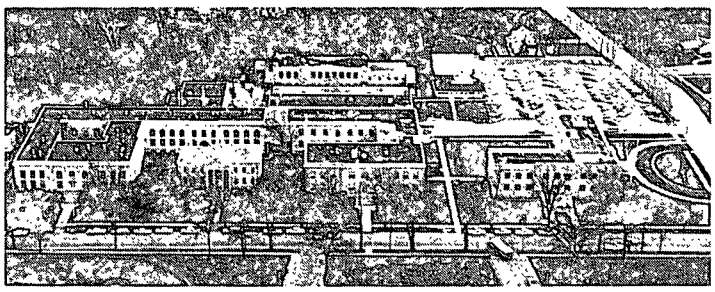


THE INSTITUTE OF PAPER CHEMISTRY, APPLETON, WISCONSIN

P A C

FOREST GENETICS

RESEARCH COMMITTEE MEETING

HANDOUTS

October 4-5, 1984

TABLE OF CONTENTS

PAC RECOMMENDATIONS, PART I

Procedural Issues

1. Acknowledge PAC recommendations
2. Itemize PAC recommendations and respond to them at the beginning of each review meeting
3. Compare performance against research plans
4. Give hypothetical basis before presenting data on an experiment

PAC RECOMMENDATIONS PART I (continued)

5. Explain statistical tests and confidence limits used to evaluate data
6. Do not present results from questionable experiments
7. Employ multiple range tests to compare treatment means
8. Supplement polyamine work with additional staff activity

PAC RECOMMENDATIONS, PART II

Technical Issues

1. Agressively publish technical progress in refereed journals
2. Make presentations at scientific and technical meetings.
3. Establish dialogue with nationally recognized institutions
4. Establish two-way exchange with staff at NC State

PAC RECOMMENDATIONS, PART II (continued)

5. Continue conventional forest genetics and silviculture as part of the IPC Funded Research Program

6. Review Project 3501 (Exploratory Research) elsewhere

7. Re-examine the hypotheses underlying the model systems approach. Additionally review our data to determine if we are using the best system available

8. Bolster work on polyamines - maintain leadership role in this area

PAC RECOMMENDATIONS, PART II (continued)

9. Employ the most reliable and sensitive analytical procedures available -- if endogenous growth regulator work is initiated

10. Limit our synthetic growth regulator research to a few instructive representative types

11. De-emphasize Objective III and IV work

12. Examine biochemical and genetic rationale associated with the use of isozymes to determine relatedness in plants

FOUR PAPERS PUBLISHED IN LAST FIVE MONTHS

- Monroe & Johnson
  Membrane bound-o-methyltransverase from Douglas-fir needle callus. (Phytochemistry).

- Feirer, Mignon and Litvay
  ADC and polyamines required for embryogenesis in wild carrot. (Science).

- Feirer, Mignon and Wann
  Effect of spermidine synthesis inhibitors on in vitro plant development. (Plant Physiology).

- Einspahr
  Tissue culture in forestry, current status. (Proc. SAF Tech. Conf.).

TWO PAPERS "IN PRESS"

- Einspahr, Litvay, Johnson and Feirer
  Challenges of somatic embryogenesis in conifer tissue culture. (Proc. International Symposium of Recent Advances in Forest Biology).

- Litvay
  The Institute of Paper Chemistry approach to propagation of forest trees using somatic embryogenesis. (Proc. TAPPI R&D Divison Conf., 1984).

TWO PAPERS "SUBMITTED FOR PUBLICATION"

- Feirer, Wann and Einspahr
  Effect of spermidine synthesis inhibitors on plant development. (Plant Growth Regulation).

- Wann and Einspahr
  Reliable plant formation from seedling explants of Populus tremuloides. (Canadian Journal of Forest Research).

CONIFER TISSUE CULTURE RESEARCH

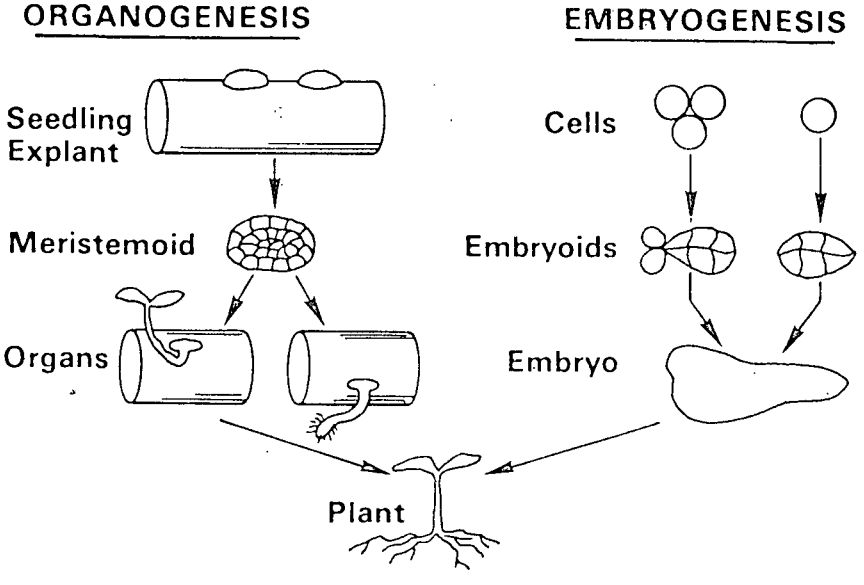
Project Goals and Objectives

- Overall - Mass production of conifer hybrids
- Near-term - Plantlets from single cells or small groups of cells

Approach

- Somatic embryogenesis

MORPHOGENESIS



Advantages of Somatic Embryogenesis

- Provides a method of greatly increasing plantlet numbers
- Opens the way for efficient use of genetic engineering techniques

MODEL SYSTEM APPROACH -- REQUIREMENTS

- Test organism that is capable of undergoing somatic embryogenesis
- Numerous similarities between dissimilar organisms
- Study "the" system histologically and biochemically
- Compare model system with systems that do not work

MODEL SYSTEM APPROACH -- METHODS

- Perturb the system and observe results

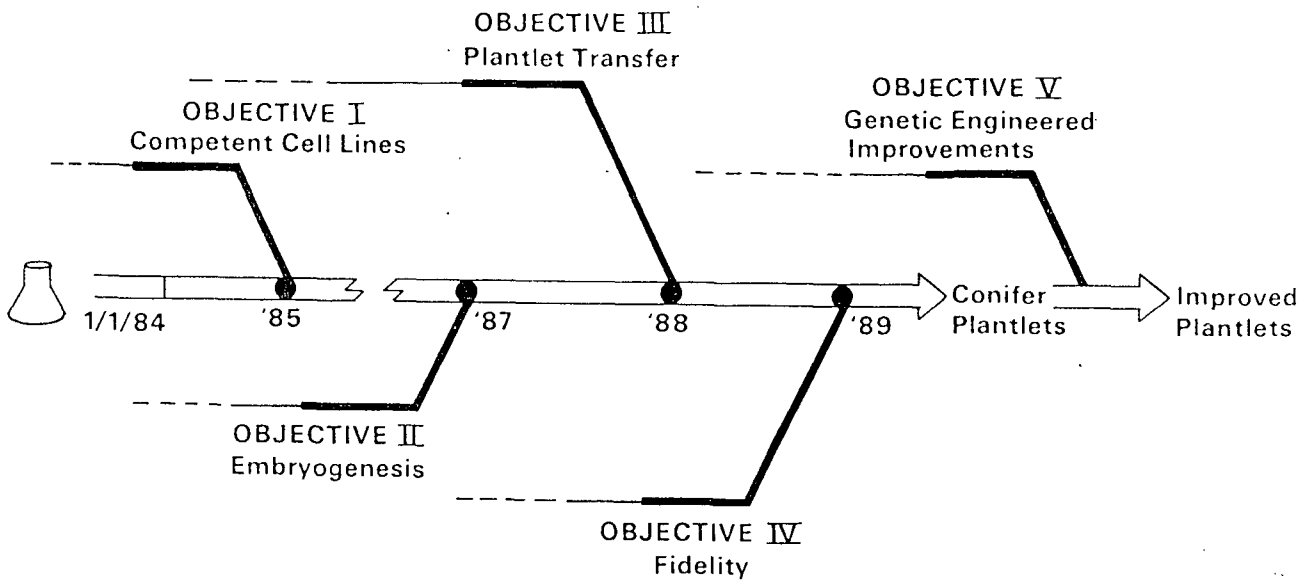
  (1) Embryogenesis
  (2) Histological markers
  (3) Biochemical markers

- Try treatments and observe results
- Modify treatments for conifers based upon dissimilarities

MODEL SYSTEM APPROACH -- ALLOWS

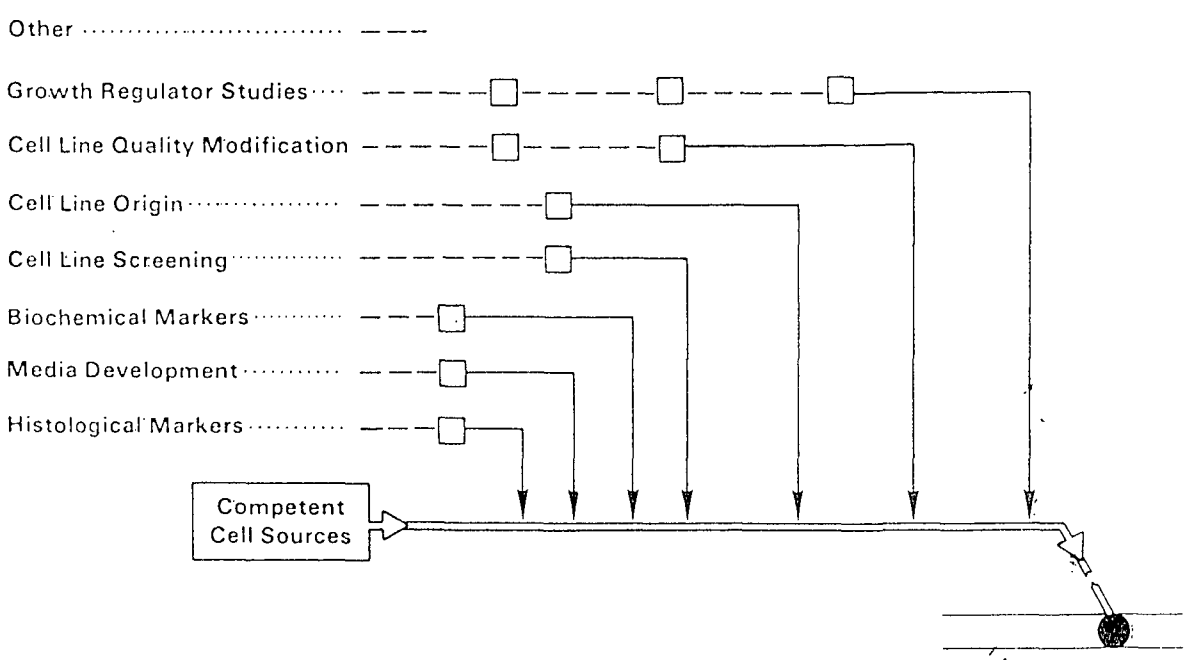
- Systematic evaluation of factors critical to embryogenesis
- Test hypotheses
- Investigate metabolic pathways
- Investigate fidelity problems
- Better utilization of the empirical approach

CONIFER TISSUE CULTURE OBJECTIVES



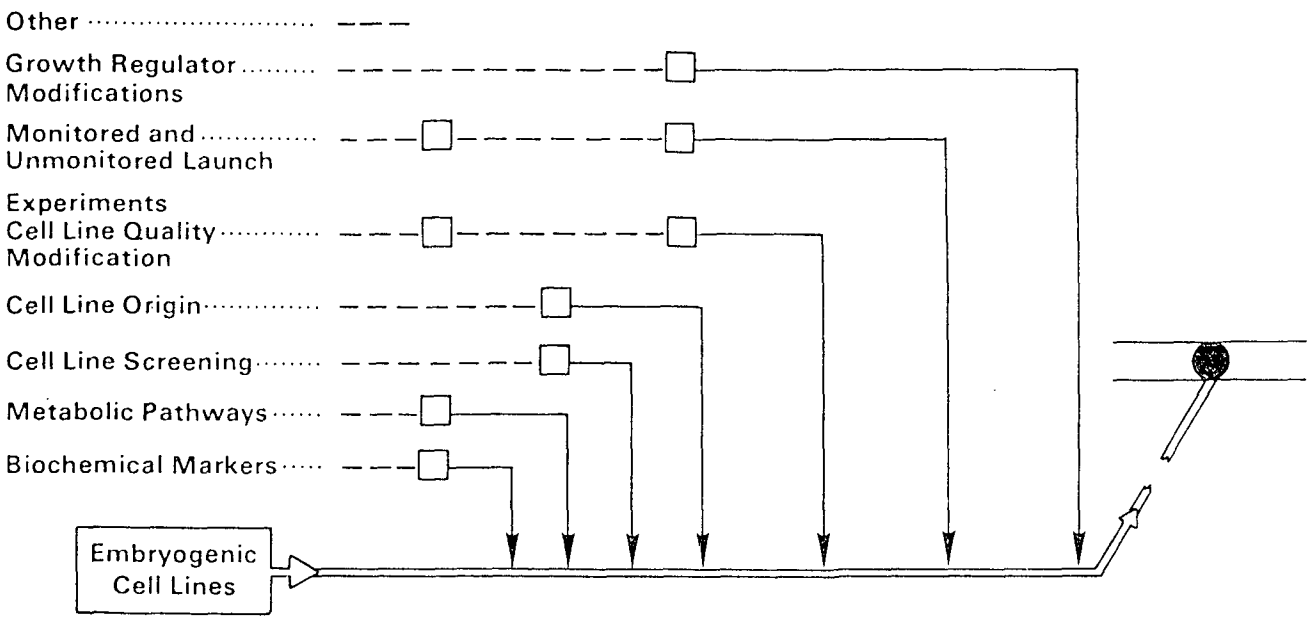
OBJECTIVE I
Competent Cell Lines

TYPE OF STUDIES



OBJECTIVE II
Embryogenesis

TYPE OF STUDIES



BIOCHEMICAL PARAMETERS TODAY

ATP

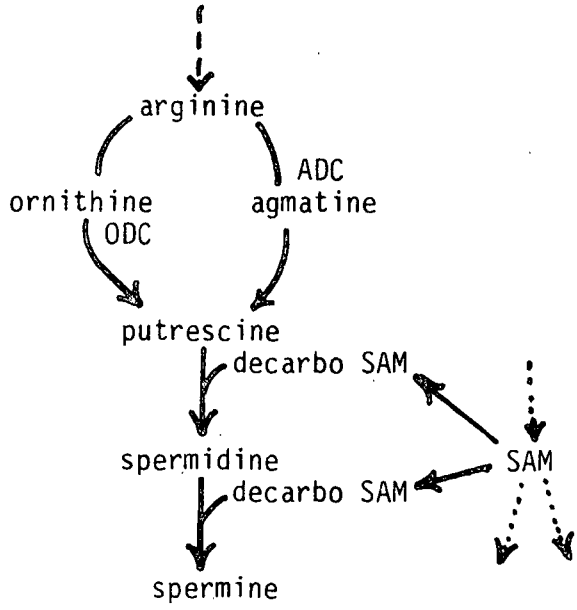
Ascorbic Acid

Polyamines

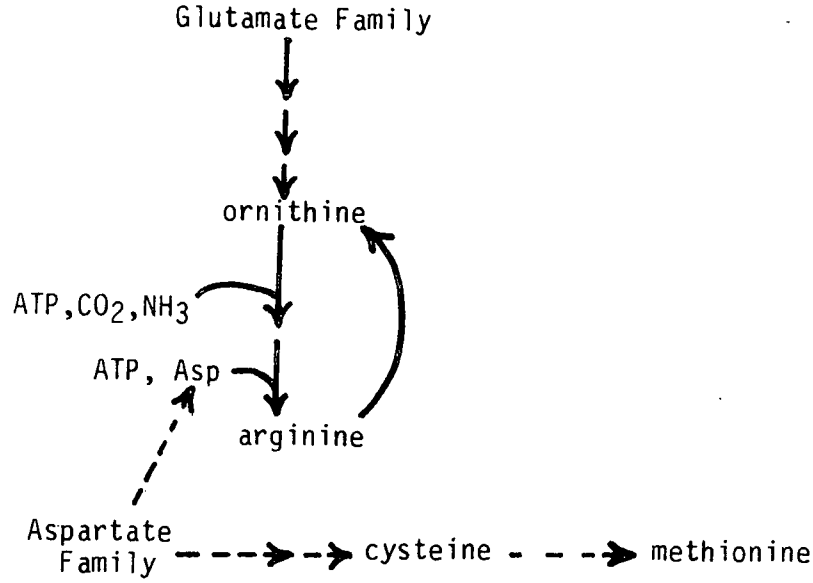
Phenolics

Methionine

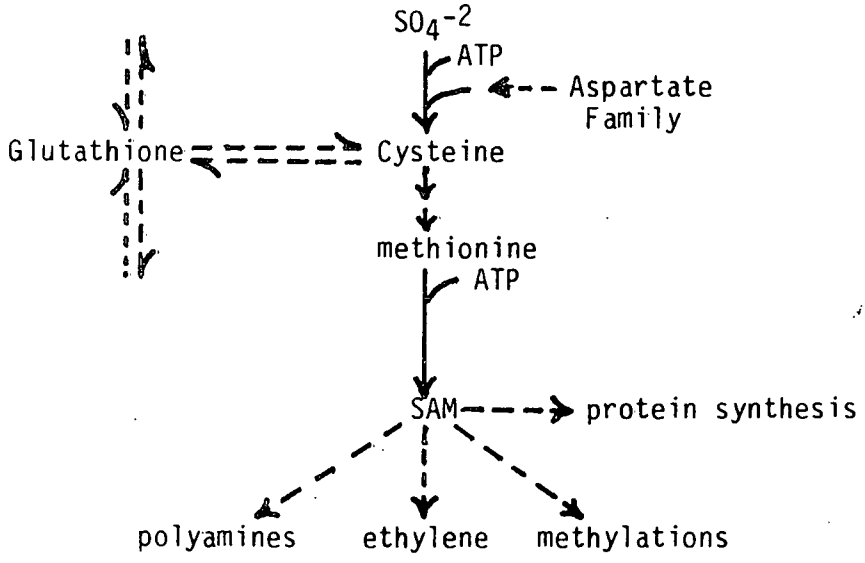
Seed Extracts



Substrate Supply for Polyamines



The Position of Arginine and Ornithine in Major Routes of Amino Acid Metabolism



The Position of SAM with Respect to Amino Acid Metabolism

METHIONINE IN PINE AND WILD CARROT CELLS

	methionine (μmoles/g. fr. wt.)
Wild Carrot Cells	
+2,4-D parental	0.12 —> 0.05 —> 0.18 (d12) —> 0.05
+2,4-D screened	0.07 —> 0.32 (d16) —> 0.06
-2,4-D screened	0.07 —> 0.12 (d8) —> 0.05
LP Cells	
+2,4-D(10-D-Cot)(parental)	t —> 0.05 (d14)[30 in CF]
+2,4-D(6L Hypo)(parental)	0 —> t
-2,4-D(cot B) (screened)	0.08 —> 0.01 (d8)
-NOAA,BAP(Cot B)(screened)	0.07 —> 0.01 (d7)

