		51210		51215		51220	
							51221	
							51222	

Apollo 15 (continued)

	LUNAR		SIMULATED LUNAR		IOWA		CONTROL	
	New-bottom	Top 2 wks	New-bottom	Top 2 wks	New-bottom	Top 2 wks	New-bottom	Top 2 wks
L.Cer.	51226	51231	51241	51236 p-3	51251	51246 p-3	51256 p-3	51261
	51227	51232 p-3	51242	51237	51252	51247	51257	51262
	51228	51234	51243	51238	51253	51248	51258	51263
	51229	51235	51244	51239	51254	51249	51259	51264
	51230		51245	51240	51255	51250	51260	51265

Marchantia

51266	51277	51287	51297
51267	51278	51288	51298
51268	51279	51289	51299 p-3
51269	51280	51290	51300
51270 p-3	51281	51291	51301
51272	51282 p-3	51292 p-3	51302
51273	51283	51293	51303
51274	51284	51294	513-4
51275	51285	51295	51305
51276	51286	51296	51306

Apollo 15 (continued)

<u>CULTURE</u>	<u>LUNAR</u>	<u>SIMULATED LUNAR</u>	<u>IOWA</u>	<u>CONTROL</u>
<u>PTA</u>				
	51311	51321	51331	51341
	51312	51322	51332	51342
	51313	51323	51333	51343
	51314	51324	51334	51344
	51315	51325	51335	51345
	51316	51326	51336	51346
	51317	51327	51337	51347
	51318	51328	51338	51348
	51319	51329	51339	51349
	51320	51330	51340	51350

Apollo 15 (continued)
CULTURE Corn (all top treated)

LUNAR			SIMULATED LUNAR			IOWA		CONTROL	
New Initiates	2nd Generation		New Initiates	2nd generation		New Initiates	2nd Generation	New Initiates	2nd Generation
51352	51351		51361	51364		51371	51373	51381	51386
51355	51353		51362	51366		51372	51374	51382	51388
51356	51354		51363	51367		51375	51379	51383	51390
51357			51365	51368		51376		51384	
51358			51369	51370		51377		51385	
51359						51378		51387	
51360						51380		51389	

Report on Work Performed
on
N.A.S.A. Contract NAS 9-10947

entitled

DEVELOPMENT OF GERM-FREE PLANTS AND TISSUE CULTURES
for the period of

March 1, 1972 - May 31, 1972

July 31, 1972

S. Venketeswaran

From March, 1972, both mission preparatory work and pine nutritional work expanded.

Lists of (1) shipments from the University of Houston to N.A.S.A., (2) work performed on maintenance of plant tissue culture in the LRL cabinet lines, and (3) plant tissue cultures required for Apollo 16 are included in this report.

Also, media protocols for two pine growth studies are included. Fresh weight and dry weight measurements were recorded periodically for each media listed. The results are tabulated and plotted. An interest in nitrogen metabolism led to the media modification used. Presently, protein and growth measurements are being made on pine cultures grown on these media. When the entire series of experiments is completed, conclusions will be made and a discussion reported. Meanwhile, only the data obtained up to this time is included in this report.

Abbreviations

I. Media: (protocols are included in report)

MM	=	Modified Murashige and Skoog (MOD MUK)
SIK	=	SV + IAA + Kinetin
SV	=	Modified Bonner
K	=	Knudson
10XW	=	10 X WHITE
1XW	=	1 X WHITE
Hoag	=	HOAGLAND'S
H	=	HELLER'S
V	=	VOTH
MIK	=	Modified MUK + IAA + Kinetin
SAB	=	Sabouraud broth
thio	=	Thioglycolate broth

II. Cultures:

PTA	=	<u>Pteridium aqualium</u>
TOB	=	<u>Todea barbara</u>
LCer	=	<u>Lycopodium cernum</u>
March	=	<u>Marchantia polymorpha</u>
Pine L.	=	<u>Pinus lambertiana</u>
Pine P.	=	<u>Pinus palustris</u>
Pine E.	=	<u>Pinus elliottii</u>
D.C.	=	<u>Daucus carota</u> (wild carrot)
Sun.	=	Sunflower = <u>Helianthus annus</u>
Soy	=	Soybean = <u>Glycine soja</u>
Hap	=	<u>Haplopappus gracilis</u>
Hab. tob	=	Habituated tobacco
tera tob	=	Teratoma tobacco

Shipments

<u>Date</u>	<u>Cultures</u>	<u>Media</u>	<u>Number taken</u>	<u>Purpose</u>	<u>Last Trans. Date</u>
March 7 1972	Corn	MM-1	50	Mission	3/ 9
March 16 1972		K	40	Research	
April 25 1972	Carrot	SV	40	Mission	4/27
	Corn	MM-1	40	Mission	

Work Performed at N.A.S.A.

<u>Date</u>	<u>Culture</u>	<u>Media</u>	<u>Number of Transfers</u>	<u>Type of Treatment</u>
3/ 7/72	Corn	MM-1	26	Control, 2nd. generation
4/14/72	D. Carrot	SV	35	Controls
4/14/72	Corn	MM-1	14	Controls
4/17/72	Marchantia	K	40	Controls
4/17/72	PTA	K	40	Controls

Apollo 16

Plant Culture Requirement

<u>Culture</u>	<u>Media</u>	<u>Number</u>
L. Cer	K	40
Marchianta	K	40
PtA	K	40
D. Carrot	SV	40
Soybean	SV	40
Sunflower	SV	40
Tobacco	10XW	40
Rice	HELLER's	40
Slash Pine	ME1	40
Corn	MM-1	40

Pine Growth Study Experiment #1

MME - Experiments: December 2, 1971 (initiation of experiment)
January 31, 1972 (subculture, start of
2nd. generation)
March 7, 1972 (termination of experiment)

MME--1	Increase thiamine HCL to 5 g/l
MM--2	Leave out nicotinic acid and pyridoxal HCL
MM--3	Increase thiamine HCL to 5 g/l Leave out nicotinic acid and pyridoxal HCL
MME--4	Change NH_4NO_3 to NH_4PO_4 (11.9 g/l) Add NH_4 citrate diam. salt 11.4 g/l
MME--5	Change KNO_3 to K_2SO_4 (8.1 g/l) Increase KH_2PO_4 monobasic (12.7 g/l)
MME--6	Change NH_4NO_3 to NH_4PO_4 (11.9 g/l) Increase KH_2PO_4 monobasic (12.7 g/l) Add NH_4 citrate diam. salt (11.4 g/l) Change KNO_3 to K_2SO_4 (8.1 g/l)
MME--control	MM Basal media

Pine Growth Study Experiment #2

MME Experiments: February 22, 1972 (initiation of experiment)

March 29, 1972 (subculture; start of
2nd. generation)

May 10, 1972 (termination of experiment)

MME-A	Only ammonium	
	Change NH_4NO_3 to NH_4PO_4	11.9 g/l
	Add NH_4 citrate diammonium salt	11.4 g/l
	Change KNO_3 to K_2SO_4	8.1 g/l
	Add KH_2PO_4 (monobasic)	12.7 g/l
	Omit asparagine	
MME-B	Ammonium and NO_3	
	Change NH_4NO_3 to NH_4PO_4	11.9 g/l
	Add NH_4 citrate diammonium salt	11.4 g/l
	Omit asparagine	
MME-G	Ammonium and asparagine	
	Change NH_4NO_3 to NH_4PO_4	11.9 g/l
	Add NH_4 citrate diammonium salt	11.4 g/l
	Change KNO_3 to K_2SO_4	8.1 g/l
	Add KH_2PO_4 (monobasic)	12.7 g/l
	Keep asparagine	
MME-D	NO_3 only	
	Omit NH_4NO_3	
	Omit asparagine	
MME-E	NO_3 only	
	Omit NH_4NO_3	
	Omit asparagine	
	Double concentration of KNO_3	

Media
Pine Growth Study Experiment #2 (continued)

MME-F NO₃ and asparagine
 Omit NH₄NO₃
 Keep asparagine
 Keep KNO₃

MME-G NO₃ and asparagine
 Omit NH₄NO₃
 Keep asparagine
 Double concentration of KNO₃

MME-H Asparagine only
 Omit NH₄NO₃
 Change KNO₃ to K₂SO₄ 8.1 g/l
 Add KH₂PO₄ (monobasic) 12.7 g/l
 Keep asparagine

MME-I Control
 MM basal media (keep NH₄NO₃, KNO₃
 and asparagine)

NOTE: pH adjustment with mono-and dibasic PO₄ salts
 purified agar used
 2 gram inoculums
 15 cultures started for each type
 2 cultures sacrificed at each weighing date

MODIFIED MURASHIGE AND SKOOG MEDIUM (M-M medium)

Basal Medium

pH 5.7 - 5.8

<u>MAJOR SOLUTION</u>		<u>STOCK SOLN.</u> <u>g/m liter</u>	<u>STOCK SOLN.</u> <u>ml/liter</u>	<u>FINAL CONC.</u> <u>mg/liter</u>
1)	NH ₄ NO ₃	16.5		1650.0
	KNO ₃	19.0		1900.0
	CaCl ₂ ·2H ₂ O	4.4	100.0	440.0
	MgSO ₄ ·7H ₂ O	3.7		370.0
	KH ₂ PO ₄	1.7		170.0
2)	Na ₂ EDTA	3.725	10.0	37.3
	FeSO ₄ ·7H ₂ O	2.785		27.8
<u>MINOR SOLUTION</u>		<u>STOCK SOLN.</u> <u>gm/liter</u>	<u>STOCK SOLN.</u> <u>ml/liter</u>	<u>FINAL CONC.</u> <u>mg/liter</u>
3)	HBO ₃	6.2		6.2
	MnSO ₄ ·7H ₂ O	22.3		22.3
	ZnSO ₄ ·4H ₂ O	8.6	1.0	8.6
	KI	0.83		0.83
	Na ₂ MoO ₄ ·2H ₂ O	0.25		0.25
	CuSO ₄ ·5H ₂ O	0.025		0.025
	CoCl ₂ ·6H ₂ O	0.025		0.025
<u>VITAMINS AND</u> <u>AMINO ACIDS</u>				
4)	Nicotinic acid	0.5		0.5
	Thiamine HCL	0.1	1.0	0.1
	Pyridoxine HCL	0.1		0.1
5)	2,4 D	5.0	1.0	5.0
6)	Inositol	10.0	10.0	100.0
7)	Asparagine	100.0	1.0	100.0
8)	Ascorbic acid	50.0	1.0	50.0
9)	Sucrose		30 gm	3%
10)	Agar		10 gm	1%

MODIFIED MURASHIGE AND SKOOG MEDIUM (MM-1)

TISSUES CULTURED ON MEDIUM: PINUS palustrisPINUS lambertiPINUS elliottii

pH 5.7-5.8

<u>Major Solution</u>		<u>Stock Solution, gm/liter</u>	<u>Stock solution, ml/liter of medium</u>	<u>Final concentration mg/liter</u>
Solution Component				
(1)	NH_4NO_3	16.5		1650.0
	KNO_3	19.0	100.0	1900.0
	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	4.4		440.0
	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	3.7		370.0
	KH_2PO_4	1.7		170.0
(2)	$\text{Na}_2\text{-EDTA}$	7.45	5	37.3
	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	5.57		27.8

<u>Minor Solution</u>		<u>Stock Solution, gm/liter</u>	<u>Stock solution, ml/liter of medium</u>	<u>Final concentration mg/liter</u>
Solution Component				
(3)	HBO_3	6.2		6.2
	$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	22.3		22.3
	$\text{ZnSO}_4 \cdot 4\text{H}_2\text{O}$	8.6	1.0	8.6
	KI	0.83		0.83
	$\text{Na}_2\text{MoO}_3 \cdot 2\text{H}_2\text{O}$	0.25		0.25
	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.025		0.025
	$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.025		0.025

MODIFIED MURASHIGE AND SKOOG MEDIUM (MM-1) (continued)

Solution Component	<u>Vitamins and Amino acids</u>	<u>Stock Solution, gm/liter</u>	<u>Stock Solution, ml/liter of medium</u>	<u>Final con- centration mg/liter</u>
(4) Nicotinic acid		0.5		0.5
Thiamine HCl		0.1	1.0	0.1
Pyridoxine HCl		0.1		0.1
Kinetin		0.5		0.5
(5) 2,4-D		5	1.0	5.0
(6) Inositol		100	1.0	100.0
(7) Asparagine		100	1.0	100.0
(8) Ascorbic acid		50	1.0	50.0
(9) Sucrose		30		3%
(10) Coconut milk		1	100	10%
(11) Yeast extract		1		1%
(12) Agar		10		1.0%

Data
Pine Growth Experiments #1 & 2

NOTE: recorded in grams

<u>Weighing date</u>	<u>Media</u>	<u>No.</u>	<u>Fresh Weight</u>	<u>Average Fresh Weight</u>	<u>Dry Weight</u>	<u>Average Dry Weight</u>
12/10/71	MME-1	1	5.0		.22	
12/10/71	MME-1	2	5.6	5.3	.29	.255
12/10/71	MME-2	1	4.7		.28	
12/10/71	MME-2	2	4.0	4.3	.24	.26
12/10/71	MME-3	1	3.1		.20	
12/10/71	MME-3	2	4.0	3.55	.26	.23
12/17/71	MME-1	1	6.1		.25	
12/17/71	MME-1	2	7.2	6.65		.295
12/17/71	MME-2	1	4.0		.29	
12/17/71	MME-2	2	5.3	4.65	.20	.245
12/17/71	MME-3	1	3.0		.19	
12/17/71	MME-3	2	4.0	3.5	.21	.20
12/28/71	MME-1	1	7.7		.23	
12/28/71	MME-1	2	6.6	7.15	.33	.28
12/28/71	MME-2	1	10.3		.26	
12/28/71	MME-2	2	5.1	7.7	.27	.265
12/28/71	MME-3	1	7.0		.36	
12/28/71	MME-3	2	6.8	6.9	.33	.345
12/28/71	control	1	2.3		.15	
12/28/71	control	2	5.6	3.95	.18	.165
12/28/71	MME-4	1	2.3		.20	
12/28/71	MME-4	2	4.5	3.4	.16	.18
12/28/71	MME-5	1	4.1		.21	
12/28/71	MME-5	2	3.5	3.8	.31	.26

Data (continued)

<u>Weighing date</u>	<u>Media</u>	<u>No.</u>	<u>Fresh Weight</u>	<u>Average Fresh Weight</u>	<u>Dry Weight</u>	<u>Average Dry Weight</u>
12/28/71	MME-6	1	3.7	3.25	.17	.195
12/28/71	MME-6	2	2.8		.22	
1/ 3/72	MME-1	1	7.7	7.15	.32	.325
1/ 3/72	MME-1	2	6.6		.33	
1/ 3/72	MME-2	1	8.7	9.55	.33	.335
1/ 3/72	MME-2	2	10.4		.34	
1/ 3/72	MME-3	1	7.0	7.95	.38	.34
1/ 3/72	MME-3	2	8.9		.30	
1/ 3/72	MME-4	1	5.6	4.75	.26	.265
1/ 3/72	MME-4	2	3.9		.27	
1/ 3/72	MME-5	1	2.8	3.2	.21	.225
1/ 3/72	MME-5	2	3.6		.24	
1/ 3/72	MME-6	1	3.6	3.5	.21	.23
1/ 3/72	MME-6	2	3.4		.25	
1/10/72	MME-1	1	10.3	7.7	.34	.29
1/10/72	MME-1	2	5.1		.24	
1/10/72	MME-2	1	6.3	6.95	.32	.315
1/10/72	MME-2	2	7.6		.31	
1/10/72	MME-3	1	6.9	6.7	.29	.275
1/10/72	MME-3	2	6.5		.26	
1/10/72	control	1	6.4	5.55	.27	.25
1/10/72	control	2	4.7		.23	
1/10/72	MME-4	1	3.4	2.9	.23	.205
1/10/72	MME-4	2	2.4		.18	
1/10/72	MME-5	1	3.2	5.45	.44	.33
1/10/72	MME-5	2	7.7		.22	

Data (continued)

<u>weighing date</u>	<u>Media</u>	<u>No.</u>	<u>Fresh Weight</u>	<u>Average Fresh Weight</u>	<u>Dry Weight</u>	<u>Average Dry Weight</u>
1/10/72	MME-6	1	5.0	4.65	.28	.295
1/10/72	MME-6	2	4.3		.31	
1/18/72	MME-1	1	7.0	6.9	.31	.31
1/18/72	MME-1	2	6.8		---	
1/18/72	MME-2	1	9.4	7.15	.32	.30
1/18/72	MME-2	2	4.9		.28	
1/18/72	MME-3	1	8.0	8.35	.35	.33
1/18/72	MME-3	2	8.7		.31	
1/18/72	MME-4	1	3.2	3.45	.21	.23
1/18/72	MME-4	2	3.7		.25	
1/18/72	MME-5	1	7.5	6.65	.35	.395
1/18/72	MME-5	2	5.8		.44	
1/18/72	MME-6	1	4.2	3.5	.29	.28
1/18/72	MME-6	2	2.8		.27	
1/25/72	MME-4	1	9.1	6.05	.29	.30
1/25/72	MME-4	2	3.0		.31	
1/25/72	MME-5	1	8.4	7.05	.45	.33
1/25/72	MME-5	2	5.7		.21	
1/25/72	MME-6	1	3.1	3.55	.31	.31
1/25/72	MME-6	2	4.0		---	

Data (continued)

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<u>Weighing date</u>	<u>Media</u>	<u>No.</u>	<u>Fresh Weight</u>	<u>Average Fresh Weight</u>	<u>Dry Weight</u>	<u>Average Dry Weight</u>
2/15	MME-1	1	1.5	2.0	.12	.145
2/15	MME-1	2	2.5		.17	
2/15	MME-2	1	1.8	1.9	.12	.115
2/15	MME-2	2	2.0		.11	
2/15	MME-3	1	2.8	3.1	.19	.215
2/15	MME-3	2	3.4		.24	
2/15	MME-4	1	1.5	1.45	.12	.115
2/15	MME-4	2	1.4		.11	
2/15	MME-5	1	1.0	1.1	.08	.08
2/15	MME-5	2	1.2		.08	
2/15	MME-6	1	2.4	2.05	.17	.145
2/15	MME-6	2	1.7		.12	
2/15	MME-control	1	5.3	4.4	.34	.305
2/15	MME-control	2	3.5		.27	
2/22	MME-1	1	3.8	3.45	.23	.215
2/22	MME-1	2	3.1		.20	
2/22	MME-2	1	2.1	2.15	.15	.145
2/22	MME-2	2	2.2		.14	
2/22	MME-3	1	4.9	5.45	.25	.27
2/22	MME-3	2	6.0		.29	
2/22	MME-4	1	3.0	2.55	.21	.195
2/22	MME-4	2	2.1		.18	

Data (continued)

<u>Weighing date</u>	<u>Media</u>	<u>No.</u>	<u>Fresh Weight</u>	<u>Average Fresh Weight</u>	<u>Dry Weight</u>	<u>Average Dry Weight</u>
2/22	MME-1	1	1.8	1.75	.14	.115
2/22	MME-5	2	1.7		.09	
2/22	MME-6	1	1.2	1.6	.14	.15
2/22	MME-6	2	2.0		.16	
2/22	MME-control	1	10.8	8.4	.43	.385
2/22	MME-control	2	6.0		.34	
2/29	MME-1	1	1.3	2.50	.11	.17
2/29	MME-1	2	3.7		.23	
2/29	MME-2	1	1.4	1.85	.11	.14
2/29	MME-2	2	2.3		.17	
2/29	MME-3	1	4.8	6.35	.22	2.5
2/29	MME-3	2	7.9		.28	
2/29	MME-4	1	1.0	1.2	.10	.11
2/29	MME-4	2	1.4		.12	
2/29	MME-5	1	1.1	1.1	.09	.085
2/29	MME-5	2	1.1		.08	
2/29	MME-6	1	1.3	2.15	.14	.185
2/29	MME-6	2	3.0		.23	
2/29	MME-control	1	3.2	3.1	.25	.265
2/29	MME-control	2	3.0		.28	
3/ 3	MME-A	1	3.1	3.15	.21	.22
3/ 3	MME-A	2	3.2		.23	

Data (continued)

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<u>Weighing date</u>	<u>Media</u>	<u>No.</u>	<u>Fresh Weight</u>	<u>Average Fresh Weight</u>	<u>Dry Weight</u>	<u>Average Dry Weight</u>
3/ 3	MME-B	1	2.8	3.05	.17	.185
3/ 3	MME-B	2	3.3		.20	
3/ 3	MME-C	1	4.0	3.75	.21	.205
3/ 3	MME-C	2	3.5		.20	
3/ 3	MME-D	1	3.0	3.55	.19	.205
3/ 3	MME-D	2	4.1		.22	
3/ 3	MME-E	1	2.1	2.55	.14	.165
3/ 3	MME-E	2	3.0		.19	
3/ 3	MME-F	1	4.0	3.55	.21	.195
3/ 3	MME-F	2	3.1		.18	
3/ 3	MME-G	1	2.7	2.7	.17	.17
3/ 3	MME-G	2	2.7		.17	
3/ 3	MME-H	1	2.9	2.7	.18	.18
3/ 3	MME-H	2	2.5		.18	
3/ 3	MME-I	1	3.6	3.4	.21	.215
3/ 3	MME-I	2	3.2		.22	
3/ 7	MME-1		6.9		.36	
3/ 7	MME-2		3.8		.25	
3/ 7	MME-3		8.7		.34	
3/ 7	MME-4		1.3		.10	
3. 7	MME-5		2.0		.15	
3/ 7	MME-6		4.0		.29	
3/ 7	MME-control		7.1		.40	

(continued)

<u>hing</u> <u>ite</u>	<u>Media</u>	<u>No.</u>	<u>Fresh</u> <u>Weight</u>	<u>Average</u> <u>Fresh</u> <u>Weight</u>	<u>Dry</u> <u>Weight</u>	<u>Average</u> <u>Dry</u> <u>Weight</u>
/13	MME-A	1	3.0		.20	
				4.05		.27
3/13	MME-A	2	5.1		.34	
3/13	MME-B	1	3.9		.24	
				4.95		.275
3/13	MME-B	2	6.0		.31	
3/13	MME-C	1	5.8		.36	
				5.2		3.45
3/13	MME-C	2	4.6		.33	
3/13	MME-D	1	4.9		.26	
				5.25		.29
3/13	MME-D	2	5.6		.32	
3/13	MME-E	1	4.1		.29	
				4.3		2.75
3/13	MME-E	2	4.5		.26	
3/13	MME-F	1	6.0		.35	
				6.1		.345
3/13	MME-F	2	6.2		.34	
3/13	MME-G	1	4.8		.25	
				5.25		.285
3/13	MME-G	2	5.7		.32	
3/13	MME-H	1	4.8		.29	
				4.2		.27
3/13	MME-H	2	3.6		.25	
3/13	MME-I	1	3.7		.21	
				3.9		.22
3/13	MME-I	2	4.1		.23	

