

LEAD CITRATE

- a) Place 1,33g Pb (NO₃)₂ and 1,7g sodium citrate, with 30ml water, into a volumetric flask.
- b) Shake for 1 min.
- c) Leave for 30 min, shaking occasionally.
- d) Add 8ml 1N NaOH.
- e) Make up to 50ml with distilled water.
- f) If stored in the cold, the stain will keep for several months.

URANYL ACETATE

A saturated solution of uranyl acetate in distilled water is well shaken until the powder has dissolved. Make up a fresh solution for each staining.

APPENDIX ivTABLE 1

NUMBER OF CELLS PER 0,1cc OF NUTRIENT BROTH UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

MEAN OF 5 SAMPLES

<u>Time</u>	<u>500 Lux</u>	<u>C Lux</u>	<u>2000 Lux</u>
<u>Hours</u>	<u>20°C</u>	<u>32°C</u>	<u>32°C</u>
24	0,7	3,7	3,7
48	3,4	16,9	15,8
72	12,5	30,0	28,5
96	28,6	57,8	51,4
120	43,5	87,7	99,1
144	70,3	101,9	113,2
168	81,0	144,7	150,8

TABLE 2

NUMBER OF CELLS PER 0,1cc OF DIFFERENT NUTRIENT SOLUTIONS
AT 2000 LUX AND 32°C

MEAN OF 6 SAMPLES

<u>Time</u> <u>Hours</u>	<u>Nutrient Broth</u>	<u>Casselton and Stacey Solution</u>	
		<u>with Glucose</u>	<u>with Sucrose</u>
24	3,7	2,7	3,8
48	15,8	37,9	51,3
56	—	41,7	84,9
72	28,5	71,7	109,5
96	51,4	137,6	146,9
120	99,1	159,1	161,6
144	113,2	—	—
168	150,8	—	—

TABLE 3

ABSORBANCE AT 460mμ BY CELLS GROWN IN DIFFERENT NUTRIENT
SOLUTIONS

MEAN OF 6 SAMPLES

<u>Time</u> <u>Hours</u>	<u>Nutrient Broth</u>	<u>Casselton and Stacey Solution</u>	
		<u>with Glucose</u>	<u>with Sucrose</u>
24	,05	,10	,00
48	,16	,17	,16
72	,16	,20	,18
96	,22	,24	,21
120	,22	,26	,25
144	,26	,29	,26
168	,35	,39	,29

TABLE 4

MINIMUM SAMPLE SIZE REQUIREMENTS FOR HEMACYTOMETER

<u>Time</u> <u>Hours</u>	<u>Nutrient Broth</u>	<u>Casselton and Stacey Solution</u>	
		<u>with Glucose</u>	<u>with Sucrose</u>
24	1	1	1
48	1	17	24
56	—	10	3
72	1	14	40
96	5	58	22
120	64	12	26
144	70	—	—
168	42	—	—

TABLE 5

MINIMUM SAMPLE SIZE REQUIREMENTS FOR SPECTROPHOTOMETER

<u>Time</u> <u>Hours</u>	<u>Nutrient Broth</u>	<u>Casselton and Stacey Solution</u>	
		<u>with Glucose</u>	<u>with Sucrose</u>
24	5	11	13
48	31	20	44
72	8	58	77
96	6	7	1
120	2	2	37
144	1	4	118
168	8	5	39

APPENDIX v

TABLE 6

pH READINGS OF HERBICIDE SOLUTIONS

<u>Herbicide</u>	<u>pH before</u> <u>Sterilisation</u>	<u>pH after</u> <u>Sterilisation</u>	<u>50% Herbicide</u> <u>50% Nutrient Broth</u>
MH	7,25	7,50	7,35
2,4,5-T	6,90	7,80	7,35

<u>Herbicide</u>	<u>pH before</u> <u>Sterilisation</u>	<u>pH after</u> <u>Sterilisation</u>	<u>50% Herbicide</u> <u>50% Nutrient Broth</u>
NAA	6,70	7,50	7,35
IAA	7,00	7,30	7,35
IBA	7,00	6,90	7,35
IPA	7,30	7,00	7,30
Kinetin	7,00	7,50	7,30
GA ₃	7,00	7,30	7,30
Simazine	7,00	7,20	7,30
Na Gold			
Salt	6,00	5,90	7,00
Coconut			
Milk	5,70	5,60	6,60

