



Photosynthetic responses of *Lessertia frutescens* to different shading conditions

AM du Toit

 **orcid.org 0000-0004-2329-123x**

Dissertation accepted in fulfilment of the requirements for the
degree *Master of Science in Botany* at the North-West
University

Supervisor: Prof JM Berner

Graduation: July 2025

PREFACE

This thesis represents the culmination of my research on the photosynthetic effects of different netting on *Lessertia frutescens*. My interest in plant physiology and environmental stress responses led me to investigate how controlled light manipulation can influence photosynthetic efficiency. Understanding these dynamics is essential for optimizing cultivation strategies, particularly for medicinal plants.

This research has been challenging and rewarding, and the hope is that the findings contribute meaningfully to the field of plant physiology and sustainable agriculture of *Lessertia frutescens*. I hope this work serves as a stepping stone for future research.

ACKNOWLEDGEMENTS

Firstly, I wish to express gratitude to my supervisor, Professor Jacques Berner, for his guidance, support, patience, and assistance throughout the completion of my master's degree. I am grateful to Dr João Silva for his expertise and assistance with the statistical analyses in my study.

My sincere thanks go to the North-West University (NWU) Geo and Spatial sciences team for collecting the weather data and making it available to me.

I want to thank Dr Philisiwe Mhlanga, (postdoctoral researcher), for her guidance and support in improving the writing of the thesis. Lastly, I want to thank my fellow master's student, Bianca Boshoff, for the camaraderie, encouragement, and the countless conversations that helped to navigate the challenges of our studies together.

ABSTRACT

Key terms: Photosynthesis, netting, *Lessertia frutescens*, chlorophyll fluorescens and plant vitality.

Light manipulation is crucial for optimizing plant growth and photosynthetic performance. This study investigated how different shading conditions influence the photosynthetic efficiency of *Lessertia frutescens*, a medicinal plant with significant pharmacological value. Plants were grown under grey, white, and green (40%) laminated shade netting and compared to an unshaded control. Key photosynthetic parameters, including chlorophyll fluorescence, PSI redox states, absorption fluxes, cross-section parameters, and chlorophyll content, were measured using a Handy-PEA fluorescence meter and a CCM-300 chlorophyll content meter.

Results showed that shading improved photosynthetic performance relative to full sunlight, likely due to reduced photoinhibition and optimized light interception. Shaded plants exhibited a higher maximum quantum yield (F_V/F_M) and total performance index (PI_{TOTAL}) values, lower minimal fluorescence (F_0), and increased PSI oxidation and re-reduction rates, suggesting enhanced photoprotection. While green laminated shade netting was expected to provide the greatest benefit, no significant ($p < 0.05$) advantage was observed among shading treatments, indicating that overall shading, rather than spectral composition, played a dominant role.

Intraspecific variation was evident, with plant group A responding more favourably to shading, while group B exhibited a decline in PSI efficiency with prolonged exposure to laminated shade netting. Chlorophyll content was higher in shaded plants, supporting improved light capture. The increase of the absorption flux per cross-section (ABS/CS_m) confirmed efficient electron transport under shading, while unshaded plants showed increased energy dissipation (DI/CS_m) as a photoprotective response.

Overall, these findings demonstrate that moderate shading enhances photosynthetic efficiency in *Lessertia frutescens* and improves plant vitality. These insights contribute to refining shading strategies for improved cultivation and stress resilience.

LIST OF ABBREVIATIONS

ABS	A beam of photons that is absorbed by a pigment molecule on energy antennae and is able to excite chlorophyll molecules (Chl*)
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
CO ₂	Carbon dioxide
CS	Cross section (Surface of the excited photosynthesising sample)
duLN	Days under laminated shade netting
F ₀	Minimum fluorescence yield
F _d	Ferredoxin
F _m	Maximum fluorescence yield
FNR	Ferredoxin-NADP ⁺ Reductase
G3P	Glyceraldehyde-3-phosphate
G3P	Glyceraldehyde-3-phosphate
Handy-PEA	Hand-held plant efficiency analyser
IRGA	Infrared gas analyser
LHC	Light harvesting complex
Mn ²⁺	Manganese (II) ion
M-PEA	Multifunctional plant efficiency analyser
MR	Modulated reflection
MR ₀	Initial Modulated Reflectance
MR _{min}	Minimum Modulated Reflectance
MVA	Mevalonate pathway
NADP ⁺	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	Nicotinamide Adenine Dinucleotide Phosphate

NAR	Near-infrared radiation
O ₂	Oxygen
OEC	Oxygen evolving complex
OTC	Open top chambers
P680 ⁺	Photosystem II primary donor
P700 ⁺	Photosystem I primary donor
PAR	Photosynthetic active radiation
PC	Plastocyanin
PI _{ABS}	Performance index (on absorption basis)
PI _{TOTAL}	Total performance index
PPFD	Photosynthetic photon flux density
PQ	Plastoquinone
PQH ₂	Plastoquinol
PSI	Photosystem I
PSII	Photosystem II
Q _A	Quinone A
RC	Reaction complex (Photosynthetically active reaction centre of PSII that are able to reduce Q _A)
ROS	Reactive oxygen species
RuBP	Ribulose biphosphate
UV	Ultraviolet
V _{ox}	P700 and PC oxidation velocity
V _{red}	P700 and PC re-reduction velocity
Z	A redox-active tyrosine residue

TABLE OF CONTENTS

CHAPTER 1	INTRODUCTION.....	1
1.1	Hypothesis.....	2
1.2	Aim.....	2
1.3	Objectives.....	3
1.4	Thesis layout.....	3
CHAPTER 2	LITERATURE REVIEW.	5
2.1	Photosynthesis: A Two-Phase Process	5
2.1.1	Light-dependent reaction: Energy conversion	6
2.1.1.1	The role of chlorophyll pigments in photosynthesis	6
2.1.1.2	The Electron Transport Chain (ETC).....	7
2.1.2	Light-Independent Reactions: Carbon Fixation.....	9
2.1.3	Chlorophyll fluorescence as a tool to investigate the electron transport chain ...	10
2.1.4	The 820 nm modulated reflection	14
2.1.5	Combining the OJIP transient and 820 nm Modulated reflection curve.....	15
2.1.6	The gas exchange rates.....	15
2.2	Shading and light manipulation	16
2.2.1	Acclimation and adaptation of plants under sun and shade.....	16
2.2.2	Morphological Changes under Shade:	17
2.2.3	Manipulation of light through netting:.....	17
2.3	Background on <i>Lessertia frutescens</i>.....	18

2.3.1	Ecological and ethnobotanical importance:	18
2.4	Implications and Applications.....	23
2.5	Research gap and possible hypothesis	24
CHAPTER 3	MATERIALS AND METHODS.....	26
3.1	Plant materials and growth conditions.....	26
3.2	Experimental setup	27
3.3	Chlorophyll content	27
3.4	The simultaneous measurements of Prompt fluorescence and 820 nm Modulated reflection	27
3.4.1	Photosystem II activity.....	28
3.4.2	Photosystem I activity.....	29
3.5	Visual assessment of plants	30
3.6	Statistical analyses	30
CHAPTER 4	RESULTS	31
4.1	Environmental conditions:	31
4.1.1	Climatic conditions:	31
4.1.2	Light intensity:	32
4.2	The photosynthetic effects on <i>Lessertia frutescens</i>:	32
4.2.1	The effect of different shading conditions on the chlorophyll content:	33
4.2.1.1	Plant group A:	33
4.2.1.2	Plant group B:	34
4.2.2	The effect of different shading conditions on the OJIP transient:	35

4.2.2.1	Plant group A	35
4.2.2.2	Plant group B:	38
4.2.3	The effect of different shading conditions on the maximum quantum yield of PSII (F_V/F_M):	40
4.2.3.1	Plant group A:	40
4.2.3.2	Plant group B:	41
4.2.4	The effect of different shading conditions on the total performance index (PI_{TOTAL}).....	42
4.2.4.1	Plant group A:	43
4.2.4.2	Plant group B	45
4.2.5	Phenomenological energy fluxes per excited cross-section (CS) of a sample ...	48
4.2.5.1	Plant group A:	49
4.2.5.2	Plant group B:	51
4.2.6	The specific energy fluxes per Q_A -Reduced PSII reaction centres (RC)	54
4.2.6.1	Plant group A:	55
4.2.6.2	Plant group B:	57
4.3	The effect of different shading conditions on the 820 nm Modulated reflection and its parameters of <i>Lessertia frutescens</i>.....	60
4.3.1	The 820 nm Modulated reflection of plant group A:	61
4.3.1.1	MR_0/MR_{min}	62
4.3.1.2	Fast phase (V_{ox}):	63
4.3.1.3	Slow phase (V_{re-red}).....	64
4.3.2	The 820 nm Modulated reflection of Plant group B:	65

4.3.2.1	MR ₀ /MR _{min}	66
4.3.2.2	Fast phase (V _{ox}):	67
4.3.2.3	Slow phase (V _{re-red}).....	68
CHAPTER 5 DISCUSSION.....		69
5.1	The effect of different laminated shade netting on the redox state of PSII through the analysis of the OJIP transient and its parameters	69
5.2	The effect of different laminated shade netting on the plant vitality of the plant group Based on the performance indexes.....	70
5.3	The effect of different laminated shade netting on the redox state of PSI through the 820 nm modulated reflection curve and associated parameters.....	71
5.4	The effect of different laminated shade netting on the plant's photosynthetic efficiency based on absorption fluxes	72
5.5	The effect of different laminated shade netting on the plant's photosynthetic efficiency based on the cross-section.....	73
5.6	The effect of different shading conditions on the chlorophyll content	73
CHAPTER 6 CONCLUSION.....		75
CHAPTER 7 RECOMMENDATIONS.....		77
7.1	Light intensities.....	85
7.2	Chlorophyll content:	86
7.3	OJIP.....	87
7.3.1	Plant group A	87
7.3.2	Plant group B	89

7.3.3	The maximum quantum yield of PSII (F_v/F_m).....	92
7.3.4	The total performance index (PI_{TOTAL}).....	93
7.4	Per cross section	99
7.4.1	Plant group A	99
7.4.2	Plant group B	101
7.5	Per reaction center.....	104
7.5.1	Plant group A	104
7.5.2	Plant group B	106
7.6	The parameters of the 820 nm modulated reflection	109
7.6.1.1	Plant group A	109
7.6.1.2	Plant group B	110

LIST OF TABLES

Table 1: The formulas and description for parameters derived from the JIP-test to analyse the chlorophyll a fluorescence	12
Table 2: A summary of the chemical constituents found in <i>Lessertia frutescens</i> and the ailments its uses.	22
Table 3: A representation of the statistics of this study.	30
Table 4: The climatic conditions in Potchefstroom during the experiment.	31
Table 5: A summary of the statistics of the OJIP transient of plant group A after 60 days under laminated shade netting (duLN). The first number refers to the average, the second to the standard deviation and the letter are denoted by significant ($p < 0.05$) differences between the different laminated shade netting at the different inclination points (Appendix: Table 23 - 27).	37
Table 6: A summary of the statistics of the OJIP transient of plant group A after 120 days under laminated shade netting (duLN). The first number refers to the average, the second to the standard deviation and the letter are denoted by significant ($p < 0.05$) differences between the different laminated shade netting at the different inclination points (Appendix: Table 23 - 27).	37
Table 7: A summary of the statistics of the OJIP transient of plant group B after 60 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant ($p < 0.05$) differences between the various laminated shade netting at different inclination points (Appendix: Table 28- 32).	39
Table 8: A summary of the statistics of the OJIP transient of plant group B after 120 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant ($p < 0.05$) differences between the various laminated shade netting at different inclination points (Appendix: Table 28 - 32).	39

- Table 9: A summary of the statistics of the parameters of PI_{TOTAL} of plant group A after 60 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant differences ($p < 0.05$) among the laminated shade netting for different parameters (Appendix: Table 35 - 39). 44**
- Table 10: A summary of the statistics of the parameters of PI_{TOTAL} of plant group A after 120 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant differences ($p < 0.05$) among the laminated shade netting for different parameters (Appendix: Table 35 - 39). 45**
- Table 11: A summary of the statistics of the parameters of PI_{TOTAL} of plant group B after 60 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant differences ($p < 0.05$) among the laminated shade netting for different parameters (Appendix: Table 35 - 39). 47**
- Table 12: A summary of the statistics of the parameters of PI_{TOTAL} of plant group B after 120 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant differences ($p < 0.05$) among the laminated shade netting for different parameters (Appendix: Table 35 - 39). 47**
- Table 13: A summary of the statistics of the phenomenological energy fluxes per excited cross-section of *Lessertia frutescens*, plant group A, after 60 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant differences ($p < 0.05$) among the laminated shade netting for different parameters (Appendix: Table 40- 44). 50**
- Table 14: A summary of the statistics of the phenomenological energy fluxes per excited cross-section of *Lessertia frutescens*, plant group A, after 120 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant differences ($p < 0.05$) among the laminated shade netting for different parameters (Appendix: Table 40- 44). 51**

Table 15: A summary of the phenomenological energy fluxes per excited cross-section (ABS/CS_m, DI₀/CS_m, TR₀/CS_m, ET₀/CS_m and RE₀/CS_m) of *Lessertia frutescens*, plant group B, after 60 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant differences (p < 0.05) among the laminated shade netting for different parameters (Appendix: Table 45 - 49). 53

Table 16: A summary of the statistics of the phenomenological energy fluxes per excited cross-section (ABS/CS_m, DI₀/CS_m, TR₀/CS_m, ET₀/CS_m and RE₀/CS_m) of *Lessertia frutescens*, plant group B, after 120 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant differences (p < 0.05) among the laminated shade netting for different parameters (Appendix: Table 45 - 49). 53

Table 17: A summary of the statistics of the specific energy fluxes per Q_A-Reduced PSII reaction centres (ABS/RC, DI₀/RC, TR₀/RC, ET₀/RC and RE₀/RC) of *Lessertia frutescens*, plant group A, after 60 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant differences (p < 0.05) among the laminated shade netting for different parameters (Appendix: Table 50 - 54). 56

Table 18: A summary of the specific energy fluxes per Q_A-Reduced PSII reaction centres (ABS/RC, DI₀/RC, TR₀/RC, ET₀/RC and RE₀/RC) of *Lessertia frutescens*, plant group A, after 120 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant differences (p < 0.05) among the laminated shade netting for different parameters (Appendix: Table 50 - 54). 57

Table 19: A summary of the statistics of the specific energy fluxes per Q_A -Reduced PSII reaction centres (ABS/RC, DI_0 /RC, TR_0 /RC, ET_0 /RC and RE_0 /RC) of *Lessertia frutescens*, plant group B, after 60 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant differences ($p < 0.05$) among the laminated shade netting for different parameters (Appendix: Table 55 - 59). 59

Table 20: A summary of the statistics of the specific energy fluxes per Q_A -Reduced PSII reaction centres (ABS/RC, DI_0 /RC, TR_0 /RC, ET_0 /RC and RE_0 /RC) of *Lessertia frutescens*, plant group B, after 120 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant differences ($p < 0.05$) among the laminated shade netting for different parameters (Appendix: Table 55 - 59). 59

Table 21: The statistical analysis of the light intensities under the different netting, with L1= outside, L2=Grey, L3= White, L4 = Green, on different times of the day, 10:00, 12:00, 15:00. 85

Table 22 : The statistical analysis of the chlorophyll content of *Lessertia frutescens* under different shading conditions. The first letter refers to the plant, the number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The number refers to the days that the plants were under laminated shade netting. 86

Table 23: The statistical analysis of the O inclination point of *Lessertia frutescens*, plant group A, under different shading conditions. The number after the L refers to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The last number refers to the number of days that the plants were under laminated shade netting. 87

Table 24: The statistical analysis of the K inclination point of *Lessertia frutescens*, plant group A, under different shading conditions. The number after the L refers to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The last number refers to the number of days that the plants were under laminated shade netting. 87

Table 25: The statistical analysis of the J inclination point of *Lessertia frutescens*, plant group A, under different shading conditions. The number after the L refers to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The last number refers to the number of days that the plants were under laminated shade netting. 89

Table 26: The statistical analysis of the I inclination point of *Lessertia frutescens*, plant group A, under different shading conditions. The number after the L refers to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The last number refers to the number of days that the plants were under laminated shade netting. 89

Table 27: The statistical analysis of the P inclination point of *Lessertia frutescens*, plant group A, under different shading conditions. The number after the L refers to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The last number refers to the number of days that the plants were under laminated shade netting. 90

Table 28: The statistical analysis of the O inclination point of *Lessertia frutescens*, plant group B, under different shading conditions. The number after the L refers to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The last number refers to the number of days that the plants were under laminated shade netting. 90

Table 29: The statistical analysis of the K inclination point of *Lessertia frutescens*, plant group B, under different shading conditions. The number after the L refers to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The last number refers to the number of days that the plants were under laminated shade netting. 91

Table 30: The statistical analysis of the J inclination point of *Lessertia frutescens*, plant group B, under different shading conditions. The number after the L refers to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The last number refers to the number of days that the plants were under laminated shade netting. 91

Table 31: The statistical analysis of the I inclination point of *Lessertia frutescens*, plant group B, under different shading conditions. The number after the L refers to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The last number refers to the number of days that the plants were under laminated shade netting. 92

Table 32: The statistical analysis of the P inclination point of *Lessertia frutescens*, plant group B, under different shading conditions. The number after the L refers to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The last number refers to the number of days that the plants were under laminated shade netting. 92

Table 33: The statistical analysis of the maximum quantum yield of PSII (F_v/F_m) of *Lessertia frutescens* under different shading conditions. The first letter refers to the plant, the number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The number refers to the days that the plants were under laminated shade netting. 93

Table 34: The statistical analysis of the total performance index (PI_{TOTAL}) of *Lessertia frutescens* under different shading conditions. The first letter refers to the plant, the number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The number refers to the days that the plants were under laminated shade netting. 94

Table 35: The statistical analysis of the efficiency of light capturing (RC/ABS) of *Lessertia frutescens* under different shading conditions. The first letter refers to the plant, the number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The number refers to the days that the plants were under laminated shade netting. 95

Table 36: The statistical analysis of the quantum efficiency of light trapping ($\phi_{Po}/(1 - \phi_{Po})$) of *Lessertia frutescens* under different shading conditions. The first letter refers to the plant, the number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The number refers to the days that the plants were under laminated shade netting. 96

Table 37: The statistical analysis of the conversion of trapped excited energy into electron transport ($\Psi_0/(1 - \Psi_0)$) of *Lessertia frutescens* under different shading conditions. The first letter refers to the plant, the number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The number refers to the days that the plants were under laminated shade netting. 97

Table 38: The statistical analysis of the performance index based on absorption (PI_{ABS}) of *Lessertia frutescens* under different shading conditions. The first letter refers to the plant, the number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The number refers to the days that the plants were under laminated shade netting. 98

Table 39: The statistical analysis of the probability to reduce end electron acceptors ($\delta_0/(1 - \delta_0)$) of *Lessertia frutescens* under different shading conditions. The first letter refers to the plant, the number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The number refers to the days that the plants were under laminated shade netting. 99

Table 40: The statistical analysis of the light absorption per cross section (ABS/CS_m) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days). 100

Table 41: The statistical analysis of the heat dissipation per cross section (DI_0/CS_m) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days). 100

Table 42: The statistical analysis of the excitation energy flux trapping per cross section (TR_0/CS_m) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days). 101

Table 43: The statistical analysis of the electron flux transported per cross section (ET_0/CS_m) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days). 101

Table 44: The statistical analysis of the re-oxidation of electron acceptors per cross section (RE_0/CS_m) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days). 102

Table 45: The statistical analysis of the light absorption per cross section (ABS/CS_m) of *Lessertia frutescens*, Plant group B, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days). 102

Table 46: The statistical analysis of the heat dissipation per cross section (DI_0/CS_m) of *Lessertia frutescens*, Plant group B, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days). 103

Table 47: The statistical analysis of the excitation energy flux trapping per cross section (TR_0/CS_m) of *Lessertia frutescens*, Plant group B, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days). 103

Table 48: The statistical analysis of the electron flux transported per cross section (ET_0/CS_m) of *Lessertia frutescens*, Plant group B, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days). 104

Table 49: The statistical analysis of the re-oxidation of electron acceptors per cross section (RE_0/CS_m) of *Lessertia frutescens*, Plant group B, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days). 104

Table 50: The statistical analysis of the light absorption per reaction centre (ABS/RC) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days). 105

Table 51: The statistical analysis of the heat dissipation per reaction centre (DI_0/RC) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days). 105

Table 52: The statistical analysis of the excitation energy flux trapping per reaction centre (TR_0/RC) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days). 106

Table 53: The statistical analysis of the electron flux transported per reaction centre (ET_0/RC) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days). 106

Table 54: The statistical analysis of the re-oxidation of electron acceptors per reaction centre (RE_0/RC) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days). 107

Table 55: The statistical analysis of the light absorption per reaction centre (ABS/RC) of *Lessertia frutescens*, Plant group B, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days). 107

Table 56: The statistical analysis of the heat dissipation per reaction centre (DI_0/RC) of *Lessertia frutescens*, Plant group B, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days). 108

Table 57: The statistical analysis of the excitation energy flux trapping per reaction centre (TR_0/RC) of *Lessertia frutescens*, Plant group B, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days). 108

Table 58: The statistical analysis of the electron flux transported per reaction centre (ET_0/RC) of *Lessertia frutescens*, Plant group B, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days). 109

Table 59: The statistical analysis of the re-oxidation of electron acceptors per reaction centre (RE_0/RC) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days). 109

Table 60: The statistical analysis of the re-oxidation of the MR_0/MR_{min} ratio of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days). 110

Table 61: The statistical analysis of the fast phase (V_{ox}) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days). 110

Table 62: The statistical analysis of the slow phase (V_{re-red}) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days). 111

Table 63: The statistical analysis of the re-oxidation of the MR_0/MR_{min} ratio of *Lessertia frutescens*, Plant group B, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days). 111

Table 64: The statistical analysis of the fast phase (V_{ox}) of *Lessertia frutescens*, Plant group B, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days). 112

Table 65: The statistical analysis of the slow phase (V_{re-red}) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days). 112

LIST OF FIGURES

- Figure 1: The light-dependent and light-independent reaction of photosynthesis
<https://www.khanacademy.org/science/hs-bio/x230b3ff252126bb6:energy-and-matter-in-biological-systems/x230b3ff252126bb6:photosynthesis/a/photosynthesis-overview>. 6
- Figure 2: An illustration of the flow of an electron through the electron transport chain (Hopkins & Hüner, 2008). 8
- Figure 3: A representation of the Calvin cycle (“The calvin cycle: How the products of light reactions, ATP and NADPH, are used to fix carbon into sugars in the second stage of photosynthesis.”, s.a.). 9
- Figure 4: A typical form of the OJIP transient, plotted on a logarithmical timescale. Highlighting the O (0.03 ms), K (0.3 ms), J (2 ms), I (30 ms) and P (300 ms) inclination points (Tsimilli-Michael & Strasser, 2008). 11
- Figure 5: The 820 nm Modulated reflection curve. 14
- Figure 6: Combined illustration of the OJIP transient and the 820 nm Modulated reflection curve. 15
- Figure 7: An Illustration of the gass exchange taking place in a plant that is measured by the IRGA https://www.freepik.com/free-vector/diagram-showing-schematic-stomata_19013687.htm#fromView=keyword&page=1&position=31&uid=45038411-d939-423a-b6d2-a3c4b60c4a92&new_detail=true&query=Plant+Respiration. 16
- Figure 8: The response of plants on different light colours (Paradiso & Proietti, 2022). 18
- Figure 9: Geographical distribution of *Lessertia frutescens* across South Africa (Mncwangi et al., 2022). This map does not include the distribution in Namibia and Botswana. 19