Data (continued)

<u>Weighing date</u>	<u>Media</u>	<u>No.</u>	<u>Fresh Weight</u>	<u>Average Fresh Weight</u>	<u>Dry Weight</u>	<u>Average Dry Weight</u>
3/23	MME-A	1	7.4	7.25	.44	.42
3/23	MME-A	2	7.1		.40	
3/23	MME-B	2	7.8	6.85	.39	.37
3/23	MME-B	1	5.9		.35	
3/23	MME-C	2	5.7	6.95	.39	.425
3/23	MME-C	1	8.2		.46	
3/23	MME-D	1	7.1	8.95	.35	.41
3/23	MME-D	2	10.8		.47	
3/23	MME-E	1	5.3	5.65	.31	.325
3/23	MME-E	2	6.0		.34	
3/23	MME-F	1	8.9	9.55	.73	.595
3/23	MME-F	2	10.2		.46	
3/23	MME-G	1	6.5	6.3	.38	.37
3/23	MME-G	2	6.1		.36	
3/23	MME-H	1	4.9	4.75	.36	.32
3/23	MME-H	2	4.6		.28	
3/23	MME-I	1	10.7	9.5	.45	.415
3/23	MME-I	2	8.3		.38	
4/ 9	MME-A	1	2.3	2.6	.21	.23
4/ 9	MME-A	2	2.9		.25	
4/ 9	MME-B	1	2.0	2.7	.16	.25
4/ 9	MME-C	1	3.0		.28	
4/ 9	MME-C	2	2.4	2.25	.22	.195
4/ 9	MME-D	1	2.4		.20	
4/ 9	MME-D	2	2.1		.19	

Data (continued)

<u>Weighing date</u>	<u>Media</u>	<u>No.</u>	<u>Fresh Weight</u>	<u>Average Fresh Weight</u>	<u>Dry Weight</u>	<u>Average Dry Weight</u>
4/ 9	MME-E	1	2.3	2.15	.20	.215
4/ 9	MME-E	2	2.0		.23	
4/11	MME-F	1	3.0	2.25	.23	.23
4/11	MME-G	1	2.0		.17	.185
4/11	MME-G	2	2.5		.20	
4/11	MME-H	1	2.3	2.15	.21	.195
4/11	MME-H	2	2.0		.18	
4/11	MME-I	1	2.8	2.9	.17	.175
4/11	MME-I	2	3.0		.18	
4/24	MME-A	1	3.0	3.05	.31	.325
4/24	MME-A	2	3.1		.34	
4/24	MME-B	1	2.6	1.7	.27	.23
4/24	MME-B	2	0.8		.18	
4/24	MME-C	1	3.2	3.6	.32	.335
4/24	MME-C	2	4.0		.35	
4/24	MME-D	1	3.9	3.45	.31	.29
4/24	MME-D	2	3.0		.27	
4/24	MME-E	1	2.2	4.15	.27	.27
4/24	MME-F	1	4.8		.30	.27
4/24	MME-F	2	3.5		.24	
4/24	MME-G	1	3.9	3.4	.37	.315
4/24	MME-G	2	2.9		.26	

Data (continued)

<u>Weighing date</u>	<u>Media</u>	<u>No.</u>	<u>Fresh Weight</u>	<u>Average Fresh Weight</u>	<u>Dry Weight</u>	<u>Average Dry Weight</u>
4/25	MME-H	1	2.5	2.65	.25	.245
4/25	MME-H	2	2.8		.24	
4/25	MME-I	1	5.1	4.75	.32	.31
4/25	MME-I	2	4.4		.30	
5/ 9	MME-A	1	3.9	4.85	.40	.43
5/ 9	MME-A	2	5.8		.46	
5/ 9	MME-B	1	3.1	4.05	.32	.365
5/ 9	MME-B	2	5.0		.41	
5/ 9	MME-C	1	4.6	4.65	.40	.40
5/ 9	MME-C	2	4.7		.40	
5/ 9	MME-D	1	5.5	5.25	.36	.355
5/ 9	MME-D	2	5.0		.35	
5/ 9	MME-E	1	5.7	6.35	.35	.38
5/ 9	MME-E	2	7.0		.41	
5/ 9	MME-F	1	5.0	5.25	.41	.35
5/ 9	MME-F	2	5.5		.29	
5/ 9	MME-G	1	3.1	4.05	.36	.385
5/ 9	MME-G	2	5.0		.41	
5/ 9	MME-H	1	3.0	2.45	.32	.245
5/ 9	MME-H	2	1.9		.17	
5/ 9	MME-I	1	8.5	8.25	.40	.37
5/ 9	MME-I	2	8.0		.34	

Data
Pine Growth Study Experiment #1
Summary

Weighing in date	Age Days	<u>Fresh Weight in Grams</u>				<u>Dry Weight in Grams</u>			
	control	MME-1	MME-2	MME-3	control	MME-1	MME-2	MME-3	
(1st generation)									
12/10/71	7	--	5.3	4.35	3.55	--	.255	.26	.23
12/17/71	14	3.5	6.65	4.65	3.5	.165	.295	.245	.20
12/28/71	25	3.95	6.5	6.25	5.45	.19	.28	.265	.345
1 / 3/72	31	4.05	7.15	9.55	7.95	.245	.325	.335	.34
1 /10/72	38	5.55	7.7	6.95	6.7	.25	.29	.315	.275
1 /18/72	46	--	6.9	7.15	8.35	--	.31	.30	.33
(2nd generation)									
2 /15/72	15	4.4	2.0	1.9	3.1	.305	.145	.115	.215
2 /22/72	22	8.4	3.45	2.15	5.45	.385	.215	.145	.27
2 /29/72	29	3.1	2.5	1.85	6.35	.265	.17	.14	.25
3 / 7/72	36	7.1	6.9	3.8	8.7	.40	.36	.25	.34

Weighing Date	Age in Days	<u>Fresh Weight in Grams</u>			<u>Dry Weight in Grams</u>		
		<u>MME-4</u>	<u>MME-5</u>	<u>MME-6</u>	<u>MME-4</u>	<u>MME-5</u>	<u>MME-6</u>
(1st generation)							
12/28/71	7	3.4	3.8	3.25	.18	.26	.195
1/ 3/72	13	4.75	3.2	3.5	.265	.225	.23
1/10/72	20	2.9	5.45	4.65	.205	.33	.295
1/18/72	28	3.45	6.65	3.5	.23	.395	.28
1/25/72	36	6.05	7.05	3.55	.30	.33	.31
(2nd generation)							
2/15/72	15	1.45	1.1	2.05	.115	.08	.145
2/22/72	22	2.55	1.75	1.6	.195	.115	.15
2/29/72	29	1.2	1.1	2.15	.11	.085	.185
3/ 7/72	36	1.3	2.0	4.0	.10	.15	.29

Pine Growth Experiment #2

Summary

Fresh Weight in Grams

Weighing Date	Days	MME-- <u>A</u>	MME-- <u>B</u>	MME-- <u>C</u>	MME-- <u>D</u>	MME-- <u>E</u>	MME-- <u>F</u>	MME-- <u>H</u>	MME-- <u>H</u>	MME-- <u>I</u>
(1st generation)										
3/ 3/72	10	3.15	3.05	3.75	3.55	2.55	3.55	2.7	2.7	3.4
3/13/72	20	4.05	4.95	5.2	5.25	4.3	6.1	5.25	4.2	3.9
3/23/72	30	7.26	6.85	6.95	8.95	5.65	9.55	6.3	4.75	9.5
(2nd generation)										
4/ 9/72	11	2.6	2.0	2.7	2.25	2.15	3.0	2.25	2.15	2.9
4/25/72	25	3.05	1.7	3.6	3.45	2.2	4.15	3.4	2.65	4.75
5/ 9/72	39	4.85	4.05	4.65	5.25	6.35	5.25	4.05	2.45	8.25

Pine Growth Experiment #2 - Summary (continued)

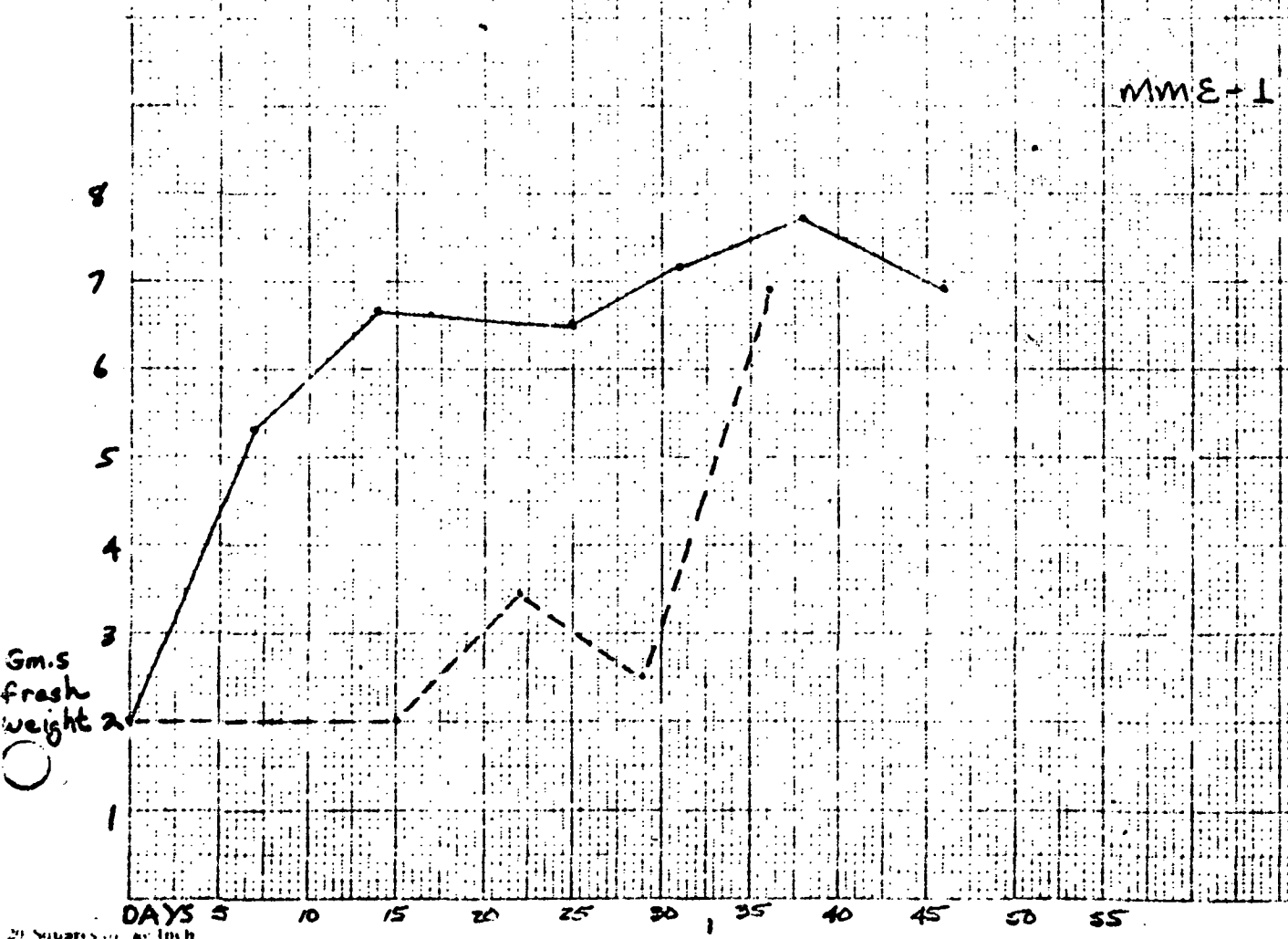
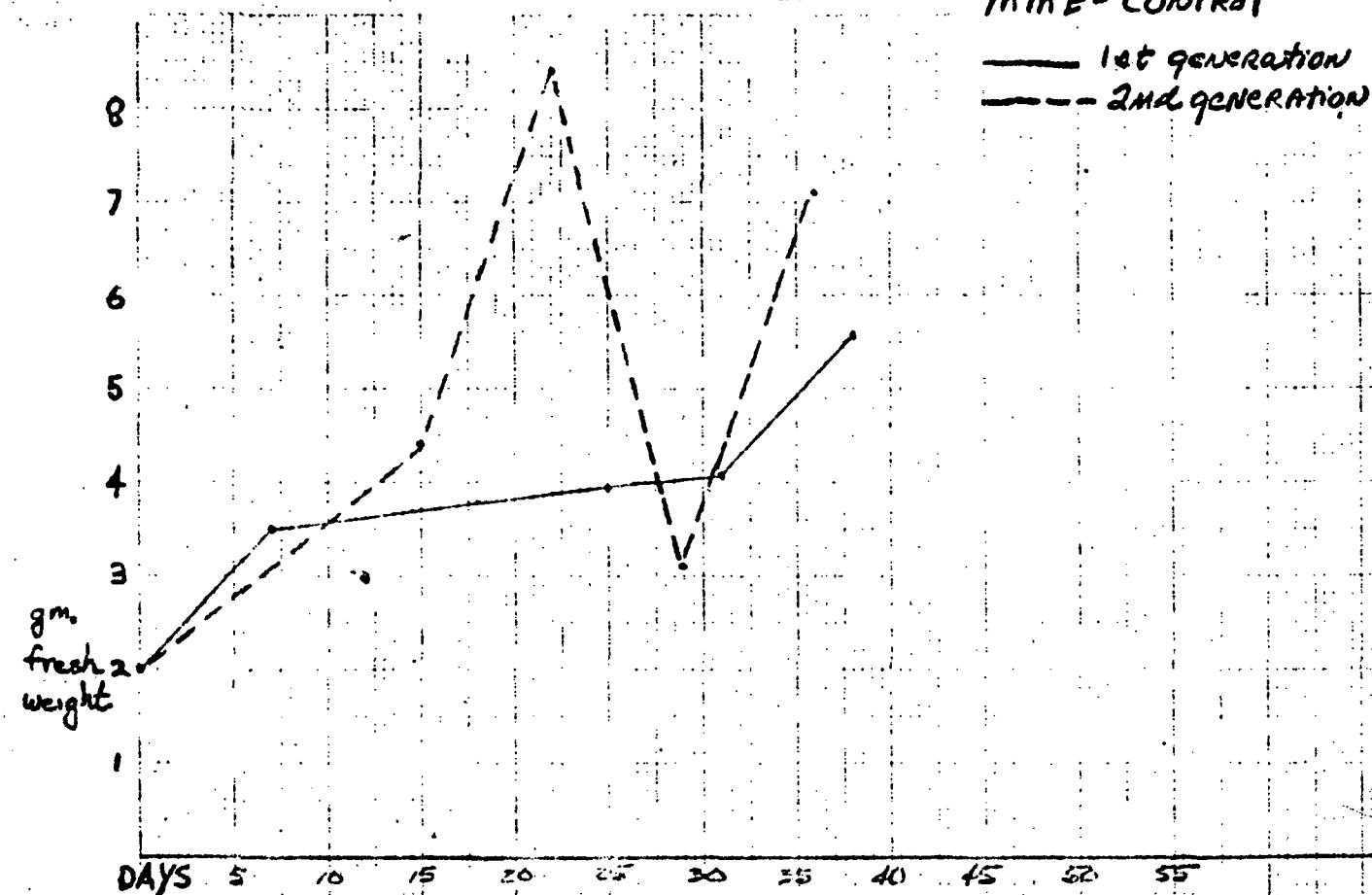
Weighing Date	Age in Days	Dry Weight in Grams								
		<u>MME-</u> <u>A</u>	<u>MME-</u> <u>B</u>	<u>MME-</u> <u>C</u>	<u>MME-</u> <u>D</u>	<u>MME-</u> <u>E</u>	<u>MME-</u> <u>F</u>	<u>MME-</u> <u>G</u>	<u>MME-</u> <u>H</u>	<u>MME-</u> <u>I</u>
(1st generation)										
3/ 3/72	10	.22	.185	.205	.205	.165	.195	.17	.18	.215
3/13/72	20	.27	.275	.345	.29	.275	.345	.285	.27	.22
3/23/72	30	.42	.37	.425	.41	.325	.595	.37	.32	.415
(2nd generation)										
4/ 9/72	11	.23	.16	.25	.195	.215	.23	.185	.195	.175
4/25/72	25	.325	.23	.335	.29	.27	.27	.315	.245	.31
5/ 9/72	39	.43	.365	.40	.355	.38	.35	.385	.245	.37

APPENDIX

A graphical illustration of the data on the experiments of nutritional studies on Pinus ellioti is attached.

MME-CONTROL

— 1st generation
 --- 2nd generation

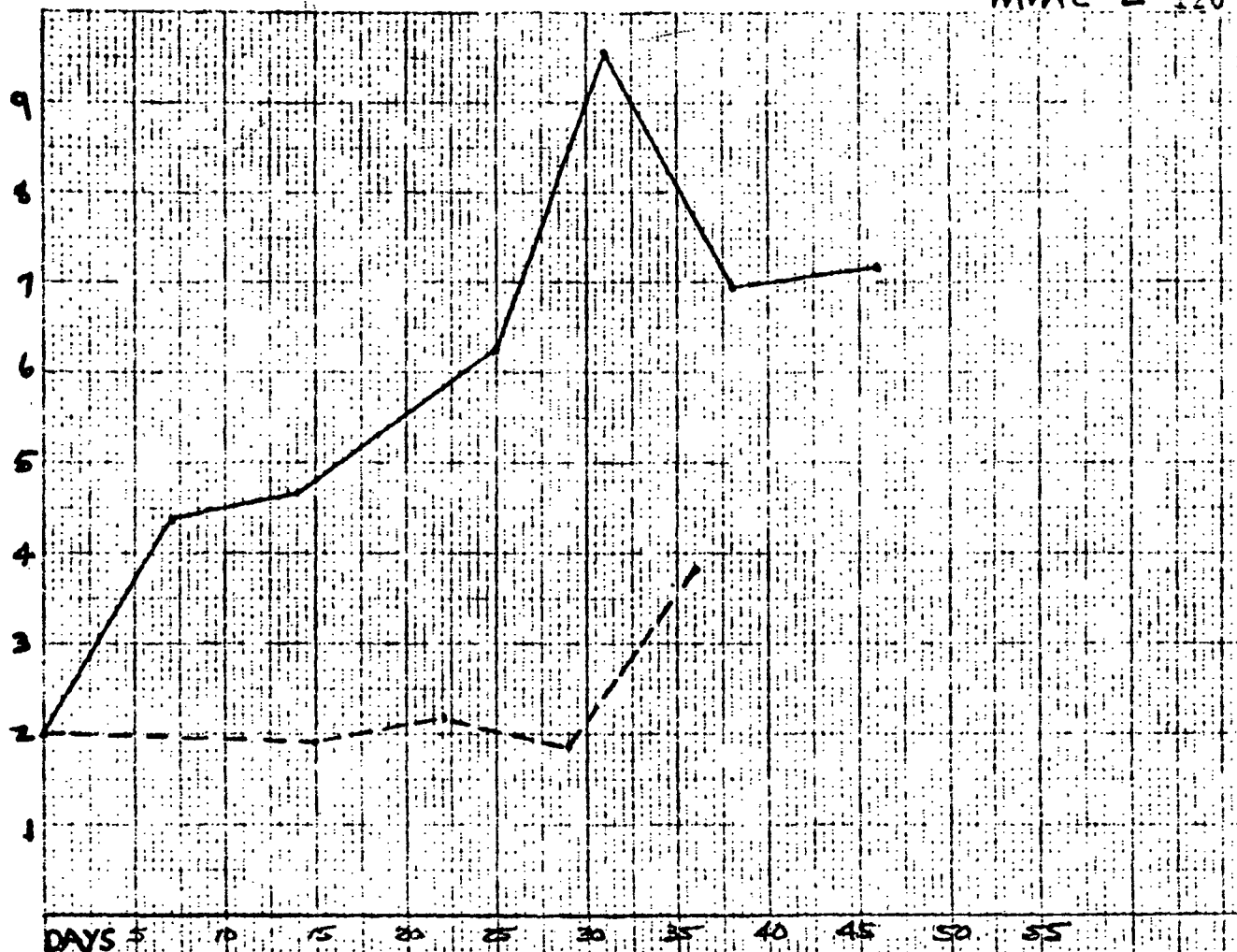


MME+1

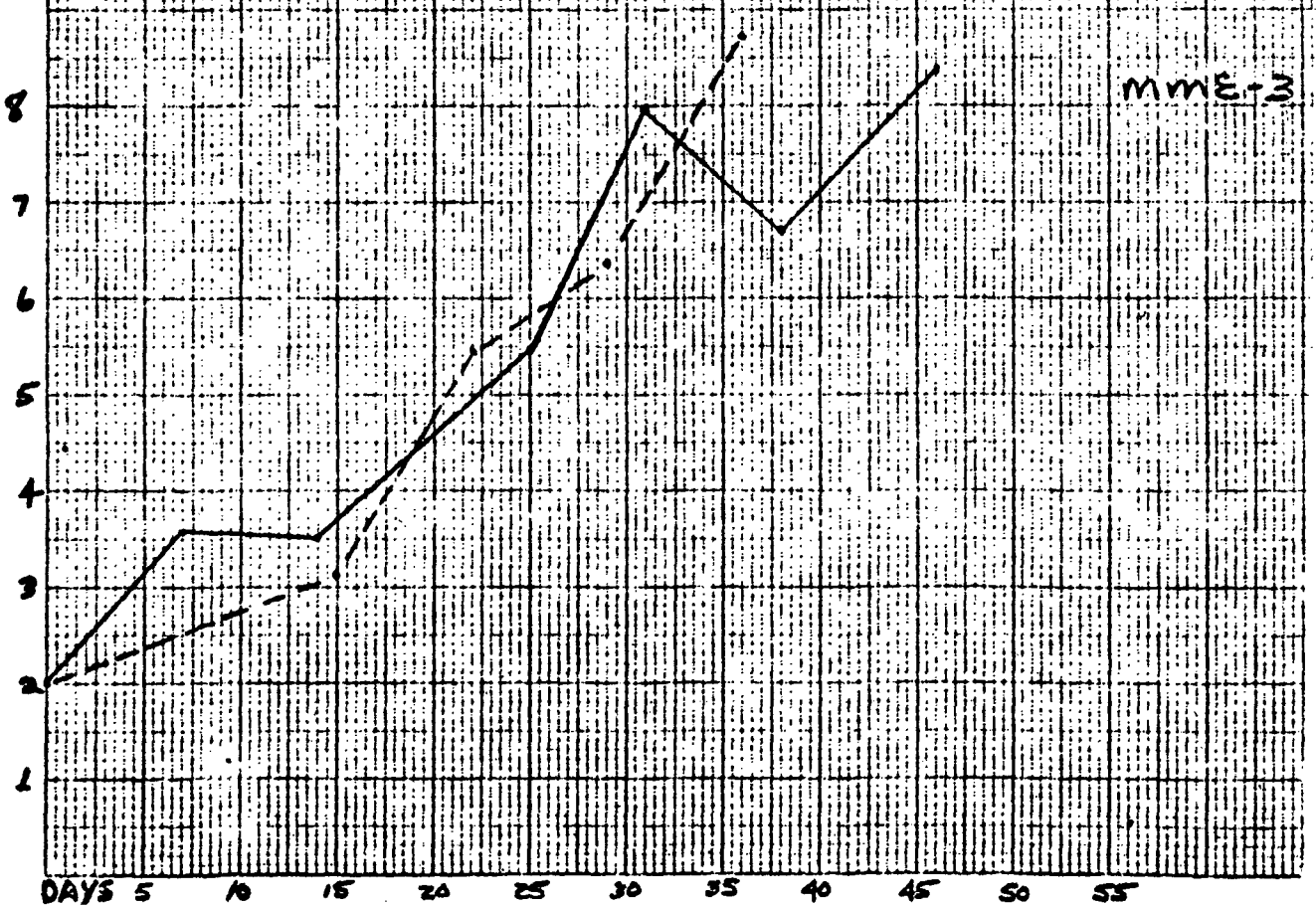
Growth Curves

MME-2 120

G.M.S
FRESH
WT.

