



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](#)

PHYSIOLOGICAL AND BIOCHEMICAL
ADAPTATION IN THE NITROGEN
NUTRITION OF SPIRODELA OLIGORRHIZA

A. R. Ferguson
Department of Cell Biology
University of Auckland

February, 1969

This is submitted in partial
fulfilment of the requirements
for the degree of Doctor of
Philosophy of the University
of Auckland.

LIST OF CONTENTS

	<u>Page</u>
<u>LIST OF CONTENTS</u>	(i)
<u>LIST OF PLATES AND FIGURES</u>	(v)
<u>ABBREVIATIONS AND NOMECLATURE</u>	(vii)
<u>ACKNOWLEDGEMENTS</u>	(viii)
<u>SUMMARY</u>	(ix)
Section 1 <u>INTRODUCTION</u>	1
Section 2 <u>LITERATURE SURVEY - The utilization of nitrogenous compounds by plants</u>	6
2.1 <u>The forms of nitrogen supporting the growth of plants</u>	6
2.2 <u>The uptake of compounds by plants</u>	6
2.3 <u>The utilization of ammonium and nitrate by plants</u>	7
2.4 <u>The assimilation of nitrate</u>	8
2.5 <u>The adaptive formation of nitrate and nitrite reductases</u>	10
2.5.1 Adaptive formation of nitrate reductase in bacteria	11
2.5.2 Adaptive formation of nitrate and nitrite reductases in fungi	11
2.5.3 Adaptive formation of nitrate and nitrite reductases in algae	11
2.5.4 Adaptive formation of nitrate and nitrite reductases in higher plants	12
2.6 <u>The inhibition by ammonium of the utilization of nitrate</u>	15
2.7 <u>The utilization of organic nitrogenous compounds by plants</u>	16

Section 3	<u>MATERIALS AND METHODS</u>	19
3.1	<u>Experimental organisms</u>	19
3.2	<u>Chemicals used</u>	19
3.2.1	Chemicals used in culture media	19
3.2.2	Chemicals used in thin-layer chromatography	19
3.2.3	Chemicals used in the estimation of nitrogenous compounds	20
3.2.4	Chemicals used in enzymatic assays	20
3.2.5	Radiochemicals used	20
3.2.6	Chemicals used in the assay of radioactivity	21
3.3	<u>Culture methods</u>	21
3.3.1	Culture media	21
3.3.2	Conditions of growth	22
3.3.3	Inoculation	22
3.3.4	Assessment of growth of <i>S. oligorhiza</i>	23
3.3.5	Photographing of <i>S. oligorhiza</i>	23
3.4	<u>Estimation of nitrogenous compounds in the medium</u>	23
3.4.1	Total nitrogen	23
3.4.2	Ammonia	24
3.4.3	Nitrate	24
3.4.4	Nitrite	24
3.4.5	Amino acids	24
3.4.6	Identification of a ninhydrin-reacting compound in the medium	25
3.5	<u>Analysis of plants of <i>S. oligorhiza</i> for nitrogenous compounds</u>	26
3.5.1	Total nitrogen	26
3.5.2	Preparation of extracts for the estimation of soluble nitrogenous compounds	26

	3.5.3	Total soluble nitrogen	26
	3.5.4	Ammonium	26
	3.5.5	Nitrate	26
	3.5.6	Nitrite	27
	3.5.7	Soluble amino acids	27
3.6		<u>Examination of plants for enzymatic activity</u>	27
	3.6.1	Preparation of plant homogenates	27
	3.6.2	Measurement of nitrate reductase activity	28
	3.6.3	Measurement of nitrite reductase activity	28
	3.6.4	Comparison of nitrate and nitrite reductase activities	29
3.7		<u>Detection and assay of radioactivity</u>	29
3.8		<u>Determination of the rates of uptake of amino acids by <i>S. oligorrhiza</i></u>	30
Section 4		<u>THE UTILIZATION OF AMMONIUM AND NITRATE</u>	31
	4.1	<u>Growth of <i>S. oligorrhiza</i> with either ammonium or nitrate as sole nitrogen source</u>	31
	4.2	<u>Chemical composition of <i>S. oligorrhiza</i> growing with either ammonium, nitrate, or ammonium together with nitrate as sources of nitrogen</u>	31
	4.3	<u>The uptake of ammonium, nitrate, and nitrite by <i>S. oligorrhiza</i></u>	34
	4.4	<u>Discussion: The utilization of ammonium and nitrate</u>	36
Section 5		<u>CONTROL OF THE ENZYMES OF NITRATE ASSIMILATION</u>	39
	5.1	<u>Development of extraction procedures allowing for the assay of nitrate reductase in homogenates of <i>S. oligorrhiza</i></u>	39
	5.2	<u>Some properties of nitrate reductase and nitrite reductase in <i>S. oligorrhiza</i></u>	40
	5.2.1	Co-enzyme specificity of nitrate reductase	40