TABLE 7

EFFECT OF DIFFERENT HERBICIDES FROM 100 TO 1000µg/ml ON
THE NUMBER OF CELLS PER 0,1cc

MEAN OF 5 SAMPLES

<u>Herbicide</u>	<u>100</u>	<u>200</u>	<u>400</u>	<u>600</u>	<u>800</u>	<u>1000µg/ml</u>
MH	56,0	52,0	52,8	54,9	53,0	55,0
2,4,5-T	57,4	50,9	54,7	52,7	54,5	57,3
NAA	48,8	51,0	55,0	55,5	59,0	53,8
IAA	42,9	30,7	25,3	24,6	21,8	21,5
IBA	33,6	28,1	21,7	23,3	23,6	21,1
IPA	30,3	29,0	22,8	24,7	23,8	21,6
GA ₃	49,9	50,8	50,4	48,6	52,1	48,8
Simazine	48,2	49,7	47,7	51,4	49,7	52,9
Na Gold						
Salt	53,4	50,9	52,4	55,1	52,9	52,3

TABLE 8

CONTROL NUMBER OF CELLS PER 0,1cc FOR THE DIFFERENT
HERBICIDES USED FROM 100 TO 1000µg/ml

MEAN OF 5 SAMPLES

<u>Herbicide</u>	<u>48 hour Control</u>	<u>96 hour Control</u>
MH	16,1	52,0
2,4,5-T	15,5	52,6
NAA	15,3	53,2
IAA	15,5	50,9
IBA	15,2	52,5
IPA	15,2	51,7
GA ₃	15,2	50,3
Simazine	15,3	51,1
Na Gold Salt	15,3	52,5

TABLE 9

EFFECT OF DIFFERENT HERBICIDES FROM 10 TO 1000µg/ml ON THE
NUMBER OF CELLS PER 0,1cc

MEAN OF 5 SAMPLES

<u>Herbicide</u>	<u>10</u>	<u>20</u>	<u>40</u>	<u>60</u>	<u>80</u>	<u>1000µg/ml</u>
Kinetin	51,2	49,5	51,0	49,4	49,1	51,1
IAA	50,7	48,8	51,2	47,2	43,6	43,8
IBA	55,5	53,4	43,2	40,9	41,1	34,1
IPA	54,5	50,0	44,6	44,1	41,7	42,0
Coconut	10%	20%	30%	40%	50%	v/v
Milk	55,0	54,9	55,7	53,5	87,3	

TABLE 10

CONTROL NUMBER OF CELLS PER 0,1cc FOR THE DIFFERENT
HERBICIDES USED FROM 10 TO 100µg/ml

MEAN OF 5 SAMPLES

<u>Herbicide</u>	<u>48 hour Control</u>	<u>96 hour Control</u>
Kinetin	15,3	51,3
IAA	15,7	55,0
IBA	15,5	54,9
IPA	15,8	54,0
Coconut Milk	15,2	55,7

TABLE 11

EFFECT OF HIGHER CONCENTRATIONS OF SOME HERBICIDES ON THE
NUMBER OF CELLS PER 0,1cc

MEAN OF 5 SAMPLES

<u>Herbicide</u>	<u>2000µg/ml</u>	<u>4000µg/ml</u>
MH	47,8	48,4
2,4,5-T	48,4	47,8
NAA	47,0	47,2
GA ₃	48,2	48,4
Simazine	49,4	51,0
Control 48 hours	14,8	
Control 96 hours	47,9	

TABLE 12

TIME CURVES FOR HERBICIDES (NUMBER OF CELLS PER 0,1cc)
 LIGHT/DARK PERIOD 7am TO 7pm

MEAN OF 3 SAMPLES

<u>Time after Addition</u>	<u>IAA</u>	<u>IBA</u>	<u>IPA</u>	<u>Control</u>
2 hours	24,2	27,0	24,8	25,3
4 hours	25,9	28,5	24,1	26,2
6 hours	27,8	29,8	27,3	27,9
8 hours	29,5	30,8	29,9	31,0
10 hours	31,3	30,8	31,4	32,2
12 hours	31,5	30,4	31,2	33,8
24 hours	32,3	30,3	32,7	43,6