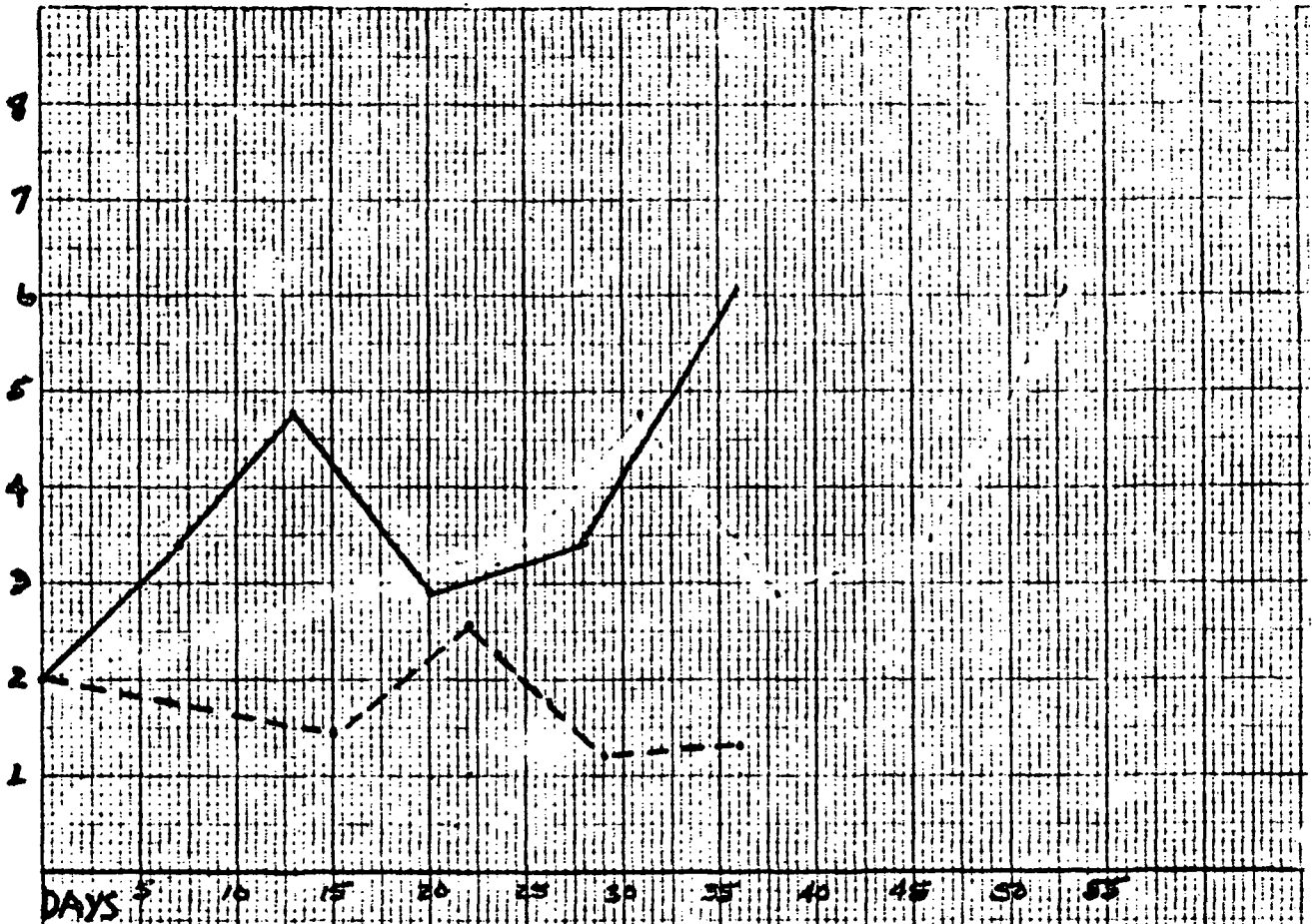


G.M.S
FRESH
WT.



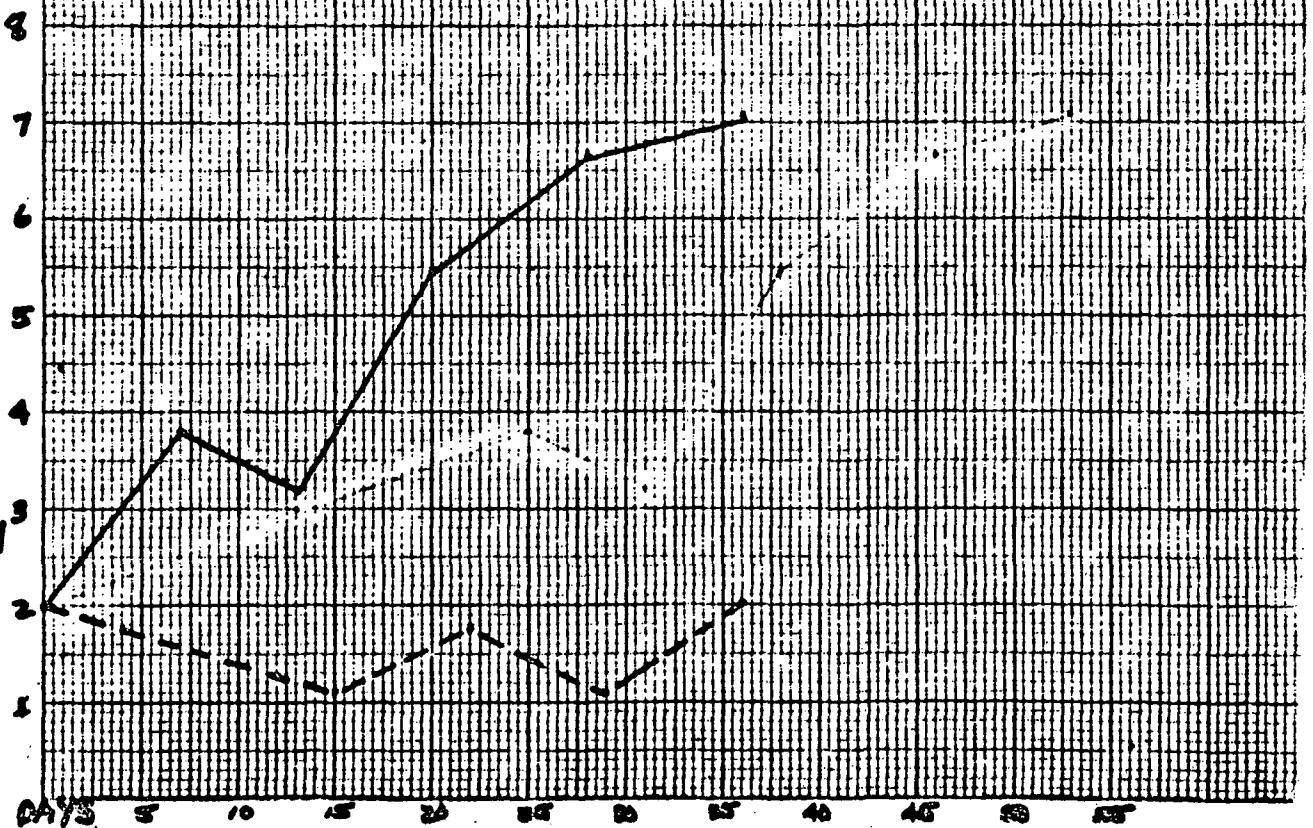
MME-3

GMS
FRESH
WT.



MM2-5

GMS
FRESH
WT.

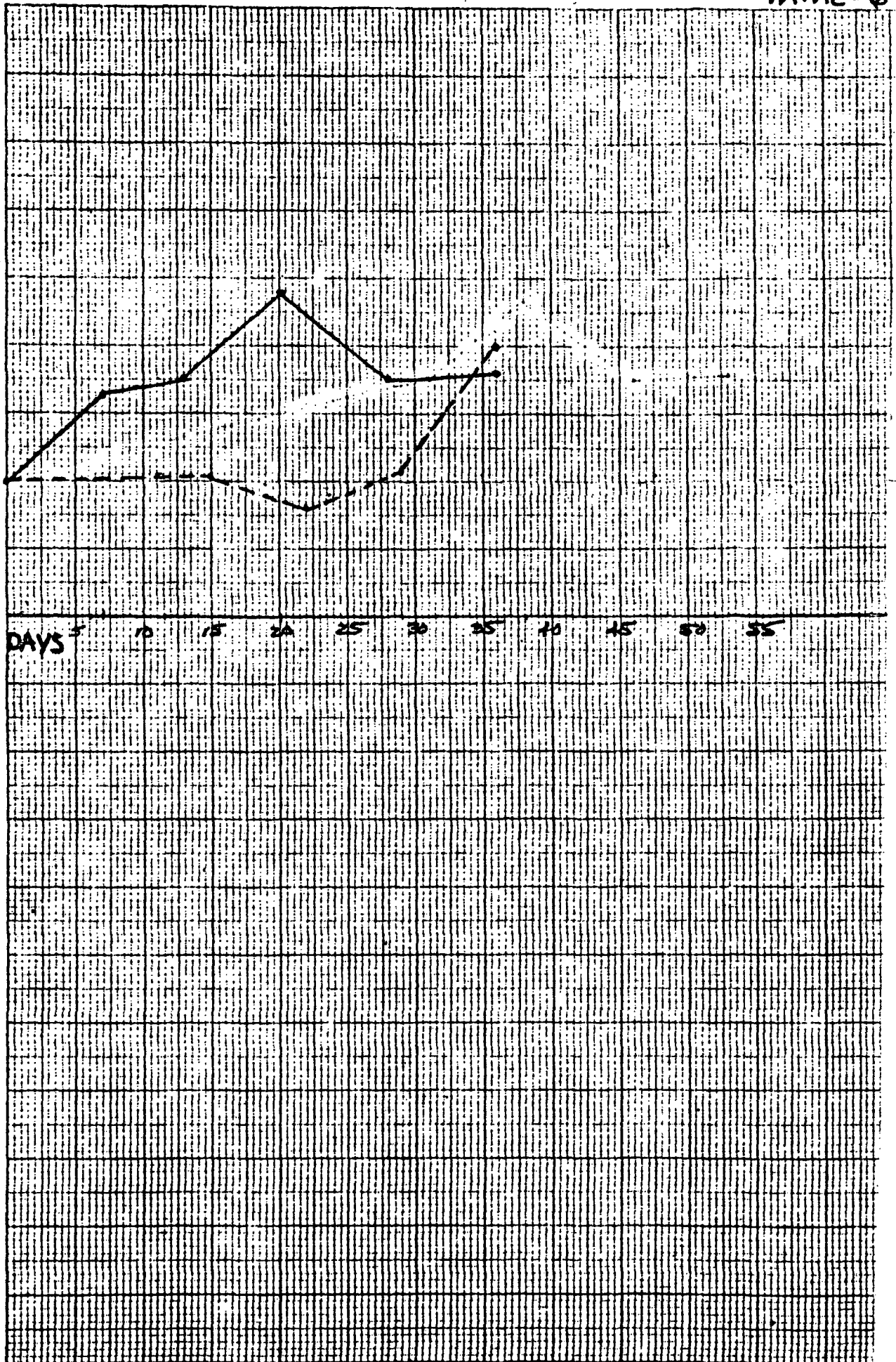


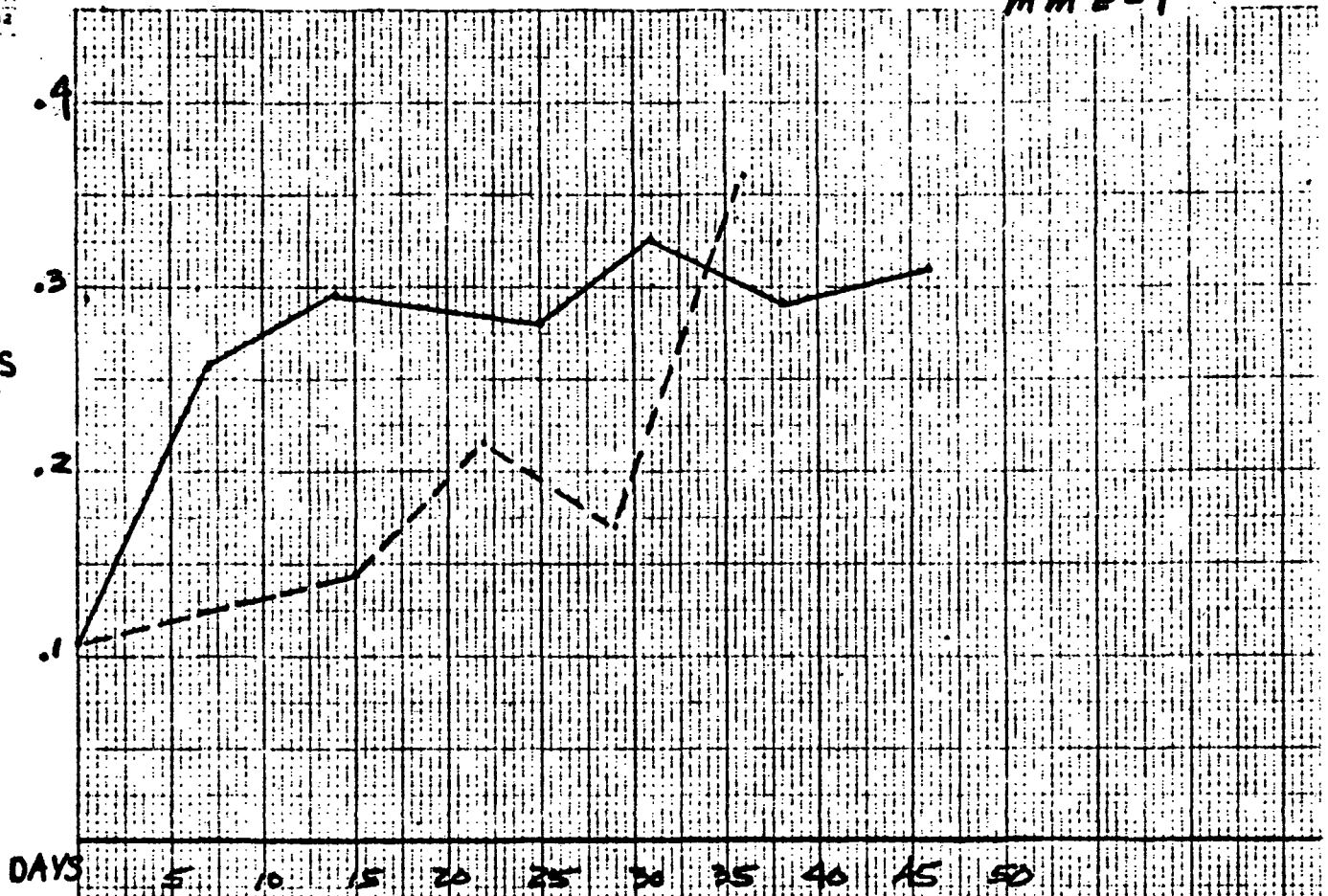
GM.S
FRESH
WT.

8
7
6
5
4
3
2
1

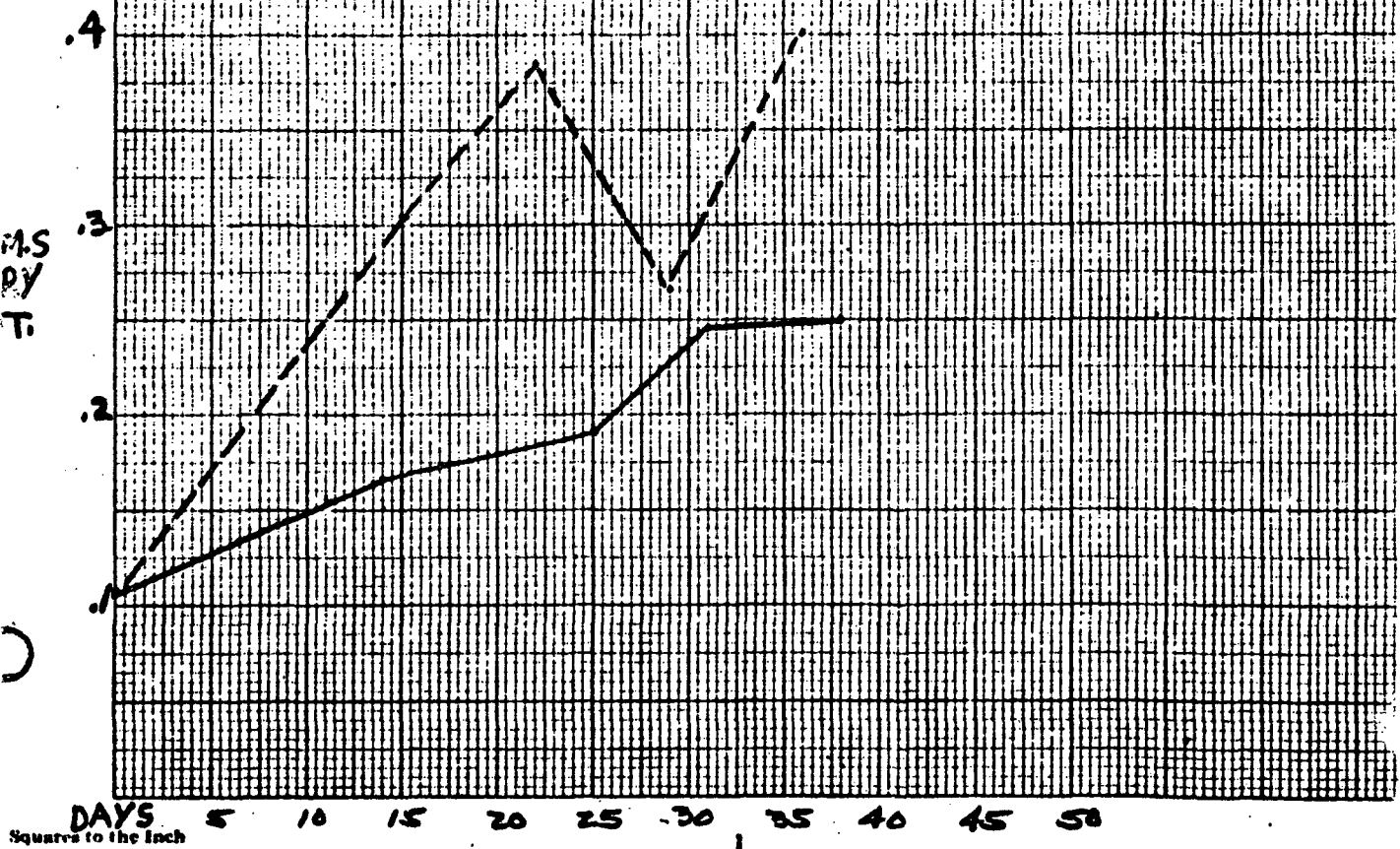
DAYS

5 10 15 20 25 30 35 40 45 50 55



M.S.
RY
T.