- Figure 10: Schematic representation of the characteristics of *Lessertia frutescens*, it's subspecies and different forms (van Wyk & Albrecht, 2008). 20**
- Figure 11: Characteristics of *Lessertia frutescens*, the pod can differ in the direction of the (a) fruit stipe and the (b) fruit bearing suture and the hairiness (c) adaxial side of apex leaf. 21**
- Figure 12: The open top chamber (OTC) at the North-West University 26**
- Figure 13: A typical polyphasic chlorophyll a fluorescence rise (OJIP transient), plotted on a logarithmic scale (0.01-1000ms). The different steps are labelled O (0.03 ms), K (0.3 ms), J (2 ms), I (30 ms) and P (300 ms). The basal fluorescence and maximal fluorescence as well as the equation for the maximum quantum yield is indicated. 28**
- Figure 14: The activity of PSI represented in an 820 nm Modulated reflection curve plotted logarithmically. The slow phase and fast phase are indicated as well as the parameters. 29**
- Figure 15: The light intensities under the different nettings and outside through the day. 32**
- Figure 16: The chlorophyll content of *Lessertia frutescens*, plant group A, under different laminated shade netting. Different letters are denoted to significant differences ($p < 0.05$). The capital letters refer to significant ($p < 0.05$) differences between the shading conditions and the lowercase letters refer to the significant ($p < 0.05$) differences that occurred over time, after 60 and 120 days under laminated shade netting (duLN). 33**
- Figure 17: The chlorophyll content of *Lessertia frutescens*, plant group B, under different laminated shade netting. Different letters are denoted to significant differences ($p < 0.05$). The capital letters refer to significant ($p < 0.05$) differences between the shading conditions and the lowercase letters refer to the significant ($p < 0.05$) differences that occurred over time, after 60 and 120 days under laminated shade netting (duLN). 34**

- Figure 18: The OJIP transient of plant group A after a) 60 days and b) 120 days under laminated shade netting (duLN). 35**
- Figure 19: The OJIP transient of *Lessertia frutescens*, plant group B, after a) 60 days and b) 120 days under laminated shade netting (duLN). 38**
- Figure 20: The maximum quantum efficiency of PSII (F_V/F_M) of *Lessertia frutescens*, plant group A, under different shading conditions. Different letters are denoted to significant differences ($p < 0.05$). The capital letters refer to differences between the different shading conditions and the lowercase letters refer to the differences that occurred over time, after 60 and 120 days under laminated shade netting (duLN). 40**
- Figure 21: The maximum quantum yield of PSII (F_V/F_M) of *Lessertia frutescens*, plant group B, under different shading conditions. Different letters are denoted to significant differences ($p < 0.05$). The capital letters refer to differences between the different shading conditions and the lowercase letters refer to the significant ($p < 0.05$) differences that occurred over time, after 60 and 120 days under laminated shade netting (duLN). 41**
- Figure 22: The total performance index (PI_{TOTAL}) of *Lessertia frutescens*, plant group A, under different shading conditions. Different letters are denoted to significant differences ($p < 0.05$). The capital letters refer to differences between the different shading conditions and the lowercase letters refer to the differences that occurred over time, after 60 and 120 days under laminated shade netting (duLN). 43**
- Figure 23: The total performance index (PI_{TOTAL}) of *Lessertia frutescens*, plant group B, under different shading conditions. Different letters are denoted to significant differences ($p < 0.05$). The capital letters refer to differences between the different shading conditions and the lowercase letters refer to the differences that occurred over time, after 60 and 120 days under laminated shade netting (duLN). 45**

- Figure 24:** The phenomenological energy fluxes per excited cross-section (ABS/CS_m , DI_0/CS_m , TR_0/CS_m , ET_0/CS_m and RE_0/CS_m) of *Lessertia frutescens*, plant group A, under different shading conditions over a period of (a) 60 days and (b) 120 days under laminated shade netting (duLN). 49
- Figure 25:** The phenomenological energy fluxes per excited cross-section (ABS/CS_m , DI_0/CS_m , TR_0/CS_m , ET_0/CS_m and RE_0/CS_m) of *Lessertia frutescens*, plant group B, under different shading conditions over a period of (a) 60 days and (b) 120 days under laminated shade netting (duLN). 51
- Figure 26:** The specific energy fluxes per Q_A -Reduced PSII reaction centres (ABS/RC , DI_0/RC , TR_0/RC , ET_0/RC and RE_0/RC) of *Lessertia frutescens*, plant group A, under different shading conditions over a period of (a) 60 days and (b) 120 days under laminated shade netting (duLN). 55
- Figure 27:** The specific energy fluxes per Q_A -Reduced PSII reaction centres (ABS/RC , DI_0/RC , TR_0/RC , ET_0/RC and RE_0/RC) of *Lessertia frutescens*, plant group B, under different shading conditions over a period of (a) 60 days and (b) 120 days under laminated shade netting (duLN). 57
- Figure 28:** The 820 nm Modulated reflection curve of *Lessertia frutescens*, plant group A, under different shading conditions over a period of (a) 60 days and (b) 120 days under laminated shade netting (duLN). 61
- Figure 29:** The MR_0/MR_{min} of the 820 nm Modulated reflection of *Lessertia frutescens*, Plant group A, under different shading conditions over a period of 60 days and 120 days under the shade netting. 62
- Figure 30:** The fast phase (V_{ox}) of the 820 nm Modulated reflection of *Lessertia frutescens*, Plant group A, under different shading conditions over a period of 60 days and 120 days under laminated shade netting (duLN). 63
- Figure 31:** The slow phase (V_{re-red}) of the 820 nm Modulated reflection of *Lessertia frutescens*, Plant group A, under different shading conditions over a period of 60 days and 120 days under laminated shade netting (duLN). 64
- Figure 32:** The 820 nm Modulated reflection curve of *Lessertia frutescens*, Plant group B, under different shading conditions over a period of (a) 60 days and (b) 120 days under laminated shade netting (duLN). 65

Figure 33: The MR_0/MR_{\min} of the 820 nm Modulated reflection of *Lessertia frutescens*, Plant group B, under different shading conditions over a period of 60 days and 120 days under laminated shade netting (duLN). 66

Figure 34: The fast phase (V_{ox}) of the 820 nm Modulated reflection of *Lessertia frutescens*, Plant group B, under different shading conditions over a period of 60 days and 120 days under laminated shade netting (duLN). 67

Figure 35: The slow phase (V_{re-red}) of the 820 nm Modulated reflection of *Lessertia frutescens*, Plant group B, under different shading conditions over a period of 60 days and 120 days under laminated shade netting (duLN). 68

CHAPTER 1 INTRODUCTION

In a world grappling with the dual challenges of climate change and increasing demand for natural remedies, medicinal plants have emerged as a cornerstone for sustainable healthcare and agriculture. Among these, *Lessertia frutescens*, a medicinal plant native to Southern Africa (van Wyk & Albrecht, 2008), holds significant promise due to its array of bioactive compounds with therapeutic properties. However, optimizing its cultivation to ensure consistent quality and yield remains a pressing challenge, particularly in the face of variable environmental conditions.

Environmental variations influence plant vitality, which is a crucial factor in crop performance and yield, and can be assessed by examining plant morphology, biochemistry, and physiology (Johnstone *et al.*, 2013). Plant vitality is directly impacted by photosynthesis, a key physiological process, which is highly sensitive to environmental changes. It is an early indicator of plant stress before visible symptoms manifest (Kalaji *et al.*, 2016). Thus, understanding and optimizing a plant's photosynthetic response is vital for enhancing plant health and productivity.

Light is one of the most significant environmental factors affecting photosynthesis and overall plant vitality (Xu, 2016) and can easily be manipulated in agriculture through shade netting to optimise plant growth and fruit production. This is possible since shade netting alters the light spectrum and intensities by filtering wavelengths (Hogewoning *et al.*, 2010), thereby influencing the photosynthetic processes in plants. In this thesis, photosynthetic efficiency refers to the plant's ability to absorb sunlight, initiate the electron transport chain, and generate ATP and NADPH, key energy molecules that drive carbon fixation and secondary metabolite biosynthesis. These metabolites, including flavonoids and phenolics, are crucial for the pharmacological efficacy of medicinal plants, but their concentrations can vary significantly in response to environmental factors, particularly shading conditions (Colling *et al.*, 2010). This variability presents challenges for sustainable wild harvesting and standardisation of medicinal products, emphasizing the need for controlled cultivation of medicinal plants (Ilić *et al.*, 2019; Milenković *et al.*, 2019).

The effect of different shading conditions has been proven to be species-dependent and has important implications for cultivation practices (Tmušić *et al.*, 2021). For medicinal plants, such as *Lessertia frutescens*, optimising growth conditions to maximise the production of bioactive compounds is critical for their pharmacological efficacy. *Lessertia frutescens* is known for its therapeutic properties, including antiproliferative, anti-viral, antidiabetic, anti-inflammatory, and antioxidant effects (Aboyade *et al.*, 2014). Its bioactive compounds include proteins, flavonoids, phenols, and L-canavanine, the formation of which depends on ATP and NADPH generated during photosynthesis. While previous studies have explored the impact of light quality on

photosynthesis in various plants, limited research has been conducted on the specific responses of *Lessertia frutescens* under controlled shading conditions.

Given the pharmacological importance of *Lessertia frutescens*, it is essential to establish standardised cultivation methods that ensure optimal and consistent concentrations of its active compounds. The use of different light qualities through shade netting offers a potential solution, as light quality affects both photosynthetic efficiency and secondary metabolite production in various medicinal plants (Hashim *et al.*, 2021; Wang *et al.*, 2018; Yang *et al.*, 2019). Therefore, understanding the effects of different shading conditions on the photosynthetic performance of *Lessertia frutescens* is vital for improving its cultivation and ensuring a reliable yield of medicinal products. This study investigated the photosynthetic responses of *Lessertia frutescens* grown under different colours of polyethylene-laminated shade netting of 40%. By examining changes in photo efficiency and chlorophyll content, this research aims to determine the optimal light quality for maximizing plant vitality and ensuring consistent production of bioactive compounds.

1.1 Hypothesis

The photosynthetic efficiency will be increased under netting rather than outside. The plants under the green netting will have a higher photosynthetic efficiency compared to the other shading conditions.

1.2 Aim

By investigating the effects of shade netting on the photosynthetic efficiency in *Lessertia frutescens*, this study aims to provide a basis for optimising its cultivation. The following objectives were developed to address this aim.

1.3 Objectives

- Assessing the changes in the redox state of PSII through the analysis of the OJIP transient and its parameters
- Evaluating plant vitality through performance indexes derived from photosynthetic metrics.
- Analysing changes in the redox state of PSI using the 820 nm modulated reflection curve and its associated parameters.
- Determining the impact of different colour shade netting on the plant's photosynthetic efficiency based on absorption fluxes.
- Investigating the effect of different colour shade netting on the plant's photosynthetic efficiency based on the cross-section.
- Assessing whether different colour shade netting cause changes in the chlorophyll content.

1.4 Thesis layout

- Chapter 1 Introduction
 - Gives a brief background on the study field. This indicates the importance of the study through the problem statement and by providing information on the objectives, hypothesis, and layout of the thesis.
- Chapter 2 Literature review
 - A brief overview of *Lessertia frutescens*, its photosynthetic efficiency and previous research on the plant. A gap in the research is identified and how this study will address this gap.
- Chapter 3 Materials and methods
 - A detailed description on the cultivation and growing conditions of *Lessertia frutescens* and the measurements to evaluate the photosynthetic efficiency of the plants to determine the effect of different coloured laminated shade netting.
- Chapter 4 Results
 - All the data was derived from this study: the prompt fluorescence, 820 nm Modulated reflection curve and related parameters.

- Chapter 5 Discussion
 - The findings on the objectives are discussed and clarified.
- Chapter 6 Conclusion
 - Concluding remarks
- Chapter 7 Recommendations
 - Identify key areas that require further investigation in future studies.

CHAPTER 2 LITERATURE REVIEW.

Photosynthesis is a fundamental biological process that sustains life on earth by converting light energy into chemical energy, forming the foundation of terrestrial ecosystems (Hopkins & Hüner, 2008). This process is critical for plants such as *Lessertia frutescens*, a medicinal shrub whose chemical composition is influenced by environmental factors, mainly light. Light intensity and quality play crucial roles in regulating photosynthesis and plant growth and metabolism.

Chlorophyll *a* fluorescence, a non-invasive technique widely used to assess photosynthetic performance, provides valuable insights into plant health and vitality by measuring the efficiency of PS II (Strasser *et al.*, 2002). This approach is particularly relevant for understanding how environmental factors impact photosynthesis, such as light modifications through shade netting. This literature review explores the impact of shade netting on shading conditions and its subsequent effects on the photosynthetic efficiency and vitality of *Lessertia frutescens*.

2.1 Photosynthesis: A Two-Phase Process

Photosynthesis consists of two interconnected stages: the light-dependent and light-independent reactions, each fulfilling a distinct role in the process. As illustrated in Figure 1, the light-dependent reactions occur in the thylakoid membrane, where light energy drives the conversion of water molecules into oxygen while generating ATP and NADPH from ADP and NADP⁺ (Shevela *et al.*, 2019). These high-energy molecules then fuel the light-independent reactions in the stroma. During this stage, carbon dioxide is assimilated and utilized in the Calvin cycle to synthesize glucose, with ATP and NADPH providing the necessary energy and electrons. As a result, ADP and NADP⁺ are regenerated, completing the cycle and ensuring the continuous operation of photosynthesis.

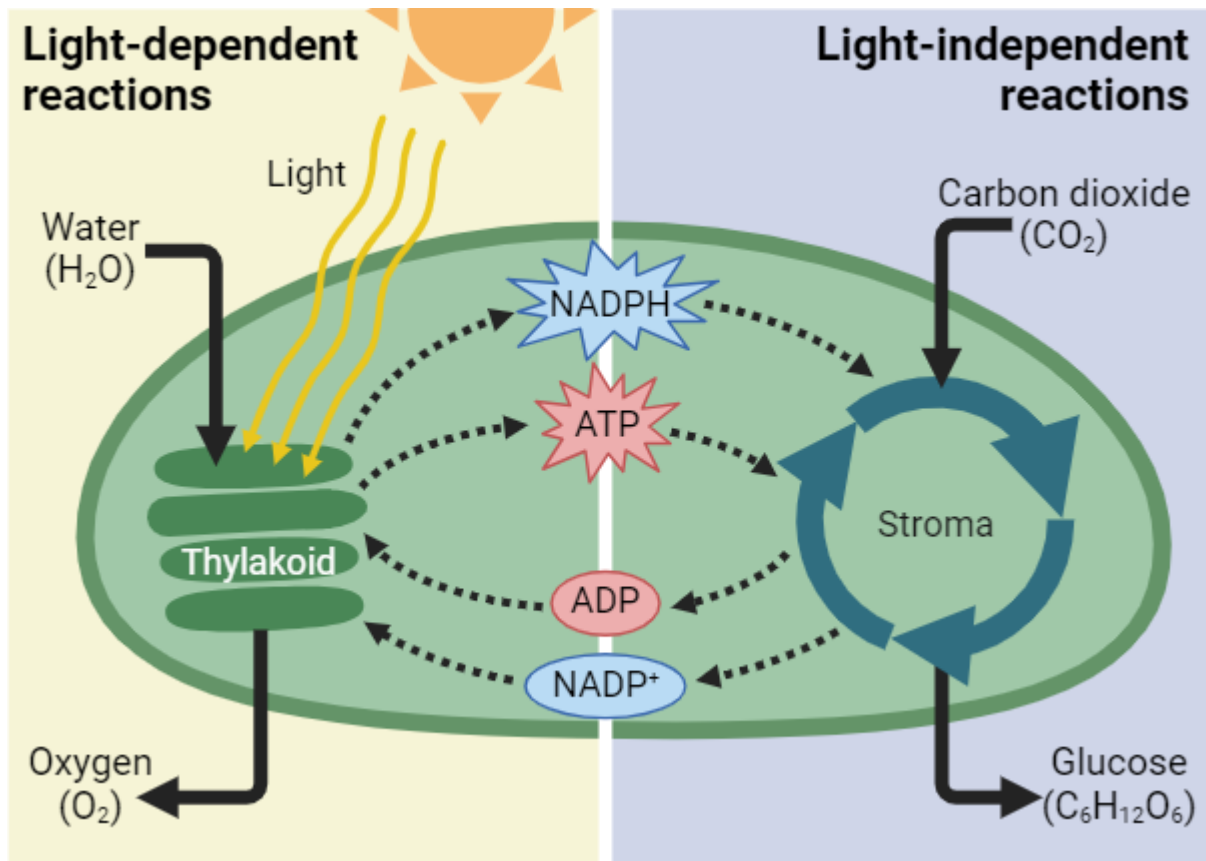


Figure 1: The light-dependent and light-independent reaction of photosynthesis
<https://www.khanacademy.org/science/hs-bio/x230b3ff252126bb6:energy-and-matter-in-biological-systems/x230b3ff252126bb6:photosynthesis/a/photosynthesis-overview>.

2.1.1 Light-dependent reaction: Energy conversion

2.1.1.1 The role of chlorophyll pigments in photosynthesis

Chlorophyll pigments, located in the chloroplast, capture light energy, which is crucial for photosynthesis. Light entering a leaf can follow one of three paths: it may be absorbed by pigments, dissipated as heat, or emitted as chlorophyll fluorescence (Strasser *et al.*, 2007). Light capture and conversion efficiencies are influenced by the organisation of light-harvesting complexes (LHC) and interactions between chlorophyll and proteins within the chloroplast (Hopkins & Hüner, 2008).

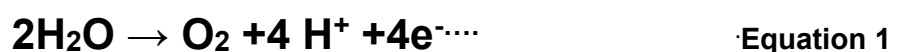
The light-harvesting complexes primarily contain chlorophyll pigments—mainly chlorophyll *a* and *b*—that absorb photons and release electrons to further photosynthetic reactions (Kume *et al.*, 2018). This function is central to the photosynthetic process, and chlorophyll content indicates photosynthetic capacity and potential plant growth (Li *et al.*, 2018).

Chlorophyll *a* absorbs light most effectively at wavelengths around 430 nm and 660 nm, while chlorophyll *b* absorbs optimally at wavelengths around 450 nm and 640 nm (Kume *et al.*, 2018). The relative content of chlorophyll *a* and *b* may vary with light intensity; plants often increase their chlorophyll *a/b* ratio under high light to maximise energy capture. Under shaded or low-shading conditions, chlorophyll *b* levels increase to improve light absorption across a broader range of wavelengths, enhancing the plant's overall light-harvesting efficiency (Khan *et al.*, 2023; Li *et al.*, 2018).

2.1.1.2 The Electron Transport Chain (ETC)

The electron transport chain (ETC) is a series of reactions that follow after light has been successfully absorbed by the chlorophyll pigments (Figure 2). It consists of three main complexes, Photosystem II (PSII), the cytochrome complex, and Photosystem I (PSI), and two mobile carriers, plastoquinone (PQ) and plastocyanin (PC).

The process begins in photosystem II (PSII), where light absorption causes a significant change in the redox potential of P680, shifting from +0.8 eV to -0.4 eV and forming the excited state P680* (Hopkins & Hüner, 2008). This excitation drives a primary photosynthetic event, resulting in charge separation and the formation of the oxidised species P680⁺ and reduced Pheophytin (Pheo⁻). This initial charge separation is critical, as it stores energy that propels further electron transport (Hopkins & Hüner, 2008; Shevela *et al.*, 2019).



In the meantime, the oxygen-evolving complex, also known as the Mn²⁺-complex, split water, as shown in Equation 1. The electrons obtained from this reaction are then used to rapidly re-reduce the oxidised P680⁺ through an intermediate electron donor known as Z (a redox-active tyrosine residue). Located in the D1 protein of PSII, Z transfers electrons from the OEC to P680⁺. The OEC operates in a cycle of oxidation states (the S-cycle) to split water molecules, producing molecular oxygen (O₂), protons, and electrons (Dekmak *et al.*, 2023). This cycle must complete four turnovers to fully oxidise two water molecules, releasing oxygen and protons into the thylakoid lumen, which creates a proton gradient essential for ATP synthesis (Hopkins & Hüner, 2008).

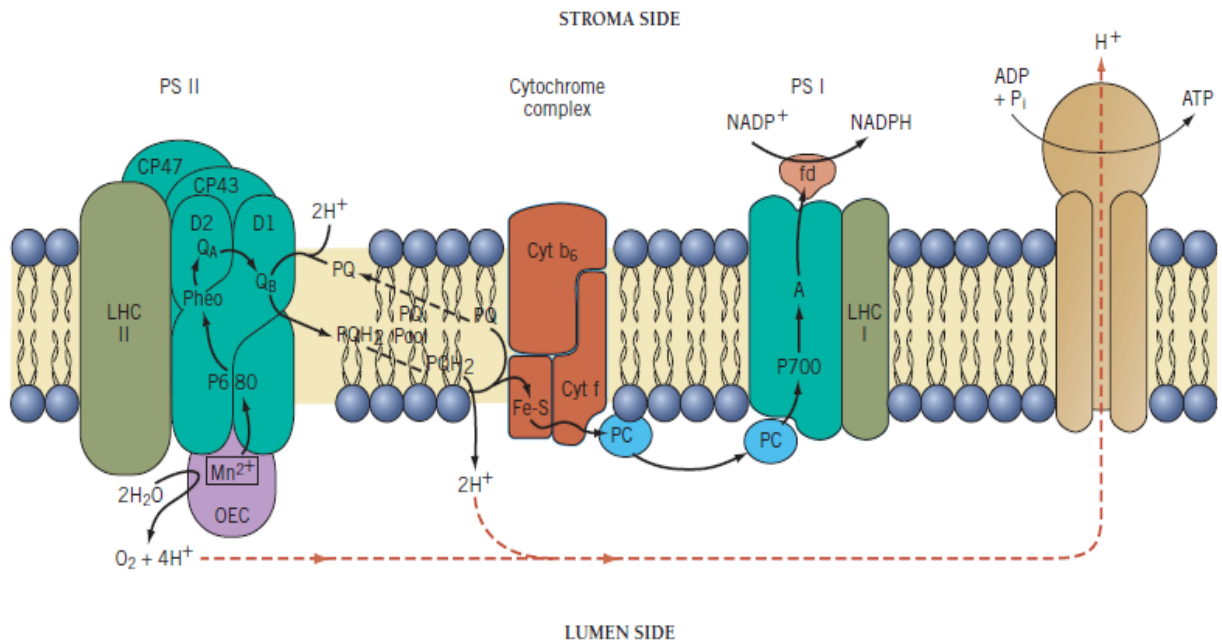


Figure 2: An illustration of the flow of an electron through the electron transport chain (Hopkins & Hüner, 2008).

Once P680* donates its electron to Pheophytin (Pheo), the electron is passed sequentially to plastoquinone Q_A , reducing it to Q_A^- (Figure 2). Thereafter, Q_A^- transfers the electron to a secondary quinone, Q_B , which is subsequently reduced to Q_B^{2-} after additional electron input. Q_B^{2-} undergoes protonation events, transforming into plastoquinol (PQH_2), which dissociates from the reaction centre. PQH_2 carries electrons from PSII to the cytochrome complex (Figure 2), where protons are released into the thylakoid lumen, further strengthening the proton gradient (Strasser *et al.*, 1995). Thereafter, the plastocyanin (PC) transfers the electrons from the cytochrome complex to PSI.

Following the principle of PSII, light absorption causes a change in the redox potential of P700 to form $P700^*$. An electron is then transported to ferredoxin (Fd) to form $P700^+$ and Fd^- . $P700^+$ is re-reduced by the electron transport from PC to PSI. Meanwhile, Fd^- interacts with the enzyme ferredoxin-NADP⁺ reductase (FNR) to reduce NADP⁺ into NADPH (Figure 2).

Meanwhile, the proton gradient generated during electron transport drives ATP synthesis via ATP synthase, a process known as photophosphorylation. The ATP and NADPH generated are then used in the Calvin cycle for carbon fixation, as illustrated in Figure 1 (Shevela *et al.*, 2019).

This entire electron transport process, driven by light and mediated by the coordinated action of PSII, the cytochrome complex, and PSI, is fundamental to energy conversion in photosynthesis, sustaining both ATP synthesis and the reduction reactions of PSI (Hopkins & Hüner, 2008).

2.1.2 Light-Independent Reactions: Carbon Fixation

The light-independent reactions, or Calvin cycle, convert carbon dioxide into organic molecules. Specifically, carbon dioxide is fixed into a three-carbon compound, glyceraldehyde-3-phosphate (G3P), which is eventually used to form glucose (Figure 3). This cycle consists of three phases: carbon fixation, reduction, and regeneration of ribulose biphosphate (RuBP) (Hopkins & Hüner, 2008). Through these steps, carbon is transformed into organic molecules, which provide energy and structure to the plant.