METHIONINE EFFECTS ON WILD CARROT SOMATIC EMBRYOGENESIS

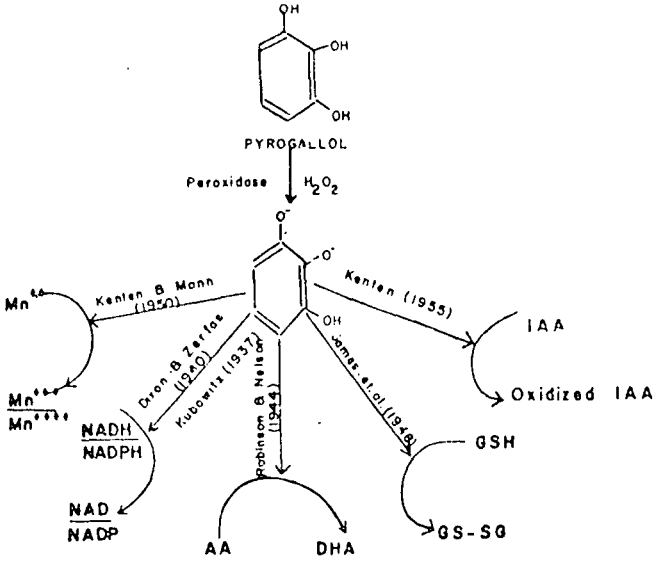
Methionine Added	Fr. Wt., mg	Embryos, #
none	158 ± 24	224 ± 26
10^{-6}M	154 ± 46	219 ± 100
10^{-5}M	197 ± 43	265 ± 74
10^{-4}M	269 ± 33	441 ± 67
10^{-3}M	133 ± 20	172 ± 56
10^{-2}M	6 ± 2	2 ± 2

RP 255D, n = 5

DINITROPHENOL EFFECTS ON WILD CARROT
AND PINE SUSPENSION CELLS

DNP Added	Wild Carrot (-GR) Fr. Wt., mg	Wild Carrot (-GR) Embryos, #	F2 Pine (+GR) Fr. Wt., mg
none	48 ± 3	37 ± 8	39 ± 4
10^{-6}M	89 ± 18	70 ± 14	53 ± 6
10^{-5}M	8 ± 7	1 ± 2	9 ± 1
10^{-4}M	0	0	2 ± 0

RP255E, n = 5



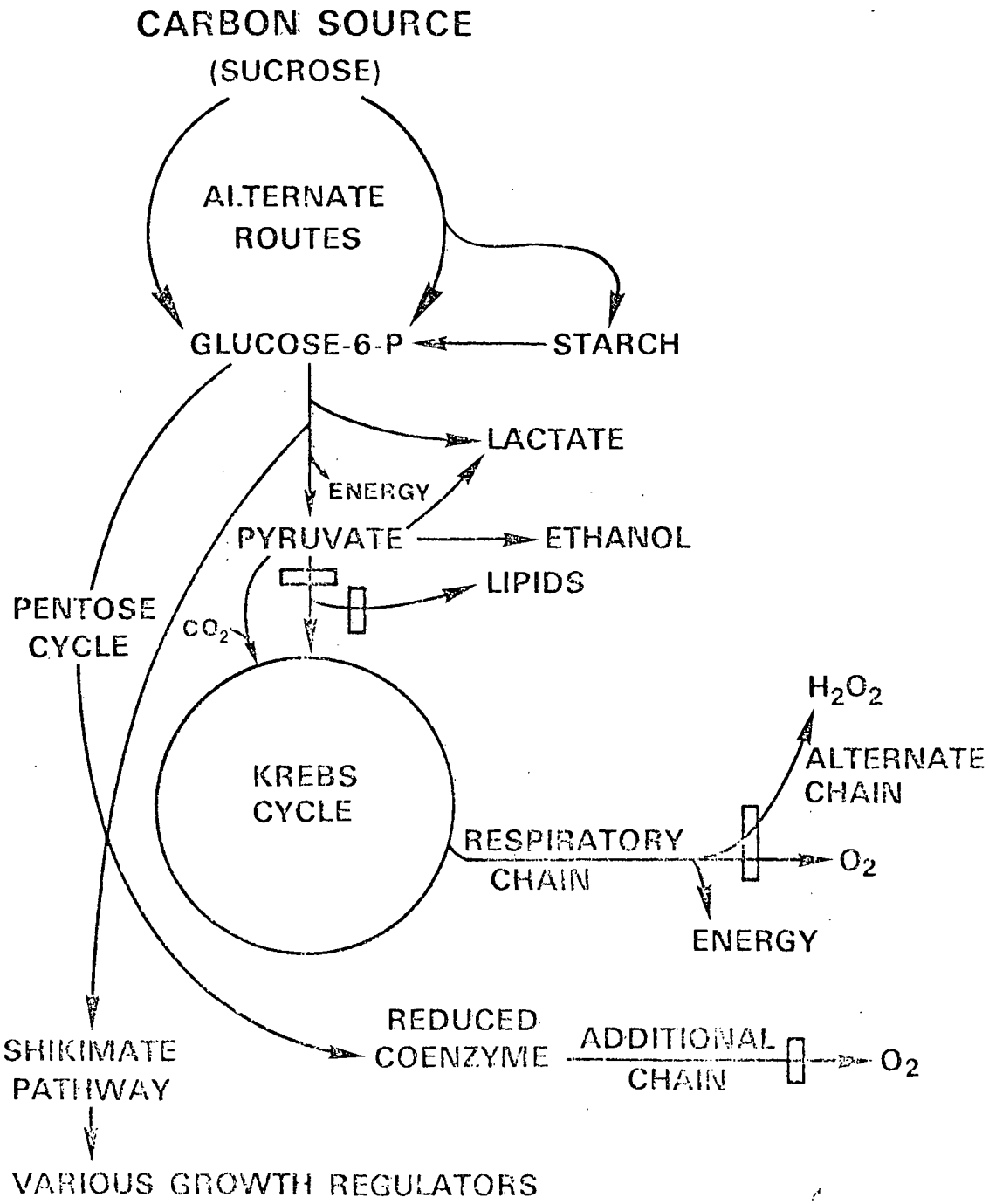
Chinoy, 1984

Proposed Role of Peroxidase and Phenolic substances in differentiation.

PHENOLIC EFFECTS ON WILD CARROT
SUSPENSION CELLS

Phenolic Added	Wild Carrot (-GR) Dry Wt., mg/mL
None	4.34 ± 0.24
10^{-4}M caffeic	3.44 ± 0.42 (brown)
10^{-5}M caffeic	4.85 ± 0.47
10^{-4}M Chlorogenic	3.12 ± 0.26 (brown)
10^{-5}M Chlorogenic	3.34 ± 0.43
10^{-4}M Cinnamic	1.80 ± 0.64
10^{-5}M Cinnamic	4.52 ± 0.24
10^{-4}M Coumaric	2.48 ± 0.61
10^{-5}M Coumaric	4.60 ± 0.54
10^{-4}M Ferulic	3.64 ± 0.64
10^{-5}M Ferulic	4.62 ± 0.31
10^{-4}M D-catechin	3.50 ± 0.40 (brown)
10^{-5}M D-catechin	4.02 ± 0.38

RP255C, n = 5

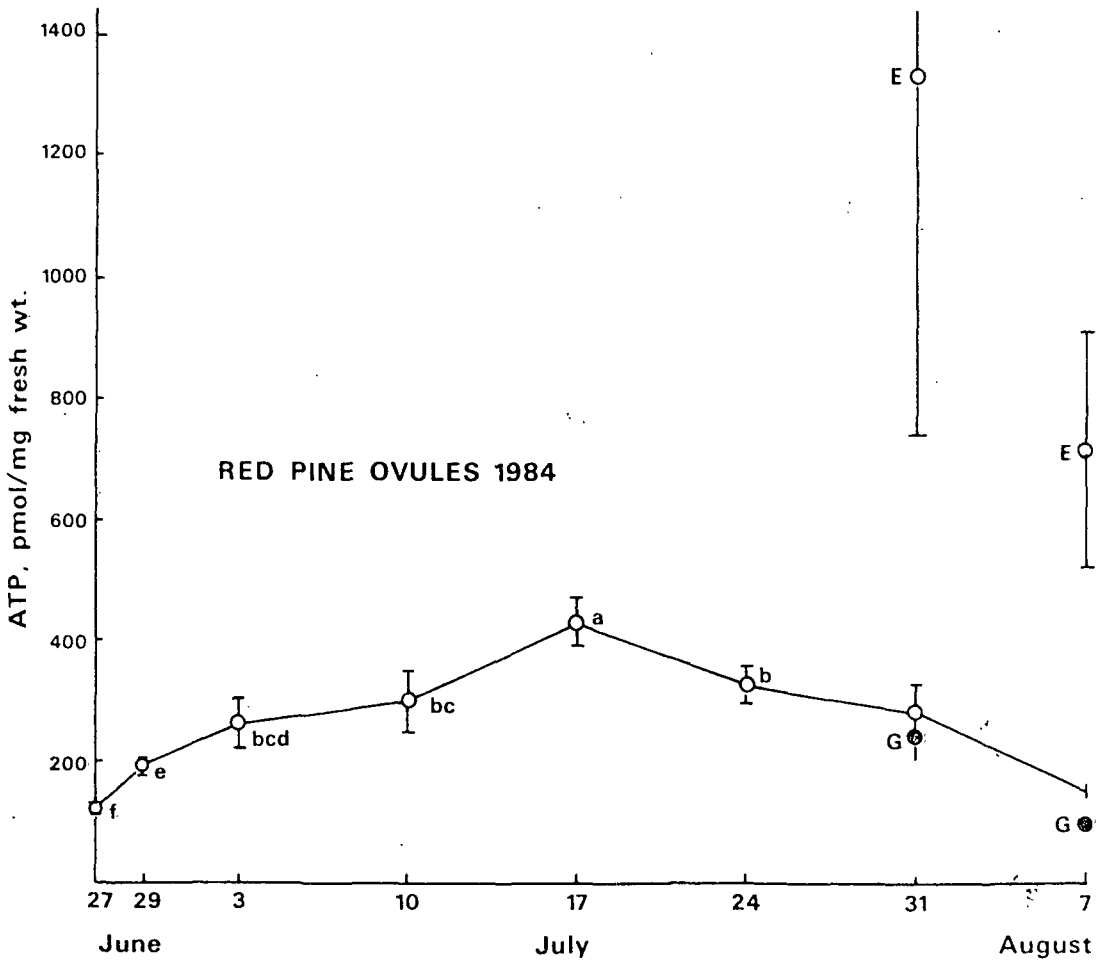


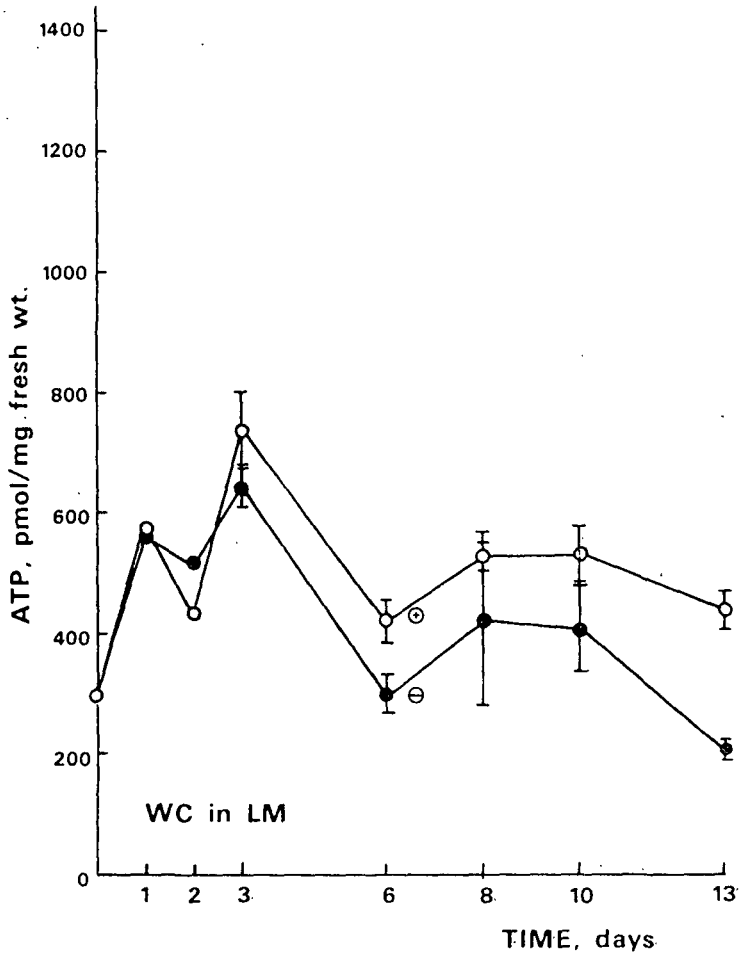
Some Major Metabolic Pathways

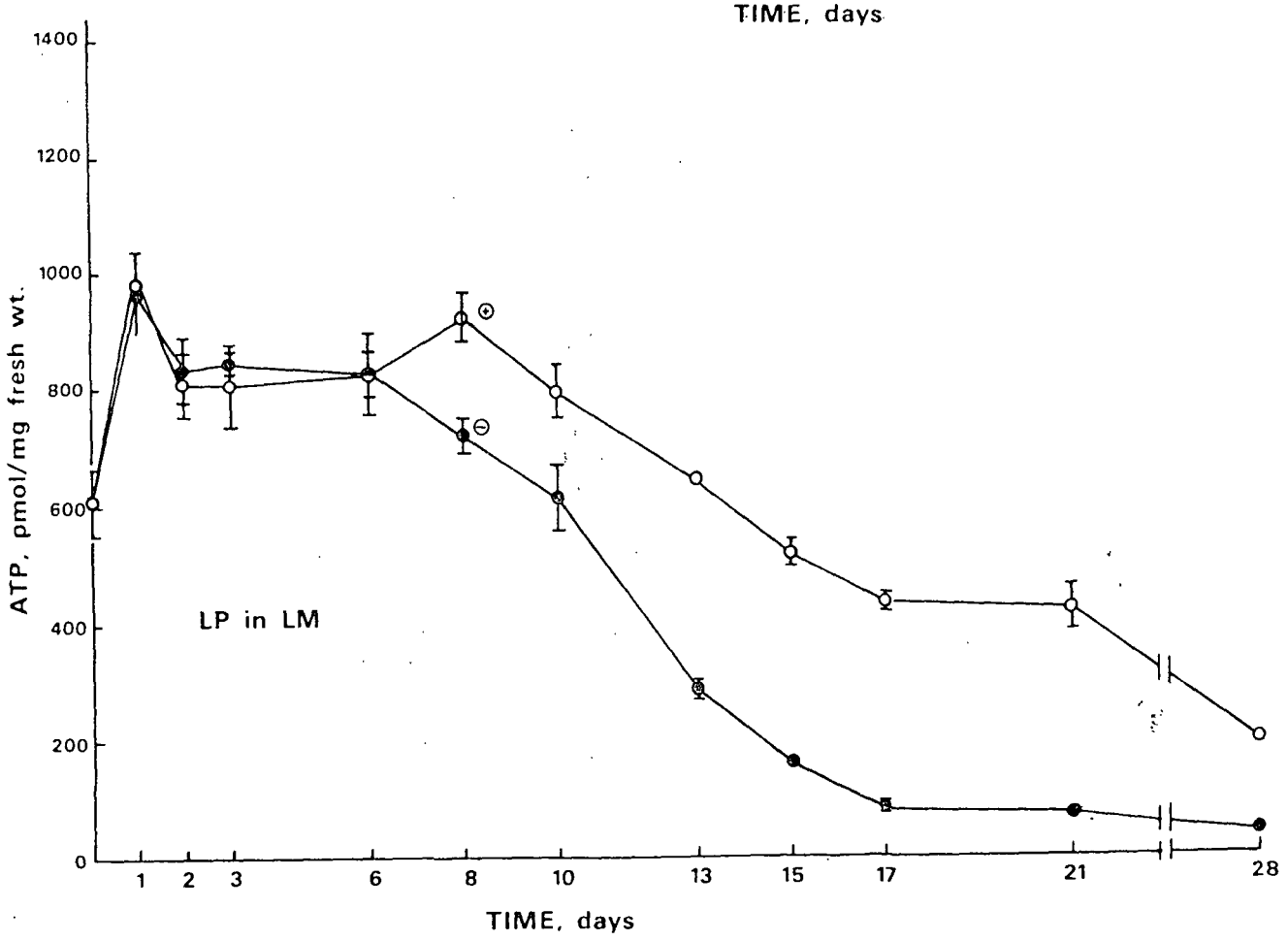
ATP and Energy Charge

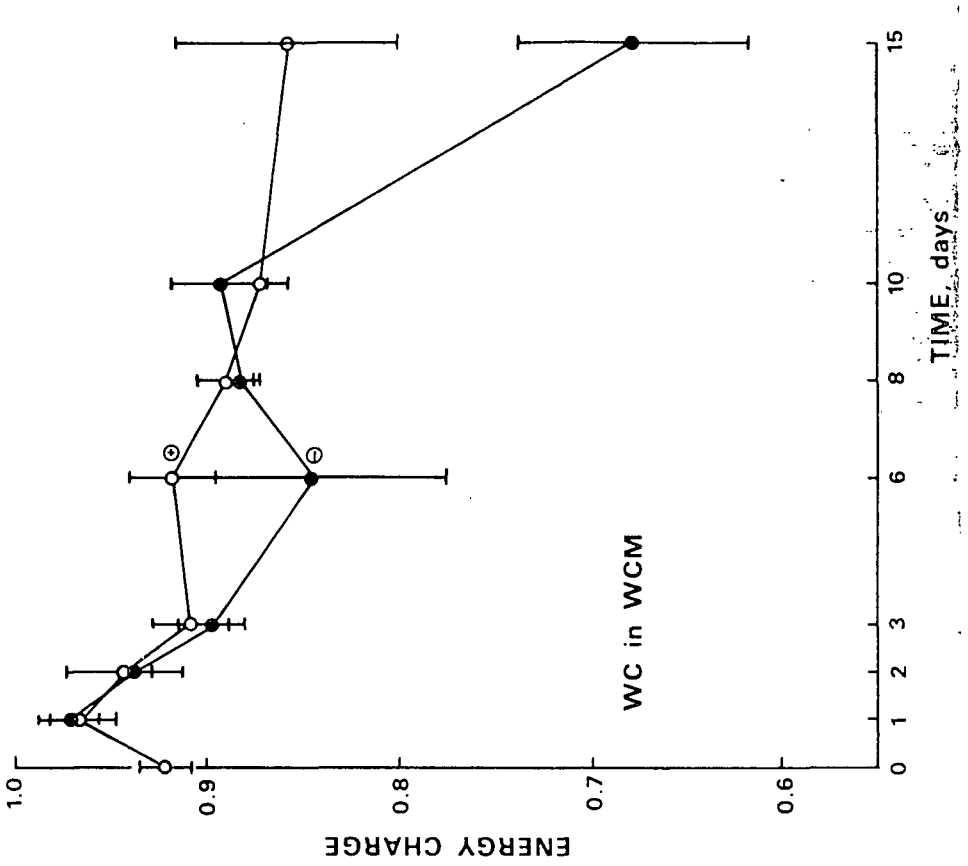
Question: Relative to the model system cells, do cultured conifer cells generate and maintain a sufficient level of ATP for their biosynthetic needs?