5.2.2	Effects of addition of FAD to assay system	41
5.2.3	Effects of pH	41
5.3	<u>Levels of activity of nitrate reductase and nitrite reductase in <i>S. oligorrhiza</i> under different conditions of growth</u>	41
5.3.1	Levels of enzyme activity in plants growing with different sources of nitrogen	41
5.3.2	Increase in levels of enzyme activity in plants transferred to medium containing nitrate	42
5.3.3	Increase in levels of enzyme activity in plants transferred to medium containing both ammonium and nitrate	42
5.3.4	Levels of enzyme activity in plants grown with nitrate and transferred to medium containing both ammonium and nitrate	42
5.3.5	Decrease in levels of enzyme activity in plants grown with nitrate and transferred to medium containing no nitrogen source	43
5.3.6	Decrease in levels of enzyme activity in plants grown with nitrate and transferred to medium containing no nitrogen source	43
5.4	<u>Studies on inhibition of nitrate reductase activity by various nitrogenous compounds</u>	44
5.5	<u>Discussion: Control of the enzymes of nitrate assimilation</u>	46
Section 6	<u>THE UTILIZATION OF AMINO ACIDS</u>	51
6.1	<u>Growth of <i>S. oligorrhiza</i> with ammonium and different amino acids as nitrogen sources</u>	51
6.2	<u>The uptake of glutamic acid and glutamine by <i>S. oligorrhiza</i></u>	53
6.3	<u>Discussion: the utilization of amino acids</u>	57
Section 7	<u>CONCLUDING DISCUSSION</u>	64

LITERATURE CITED

LIST OF PLATES AND FIGURES

The following plates and figures are after page 35:

- Plate 1 Appearance of plants of *S. oligorrhiza* growing with different sources of nitrogen (ammonium and nitrate).
- Figure 1 Utilization of ammonium during growth of *S. oligorrhiza*.
- Figure 2 Utilization of ammonium and nitrate during growth of *S. oligorrhiza*.
- Figure 3 Utilization of nitrate by *S. oligorrhiza* growing with either nitrate alone, or nitrate together with ammonium as nitrogen sources.
- Figure 4 Utilization of nitrate by *S. oligorrhiza* growing with either nitrate alone, or nitrate together with nitrite as nitrogen sources.
- Figure 5 Utilization of nitrite by *S. oligorrhiza* growing with either nitrite alone, or nitrite together with ammonium as nitrogen sources.

The following figures are after page 45:

- Figure 6 Effects of pH on nitrate reductase activity in homogenates of *S. oligorrhiza*.
- Figure 7 Levels of nitrate and nitrite reductases in plants of *S. oligorrhiza* grown with ammonium, and then supplied with medium containing nitrate.
- Figure 8 Levels of nitrate and nitrite reductases in plants of *S. oligorrhiza* grown with ammonium, and then supplied with medium containing both ammonium and nitrate.
- Figure 9 Levels of nitrate and nitrite reductases in plants of *S. oligorrhiza* grown with nitrate, and then supplied with medium containing both ammonium and nitrate.
- Figure 10 Ratios of the levels of nitrate and nitrite reductases in plants of *S. oligorrhiza* grown with nitrate, and then supplied with medium containing ammonium and nitrate, to the levels in plants then supplied with medium containing nitrate.
- Figure 11 Levels of nitrate and nitrite reductases in plants of *S. oligorrhiza* grown with nitrate, and then supplied with medium containing no nitrogen source.

- Figure 12 Ratios of the levels of nitrate and nitrite reductases in plants of *S. oligorrhiza* grown with nitrate, and then supplied with medium containing no nitrogen source, to the levels in plants then supplied with medium containing nitrate.
- Figure 13 Total amounts of nitrate and nitrite reductases in plants of *S. oligorrhiza* grown with nitrate, and then supplied with medium containing no nitrogen source.
- Figure 14 Levels of nitrate and nitrite reductases in plants of *S. oligorrhiza* grown with nitrate, and then supplied with medium containing only ammonium.
- Figure 15 Total amounts of nitrate and nitrite reductases in plants of *S. oligorrhiza* grown with nitrate and then supplied with medium containing ammonium.
- Figure 16 Correlation between the concentrations of nitrate in plants of *S. oligorrhiza* and the levels of nitrate reductase that they contain.

The following plates and figures are after page 56:

- Plate 2 Appearance of plants of *S. oligorrhiza* growing with different sources of nitrogen (ammonium, glutamic acid, glutamine, and ammonium and glutamic acid).
- Plate 3 Appearance of plants of *S. oligorrhiza* growing with different sources of nitrogen (ammonium, aspartic acid, asparagine, and ammonium and aspartic acid).
- Figure 17 Utilization of L-glutamic acid during growth of *S. oligorrhiza*.
- Figure 18 Utilization of ammonium and L-glutamic acid during growth of *S. oligorrhiza*.
- Figure 19 Utilization of ammonium and L-aspartic acid during growth of *S. oligorrhiza*.
- Figure 20 Utilization of L-glutamic acid and L-glutamine during growth of *S. oligorrhiza*.
- Figure 21 Relationship between the rate of uptake of L-glutamic acid by *S. oligorrhiza* and the concentration of glutamic acid in the medium.
- Figure 22 Relationship between the rate of uptake of L-glutamine by *S. oligorrhiza* and the concentration of glutamine in the medium.