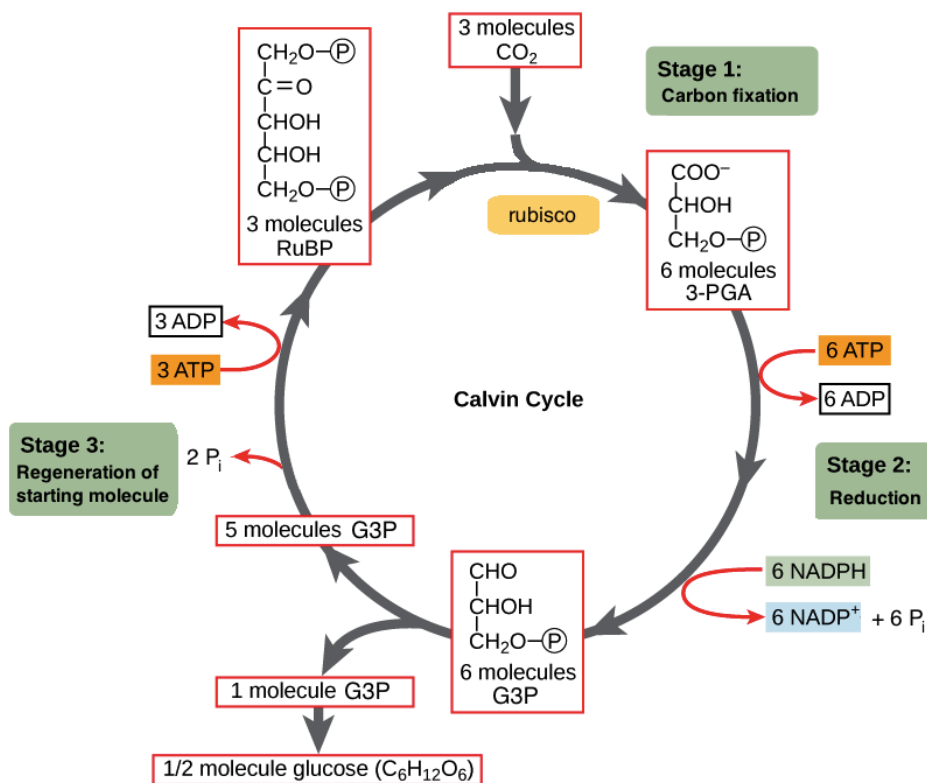


Figure 3: A representation of the Calvin cycle (“The calvin cycle: How the products of light reactions, ATP and NADPH, are used to fix carbon into sugars in the second stage of photosynthesis.”, s.a.).

2.1.3 Chlorophyll fluorescence as a tool to investigate the electron transport chain

Chlorophyll fluorescence is commonly assessed after a period of dark adaptation, during which all photosystem II (PSII) reaction centres are fully oxidized (Strasser *et al.*, 2002). This is followed by the application of a saturating pulse of light, allowing researchers to evaluate photosynthetic efficiency and the activity of the electron transport chain. This response, known as the Kautsky effect, reflects dynamic changes in fluorescence emission as photochemical and non-photochemical quenching processes are initiated (Kautsky & Hirsch, 1931). Instruments such as the Handy-PEA and M-PEA fluorometers are commonly used for this purpose. These devices are equipped with light-emitting diodes and a detector within a closed environment to measure the plant's response to light precisely. Chlorophyll *a* fluorescence is represented by an OJIP transient, a curve that maps specific phases of the photosynthetic electron transport chain (Figure 4).

The OJIP transient consists of two primary phases: a single turnover event, lasting around the first 2 milliseconds and multiple turnover events that span from 2 to 1000 milliseconds (Figure 4). The single turnover event represents a brief period where each PSII reaction centre (P680) undergoes a single electron excitation and transfer, capturing the initial photochemistry. During this phase, P700 in PSI remains primarily inactive. In contrast, the multiple turnover events involve successive excitation cycles where the PSII reaction centres are repeatedly excited. This leads to a continuous reduction of electron carriers and a progressive activation of the P700 reaction centre in PSI. These phases provide insights into the efficiency and capacity of the photosynthetic apparatus under conditions of varying electron flow demand.

The OJIP transient is typically divided into four distinct steps, which correspond to specific stages of the electron transport chain:

- **O-step:** This represents the minimal chlorophyll *a* fluorescence yield (highest photosynthetic yield) and serves as the initial baseline before any electron transfer occurs.
- **O to J transition:** During this phase, Q_A , the primary quinone electron acceptor, is reduced to Q_A^- . This step is primarily photosynthetic and corresponds to the rapid electron transfer within PSII.
- **J to I transition:** This phase represents the non-photosynthetic phase where intermediate electron carriers are reduced, slowing the overall electron flow as energy dissipation mechanisms occur.

- **I to P transition:** This phase involves the reduction of the end electron acceptors, specifically those at PSI, and indicates the full activation of the entire electron transport chain.

By analysing these steps, researchers gain detailed insights into the performance and potential limitations within the photosynthetic electron transport chain and the plant's adaptation to environmental conditions (Strasser et al., 1995, 2007).

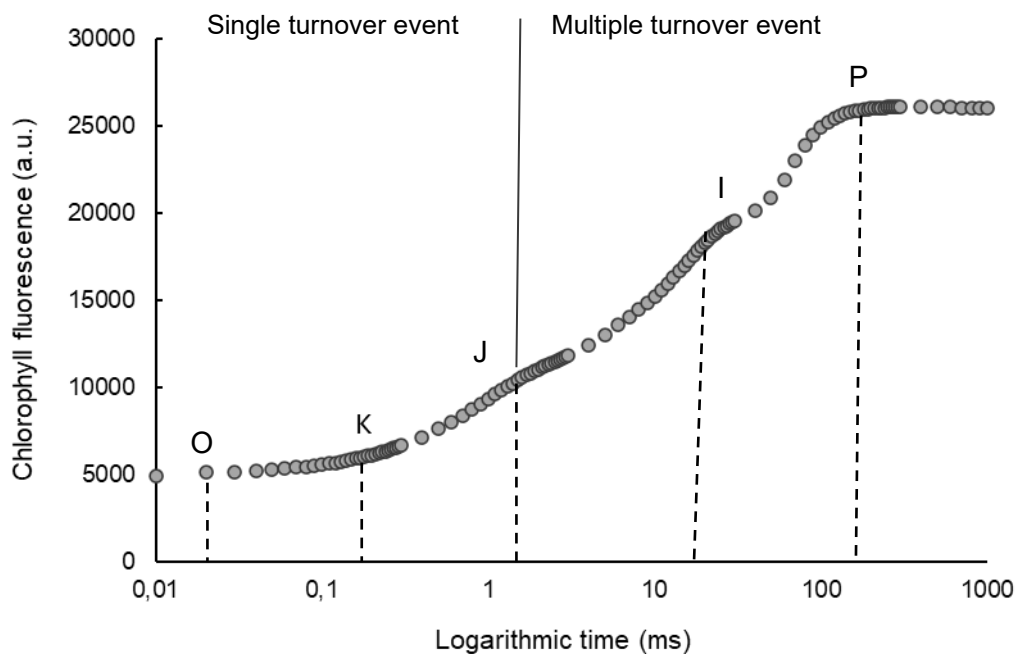


Figure 4: A typical form of the OJIP transient, plotted on a logarithmic timescale. Highlighting the O (0.03 ms), K (0.3 ms), J (2 ms), I (30 ms) and P (300 ms) inclination points (Tsimilli-Michael & Strasser, 2008).

Several parameters can be derived from the OJIP transient that investigate different parts of the electron transport chain and is summarised in Table 1.

Table 1: The formulas and description for parameters derived from the JIP-test to analyse the chlorophyll a fluorescence

Fluorescence parameters derived from measured data		
F_0	Basal Fluorescence	The fluorescence intensity emitted where all the active PSII are open
F_M	Maximum fluorescence	Maximal fluorescence when all PSII RC are closed
F_V	$= F_M - F_0$	Maximal variable fluorescence
F_V/F_M		Maximum quantum yield of primary photosynthetic reactions (at $t = 0$)
F_V/F_0		Maximum efficiency of the water diffusion reaction on the donor side of PSII
F_0/F_M		Quantum yield of basal non-photosynthetic process in PSII
S_M	$= (A_M)/(F_M - F_0)$	Normalised total area above the OJIP transient
N	$= S_M M_0 (1/V_j)$	Turnover number, indicates the number of times Q_A was reduced from time 0 to t_{Fm}
M_0	$= (\Delta V/\Delta t)_0 = TR_0/RC - ET_0/RC$	Approximate initial slope of the fluorescence transient Reflect the rate that at which PSII RC are closed
Performance index		
PI_{TOTAL}	$= PI_{ABS} + \delta_{Ro}/1 - \delta_{Ro}$	Total performance index
PI_{ABS}	$= RC/ABS + \phi_o/(1 - \phi_o) + \psi_o/(1 - \psi_o)$	PSII performance index based on absorption
RC/ABS	$= Chl_{RC}/(1 - Chl_{RC})$	Efficiency index, expressed as the density of RCs per chlorophyll

Phenomenological energy fluxes per excited cross-section (CS) of a sample		
ABS/CS _m		Absorption of energy per excited CS _m of a sample
DI ₀ /CS _m	= (ABS/CS) - (TR ₀ /CS _m)	Heat dissipation of excitation energy by PSII of a photosynthesising CS _m at t=0
TR ₀ /CS _m	= $\phi_{P0} \cdot (ABS/CS_m)$	Excitation energy flux trapped by PSII of a photosynthesising CS _m at t=0
ET ₀ /CS _m	= $\phi_{E0} \cdot (ABS/CS_m)$	Electron flux transported by PSII of a photosynthesising CS _m at t=0
RE ₀ /CS _m		Re-oxidation of electron acceptors per cross-section
Specific energy fluxes per Q _A -Reduced PSII reaction centres (RC)		
ABS/RC	= $M_0(1/V_j)(1/\phi_{P0})$	Absorption flux per one active reaction centre
DI ₀ /RC	= (ABS/RC) - (TR ₀ /RC)	Energy flux not intercepted by a RC.
TR ₀ /RC	= $M_0(1/V_j)$	Trapped energy flux per one active reaction centre (at t=0)
ET ₀ /RC	= $M_0(1/V_j)\Psi_0$	Electron transport flux per reaction centre (at t=0)
RE ₀ /RC	= $M_0(1/V_j)(1-V_j)$	The electron flux transferred per active reaction centre and reducing terminal acceptors on the acceptor side of PSI (at t=0)
Yields or energy flux ratios		
Ψ_0	= $ET_0/TR_0 = (1-V_j)$	Probability (at t=0) of electron transport further than Q _A ⁻
ϕ_{P0}	= $TR_0/ABS = F_v/F_M$	Maximum quantum yield of primary photosynthetic reactions

(Goltsev *et al.*, 2016; Kalaji *et al.*, 2017).

2.1.4 The 820 nm modulated reflection

The 820 nm modulated reflection curve (Figure 5) investigates the electron transport chain and is simultaneously measured with chlorophyll fluorescence. The modulated reflection curve focuses on PSI, specifically the oxidation and reduction (Hopkins & Hüner, 2008).

The typical 820 nm MR curve includes a decreasing phase followed by an increasing phase. This represents the ratio between PSI and PC's reduction to oxidation rate (Shu *et al.*, 2024). The oxidation rate is higher during the decreasing phase, while the reduction rate is higher during the increasing phase. Therefore, these slopes are referred to as the oxidation and re-reduction phase. At the turning point, MR_0 , the oxidation and reduction rate are equal (Strasser *et al.*, 2010).

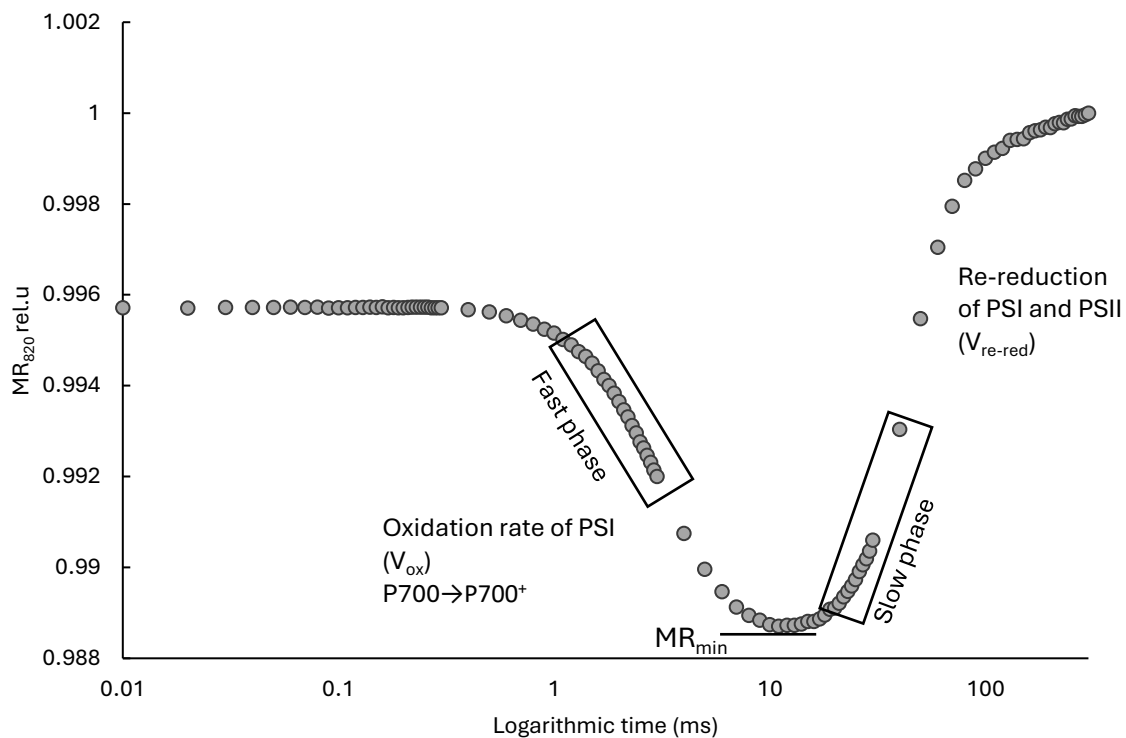


Figure 5: The 820 nm Modulated reflection curve (Shu *et al.*, 2024, Strasser *et al.*, 2010).

2.1.5 Combining the OJIP transient and 820 nm Modulated reflection curve

Both can be plotted on a single graph to illustrate better the simultaneous reactions and overlapping dynamics of the OJIP transient and the 820 nm modulated reflectance (MR) signal (Figure 6). This representation depicts the relationship between PSII and PSI, showing how electron flow initiated at PSII affects PSI activity and leads to the delayed response observed in the MR signal.

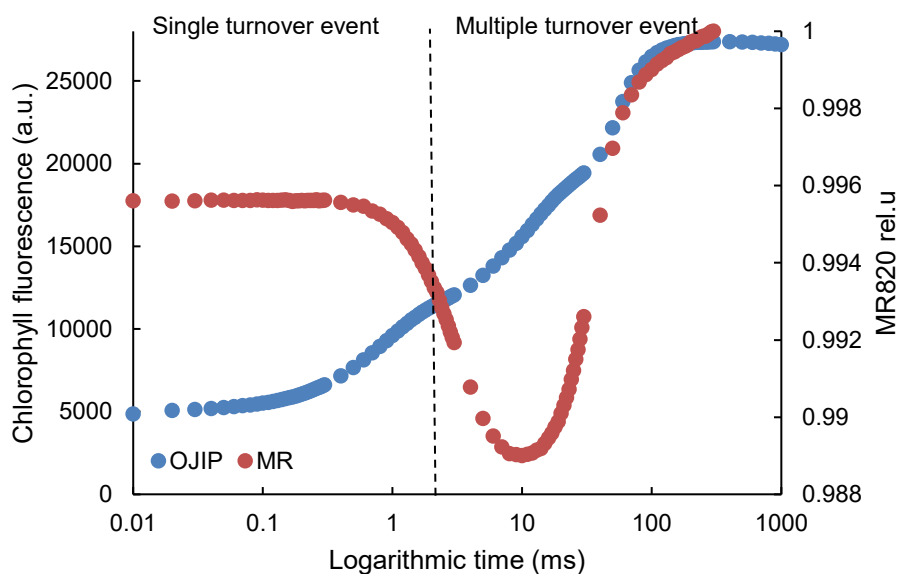


Figure 6: Combined illustration of the OJIP transient and the 820 nm Modulated reflection curve (Kalaji *et al.*, 2016, Liu *et al.*, 2018).

2.1.6 The gas exchange rates

Gas exchange rates provide valuable insights into photosynthetic efficiency. The Infrared Gas Analyzer (IRGA) is a tool used to measure a plant's respiration and transpiration rates (Moualeu-Ngangue *et al.*, 2017). By detecting oxygen (O_2) released through the stomata and carbon dioxide (CO_2) uptake (Figure 7) essential for the Calvin cycle, the IRGA serves as an effective indicator of changes in photosynthesis (Figure 3). This process can be influenced by light variation.

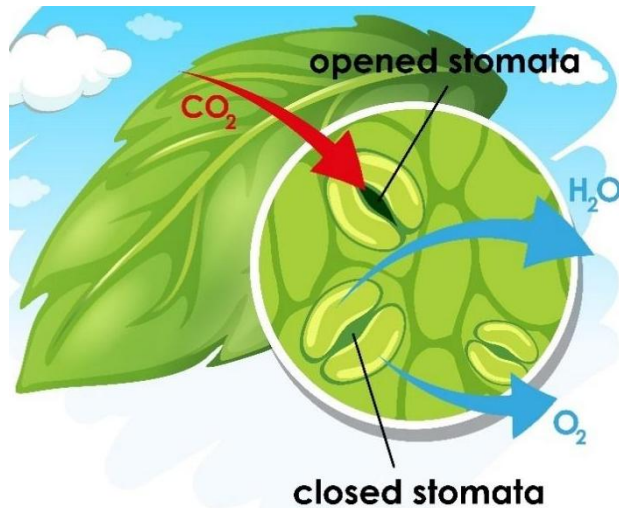


Figure 7: An Illustration of the gass exchange taking place in a plant that is measured by the IRGA https://www.freepik.com/free-vector/diagram-showing-schematic-stomata_19013687.htm#fromView=keyword&page=1&position=31&uuid=45038411-d939-423a-b6d2-a3c4b60c4a92&new_detail=true&query=Plant+Respiration.

2.2 Shading and light manipulation

2.2.1 Acclimation and adaptation of plants under sun and shade.

Acclimation and adaptation are two distinct processes that enable plants to endure and thrive in varying environmental conditions. Acclimation refers to a plant's capacity to adjust to and survive under stress. It is a genetically determined trait involving non-heritable changes that are reversible once the stress is removed (Hopkins & Hüner, 2008). In contrast, adaptation refers to the heritable modifications in structure or function that enhance an organism's fitness in a stressful environment resulting from natural selection. For instance, shade-adapted plants exhibit minimal capacity to acclimate to high-shading conditions. The leaves of these plants are thinner, with higher chlorophyll content and lower chlorophyll *a/b* ratios, indicative of an increased presence of light-harvesting complexes (LHC) (Wang *et al.*, 2024).

Under varying shading conditions, plants exhibit notable acclimation responses, collectively termed photo acclimation, which include changes in pigment composition. Plants in low light increase the total chlorophyll to optimise light absorption, while high light boosts photosynthetic capacity, Rubisco content and light saturation rate (Hopkins & Hüner, 2008). High light also increases the chlorophyll *a/b* ratio, Calvin cycle activity and growth rates, though excessive irradiance may cause photoinhibition, reducing photosynthetic efficiency. These imbalances are sensed through the redox state of the plastoquinone (PQ) pool. Additionally, leading to heat stress and stomatal closure (Hopkins & Hüner, 2008; Ilić & Fallik, 2017).

In sun-adapted plants, acclimation to light involves maintaining homeostasis through feedback mechanisms that stabilise their internal environment despite fluctuating light intensities (Hopkins & Hüner, 2008). Controlled environments, such as those using coloured netting, effectively simulate shading effects and can offer valuable insights into plants' adaptive and acclimatized responses under modified shading conditions.

2.2.2 Morphological Changes under Shade:

Plants adjust their physiological and morphological traits to optimise photosynthesis in response to environmental conditions (Li *et al.*, 2018). Light spectral quality and intensity affect the plant's microclimate, subsequently impacting growth and yield (Ilić & Fallik, 2017). At a lower light intensity, plants often increase leaf area while reducing leaf thickness to expand the surface area exposed to light, enhancing light absorption and maximising photosynthetic efficiency (Huang *et al.*, 2016; Lobos *et al.*, 2012). Additionally, chlorophyll content per leaf tends to increase under low light, along with a decrease in the chlorophyll *a/b* ratio, further improving the plant's ability to capture and utilise available light for photosynthesis (Huang *et al.*, 2016).

2.2.3 Manipulation of light through netting:

In agriculture, shading is often used to control light intensity and quality. Shade nets, commonly employed in crop production, create a controlled microclimate by modifying airflow, temperature, humidity, and both photosynthetically active radiation (PAR) and near-infrared radiation (NAR) (Ahemd *et al.*, 2016). These nets protect crops from environmental stressors such as high light intensity, pests, diseases and hail (Manja & Aoun, 2019). Different coloured nets manipulate light by refracting or reflecting specific wavelengths, altering the plants' light quality and spectral composition. This can influence critical physiological processes, including photosynthesis, and is used to enhance crop quality and productivity. For example, altering light quality with shade nets has been shown to affect fruit ripening, improve yield, and regulate the plant life cycle (Ilić & Fallik, 2017). How different light quality can influence the plant is summarised in Figure 8.


Wavelengths of light	Photoreceptors	Plant responses
 Red, Far-red (600-750 nm)	PHYs	Germination, De-etiolation, Shade avoidance, Inhibition of stem and petiole elongation, Leaf expansion and flattening, Circadian rhythms, Flowering, Branching.
Green (530-570 nm)	CRYs	De-etiolation, Inhibition of stem and petiole elongation, Leaf expansion, Circadian rhythms, Flowering, Flavonoid biosynthesis. Response to shade by B/G photoperception.
Blue (390-500 nm)	PHOTs	Leaf flattening, Phototropism, Stomatal opening, Chloroplast relocation.
UV-A (320-390 nm)	ZTL/FKF1/LKP2	Circadian rhythms, Flowering.
UV-B (290-315 nm)	UVR8	De-etiolation, Flavonoid biosynthesis.

Figure 8: The response of plants to different light colours (Paradiso & Proietti, 2022).

Studies demonstrate that the strategic manipulation of light using shading nets can enhance plant vitality by optimising photosynthetic efficiency and triggering stress-related pathways that lead to increased production of secondary metabolites (Thoma *et al.*, 2020; Tmušić *et al.*, 2021b; Yang *et al.*, 2019). For instance, light environments created by coloured shading nets have been shown to improve plant health, resilience, and yield, making this an effective strategy for cultivation.

The manipulation of light through shade netting is an effective tool for modulating light quality and intensity. This approach ensures the production of high-quality medicinal plants, such as *Lessertia frutescens*. By promoting consistent chemical profiles and improved plant vitality, shade netting ultimately enhances the pharmacological value of these plants.

2.3 Background on *Lessertia frutescens*

2.3.1 Ecological and ethnobotanical importance:

Widely distributed across southern Africa, *Lessertia frutescens* is found in the Western, Eastern and Northern Cape provinces, parts of KwaZulu-Natal, southern Namibia and the southeastern corner of Botswana (Aboyade *et al.*, 2014; Fernandes *et al.*, 2004; van Wyk & Albrecht, 2008). A distribution map of *Lessertia frutescens* in South Africa is presented in Figure 9. The regions where the plant is commonly found typically experience low annual rainfall, high light intensities and extreme temperature fluctuations. *Lessertia frutescens* is well adapted to thrive in sandy or

rocky soils with excellent drainage, allowing it to withstand the arid and semi-arid conditions characteristic of its native habitat (SANBI, s.a.).

