



<http://researchspace.auckland.ac.nz>

### *ResearchSpace@Auckland*

#### **Copyright Statement**

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- Any use you make of these documents or images must be for research or private study purposes only, and you may not make them available to any other person.
- Authors control the copyright of their thesis. You will recognise the author's right to be identified as the author of this thesis, and due acknowledgement will be made to the author where appropriate.
- You will obtain the author's permission before publishing any material from their thesis.

To request permissions please use the Feedback form on our webpage.

<http://researchspace.auckland.ac.nz/feedback>

#### **General copyright and disclaimer**

In addition to the above conditions, authors give their consent for the digital copy of their work to be used subject to the conditions specified on the Library Thesis Consent Form.

CYTOKININS AND THE GROWTH OF SPIRODELA OLIGORRHIZA

A thesis submitted  
for the degree of  
Doctor of Philosophy

by

P.J.A. McCOMBS

Department of Cell Biology,  
University of Auckland.

December 1971

SUMMARY

- 1 Spirodela oligorrhiza on sterile glucose-mineral medium ceased to grow three days after transfer into darkness.
- 2 Cytokinins, supplied in the medium, allowed continuous growth of Spirodela after transfer to darkness. Other plant growth substances, or adenine analogues, were ineffective.
- 3 Kinetin stimulated production of new fronds after a 24 hour lag period when added to dormant cultures although it was rapidly taken up. Kinetin reached a constant concentration in the plantlets within 30 to 60 minutes of addition to the medium.
- 4 In the absence of cytokinin, dormancy continued for three or four weeks after which growth spontaneously resumed in darkness. The growth rate then reached almost half that achieved with optimum kinetin concentrations in darkness. Growth continued in darkness for at least eight weeks without cytokinin.
- 5 Pretreatment in the light with either metabolic inhibitors, kinetin, abscisic acid, or high or low temperature, essentially eliminated the period of dormancy of Spirodela transferred to darkness in the absence of cytokinin. Growth was reduced 50% to 90% during the pretreatments.
- 6 The pretreatments designed to affect plastid RNA, protein or ATP production were the most effective. The fastest growth rate achieved in darkness without cytokinin after pretreatment was 10% less than that promoted by optimum kinetin.

- 7 Dormant Spirodela in darkness continued to incorporate precursors into RNA, DNA and protein at a rate 50% that in growing (plus kinetin) cultures. Net rates of macromolecule accumulation were extremely slow, indicating extensive degradation.
- 8 Addition of kinetin to non-growing Spirodela in darkness stimulated the synthesis of RNA, DNA and protein simultaneously after a lag of approximately one hour. The rates of precursor incorporation increased to equal those in continuously growing cultures.
- 9 Non-growing Spirodela in darkness rapidly accumulated starch. Kinetin had little or no effect on accumulation and mobilization of starch.
- 10  $^{14}\text{C}$ -glucose was taken up by growing (plus kinetin) and non-growing (minus kinetin) Spirodela in darkness, and was metabolized equally in each. Three times as much  $^{14}\text{C}$ -glucose entered starch in the non-growing cultures.
- 11 A model scheme for the control of dormancy in Spirodela is proposed based on an inhibitory mediator. The mediator may be similar to the hypothetical mediator of the pleiotypic response shown by animal cells.

CYTOKININS AND THE GROWTH OF SPIRODELA OLIGORRHIZATABLE OF CONTENTS

Page		
i	Summary	
iii	Table of Contents	
iv	Index	
xii	Acknowledgments	
xiii	Abbreviations and trivial names	
1	Chapter 1	General Introduction
26	Chapter 2	Materials
SECTION I		
29	Chapter 3	Introduction: Growth of Lemnaceae
34	Chapter 4	Methods I
39	Chapter 5	Growth of Spirodela in darkness
62	Chapter 6	Discussion: Growth of Spirodela
SECTION II		
70	Chapter 7	Introduction: Plant hormones and metabolism
76	Chapter 8	Methods II
102	Chapter 9	Protein Metabolism in Spirodela in darkness
115	Chapter 10	Nucleic acid metabolism in Spirodela in darkness
144	Chapter 11	Uptake and metabolism of kinetin by Spirodela in darkness
149	Chapter 12	Carbohydrate Metabolism in Spirodela in darkness
161	Chapter 13	Discussion: Metabolism of Spirodela
SECTION III		
165	Chapter 14	Concluding Discussion
xiv	Appendix I	Media
xv	Appendix II	Method for determination of sulphate
xix	Bibliography	