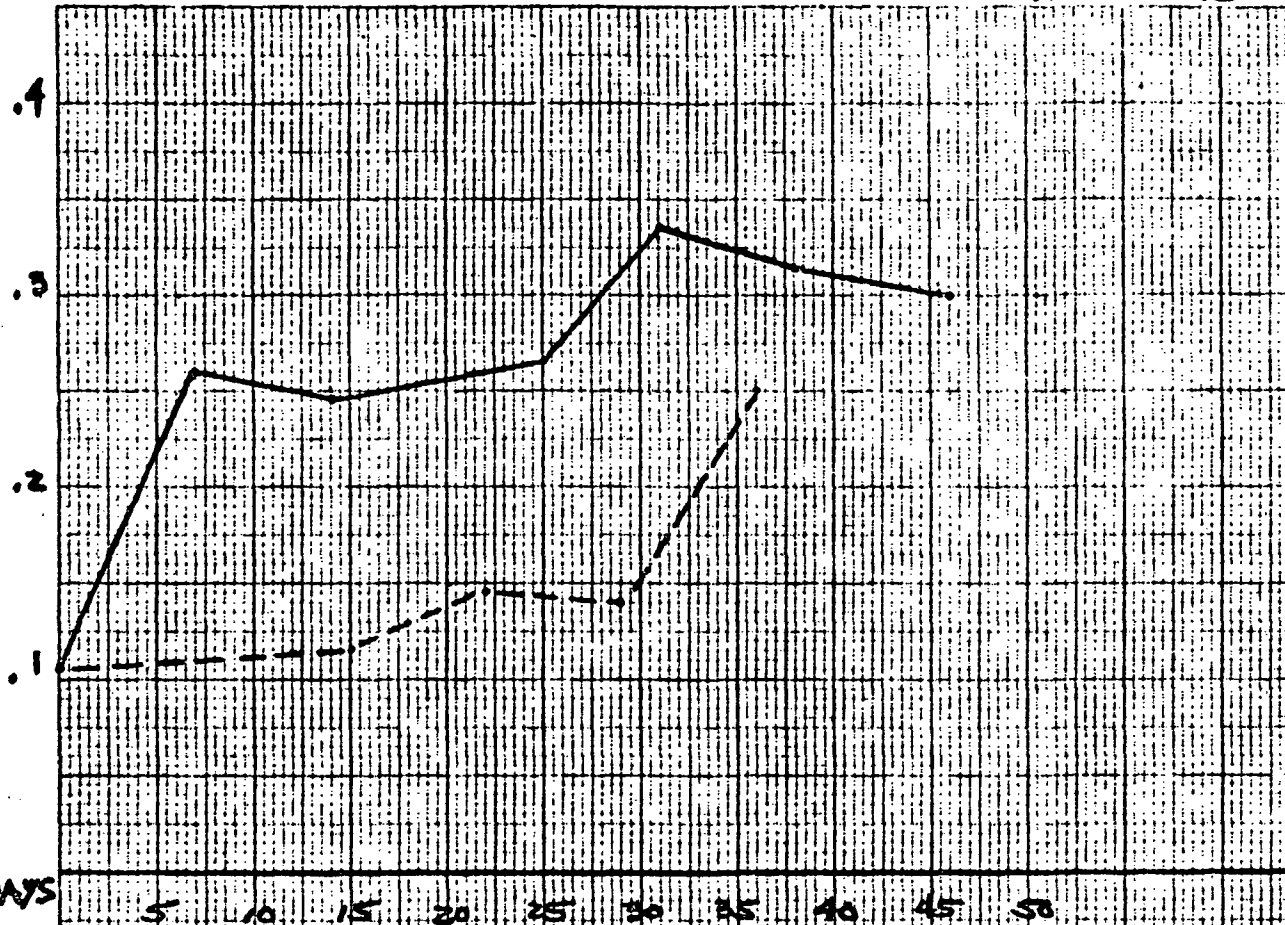
MME-CONTROL

M.S.
RY
T.

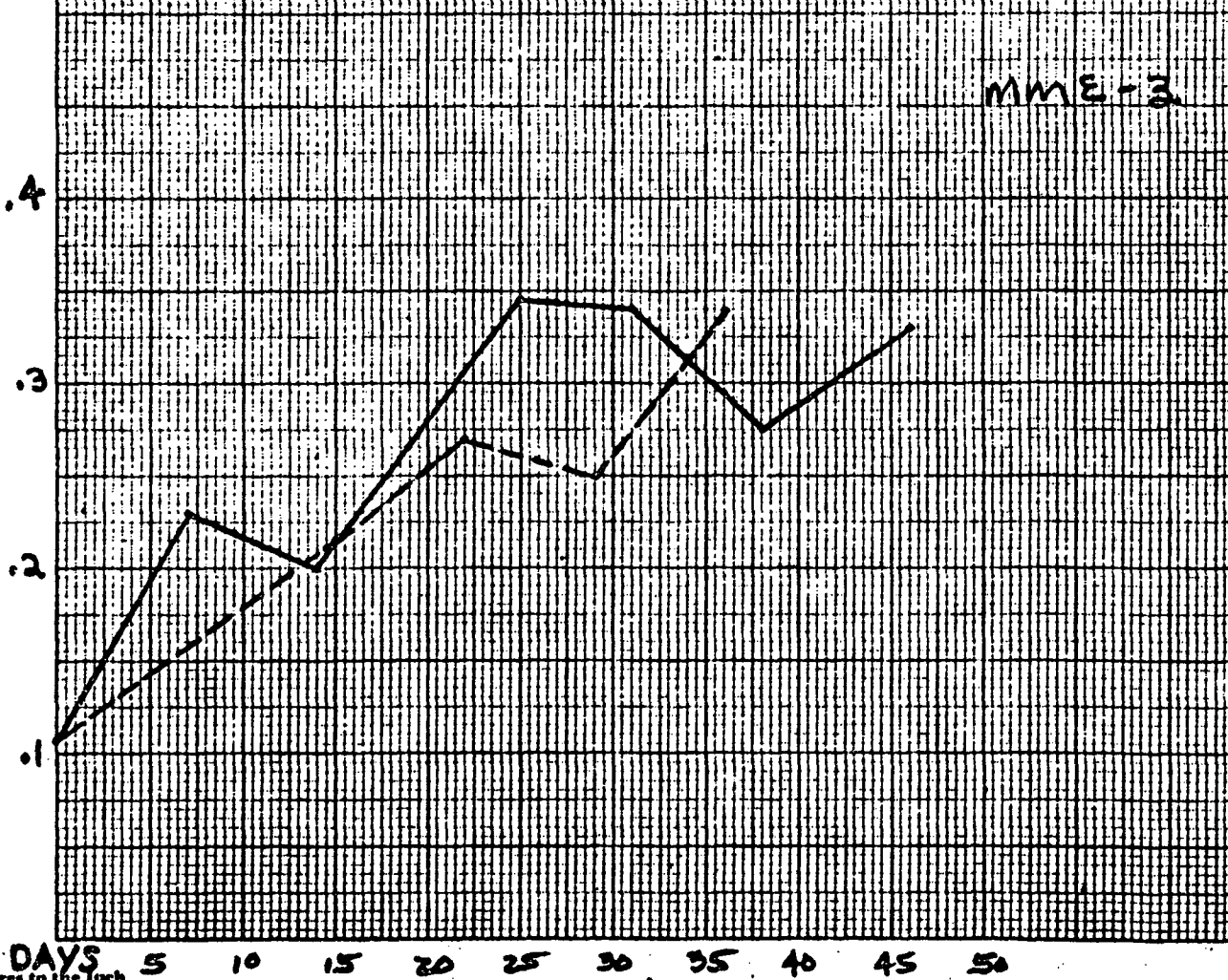
G.M.S
DRY
WT

DAYS

G.M.S
DRY
WT.



MME-3



MS
BY
T.

DAYS

mm 6-5

S
1

DAYS
Squares to the Inch





126

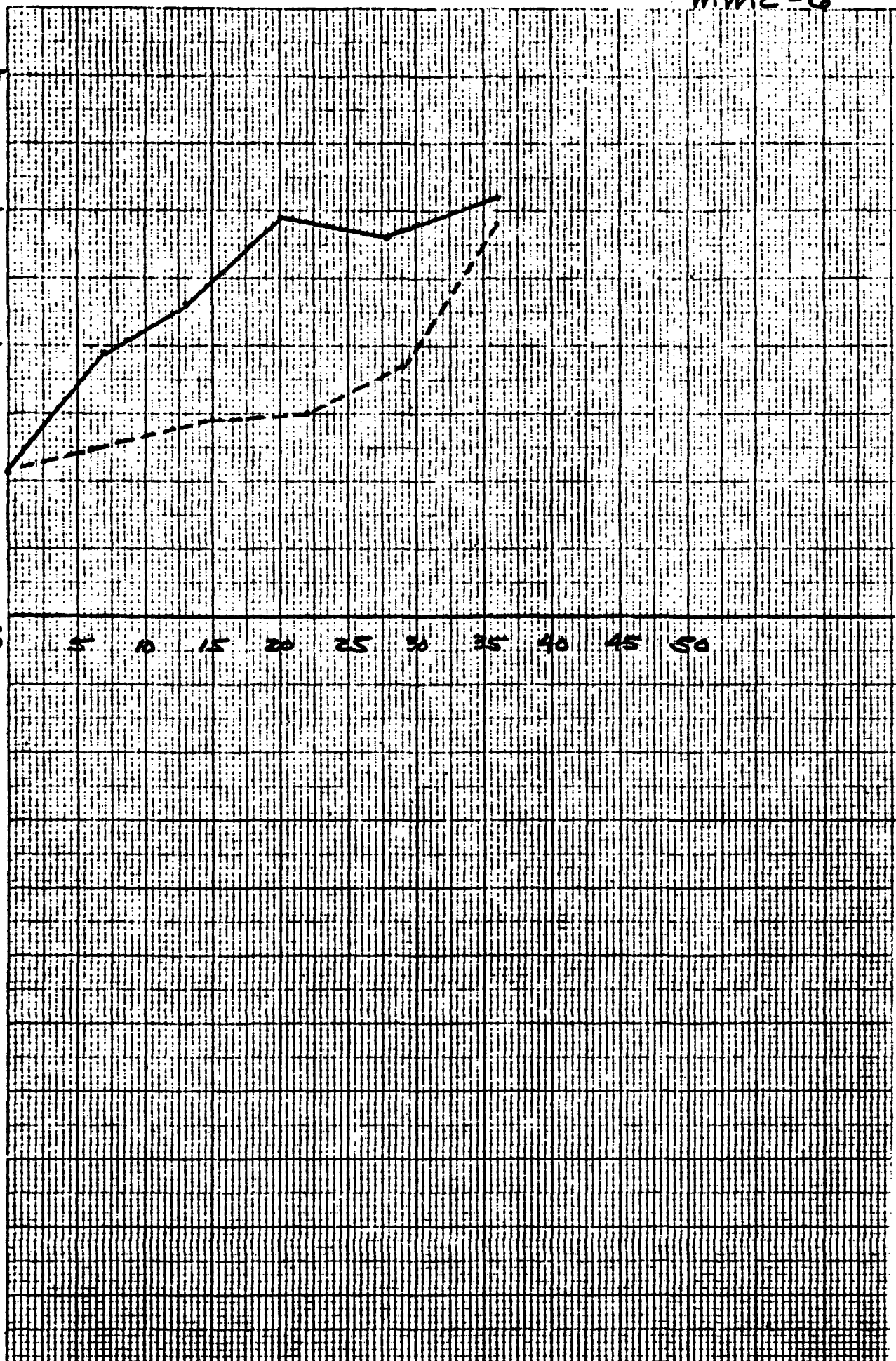
MME-6

GMS
DRY
WT.

DAYS

.4
.2
.2
.1

5 10 15 20 25 30 35 40 45 50



Growth Curves

127

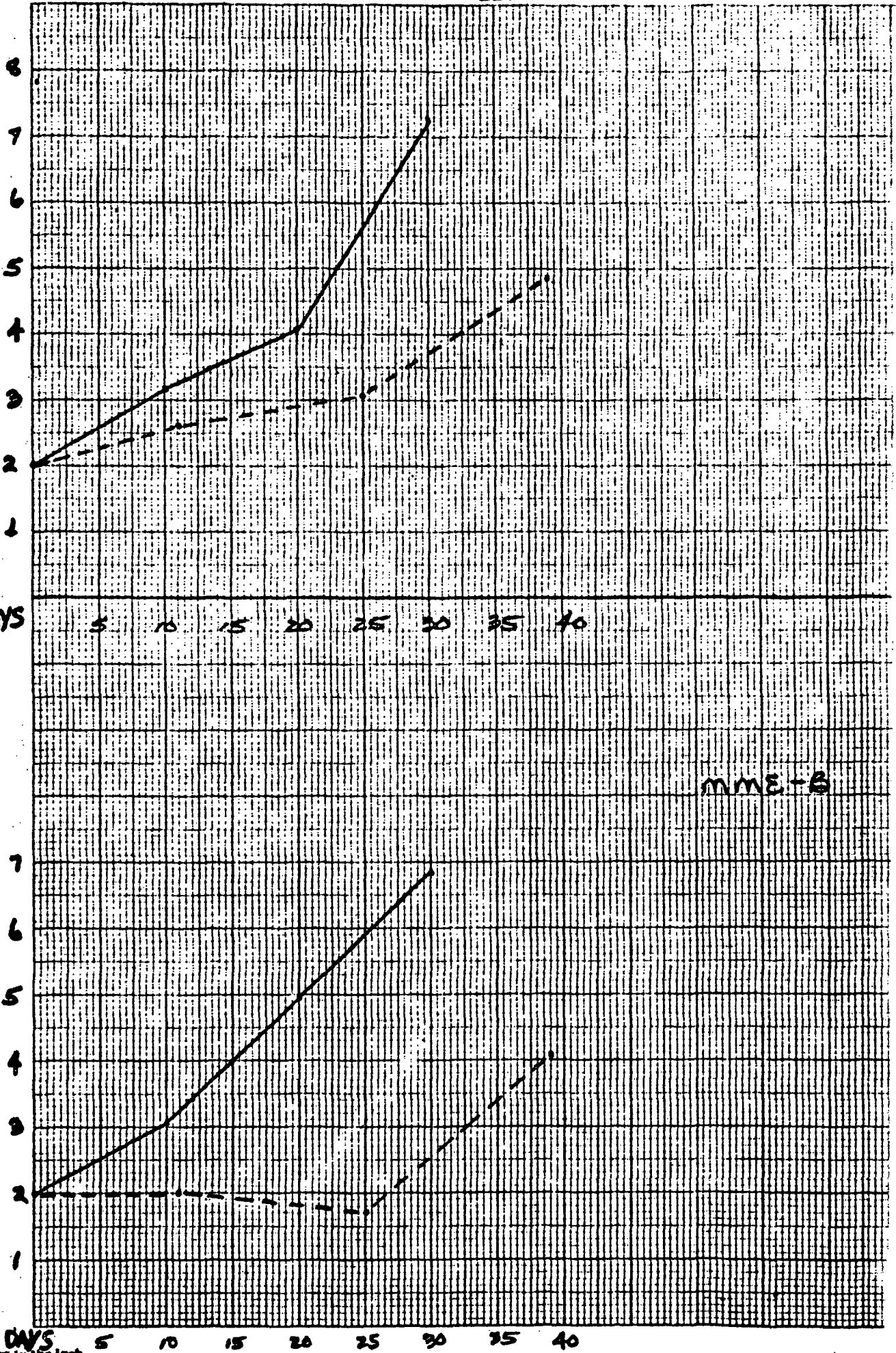
MME-A

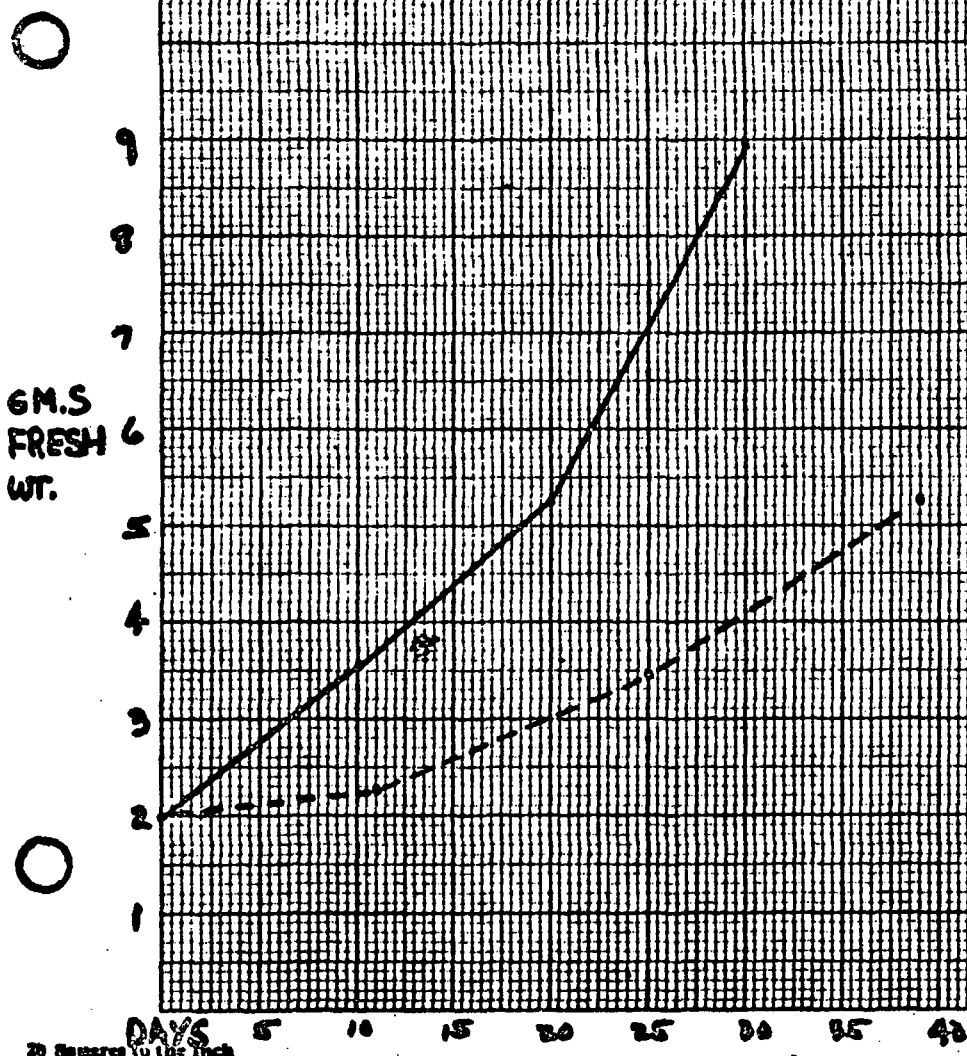
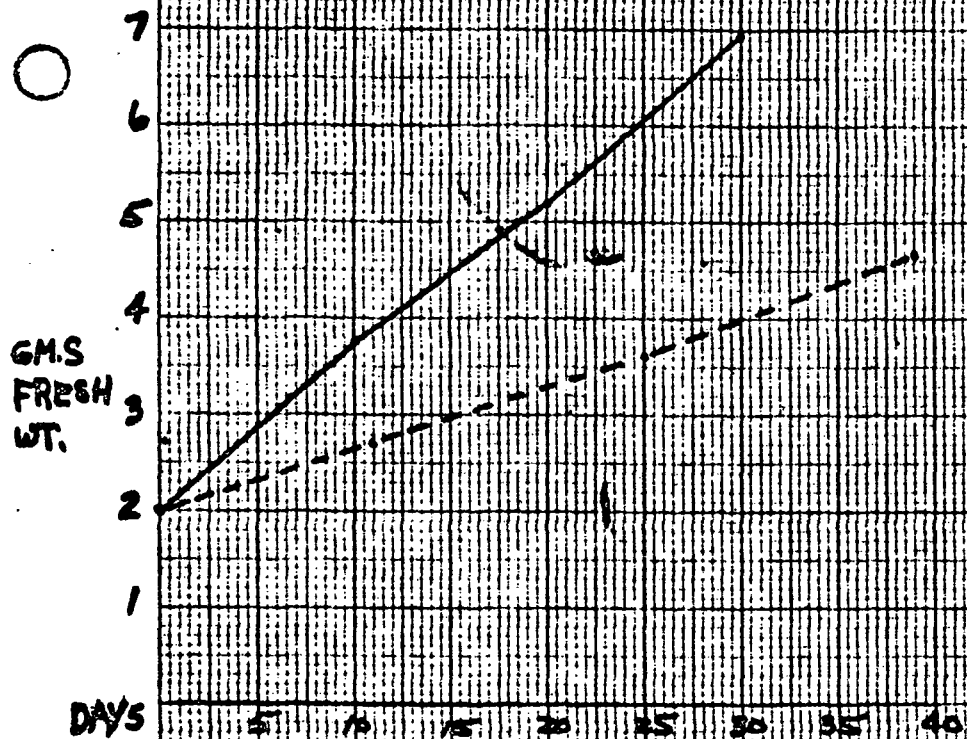
GMS
FRESH
WT.