Historically, six *Sutherlandia* species were recognised, but enzyme electrophoresis studies led to the recognition of only two species, *Sutherlandia frutescens* and *Sutherlandia tomentosa* (Moche, 1998). Other taxa are now considered subspecies or forms (SANBI, s.a.). *Sutherlandia frutescens* includes four forms: typical, hairy, dwarf, and high-altitude, as represented by the key in Figure (van Wyk & Albrecht, 2008). These different forms reflect the plant's geographical distribution and morphological variation (Goldblatt & Manning, 2003). *Sutherlandia* is no longer recognized and the species are now known as *Lessertia frutescens* and *Lessertia canescens* (Goldblatt & Manning, 2003; SANBI, s.a.).

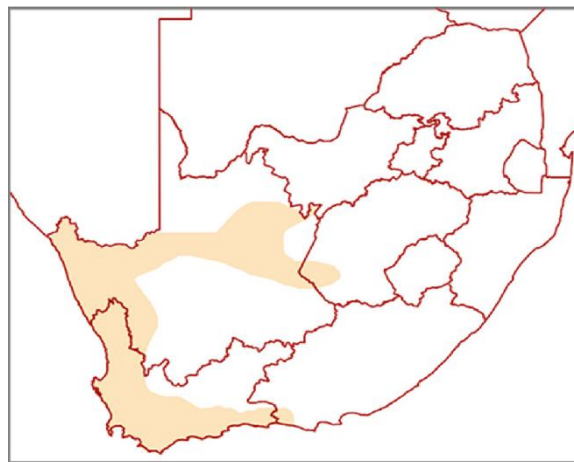


Figure 9: Geographical distribution of *Lessertia frutescens* across South Africa (Mncwangi *et al.*, 2022). This map does not include the distribution in Namibia and Botswana.

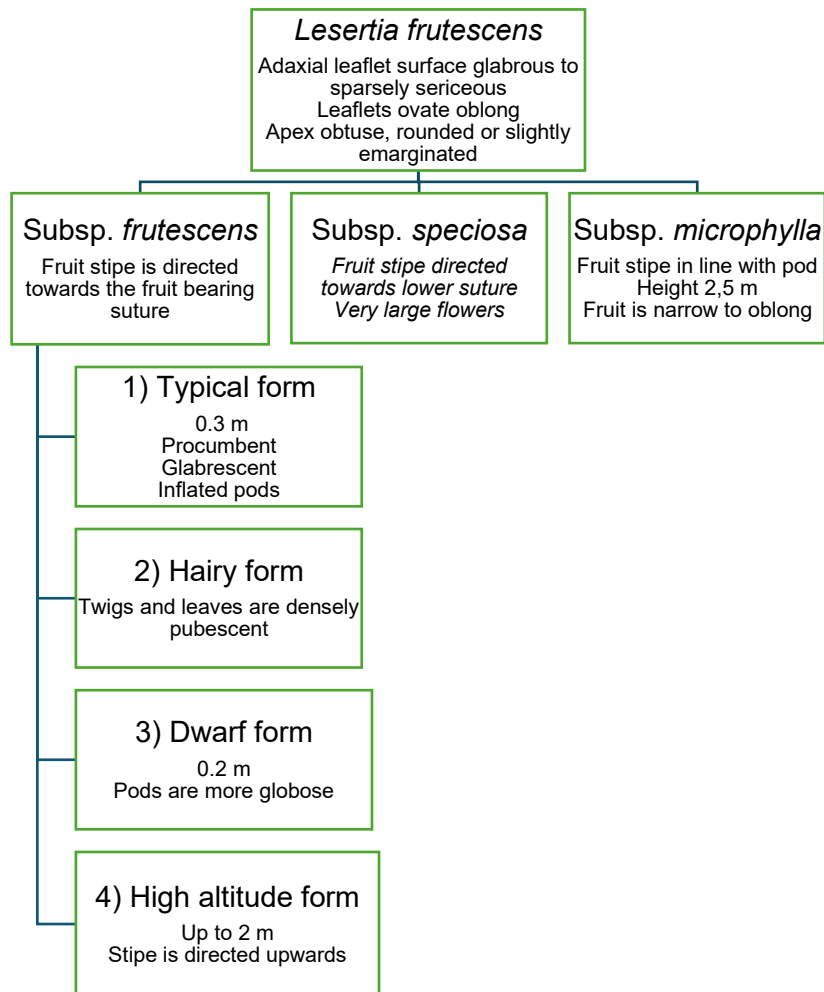


Figure 10: Schematic representation of the characteristics of *Lessertia frutescens*, its subspecies and different forms (van Wyk & Albrecht, 2008).

As a member of Fabaceae, *Lessertia frutescens* is a nitrogen fixer, with nodules on its roots and seeds contained within pods (Goldblatt & Manning, 2003). The plant is distinguished by its vibrant red flowers. Its compound pinnately arranged leaves consist of 6-18 pairs of small (1-2 cm) oval leaflets that emits a bitter odour when crushed. The leaves are covered with fine hairs (Figure 11c), giving them a green-greyish hue (van Wyk & Albrecht, 2008).

Subspecies of *Lessertia frutescens* are differentiated based on the direction of the fruit stipe and the hairiness of the stalk and leaves (Figure 11). In *Subsp. speciosa*, the fruit stipe is oriented toward the lower suture, whereas in *Subsp. microphylla*, it aligns with the pod, and in *Subsp. frutescens*, it points toward the fruit-bearing suture. The identification of the species to form level is very simplified in Figure 10, but the hairiness and height of the plant group are fundamental characteristics.

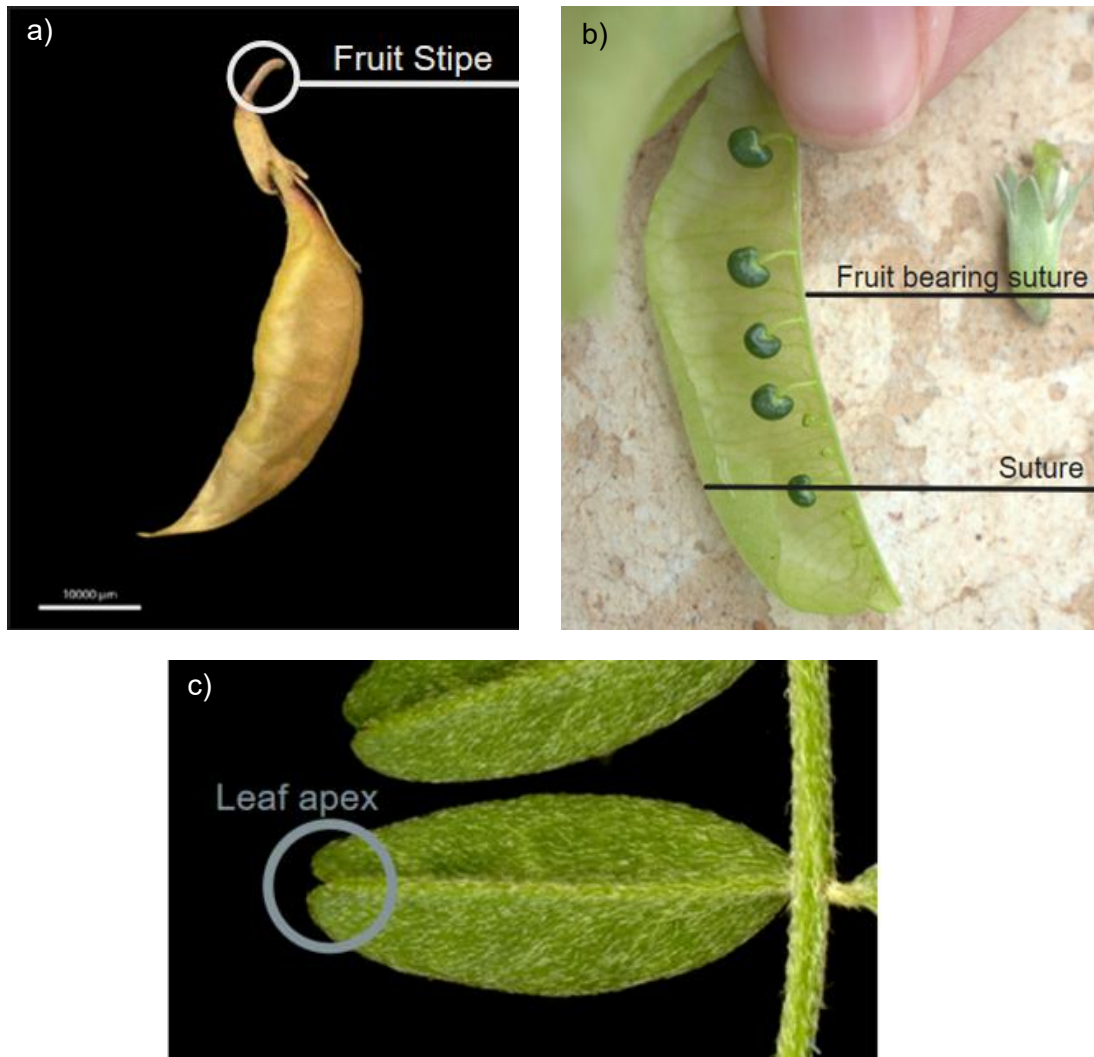
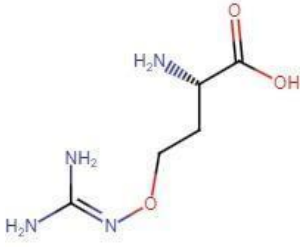
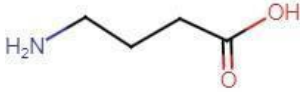
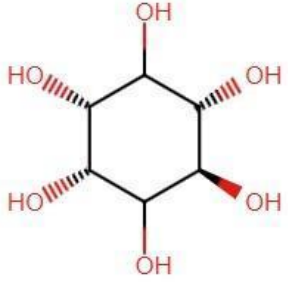
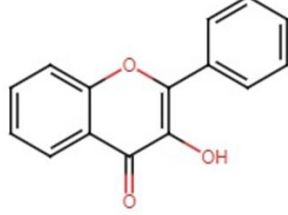
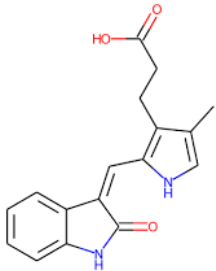


Figure 11: Characteristics of *Lessertia frutescens*, the pod can differ in the direction of the (a) fruit stipe and the (b) fruit bearing suture and the hairiness (c) adaxial side of apex leaf.

Lessertia frutescens holds medicinal value among various cultural groups, including the Zulu, Xhosa, Sotho, Khoisan, and Cape-Dutch. It is traditionally used to treat ailments such as stomach aches, diabetes, cancer, stress, anxiety, influenza, back pain, fever, and cleansing (van Wyk & Albrecht, 2008). Research by Aboyade *et al.* (2014) has sought to bridge traditional knowledge with scientific understanding, investigating the plant's chemical composition and medicinal properties. A summary of these chemical compounds is shown in Table 2.

Table 2: A summary of the chemical constituents found in *Lessertia frutescens* and the ailments its uses.

Compound	Pathways	Ailments	Chemical composition	References
L-canavanine	L-arginine biosynthesis and L-canavanine biosynthesis	Cancer, anti-viral		(Bence <i>et al.</i> , 2002; Crooks & Rosenthal, 1996; Swaffer <i>et al.</i> , 1995; van Wyk & Albrecht, 2008)
GABA (inhibitory neurotransmitter)	Glutamate Decarboxylation Pathway	Anxiety, Stress Inhibit tumour cell migration		(Aboyade <i>et al.</i> , 2014; Ortega, 2003; van Wyk & Albrecht, 2008)
Pinitol	Pinitol biosynthesis pathway	Diabetes and inflammation Reversal of Wasting in Cancer and AIDS Play a role in regulating cellular energy		(Aboyade <i>et al.</i> , 2014; van Wyk & Albrecht, 2008)
Flavonoids	Acetate pathway	Antioxidant, anti-inflammatory, antimicrobial, anti-tumoral, anti-thrombogenic, anti-viral and	 Flavonoids	(Umesh <i>et al.</i> , 2018)

		anti-allergic properties		
SU1 – cycloartane-type triterpene glycoside	Mevalonate (MVA) pathway and the cycloartenol biosynthesis pathway.	Cancer Chemo preventive activity		(van Wyk & Albrecht, 2008)

2.4 Implications and Applications

The findings from light manipulation studies reveal important implications for both agricultural research and practical applications. Shading nets are valuable tools for enhancing crop yields and addressing environmental stressors. By creating favourable microclimates, nets can boost plant health and productivity while influencing the production of secondary metabolites in medicinal plants such as *Lessertia frutescens* (Tmušić *et al.*, 2021a). For *Lessertia frutescens*, controlled shading conditions are particularly promising, as they could help standardise the production of its medicinal compounds despite its susceptibility to environmental variability.

In sustainable agriculture, shading nets have demonstrated utility in mitigating climatic extremes, and fostering resilience in crops grown in challenging environments (Ahemd *et al.*, 2016). Coloured nets enable precise manipulation of the light spectrum, allowing for targeted physiological responses such as improved photosynthetic efficiency or enhanced metabolite synthesis (Paradiso & Proietti, 2022). These strategies offer the flexibility to optimise outcomes based on the specific objectives of cultivation, whether for food production, medicinal use or other applications (Ilić & Fallik, 2017).

Tailored shading practices further maximize productivity and quality (Milenkovic *et al.*, 2012) and Ilić *et al.*, 2015). Integrating shading systems with other technologies, such as plastic coverings or dynamic light control, could enhance their effectiveness, providing a robust framework for modern agricultural systems.

For *Lessertia frutescens*, adopting species-specific shading configurations could support consistent, high-quality yields commercially. This approach bridges traditional medicinal knowledge with contemporary agricultural practices, opening pathways for its use in pharmaceutical and nutraceutical industries. Moreover, it reinforces the importance of preserving ecological and cultural value while promoting sustainable utilization.

2.5 Research gap and possible hypothesis

Research on the cultivation of *Lessertia frutescens* remains limited, with recent studies emphasizing key knowledge gaps. Zonyane *et al.* (2019, 2020) investigated the chemotypic variation in wild populations of *Lessertia frutescens*, focusing on antioxidant levels. Additionally, (Korth (2021) reviewed the species' taxonomy, medicinal properties, and germination, emphasizing further research on its invasive potential as a crop and detailed cultivation requirements, including soil pH, light, fertilizers, and temperatures.

Shading studies on leguminous species emphasize species-specific responses to light manipulation. For instance, Raai *et al.* (2020) found that *Psophocarpus tetragonolobus* exhibited a fourfold increase in photosynthesis under full sunlight compared to shaded conditions, aligning with its preference for high light. Conversely, Khalid *et al.* (2019) showed that light shading improved soybean yield through enhanced stomatal efficiency.

Research on other crops under shade nets has provided valuable insights into light modulation effects. Milenkovic *et al.* (2012) and Ilić *et al.* (2015) found that the same shading conditions elicited varying growth responses depending on the species. For instance, peppers and tomatoes responded differently to identical shade net treatments, emphasizing the importance of tailoring shade configurations for specific crops. Combining shade nets with plastic coverings was also shown to optimize light distribution and productivity further.

Coloured shade nets have garnered significant attention due to their ability to manipulate radiation spectra and increase light scattering by 50% or more (Ilić *et al.*, 2019). These nets enable precise control of light quality and intensity, creating conditions conducive to growth for a wide range of crops.

Chlorophyll content has also been shown to increase under shaded conditions, enhancing a plant's capacity to harvest and utilize available light. This increase is primarily due to an expansion in peripheral light-harvesting antenna complexes, which have the highest chlorophyll content but the lowest chlorophyll *a/b* ratio. These findings underscore the adaptability of chlorophyll

dynamics in optimizing photosynthetic efficiency under low-light environments (Huang *et al.*, 2016)

Finally, comparative studies on shading densities and net colours highlight the effects of light quality and intensity on different species. For instance, Lobos *et al.*, (2009) examined *Vaccinium corymbosum* (blueberries) grown under no shade, white, red, and black nets at varying densities (25%, 50%, and 75%). Their results showed that higher shading densities generally reduced light intensity, with photosynthetic responses aligning with the plant's adaptability from full sun to partial shade.

In this study, *Lessertia frutescens* will be cultivated under 40% green, white, and grey shade nets and in outdoor conditions without shading. The photosynthetic performance and growth of *Lessertia frutescens* are anticipated to differ significantly across these treatments due to light quality and intensity variations. Coloured shade nets are expected to modulate the spectral composition of light, influencing chlorophyll content, photosynthetic efficiency, and overall plant vitality. Specifically, green nets may enhance light-harvesting efficiency by favouring wavelengths that optimize photosynthesis. In contrast, grey nets are likely to reduce light intensity, potentially resulting in lower photosynthetic rates and diminished growth.

CHAPTER 3 MATERIALS AND METHODS

3.1 Plant materials and growth conditions

Lessertia frutescens plants were obtained in different ways. Firstly, seeds were sown in 6 cm pots containing hygromix (Hygrotech SA). The seedlings were cultivated in a greenhouse where the maximum temperature was set to 30 °C and the minimum to 16 °C. To supplement natural light and to induce a daylength of 16 hours, plants were exposed to a photosynthetic photon flux density (PPFD) of 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ through fluorescent growth tubes. After 1 month, the plants were repotted into 12 cm pots with a mixture of soil and vermiculite (Sun Silicates (Pty) Ltd, South Africa) (2:1 ratio). At 5 months the plants were further transplanted into 30 cm pots containing soil, vermiculite (Sun Silicates (Pty) Ltd, South Africa) and Vermicompost (Ecolife, South Africa) in the ratio of 2:1:1. A slow-release fertilizer, 10 g of Osmocote Pro 3-4 months (Osmcote, Netherlands) with an NPK ratio of 19:9:10 + 2 Mg, were added to the mixture. During cultivation, all plants were watered to field capacity to ensure uniform soil moisture conditions across treatments. The plants cultivated from seeds were referred to as plant group A. Additionally, vegetatively propagated *Lessertia frutescens* purchased from a commercial nursery were transplanted in the 30 cm pots described above, referred to as plant group B. The plants were kept separate as visual differences were observed. After repotting, the plants were placed either outside or on the open tops, which were covered with grey, white, or green netting (Figure 12).



Figure 12: The open top chamber (OTC) at the North-West University

3.2 Experimental setup

The experiment was conducted using open-top chambers (OTC) at the North-West University during the summer months of January to April 2024. Initially designed for pollution studies, the OTC was modified for this experiment to assess the impact of different coloured laminated shade netting. Each cylindrical OTC (2.2 m height, 1.7 m diameter) was equipped with a roof for air circulation and a 3 kW, 380 V large axial fan motor (Trojan Fans, Kempton Park, South Africa) for mechanical ventilation. Twelve OTC were covered with either grey, white, or green Knipol-laminated 40% shade netting (Figure 12). The photosynthetic photon flux density (PPFD) beneath each shading condition was measured, at canopy level using a full spectrum quantum meter (MQ-500, Apogee, USA).

3.3 Chlorophyll content

The chlorophyll content of *Lessertia frutescence* was measured with the CCM-300 chlorophyll content meter (Opti-Sciences, USA). This is a non-invasive method to measure the chlorophyll content of plants. The Gitelson equation determines the chlorophyll content ($\text{mg}\cdot\text{m}^{-2}$) through the emission ratio of 700 to 735 nm, which is measured and calculated by the CCM-300 (Opti-Sciences, s.a.). For each plant, 5 leaves were measured.

3.4 The simultaneous measurements of Prompt fluorescence and 820 nm Modulated reflection

The Multifunctional plant efficiency analyser (M-PEA) fluorometer (Hansatech Instruments, King's Lynn, Norfolk, UK) was used to investigate PSII through the prompt fluorescence and PSI through the 820 nm transmission transient. The M-PEA consist of a control unit and an optical sensor. The Optical sensor is equipped with three light diodes, an actinic with a dominant wavelength of 625 nm, a far-red light and an optical filtered pulse at 820 nm, and detectors that measure the response (Hansatech Instruments, s.a.). Measurements were taken on 5 different leaves of each plant group After one hour of dark adaption. Thereafter, the M-PEA exposed the leaf to a light of $3500 \mu\text{mol}\cdot\text{mol}^{-1}$ through the light-emitting diodes.

3.4.1 Photosystem II activity

The M-PEA measured the kinetics of the polyphasic prompt fluorescence rise, while the M-PEA plus (v 1.10) software calculated the chlorophyll *a* fluorescence. This data is then used to calculate the parameters through complex formulas.

The OJIP transient was analysed at different inclination points: O point at 0.03 ms, K point at 0.3 ms, J at 2 ms, I at 30 ms and P at 300 ms (Figure 13). The maximum quantum yield of primary photosynthetic reactions (F_v/F_M) reflects the efficiency of PSII, which is the efficiency of light energy transformed into chemical energy. It is calculated by dividing the difference between the maximal and basal fluorescence by the maximal fluorescence, as explained in Figure 13.

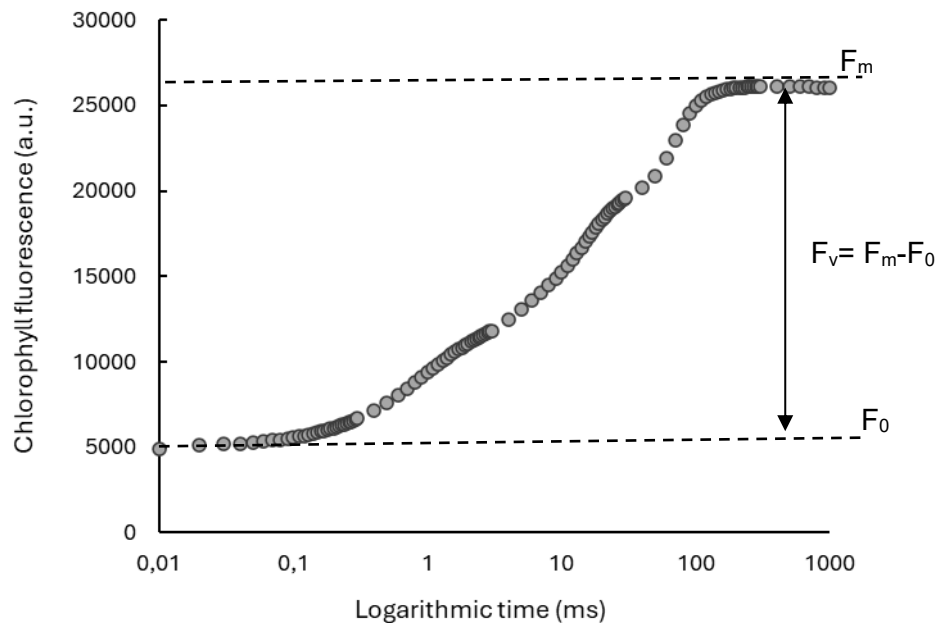


Figure 13: A typical polyphasic chlorophyll *a* fluorescence rise (OJIP transient), plotted on a logarithmic scale (0.01-1000ms). The different steps are labelled O (0.03 ms), K (0.3 ms), J (2 ms), I (30 ms) and P (300 ms). The basal fluorescence and maximal fluorescence as well as the equation for the maximum quantum yield is indicated.