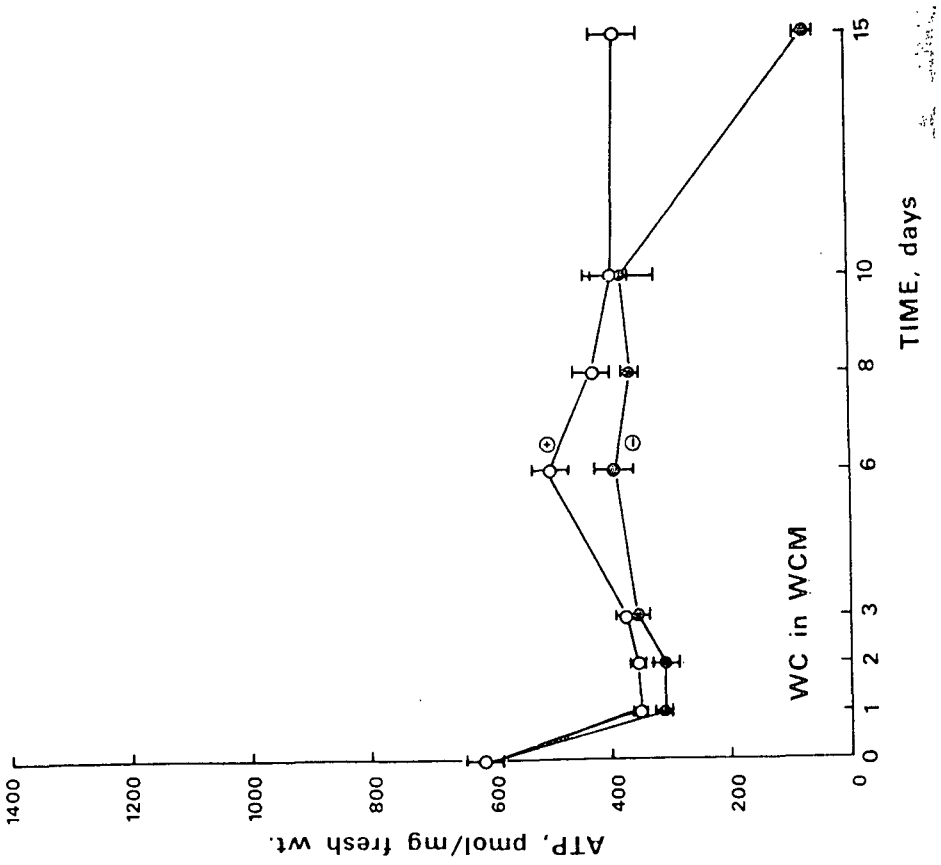
Answer: Cultured pine cells seem to have plenty of ATP available; in fact, the data indicates that they have it but don't use it.

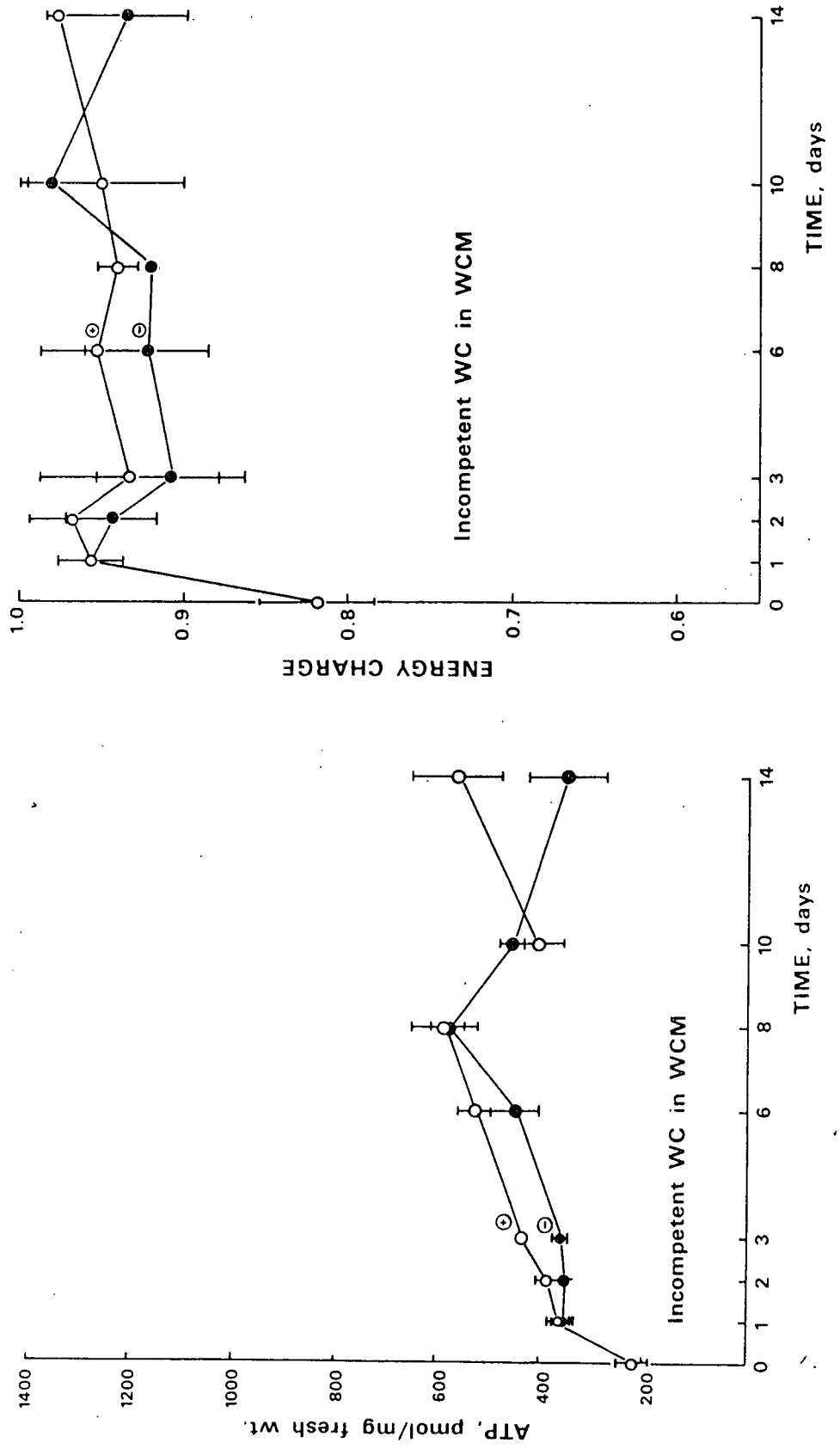


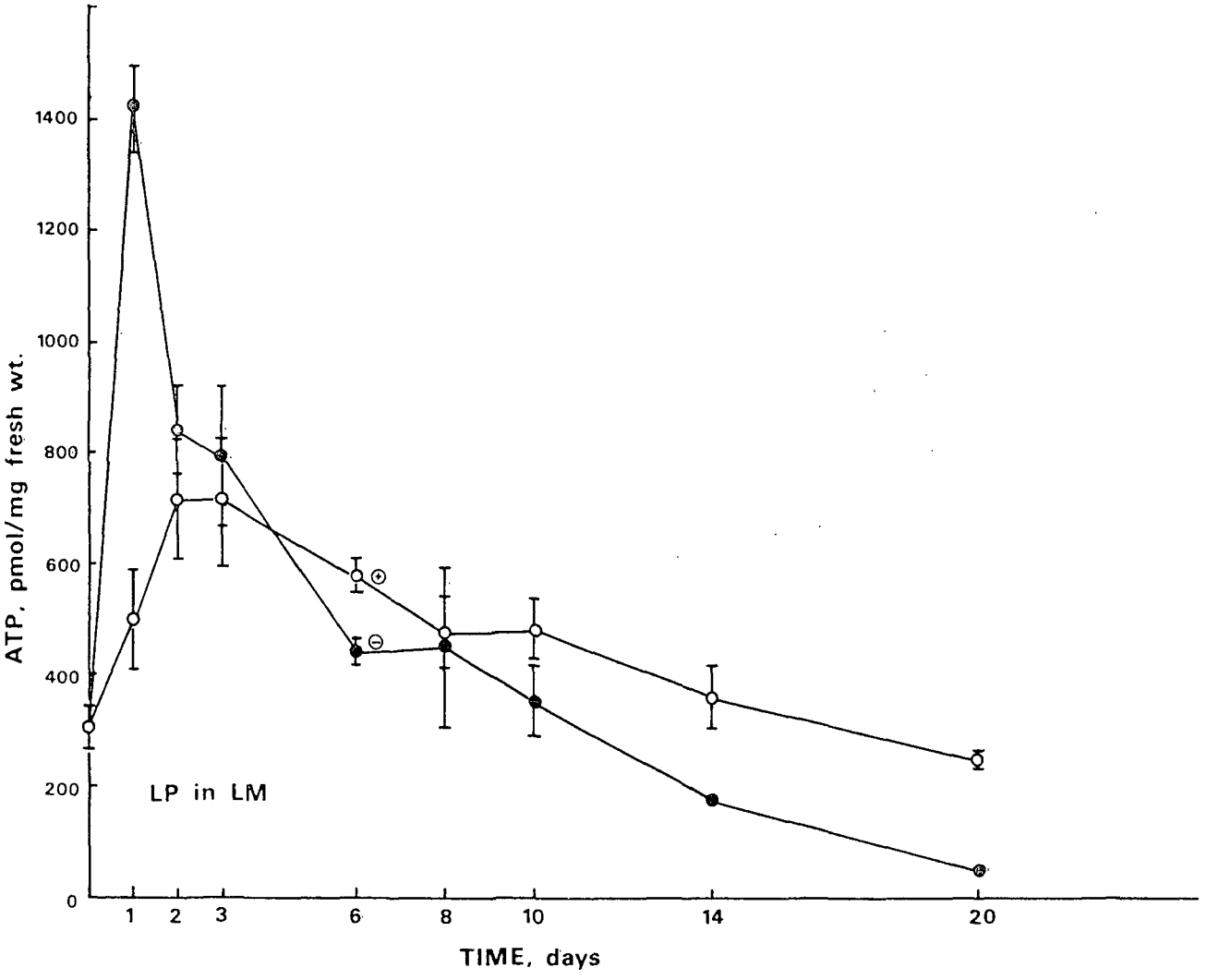

1400
1200
1000
800
600
400
200
0
ATP, pmol/mg fresh wt.
WC in LM
1
2
3
6
8
10
13
TIME, days

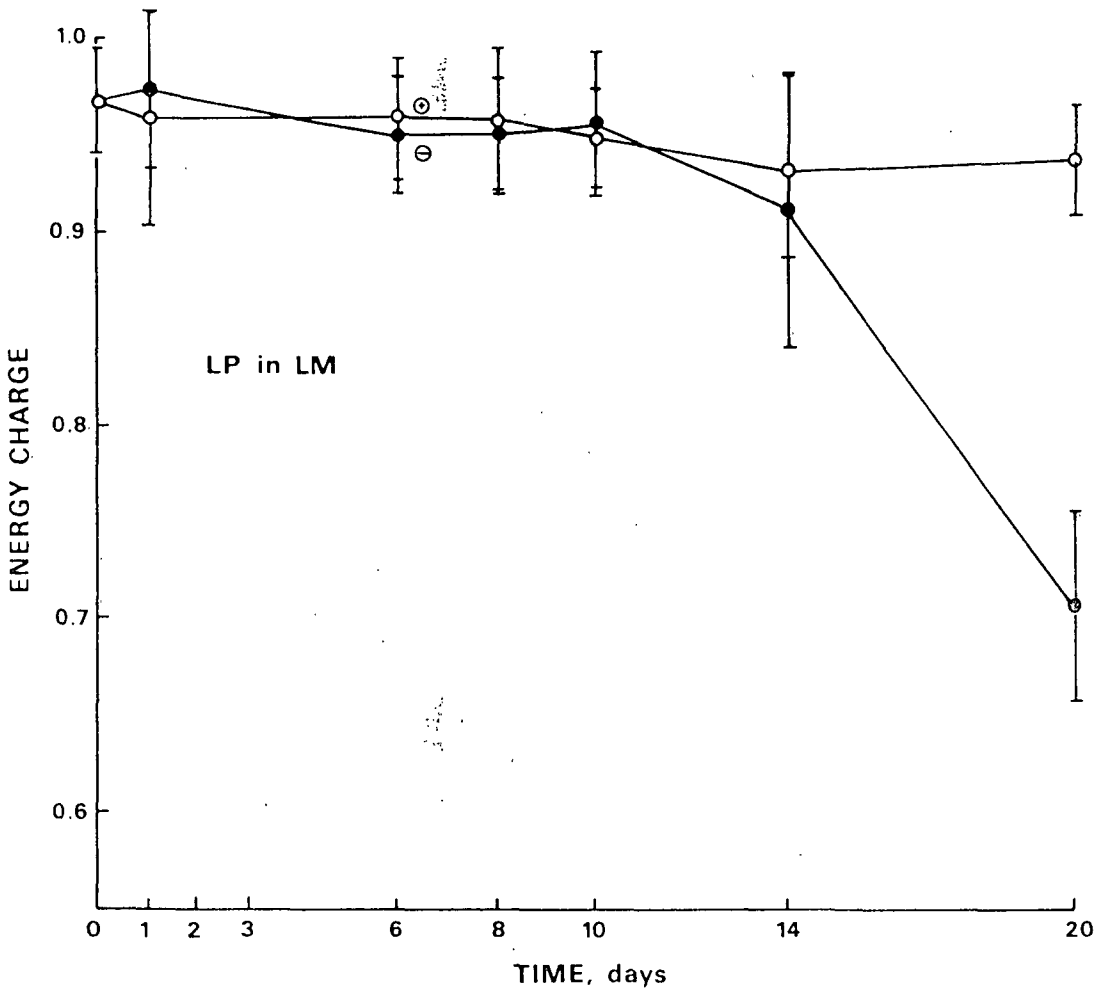

1400
1200
1000
800
600
400
200
0
ATP, pmol/mg fresh wt.
LP in LM
1
2
3
6
8
10
13
15
17
21
28
TIME, days


ENERGY CHARGE
1.0
0.9
0.8
0.7
0.6
WC in WCM
0 1 2 3 6 8 10 15
TIME, days


ATP, pmol/mg fresh wt.
1400
1200
1000
800
600
400
200
WC in WCM
0 1 2 3 6 8 10 15
TIME, days


ENERGY CHARGE
1.0
0.9
0.8
0.7
0.6
Incompetent WC in WCM
0 1 2 3 6 8 10 14
TIME, days
ATP, pmol/mg fresh wt.
1400
1200
1000
800
600
400
200
Incompetent WC in WCM
0 1 2 3 6 8 10 14
TIME, days

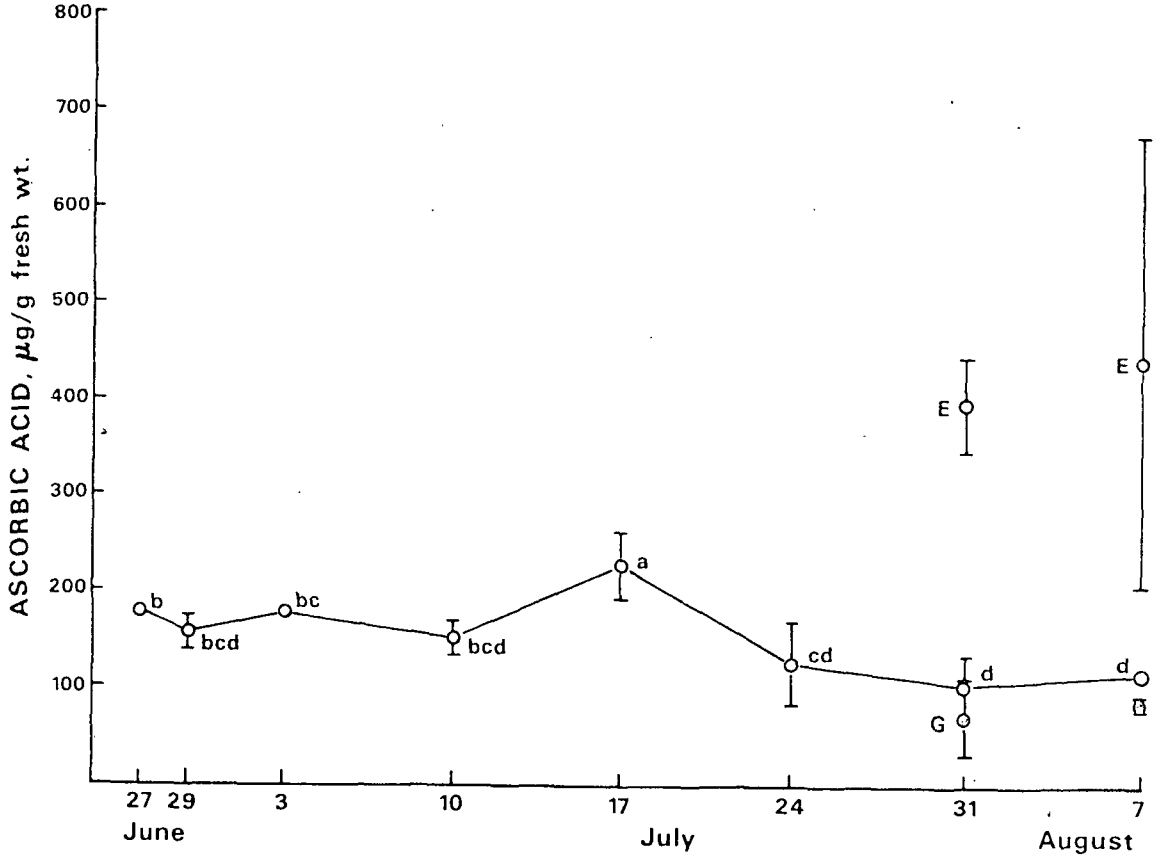

ATP, pmol/mg fresh wt.
1400
1200
1000
800
600
400
200
0
LP in LM
1
2
3
6
8
10
14
20
TIME, days