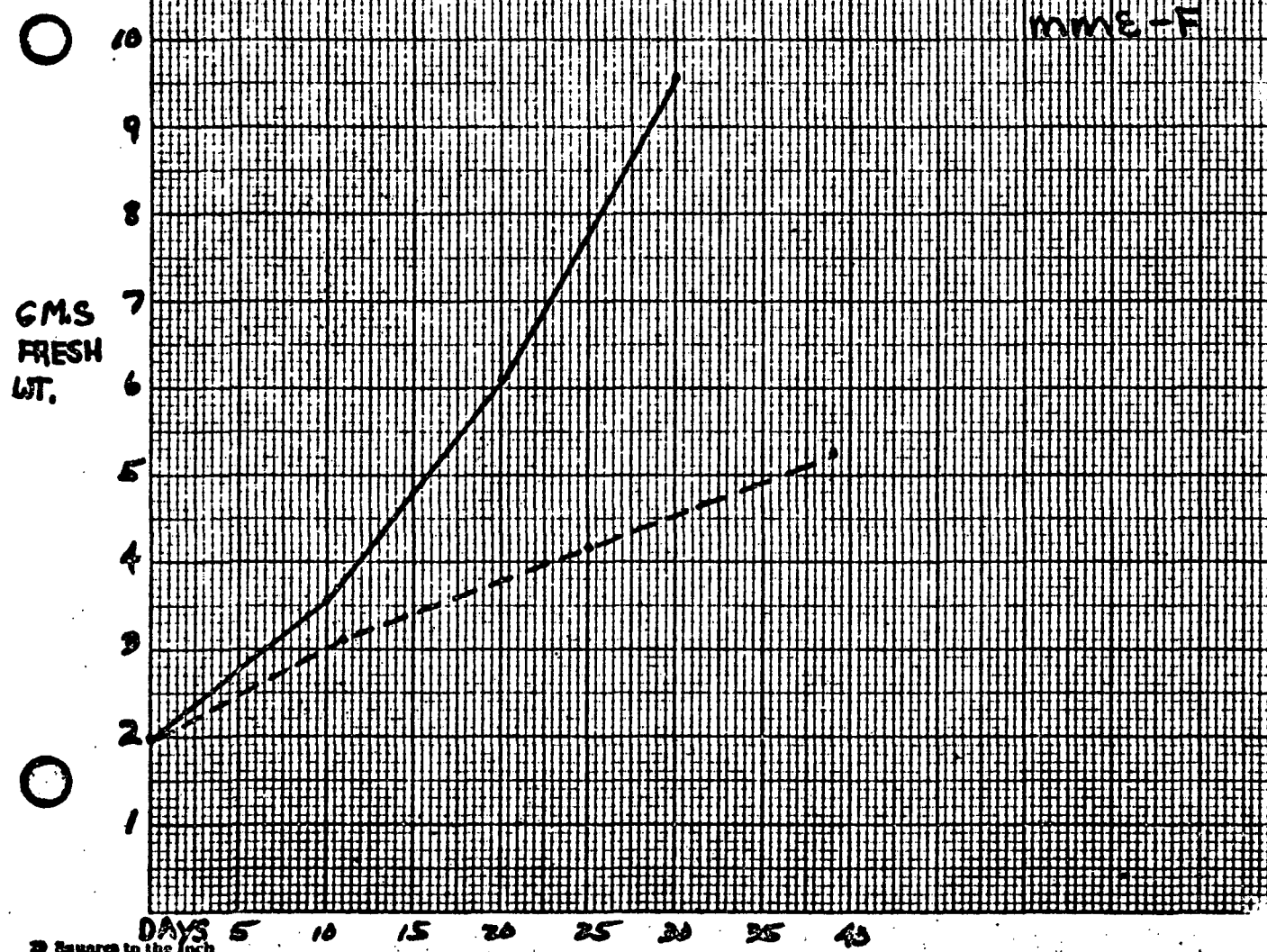
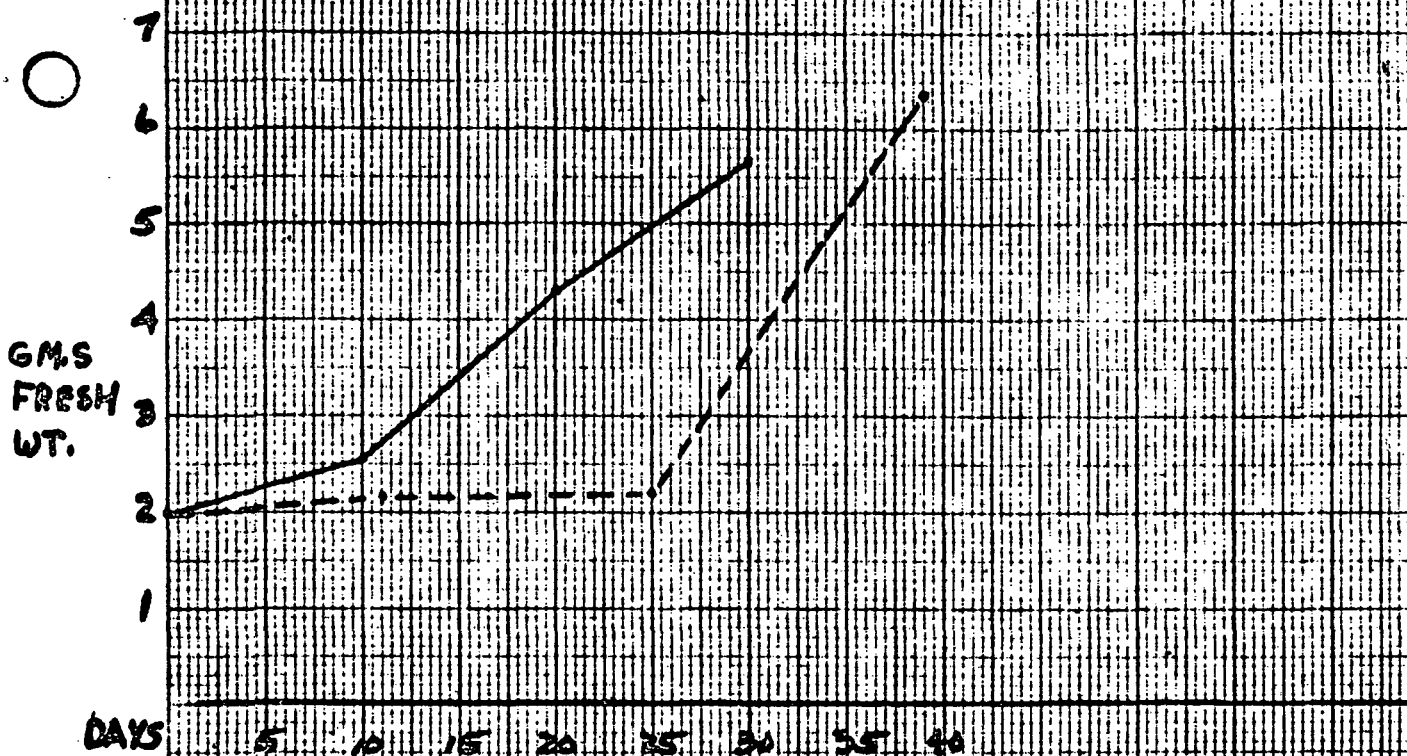
DAYS

MME-B

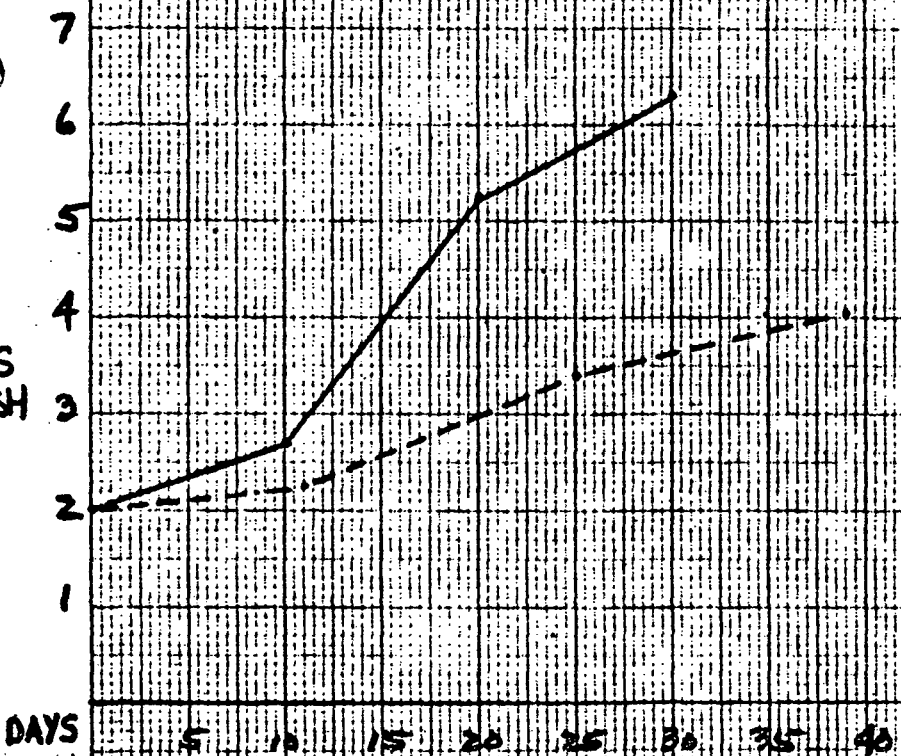
GMS
FRESH
WT.





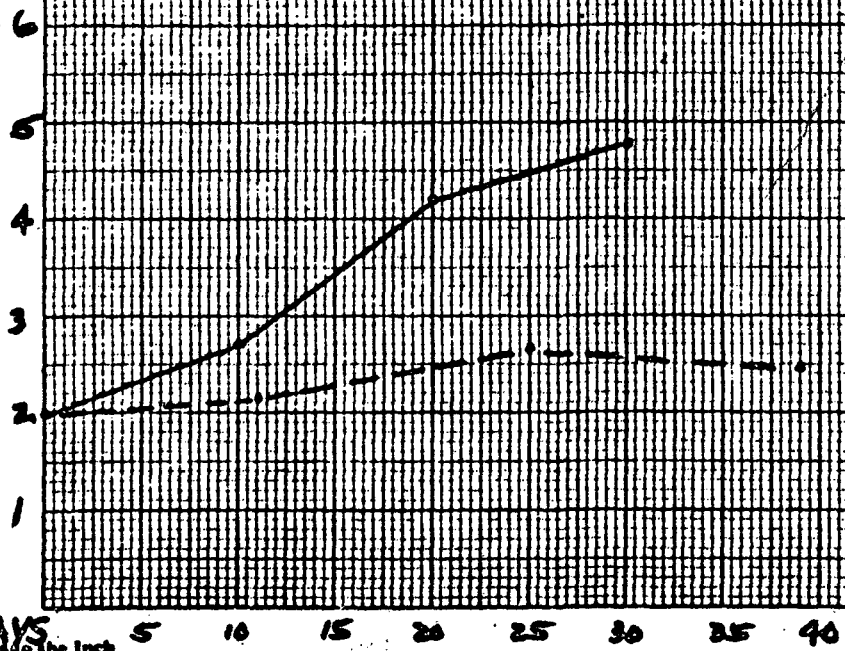


GM.S
FRESH
WT.



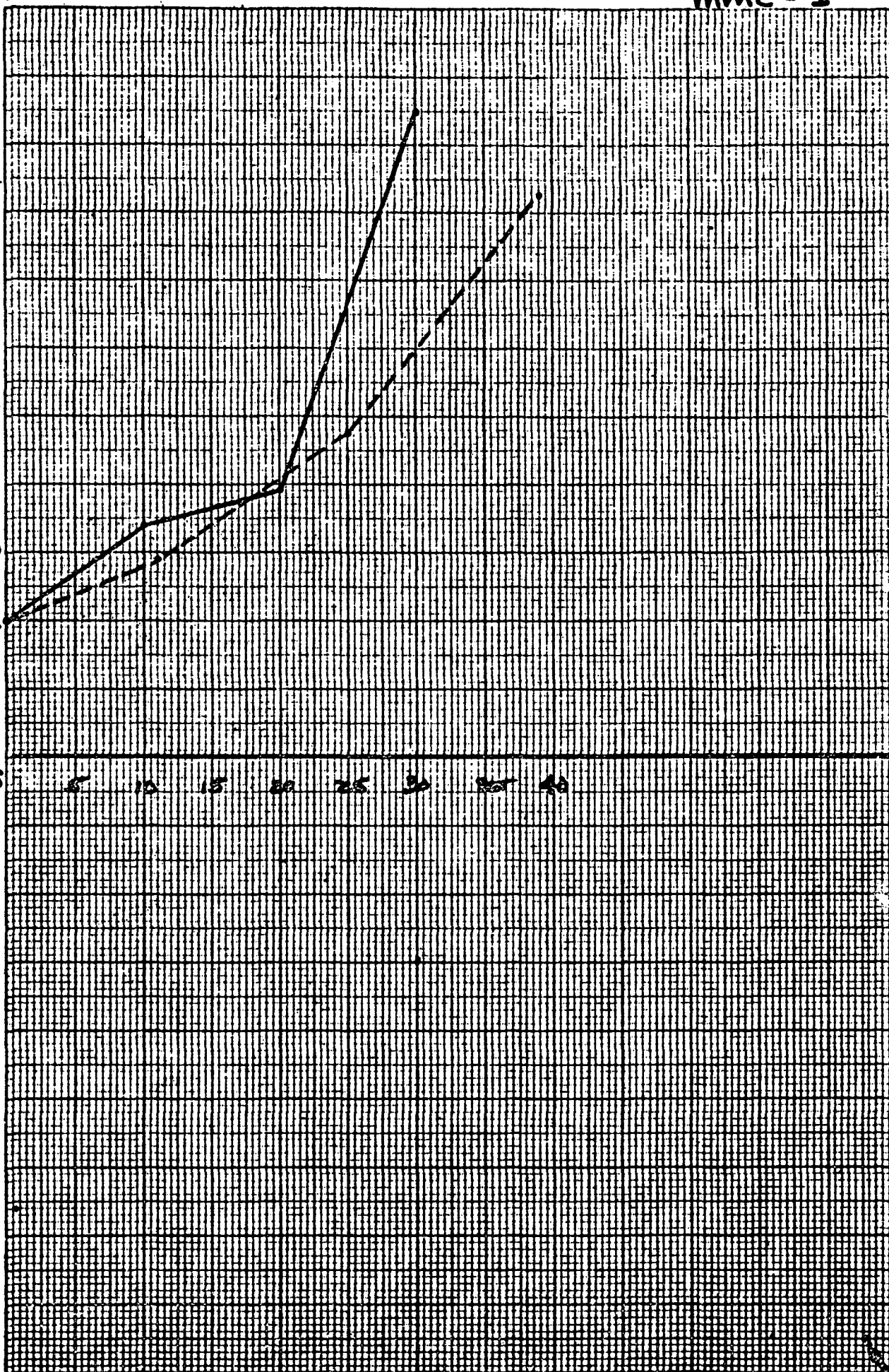
GM.S
FRESH
WT.

mme-H



GMS
FRESH
WT.

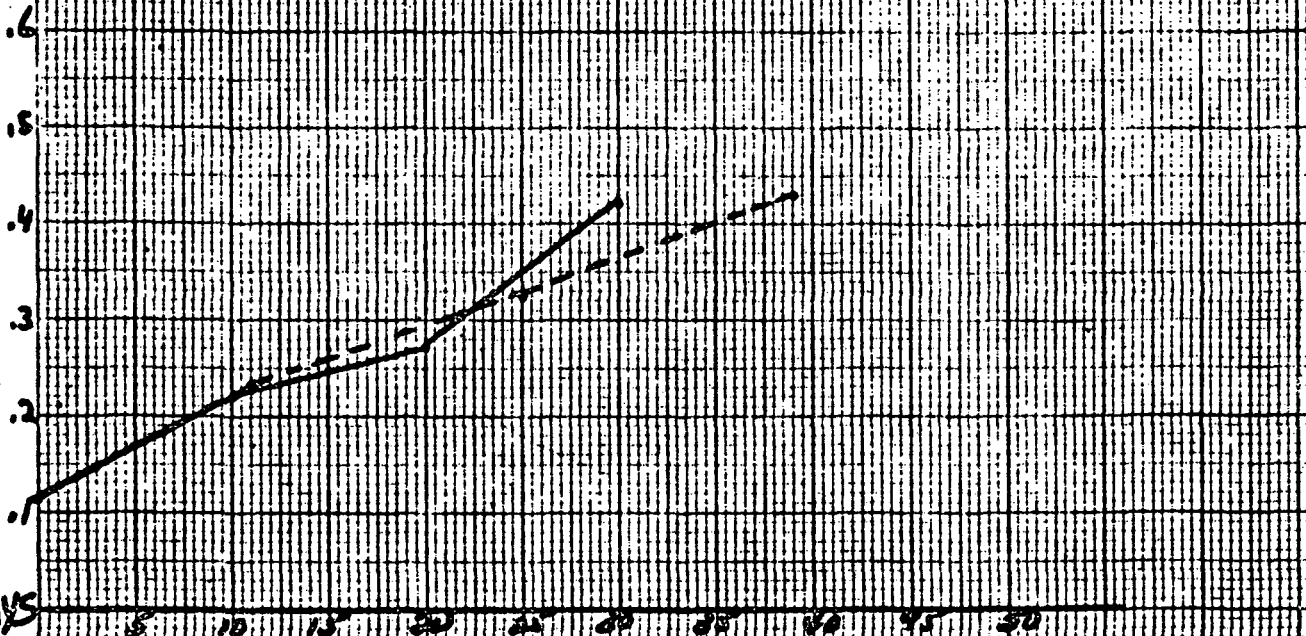
DAYS



GMS
DRY
WT.

DAYS

MME-A



GMS
DRY
WT.

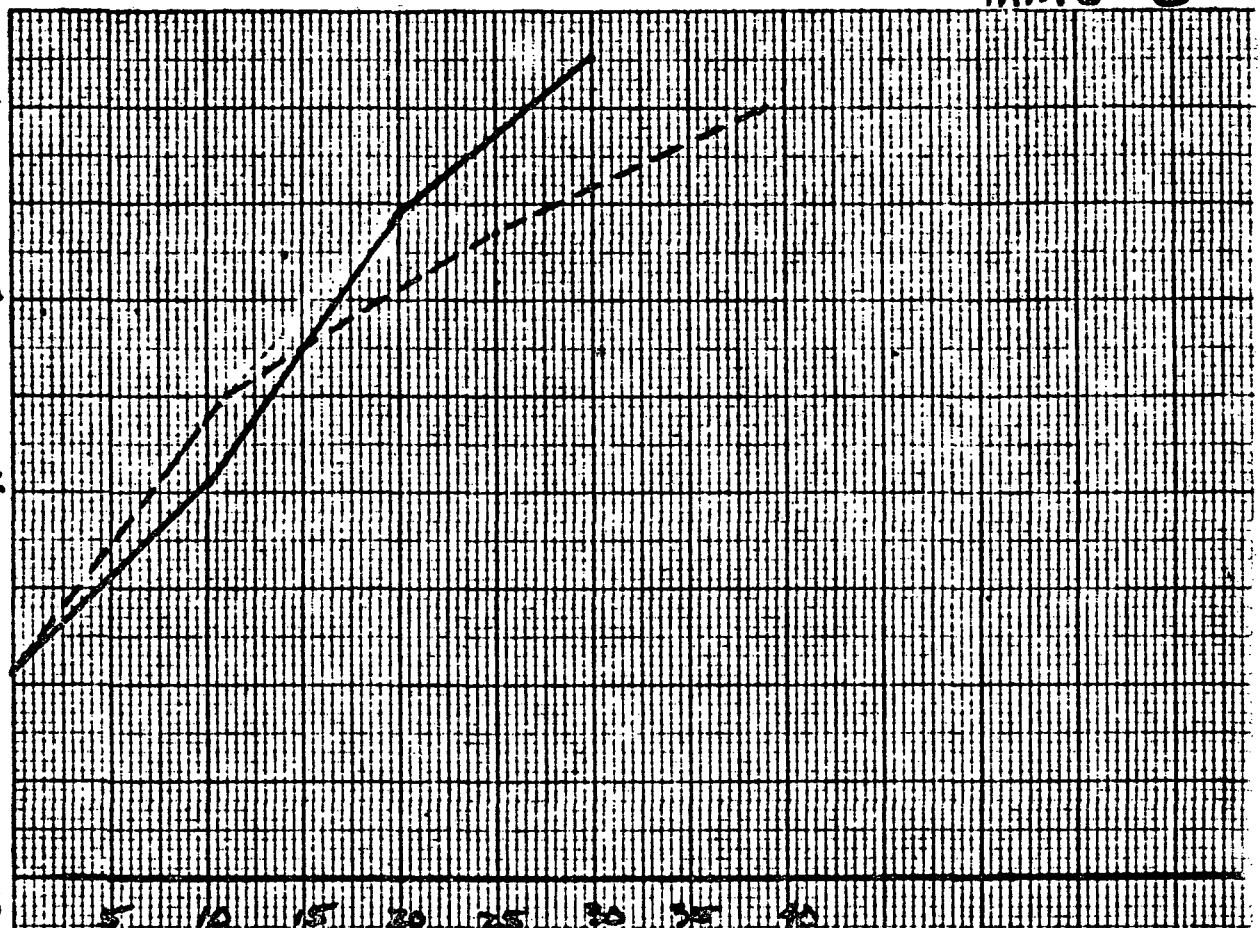
DAYS

MME-B



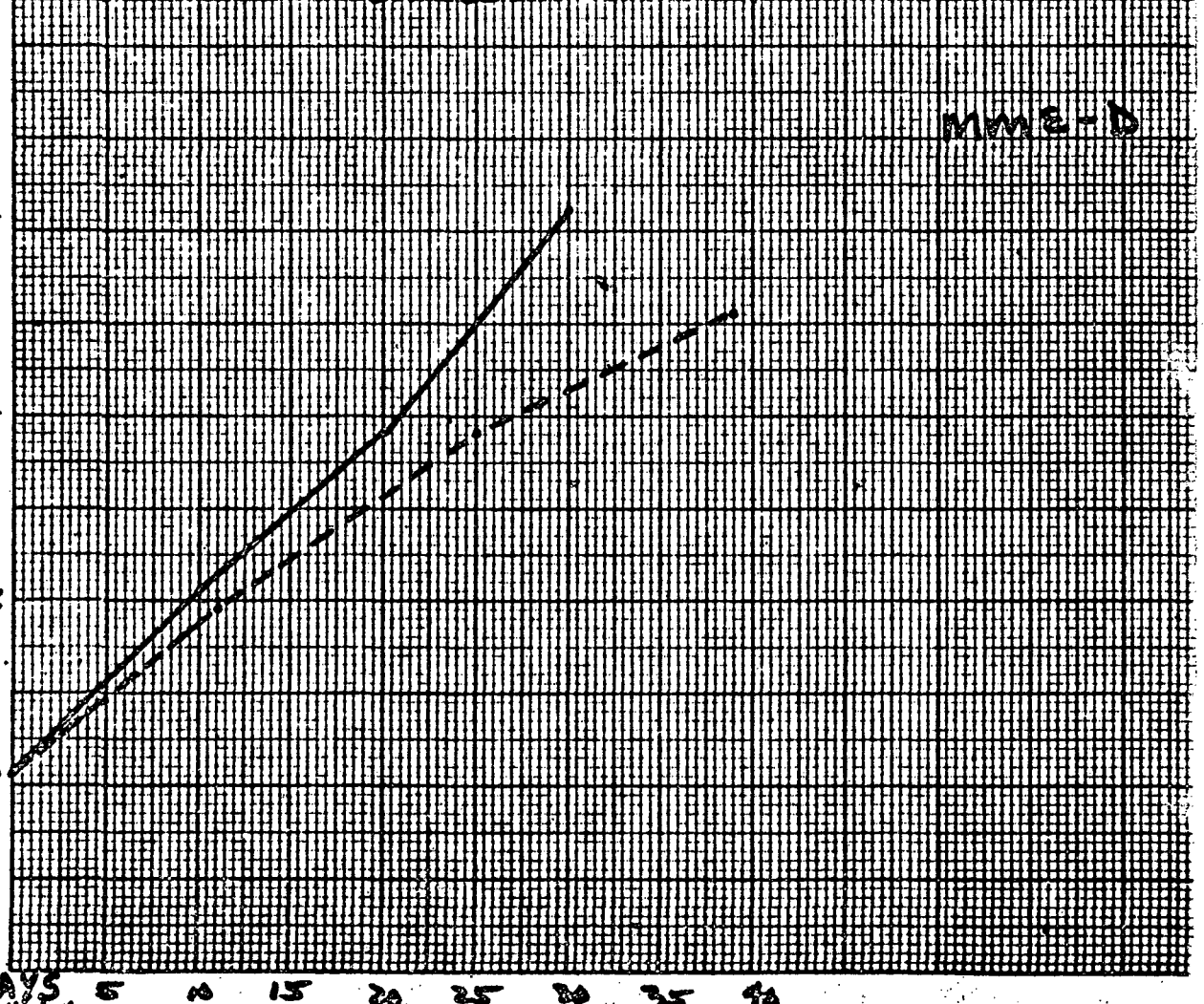
GM.S
DRY
WT.