3.4.2 Photosystem I activity

The 820 nm Modulated reflection curve were calculated by the M-PEA plus (v 1.10) software. Which give insight to the efficiency of PSI. The data retrieved were used to calculate the following parameters in excel:

$$Slope = \frac{\Delta y}{\Delta x}$$

- Fast phase (V_{ox}) = Slope between 1-3 ms
- Slow phase (V_{re-red}) = Slope between 20-40 ms
- MR_{min} = Minimum for each graph calculated through Excel
- MR_0 = The value at onset of actinic illumination (0.7 ms)

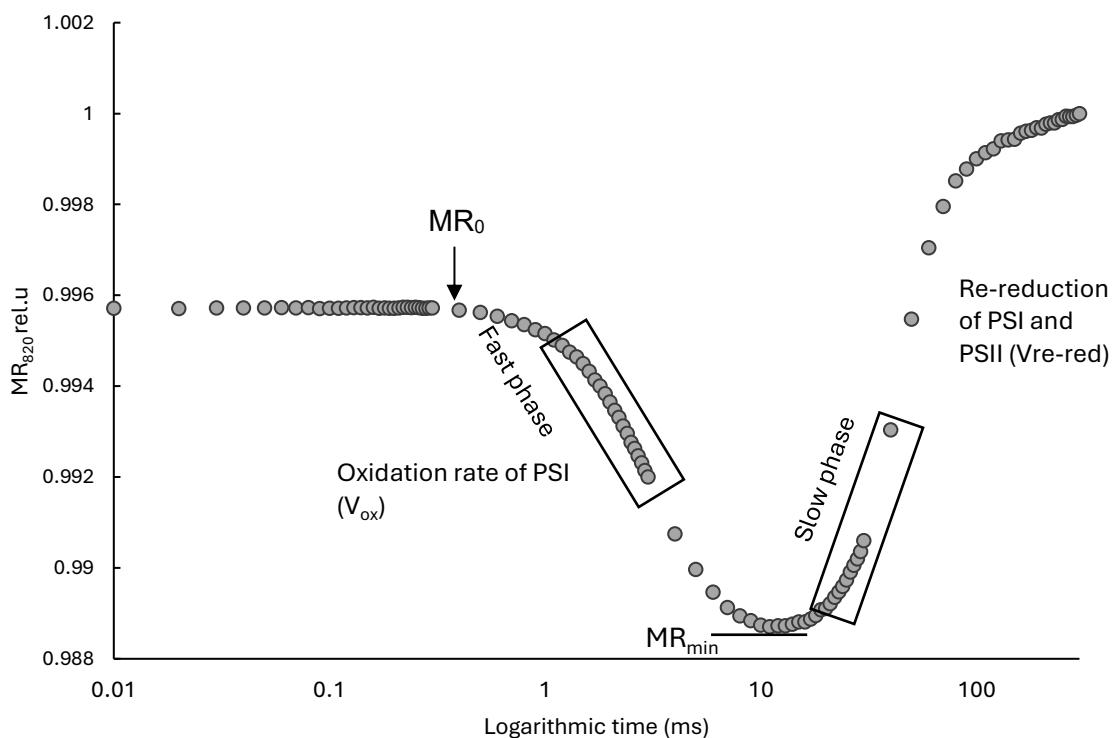


Figure 14: The activity of PSI represented in an 820 nm Modulated reflection curve plotted logarithmically. The slow phase and fast phase are indicated, as well as the parameters.

3.5 Visual assessment of plants

Photos were taken with a Cannon EOS800D (Canon, Japan) to illustrate the environment and overall appearance of the plant and experimental setup. The differences between the group A and B plants were visually illustrated with photos. The Nikon AZ100M stereomicroscope equipped with a Nikon DSFI3 camera and Nis elements D 5.42.13 software (Nikon, Japan) was used to capture the abaxial and adaxial leaf surface details.

3.6 Statistical analyses

The statistical analyses were implemented by Statistica. The normality of the datasets was done with a Shapiro Wilk test. Thereafter, the data was subjected to a two-way analysis of the variance of repeated measures with a significance level of 95%, followed by a Tukey test. A summary of the statistics, like the degrees of freedom, are summarised in Table 3.

Table 3: A representation of the statistics of this study.

Plants used	Different plant origins	Treatments	Replications	Leaves used per plant	Degrees of freedom
24+24	2	4	2	5	$= (2 \times 4 \times 2) - 1$ $= 15$

CHAPTER 4 RESULTS

At the beginning of this study, we initially considered Plant group A and Plant group B to be the same, as no distinguishing characteristics were observed. However, differences emerged in their origin and cultivation methods, prompting us to maintain separate datasets. As the trial progressed, distinct physiological responses became evident, further justifying their classification as Plant Group A and Plant Group B.

4.1 Environmental conditions:

4.1.1 Climatic conditions:

The climatic data for the experiment, conducted from January to April 2024, were obtained from a weather station located near the study site, operated by the North-West University's Department of Geographical and Spatial Sciences. The key climatic conditions during the experimental period are summarized in Table 4.

Table 4: The climatic conditions in Potchefstroom during the experiment.

	Maximum temperature T _{dry} (°C)	Minimum temperature (°C)	Relative humidity (%)	Day length (beginning of each month)
January	32.95	10.20	74.68	13h 50 min
February	34.32	11.3	69.94	13h 17 min
March	36.61	12.25	66.49	12h 17 min
April	30.61	5.98	74.48	11h 10 min

NWU Geographical and Spatial sciences

4.1.2 Light intensity:

The light intensity to which the plants are exposed is manipulated through the different coloured laminated shade netting and can influence photosynthesis.

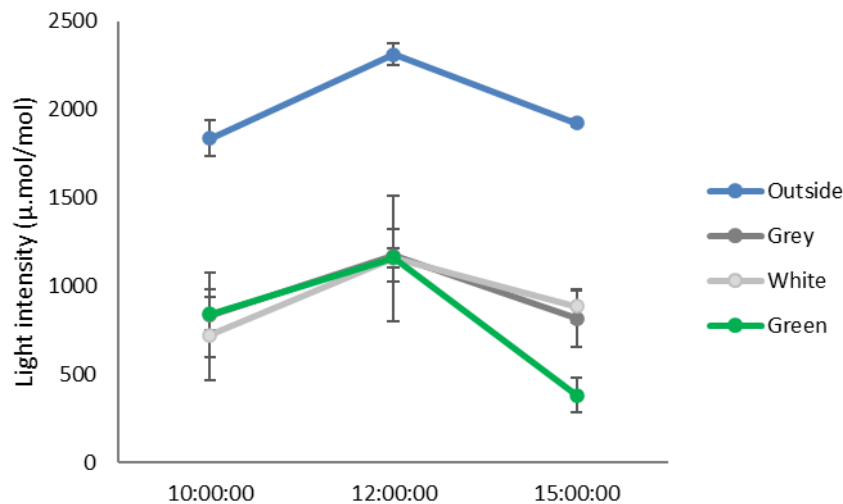


Figure 15: The light intensities under the different nettings and outside through the day.

The light intensities, at canopy level, outside were consistently higher than those recorded under the laminated shade netting across all three measurement times (10:00, 12:00, and 15:00) (Figure 15), with significant ($p < 0.05$) differences observed (Appendix: Table 21). The expected diurnal trend is depicted in Figure 15, showing an increase in light intensity until midday, followed by a decrease in the afternoon. Notably, under the green laminated shade netting at 15:00, the light intensity was significantly ($p < 0.05$) lower compared to both the white laminated shade netting at 15:00 and the green laminated shade netting at 12:00. Within each netting type, light intensity did not differ significantly ($p < 0.05$) throughout the day, except for the green laminated shade netting at 15:00, which showed significantly lower values than at 10:00 and 12:00 (Appendix: Table 21).

4.2 The photosynthetic effects on *Lessertia frutescens*:

The results gathered of *Lessertia frutescens* under different laminated shade netting is investigated, while plants A and B are kept separated as a difference in the physiology and morphology of the plants were observed.

4.2.1 The effect of different shading conditions on the chlorophyll content:

Chlorophyll is the primary photosynthetic pigment in the plant. As plants are subjected to different environments, their chlorophyll content and ratios are adjusted to optimize light absorption and photosynthesis (Li *et al.*, 2018).

4.2.1.1 Plant group A:

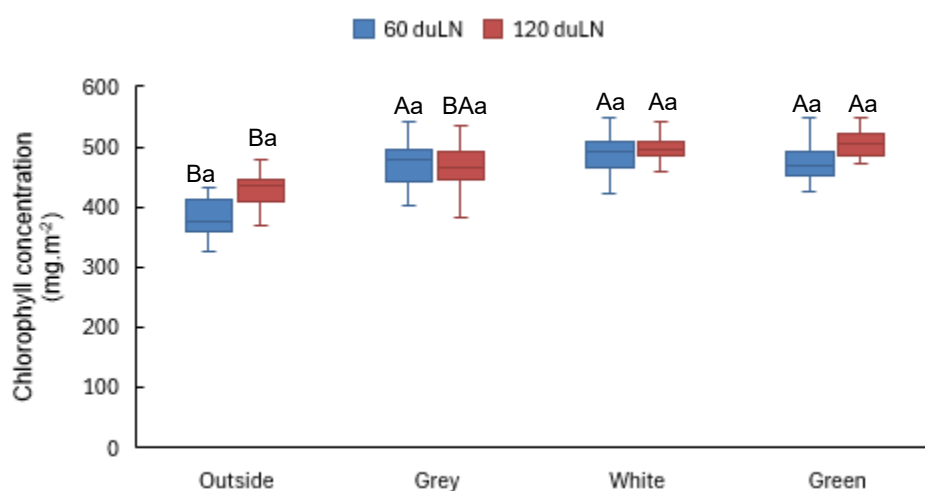


Figure 16: The chlorophyll content of *Lessertia frutescens*, plant group A, under different laminated shade netting. Different letters are denoted by significant differences ($p < 0.05$). The capital letters refer to significant ($p < 0.05$) differences between the shading conditions and the lowercase letters refer to the significant ($p < 0.05$) differences that occurred over time after 60 and 120 days under laminated shade netting (duLN).

The chlorophyll content of plants under laminated shade netting for 60 days was significantly ($p < 0.05$) higher compared to those outside (Figure 16). After 120 days, plants under the white laminated shade netting exhibited a significantly ($p < 0.05$) higher chlorophyll content than those grown outside. However, no significant ($p < 0.05$) differences were observed between the two-time points (60 and 120 days) for plants under the same conditions (Appendix: Table 22).

4.2.1.2 Plant group B:

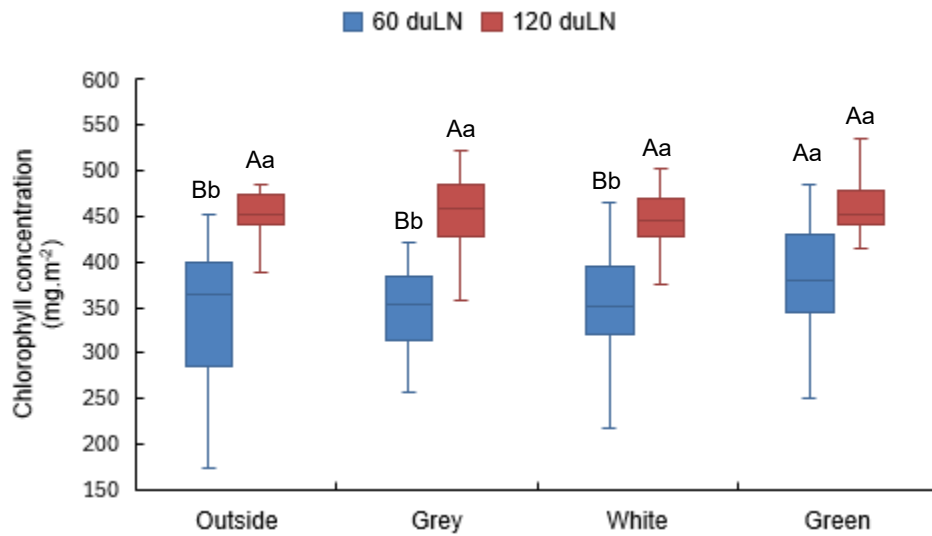


Figure 17: The chlorophyll content of *Lessertia frutescens*, plant group B, under different laminated shade netting. Different letters are denoted by significant differences ($p < 0.05$). The capital letters refer to significant ($p < 0.05$) differences between the shading conditions and the lowercase letters refer to the significant ($p < 0.05$) differences that occurred over time, after 60 and 120 days under laminated shade netting (duLN).

The chlorophyll content of plants under the green laminated shade netting was significantly ($p < 0.05$) higher than that of the other shading conditions after 60 days (Figure 17, Appendix: Table 22). In contrast, no significant ($p < 0.05$) differences were observed among the outside, grey, and white laminated shade netting during this period. After 120 days, there were no significant ($p < 0.05$) differences in chlorophyll content between the various light treatments (Appendix: Table 22). However, the chlorophyll content increased significantly ($p < 0.05$) from 60 to 120 days for plants exposed to the outside, grey, and white laminated shade netting. Although an increase in chlorophyll content was also observed under the green laminated shade netting during this period, it was not statistically significant ($p < 0.05$) (Appendix: Table 22). The increased chlorophyll content in plants grown under laminated shade netting indicates that these plants adjusted their chlorophyll levels to optimize light absorption under reduced shading conditions.

4.2.2 The effect of different shading conditions on the OJIP transient:

The OJIP transient provides valuable insights into the photosynthetic performance of plants, especially under different environmental conditions (Kautsky & Hirsch, 1931). Investigating the fluorescence response at specific inclination points (O, K, J, I, and P) allows the assessment of electron transport efficiency and photosynthetic activity. These points can provide insights into stress-related changes in photosystem II (PSII) (Tsimilli-Michael & Strasser, 2008). In this section, the effects of different shading conditions on the OJIP transient of Plant group A and Plant group B over 60 and 120 days were investigated, emphasising differences observed at the inclination points.

4.2.2.1 Plant group A

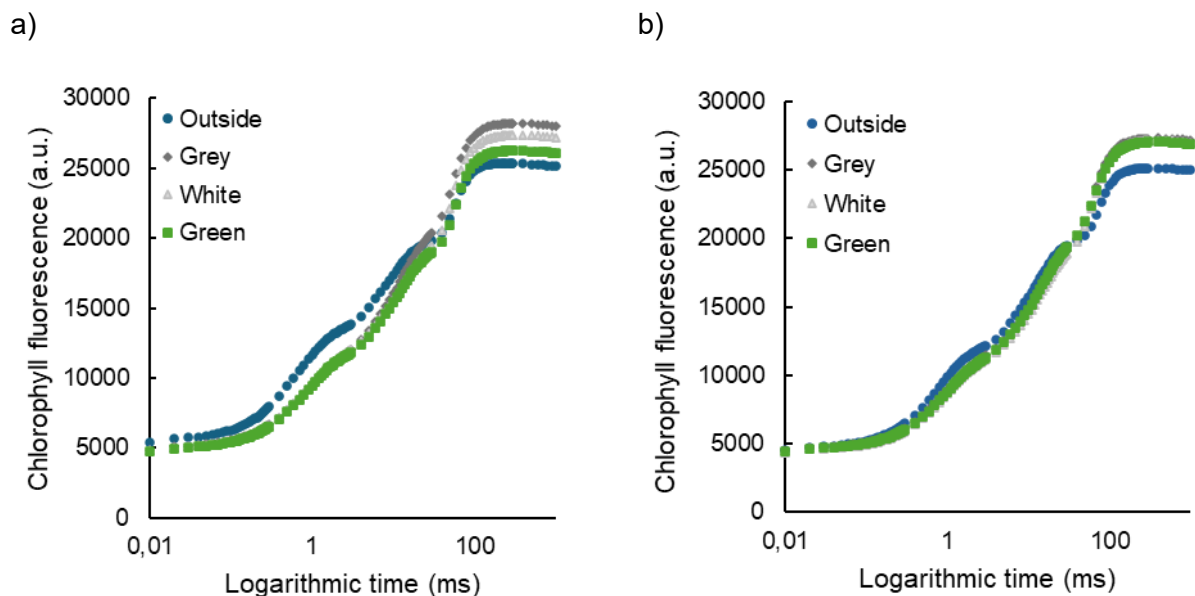


Figure 18: The OJIP transient of plant group A after a) 60 days and b) 120 days under laminated shade netting (duLN).

Differences in the OJIP transient of Plant group A were observed, influenced by the varying shading conditions (Figure 18). After 60 days under laminated shade netting (duLN), plants grown outside exhibited higher chlorophyll fluorescence than those under laminated shade netting during the O to J inclination points, also known as the single turnover event of the transient curve (Figure 18a). An increase in chlorophyll fluorescence at the single turnover event can indicate damage to the D1 protein, reduced water-splitting efficiency, and electron accumulation at quinone A. The only significant ($p < 0.05$) difference between the outside plants and those under laminated shade netting at this stage was at the J inclination point, representing the accumulation of electrons at quinone A. At the P inclination point, however, outside plants showed significantly ($p < 0.05$) lower fluorescence intensity than plants under the netting. Additionally, differences among shading treatments were observed at the P inclination point, where chlorophyll fluorescence under the green netting was significantly ($p < 0.05$) lower than under the grey netting (Table 5).

After 120 days under laminated shade netting (duLN), further differences in the OJIP transient of plant group A were observed (Figure 18b). At the P inclination point, plants outside the netting again exhibited significantly ($p < 0.05$) lower chlorophyll fluorescence compared to those under laminated shade netting (Table 6).

The duration of exposure had a significant ($p < 0.05$) impact on the OJIP transient (Table 23 - 27). Over time, plants under laminated shade netting appeared to stabilize, with chlorophyll fluorescence values converging across the treatments. In contrast, plants grown outside consistently showed lower chlorophyll fluorescence, even after 120 days. This suggests that the high light intensity outside the netting may be causing persistent stress, potentially exceeding the plants' photoprotective capacity.

Table 5: A summary of the statistics of the OJIP transient of plant group A after 60 days under laminated shade netting (duLN). The first number refers to the average, the second to the standard deviation and the letter is denoted by significant ($p < 0.05$) differences between the different laminated shade netting at the different inclination points (Appendix: Table 23 - 27).

120 duLN	O (0.03 ms)	K (0.3 ms)	J (2 ms)	I (30 ms)	P (300 ms)
Outside	4785±188 a	6435±477 a	11540±1045 a	19356±1719 a	25012±1904 a
Grey	4658±315 a	5908±512 a	10461±968 a	18666±1753 a	27275±1398 b
White	4627±258 a	5891±438 a	10428±795 a	18865±1133 a	27142±1308 b
Green	4729±384 a	6050±607 a	10619±1091 a	19346±1883 a	27012±1271 b

Table 6: A summary of the statistics of the OJIP transient of plant group A after 120 days under laminated shade netting (duLN). The first number refers to the average, the second to the standard deviation and the letter is denoted by significant ($p < 0.05$) differences between the different laminated shade netting at the different inclination points (Appendix: Table 23 - 27).

120 duLN	O (0.03 ms)	K (0.3 ms)	J (2 ms)	I (30 ms)	P (300 ms)
Outside	4785±188 a	6435±477 a	11540±1045 a	19356±1719 a	25012±1904 a
Grey	4658±315 a	5908±512 a	10461±968 a	18666±1753 a	27275±1398 b
White	4627±258 a	5891±438 a	10428±795 a	18865±1133 a	27142±1308 b
Green	4729±384 a	6050±607 a	10619±1091 a	19346±1883 a	27012±1271 b

4.2.2.2 Plant group B:

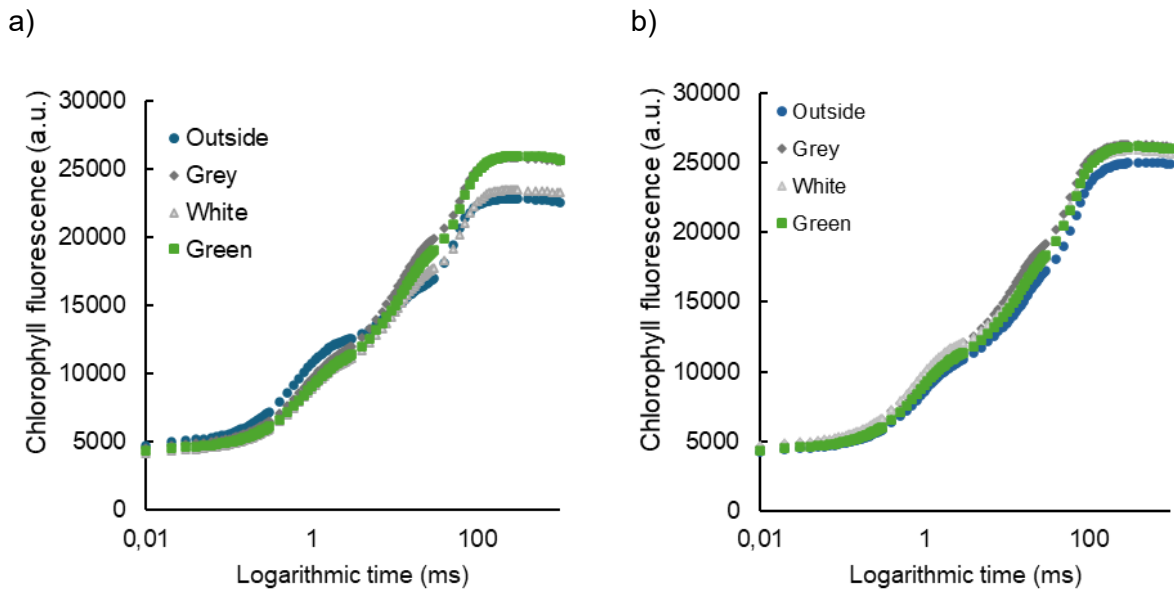


Figure 19: The OJIP transient of *Lessertia frutescens*, plant group B, after a) 60 days and b) 120 days under laminated shade netting (duLN).

Different shading conditions influenced the OJIP transient of Plant group B (Figure 19). However, these effects diminished over time. After 60 days under laminated shade netting, outside plants exhibited higher chlorophyll fluorescence at the single turnover event (Figure 19a), although this difference was not statistically significant ($p > 0.05$) (Table 7). At the multiple turnover event, chlorophyll fluorescence at the I and P inclination points was significantly ($p < 0.05$) lower in outside plants and those under the white laminated shade netting compared to plants under the green and grey laminated shade netting (Table 7). By 120 days, no significant ($p < 0.05$) differences were observed between shading conditions at any of the inclination points (Figure 19b, Table 8). However, outside plants still exhibited slightly lower chlorophyll fluorescence at the P inclination point than those under laminated shade netting.

The I inclination point represents electron transfer *further than* plastoquinone, while the P inclination point reflects the production of end electron acceptors and the maximum photosynthetic activity of PSII (Strasser *et al.*, 2004). The observed differences at these points suggest that outside plants and those under the white netting experienced reduced efficiency in electron transport and overall photosynthetic activity under high shading conditions.

Table 7: A summary of the statistics of the OJIP transient of plant group B after 60 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant ($p < 0.05$) differences between the various laminated shade netting at different inclination points (Appendix: Table 28- 32).

60 duLN	O	K	J	I	P
Outside	5101±801 a	7233±1676 a	12226±2085 a	17031±1329 a	22855±884 a
Grey	4686±367 a	6292±551 a	11127±892 a	19639±1319 b	25397±1544 b
White	4459±378 a	5980±604 a	10564±1155 a	17780±2083 a	23490±2489 a
Green	4658±343 a	6162±512 a	10849±871 a	19221±1541 b	26022±1536 b

Table 8: A summary of the statistics of the OJIP transient of plant group B after 120 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant ($p < 0.05$) differences between the various laminated shade netting at different inclination points (Appendix: Table 28 - 32).

120 duLN	O	K	J	I	P
Outside	4509±298 a	5831±460 a	10268±963 a	17244±1371 a	24978±1431 a
Grey	4846±229 a	6500±469 a	11363±627 a	19201±1341 b	26374±1365 a
White	4944±340 a	6661±590 a	11585±983 a	18323±1270 ab	25952±1430 a
Green	4590±4838 a	6054±4435 a	10837±3143 a	18578±1715 ab	26337±1784 a

The results indicate that shading conditions significantly ($p < 0.05$) influenced the OJIP transient of both Plant group A and Plant group B, particularly at the I and P inclination points, which reflect electron transport efficiency and the production of end electron acceptors, respectively. Outside plants generally exhibited reduced photosynthetic activity, likely due to high light intensity, while green and grey netting provided more favourable conditions for maintaining PSII functionality.

Over time, the effects of laminated shading diminished, suggesting an acclimation process. These findings highlight the importance of optimized shading conditions for efficient photosynthetic performance under varying light environments.

4.2.3 The effect of different shading conditions on the maximum quantum yield of PSII (F_v/F_M):

The maximum quantum yield of Photosystem II (F_v/F_M) is a vital indicator of plant health. It provides a measure of the photochemical efficiency of photosynthesis and the sensitivity of PSII to environmental stimuli (Murchie & Lawson, 2013). Healthy unstressed plants usually has an F_v/F_M value of approximately 0.83 (Bjorkman & Demmig, 1987), while plants under stress has lower values. This parameter offers valuable insights into the physiological responses of *Lessertia frutescens* to environmental factors. The following section investigates how different shading conditions and exposure durations affect the maximum quantum yield of PSII (F_v/F_M).