1.0
0.9
0.8
0.7
0.6
ENERGY CHARGE
LP in LM
⊙
⊖
0
1
2
3
6
8
10
14
20
TIME, days

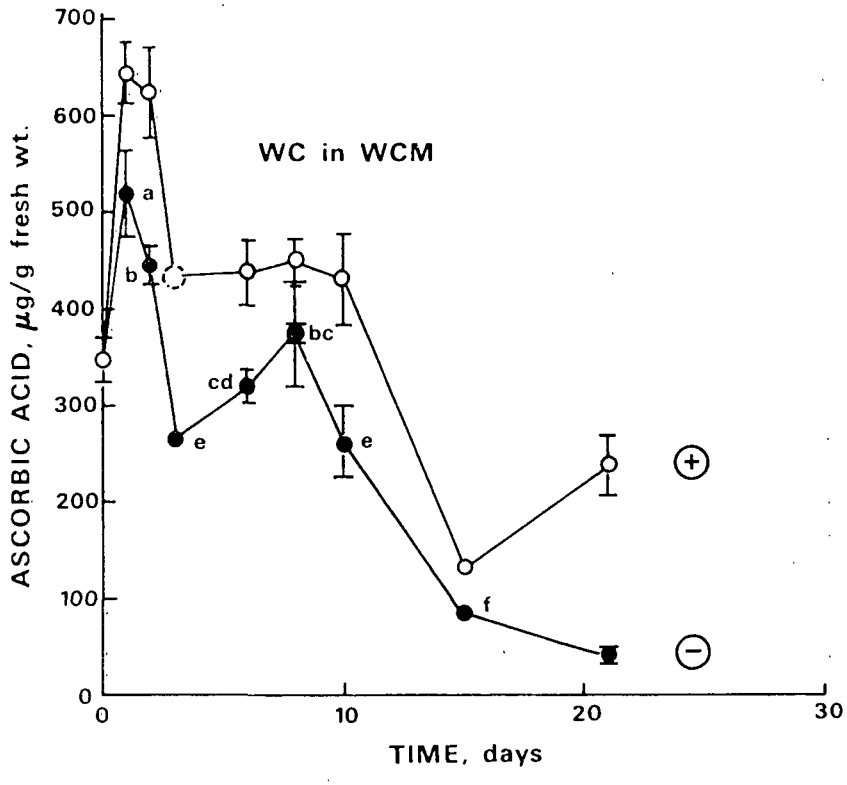
Ascorbic Acid

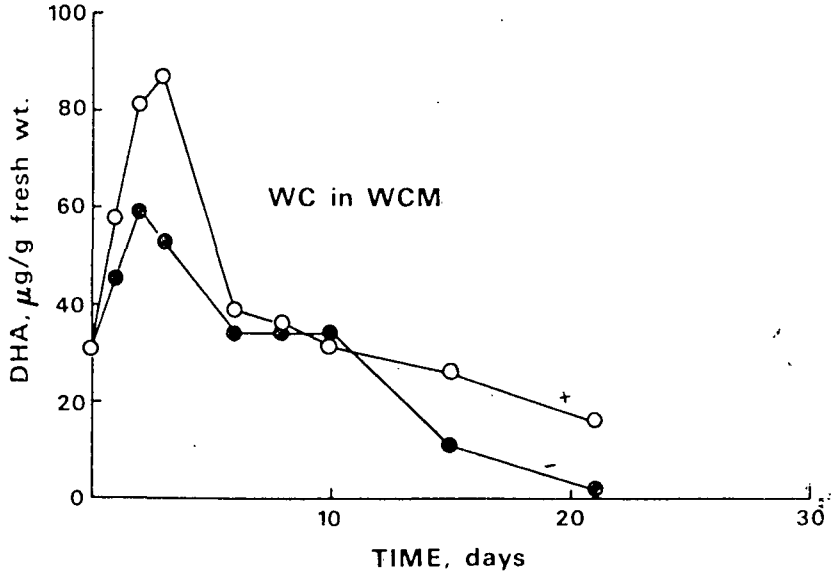
Question: Beyond the likelihood that ascorbic acid has specific functions in cell physiology, the oxidation state of endogenous ascorbic acid should reflect the internal redox status of cells. Do the ascorbic/dehydroascorbic levels in cultured conifer cells fluctuate in a manner similar to their behavior in model systems?

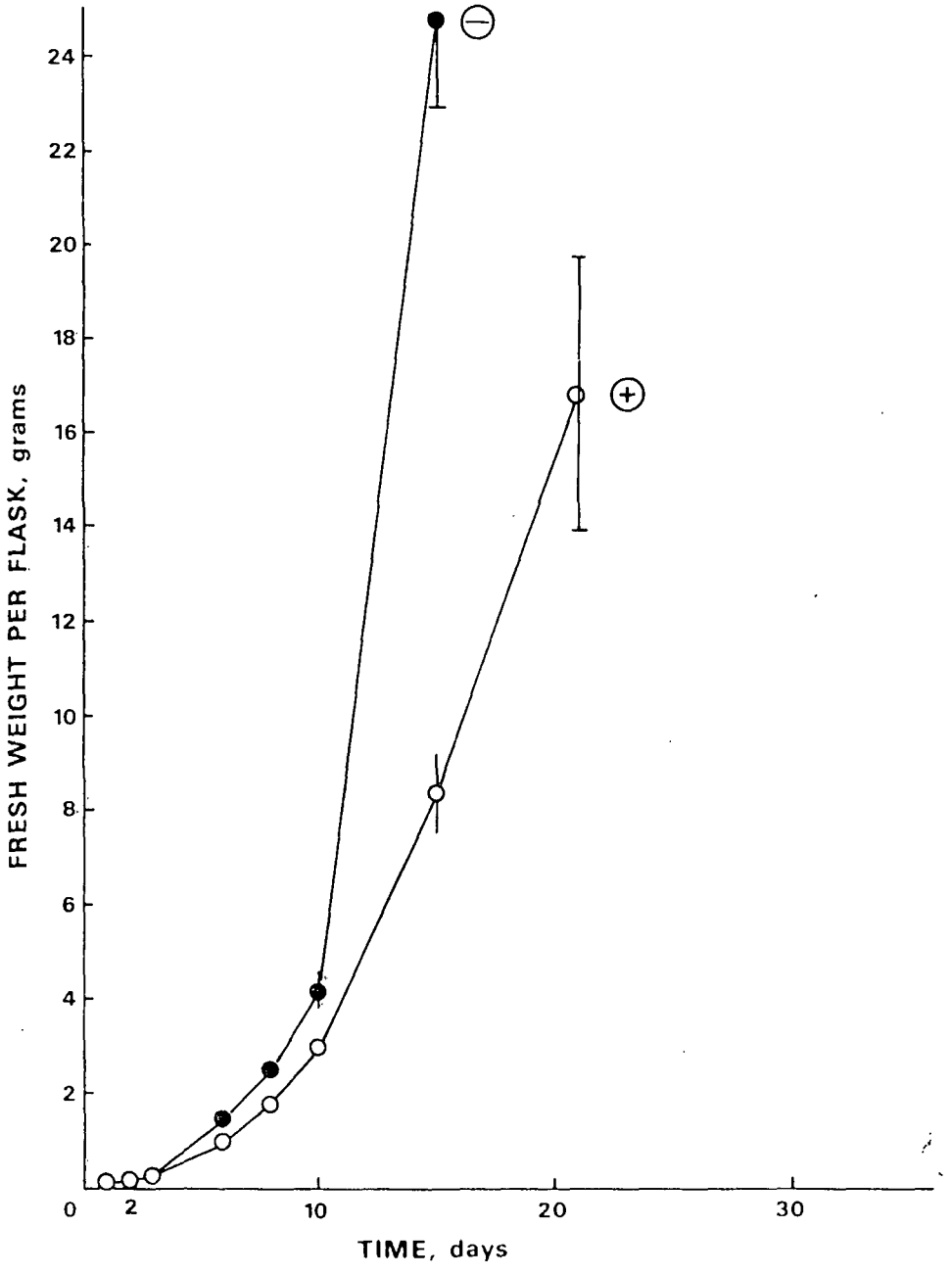
Answer: The data collected to date indicates that cultured conifer cells are too oxidizing during a time period crucial to development.



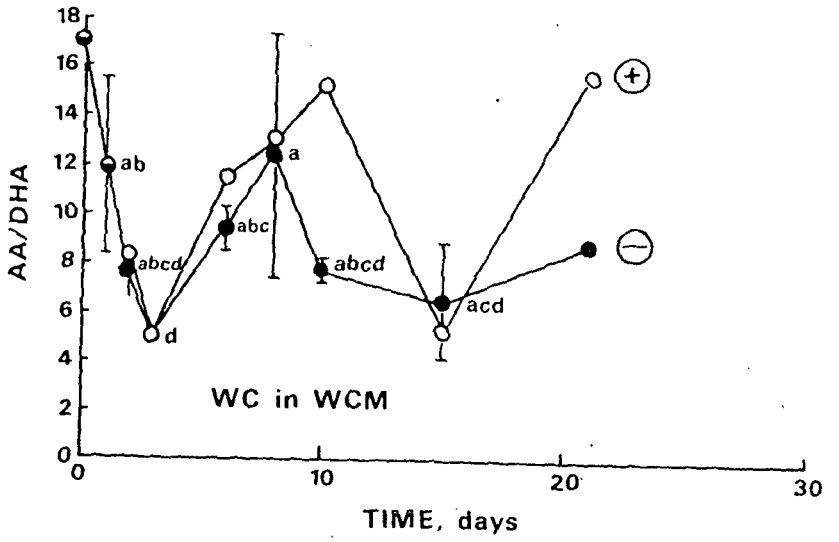
Red Pine Ovules 1984

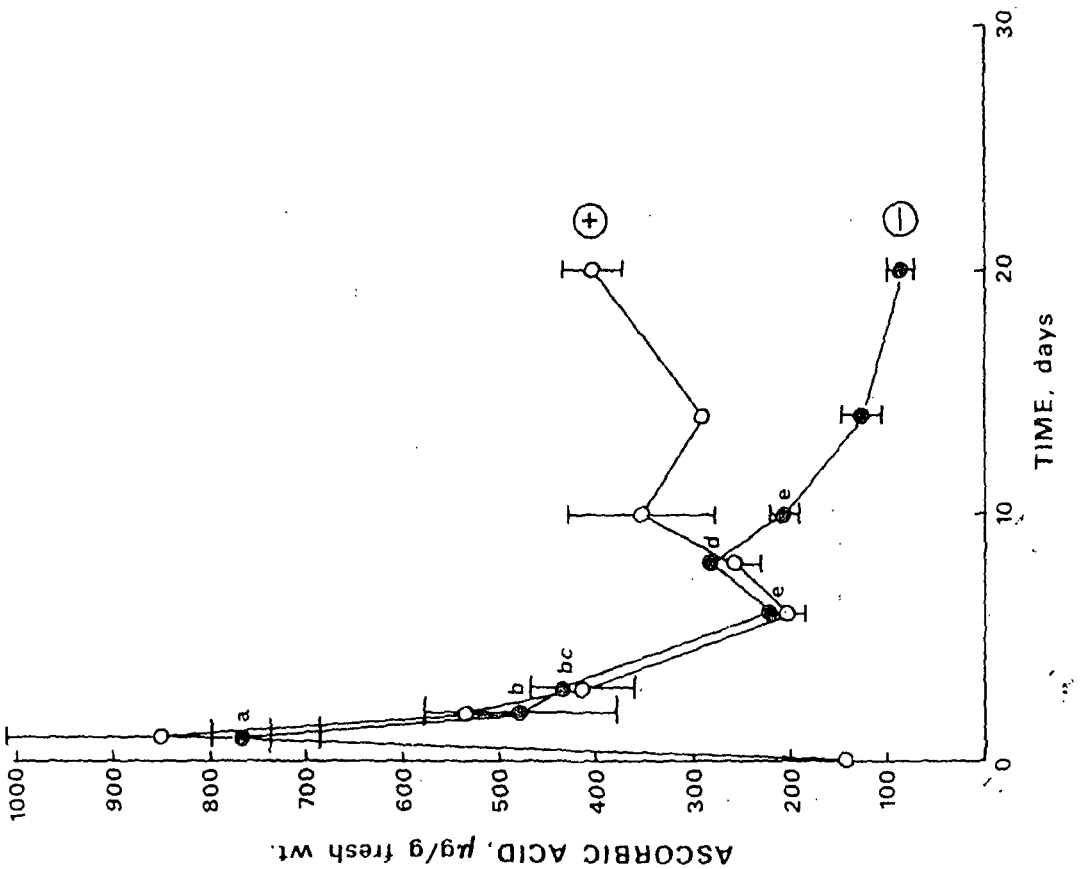

WC in WCM
ASCORBIC ACID, μg/g fresh wt.
TIME, days
700
600
500
400
300
200
100
0
0
10
20
30
a
b
cd
e
bc
e
f
+
−

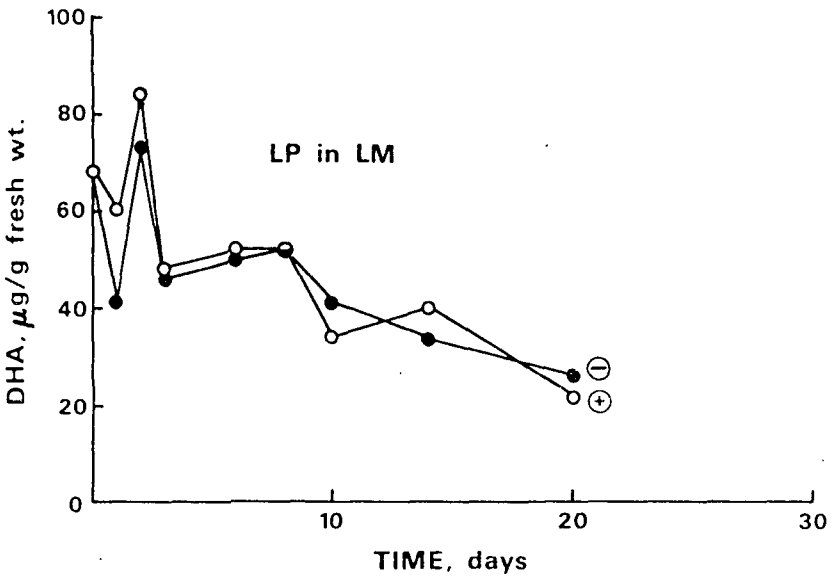

WC in WCM
DHA, μg/g fresh wt.
TIME, days
100
80
60
40
20
0
10
20
30
+
−

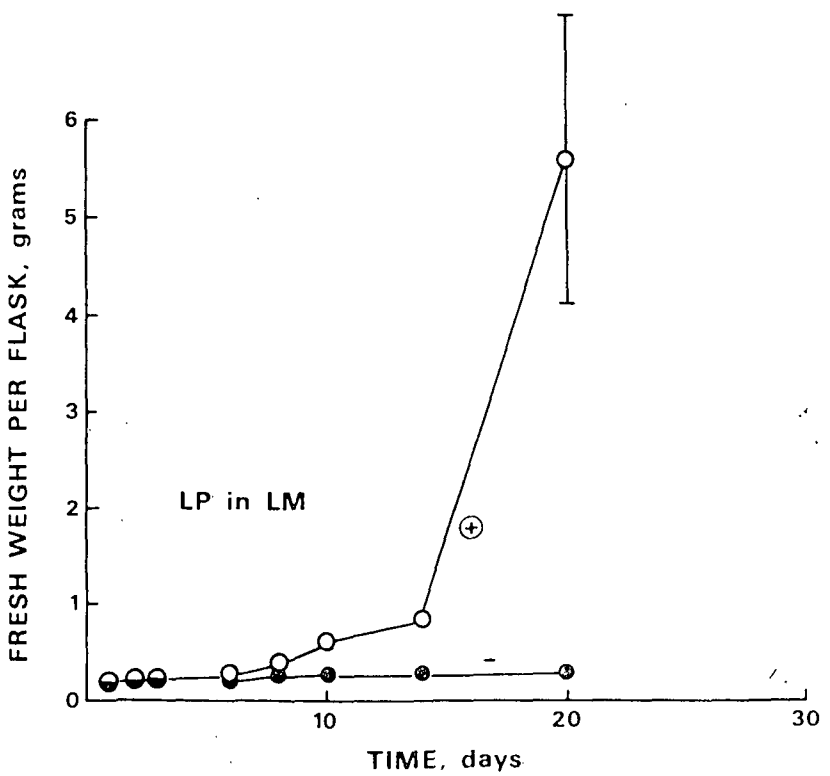

FRESH WEIGHT PER FLASK, grams
0
2
4
6
8
10
12
14
16
18
20
22
24
2
10
20
30
TIME, days
⊖
⊕

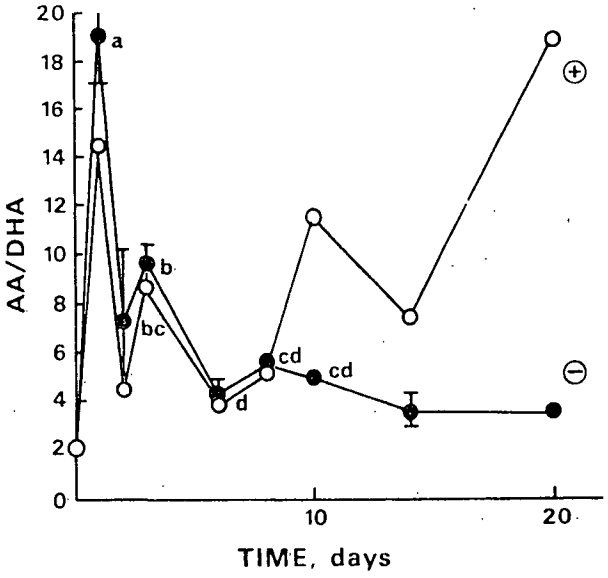
WC in WCM


AA/DHA
18
16
14
12
10
8
6
4
2
0
ab
abcd
d
abc
a
abcd
acd
+
−
WC in WCM
0
10
20
30
TIME, days

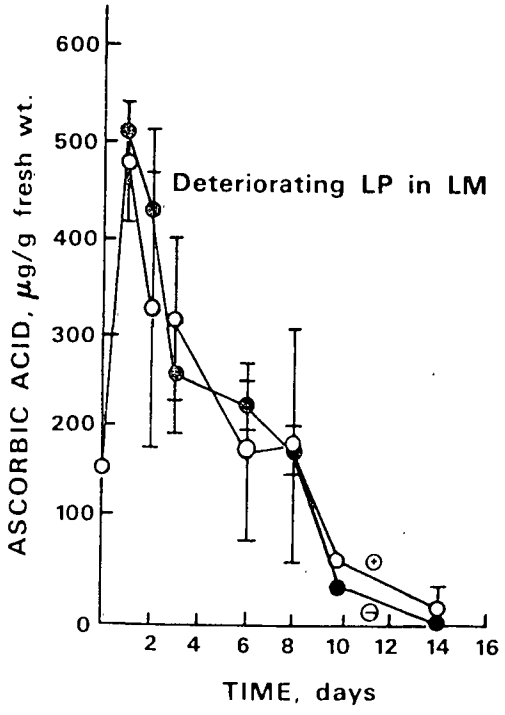

ASCORBIC ACID, μg/g fresh wt.
1000
900
800
700
600
500
400
300
200
100
0
a
b
bc
d
e
e
+
−
0
10
20
30
TIME, days
LP in LM