ABBREVIATIONS AND NOMENCLATURE

The abbreviations used in this thesis are based on the revised tentative rules (1965) recommended by the IUPAC-IUB Combined Commission of Biochemical Nomenclature for chemical names of special interest in biological chemistry (*Biochem.* 5 (1965): 1445-1453 and subsequently).

The following abbreviations not listed by the commission were also used:

EDTA ethylenediaminetetraacetic acid

PVP polyvinylpyrrolidone

In some literature citations, the forms DPN and TPN are used instead of the recommended abbreviations, NAD and NADP.

The term "ammonium" is used without implying whether free ammonium ions or undissociated ammonium compounds (e.g. ammonium hydroxide) are actually present.

The term "nitrate" is taken as meaning nitrate ions.

All amino acids are, unless otherwise stated, the *L*-isomers.

ACKNOWLEDGEMENTS

I would like to thank the following people:

Mr. J. D. Atkinson, Director, Fruit Research Division, Department of Scientific and Industrial Research, for allowing me to carry out this work while a member of his staff.

Professor R.E.F. Matthews, Department of Cell Biology.

My supervisor, Dr. E.G. Bollard, Fruit Research Division, for his advice and encouragement, and for his detailed criticism of the manuscript.

Dr. R.L. Bielecki, Fruit Research Division, for his advice on the planning of some of the experiments described in Section 6, and for reading Section 4.

Mr. N.A. Turner, Fruit Research Division, for advice on experimental techniques, and for reading Section 3.

Mr. A. Underhill, Plant Diseases Division, who took the photographs.

Mrs. P. Bond, who xeroxed the graphs.

Miss J. Irwin, who typed the manuscript.

SUMMARY

1. A study was made of some aspects of the utilization by *Spirodela oligorrhiza* of ammonium, nitrate, nitrite, and organic nitrogenous compounds as sole sources of nitrogen.
2. *S. oligorrhiza* was grown in axenic culture under carefully defined conditions, and the only factors to be consciously varied were those relating to the nitrogen source.
3. Ammonium, or some product of its assimilation, inhibited the utilization of nitrate by inhibiting, at least partially, the uptake of nitrate, and by inhibiting almost completely the reduction of nitrate to nitrite.
4. Nitrite also inhibited the utilization of nitrate.
5. Ammonium and nitrite were taken up and assimilated simultaneously when they were supplied together in the medium.
6. Nitrate reductase and nitrite reductase were found to be adaptive enzymes, being present in *S. oligorrhiza* only when it was supplied with nitrate or nitrite. There was good correlation between the concentration of nitrate in plants and the levels of nitrate reductase that they contained.
7. When plants not containing nitrate and nitrite reductases were supplied with nitrate, there was a rapid increase in the levels of both enzymes and in the concentration of nitrate in the plants. This increase was retarded but not prevented by ammonium.
8. When nitrate-grown plants were depleted of nitrogen, there was a rapid decrease in the levels of nitrate and nitrite reductases. This was a real loss of enzyme activity, and not simply dilution by growth.

9. The utilization of ammonium prevented the assimilation of nitrate even by plants that contained high levels of nitrate and nitrite reductases. It was therefore concluded that ammonium, or the processes or products of its assimilation, must inhibit the activity of nitrate reductase.
10. Plants utilizing ammonium contained higher levels of ammonium, arginine, asparagine, and glutamine than did those utilizing nitrate. The concentrations of all other amino acids were very similar.
11. None of these compounds, tested either separately or together, had any effect on the activity *in vitro* of nitrate reductase. Cyanate was an inhibitor of nitrate reductase, but it is not known whether this inhibition has any physiological significance.
12. *S. oligorhiza* can use asparagine and glutamine as sole sources of nitrogen. These compounds were taken up as such and did not undergo any prior amidhydrolysis in the medium.
13. Plants supplied with any two of a number of organic nitrogenous compounds, each capable by itself of serving as a sole source of nitrogen, usually took both up from the medium, although at different rates. Thus glutamine was taken up more rapidly than was glutamic acid when they were supplied together in the medium.
14. Ammonium was taken up more rapidly than glutamic acid when they were supplied together in the medium. Similarly, ammonium was taken up more rapidly than aspartic acid.
15. Asparagine, *D*-glutamine, and glutamic acid did not inhibit the rate of uptake of glutamine as measured over short periods.
16. Asparagine, aspartic acid, and *L*-glutamine strongly inhibited the rate of uptake of glutamic acid, but *D*-glutamine and ammonium had no effect.