4.2.3.1 Plant group A:

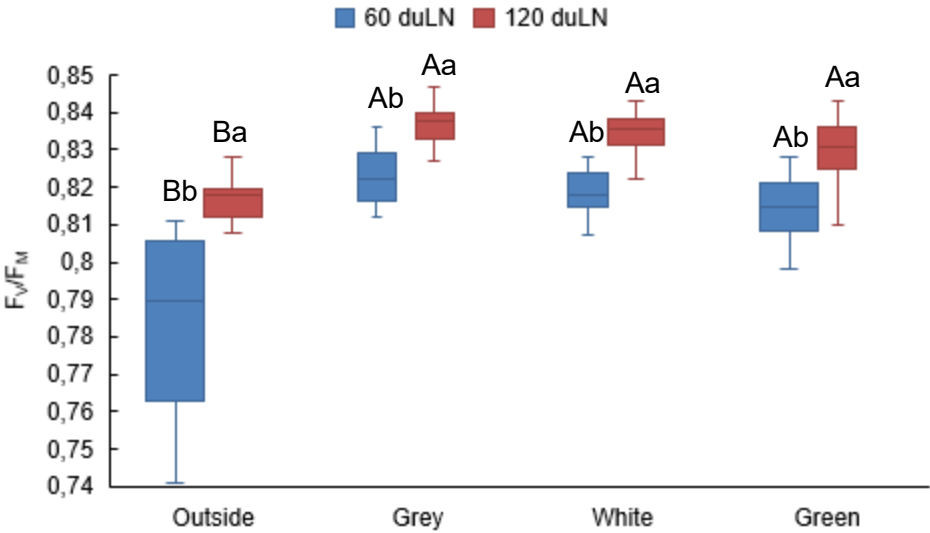


Figure 20: Under different shading conditions, the maximum quantum efficiency of PSII (F_v/F_M) of *Lessertia frutescens*, plant group A. Different letters are denoted by significant differences ($p < 0.05$). The capital letters refer to differences between the different shading conditions and the lowercase letters refer to the differences that occurred over time, after 60 and 120 days under laminated shade netting (duLN).

After 60 days under laminated shade netting (duLN), the outside plants of plant group A had the lowest mean maximum quantum yield of PSII (F_V/F_M) value of 0.79, which increased to 0.82 after 120 days. At both time points, the F_V/F_M values of the outside plants were significantly ($p < 0.05$) lower than those of plants grown under laminated shade netting. Additionally, a significant ($p < 0.05$) increase in F_V/F_M values was observed from 60 to 120 days across all shading treatments, including the grey, white, and green laminated shade netting, as well as the outside plants (Figure 20, Appendix: Table 33).

4.2.3.2 Plant group B:

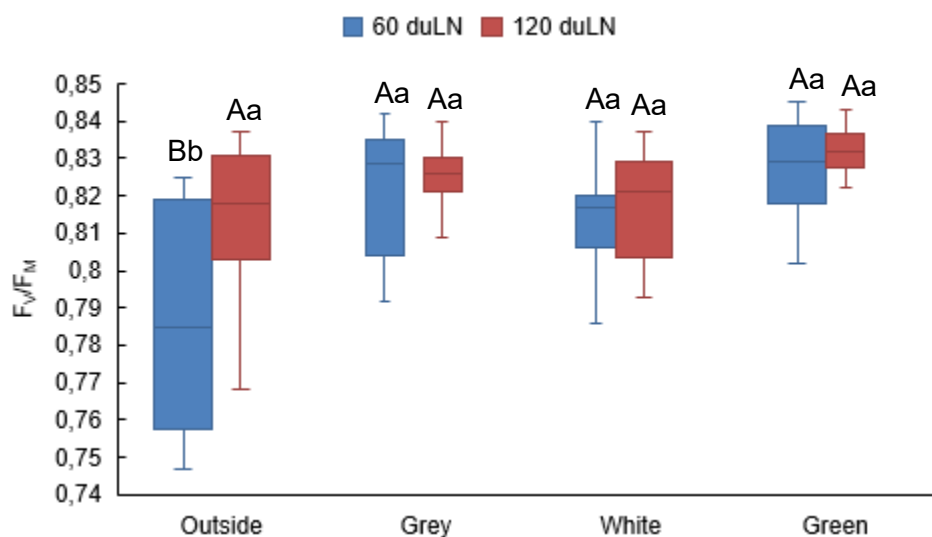


Figure 21: Under different shading conditions, the maximum quantum yield of PSII (F_V/F_M) of *Lessertia frutescens*, plant group B. Different letters are denoted by significant differences ($p < 0.05$). The capital letters refer to differences between the different shading conditions and the lowercase letters refer to the significant ($p < 0.05$) differences that occurred over time, after 60 and 120 days under laminated shade netting (duLN).

The maximum quantum yield (F_V/F_M) of the plants under the netting was significantly ($p < 0.05$) higher than the outside plants after 60 days under laminated shade netting (duLN) (Figure 21, Appendix: Table 25). However, no significant ($p < 0.05$) differences or trends were observed among the different shading conditions after 120 days. The maximum quantum yield (F_V/F_M) of the outside plants increased significantly ($p < 0.05$) from 60 to 120 days under laminated shade netting (duLN) (Appendix: Table 25).

The maximum quantum yield (F_V/F_M) was significantly ($p < 0.05$) influenced by shading treatments, with plants under laminated shade netting consistently exhibiting higher F_V/F_M values than those grown outside. This suggests that shaded conditions reduced stress levels. However, the consistently high F_V/F_M values (> 0.74) indicated that the stress experienced by the plants was not severe enough to cause permanent damage to PSII. Over time, outside plants' F_V/F_M values increased, reflecting an adaptation to high light intensity. These findings highlight the role of shading in mitigating stress and improving the photosynthetic efficiency of *Lessertia frutescens*, particularly during extended exposure to varying shading conditions.

4.2.4 The effect of different shading conditions on the total performance index (PI_{TOTAL})

The total performance index (PI_{TOTAL}) is a commonly used parameter for detecting plant stress due to its high sensitivity. It is derived from a multiparametric equation consisting of four independent parameters: the efficiency of light energy capture (RC/ABS), the quantum efficiency of energy trapping ($\phi_{P_0}/(1 - \phi_{P_0})$), the conversion of trapped excitation energy into electron transport [$\Psi_0/(1 - \Psi_0)$], and the probability of reducing an end electron acceptor ($\delta_0/(1 - \delta_0)$) (Hopkins & Hüner, 2008; Kalaji *et al.*, 2017; Strasser *et al.*, 2010).

4.2.4.1 Plant group A:

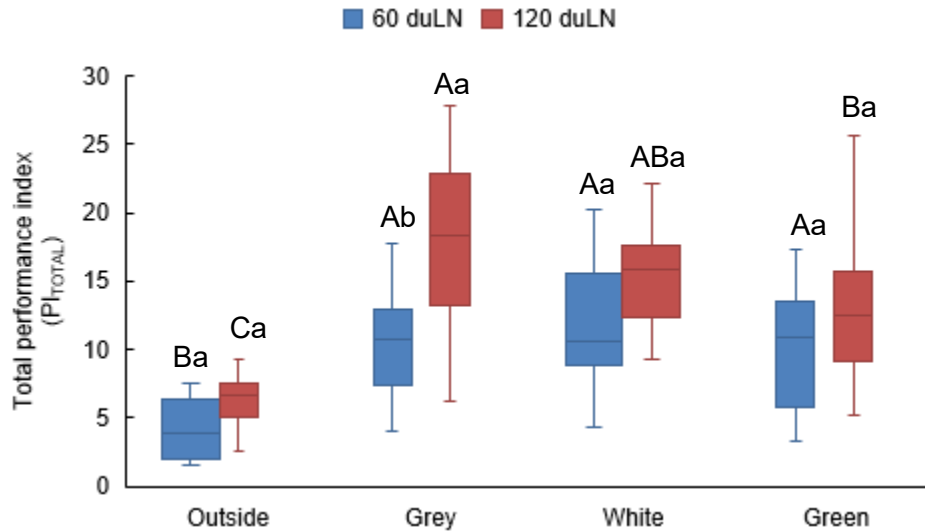


Figure 22: Under different shading conditions, the total performance index (PI_{TOTAL}) of *Lessertia frutescens*, plant group A. Different letters are denoted by significant differences ($p < 0.05$). The capital letters refer to differences between the different shading conditions and the lowercase letters refer to the differences that occurred over time, after 60 and 120 days under laminated shade netting (duLN).

The total performance index (PI_{TOTAL}) of the outside plants was significantly ($p < 0.05$) lower than that of plants under laminated shade netting after both 60 and 120 days. After 120 days, the total performance index (PI_{TOTAL}) of plants under the grey and white netting was significantly ($p < 0.05$) higher than that of plants under the green netting. A general trend of increasing PI_{TOTAL} from 60 to 120 days was observed (Figure 22); however, the only statistically significant ($p < 0.05$) increase occurred under the green netting (Appendix: Table 34).

After 60 days under laminated shade netting (duLN), the efficiency of light capturing (RC/ABS), the conversion of trapped excitation energy into electron transport ($\Psi_0/(1 - \Psi_0)$) and the probability of reducing an end electron acceptor ($\delta_0/(1 - \delta_0)$) were not significantly ($p < 0.05$) influenced by the different shading conditions (Table 9). Although the quantum efficiency of light trapping ($\phi_{P_0}/(1 - \phi_{P_0})$) and the performance index based on the absorption (PI_{ABS}) of the outside, unshaded plants had a significantly ($p < 0.05$) lower value than laminated shade netting (grey, white and green).

After 120 days under laminated shade netting (duLN), the efficiency of light capturing (RC/ABS), the quantum efficiency of light trapping ($\phi_{P_0}/(1 - \phi_{P_0})$) and the conversion of trapped excitation energy into electron transport ($\Psi_0/(1 - \Psi_0)$) of the outside, unshaded, plants had a significantly ($p < 0.05$) lower value than the plants under laminated shade netting (grey, white and green). The outside, unshaded plants had the lowest performance index based on absorption (PI_{ABS}) and the plants under the white netting had a significantly ($p < 0.05$) higher value than those under the green netting. The probability to reduce an end electron acceptor ($\delta_0/(1 - \delta_0)$) was the lowest (6.597) outside and highest (17.870) under the grey netting. Additionally, values for unshaded, white, and green netting treatments were all significantly lower ($p < 0.05$) than those observed under grey netting (Table 10).

Table 9: A summary of the statistics of the parameters of PI_{TOTAL} of plant group A after 60 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant differences ($p < 0.05$) among the laminated shade netting for different parameters (Appendix: Table 35 - 39).

60 duLN	RC/ABS	$\phi_{P_0}/(1 - \phi_{P_0})$	$\Psi_0/(1 - \Psi_0)$	PI_{ABS}	$(\delta_0/(1 - \delta_0))$
Outside	0,763±0,133 a	3,675±0.568 b	1,577±0.356 a	4,810±2.441 b	0,843±0.140 a
Grey	0,933±0.067 a	4,656±0.235 a	2,727±0.323 a	12,013±2.634 a	0,859±0.130 a
White	0,958±0.084 a	4,521±0.166 a	2,542±0.286 a	11,127±2.359 a	1,013±0.249 a
Green	0,941±0.104 a	4,400±0.247 a	2,426±2.694 a	10,252±2.694 a	0,931±0.231 a

Table 10: A summary of the statistics of the parameters of PI_{TOTAL} of plant group A after 120 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant differences ($p < 0.05$) among the laminated shade netting for different parameters (Appendix: Table 35 - 39).

120 duLN	RC/ABS	$\phi_{Po}/(1 - \phi_{Po})$	$\Psi_o/(1 - \Psi_o)$	PI_{ABS}	$\delta_o/(1 - \delta_o)$
Outside	4,499±0,209 b	2,047±0,326 b	8,992±2.293 b	0,735±0.100 ab	6,597±2.163 a
Grey	5,079±0,341 a	2,909±0,413 a	16,349±3.838 a	0,980±0.234 ab	17,870±5.747 b
White	5,045±0,211 a	2,813±0.310 a	15,247±2.863 a	1,013±0.147 a	15,180±3.444 c
Green	4,886±0,277 a	2,758±0.412 a	14,013±3.418 a	0,914±0.198 b	13,322±5.871 c

4.2.4.2 Plant group B

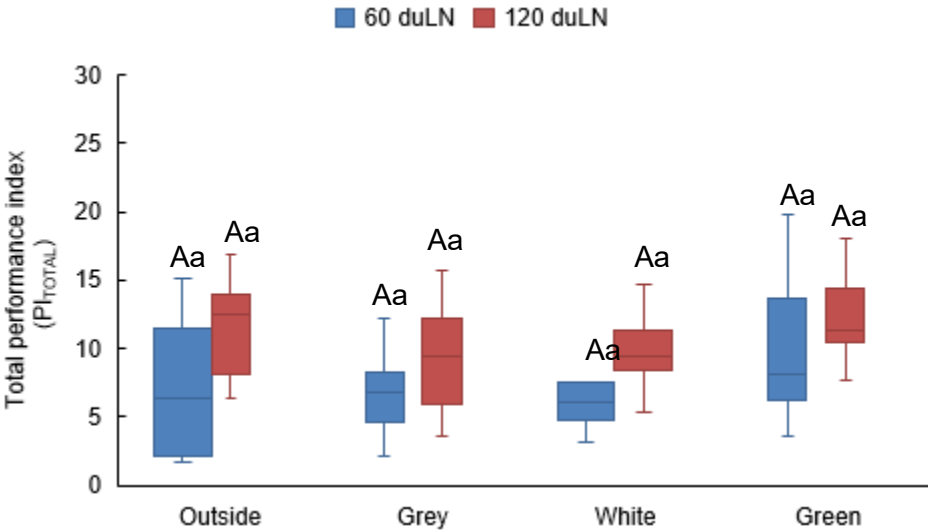


Figure 23: The total performance index (PI_{TOTAL}) of *Lessertia frutescens*, plant group B, under different shading conditions. Different letters are denoted to significant differences ($p < 0.05$). The capital letters refer to differences between the different shading conditions and the lowercase letters refer to the differences that occurred over time, after 60 and 120 days under laminated shade netting (duLN).

The total performance index (PI_{TOTAL}) remains relatively constant across all shading conditions and time points. No statistically significant ($p < 0.05$) differences were observed between the

different shading conditions, outside, grey, white, and green, or the two time periods (60 and 120 days) (Appendix: Table 34). The variability within each treatment group is minimal, indicating consistency in PI_{TOTAL} regardless of the shading condition or duration of exposure.

After 60 days under laminated shade netting (duLN), the efficiency of light capturing (RC/ABS), the conversion of trapped excitation energy into electron transport ($\Psi_o/(1 - \Psi_o)$) and the probability of reducing an end electron acceptor ($\delta_o/(1 - \delta_o)$) were not significantly ($p < 0.05$) influenced by the different shading conditions (Table 11). The quantum efficiency of light trapping ($\phi_{Po}/(1 - \phi_{Po})$) of the outside, unshaded plants had a significantly ($p < 0.05$) lower value than the plants under the netting. For the performance index based on absorption (PI_{ABS}) the green netting had a significantly ($p < 0.05$) higher value than outside, unshaded plants (Appendix: Table 38).

After 120 days under laminated shade netting (duLN), the efficiency of light capturing (RC/ABS), the conversion of trapped excitation energy into electron transport ($\Psi_o/(1 - \Psi_o)$) and the probability to reduce an end electron acceptor ($\delta_o/(1 - \delta_o)$) followed the same trend. The outside, unshaded plants had a significantly ($p < 0.05$) lower value than those under laminated shade netting (Table 12) and plants under the green netting exhibited the highest value that was also significantly ($p < 0.05$) higher than the outside, grey and white netting (Appendix: Table 35). The quantum efficiency of light trapping ($\phi_{Po}/(1 - \phi_{Po})$) was significantly higher in the outside plants than those under laminated shade netting. The performance index based on the absorption was not significantly ($p < 0.05$) influenced by the different shading conditions (Appendix: Table 38).

Table 11: A summary of the statistics of the parameters of PI_{TOTAL} of plant group B after 60 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant differences ($p < 0.05$) among the laminated shade netting for different parameters (Appendix: Table 35 - 39).

60 duLN	RC/ABS	$\varphi_{Po}/(1 - \varphi_{Po})$	$\Psi_o/(1 - \Psi_o)$	PI_{ABS}	$\delta_o/(1 - \delta_o)$
Outside	0,804±0.225 a	3,806±0.823 a	1,542±0.639 a	5,752±4.396 a	1,227±0.245 a
Grey	0,919±0.067 a	4,669±0.505 bc	2,186±0.401 a	9,646±3.011 ab	0,681±0.103 a
White	0,917±0.078 a	4,483±0.457 b	2,084±0.544 a	8,931±4.208 ab	0,771±0.120 a
Green	0,949±0.076 a	4,820±0.401 c	2,416±0.385 a	11,261±3.088 b	0,827±0.223 a

Table 12: A summary of the statistics of the parameters of PI_{TOTAL} of plant group B after 120 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant differences ($p < 0.05$) among the laminated shade netting for different parameters (Appendix: Table 35 - 39).

120 duLN	RC/ABS	$\varphi_{Po}/(1 - \varphi_{Po})$	$\Psi_o/(1 - \Psi_o)$	PI_{ABS}	$\delta_o/(1 - \delta_o)$
Outside	0,942±0.103 a	4,403±0.632 a	2,320±0.431 a	10,087±3.881 a	1,206±0.200 a
Grey	4,674±0.371 bc	2,256±0.305 b	9,848±2.683 bc	9.848±2.683 a	9,482±3.890 b
White	4,482±0.432 b	2,134±0.377 b	8,688±2.755 b	8.688±2.755 a	9,798±2.374 bc
Green	4,956±0.231 c	2,441±0.268 b	12,035±2.154 c	12.035±2.154 a	12,401±3.412 c

The total performance index (PI_{TOTAL}) indicated a stress response in *Lessertia frutescens*. For Plant group A, shading significantly alleviated stress, as evidenced by the consistently higher PI_{TOTAL} values in plants under netting than those grown outside. In contrast, Plant group B displayed stable PI_{TOTAL} values across all shading conditions and time points, suggesting minimal stress and reduced sensitivity to variations in light intensity. These results reinforce the role of shading in enhancing the photosynthetic performance of *Lessertia frutescens* under high shading conditions.

4.2.5 Phenomenological energy fluxes per excited cross-section (CS) of a sample

The parameters ABS/CS_m , DI_0/CS_m , TR_0/CS_m , ET_0/CS_m , and RE_0/CS_m provide valuable insights into the photosynthetic efficiency and energy allocation within photosystem II (PSII). These parameters are normalized to the cross-section (CS_m) at maximum fluorescence (F_M) (Goltsev *et al.*, 2016)

The absorption energy flux per cross-section (ABS/CS_m) reflects the light energy absorbed per unit cross-section in PSII. It represents the photosynthesizing object at maximum fluorescence (Goltsev *et al.*, 2016). The heat dissipation per cross-section (DI_0/CS_m), reflects the energy lost as thermal energy through non-photosynthetic quenching (Goltsev *et al.*, 2016). The excitation energy flux trapped by PSII (TR_0/CS_m) describes the fraction of absorbed energy that is successfully trapped by PSII reaction centres for photosynthetic activity. In contrast, the electron flux transported by PSII (ET_0/CS_m) represents the subsequent transfer of this energy through the electron transport chain. Finally, the re-oxidation of electron acceptors per cross-section (RE_0/CS_m) measures the efficiency of electron transport reaching the end acceptors in the photosynthetic chain, providing a broader indicator of overall photosynthetic performance (Goltsev *et al.*, 2016).

By analysing these parameters, the impact of shading and unshaded conditions on the photosynthetic efficiency of *Lessertia frutescens* can be evaluated. Shading conditions influence light absorption, energy trapping, and electron transport dynamics, revealing plants' stress response and acclimation capacity. The following data explores how shading treatments affect these parameters over 60 and 120 days under laminated shade netting (duLN).

4.2.5.1 Plant group A:

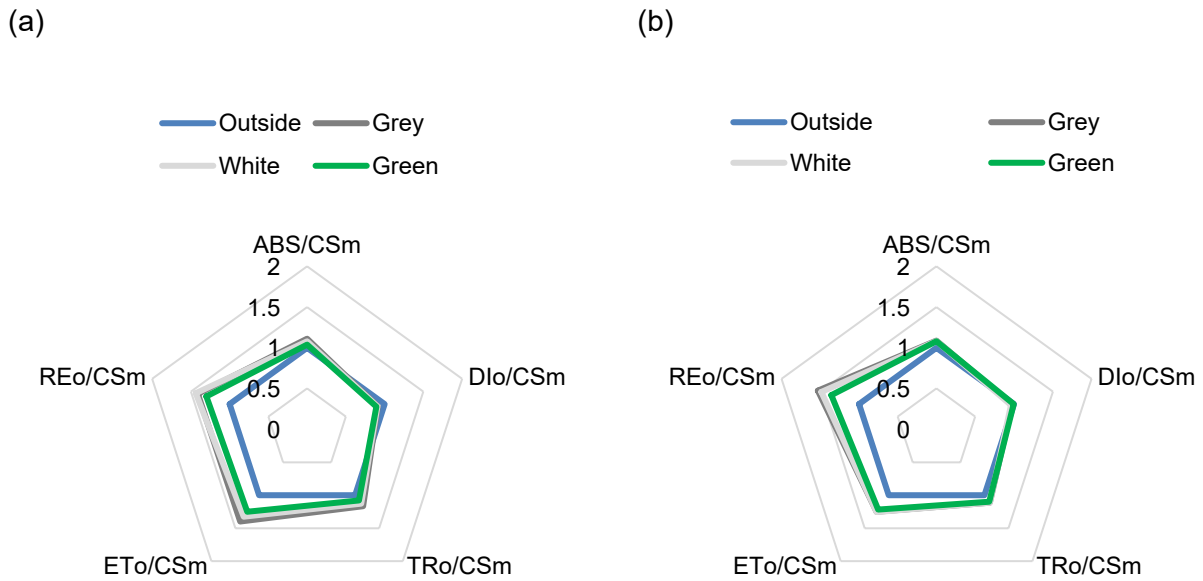


Figure 24: The phenomenological energy fluxes per excited cross-section (ABS/CS_m , DI_o/CS_m , TR_o/CS_m , ET_o/CS_m and RE_o/CS_m) of *Lessertia frutescens*, plant group A, under different shading conditions over a period of (a) 60 days and (b) 120 days under laminated shade netting (duLN).

The general trend observed in Plant group A after 60 and 120 days under laminated shade netting (duLN) is that outside, unshaded plants exhibited consistently lower physiological performance values than those under netting, except for heat dissipation (DI_o/CS_m).

After 60 days, outside, unshaded plants showed significantly ($p < 0.05$) lower absorption energy flux per cross-section (ABS/CS_m) than those under grey and white laminated shade netting, demonstrating reduced light capture (Table 13). In contrast, dissipation heat (DI_o/CS_m) was significantly ($p < 0.05$) higher in outside, unshaded plants, reflecting greater photoprotective energy loss as a result of high light intensity exposure (Table 13). Among the netting treatments, plants under green laminated shade netting had significantly ($p < 0.05$) lower dissipation heat (DI_o/CS_m) compared to those under grey laminated netting. The quantum efficiency of energy trapping (TR_o/CS_m) in outside, unshaded plants was significantly ($p < 0.05$) lower than in plants under grey and white netting (Table 13). Similarly, the electron transport (ET_o/CS_m) and re-oxidation of end electron acceptors (RE_o/CS_m) were significantly ($p < 0.05$) lower in outside, unshaded plants relative to plants under laminated shade netting (Table 13). The reduced

RE₀/CS_m values observed in outside, unshaded plants further emphasize their lower electron transport efficiency to end acceptors (Figure 24).

After 120 days, the benefits of shading became more apparent. Outside, unshaded, plants continued to exhibit significantly ($p < 0.05$) lower absorption (ABS/CS_m) values compared to the plants under the laminated shade netting, reaffirming the lower capacity to capture light effectively. Unlike the results at 60 days, there were no significant ($p < 0.05$) differences among the dissipation heat (DI₀/CS_m), suggesting possible acclimation of PSII to prolonged high-light exposure (Table 10). However, the outside, unshaded plants still had significantly ($p < 0.05$) lower quantum efficiencies for energy trapping (TR₀/CS_m) and electron transport (ET₀/CS_m), indicating persistent photosynthetic inefficiency under unshaded conditions (Table 14).

Interestingly, plants under grey laminated shade netting consistently displayed the highest RE₀/CS_m values at both time points, reflecting enhanced electron transport to end acceptors. Over time, RE₀/CS_m values under netting increased, indicating improved long-term photosynthetic efficiency and reduced stress under shaded conditions.