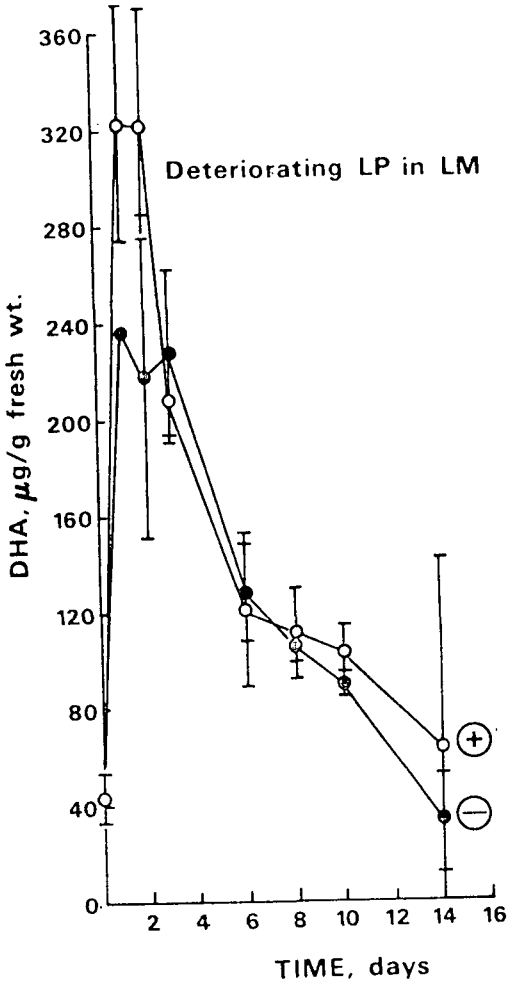

100
80
60
40
20
0
DHA, μg/g fresh wt.
LP in LM
⊖
⊕
10
20
30
TIME, days

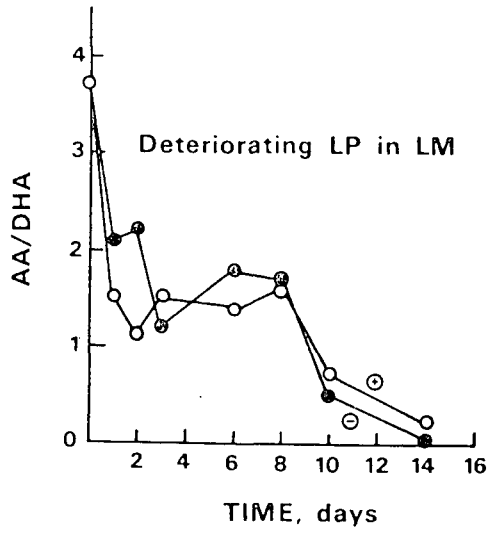

6
5
4
3
2
1
0
FRESH WEIGHT PER FLASK, grams
LP in LM
⊕
10
20
30
TIME, days

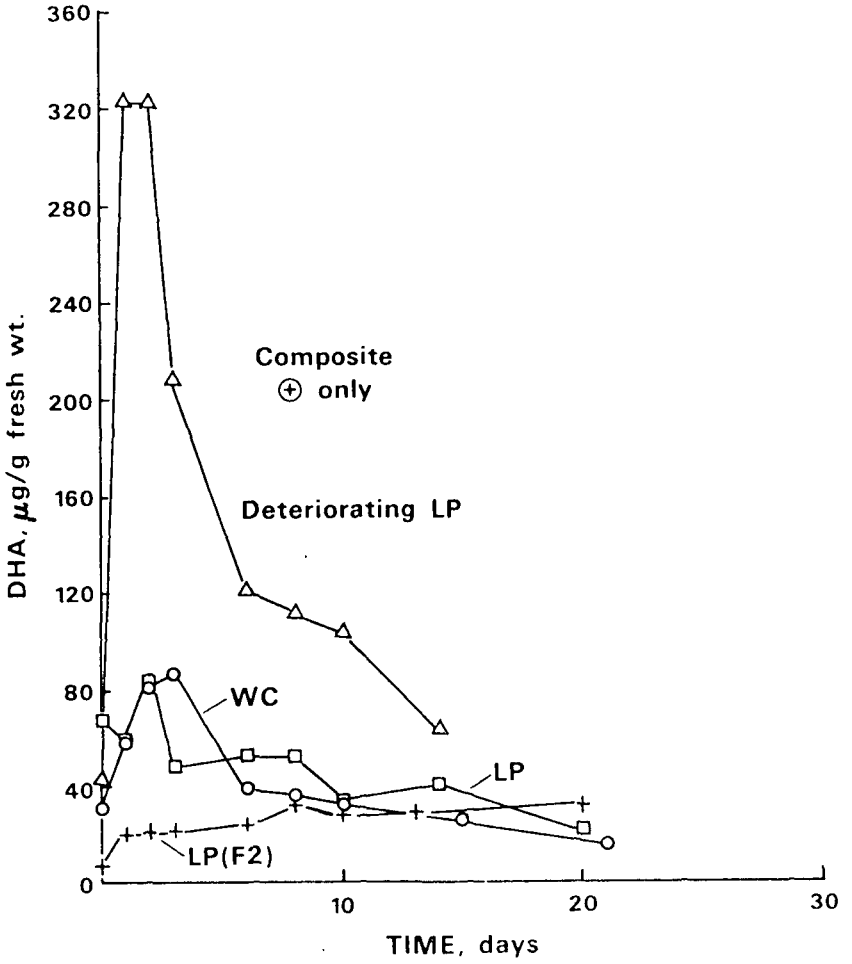

AA/DHA
TIME, days
a
b
bc
cd
cd
d
⊕
⊖

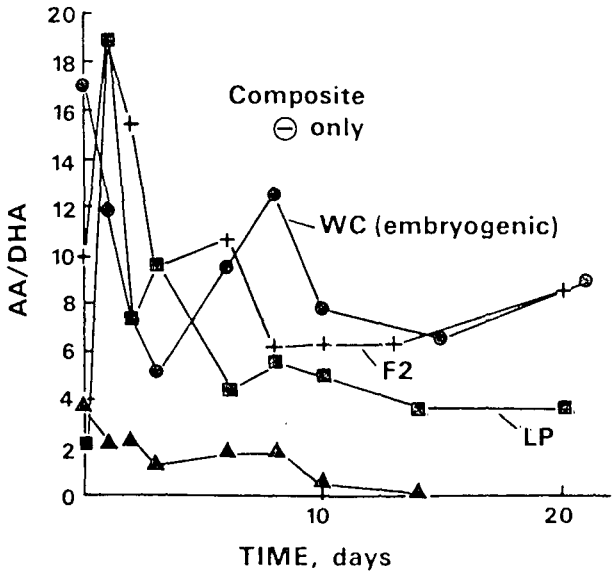
LP in LM

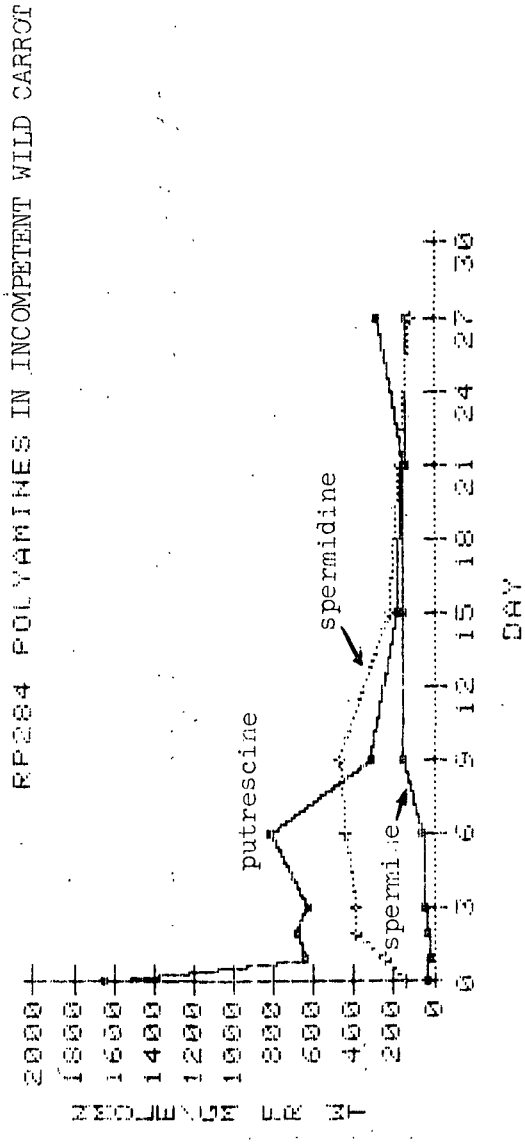

ASCORBIC ACID, μg/g fresh wt.
TIME, days
Deteriorating LP in LM
⊙
⊖


Deteriorating LP in LM
DHA, µg/g fresh wt.
360
320
280
240
200
160
120
80
40
0
2
4
6
8
10
12
14
16
TIME, days
+
−

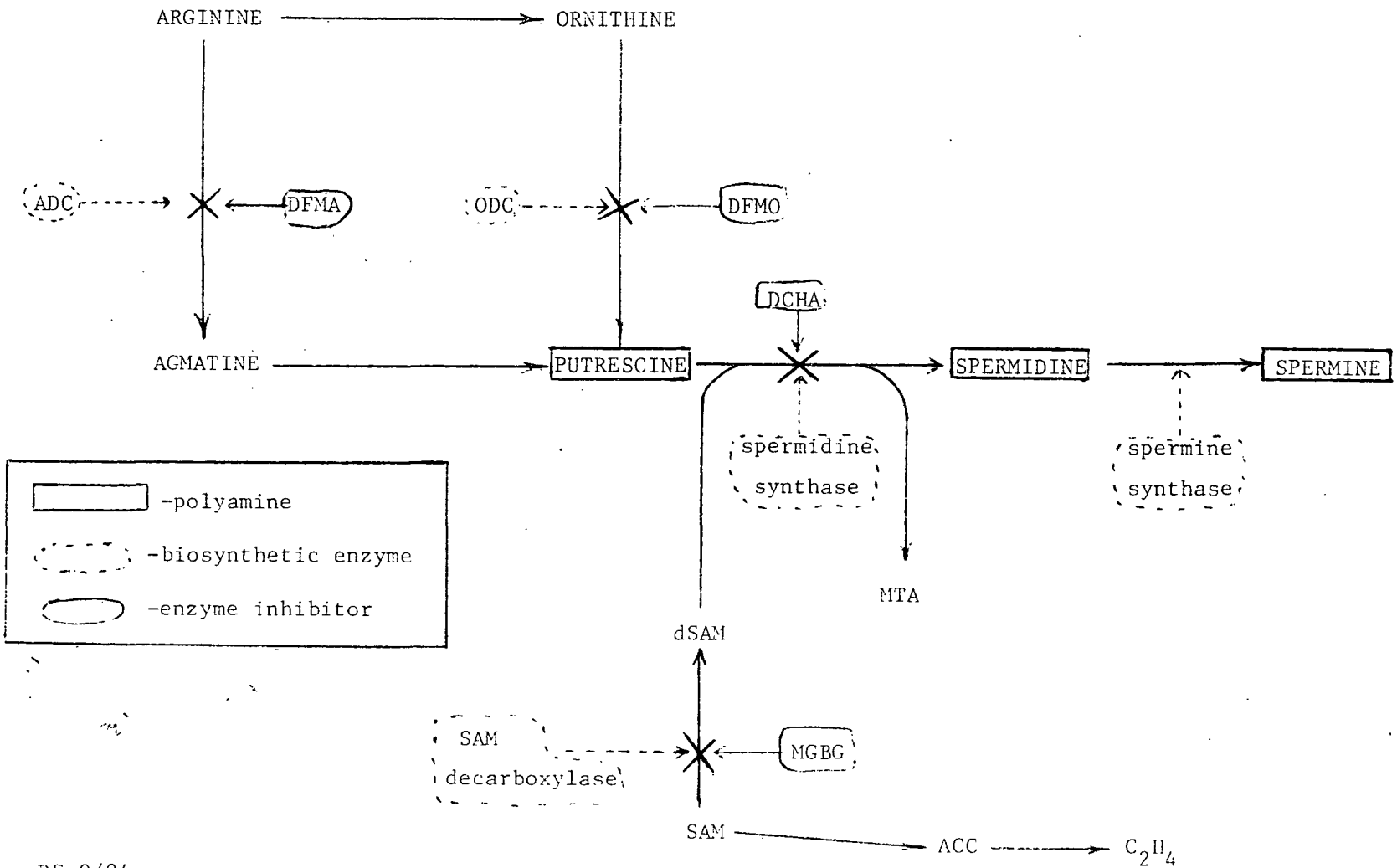

Deteriorating LP in LM
AA/DHA
4
3
2
1
0
2
4
6
8
10
12
14
16
TIME, days


360
320
280
240
200
160
120
80
40
0
DHA, μg/g fresh wt.
Composite
⊕ only
Deteriorating LP
WC
LP
LP(F2)
10
20
30
TIME, days


20
18
16
14
12
10
8
6
4
2
0
AA/DHA
Composite
⊖ only
WC (embryogenic)
F2
LP
10
20
TIME, days


EP284 POLYAMINES IN INCOMPETENT WILD CARROT
putrescine
spermidine
spermine
DAY
0
3
6
9
12
15
18
21
24
27
30
200
400
600
800
1000
1200
1400
1600
1800
2000

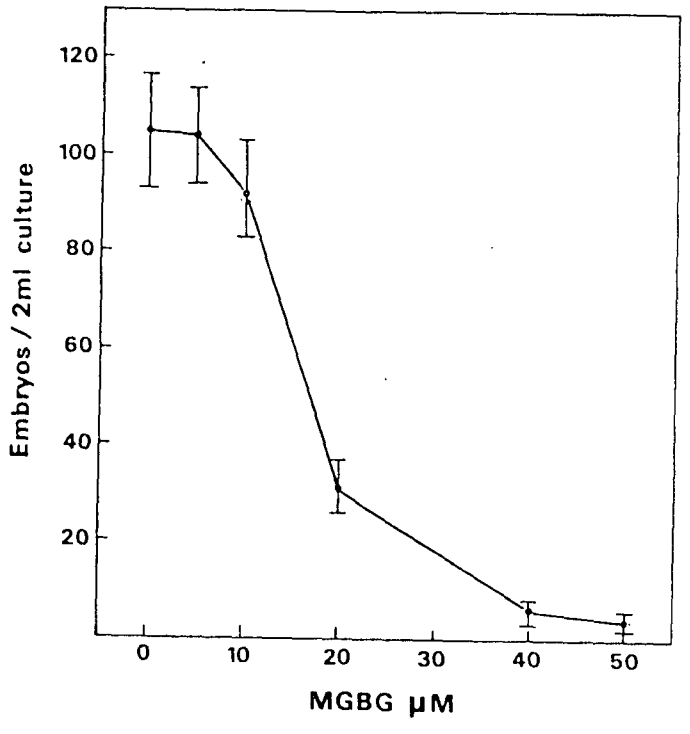
POLYAMINE METABOLISM IN PLANTS AND INHIBITORS OF BIOSYNTHETIC ENZYMES



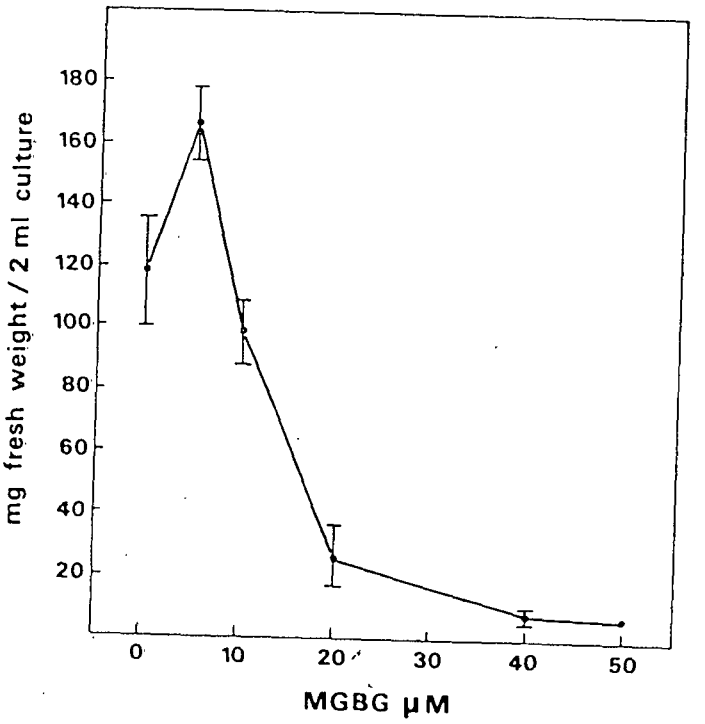
RF 9/84

Table 1. Effect of DFMA on wild carrot polyamine concentrations. Polyamines were determined in 5 percent perchloric acid extracts of tissue from 6-day-old cultures. We previously determined that polyamine concentrations in our wild carrot cultures are elevated on day 6. Embryogenesis was initiated as described in the legend to Fig. 1, except that 250-ml Erlenmeyer flasks containing 50 ml of medium were used to yield an adequate amount of tissue for analysis. Benzoylated derivatives of the polyamines were separated and measured by high-performance liquid chromatography (9, 21). Values are means ± standard deviations of quadruplicate determinations and are expressed as nanomoles per gram (fresh weight). N.D., none detected.

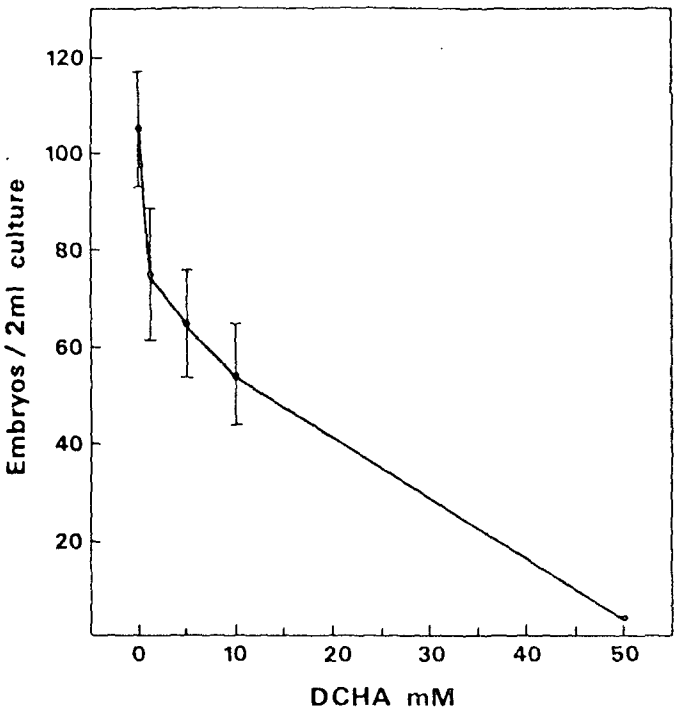
Treatment	Putrescine	Spermidine	Spermine
Control	890 ± 121	506 ± 46	66 ± 25
DFMA (1.0 m*M*)	N.D.	39 ± 16	260 ± 38
DFMA (1.0 m*M*) + putrescine (0.1 m*M*)	185 ± 18	463 ± 143	115 ± 18
DFMO (1.0 m*M*)	645 ± 69	415 ± 17	66 ± 10



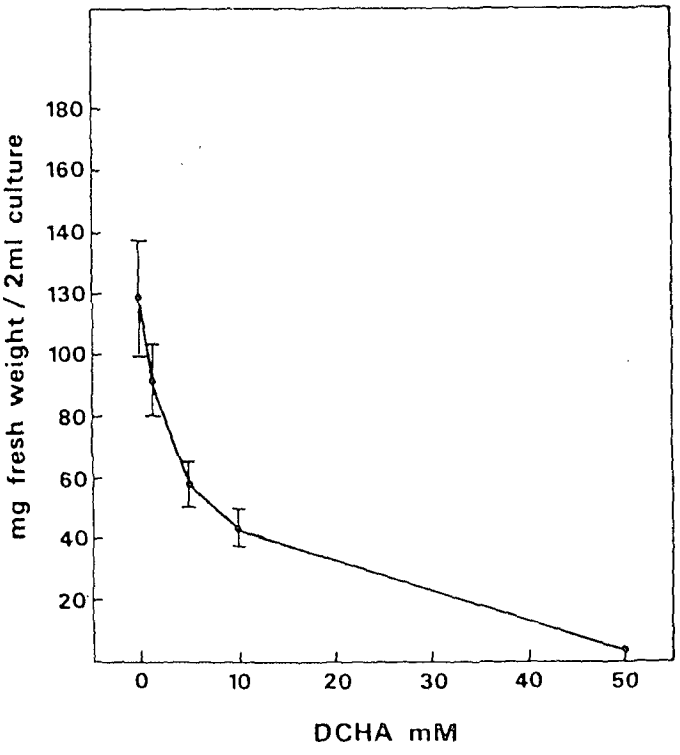
Effect of MGBG on embryogenesis of wild carrot suspension cultures.