DAYS



GM.S
DRY
WT.

DAYS

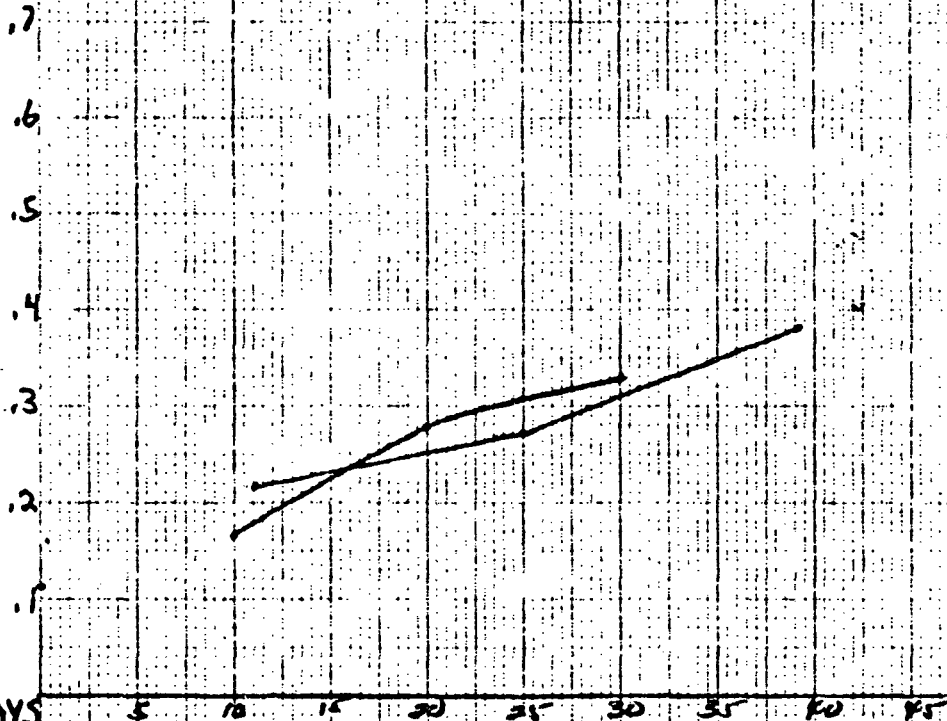


MME-D

GM.S
DRY
WT.

DAYS

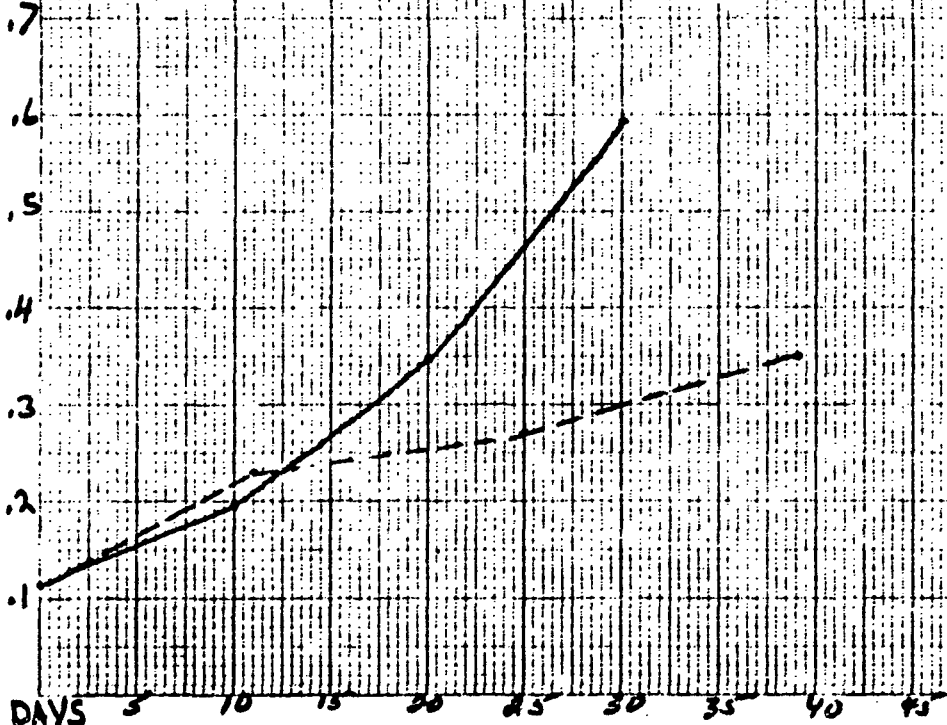
MME-E

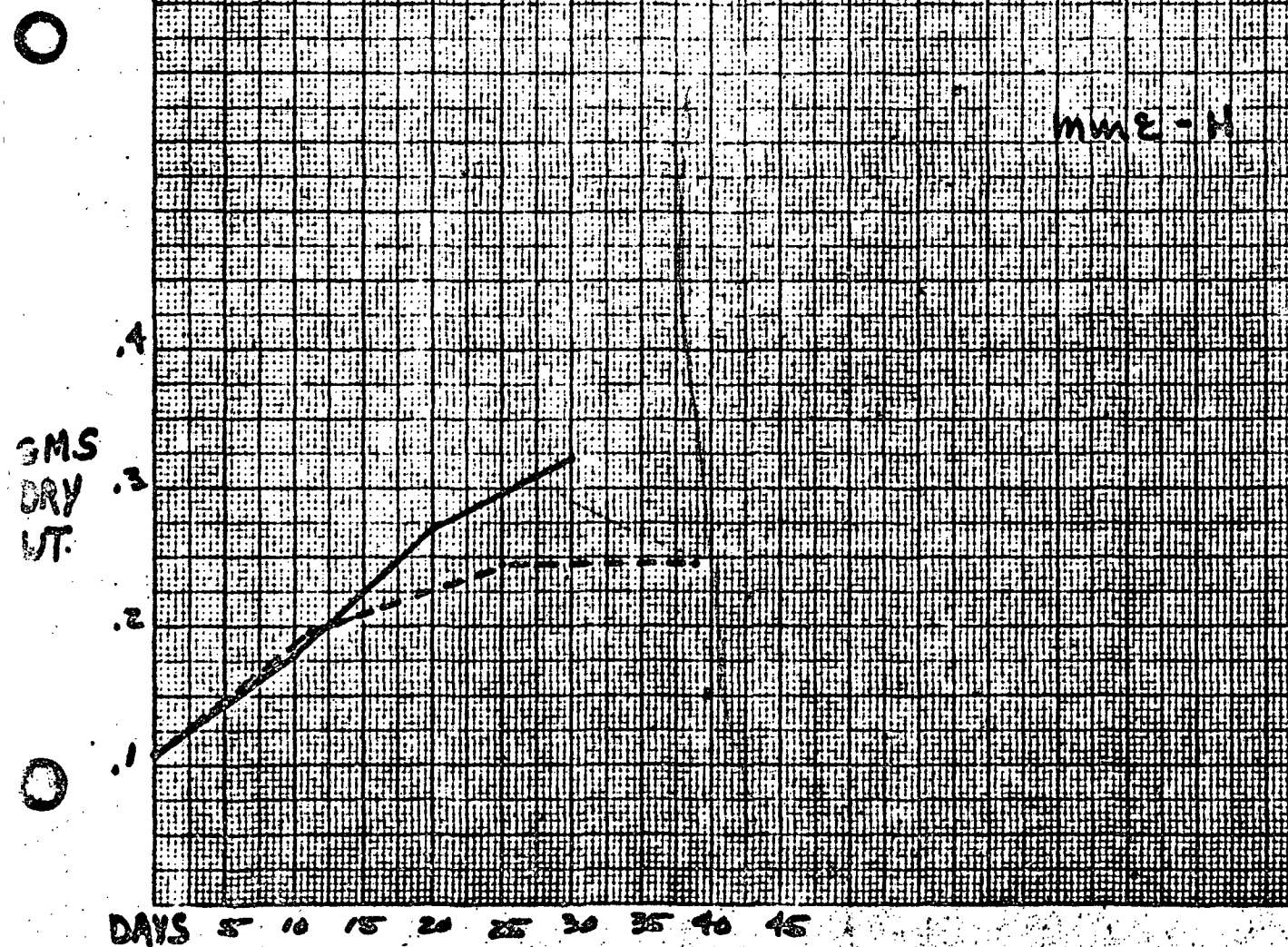
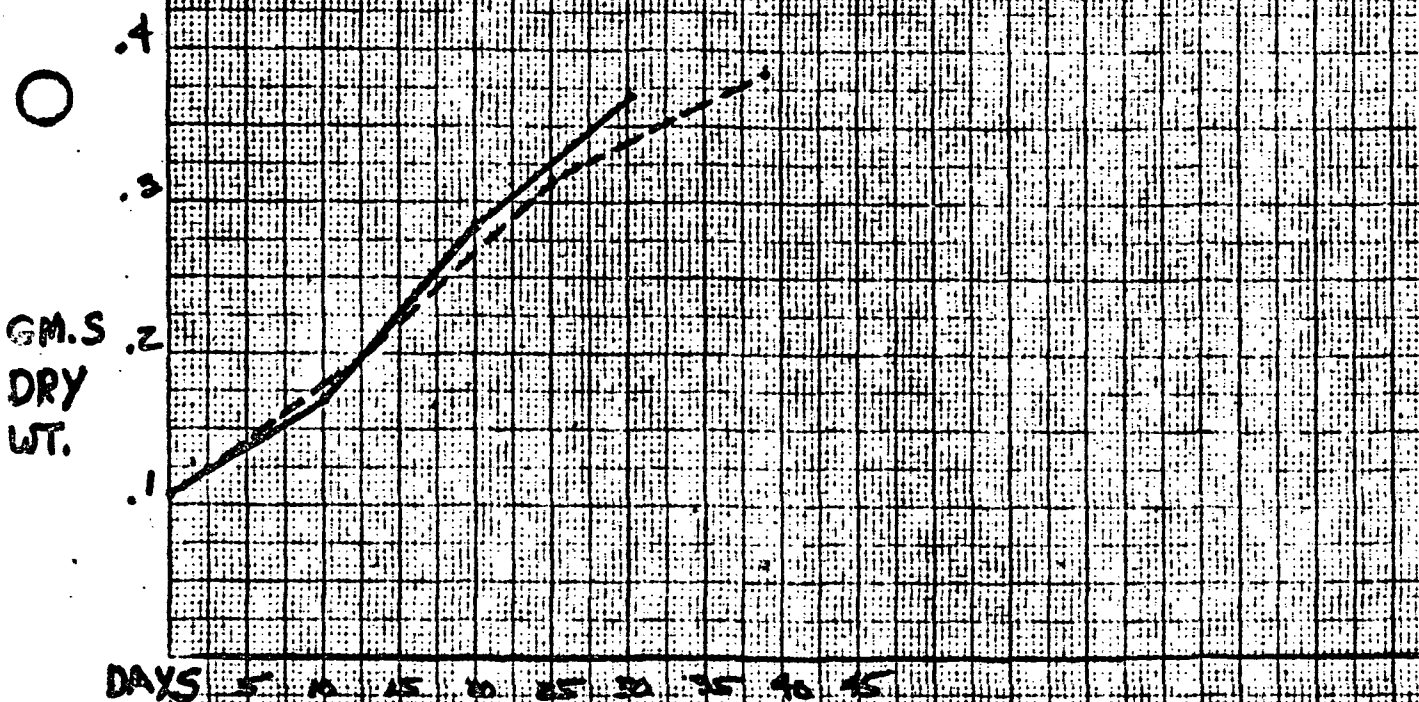


GM.S
DRY
WT.

DAYS

MME-F

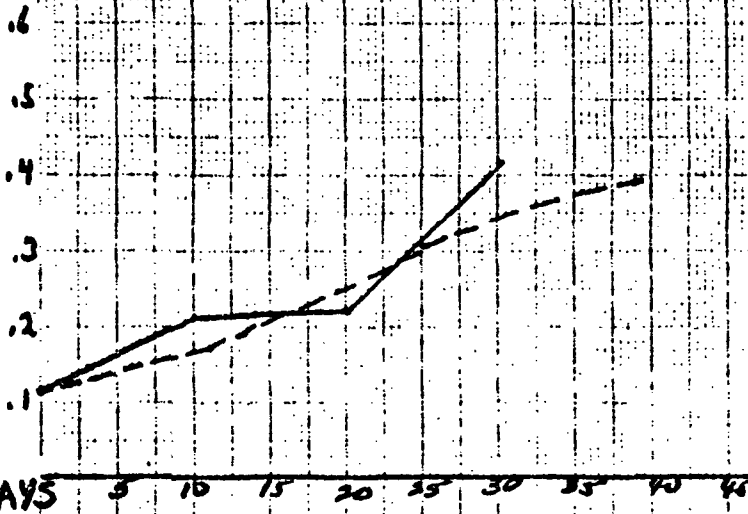




MMG-I

GM.S
DRY
WT.

DAYS



PROGRESS REPORT

June 1, 1972 - October 31, 1972

ON

N.A.S.A. CONTRACT NAS 9-10947

DEVELOPMENT OF GERM-FREE PLANTS AND TISSUE CULTURES

S. Venkateswaran
Associate Professor

March, 1973

During the months of June through October, efforts were directed toward nutritional experiments with pine tissue cultures. These experiments were based on the effects of media modification. growth rates (fresh weight and dry weight) and protein concentration of periods of growth. Included is a special report on this project.

Regular maintenance of plant tissue cultures continued. This entailed the supply to N.A.S.A. each month of media to enter into the barrier and subculture of the plant tissues there. Approximately, 500 cultures were transferred each month at N.A.S.A. alone. Back-up cultures were maintained in our laboratories for support purposes. A list of the extensive preparation of media prior to termination of contract is included in this report.

Apollo 17 Media Preparation

The following media was prepared in our laboratory for mission experiments and maintenance of the tissue cultures at N.A.S.A.

<u>Media</u>	<u># Small Jars*</u>	<u># Mason Jars*</u>	<u>Cultures</u>
Knudson's	300 (9ℓ)	150 (6ℓ)	PTA, L. Cer, March.
SV's	300 (9ℓ)	150ℓ (6ℓ)	Soy., Sun., Carrot
Heller's	100 (3ℓ)	50 (2ℓ)	Rice
MOD MUK.	100 (3ℓ)	50 (2ℓ)	Pine
MM-1A	100 (3ℓ)	50 (2ℓ)	Corn
10 X W	100 (3ℓ)	50 (2ℓ)	Tob.

* ℓ = liters

NUTRITIONAL STUDY - GROWTH OF PROTEIN DATA
ON PINUS ELLIOTTI TISSUE CULTURES

Cultures were initiated on the following medias:

AA 1 through AA 7

MM*

MM 1*

MM E-E

MME-3

The cultures were initiated on two different dates. AA 1 through AA 6 were initiated on 6/9/72, the others were initiated (AA 7, MM *, MM 1*, MME-E, and MME-3) on 6/15/72. Fifteen cultures of Pine E. were initiated. Two grams of pine were initiated on each media.

Every 5 days 2 cultures were sacrificed from each media and weighed, then assayed for the protein content. When assaying for protein, 0.5 gms of culture was taken from each culture (the rest of the culture was frozen and then freeze-dried) and combined with the 0.5 gms of the other culture of the same media then assayed.

The cultures were homogenized with 5.0 mls of 7.5 pH Tris buffer. They were centrifuged for 15 minutes at 17,500 r.p.m. The supernatant was used, 1 ml was combined with 4.0 mls of Biuret solution, was shaken, and set aside for 30 minutes. Then the samples were read on the Beckman DB at 550 mμ.

Calculations and data are on the following pages:

The dates on the following tables refer to the days the tissues were harvested. The corresponding ages of the tissues are listed below for each date.

6/14/72 5 day old cultures harvested
6/19/72 10 days old cultures harvested
6/24/72 15 days old cultures harvested
6/29/72 20 days old cultures harvested
7/ 4/72 25 days old cultures harvested
7/ 9/72 30 days old cultures harvested

6/20/72 5 day old cultures harvested
6/25/72 10 days old cultures harvested
6/30/72 15 days old cultures harvested
7/ 5/72 20 days old cultures harvested
7/10/72 25 days old cultures harvested
7/15/72 30 days old cultures harvested

6/4/72 - Growth Samples AA 1-6

Sample		fresh wt.(mg)	amt. fresh wt. used for protein(mg)	amt. frozen(mg)	dry wt. (frozen)
AA1	a	2.975	0.5	2.475	.17
AA1	b	3.18	0.5	2.680	.19
AA2	a	2.25	0.5	1.750	.14
AA2	b	1.98	0.5	1.480	.11
AA3	a	2.41	0.5	1.910	.13
AA3	b	2.625	0.5	2.125	.15
AA4	a	2.32	0.5	1.820	
AA4	b	2.165	0.5	1.665	.115
AA5	a	2.105	0.5	1.605	.11
AA5	b	2.75	0.5	2.250	.16
AA6	a	1.995	0.5	1.495	.13
AA6	b	2.315	0.5	1.815	.145
AA1	a				.20
AA1	b				.225
AA2	a				.180
AA2	b				.146
AA3	a				.163
AA3	b				.184
AA4	a				.165
AA4	b				.148
AA5	a				.143
AA5	b				.195
AA6	a				.173
AA6	b				.184

6/19/72 - Growth Samples AA 1-6

Sample	Fresh wt. (mg)	Amount Fresh wt. Used for Protein	Amount Frozen wt. (mg)	Dry wt. Frozen	Dry wt. Total
AA 1 a.	3.34	0.5	2.840	.24	.282
AA 1 b.	2.905	0.5	2.405	.18	.217
AA 2 a.	2.685	0.5	2.185	.185	.227
AA 2 b.	2.94	0.5	2.440	.205	.246
AA 3 a.	3.52	0.5	3.020	.205	.238
AA 3 b.	3.635	0.5	3.135	.185	.214
AA 4 a.	2.26	0.5	1.760	.125	.160
AA 4 b.	2.06	0.5	1.560	.11	.144
AA 5 a.	2.42	0.5	1.920	.14	.176
AA 5 b.	2.79	0.5	2.290	.195	.237
AA 6 a.	2.12	0.5	1.62	.15	.196
AA 6 b.	1.765	0.5	1.265	.11	.153

6/24/72 - Growth Samples AA 1 + AA 6

Sample	Fresh wt. (mg)	Amount Fresh wt. Used for Protein	Amount Frozen wt. (mg)	Dry wt. Frozen	Dry wt. Total
AA 1 a.	3.845	0.5	3.345	.245	.281
AA 1 b.	4.07	0.5	3.570	.25	.284
AA 2 a.	2.61	0.5	2.110	.175	.216
AA 2 b.	2.47	0.5	1.970	.165	.206
AA 3 a.	4.27	0.5	3.770	.215	.243
AA 3 b.	5.73	0.5	5.230	.27	.295
AA 4 a.	2.67	0.5	2.170	.225	.276
AA 4 b.	2.17	0.5	1.670	.135	.174
AA 5 a.	2.13	0.5	1.630	.17	.222
AA 5 b.	3.70	0.5	3.200	.215	.248
AA 6 a.	1.53	0.5	1.030	.09	.133
AA 6 b.	2.31	0.5	1.810	.15	.191

6/29/72 - Growth Samples AA 1 → AA 6

Sample	Fresh wt. (mg)	Amount Fresh wt. Used for Protein	Amount Frozen wt. (mg)	Dry wt. (mg)	Dry wt. Total
AA 1 a.	4.94	0.5	4.440	.355	.372
AA 1 b.	4.63	0.5	4.130	.31	.347
AA 2 a.	3.01	0.5	2.510	.21	.251
AA 2 b.	3.77	0.5	3.270	.26	.299
AA 3 a.	4.64	0.5	4.140	.275	.308
AA 3 b.	7.04	0.5	6.540	.28	.301
AA 4 a.	3.15	0.5	2.650	.26	.309
AA 4 b.	4.89	0.5	4.390	.295	.328
AA 5 a.	3.56	0.5	3.060	.23	.267
AA 5 b.	2.08	0.5	1.580	.16	.210
AA 6 a.	1.98	0.5	1.480	.13	.173
AA 6 b.	2.47	0.5	1.970	.17	.212

7/4/72 - Growth Samples AA 1 → AA 6

Sample	Fresh wt. (mg)	Amount Fresh wt. Used for Protein	Amount Frozen wt. (mg)	Dry wt. (mg)	Dry wt. Total
AA 1 a.	5.61	0.5	5.110	.32	.351
AA 1 b.	4.55	0.5	4.050	.37	.415
AA 2 a.	3.87	0.5	3.370	.24	.275
AA 2 b.	5.18	0.5	4.680	.305	.337
AA 3 a.	5.08	0.5	4.580	.28	.310
AA 3 b.	3.00	0.5	2.500	.20	.240
AA 4 a.	2.74	0.5	2.240	.20	.244
AA 4 b.	6.06	0.5	5.560	.33	.359
AA 5 a.	3.85	0.5	3.360	.24	.275
AA 5 b.	3.94	0.5	3.440	.25	.286
AA 6 a.	1.99	0.5	1.490	.15	.200
AA 6 b.	2.13	0.5	1.630	.165	.215

7/9/72 - Growth Samples AA 1 + AA 6

Sample	Fresh wt. (mg)	Amount Fresh wt. Used for Protein	Amount Frozen wt. (mg)	Dry wt. (mg)	Dry wt. Total
AA 1 a.	6.02	0.5	5.520	.33	.359
AA 1 b.	5.23	0.5	4.730	.345	.381
AA 2 a.	4.35	0.5	3.850	.55	.621
AA 2 b.	5.53	0.5	5.030	.36	.395
AA 3 a.	9.67	0.5	9.170	.60	.632
AA 3 b.	7.60	0.5	7.100	.92	.984
AA 4 a.	7.17	0.5	6.670	.34	.365
AA 4 b.	6.23	0.5	5.730	.34	.369
AA 5 a.	3.33	0.5	2.830	.26	.305
AA 5 b.	4.73	0.5	4.230	.275	.307
AA 6 a.	2.33	0.5	1.830	.18	.228
AA 6 b.	1.82	0.5	1.32	.13	.178