These findings emphasize the cumulative advantages of shading, particularly under grey and white netting, in improving photosynthetic performance and energy transfer efficiency over time, compared to green netting or outside, unshaded conditions.

Table 13: A summary of the statistics of the phenomenological energy fluxes per excited cross-section of *Lessertia frutescens*, plant group A, after 60 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant differences ($p < 0.05$) among the laminated shade netting for different parameters (Appendix: Table 40- 44).

60 duLN	ABS/CS _m	DI ₀ /CS _m	TR ₀ /CS _m	ET ₀ /CS _m	RE ₀ /CS _m
Outside	25359±1288 a	5474±491 a	19885±1601 a	12104±2010 a	5521±1185 a
Grey	28194±947 b	4991±225 b	23203±860 b	16933±902 b	7785±830 b
White	27382±1322 bc	4966±308 bc	22416±1060 bc	16043±797 b	7932±902 b
Green	26352±1286 ac	4890±347c	21461±1045 ac	15140±838 b	7179±1044 b

Table 14: A summary of the statistics of the phenomenological energy fluxes per excited cross-section of *Lessertia frutescens*, plant group A, after 120 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant differences ($p < 0.05$) among the laminated shade netting for different parameters (Appendix: Table 40- 44).

120 duLN	ABS/CS _m	Dl ₀ /CS _m	TR ₀ /CS _m	ET ₀ /CS _m	RE ₀ /CS _m
Outside	25020±1898 a	4603±169 a	20417±1753 a	13480±1367 a	5664±727 a
Grey	27239±1383 b	4491±285 a	22748±1280 b	16868±1285 b	8673±840 b
White	27108±1269 b	4423±241 a	22686±1099 b	16917±937 b	8418±650 b
Green	27033±1260 b	4583±360 a	22450±993 b	16414±828 b	7687±1093 b

4.2.5.2 Plant group B:

(a)

(b)

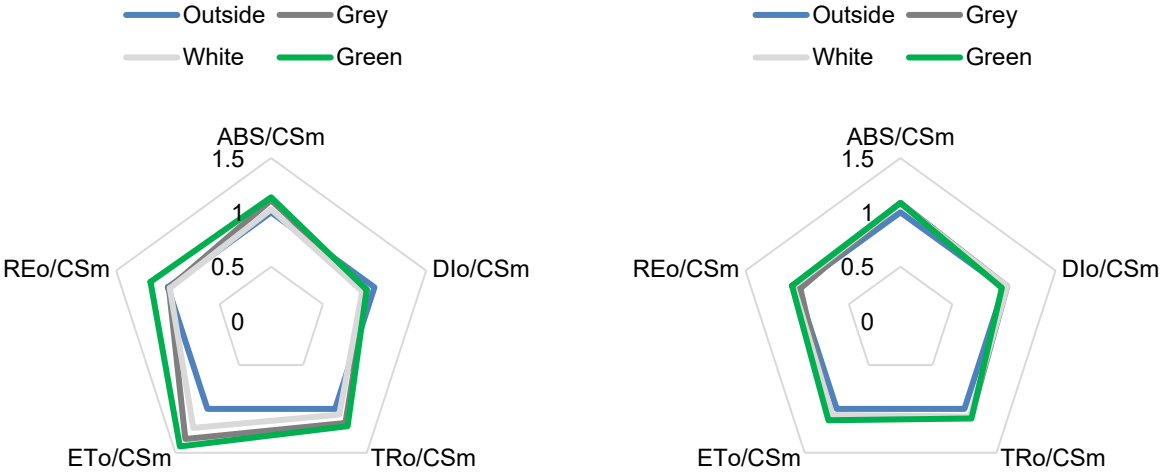


Figure 25: The phenomenological energy fluxes per excited cross-section (ABS/CS_m, Dl₀/CS_m, TR₀/CS_m, ET₀/CS_m and RE₀/CS_m) of *Lessertia frutescens*, plant group B, under different shading conditions over a period of (a) 60 days and (b) 120 days under laminated shade netting (duLN).

After 60 days under laminated shade netting (duLN), the absorption (ABS/CS_m) in outside, unshaded plants was significantly ($p < 0.05$) lower than in plants under the grey and green laminated shade netting, indicating reduced light capture outside (Table 15). Heat dissipation (DI_0/CS_m) under the white laminated shade netting was significantly ($p < 0.05$) lower compared to the outside, unshaded plants and those under green and grey laminated shade netting (Table 15). The quantum efficiency of energy trapping (TR_0/CS_m) in outside, unshaded plants was significantly ($p < 0.05$) lower than plants under grey and green laminated shade netting. Electron transport (ET_0/CS_m) values were similar across all treatments, showing no statistically significant ($p < 0.05$) differences (Table 15). For the electron transport to end acceptors (RE_0/CS_m), outside, unshaded plants showed no significant ($p < 0.05$) differences compared to other treatments. However, plants under green laminated shade netting had numerically higher values (Figure 25a, Table 15).

After 120 days under the laminated shade netting (duLN), the absorption (ABS/CS_m) values in outside, unshaded plants remained slightly lower than those under the laminated shade netting. Still, no significant ($p < 0.05$) differences were observed. Heat dissipation (DI_0/CS_m) showed no significant ($p < 0.05$) differences between treatments (Table 16). The quantum efficiency of energy trapping (TR_0/CS_m) and electron transport (ET_0/CS_m) did not differ significantly ($p < 0.05$) between treatments, indicating a level of acclimation over time. However, electron transport to end acceptors (RE_0/CS_m) was numerically higher in plants under green and white laminated shade netting, though these differences were not statistically significant ($p < 0.05$) (Figure 25b, Table 16).

These observations suggest that shading, particularly with grey and green laminated shade netting, offers minor improvements in photosynthetic efficiency and light energy utilization over time, while outside, unshaded plants exhibit signs of acclimation to unshaded conditions.

Table 15: A summary of the phenomenological energy fluxes per excited cross-section (ABS/CS_m, DI₀/CS_m, TR₀/CS_m, ET₀/CS_m and RE₀/CS_m) of *Lessertia frutescens*, plant group B, after 60 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant differences (p < 0.05) among the laminated shade netting for different parameters (Appendix: Table 45 - 49).

60 duLN	ABS/CS _m	DI ₀ /CS _m	TR ₀ /CS _m	ET ₀ /CS _m	RE ₀ /CS _m
Outside	22864±884 a	4856±697 a	18009±1442 a	10638±2671 a	5833±1591 a
Grey	25410±1540 b	4504±345 a	20906±1546 b	14283±1703 a	5771±989 a
White	23501±2481 a	4286±357 b	19215±22264 a	12937±2249 a	5721±1110 a
Green	26033±1534 b	4487±325 a	21546±1410 b	15184±1454 a	6812±1453 a

Table 16: A summary of the statistics of the phenomenological energy fluxes per excited cross-section (ABS/CS_m, DI₀/CS_m, TR₀/CS_m, ET₀/CS_m and RE₀/CS_m) of *Lessertia frutescens*, plant group B, after 120 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant differences (p < 0.05) among the laminated shade netting for different parameters (Appendix: Table 45 - 49).

120 duLN	ABS/CS _m	DI ₀ /CS _m	TR ₀ /CS _m	ET ₀ /CS _m	RE ₀ /CS _m
Outside	24255±2051 a	4513±318 a	19743±2118 a	13744±1998 a	7410±706 a
Grey	26387±1360 a	4659±203 a	21728±1382 a	15025±1491 a	7186±1061 a
White	26128±1499 a	4656±302 a	21472±1490 a	14686±1574 a	7674±549 a
Green	26361±1292 a	4427±168 a	21934±1193 a	15524±998 a	7784±638 a

4.2.6 The specific energy fluxes per Q_A -Reduced PSII reaction centres (RC)

The photosynthetic performance of plants can be assessed through the energy fluxes on a per reaction centre basis (ABS/RC , DI_0/RC , TR_0/RC , ET_0/RC , and RE_0/RC). These parameters provide a more focused perspective on the functionality of the individual reaction centres within Photosystem II (PSII), enabling a detailed understanding of how plants respond to varying light environments at the photosynthetic level.

The absorption flux per reaction centre (ABS/RC) represents the light energy absorbed by each active PSII reaction centre. This parameter reflects the light-harvesting efficiency and provides insights into the extent of energy capture under different conditions (Strasser *et al.*, 2010).

The energy dissipated as heat per reaction centre (DI_0/RC) represents the fraction of energy lost through non-photosynthetic quenching (Kalaji *et al.*, 2017; Strasser *et al.*, 2010). This parameter is crucial for understanding photoprotective mechanisms, particularly under high-shading conditions, where the risk of photodamage increases

The trapped energy flux per reaction centre (TR_0/RC) refers to the energy effectively trapped by PSII reaction centres for photosynthetic use. This parameter is a key indicator of PSII efficiency in capturing and processing light energy (Goltsev *et al.*, 2016; Kalaji *et al.*, 2017).

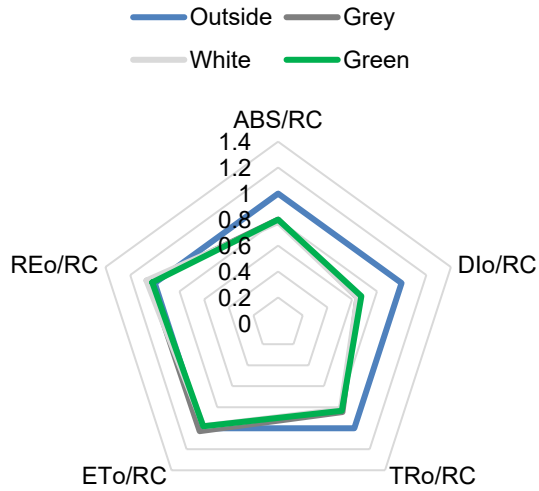
The electron transport flux per reaction centre (ET_0/RC) measures the energy transferred through the electron transport chain, reflecting the ability of PSII to maintain downstream electron flow. This is an important parameter for assessing the overall efficiency of the photosynthetic electron transport chain under different treatments (Goltsev *et al.*, 2016; Kalaji *et al.*, 2017).

Finally, the re-oxidation of electron acceptors per reaction centre (RE_0/RC) indicates the electron flow from PSII to end acceptors in the photosynthetic pathway. This parameter provides insight into the capacity for energy transfer to subsequent photosynthetic processes (Goltsev *et al.*, 2016; Kalaji *et al.*, 2017).

By analysing these parameters, it is possible to gain a deeper understanding of how shading treatments influence the functionality and efficiency of PSII at the reaction-centre level, highlighting the plant's ability to adapt to varied shading conditions.

4.2.6.1 Plant group A:

(a)



(b)

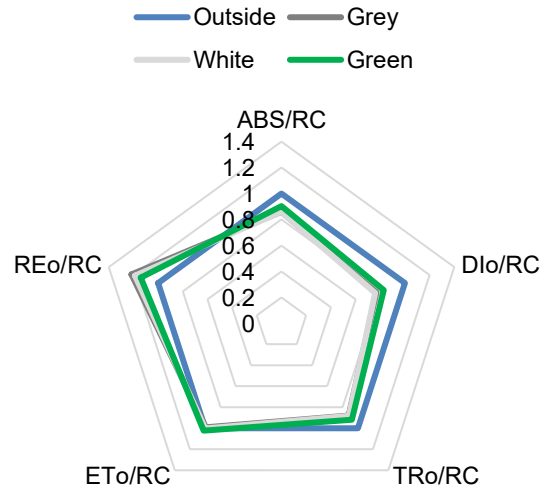


Figure 26: The specific energy fluxes per Q_A -Reduced PSII reaction centres (ABS/RC, DI_o /RC, TR_o /RC, ET_o /RC and RE_o /RC) of *Lessertia frutescens*, plant group A, under different shading conditions over a period of (a) 60 days and (b) 120 days under laminated shade netting (duLN).

After 60 days under laminated shade netting (duLN), the absorption flux per active reaction centre (ABS/RC) was significantly ($p < 0.05$) higher in outside plants compared to those under netting, suggesting that unshaded plants absorbed more light per reaction centre (Figure 26a, Table 17). The dissipation of excess energy as heat (DI_o /RC) was also significantly ($p < 0.05$) higher in outside, unshaded plants, reflecting increased energy loss due to higher light intensity. In contrast, the trapped energy flux per reaction centre (TR_o /RC) was significantly ($p < 0.05$) greater in plants under netting, highlighting improved photosynthetic activity under shaded conditions (grey, white and green laminated shade netting) (Table 17).

The electron transport flux per reaction centre (ET_o /RC), which measures energy transfer through the electron transport chain, followed a trend similar to TR_o /RC. The plants under the netting generally performed better, though the differences were not always significant ($p < 0.05$). Meanwhile, the re-oxidation of electron acceptors per reaction centre (RE_o /RC) showed no significant ($p < 0.05$) differences among treatments, indicating comparable electron flow to the end acceptors across all conditions.

After 120 days under laminated shade netting (duLN), the ABS/RC of the outside, unshaded plants remained significantly ($p < 0.05$) higher than the plants under shade laminated netting (Figure 26b, Table 18). The DI_0/RC for the outside, unshaded plants decreased but were still higher than those for plants under grey and white laminated shade netting, suggesting some acclimation to high light intensity over time (Table 18). The TR_0/RC of the outside, unshaded plants was significantly ($p < 0.05$) higher than those under the grey laminated shade netting. The ET_0/RC under the green netting had the highest value although the increase was not significant ($p < 0.05$) (Table 14). Unlike at 60 days, the RE_0/RC under the grey and white netting were significantly ($p < 0.05$) higher than the outside, unshaded plants, emphasizing improved electron transport efficiency under shaded conditions over time (Table 18).

These results highlight the beneficial effects of netting on PSII performance at the reaction centre level, with grey and white laminated shade netting consistently supporting better energy use and reduced dissipation compared to the outside. The differences observed between 60 and 120 days also suggest potential acclimation of the outside, unshaded plants, although shading still provided a more favourable environment for photosynthetic efficiency.

Table 17: A summary of the statistics of the specific energy fluxes per Q_A -Reduced PSII reaction centres (ABS/RC, DI_0/RC , TR_0/RC , ET_0/RC and RE_0/RC) of *Lessertia frutescens*, plant group A, after 60 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant differences ($p < 0.05$) among the laminated shade netting for different parameters (Appendix: Table 50 - 54).

60 duLN	ABS/RC	DI_0/RC	TR_0/RC	ET_0/RC	RE_0/RC
Outside	1.347±0.234 a	0.297±0.086 a	1.050±0.150 a	0.628±0.044 a	0.284±0.013 a
Grey	1.077±0.081 b	0.191±0.02 b	0.886±0.062 b	0.645±0.03 ab	0.296±0.019 a
White	1.052±0.097 b	0.191±0.021 b	0.861±0.077 b	0.616±0.045 a	0.303±0.026 a
Green	1.076±0.126 b	0.201±0.032 b	0.876±0.095 b	0.616±0.049 ac	0.290±0.024 a

Table 18: A summary of the specific energy fluxes per Q_A-Reduced PSII reaction centres (ABS/RC, DI₀/RC, TR₀/RC, ET₀/RC and RE₀/RC) of *Lessertia frutescens*, plant group A, after 120 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant differences (p < 0.05) among the laminated shade netting for different parameters (Appendix: Table 50 - 54).

120 duLN	ABS/RC	DI ₀ /RC	TR ₀ /RC	ET ₀ /RC	RE ₀ /RC
Outside	1.07±0.12 a	0.20±0.03 a	0.88±0.09 a	0.58±0.04 a	0.24±0.02 a
Grey	0.92±0.07 b	0.15±0.02 b	0.77±0.005 b	0.57±0.02 a	0.29±0.03 b
White	0.92±0.06 a	0.15±0.01 c	0.77±0.05 ab	0.58±0.03 a	0.29±0.02 b
Green	0.97±0.07 a	0.16±0.02 abc	0.81±0.05 ab	0.59±0.02 a	0.28±0.04 ab

4.2.6.2 Plant group B:

(a)

(b)

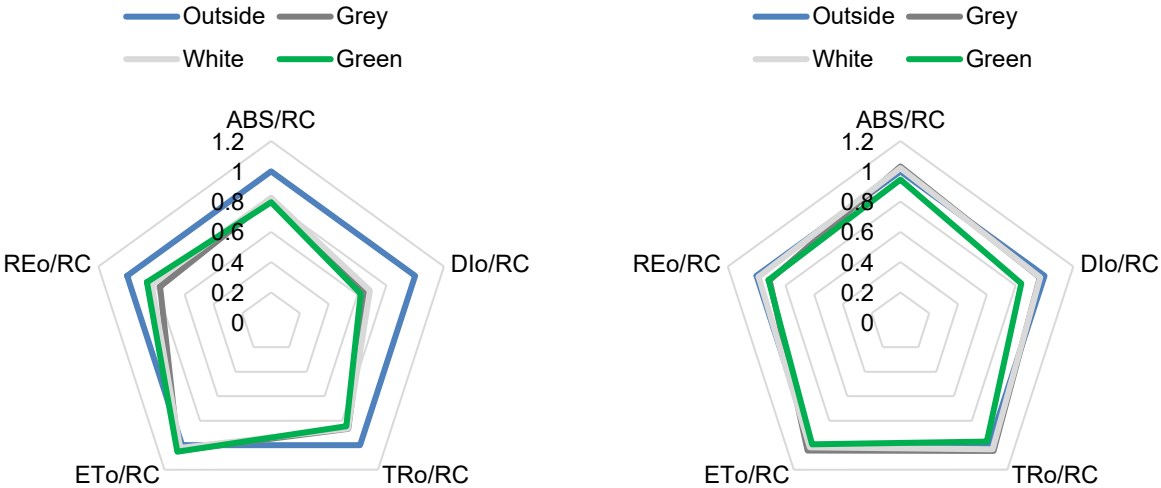


Figure 27: The specific energy fluxes per Q_A-Reduced PSII reaction centres (ABS/RC, DI₀/RC, TR₀/RC, ET₀/RC and RE₀/RC) of *Lessertia frutescens*, plant group B, under different shading conditions over a period of (a) 60 days and (b) 120 days under laminated shade netting (duLN).

After 60 days, the absorption flux per active reaction centre (ABS/RC) was significantly ($p < 0.05$) higher in outside, unshaded plants compared to those under laminated shade netting (Table 19). This suggests that outside plants were absorbing more light per reaction centre due to the high light intensity exposure.

The dissipation energy flux per reaction centre (DI_0/RC) of the outside, unshaded plants was significantly ($p < 0.05$) higher compared to the plants under the laminated shade netting. The increased heat dissipation highlights the higher photoprotective energy loss of the outside, unshaded plants due to excess light stress. Among the shading conditions, grey and white laminated shade netting resulted in comparable dissipation levels, while green laminated shade netting showed the lowest DI_0/RC values (Figure 27a, Table 19).

The trapped energy flux per reaction centre (TR_0/RC), reflecting the energy captured for photosynthetic reactions, was significantly ($p < 0.05$) higher in plants under the grey, white, and green laminated shade netting compared to outside, unshaded plants (Table 19). This indicates enhanced photosynthetic efficiency under shaded conditions. Similarly, electron transport flux per reaction centre (ET_0/RC), representing energy transfer through the electron transport chain, showed a trend of higher performance in shaded plants. However, the differences were not always statistically significant ($p < 0.05$) (Table 19).

The re-oxidation of electron acceptors per reaction centre (RE_0/RC), indicating electron flow to the end acceptors, revealed significant ($p < 0.05$) differences between the different shading conditions. Outside, unshaded plants had a significantly ($p < 0.05$) higher RE_0/RC compared to the plants under the netting (Table 19). These results suggest that shading influenced the efficiency of electron transfer processes, with grey and white laminated shade netting showing the most consistent performance.

After 120 days, the trends remained similar, with shaded conditions generally maintaining lower ABS/RC and DI_0/RC values compared to outside, unshaded plants, reflecting improved plant vitality under netting (Figure 27b, Table 20). The TR_0/RC and ET_0/RC values were slightly higher under shaded conditions, particularly under grey laminated shade netting, indicating sustained photosynthetic activity. The RE_0/RC varied somewhat, with plants under the green laminated shade netting showing marginally lower values than plants under the grey and white laminated shade netting (Table 20), further supporting the advantage of shading in enhancing electron transport efficiency over prolonged periods.

Overall, these observations emphasize the beneficial effects of shading on the photosynthetic performance of *Lessertia frutescens*, with grey and white netting consistently promoting more efficient energy use and reduced photoprotective losses.

Table 19: A summary of the statistics of the specific energy fluxes per Q_A -Reduced PSII reaction centres (ABS/RC, DI_0 /RC, TR_0 /RC, ET_0 /RC and RE_0 /RC) of *Lessertia frutescens*, plant group B, after 60 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant differences ($p < 0.05$) among the laminated shade netting for different parameters (Appendix: Table 55 - 59).

60 duLN	ABS/RC	DI_0 /RC	TR_0 /RC	ET_0 /RC	RE_0 /RC
Outside	1.33±0.38 a	0.30±0.13 a	1.04±0.25 a	0.58±0.04 a	0.32±0.02 a
Grey	1.09±0.08 b	0.20±0.03 b	0.90±0.05 b	0.61±0.04 a	0.25±0.03 b
White	1.10±0.09 b	0.20±0.03 b	0.90±0.06 b	0.60±0.02 a	0.26±0.03 b
Green	1.06±0.08 b	0.18±0.03 b	0.88±0.06 b	0.62±0.04 a	0.28±0.05 b

Table 20: A summary of the statistics of the specific energy fluxes per Q_A -Reduced PSII reaction centres (ABS/RC, DI_0 /RC, TR_0 /RC, ET_0 /RC and RE_0 /RC) of *Lessertia frutescens*, plant group B, after 120 days under laminated shade netting (duLN). The first value represents the average, followed by the standard deviation, while the letters indicate significant differences ($p < 0.05$) among the laminated shade netting for different parameters (Appendix: Table 55 - 59).

120 duLN	ABS/RC	DI_0 /RC	TR_0 /RC	ET_0 /RC	RE_0 /RC
Outside	1.07±0.12 ab	0.20±0.05 ab	0.87±0.08 ab	0.60±0.02 a	0.33±0.03 ab
Grey	1.11±0.12 ab	0.20±0.03 ab	0.91±0.09 ab	0.63±0.05 a	0.30±0.01 ab
White	1.10±0.10 a	0.20±0.03 a	0.90±0.07 a	0.61±0.04 a	0.32±0.03 a
Green	1.02±0.05 a	0.17±0.01 b	0.84±0.04 b	0.60±0.02 a	0.30±0.03 b

4.3 The effect of different shading conditions on the 820 nm Modulated reflection and its parameters of *Lessertia frutescens*.

The 820 nm modulated reflection (MR) technique is a sensitive tool for investigating the redox state of photosystem I (PSI) and its associated electron transport processes. This method focuses on measuring the electrons absorbed by PSI, which drive the oxidation and reduction of P700, the primary electron donor in PSI. By analysing the MR signal, valuable insights can be gained into the response of PSI under various environmental conditions (Liu *et al.*, 2018; Shu *et al.*, 2024)

A key parameter in MR analysis is the MR_0/MR_{min} ratio, which reflects the initial redox state of P700 (Schansker *et al.*, 2003; Shu *et al.*, 2024). The MR signal is characterized by two distinct phases: the fast and slow phases. The rapid phase represents the oxidation of P700 upon exposure to light, providing information on the immediate response of PSI to light. In contrast, the slow phase corresponds to the re-reduction of P700 via electron carriers, offering insights into the long-term electron transport capacity and the interactions between PSI and other components of the electron transport chain (Strasser *et al.*, 2010).

The following section examines the effects of different shading treatments and time intervals on the MR signal of *Lessertia frutescens*.