Effect of MGBG on growth of wild carrot suspension cultures.



Effect of DCHA on embryogenesis of wild carrot cultures.



Effect of DCHA on growth of wild carrot cultures.

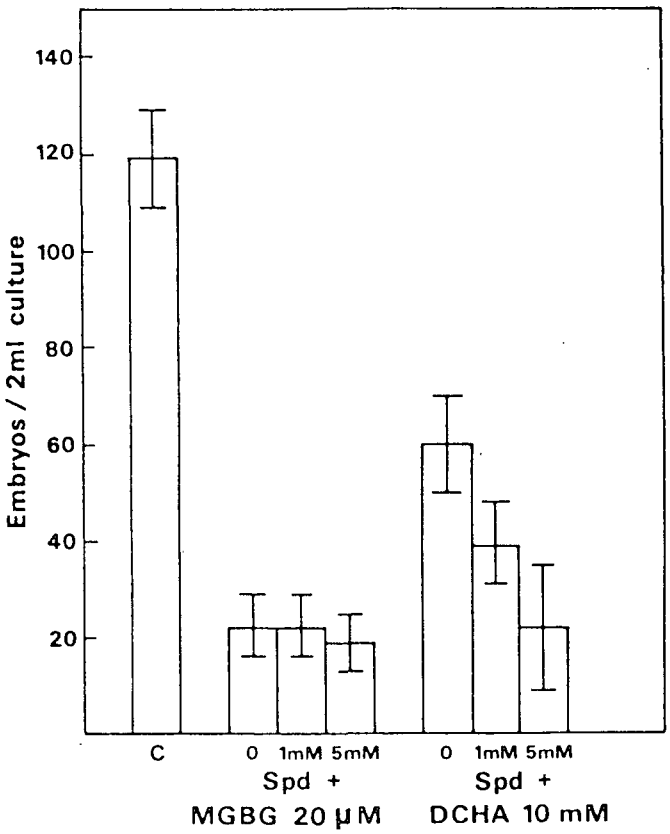
RP 283: EFFECT OF MGBG AND DCHA ON WILD CARROT POLYAMINES: PRELIMINARY RESULTS

Treatment	Put	Spd	Spm
Control	214 ± 68	561 + 135	119 + 23
MGBG 20 μM	1026 ± 260	294 ± 58	111 ± 22
MGBG 40 μM	808 ± 191	185 ± 39	101 ± 16
DCHA 5 mM	514 ± 85	1097 ± 197	153 ± 18
DCHA 10 mM	665 ± 127	2037 ± 403	207 ± 35

Samples collected on Day 9
n = 4
50 mL in 250 mL flask -2,4-D

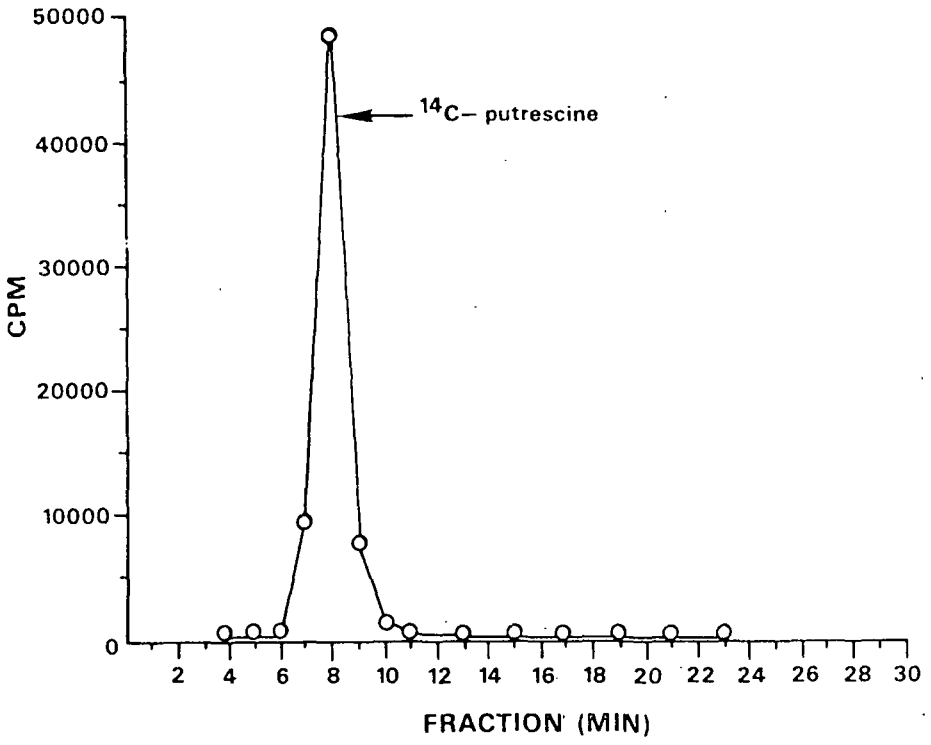
EFFECT OF MGBG, DCHA AND SPERMIDINE ON WILD CARROT POLYAMINES

Treatment	Put	Spd	Spm
	nmole/gram fresh weight		
Control	266 ± 64	387 + 57	29 + 8
MGBG 20 μM	194 ± 34	213 ± 30	40 ± 26
MGBG 20 μM + Spd 1 mM	461 ± 77	2507 ± 693	27 ± 15
MGBG 20 μM + Spd 5 mM	518 ± 99	4300 ± 754	16 ± 10
DCHA 10 mM	837 ± 88	130 ± 7	95 ± 9
DCHA 10 mM + Spd 1 mM	988 ± 160	946 ± 117	17 ± 2
DCHA 10 mM + Spd 5 mM	941 ± 216	1801 ± 185	14 ± 8

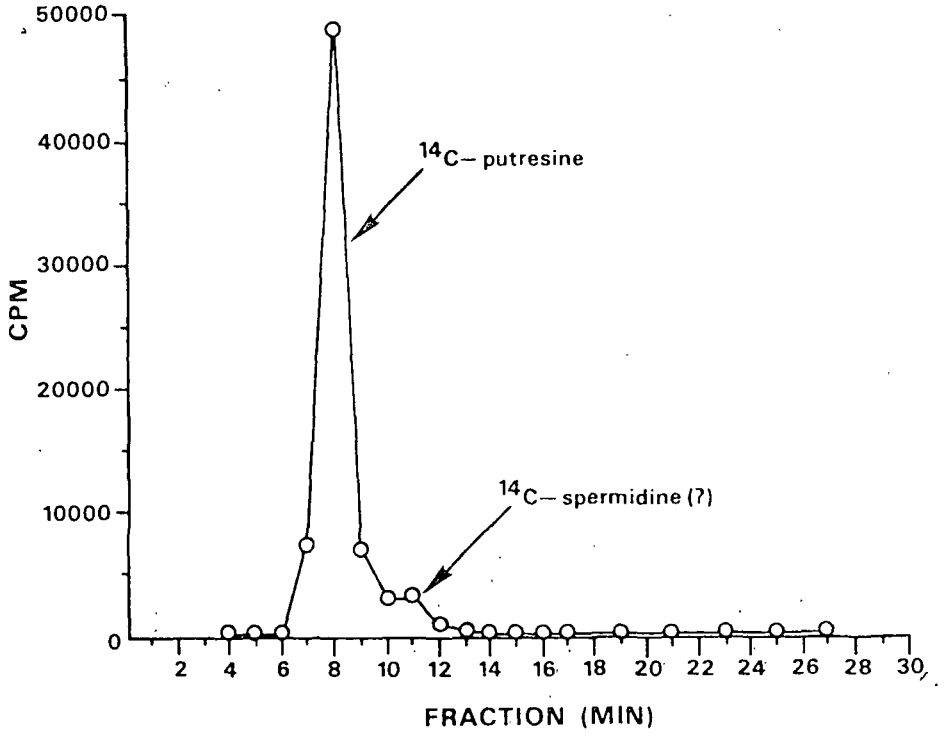


Effect of MGBG, DCHA and spermidine on wild carrot embryogenesis.

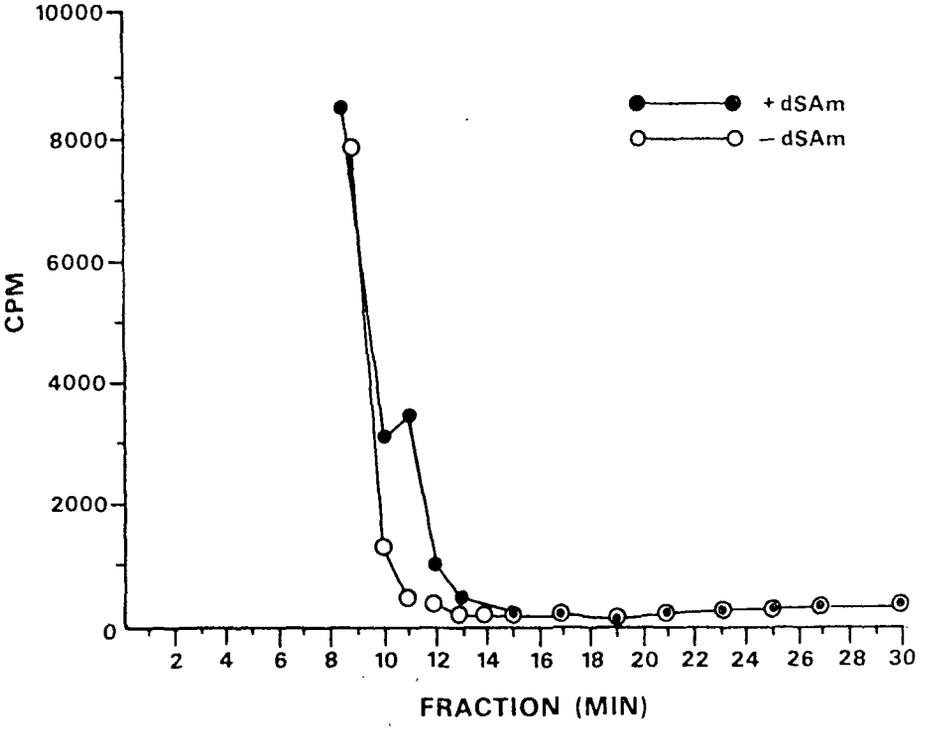
Conversion of ^{14}C– putrescine to ^{14}C– spermidine by wild carrot:– dSAm



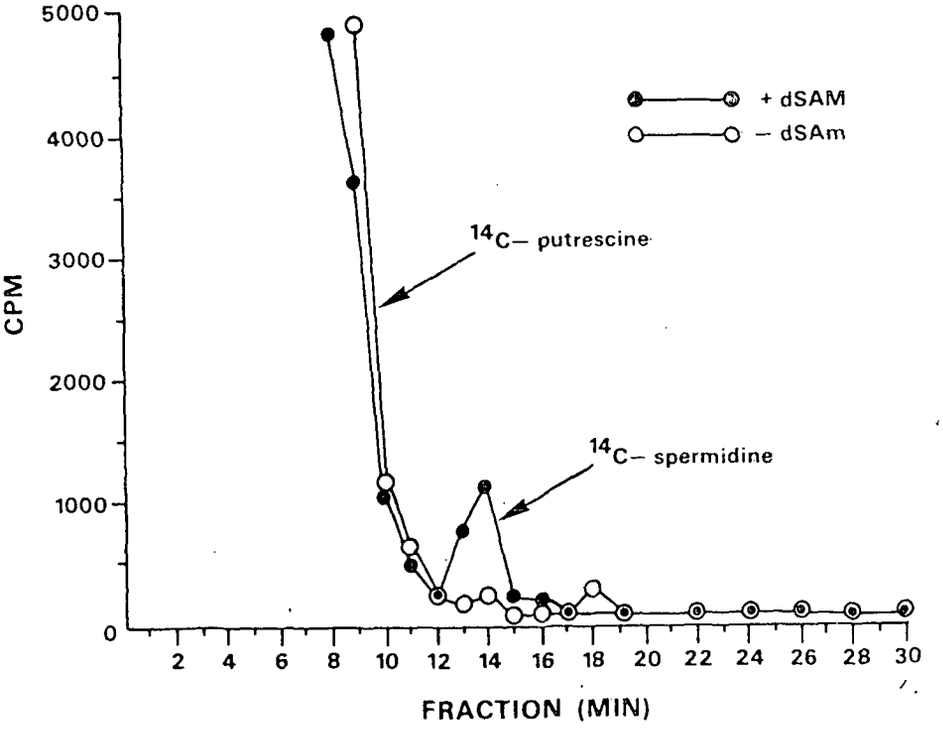
Conversion of ^{14}C– putrescine by wild carrot: + dSAm



Conversion of ^{14}C– putrescine to 14C– spermidine by wild carrot



Conversion of ^{14}C– putrescine to ^{14}C– spermidine by wild carrot



EFFECT OF DFMA AND DFMO ON LOBLOLLY PINE (SUSPENSION CULTURES) POLYAMINES

Treatment	Put	Spd	Spm
Control	287 ± 105	155 ± 10	31 ± 11
Arg	257 ± 86	141 ± 9	29 ± 6
DFMA	9 ± 4	85 ± 36	178 ± 106
DFMO + Arg	20 ± 6	91 ± 27	124 ± 77
DFMO	9 ± 3	134 ± 8	85 ± 22
DFMO + Arg	27 ± 2	146 ± 50	67 ± 38

Sample collected on Day 6
n = 3
Arg at 2.5 mM
DFMA/DFMO at 1 mM
125 mL flasks -G.R.

ENZYME ACTIVITY IN LOBLOLLY PINE OVULES: PRELIMINARY RESULTS

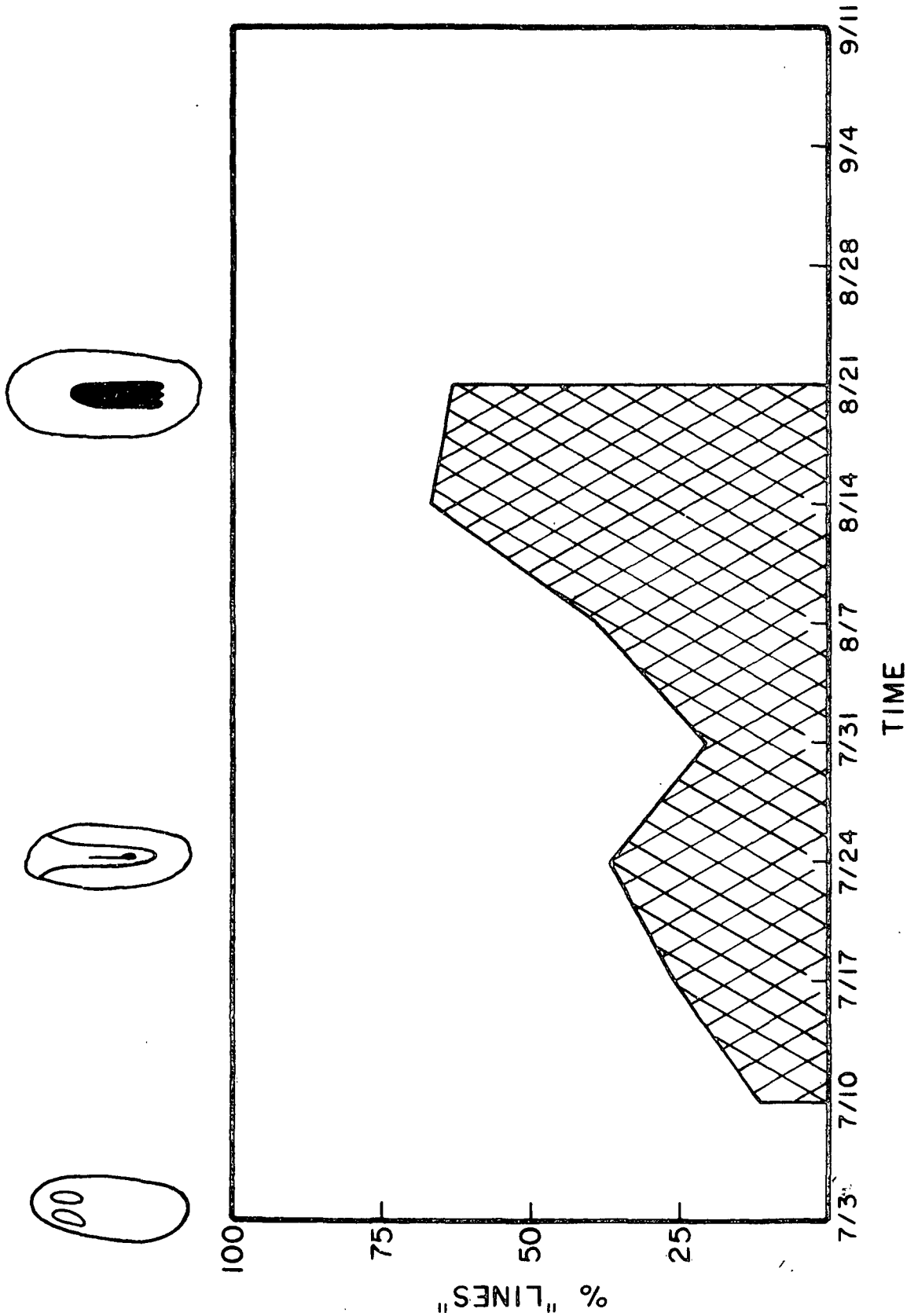
Substrate	Treatment	nmol CO_2 per g fr wt·hr
^{14}C-arginine (presumed ADC)	control	.24 ± .01
	+ DFMA	.22 ± .01
	+ DFMO	.17 ± .03
^{14}C-ornithine (presumed ODC)	control	.68 ± 0
	+ DFMA	.91
	+ DFMO	.09