TABLE 13

TIME CURVES FOR HERBICIDES (NUMBER OF CELLS PER 0,1cc)
 LIGHT/DARK PERIOD 1am TO 1pm

MEAN OF 3 SAMPLES

<u>Time after Addition</u>	<u>IAA</u>	<u>IBA</u>	<u>IPA</u>	<u>Control</u>
2 hours	26,9	26,2	25,3	25,7
4 hours	28,7	27,9	26,9	27,7
6 hours	31,2	30,2	29,8	30,3
8 hours	31,9	33,8	31,0	31,4
10 hours	34,3	34,8	34,0	34,4
12 hours	35,6	34,4	34,0	36,3
24 hours	36,4	34,2	35,0	46,2

TABLE 14

PERCENTAGE INHIBITION OF *PROTOTHECA WICKERHAMII* WITH
DIFFERENT HERBICIDES FROM 100 TO 1000 μ g/ml

<u>Herbicide</u>	<u>100</u>	<u>200</u>	<u>400</u>	<u>600</u>	<u>800</u>	<u>1000μg/ml</u>
MH	-12,9	0	-2,6	-9,4	-3,2	-9,7
2,4,5-T	-15,2	6,9	-5,7	-0,3	-6,0	-14,9
NAA	13,7	6,8	-5,6	-7,1	-18,0	-1,9
IAA	26,8	67,6	85,6	88,0	97,3	98,3
IBA	60,0	77,5	97,7	92,7	84,4	99,7
IPA	69,7	73,9	94,1	87,9	90,9	98,1
GA ₃	1,4	-1,7	-0,3	5,8	-6,1	5,1
Simazine	9,6	4,7	11,3	-1,0	4,7	-6,0
Na Gold Salt	-2,9	5,1	0,3	-8,3	-1,3	1,0

TABLE 15

PERCENTAGE INHIBITION OF *PROTOTHECA WICKERHAMII* WITH
DIFFERENT HERBICIDES FROM 10 TO 100 μ g/ml

<u>Herbicide</u>	<u>10</u>	<u>20</u>	<u>40</u>	<u>60</u>	<u>80</u>	<u>100μg/ml</u>
Kinetin	0,3	5,9	1,0	6,3	7,3	0,7
IAA	12,7	18,2	11,2	22,9	33,5	32,9
IBA	-1,8	4,4	34,5	41,3	35,4	61,4
IPA	-1,2	12,1	28,5	30,0	37,3	36,4
Coconut	10%	20%	30%	40%	50%	v/v
Milk	2,0	2,3	0	6,3	-91,1	

TABLE 16

PERCENTAGE INHIBITION WITH HIGHER CONCENTRATIONS OF SOME
HERBICIDES

<u>Herbicide</u>	<u>2000μg/ml</u>	<u>4000μg/ml</u>
MH	4,3	2,1
2,4,5-T	2,1	4,3

<u>Herbicide</u>	<u>2000µg/ml</u>	<u>4000µg/ml</u>
NAA	7,1	6,4
GA ₃	2,9	2,1
Simazine	-1,4	-7,1

APPENDIX viTABLE 17TIME CURVE FOR IAA AT 400µg/ml (NUMBER OF CELLS PER 0,1cc)MEAN OF 3 SAMPLES

<u>Time after Addition</u>	<u>IAA</u>	<u>Control</u>
2 hours	34,5	35,8
4 hours	36,0	36,8
6 hours	36,1	37,3
8 hours	37,1	37,7
10 hours	38,7	38,0
12 hours	38,4	38,5
24 hours	38,5	42,2

APPENDIX viiTABLE 22EFFECT OF HERBICIDES ON THE CELLULAR COMPONENT SIZES (µm)
OF PROTOTHECA WICKERHAMII

Normal Cells			
Nucleus	3,7	±	1,1
Mitochondrion	1,3	±	0,6
Cell	10,9	±	4,9
Herbicide Treated Cells			
Nucleus	3,6	±	0,7
Mitochondrion	1,3	±	0,5
Cell	8,9	±	2,7

TABLE 23

DIFFERENCE BETWEEN MEANS TEST FOR NORMAL AND HERBICIDE
TREATED CELLS

Nucleus diameter difference		t = ,241
At 26 d.f.	p < 0,1	
Mitochondria length difference		t = ,091
At 127 d.f.	p < 0,1	
Cell size difference		t = ,799
At 24 d.f.	p < 0,1	

TABLE 24

PERCENTAGE DIVIDING AND DEGENERATING CELLS AS AFFECTED BY
HERBICIDE TREATMENT (FIVE GRIDS)