Volume in Ml's of Samples after Centrifuging

AA 7, MM*, MM1*, MME-3, MME-E

<u>5. Day Old Cultures</u>	<u>10 Day Old Cultures</u>	<u>15 Day Old Cultures</u>
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1. AA 7 = 5.1	AA 7 = 5.1	AA 7 = 5.0
2. MM* = 5.1	MM* = 5.2	MM* = 5.1
3. MM1* = 5.1	MM1* = 5.2	MM1* = 5.1
4. MME-E = 5.2	MME-E = 5.1	MME-E = 5.0
5. MME-3 = 5.2	MME-3 = 5.1	MME-3 = 5.1

<u>20 Day Old Cultures</u>	<u>25 Day Old Cultures</u>	<u>30 Day Old Cultures</u>
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1. AA 7 = 5.1	AA 7 = 5.2	AA 7 = 5.2
2. MM* = 5.2	MM* = 5.1	MM* = 5.2
3. MM1* = 5.2	MM1* = 5.1	MM1* = 5.2
4. MME-E = 5.0	MME-E = 5.1	MME-E = 5.1
5. MME-3 = 5.1	MME-3 = 5.2	MME-3 = 5.1

6/20/72 - Growth Samples AA-7, MM*, MM1*, MME-E, MME-3
(5 day old cultures)

			Fresh wt.	Amount Fresh wt. Used for	Amount Frozen wt.	Dry wt.	Dry wt.
Sample			(mg)	Protein	(mg)	(mg)	Total
1	AA 7	a.	2.29	0.5	1.790	.195	.249
		b.	2.16	0.5	1.660	.185	.240
2	MM*	a.	3.27	0.5	2.770	.195	.229
		b.	2.22	0.5	1.720	.12	.154
3	MM1*	a.	2.32	0.5	1.820	.14	.178
		b.	2.935	0.5	2.435	.16	.192
4	MME-E	a.	2.27	0.5	1.770	.11	.140
		b.	2.17	0.5	1.670	.15	.194
5	MME-3	a.	2.57	0.5	2.070	.12	.148
		b.	2.91	0.5	2.410	.13	.156

6/25/72 - Growth Samples AA 7, MM*, MM1*, MME-E, MME-3
(10 day old cultures)

			Fresh wt.	Amount Fresh wt. Used for	Amount Frozen wt.	Dry wt.	Dry wt.
Sample			(mg)	Protein	(mg)	(mg)	Total
1	AA 7	a.	1.92	0.5	1.420	.16	.216
		b.	2.43	0.5	1.930	.22	.276
2	MM*	a.	3.09	0.5	2.590	.19	.226
		b.	3.30	0.5	2.800	.215	.253
3	MM1*	a.	2.92	0.5	2.420	.175	.211
		b.	3.43	0.5	2.930	.215	.251
4	MME-E	a.	2.37	0.5	1.870	.18	.227
		b.	2.81	0.5	2.310	.16	.194
5	MME-3	a.	3.12	0.5	2.620	.155	.184
		b.	2.30	0.5	1.800	.10	.127

6/30/72 Growth Samples - AA 7, MM*, MM1*, MME-E, MME-3
(15 day old cultures)

Sample		Fresh wt. (mg)	Amount Fresh wt. Used for Protein	Amount Frozen wt (mg)	Dry wt. (mg)	Dry wt. Total
1	AA 7	a. 1.73	0.5	1.230	.13	.182
		b. 1.44	0.5	0.940	.10	.153
2	MM*	a. 3.30	0.5	2.800	.21	.247
		b. 4.26	0.5	3.760	.26	.294
3	MM1*	a. 3.16	0.5	2.660	.205	.243
		b. 3.66	0.5	3.160	.215	.248
4	MME-E	a. 2.60	0.5	2.100	.20	.247
		b. 2.41	0.5	1.910	.20	.252
5	MME-3	a. 5.68	0.5	5.180	.27	.295
		b. 5.99	0.5	5.490	.305	.332

7/5/72 - Growth Samples AA 7, MM*, MM1*, MME-E, MME-3
(20 day old cultures)

Sample		Fresh wt. (mg)	Amount Fresh wt. Used for Protein	Amount Frozen wt. (mg)	Dry wt. (mg)	Dry wt. Total
1.	AA 7	a. 1.87	0.5	1.370	.155	.210
		b. 1.82	0.5	1.320	.15	.199
2	MM*	a. 7.92	0.5	7.420	.385	.410
		b. 4.94	0.5	4.440	.28	.311
3	MM1*	a. 3.30	0.5	2.800	.19	.223
		b. 6.69	0.5	6.190	.34	.367
4	MME-E	a. 2.46	0.5	1.960	.16	.200
		b. 3.20	0.5	2.700	.23	.272
5	MME-3	a. 5.04	0.5	4.540	.25	.277
		b. 5.27	0.5	4.770	.27	.298

7/10/72 Growth Samples AA 7, MM*, MM1*, MME-E, MME-3
(25 day old cultures)

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Sample		Fresh wt. (mg)	Amount Fresh wt. used for Protein	Amount Frozen wt. (mg)	Dry wt. (mg)	Dry wt. Total
1	AA 7	a. 1.80	0.5	1.300	.15	.207
		b. 2.09	0.5	1.590	.17	.223
2	MM*	a. 6.06	0.5	5.560	.32	.348
		b. 9.40	0.5	8.900	.655	.691
3	MM1*	a. 4.89	0.5	4.390	.27	.300
		b. 4.72	0.5	4.220	.33	.368
4	MME-E	a. 1.86	0.5	1.360	.16	.218
		b. 2.86	0.5	2.360	.24	.290
5	MME-3	a. 5.94	0.5	5.440	.31	.338
		b. 7.27	0.5	6.770	.36	.386

7/15/72 - Growth Samples AA 7, MM*, MM1*, MME-E, NNE-3
(30 day old cultures)

Sample		Fresh wt. (mg)	Amount Fresh wt. Used for Protein	Amount Frozen wt. (mg)	Dry wt. (mg)	Dry wt. Total
1	AA 7	a. 2.09	0.5	1.590	.195	.255
		b. 2.03	0.5	1.530	.18	.238
2	MM*	a. 6.15	0.5	5.650	.30	.326
		b. 5.08	0.5	4.580	.25	.277
3	MM1*	a. 6.90	0.5	6.400	.985	1.061
		b. 7.59	0.5	7.090	.44	.470
4	MME-E	a. 3.63	0.5	3.130	.26	.301
		b. 2.25	0.5	1.750	.215	.275
5	MME-3	a. 5.85	0.5	5.350	.31	.338
		b. 7.394	0.5	6.894	.36	.385

Extraction Procedure

1. Take Pine E. sample (1 gram sample)
2. Use 5 mls of TRIS buffer per gram of cells.
3. Homogenize 2-3 minutes.
4. Centrifuge at 17,500 RPM's for 15 minutes.
5. Save supernatant for protein measurements.
6. Assay by Biuret method - Run in duplicate.

Dilution:

Biuret Method

1. To 1.0 mls protein sample add 4.0 mls of Biuret solution.
2. Shake well.
3. Allow to stand for 30 minutes.
4. Read at 550 mμ.

Biuret Solution

1. (a) Dissolve 1.5 gm $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (cupric sulfate)
- (b) Add 6.0 gm $\text{NaKC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ (sodium potassium tartrate) in
500 mls of H_2O
2. Add 300 mls of 10% NaOH (10 gm/100 mls)
3. Dilute to 1 liter with distilled H_2O
4. Store in a dark container.

TRIS. Buffer

0.1 M Tris. pH 7.5

1. Use 12.1 grains of Tris (m.w. 121.1)
2. Dissolve in 900 mls of (D.D.) H_2O
3. Adjust the pH with concentrated HCl to 7.5
4. Bring the total volume to 1 liter

Biuret-Standard Curve Method

1. Use a 10 mg/ml stock solution for the assay (10 mg/ml = 0.5 g/50 mls) (use BSA for protein)
2. Pipette as follows for the given concentrations:

<u>Stock Solution</u>	<u>Dist. H₂O</u>	<u>Concentration</u>
1.0 mls	0	10 mg/ml
0.8 mls	0.2 mls	8 mg/ml
0.6 mls	0.4 mls	6 mg/ml
0.4 mls	0.6 mls	4 mg/ml
0.2 mls	0.8 mls	2 mg/ml
0.1 mls	0.1 mls	1 mg/ml

3. To these add 4.0 mls of biuret solution
4. Shake well
5. Wait 30 minutes (only, no more) and read at 550 mμ
6. Plot absorbance vs mg protein/ml

Procedure for Calculations

1. Standard Curve of Biuret Method.

Ave. of Sample Absorbance - Ave. of Blank Absorbance
= Absorbance.

2. Protein Determination

(X) mls of total supernatant volume

X(X) mgs/ml Concentration

(X) mgs of total protein in 1 gram of sample

(X) grams fresh weight

(X) mgs of total protein in the sample

3. Total Dry weight Determination

$$\frac{\text{dry weight (from frozen protein)}}{\text{fresh weight- amount used for assay}} = \text{X} \frac{\text{(total dry weight)}}{\text{fresh weight}}$$

4. Percent increase of the fresh weight.

Ave of fresh weight of samples
-(X) grams (original initiated weight)

(X) answer



$$\frac{(X) \times 100}{(X) \text{ answer}} = (X)\% \text{ increase in fresh weight}$$

initial fresh weight

5. Percent increase of protein concentration.

Final protein concentration (ave.)

- Initial protein concentration (ave.)

$$\frac{\text{protein conc. (ave.)} - \text{initial protein conc. (ave.)}}{\text{initial protein conc. (ave.)}} \times 100 =$$

Percent increase of protein concentration.

Data on 2 gram Initiation Sample

Volume of Supernatant

	<u>mls</u>
1.	5.2
2.	5.0

Readings of the Initiation Samples

<u>Sample</u>	<u>Absorbance</u>	
Blank a.	.075	
b.	.075	
1. a.	.22	= .145
b.	.22	
2. a.	.19	=.115
b.	.19	

Protein Content

Total protein of 1 gm

1. 15.340 mg Ave

2. 11.250 mg Ave

Total protein of the Sample

30.680 mg Ave

22,500 mg Ave

Ave. of Total protein

26.590

Biuret Standards

Standard Curve #1

<u>Conc.</u>	<u>Absorbance</u>	<u>Ave</u>	<u>Ave. Bl.</u>
10 mg/ml	.54 .53	.535 =	.47
8 mg/ml	.455 .45	.45 =	.39
6 mg/ml	.36 .36	=	.30
4 mg/ml	.265	=	.20

Biuret Standards

Standard Curve #1 (contd.)

<u>Conc.</u>	<u>Absorbance</u>	<u>Ave</u>	<u>Ave.</u> <u>Bl.</u>
-	.265		
2 mg/ml	.165	=	.10
	.165		
1 mg/ml	.115	=	.05
	.115		
Blanks	.06		
	.06		

Biuret Standards

Standard Curve #2

<u>Conc.</u>	<u>Absorbance</u>	<u>Ave</u>	<u>Ave.</u> <u>(to be plotted)</u>
10 mg/ml	.53	=	.53
	.54		=
8 mg/ml	.45		.47
	.45		=
6 mg/ml	.36		.39
	.36		=
4 mg/ml	.26		.30
	.26		=
2 mg/ml	.165		.20
	.165		=
1 mg/ml	.11		.10
	.115	=	.11
			=
Blank	.065		.05
	.065		

6/14/72 - Protein of Samples AA 1 → AA 6 153

Total Protein in 1 gm Total Protein of the Sample

AA 1	17.550	Ave	54.001	Ave
AA 2	20.670	Ave	43.717	Ave
AA 3	22.790	Ave	57.362	Ave
AA 4	7.280	Ave	16.321	Ave
AA 5	6.240	Ave	15.144	Ave
AA 6	17.160	Ave	36.979	Ave

6/19/72 - Protein of Samples AA 1 → AA 6

Total Protein in 1 gm Total Protein of the Sample

AA 1	15.000	Ave	48.391	Ave
AA 2	20.000	Ave	56.240	Ave
AA 3	13.750	Ave	49.183	Ave
AA 4	9.500	Ave	20.520	Ave
AA 5	15.300	Ave	39.856	Ave
AA 6	9.000	Ave	17.478	Ave

6/24/72 - Protein of Samples AA 1 → AA 6

Total Protein in 1 gm Total Protein of Sample

AA 1	17.160	Ave	67.902	Ave
AA 2	16.640	Ave	42.265	Ave
AA 3	15.500	Ave	77.500	Ave
AA 4	7.540	Ave	36.493	Ave
AA 5	13.000	Ave	37.895	Ave
AA 6	10.200	Ave	19.200	Ave

Total Protein in 1 gm Total Protein of the Sample

AA 1	20.250	Ave	95.896	Ave
AA 2	16.830	Ave	57.053	Ave
AA 3	15.300	Ave	89.352	Ave
AA 4	19.000	Ave	79.380	Ave
AA 5	12.240	Ave	34.516	Ave
AA 6	9.945	Ave	22.127	Ave

6/20/72 - Protein of Samples AA 7, MM*, MM1*, MME-E, MME-3

Total Protein in 1 gm			Total Protein of the Sample	
AA 7	6.630	Ave	14.751	Ave
MM*	11.985	Ave	32.898	Ave
MM1*	10.965	Ave	28.805	Ave
MME-E	15.600	Ave	34.632	Ave
MME-3	13.520	Ave	37.044	Ave

6/25/72 - Protein of Samples AA 7, MM*, MM1*, MME-E, MME-3

Total Protein in 1 gm			Total Protein of the Sample	
AA 7	7.140	Ave	15.529	Ave
MM*	11.180	Ave	35.720	Ave
MM1*	12.220	Ave	38.798	Ave
MME-E	13.260	Ave	34.343	Ave
MME-3	10.965	Ave	29.715	Ave

6/30/72 - Protein of Samples AA 7, MM*, MM1*, MME-E, MME-3

Total Protein in 1 gm			Total Protein of the Sample	
AA 7	3.850	Ave	6.102	Ave
MM*	17.850	Ave	67.473	Ave
MM1*	9.690	Ave	33.042	Ave
MME-E	16.500	Ave	41.332	Ave
MME-3	15.300	Ave	89.275	Ave

7/5/72 - Protein of Samples AA 7, MM*, MM1*, MME-E, MME-3

Total Protein in 1 gm			Total Protein of the Sample	
AA 7	7.905	Ave	14.584	Ave
MM*	14.040	Ave	90.277	Ave
MM1*	16.120	Ave	80.519	Ave
MME-E	17.750	Ave	50.232	Ave
MME-3	14.790	Ave	76.242	Ave

7/4/72 - Protein of Samples AA 1 → AA 6

Total Protein in 1 gm			Total Protein of the Sample	
AA 1	16.640	Ave	84.531	Ave
AA 2	20.280	Ave	91.767	Ave
AA 3	11.220	Ave	45.328	Ave
AA 4	16.830	Ave	74.052	Ave
AA 5	13.260	Ave	51.647	Ave
AA 6	13.770	Ave	28.366	Ave

7/9/72 - Protein of Samples AA 1 → AA 6

Total Protein in 1 gm			Total Protein of the Sample	
AA 1	22.440	Ave	126.225	Ave
AA 2	21.930	Ave	108.334	Ave
AA 3	15.600	Ave	134.706	Ave
AA 4	15.600	Ave	104.520	Ave
AA 5	13.520	Ave	54.485	Ave
AA 6	15.810	Ave	32.805	Ave

7/10/72 - Protein of Samples AA 7, MM*, MM1*, MME-E, MME-3

Total Protein of 1 gm			Total Protein of the Sample	
AA 7	9.672	Ave	18.812	Ave
MM*	12.903	Ave	99.740	Ave
MM1*	15.300	Ave	73.516	Ave
MME-E	22.287	Ave	73.212	Ave
MME-3	13.780	Ave	91.016	Ave

7/15/72 - Protein of Sample AA 7, MM*, MM1*, MME-E, MME-3

Total Protein of 1 gm			Total Protein of the Sample	
AA 7	5.200	Ave	10.7120	Ave
MM*	13.000	Ave	72.9950	Ave
MM1*	12.220	Ave	88.5339	Ave
MME-E	16.575	Ave	48.7305	Ave
MME-3	11.985	Ave	79.3646	Ave

Percent Increase of Protein and Fresh Weight
AA 7, MM*, MM1*, MME-E, MME-3

		<u>Percent</u>
AA 7	Protein	59.7%
	Fresh Weight	7.7%
MM*	Protein	174.5%
	Fresh Weight	221.5%
MM1*	Protein	232.9%
	Fresh Weight	149.7%
MME-E	Protein	83.2%
	Fresh Weight	41.5%
MME-3	Protein	198.4%
	Fresh Weight	157.7%

**REPORT ON PLANT TISSUE CULTURES
FOR APOLLO 14 MISSION**

- A. Report -**
- B. Memo - Conditions of mission-required
plant tissue cultures - January 11, 1971**
- C. Table I: Required Plant Tissue Cultures
for Apollo 14**
- D. Table II: Needed Mission-Ready Cultures as
Replacements, Supplements or Additions**
- E. Contamination Checks #1,2,3; Methods, Dates**

**Report on Plant Tissue Culture
for Apollo 14 Mission**

By September 21, 1970, the required plant tissue cultures had been delivered to N.A.S.A. for use in the Apollo 14 mission (Table I). The cultures were prepared in pint-size Mason jars, sealed tightly with the Mason jar lids, labeled with the name of the species, the media, the number of the culture, and the last transfer date. The plant tissue was purposely used in such abundance as to cover the surface of the media in each jar. In this way, the required number of cultures (Table I) could be easily subcultured from each pint size culture; and only a few, approximately fifty, Mason jar cultures would have to go through the gas decontamination in the cabinet lines. The cultures had been through two contamination checks using thioglycolate and sabouraud media, and according to such checks had been accepted as free from bacteria and fungus. Only those cultures showing no contamination on both checks were delivered to N.A.S.A. (The data on the two contamination checks included at the end of the report).

Visual contamination later appeared in the following pint-size culture: 2-carrot, 2-todea barbara, 1-pteridium, 1-lycopodium. Except for todea, no corresponding contamination appeared in the sabouraud and thioglycolate checks. The contaminated cultures were removed from the cabinet lines. The remaining cultures were to be maintained using media prepared by the University of Houston.

Report on Plant Tissue Culture
for Apollo 14 Mission (continued)

By December, all pine cultures in the cabinet lines were dead, the Marchantia was dead. Haplopappus was no longer green, but rather tan-colored. According to Dr. C. Walkinshaw and Jo Ann Fowler the condition of the cultures was as follows:

<u>in acceptable condition</u>	<u>to be replaced</u>	<u>to be supplemented</u>	<u>to be added</u>
Soybean	<u>Marchantia</u>	Haplopappus	Carrot
Sunflower	<u>Pinus elliotii</u>	Lettuce	Corn
Okra	<u>Pinus lambertiana</u>	D. Carrot	Euphorbia
Rice	<u>Pinus palustris</u>	PTA	
Tobacco		TDB	
		L. Cer	

The needed cultures were put through a contamination check (#3) with sabouraud and thioglycolate media and were prepared for delivery to N.A.S.A. by the deadline set for January 11, 1971. On Thursday, January 7, 1971, a postponement on the primary mission tissue cultures was decided upon. This included carrot, corn, euphorbia, and pinus elliotii - all of which had already been prepared. Thus, the delivery made on January 11, 1971, included only the culture needed for the west-side cabinet lines in R 1-104 of the L.R.L. A report (memo) was submitted to Dr. Walkinshaw from Jo Ann Fowler on the conditions existing at that time. (Refer to respective report: Mission-Required Plant Tissue Cultures).

Report on Plant Tissue Culture
for Apollo 14 Mission (continued)

Subsequently, a second deadline was set for January 25, 1971 for carrot, euphorbia, and pinus elliotii - all to enter the east-side cabinet lines. The following were delivered to N.A.S.A. on January 24, 1971 to meet the deadline:

<u>species</u>	<u>media</u>	<u>number</u>
Carrot	SV	40
Euphorbia	SV	40
<u>Pinus elliotii</u>	MM-1	40

All cultures were as follows:

1. Sealed with Mason jar lids.
2. Labeled on lid for maintenance purposes (name of culture, number of culture for contamination check, last transfer date).
3. Without white gum label due to request by Dr. Walkinshaw.
4. Except for carrot, all cultures presently undergoing their contamination check.

Therefore:

1. Lids on carrot cultures should be removed as soon as gas decontamination is terminated.
2. Lids on Euphorbia and Pinus eliotii should remain sealed until termination of contamination check.

M E M O

Dr. C. H. Walkinshaw

January 11, 1971

All cultures are as follows:

1. Freshly transferred in 1/2 pint Mason jars.
2. Sealed with Mason jar lids.
3. Labeled with white gum labels for photography purposes (name of culture and media).
4. Labeled on lid for maintenance purposes (name of culture, number of culture for contamination check, last transfer date).
5. Presently undergoing their contamination check.

Therefore:

1. D. Carrot, haplopappus, lettuce, pinus lambertiana, and pinus lambertiana, and pinus palustris will need transferring after approximately four weeks from last transfer date.
2. Marchantia, pteridium, and lycopodium will not need any transferring before lunar treatments.
3. Todea should supplement the Todea in the cabinet lines to be subcultured into forty cultures. Only one Todea culture of sixty tested was free of contamination by required checks. It is now ready for subculturing.
4. Except for Todea culture jar, no Mason jar seal should be broken until the contamination check is complete. If any contamination appears either in the checks at the University of Houston or visually in the culture, then the respective Mason jar can be discarded from the cabinet lines.
5. The pteridium and lycopodium may be removed from the cabinet lines and returned to Jo Ann Fowler to be replaced, rather than supplemented, with the newly-delivered cultures.
6. The Okra from the east side of room 104 needs to be sealed properly, moved to the next side, and subcultured to give twenty good quality cultures.
7. The lettuce from the east side of room 104 should be removed from the cabinet line and returned to Jo Ann Fowler.