INDEX

## CHAPTER 1: GENERAL INTRODUCTION

1.1	Research described in this thesis	1
1.2	Experimental organism	1
2.1	Advantages of <u>S.oligorrhiza</u>	2
2.2	Previous studies using the Lemnaceae	4
1.3	Cytokinins	5
3.1	Historical	5
3.2	Occurrence	6
3.3	Structural requirements for cytokinin activity	6
3.4	Biological effects	7
1.4	Control of metabolism in micro-organisms and animals	9
4.1	Control of transcription	9
4.2	Control of translation	11
4.3	mRNA stability	12
4.4	Enzyme activity and turnover	12
4.5	Hormonal control in animals	13
4.6	Pleiotypic control	14
1.5	Control of growth and metabolism in plants	16
5.1	Developmental and environmental control	16
5.2	Effects of light	17
5.3	Dormancy	20
5.4	Plant growth substances	24

## CHAPTER 2: MATERIALS

2.1	General chemicals	26
2.2	Specific chemicals	26
2.3	Radiochemicals	27
2.4	Drugs	27
2.5	Enzymes	28
2.6	Chromatographic materials	28

CHAPTER 3: INTRODUCTION: GROWTH OF LEMNACEAE		
3.1	Ontogenesis of fronds in <u>S.oligorrhiza</u>	29
3.2	Growth of Lemnaceae in darkness	31
3.3	Effect of growth substances on Lemnaceae	32
3.1	Auxins	32
3.2	Gibberellins	33
3.3	Abscisic Acid	33
CHAPTER 4: METHODS I		
4.1	Experimental organism	34
1.1	Sterilization of Spirodela	34
4.2	Culture conditions	35
2.1	Medium	35
2.2	Stock cultures	35
2.3	Experimental cultures	36
2.4	Transfers and additions to flasks	36
4.3	Assessment of growth	37
3.1	Frond number	37
3.2	Weight	37
4.4	Photography	38
CHAPTER 5: GROWTH OF SPIRODELA IN DARKNESS		
5.1	Growth in darkness with and without kinetin	39
5.2	Growth in darkness on different kinetin concentrations	40
5.3	Ability of other compounds to allow growth in darkness	42
3.1	Plant growth substances	43
3.2	Acetylcholine	43
3.3	9-substituted-6-benzyladenine	45
3.4	CyclicAMP	46
5.4	Competition of 6-benzyladenine and 3-benzyladenine	47
5.5	Initiation of growth in darkness by kinetin	48
5.6	Effects of metabolic inhibitors on growth	48
6.1	Chloramphenicol	49
6.2	Methotrexate	50

6.3	5-Fluorodeoxyuridine	51
6.4	Effects of abscisic acid, actidione and 5-methyl-tryptophan	51
5.7	Growth with or without kinetin after extended periods in darkness	53
5.8	Growth in darkness without kinetin following pretreatment with chloramphenicol	54
8.1	Growth after seven days CAP treatment	54
8.2	Effect of length of CAP pretreatment on subsequent dark growth without kinetin	56
8.3	Effect of light on CAP pretreatment	56
8.9	Growth in darkness without kinetin following inhibition of growth by other agents	57
5.10	Growth in darkness after pretreatment with kinetin	60
CHAPTER 6: DISCUSSION: SECTION I: GROWTH OF SPIRODELA		
6.1	Effect of cytokinins on growth in darkness	62
6.2	Spontaneous resumption of growth in darkness without cytokinin	66
CHAPTER 7: INTRODUCTION: PLANT HORMONES AND METABOLISM		
7.1	Effects of plant hormones on protein metabolism	70
7.2	Effects of plant hormones on nucleic acid synthesis	71
7.3	Metabolism of cytokinins	73
CHAPTER 8: METHODS II		
I	GENERAL METHODS	
8.1	General preparative and analytical procedures	76
1.1	Centrifugation	76
1.2	Spectroscopy	76
1.3	Measurement of radioactivity	77
1.4	Chromatography	77
	(a) Paper chromatography	77
	(b) Thin-layer chromatography	78