Ovules from cones received 8-8-84

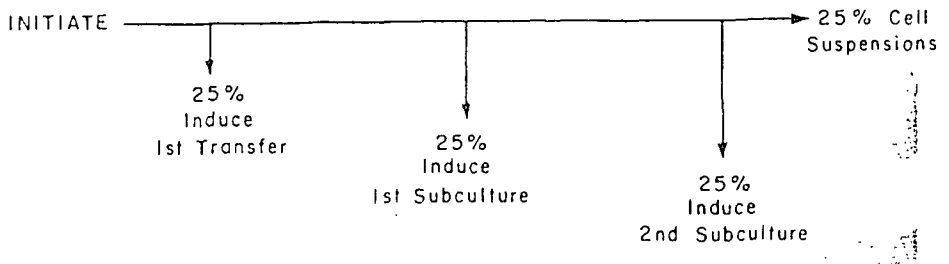
RP 281: ENZYME ACTIVITY IN SELECTED SEEDLINGS

	^{14}C-Arg (ADC)	+DFMA	^{14}C-Orn (ODC)	+DFMA
White Pine	.04 ± .06	.04 ± .04	N.D.	N.D.
Loblolly Pine	.76 ± .29	.26 ± .04	.34 ± .06	.06 ± .01
Jack Pine	.26 ± .1	.33 ± .04	.07 ± .02	.05 ± .01
Larch	N.D.	N.D.	N.D.	N.D.
Aspen	3.9 ± .2	1.1 ± .2	1.0 ± .2	.3 ± .1
Wild Carrot	33.4 ± 3.9	40.6 ± .3	6.9 ± .3	2.9 ± .4

N.D. = None Detected
DFMO or DFMA = 10 mM
Enzyme activity = nmol $^{14}CO_2$ released/g fr wt·hr


100
75
50
25
% "LINES"
7/3
7/10
7/17
7/24
7/31
8/7
8/14
8/21
8/28
9/4
9/11
TIME

Collection Date	Reps	% 1st E.V.	Growth Regulator Treatments % 1	2	3	4	5	6	7	8	9	10	11	12
7-3	0													
7-10	36	11	8°	14	6	6	3	21	14	3	14	14	30	6
7-17	36	27	11	28	22	25	50	25	20	17	61	20	28	14
7-24	32	37	41	44	47	63	56	41	28	34	22°	13°	38	22°
7-31	28	20	14	29	14	43	36	29	7	18	18	7	11	11
8-7	28	37	21	39	29	18	32	25	25	29	25	25	32	29
8-14	24	68	71	83	67	50	46	79	75	90	75	51	83	83
8-21	32		88	88	91	44	94	59	88	88	88	84	88	63
8-28														
9-4														
9-11														
Total	3000	Early	19	28	24	30	36	28	20	17	23	15	40	13
		Post	49	60	51	38	54	47	49	62	51	46	54	53
		Overall	35	44	38	34	45	38	35	40	38	31	47	34



GROWTH REGULATOR

Concentration	1	2	3	4	5
0					
.001					
.01					
.1					
1					
10					

(1) Auxin Initiation (2) Dilute out Auxin	(1) Cytokinin Initiation (2) Dilute out Cytokinin
Carrot Alfalfa Corn Soybean etc.	Gymnosperm organogenesis Walnut embryogenesis

PHENOLICS AS A MEASURE OF CELL LINE QUALITY

TABLE I

PHENOLICS EXTRACTED FROM LOBLOLLY PINE CELL LINES

Phenolic	Line 3410 nM/g FW
Gentisic	90.5
Caffeic	2.1
Salicylic	15.2
p-Coumaric	1.6
Cinnamic	3.8
	Line F2-A
Gentisic	56.9

TABLE II

PHENOLICS EXTRACTED FROM WILD CARROT CULTURES

	Day 7 nM/g FW			Day 16 nM/g FW		
Phenolic	+ 2,4-D	- 2,4-D	Parent	+ 2,4-D	- 2,4-D	Parent
Gentisic	3759	1378	833	1061	778	683
p-OH Benzoic	14.6	low	7.8	9.73	8.3	38.4
Caffeic	9.4	22.8	3.9	6.44	15.0	9.7
Ferulic				1.40	6.3	5.1
Protocatechuic				2.00		
Chlorogenic					3.1	

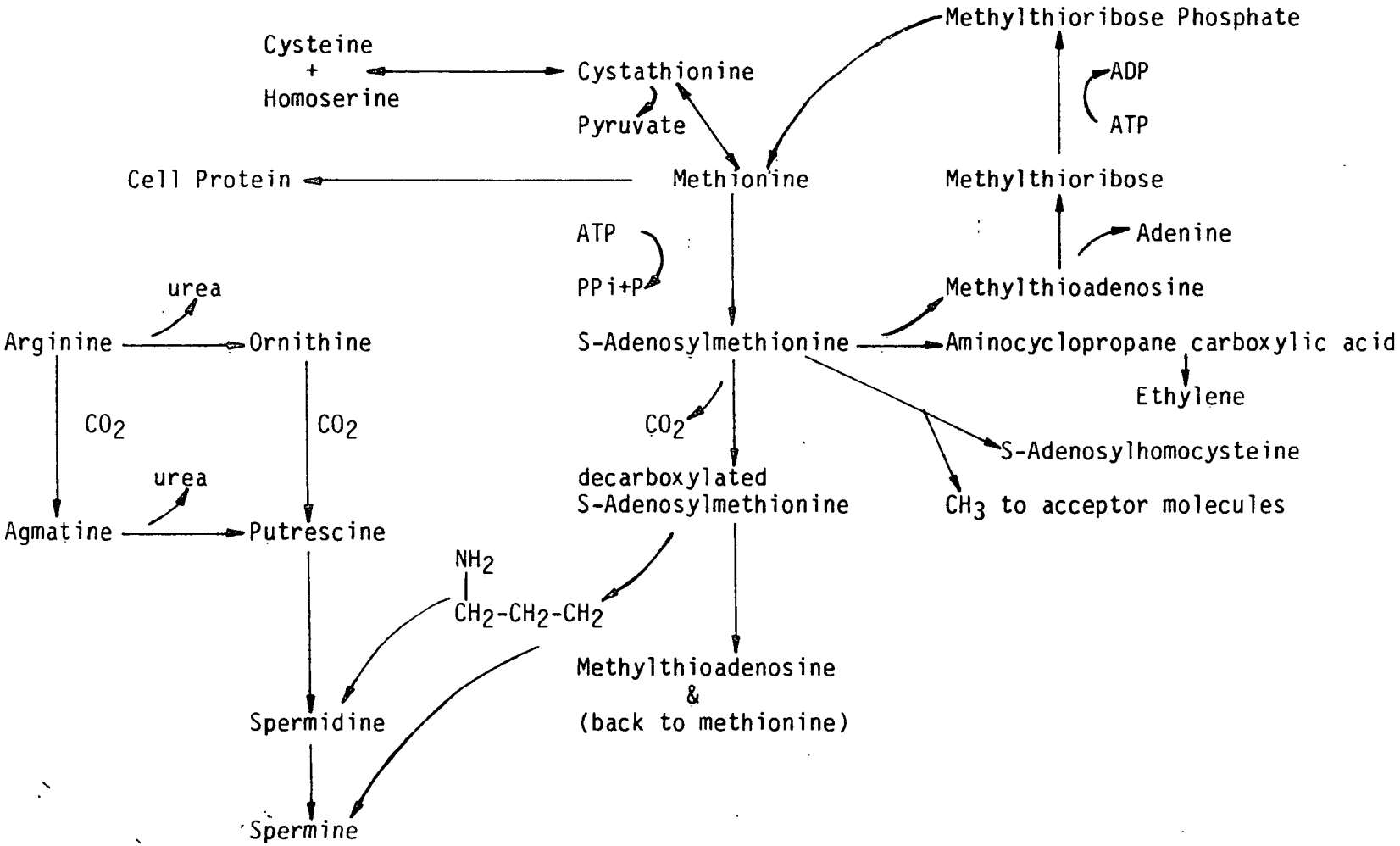
PHENOLIC STANDARDS USED IN IDENTIFYING UNKNOWNS

Phenolic Standards List 1	RT*	[conc.]
1. Gallic Acid	3.00	1×10^{-4}
2. Gentisic Acid	4.00	5×10^{-4}
3. Protocatechuic Acid	5.05	1×10^{-4}
4. Protocatechuic Aldehyde	7.26	1×10^{-4}
5. D-Catechin	8.24	1×10^{-4}
6. Chlorogenic Acid	10.50	2.5×10^{-4}
7. Caffeic Acid	12.64	2.5×10^{-4}
8. Syringic Acid	14.64	1×10^{-4}
9. Vanillin	15.96	1×10^{-4}
10. p-Coumaric Acid	18.48	1×10^{-4}
11. Ferulic Acid	20.88	1×10^{-4}
12. Benzoic Acid	21.78	5×10^{-4}
13. Cinnamic Acid	32.02	1×10^{-4}

Phenolic Standards List 2	RT	[conc.]
1. p-OH Benzoic	8.40	1×10^{-4}
2. Salicylic Acid	13.22	5×10^{-4}

*Mean retention time on HPLC chromatogram

METABOLIC INTERRELATIONS BETWEEN METHIONINE AND ITS DERIVATIVES

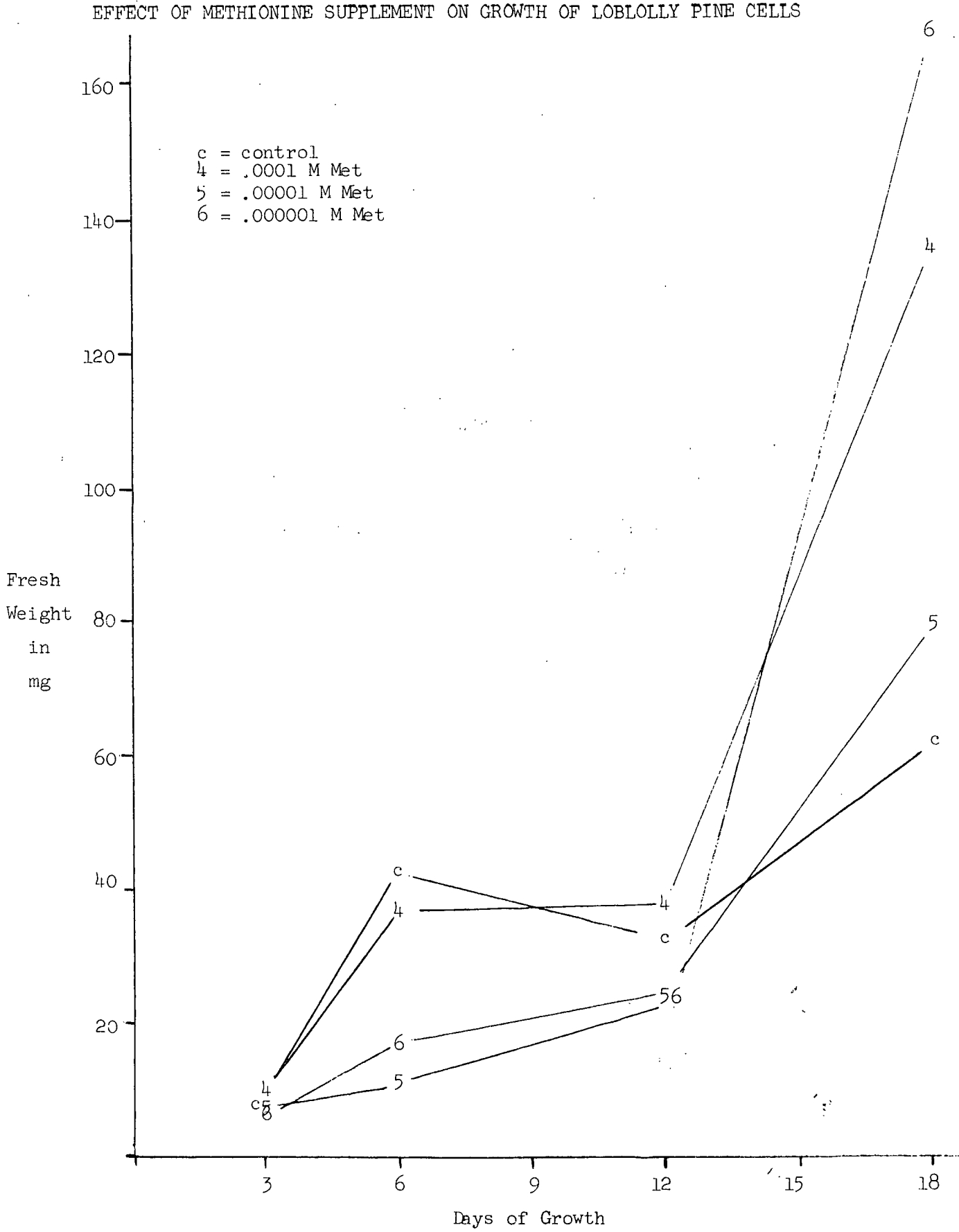


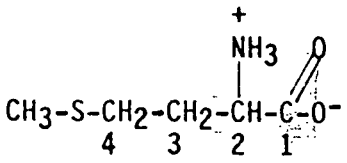
EFFECT OF METHIONINE SUPPLEMENT ON DRY WEIGHT OF LOBLOLLY PINE CELLS

Treatment	Dry Weight in mg
Control*	39.08 ± 3.97
+ 10^{-6} M Met	54.28 ± 16.59
+ 10^{-5} M Met	36.28 ± 5.17
+ 10^{-4} M Met	48.98 ± 9.94
+ 10^{-3} M Met	17.02 ± 1.41
+ 10^{-2} M Met	8.48 ± 0.34

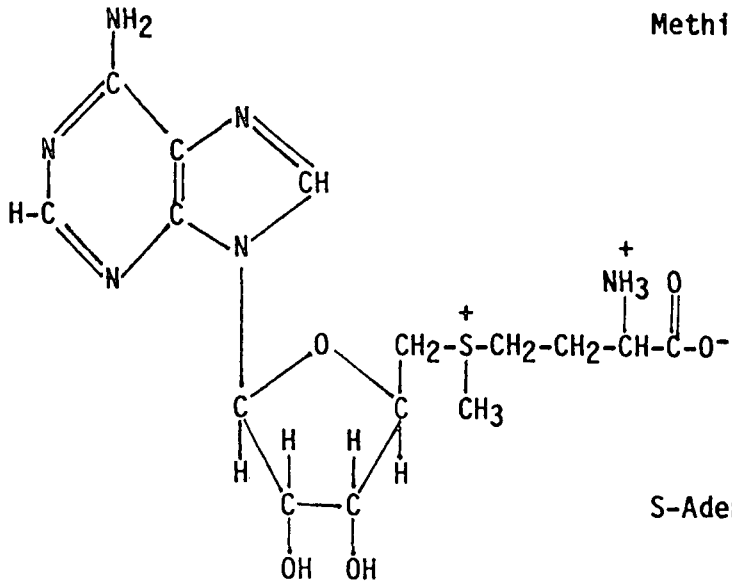
* F-2 line of cells inoculated at 10 µl / ml into 10 ml of LM3
Growth period = 14 days on roller drums in darkness.

EFFECT OF METHIONINE SUPPLEMENT ON GROWTH OF LOBLOLLY PINE CELLS

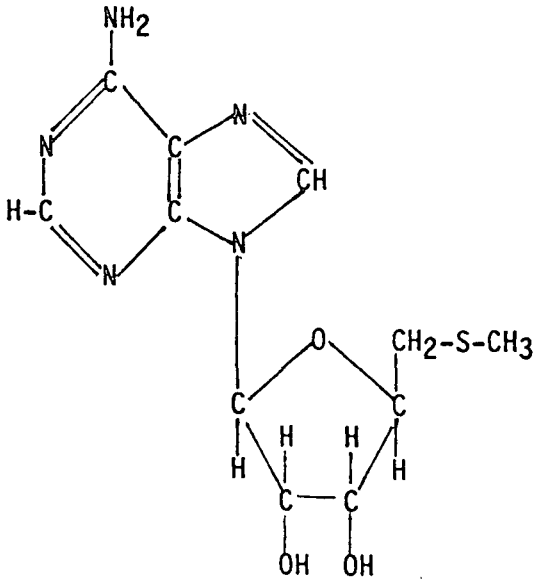




Methionine (Met)



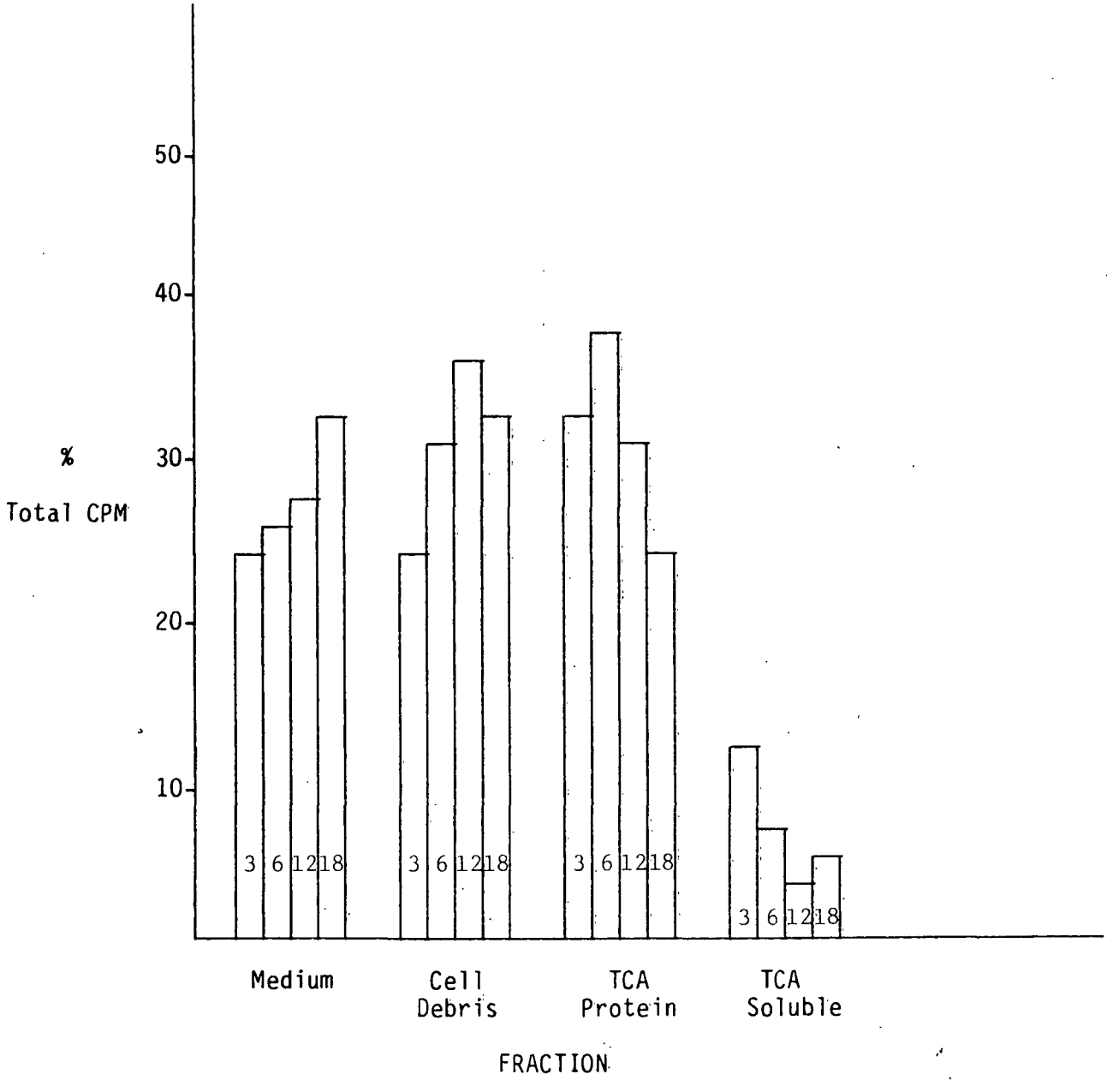
S-Adenosylmethionine (SAM)