<u>Treatment</u>	<u>% Dividing</u>	<u>% Degenerating</u>
Normal cells	13,19 $\pm$ 2,19	5,16 $\pm$ 0,63
Indoleacetic acid	11,06 $\pm$ 2,33	37,65 $\pm$ 9,92
Indolebutyric acid	9,23 $\pm$ 4,10	38,74 $\pm$ 9,18
Indolepropionic acid	13,46 $\pm$ 3,66	43,39 $\pm$ 2,65

APPENDIX viii

TABLE 26

INHIBITION MECHANISM STUDIES WITH REPLACEMENT OF HERBICIDE
BY NUTRIENT BROTH (NUMBER OF CELLS PER 0,1cc)

MEAN OF 3 SAMPLES

<u>Time (Hours)</u>	<u>x</u>	<u>10</u>	<u>then</u>	<u>24</u>	<u>48</u>	<u>72</u>	<u>96</u>
IAA	34,4	38,9	47,0	52,6	60,2	73,8	
Control	35,9	39,7	45,3	51,4	60,2	78,4	
<u>Time (Hours)</u>	<u>x</u>	<u>15</u>	<u>then</u>	<u>24</u>	<u>48</u>	<u>72</u>	<u>96</u>
IAA	34,9	36,7	42,6	47,6	55,3	69,1	
Control	36,3	43,0	52,3	53,4	62,3	78,2	

<u>Time (Hours)</u>	<u>x</u>	<u>20</u>	<u>then 24</u>	<u>48</u>	<u>72</u>	<u>96</u>
IAA	33,4	35,3	41,0	43,7	56,0	63,3
Control	34,1	40,1	50,1	57,7	71,9	83,0

TABLE 27

MODIFIED ENZYME KINETIC TYPE OF STUDY FOR SUBSTRATE
CONCENTRATIONS 1,0; 0,75; 0,5 AND 0,25

MEAN OF 3 SAMPLESWITH 1000µg/ml IAA

$\frac{1}{S}$	$\frac{1}{V}$	<u>10 Hours</u>	<u>15 Hours</u>	<u>20 Hours</u>
4,0		0,50	0,77	1,08
2,0		0,52	0,71	1,10
1,3		0,54	0,69	0,92
1,0		0,61	0,90	1,49

WITHOUT INHIBITOR

$\frac{1}{S}$	$\frac{1}{V}$	<u>10 Hours</u>	<u>15 Hours</u>	<u>20 Hours</u>
4,0		0,49	0,80	0,96
2,0		0,58	0,71	0,79
1,3		0,46	0,68	1,00
1,0		0,65	0,88	1,21

TABLE 28

MODIFIED ENZYME KINETIC TYPE OF STUDY FOR SUBSTRATE
CONCENTRATIONS 1,0; 0,1; 0,01 AND 0,001

MEAN OF 3 SAMPLESWITH 1000µg/ml IAA

$\frac{1}{S}$	$\frac{1}{V}$	<u>10 Hours</u>	<u>15 Hours</u>	<u>20 Hours</u>
1		0,20	0,30	0,39
10		0,20	0,29	0,37
100		0,29	0,41	0,54
1000		1,32	2,27	2,27

WITHOUT INHIBITOR

$\frac{1}{S}$	$\frac{1}{V}$	<u>10 Hours</u>	<u>15 Hours</u>	<u>20 Hours</u>
1		0,19	0,26	0,38
10		0,22	0,28	0,33
100		0,27	0,37	0,45
1000		0,99	1,11	1,39

TABLE 29

SIMPLE LINEAR REGRESSION ANALYSIS ON DATA FOR SUBSTRATE
CONCENTRATIONS 1,0; 0,1; 0,01 AND 0,001

<u>Treatment</u>	<u>Y Intercept</u> $\frac{1}{V}$	$\bar{Y}$	<u>Slope</u>	<u>Correlation</u> <u>Coefficient</u>
10 Hours				
With 1000ug/ml IAA	0,19	0,50	0,001	1,000
Without inhibitor	0,20	0,42	0,001	1,000
15 Hours				
With 1000ug/ml IAA	0,26	0,82	0,002	0,999
Without inhibitor	0,27	0,51	0,001	1,000
20 Hours				
With 1000ug/ml IAA	0,36	0,89	0,002	1,000
Without inhibitor	0,35	0,64	0,001	0,999

$\bar{X}$ is 277,75. All r values significant ($p < 0,001$).

Author Henning Penelope Anne

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