	(c) Column chromatography	78
	(d) Solvents	78
1.5	Electrophoresis	78
	(a) Paper electrophoresis	78
	(b) Thin-layer electrophoresis	79
1.6	Radioautography	79
8.2	Experimental procedures involving Spirodela cultures	79
2.1	Experimental conditions	79
2.2	Sampling and additions	79
2.3	Treatment of samples	79
II	EXTRACTION AND ESTIMATION OF MACROMOLECULES	
8.3	Extraction and estimation of protein	81
3.1	Estimation of soluble protein	81
3.2	Determination of protein-associated radioactivity	81
8.4	Extraction of nucleic acid	82
4.1	Estimation of RNA	82
	(a) Orcinol reaction	82
	(b) Total RNA extraction	82
4.2	Estimation of DNA	83
4.3	MAK column chromatography	84
4.4	Determination of nucleic acid-associated radioactivity	84
	(a) Determination of conditions for alkaline hydrolysis of RNA	85
	(b) Confirmation of the nature of alkali-labile material	85
8.5	Extraction of starch	86
5.1	Extraction and estimation of starch	86
5.2	Determination of starch-associated radioactivity	86
III	MACROMOLECULES IN SPIRODELA CULTURES IN DARKNESS	
8.6	Changes in macromolecule content	87
6.1	Nucleic acids and soluble protein	87
6.2	Starch	88

	(a) Starch accumulation in growing and non-growing cultures	88
	(b) Mobilization of starch in the absence of glucose	89
IV	UPTAKE AND INCORPORATION OF PRECURSORS INTO MACROMOLECULES	
8.7	Uptake and incorporation of $^{35}\text{S}$ -sulphate into protein	89
	7.1 Rates of uptake and incorporation of $^{35}\text{S}$ -sulphate	89
	7.2 Time required for the stimulation of protein synthesis by kinetin	90
8.8	Uptake and incorporation of $^3\text{H}$ -uridine into RNA	91
	8.1 Rates of uptake and incorporation of $^3\text{H}$ -uridine	91
	8.2 Chromatography of $^3\text{H}$ -uridine labelled RNA	92
	8.3 Time required for the stimulation of RNA synthesis by kinetin	92
8.9	Uptake and incorporation of $^3\text{H}$ -thymidine into DNA	93
	9.1 Rates of uptake and incorporation of $^3\text{H}$ -thymidine	93
	9.2 Time required for the stimulation of DNA synthesis by kinetin	93
8.10	Column chromatography of nucleic acid following addition of kinetin to non-growing Spirodela	94
8.11	Specific activity of $^3\text{H}$ -uridine labelled ribosomes	95
8.12	Synthesis and turnover of RNA in growing and non-growing Spirodela	95
8.13	Estimation of RNA base composition	96
8.14	Estimation of ratio of free to membrane-bound ribosomes	97
8.15	Estimation of precursor pool specific activities	98
	15.1 Specific activities of sulphate pools	98
	15.2 Specific activities of phosphate pools	99
8.16	Uptake and metabolism of $^{14}\text{C}$ -glucose	99
	16.1 Uptake and incorporation of $^{14}\text{C}$ -glucose	99
	16.2 Metabolism of $^{14}\text{C}$ -glucose	100

## CHAPTER 9: PROTEIN METABOLISM IN SPIRODELA IN DARKNESS

A	INTRODUCTION	102
B	EXPERIMENTAL RESULTS	103
9.1	Growth of Spirodela on low-sulphate medium	103
9.2	Changes in the soluble protein content of Spirodela on basal medium in darkness	103
9.3	Uptake and incorporation of $^{35}\text{S}$ -sulphate into protein	104
9.4	Effects of inhibitors of protein synthesis on $^{35}\text{S}$ -sulphate incorporation into protein by non-growing Spirodela	104
9.5	Specific activities of sulphate pools	106
9.6	Time required for the stimulation of protein synthesis by kinetin	107
C	DISCUSSION	109

## CHAPTER 10: NUCLEIC ACIDS METABOLISM IN SPIRODELA IN DARKNESS

A	INTRODUCTION	115
B	EXPERIMENTAL RESULTS	116
10.1	Extraction and hydrolysis of nucleic acids	116
1.1	Methods for extraction of nucleic acids	116
1.2	Estimation of total nucleic acids	117
1.3	Alkaline hydrolysis of RNA	118
10.2	Base composition of RNA	118
10.3	Changes in nucleic acid content of Spirodela on basal medium in darkness	120
10.4	Uptake and incorporation of $^3\text{H}$ -uridine	121
4.1	Rates of uptake and incorporation	121
4.2	Column chromatography of $^3\text{H}$ -uridine-labelled nucleic acid	122
10.5	Time required for the stimulation of RNA synthesis by kinetin	124
10.6	Uptake and incorporation of $^3\text{H}$ -thymidine	125
10.7	Stimulation of DNA synthesis by kinetin	126
7.1	Time required for the stimulation of DNA synthesis	126
7.2	Sites of $^{14}\text{C}$ -thymidine incorporation	127