- 3 -

M E M O

Dr. C. H. Walkinshaw

January 11, 1971

8. The haplopappus on the east side of room 104 will be either moved to the west side or returned to Jo Ann Fowler, depending on the decision of Dr. Walkinshaw.
9. After contamination check is complete, the Mason jar lids will be removed from the cultures and replaced with polypropylene.

Respectfully,

Jo Ann Fowler

Jo Ann Fowler

CPW

JAF:ec

TABLE I. Required Plant Tissue Culture for Apollo 14

<u>Species</u>	<u>Media</u>	<u>Number Prepared</u>	<u>Required Number of Cultures</u>
Soybean	SV	3	40
Sunflower	SV	3	40
Carrot	SV	3	40
Okra	SV	3	40
Lettuce	SV	2	20
Euphorbia	SV	0	20
Haplopappus	SV	2	20
D. Carrot	SIK	2	20
Hab. tobacco	10XW	3	40
Rice	HELLER'S	3	40
Corn	1XW	0	40
Pine L.	MM-1	2	20
Pine E.	MM-1	2	40
Pine P.	MM-1	2	20
TDB	K	3	40
PTA	K	3	40
L Cer	K	2	40
Marchantia	V-5	3	40

NOTE:

1. Euphorbia and corn were not ready for entry into the cabinet lines.
2. Requirement changes were made at a later date:

<u>Species</u>	<u>Required Number of Cultures</u>
Okra	20
Euphorbia	40
Marchantia	80

Report on Plant Tissue Cultures
for Apollo 14 Mission (continued)

TABLE I. Needed Mission-Ready Cultures as Replacements,
Supplements, or Additions

<u>Species</u>	<u>Media</u>	<u>Numbers</u>
Carrot	SV	40
Corn	1XW	40
Euphorbia	SV	40
Haplopappus	SV	20
Lettuce	SV	20
D. Carrot	SIK	20
PTA	K	40
TDB	K	40
L Cer	K	40
Marchantia	V-5	80 (two age groups)
Pinus elliotii	MM-1	40
Pinus L.	MM-1	20
Pinus P.	MM-1	20

Contamination Check #1

(August, 1970)

Each culture selected for subculturing was checked with thioglycolate and sabouraud. Test tubes of the contamination check media were prepared at N.A.S.A. and given to the University of Houston (Jo Ann Fowler). Two samples from each selected culture were taken, one of which was placed in thioglycolate and the other of which was placed in sabouraud. Ten days were allowed for contamination to appear. If no contamination appeared, the subcultures from the originally selected culture were considered germ-free by test and mission-ready. If contamination did appear, the respective subcultures were discarded.

Report on Plant Tissue Cultures
for Apollo 14 Mission (continued)

Contamination Check #1

Data

Name of Culture	<u>Hab</u>	<u>Sun</u>	<u>Soy</u>	<u>Hap</u>	<u>Rice</u>	<u>Carrot</u>	<u>Tomato</u>	<u>PTA</u>	<u>TDB</u>	<u>L Cer</u>	<u>Marchantia</u>
Number of cultures used for sub-culturing	8	9	9	2	24	23	11	22	21	21	8
Number of cultures contaminated in sab.	0	1	0	1	1	1	0	3	11	1	0
% contamination in sab.	0	11	0	50	42	4.3	0	13.6	52.4	4.8	0
Number of cultures contaminated in thio.	0	2	1	1	2	3	0	0	9	0	0
% contamination in thio.	0	22	11	50	8.3	13	0	0	42.8	0	0
Total % contamination	0	22	11	50	8.3	13	0	13.6	52.4	4.8	0

NOTE: 1. Corn, euphorbia, and lettuce were not ready for testing.

2. The above data was not obtained on all pine species, okra, and ... since the contamination check test tubes were discarded at ... noting the number of cultures used for subculturing.

3. All cultures showing contamination were discarded.

**Report on Plant Tissue Cultures
for Apollo 14 Mission (continued)**

Contamination Check #2

(September, 1970)

Method:

Only those cultures shown to be germ-free by first contamination check were used in this test. In this case several selected cultures were placed together in a pint-size Mason jar. Again, two samples from each selected culture were taken, one placed in sabouraud, and the other in thio-glycolate. Ten days time allowance was given for contamination to appear. If so, the respective culture was discarded. If not, the culture was considered germ-free by test and mission-ready.

After this test, the cultures were placed in the cabinet bins.

**Report on Plant Tissue Cultures
for Apollo 14 Mission (continued)****Contamination Check #2**

<u>Species tested</u>	<u>Percent Contamination in</u>	
	<u>Sabouraud</u>	<u>Thioglycolate</u>
Hab	0	0
Rice	0	0
Soybean	0	0
Sunflower	0	0
Haplopappus	0	0
D. Carrot	0	0
Carrot	0	0
Okra	0	0
Pine E.	0	0
Pine L.	0	0
Pine P.	0	0
Lettuce	0	0
PTA	0	0
TDB	66	66
L Car	0	0

1. Corn and euphorbia were not ready for testing.
2. Lettuce was included for the first time.
3. Tomato was discarded from the mission program.

**Report on Plant Tissue Cultures
for Apollo 14 Mission (continued)**

Contamination Check #3

Method:

Each culture selected for subculturing was checked with thioglycolate and sabouraud or try. Test tubes of the contamination check media were prepared at the University of Houston. Two samples from each new subculture were taken, one of which was placed in thioglycolate and the other of which was placed in sabouraud. Ten days was allowed as the incubation period at room temperature. If any contamination appeared in the test tubes, the respective culture was discarded from the program.

Contamination
check #3 - contd. -
Data:

Name of Culture	L Cor	FW	TDB	Hap	Lettuce	C. Prof.	W. P.	Pine L.	Pine E.	Euph	B. Corrob
Number of new subcultures	60	60	60	30	30	60	30	80	60	60	30
Number of cultures contaminated in sab	0	5	59	25	0	0	2	12	0	60	1
% contaminated in sab	0	8.3	98.3	83.3	0	0	22	15	0	100%	3.3
Number contaminated in this	0	8	59	30	0	2	10	20	0	60	1
% contaminated in this	0	13.3	98.3	100%	0	3.3	25.3	35	0	100%	3.3
Total % contamination	0	13.3	98.3	100%	0	3.3	33.3	35	0	100%	3.3

A repeat contamination check had to be done on more haploppus, euphorbia, pinus lambertiana, and todea data:

Name of Culture	Hap	Euph	Pine L.	TDB
Number of new subcultures		60	30	30
Number of cultures contaminated in sab	0	0	0	0
% contaminated in sab	0	0%	0	0
Number contaminated in this	0	0	0	0
% contaminated in this	0	0%	0	0
Total % contamination	0	0%	0	0

Note: Incubation of Euphorbia, TDB, Pine L. and Pine E. is still in progress. A memo will be submitted at the end of the period to verify the data.

REPORT OF STUDIES ON
MARCHANTIA POLYMORPHA

MEDIA PROTOCOLS FROM
FIRST STUDY OF MARCHANTIA POLYMORPHA

Report on Marchantia Polymorpha

Nutritional studies of Marchantia polymorpha were carried out in order to improve the growth of the plant on the culture medium, and subsequently to use during Apollo 14 the medium found to be optimal for growth.

Correspondence was held with Dr. Morton W. Miller at Rochester, New York. Using his suggestions, the following media were employed into the study:

1. Voth medium
2. Voth minus sucrase
3. Voth #1 minus sucrase (this has #5 solution for the microelements according to Voth's paper)
4. Voth #2 (same as Voth #1 with $\frac{1}{2}$ (Ca(NO₃)), $\frac{1}{2}$ (Mg(NO₂)), $\frac{1}{2}$ (Mg(SO₄)).
5. Hoagland medium
6. Hoagland minus sucrose
7. Miller's Voth

Note: Protocols for each media are submitted at the end of this report.

Refer to respective media protocols.

This growth study began on September 23, 1970. Differences have been noted in the cultures to the present date. The fastest growing and the greenest (by visual observation) were those grown on Miller's Voth and Voth #1. Most of the gemmae put on Voth #2 and Voth did not germinate. These cultures were discarded after two weeks. Hoagland minus sucrose

maintained healthy green growth while Hoagland's medium (with sucrose) resulted in the typical reddening of the plant. Voth minus sucrose was similar to Hoagland's minus sucrose. Of all the cultures, now four months old, those that appear healthy are growing on Voth minus sucrose, Voth #1, Hoagland minus sucrose, and Miller's Voth.

A second study on Marchantia polymorpha was initiated on November 2, 1970 with the assistance of Dr. Miller visiting the University of Houston laboratory. It was found that errors existed in molar concentrations of the salts used in the media in the first study. These concentrations were corrected, and the following media were tested:

1. Miller's Voth #5 (from Miller's bottles)
2. Miller's Voth #5 plus NH_4NO_3
3. Voth #5 (newly made at the University of Houston Lab.)
4. Voth (old formula, pH not adjusted)
5. Voth (old formula, pH adjusted = Voth minus sucrose in first study)
6. Voth (old formula plus microelements = Voth #1 in first study)

Note: Refer to respective media protocols.

After two weeks, the largest growth was seen in Miller's Voth #5 plus NH_4NO_3 and Voth #1. The gemmae in all the cultures had germinated and begun to grow. No distinction could be seen between those differing in pH adjustment only.

Growth has continued very well, reddening appearing in no cultures even after three months. The primary difference

maintained healthy green growth while Hoagland's medium (with sucrose) resulted in the typical reddening of the plant. Voth minus sucrose was similar to Hoagland's minus sucrose. Of all the cultures, now four months old, those that appear healthy are growing on Voth minus sucrose, Voth #1, Hoagland minus sucrose, and Miller's Voth.

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5. Voth (old formula, pH adjusted = Voth minus sucrose in first study)
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Note: Refer to respective media protocols.

After two weeks, the largest growth was seen in Miller's Voth #5 plus NH_4NO_3 and Voth #1. The gemmae in all the cultures had germinated and begun to grow. No distinction could be seen between those differing in pH adjustment only.

Growth has continued very well, reddening appearing in no cultures even after three months. The primary difference

in all the cultures after this period of time is size. Maintenance of the green thalli and continued increase in size can be presently observed in all cultures.

For mission use, it was decided to grow and maintain *Marchantia* on Miller's Voth #5 plus NH_4NO_3 . For simplicity, this medium is now referred to as Voth #5. All other stock solutions and media have been discarded totally.

VOTH MEDIUM

TISSUES CULTURED ON MEDIUM: MARCHANTIA

pH 6.5 - 7.5

	<u>Major Solution</u>	<u>Stock Solution, gm/liter</u>	<u>Stock Solution ml/liter of medium</u>
(1)	KNO ₃	50.56	1.66
(2)	Ca (NO ₃) ₂	164.16	1.44
(3)	Mg (NO ₃) ₂	148.41	1.2
(4)	KH ₂ PO ₄	68.04	0.8
(5)	Mg SO ₄	120.50	1.6
(6)	Sucrose	20	
(7)	Agar	10	
	H ₂ O	to make 1 liter	

Reference:

Voth, Paul D. Effects of Nutrient-Solution Concentration on the Growth of Marchantia Polymorpha. The Botanical Gazette. 104, No. 4, 591 - 601.

NOTE: Same protocol without sucrose is the second media listed.

VOTH #1 MEDIUM

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TISSUE CULTURE ON MEDIUM: MARCHANTIA

pH = 5.9

<u>Major Solution</u>	<u>Stock Solution gm/liter</u>	<u>Stock Solution ml/liter of medium</u>	<u>Small concentration mg/liter</u>
(1) K NO ₃	50.56	1.66	
(2) Ca(NO ₃) ₂	164.16	1.44	
(3) Mg(NO ₃) ₂	148.14	1.20	
(4) K H ₂ PO ₄	68.4	0.8	
(5) Mg SO ₄	120.50	1.60	

<u>Minor Solution</u>			
(6) Mn SO ₄ · H ₂ O	.224 g/l	1.0	0.22
(7) Zn Cl ₂	.2 g/l	1.0	0.2
(8) Na ₂ B ₄ O ₇	.2 g/l	1.0	0.2
(9) FeSO ₄	.02 g/l	1.0	0.02
(10) Agar	10 g/l		10

H₂O to make 1 liter

VOTH #2 MEDIUM

TISSUE CULTURE ON MEDIUM: MARCHANTIA

pH = 5.9

<u>Major Solution</u>	<u>Stock Solution gm/liter</u>	<u>Stock Solution ml/liter of medium</u>	<u>Final concentration mg/liter</u>
(1) K NO ₃	50.56	1.66	
(2) Ca (NO ₃) ₂	164.16	.72	
(3) Mg (NO ₃) ₂	148.14	.60	
(4) K H ₂ PO ₄	68.04	.80	
(5) Mg SO ₄	120.50	.80	

Minor Solution

(6) Mn SO ₄ · H ₂ O	.224 g/l	1.0	0.22
(7) Zn Cl ₂	.2 g/l	1.0	0.2
(8) Na ₂ B ₄ O ₇	.2 g/l	1.0	0.2
(9) Fe SO ₄	.02 g/l	1.0	0.02
(10) Agar	10 g/l		10

H₂O to make 1 liter

HOAGLAND'S MEDIUM ($\frac{1}{4}$ STRENGTH)TISSUES CULTURED ON MEDIUM: MARCHANTIA

pH 6.5-7.5

<u>Major Solution</u>	<u>Stock Solution, gm/liter</u>	<u>Stock Solution ml/liter of medium</u>	<u>Final Concentration mg/liter</u>
(1) $\text{Ca}(\text{NO}_3)_2$	164	1.25	205
(2) KNO_3	101	1.25	126.25
(3) KH_2PO_4	136	0.5	68
(4) MgSO_4	120	0.25	40
(5) $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	1.0	1.0	1.0
<u>Minor Solution</u>			
(6) H_3BO_3	2.86		2.86
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	1.81	1.0	1.81
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.22		0.22
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.08		0.08
$\text{Na}_2\text{MoO}_4 \cdot \text{H}_2\text{O}$	0.02		0.02
(7) Sucrose	20		20
(8) Agar	10		10
H_2O			to make 1 liter

NOTE: this same protocol without sucrose is listed as #4 in the report.

MEDIA PROTOCOLS FROM
SECOND STUDY OF MARCHANTIA POLYMORPHA

VOTH PLUS MICRONUTRIENTS

(old formula - 5/27/1970, same as VOTH #1)

	<u>Major Solution</u>	<u>Stock Solution</u> <u>gm/l</u>	<u>Stock Solution</u> <u>ml/l of medium</u>	<u>Final</u> <u>Concentration</u> <u>mg/l</u>
(1)	KNO ₃	50.56	1.66	
(2)	Ca(NO ₃) ₂	164.16	1.44	
(3)	Mg(NO ₃) ₂	148.14	1.20	
(4)	KH ₂ PO ₄	68.04	0.8	
(5)	MgSO ₄	120.50	1.60	
	<u>Minor Solution</u>			
(6)	MnSO ₄ ·H ₂ O	0.224	1.0	0.22
(7)	ZnCl ₂	0.2	1.0	0.2
(8)	Na ₂ B ₄ O ₇	0.2	1.0	0.2
(9)	FeSO ₄	0.02	1.0	0.02
(10)	Agar	10		1%

H₂O up to 1 liter

no pH adjustment

VOTH (old formula 5/27/1970)
minus sucrose

<u>Major Solution</u>	<u>Stock Solution</u> <u>gm/l</u>	<u>Stock Solution ml/l</u> <u>of Medium</u>
(1) KNO_3	50.56	1.66
(2) $\text{Ca}(\text{NO}_3)_2$	164.16	1.44
(3) $\text{Mg}(\text{NO}_3)_2$	148.41	1.2
(4) KH_2PO_4	68.04	0.8
(5) MgSO_4	120.50	1.6
(6) Agar	10 g/l	

pH not adjusted
 H_2O up to 1 liter

or

pH adjusted to 7.0
with 1 N NaOH (2 drops)

REPORT OF STUDIES ON
ZEALAND

Report on Zea Mays

Attempts have been made at reproducing work that led to the callus formation of Zea mays in cultures. After discussions including Walter Horn, Dr. C. Walkinshaw, Dr. S. Venketeswaran and Jo Ann Fowler, it was decided that various modifications of White's medium should be tested for their ability to establish callus growth of the corn.

Seeds were (Hybrid Field Corn, variety A204-MF 3) sterilized under normal conditions using 10% chlorox. The sterile seeds were then placed on agar-medium containing only the major and minor elements found in White's IX protocol. Germination occurred in two days and within a week there were large root systems and stems. Both root and stem explants were aseptically placed on the following test medium:

WHITE'S 1 X

WHITE'S #1

WHITE'S #2

WHITE'S #3

WHITE'S #4

These differ in concentration of 2,4-D, NAA, coconut milk, vitamins and amino acids.

NOTE: Refer to media protocols.

After four months there was no callus formation on White's media #1-4. Here the explants either swelled and died or grew extension root systems. However, the root explants placed on

White 1 X showed small callus formation at the nodules. These small callus areas have been placed on fresh White 1 X media in order to establish more growth. Results show growth to be minimal. Further attempts at establishing callus corn cultures are being continued.

WHITES MEDIUM #1

<u>Elements</u>		<u>Stock Solution g/l</u>	<u>Stock Solutions ml/l of medium</u>	<u>Final Concentration mg/l</u>
(1)	Ca(NO ₃) ₂	2.0		200
	Na ₂ SO ₄	2.0		200
	KNO ₃	0.8	100	80
	KCl	0.65		65
	NaH ₂ PO ₄	0.165		16.5
(2)	MgSO ₄	36.0	10	360
(3)	MnSO ₄	0.45		4.5
	ZnSO ₄	0.15		1.5
	H ₃ BO ₃	0.15	10	1.5
	KI	0.075		0.75
	CuSO ₄	0.002		0.02
	Na ₂ MoO ₄	0.021		0.21
(4)	Fe ₂ (SO ₄) ₃	0.25	10	2.5
(5)	Glycine	0.3		3.0
	Thiamine	0.01	10	0.1
	Pyridoxine	0.01		0.1
	Nicotinic Acid	0.05		0.5
(6)	2,4-D	.214	suggested 1 ml	1 x 10 ⁻⁶ M
(7)	NAA	.186	by Horne/SV 1 ml	1 x 10 ⁻⁶ M
(8)	Coconut milk		100 ml/l	
(9)	Sucrose			20 g/l
(10)	Agar			10 g/l

White's Medium #2

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(BBL No. 71-073R)

pH = 4.6-5.0

<u>Elements</u>		Final Concentration mg/l	
Ca (NO ₃) ₄		200	
MgSO ₄ · 7H ₂ O		360	
Na ₂ SO ₄	100	200	
KNO ₃		80	
KCl		65	
NaH ₂ PO ₄		16.5	
KI		0.75	
Fe(SO ₄) ₃ · 6H ₂ O		2.5	
MnSO ₄ · H ₂ O		4.5	
ZnSO ₄		1.5	
H ₃ BO ₃		1.5	
Glycine	0.3	3.0	
Thiamine	0.01	0.1	
Niacin	0.01	0.5	
Pyridoxine	0.05	0.1	
Sucrose		20 g/l	
2,4-D	.214 g/l	1 ml	1 x 10 ⁻⁶ M
NAA	.186 g/l	as suggested by Horne/SV 1ml	1 x 10 ⁻⁶ M
Coconut milk		10%	100 ml/l
Agar		10 g/l	

Reference: tissue culture - A Manual of Materials and Methods.
Bio. guest Division p. 25

WHITE'S MEDIUM #3

pH 6.1 - 7.0

	<u>Major Solution</u>	<u>Stock Solution g/l</u>	<u>Stock Solution ml/l of medium</u>	<u>Final Concentration mg/l</u>
(1)	Ca(NO ₃) ₂	6.0		300.0
	Na ₂ SO ₄	4.0	5.0	200.0
	KNO ₃	1.6		80.0
	KCl	1.3		65.0
	NaH ₂ PO ₄ ·H ₂ O	0.33		16.5
	MgSO ₄ ·7H ₂ O	14.4		720.0
	<u>Minor Solution</u>			
(2)	ZnSO ₄ ·7H ₂ O	3.0		3.0
	MnSO ₄ ·4H ₂ O	7.0		7.0
	H ₃ BO ₃	1.5	1.0	1.5
	KI	0.75		0.75
	CuSO ₄ ·5H ₂ O	0.001		0.001
	Na ₂ MoO ₄ ·2H ₂ O	0.25		0.25
(3)	FeCl ₃	1.5	1.0	1.5
(4)	2,4-D	1.0	1 ml	1.0
(5)	NAA	1.0	1 ml	1.0
(6)	Sucrose	20		2%
(7)	Agar	10		1%

Reference: Protocol in N.A.S.A. contract minus vitamins, amino acids, coconut milk, yeast extract and IAA.

WHITE'S MEDIUM #4

pH 6.1 - 7.0

	<u>Major Solution</u>	<u>Stock Solution g/l</u>	<u>Stock Solution ml/l of medium</u>	<u>Final Concentration mg/l</u>
(1)	Ca (NO ₃) ₂ ·4H ₂ O	6.0		300.0
	Na ₂ SO ₄	4.0		200.0
	KNO ₃	1.6	50.0	80.0
	KCl	1.3		65.0
	NaH ₂ PO ₄ ·H ₂ O	0.33		16.5
	MgSO ₄ ·7H ₂ O	14.4		720.0
	<u>Minor Solution</u>			
(2)	ZnSO ₄ ·7H ₂ O	3.0		3.0
	MnSO ₄ ·4H ₂ O	7.0		7.0
	H ₃ BO ₄	1.5	1.0	1.5
	KI	0.75		0.75
	CuSO ₄ ·5H ₂ O	0.001		0.001
	Na ₂ MoO ₄ ·2H ₂ O	0.25		0.25
(3)	FeCl ₃	1.5	1.0	1.5
(4)	2,4-D	.214 g/l	1.0	1 x 10 ⁻⁶ M
(5)	NAA	.186 g/l	1.0	1 x 10 ⁻⁶ M
(6)	Sucrose	20		2%
(7)	Agar	10		1%

Reference: Protocol in N.A.S.A. contract minus vitamins, amino acids, coconut milk, IAA, and yeast extract, and with changes of concentrations in 2,4-D and NAA.