Methylthioadenosine (MTA)

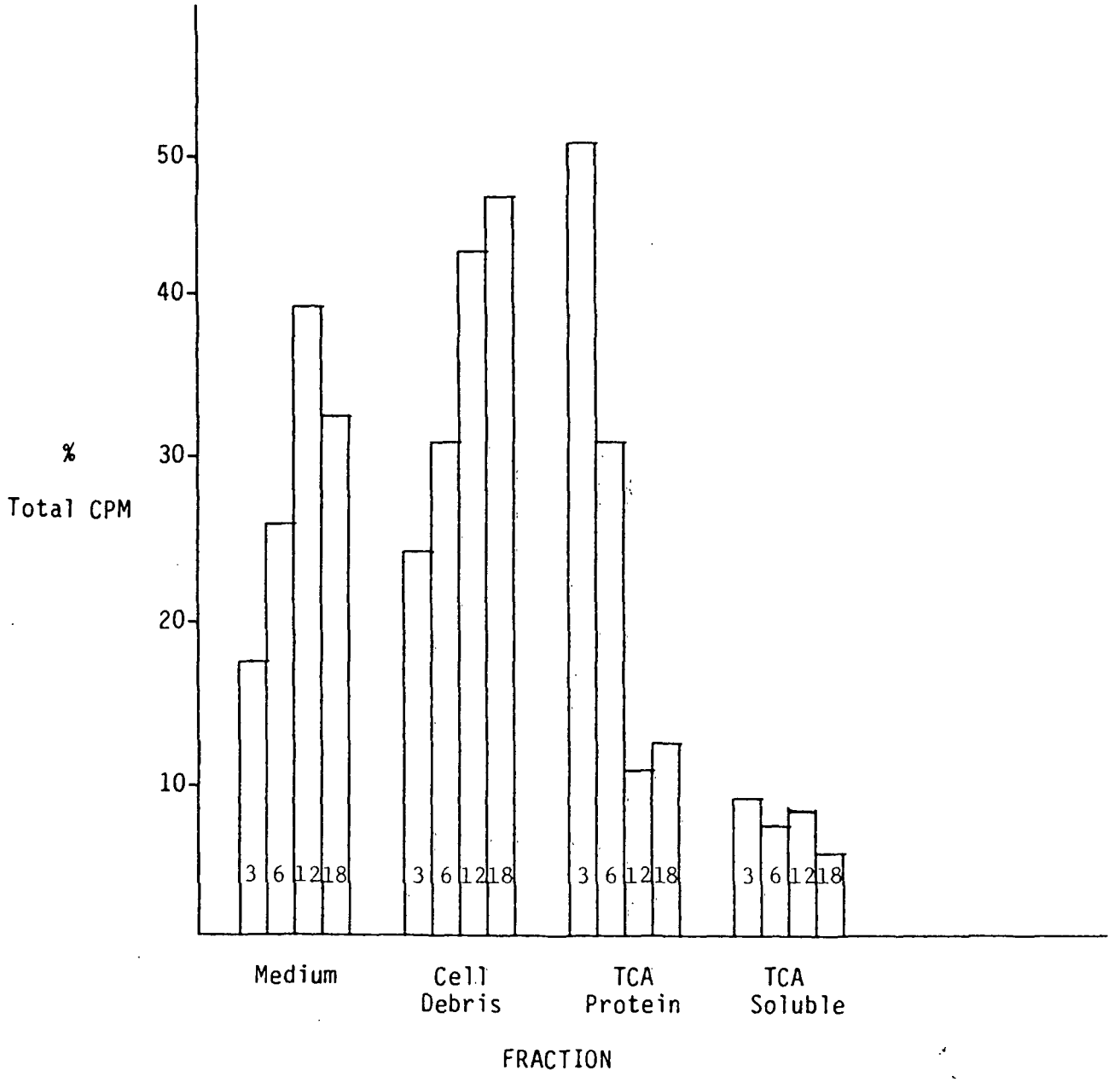
DISTRIBUTION OF ^{35}S-LABEL IN LOBLOLLY PINE CELLS

Conditions: Control, + 2,4-D



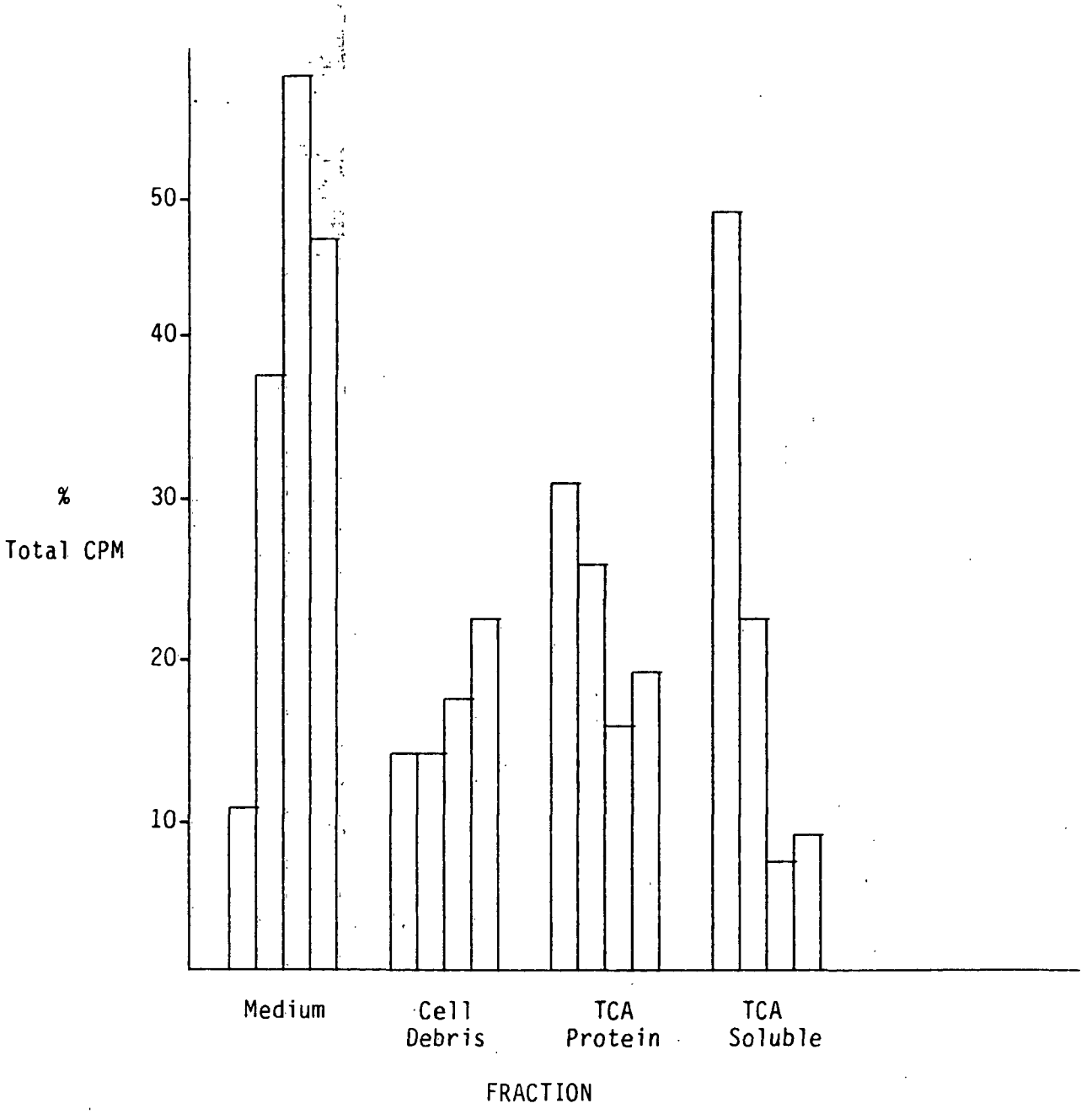
DISTRIBUTION OF ^{35}S-LABEL IN LOBLOLLY PINE CELLS

Conditions: Control, - 2,4-D



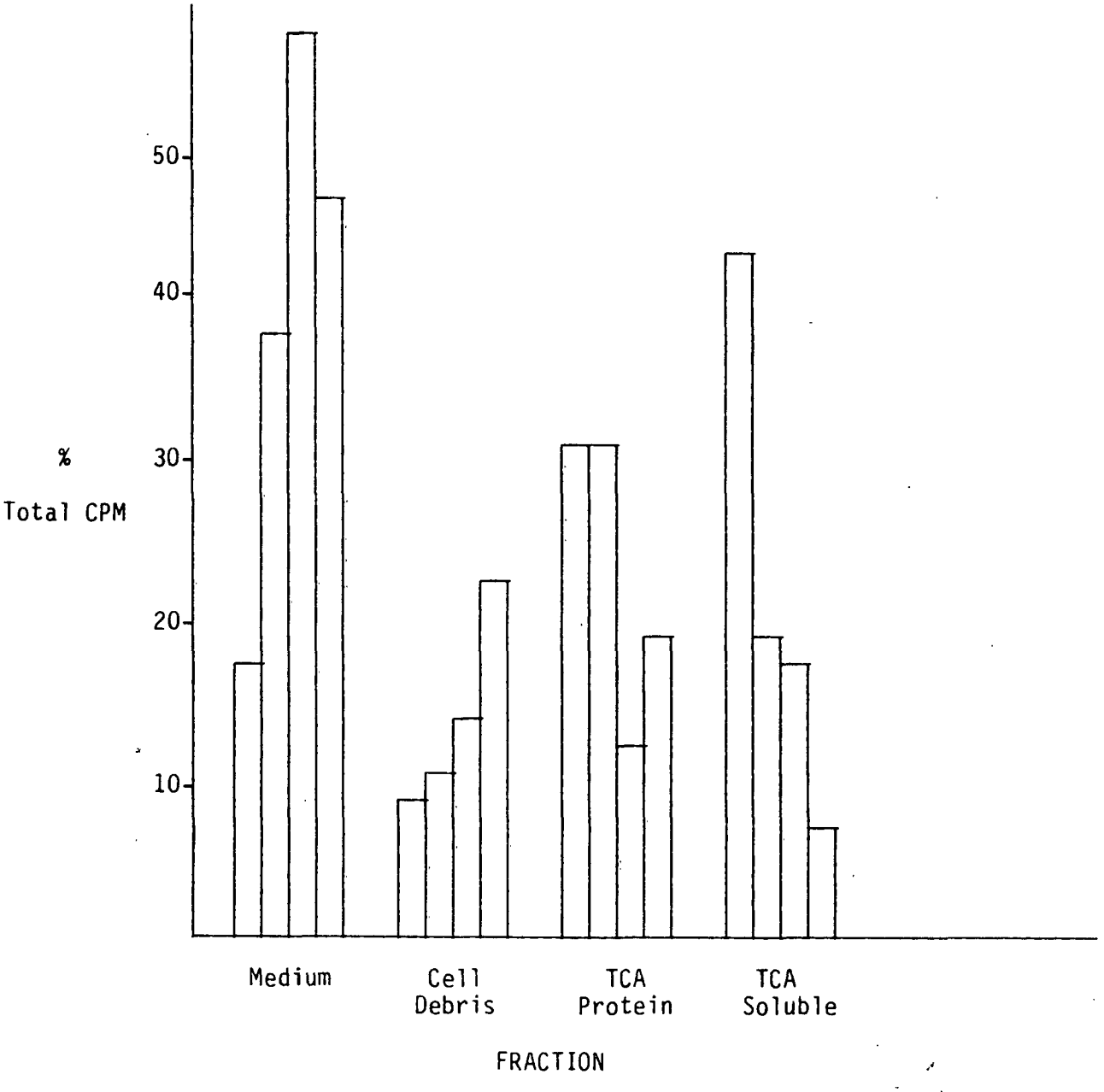
DISTRIBUTION OF ^{35}S-LABEL IN LOBLOLLY PINE CELLS

Conditions: $+10^{-4}$ M Methionine, + 2,4-D



DISTRIBUTION OF ^{35}S-LABEL IN LOBLOLLY PINE CELLS

Conditions: $+10^{-4}$ M Methionine, - 2,4-D



Extracts of Immature Pine Seeds

Question: Where do we stand at the present time on the use of extracts of immature pine seeds to promote somatic embryogenesis?

Answer: Since extracts of 1984 seeds stimulated wild carrot somatic embryogenesis as did extracts of 1983 seeds, we are now in a position to use these extracts in conifer cell launch experiments and to begin isolation of the responsible substance(s).

EFFECT OF LP SEED EXTRACT ON WILD CARROT SUSPENSION CELL GROWTH

Treatment	Pooled Cell Dry Wt. at 14 Days, mg	
2,4-D Buffer Control	0.4	
2,4-D + Extract	20.9	
No GR Buffer Control	6.3	(embryos)
No GR + Extract	40.1	(embryos)
2,4-D Water Control	1.4	
No GR Water Control	13.2	(embryos)

EFFECTS OF LP SEED EXTRACTS ON LP SUSPENSION CELLS[a]

Treatment	Fr. Wt.[b], mg/flask	Tannin[b], mg/g fr. wt.	Protein[b], ug/mL	Polyamines,[c] nmol/g fr. wt.			Enzyme Activity[c], n mol CO_2/g fr. wt./hr.	
				Put	Spd	Spm	ADC	ODC
No GR Control	494 ± 235	183 ± 20	186 ± 68	226 ± 62	207 ± 53	44 ± 29	2.3 ± 0.5	1.6 ± 0.2
No GR + Extract	249 ± 37	234 ± 6	314 ± 117	134 ± 21	216 ± 74	81 ± 35	5.7 ± 0.9	3.0 ± 0.3
2,4-D Control	1286 ± 197	62 ± 21	280 ± 95	492 ± 147	299 ± 49	41 ± 7	1.3 ± 0.1	0.3 ± 0
2,4-D + Extract	2054 ± 52	24 ± 7	127 ± 13	583 ± 236	299 ± 96	56 ± 24	1.3 ± 0.2	0.3 ± 0

[a] 34 - 10
[b] 14 days
[c] 6 days

EFFECTS OF LP SEED EXTRACT ON LP SUSPENSION CELLS[a]

Treatment	Enzyme Activity[b], nmol CO_2/g fr. wt./hr.	
	ADC	ODC
No GR Control	0.8 ± 0.1	1.2 ± 0.2
No GR + Extract	0.4 ± 0.1	0.4 ± 0.1
2,4-D Control	2.4 ± 0.2	0.3 ± 0
2,4-D + Extract	2.7 ± 0.2	0.2 ± 0
Extract only[c]	0.2 ± 0.1	0.4 ± 0.1

[a]F-2
[b]±S.D. (n = 5)
[c]per mL

EFFECTS OF LP SEED EXTRACTS ON WILD CARROT SOMATIC EMBRYOGENESIS

Sample	Embryos, numbers/tube[a]	Fresh wt., mg/tube[a]
Buffer Control	31.4 ± 11.2	23.8 ± 6.1
1983 Extract in Buffer	127.0 ± 30.5	119.2 ± 21.3
1984 Extract in Buffer	54.8 ± 15.2	52.1 ± 9.4
1984 Extract in Water	121.0 ± 23.8	120.2 ± 22.1
Water Control	44.5 ± 8.5	35.3 ± 5.2

[a]± S.D. (n = 5 except water control where n = 4)

Student Research

M.S. Level

Robert Erickson	Computer programming of x-ray Linescan data for application in the pulp and paper industry.
Peter Ryan	An investigation of methyleneoxindole and its metabolism in conifers.
Jon Saatvedt	Mycelial papers; an evalution of the properties of paper containing selected fungal mycelia.
Kathleen Turkowski	Determination of chlorine distribution in wood fibers using the scanning electron microscope and energy-dispersive spectroscopy.

Student Research

Ph.D. Level

Brent Earnshaw	Redox factors in growth and development of wild carrot
Tom Heazel	The influence of sulfonation on sulfite CMP properties
Steven Wann	Selection of mammatoxin resistant aspen via plant tissue culture

SUMMARY OF RECENT PROGRESS

- Seed extracts improved wild carrot embryogenesis
- Seed extracts improved polyamine markers when used on loblolly pine
- A new batch of seed extract has been produced and tested

SUMMARY OF RECENT PROGRESS

- Polyamine inhibitor studies again demonstrated importance of polyamines in plant development (spermidine may be the polyamine most involved)
- Energy levels in wild carrot and pine cultures indicate energy availability is no problem
- Ascorbic acid studies indicate wild carrot cells have more control of their redox status then pine cells

SUMMARY OF RECENT RESULTS

- Screening synthetic auxins turned up only one or two promising compounds
- New loblolly cell lines have been initiated from immature embryos -- some will be used in "launch" experiments
- Additional red pine cone collections made in 1984 in studies on biochemical markers associated with natural embryogenesis (polyamines, proteins, ascorbic acid, energy and glutathione monitored)

PLANS - MODEL SYSTEM RESEARCH*

Research Topic	Natural Pine Embryogenesis	Wild Carrot Somatic Embryogenesis	Loblolly Pine Cell Suspensions	Manipulation
Polyamines	X (RP)	X (RP)	X (RP)	X RP
Phenolics	X -	X (RP)	X (RP)	X (RP)
Energy	- (RP)	X (RP)	X (RP)	- RP
Growth Regulators I (Endogenous)	X (RP)	X (RP)	X (RP)	- (RP)
Growth Regulators II (Synthetic)	- -	X (RP)	X (RP)	- RP
Mitotic Index	- RP	X (RP)	X RP	- RP

* X = Data available or work underway

RP = Research planned 1984/85

RP = Work underway

PLANS - OBJECTIVE I RESEARCH

- Generate new cell lines from immature embryos, protoplasts and microsporophyll tissue
- Determine the influence of nitrogen sources, polyamines and growth regulators on cell line quality
- Determine the influence of natural conifer extracts on cell line quality
- Examine the importance of light on cell line quality

PLANS - OBJECTIVE II RESEARCH

- Run monitored launch experiments with established cell lines - to correct apparent deficiencies and reduce inhibitors
- Conduct occasional unmonitored launch experiments using promising new ideas
- Conduct monitored launch experiments using promising new cell lines
- Determine the usefulness of natural extracts, new growth regulators, and stress as ways of triggering embryogenesis

PLANS - OBJECTIVE III AND IV RESEARCH

- Determine the importance of light and embryo size on growth and survival in soil
- Try embryo encapsulation as method of improving survival and growth of newly planted embryos
- Modify larch isozyme procedures for use in making plant fidelity checks