10.8	Column chromatography of $^{32}\text{P}$ -phosphate labelled nucleic acid	128
8.1	Effect of kinetin on the specific activity of phosphate pools	129
8.2	Column chromatography of $^{32}\text{P}$ -labelled nucleic acid following stimulation by kinetin	130
10.9	Specific activity of $^3\text{H}$ -uridine-labelled ribosomes	132
10.10	Turnover of RNA in growing and non-growing Spirodela	134
10.11	Ratio of free to membrane-bound ribosomes in growing and non-growing Spirodela	136
C	DISCUSSION	138
	(i) Nucleic acids in Spirodela	138
	(ii) Nucleic acid metabolism in growing and non-growing cultures	139
	(iii) Stimulation of nucleic acid by kinetin	141
CHAPTER 11: UPTAKE AND METABOLISM OF KINETIN BY SPIRODELA IN DARKNESS		
A	INTRODUCTION	144
B	METHODS AND RESULTS	144
11.1	Extraction and chromatography of $^{14}\text{C}$ -kinetin	144
11.2	Rates of uptake and metabolism of $^{14}\text{C}$ -kinetin	146
C	DISCUSSION	147
CHAPTER 12: CARBOHYDRATE METABOLISM IN SPIRODELA IN DARKNESS		
A	INTRODUCTION	149
B	EXPERIMENTAL RESULTS	150
12.1	Effects of kinetin and FUDR on starch accumulation in darkness	150
12.2	Uptake and incorporation of $^{14}\text{C}$ -glucose	153
2.1	Rates of uptake and loss of $^{14}\text{C}$ -radioactivity following transfer to $^{14}\text{C}$ -glucose medium	153
2.2	Distribution of $^{14}\text{C}$ -radioactivity following uptake of $^{14}\text{C}$ -glucose by growing and non-growing cultures	154
C	DISCUSSION	155

## CHAPTER 13: DISCUSSION: SECTION II: METABOLISM OF SPIRODELA

- 13.1 Effects of kinetin on the metabolism of Spirodela 161
- 13.2 Similarities between control of metabolism in Spirodela in darkness and other step-down cultures 162

## CHAPTER 14: CONCLUDING DISCUSSION

- 14.1 The control of dormancy in Spirodela 165
- 14.2 Effects of cytokinin on the metabolism of Spirodela in darkness 167
- 14.3 A model for the control of dormancy in Spirodela. 169
- 14.4 Suggestions for further work arising from this investigation 173

ACKNOWLEDGMENTS

In accomplishing this work I wish to thank the following persons:

Professor R.K. Ralph for supervision, advice and guidance throughout the work, and for editing the thesis.

Dr D.S. Letham, and other staff of the Fruit Research Division, DSIR, for supplying *Spirodela inocula*, and for helpful advice.

Dr R.L. Bielecki and Mr J.R. Bedbrook for assisting in the identification of chromatographed compounds in Chapter 12.

Professor S. Bullivant for preparing material for electron microscope examinations.

The staff and students of the Department of Cell Biology for assistance and discussions throughout the course of this work, especially Dr M.V. Berridge, who also assisted with Chapter 1.

ABBREVIATIONS AND TRIVIAL NAMES

ABA	abscisic acid
Ach	acetylcholine
3BA	3-benzyladenine
6BA	6-benzylaminopurine
BSA	bovine serum albumen
CAP	chloramphenicol
<u>C</u>	Curie
DEAE	diethylaminoethyl
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
f.w.	fresh weight
FUDR	5-fluorodeoxyuridine
g	acceleration due to gravity
GA	gibberellic acid
gm	gram
IAA	indoleacetic acid
kinetin	6-furfurylaminopurine
m, u, n	milli ( $10^{-3}$ ), micro ( $10^{-6}$ ), nano ( $10^{-9}$ ) respectively
<u>M</u>	molar
MAK	methylated albumen bound to celite
ml, ul	millilitre, microlitre respectively
NDS	naphthalene disulphonate
nm	nanometres
OD	optical density
PVP	polyvinylpyrrolidone
rRNA, tRNA, mRNA	ribosomal, transfer and messenger RNA respectively
RNA	ribonucleic acid
SDS	sodium dodecyl sulphate
Spec. act.	specific activity
TCA	trichloroacetic acid
Tris	tris(hydroxymethyl)aminomethane
uv	ultraviolet