4.3.1 The 820 nm Modulated reflection of plant group A:

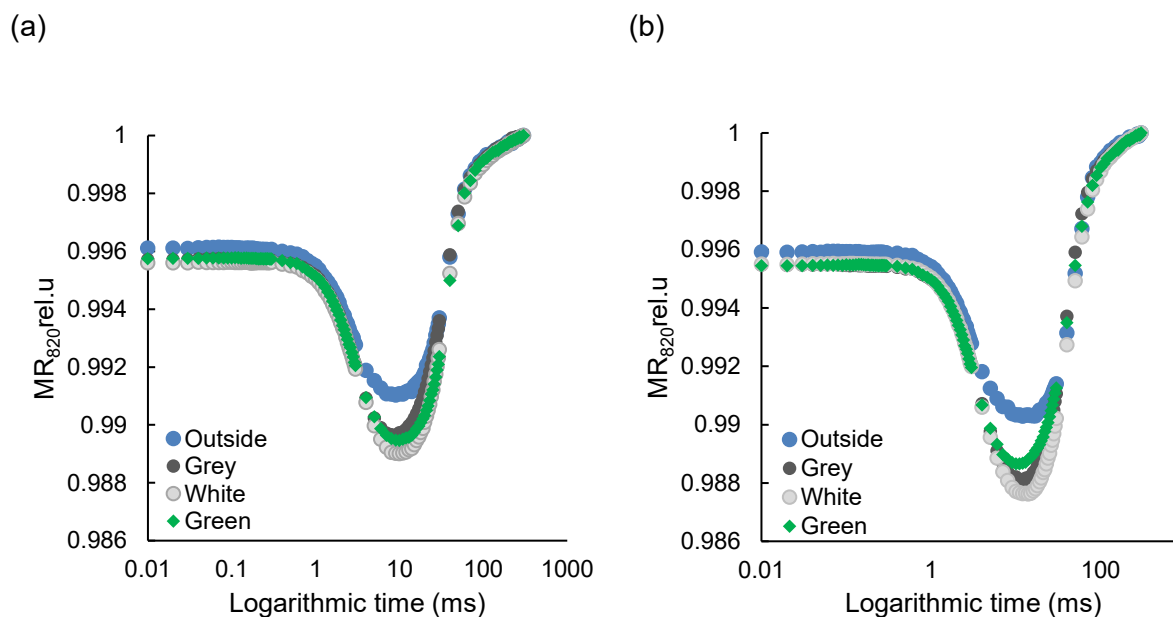


Figure 28: The 820 nm Modulated reflection curve of *Lessertia frutescens*, plant group A, under different shading conditions over a period of (a) 60 days and (b) 120 days under laminated shade netting (duLN).

The different shading conditions influenced plant group A's 820 nm modulated reflection signal (Figure 28). After both 60 and 120 days, it was observed that the outside, unshaded plants had a shallower basin compared to those under the netting. This difference indicates less efficient oxidation and reduction of P700. The variations were further analysed using specific parameters.

4.3.1.1 MR_0/MR_{min}

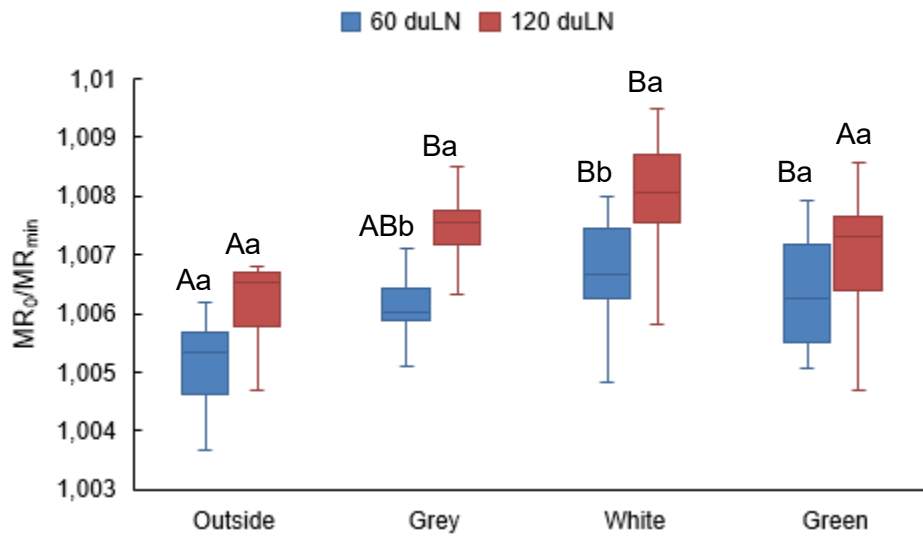


Figure 29: The MR_0/MR_{min} of the 820 nm Modulated reflection of *Lessertia frutescens*, Plant group A, under different shading conditions over a period of 60 days and 120 days under the shade netting.

The different shading conditions influenced the MR_0/MR_{min} ratio of Plant group A (Figure 29). After 60 days under laminated shade netting (duLN), the outside, unshaded plants exhibited the lowest value, which was significantly ($p < 0.05$) lower than that observed under the white and green laminated shade netting (Appendix: Table 60). After 120 days, the plants outside continued to show the lowest value, significantly ($p < 0.05$) lower than those under the white and green laminated shade netting (Appendix: Table 51). The duration of exposure to shading conditions also affected the MR_0/MR_{min} . A trend was observed where the ratio increased from 60 to 120 days under laminated shade netting (duLN). This increase was significant ($p < 0.05$) under the grey and white laminated shade netting (Appendix: Table 60).

4.3.1.2 Fast phase (V_{ox}):

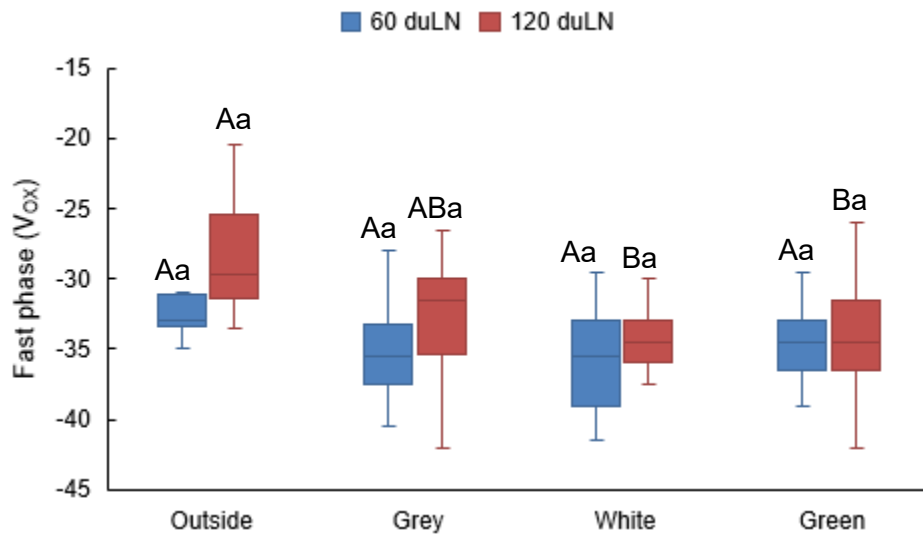


Figure 30: The fast phase (V_{ox}) of the 820 nm Modulated reflection of *Lessertia frutescens*, Plant group A, under different shading conditions over a period of 60 days and 120 days under laminated shade netting (duLN).

The different shading conditions influenced the fast phase, also referred to as the oxidation phase (Figure 30). After 60 days under the laminated shade netting (duLN), no significant ($p < 0.05$) differences were observed (Appendix: Table 61); however, the outside, unshaded plants displayed the highest value (Figure 30). After 120 days, the plants under the white laminated shade netting exhibited a significantly ($p < 0.05$) lower value compared to the outside, unshaded plants (Appendix: Table 61). A lower V_{ox} value typically indicates more efficient oxidation of P700 and plastocyanin (PC).

4.3.1.3 Slow phase (V_{re-red})

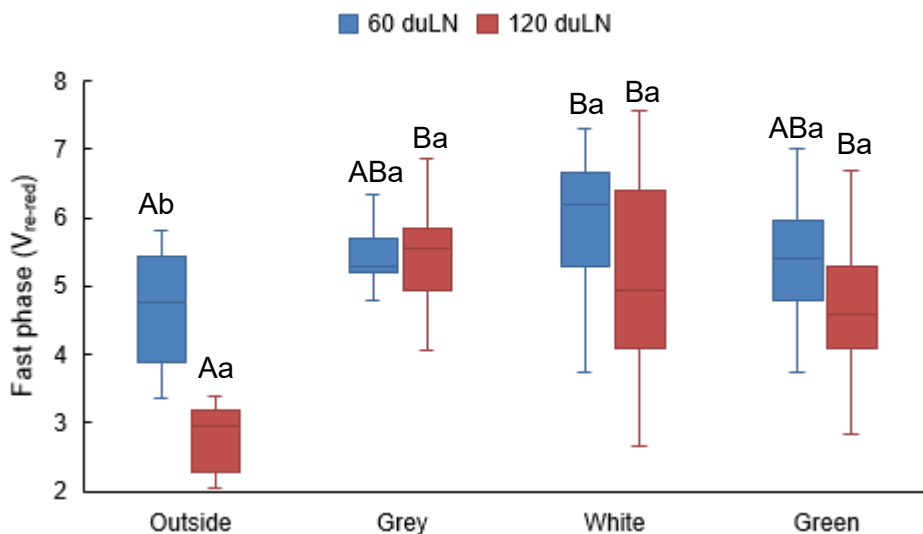


Figure 31: The slow phase (V_{re-red}) of the 820 nm Modulated reflection of *Lessertia frutescens*, Plant group A, under different shading conditions over a period of 60 days and 120 days under laminated shade netting (duLN).

The different shading conditions affected the slow phase, also referred to as the re-reduction phase, of the MR signal (Figure 31). After 60 days under laminated shade netting (duLN), the plants under the white laminated shade netting exhibited significantly ($p < 0.05$) higher values compared to those outside (Appendix: Table 62). After 120 days, the plants under the grey, white, and green laminated netting showed significantly ($p < 0.05$) higher values than the outside, unshaded plants (Appendix: Table 62). The only significant ($p < 0.05$) difference observed between the durations occurred in the outside, unshaded plants, where the value after 60 days was significantly ($p < 0.05$) lower than after 120 days. A decrease in the slow phase would usually refer to the ineffective re-reduction of P700 and plastocyanin (PC).

4.3.2 The 820 nm Modulated reflection of Plant group B:

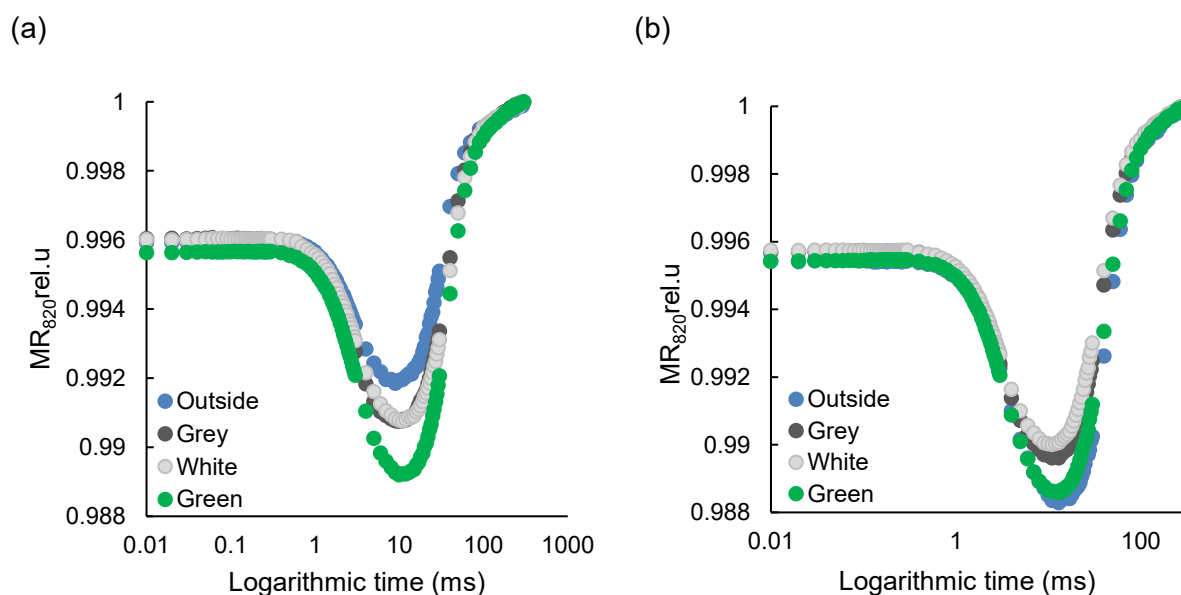


Figure 32: The 820 nm Modulated reflection curve of *Lessertia frutescens*, Plant group B, under different shading conditions over a period of (a) 60 days and (b) 120 days under laminated shade netting (duLN).

The different shading conditions resulted in variations in the 820 nm modulated reflection signal of Plant group B (Figure 28). After 60 days under laminated shade netting (duLN), plants under the green netting exhibited the deepest basin, while those outside had the shallowest (Figure 28a). This suggests that the PSI of plants under the green laminated shade netting operated with greater efficiency. However, after 120 days, the basin for plants outside became even more profound than that of the green laminated shade netting (Figure 28b), indicating a potential adaptation of Plant group B to the high-shading conditions outside. These variations were further analysed using specific parameters.

4.3.2.1 MR_0/MR_{min}

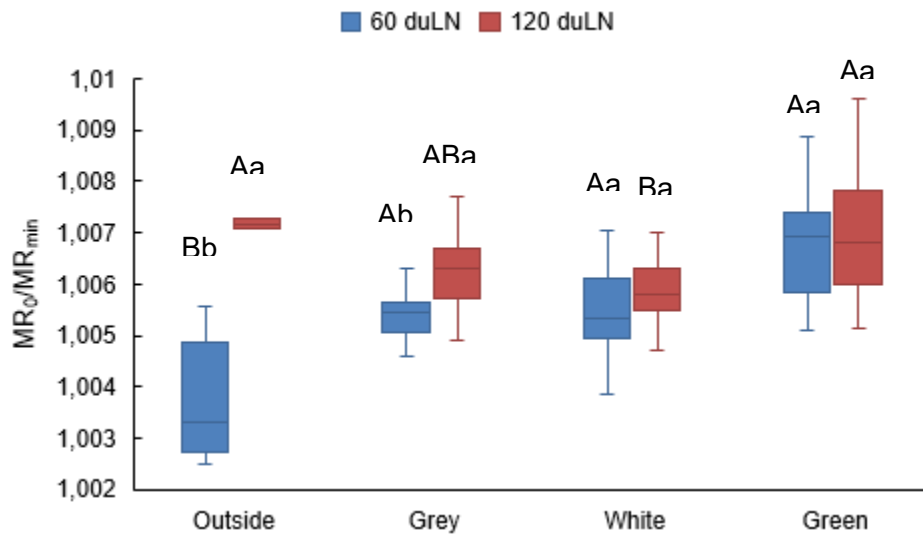


Figure 33: The MR_0/MR_{min} of the 820 nm Modulated reflection of *Lessertia frutescens*, Plant group B, under different shading conditions over a period of 60 days and 120 days under laminated shade netting (duLN).

The different shading conditions and durations influenced the MR_0/MR_{min} ratio of Plant group B (Figure 33). After 60 days under laminated shade netting (duLN), the plants under the netting exhibited a significantly ($p < 0.05$) higher value compared to those outside (Appendix: Table 63). However, after 120 days, the plants under the white laminated shade netting had a significantly ($p < 0.05$) lower value than the plants outside and those under the green and grey laminated shade netting (Appendix: Table 63).

4.3.2.2 Fast phase (V_{ox}):

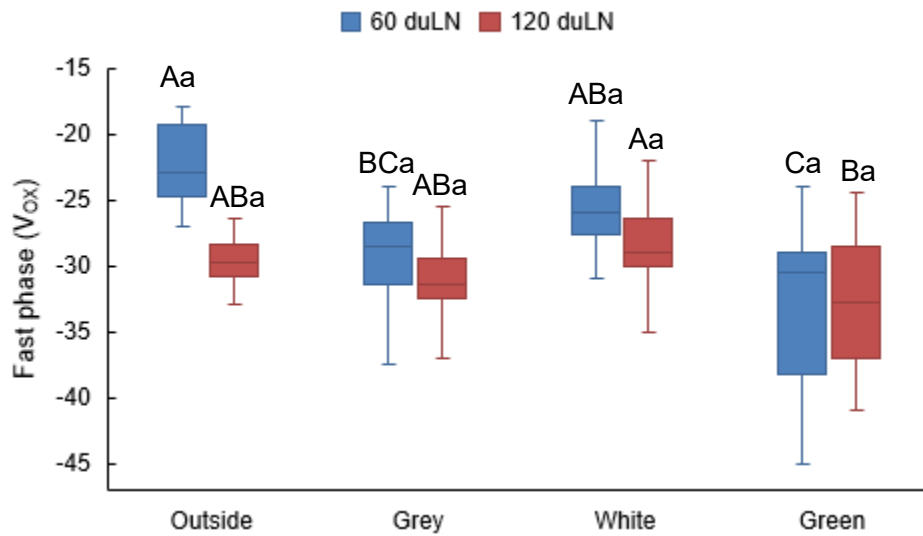


Figure 34: The fast phase (V_{ox}) of the 820 nm Modulated reflection of *Lessertia frutescens*, Plant group B, under different shading conditions over a period of 60 days and 120 days under laminated shade netting (duLN).

The different shading conditions influenced the fast phase of Plant group A (Figure 34). After 60 days under laminated shade netting (duLN), the outside plants exhibited a significantly ($p < 0.05$) higher value compared to those under the grey and green laminated shade netting. Additionally, the plants under the white laminated shade netting had a significantly ($p < 0.05$) higher value than those under the green laminated shade netting (Appendix: Table 64). After 120 days, the plants under the white netting exhibited a significantly ($p < 0.05$) higher value than those under the green netting (Appendix: Table 64). Although no significant ($p < 0.05$) differences were observed over time, the values generally decreased from 60 to 120 days. A lower V_{ox} value typically indicates more efficient oxidation of P700 and plastocyanin (PC)

4.3.2.3 Slow phase (V_{re-red})

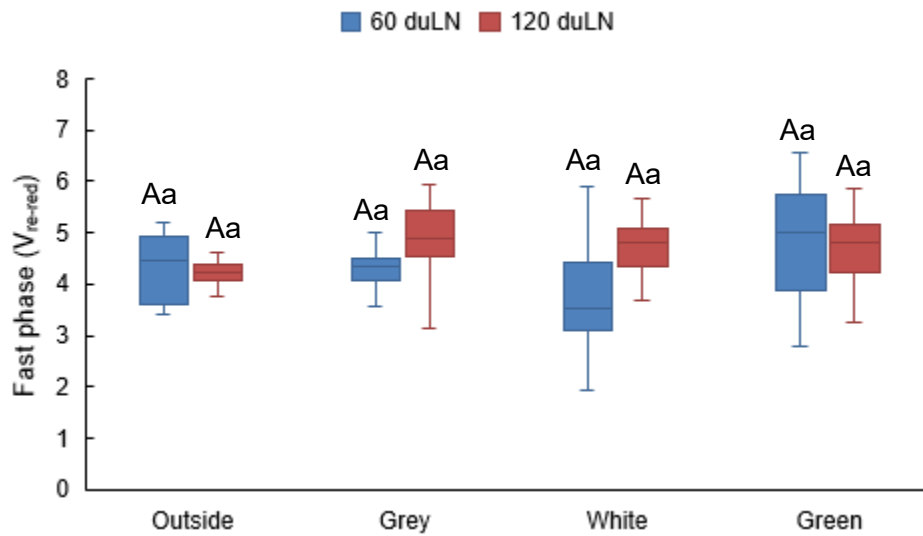


Figure 35: The slow phase (V_{re-red}) of the 820 nm Modulated reflection of *Lessertia frutescens*, Plant group B, under different shading conditions over a period of 60 days and 120 days under laminated shade netting (duLN).

The different shading conditions influenced the MR signal's slow phase (Figure 35). Although the slow phase appears to vary across shading conditions and the duration of exposure causes observable changes in V_{re-red} values, no significant ($p < 0.05$) differences were detected in either case (Appendix: Table 65). Among the shading treatments, the green laminated shade netting exhibited the highest V_{re-red} values (Figure 35). Typically, a lower V_{re-red} value indicates less effective re-reduction of P700 and plastocyanin (PC).

CHAPTER 5 DISCUSSION

Understanding how environmental factors influence photosynthetic efficiency is essential for optimising growth conditions, specifically for medicinal species, such as *Lessertia frutescens*. This study examined the effects of different colours of laminated shade netting on *Lessertia frutescens* by assessing its photosynthetic responses. Although the species has applications in treating various ailments, further research is needed to improve its cultivation and efficacy. A crucial first step in this process is determining how environmental variation affects plant vitality. This was investigated by analysing key photosynthetic parameters, including the redox state of PSII, performance indices, the redox state of PSI, absorption fluxes, cross-section and chlorophyll content.

5.1 The effect of different laminated shade netting on the redox state of PSII through the analysis of the OJIP transient and its parameters

Chlorophyll *a* fluorescence is generally expected to increase under shaded conditions due to reduced radiation stress and the subsequent decrease in reactive oxygen species (ROS) formation (Hopkins & Hüner, 2008). In theory, plants under green laminated shade netting should show the highest chlorophyll *a* fluorescence. A study by Blanke (2009) found that green netting reduces photosynthetically active radiation (PAR) by 13%, while white and grey netting reduces PAR by 8.4% and 11.6%, respectively. This reduction in PAR helps minimise the risk of photodamage. However, in this study, chlorophyll *a* fluorescence under green netting was not significantly ($p < 0.05$) higher than under the other shading treatments. This suggests that other factors, such as the net's structure or specific spectral filtering effects, may have influenced the fluorescence responses.

The OJIP transient retained its characteristic shape and distinct inclination points throughout all shading treatments (Figures 18 and 19). Notably, the outside, unshaded plants exhibited higher chlorophyll *a* fluorescence at the single-turnover event compared to shaded plants; however, their overall fluorescence remained lower than that of plants grown under netting. This indicates that while outside, unshaded plants may experience transiently higher excitation energy, they likely endure greater photoprotective stress, leading to lower fluorescence levels. To further analyse the OJIP transient, various fluorescence parameters were examined. The minimal fluorescence

(F_0) of the outdoor plants of Plant group A was higher than that of the shaded plants, suggesting photoinhibition or damage to PSII due to high light intensities. However, these variations were minor, aligning with findings by Umesh *et al.*, (2022), who observed similar trends in legumes exposed to different shading conditions. On the contrary, the maximum fluorescence (F_M) differed significantly ($p < 0.05$), with the outside, unshaded plants exhibiting the lowest values, as expected, an outcome also reported by Umesh *et al.*, (2022). Notably, while green netting was expected to enhance fluorescence due to its light-scattering properties, its F_M values did not significantly exceed those of other shading treatments, indicating that spectral composition alone does not entirely predict fluorescence outcomes.

The maximum quantum yield of PSII (F_V/F_M) remained above 0.8 in shaded plants (Bjorkman & Demmig, 1987; Medina *et al.*, 2002), indicating optimal PSII functionality. In contrast, outside, unshaded plants had lower values, likely due to high light stress. This trend has been consistently observed in previous studies in which shaded plants demonstrated significantly higher F_V/F_M values (Umesh *et al.*, 2022). These findings collectively suggest that shading mitigates photodamage and enhances PSII efficiency. However, differences between shading treatments indicate that multiple environmental and physiological factors influence fluorescence responses beyond light scattering effects.

5.2 The effect of different laminated shade netting on the plant vitality of the plant group Based on the performance indexes

The total performance index (PI_{TOTAL}) of plants grown under moderate shade netting, such as those used in this study, is expected to increase under shading due to the enhanced efficiency of chlorophyll *a* fluorescence. This trend was observed in plant group A, where shaded plants exhibited a higher performance index than outside, unshaded plants. In plant group B, PI_{TOTAL} showed some variation, but the changes were not statistically significant ($p < 0.05$). However, it is important to note that the index increased from 60 to 120 duLN.

In contrast, Yao *et al.*, (2017) reported a decrease in PI_{TOTAL} under shade netting, attributing this decline to reduced quantum efficiency from PSII to PSI. Since this was not the case in our study, the shading treatments did not appear to inhibit the photosynthetic performance of *Lessertia frutescens*, suggesting that the species effectively acclimates to moderate shading without significant ($p < 0.05$) reductions in overall photosynthetic efficiency.

The PI_{ABS} is the sum of the efficiency of light capturing (RC/ABS), the conversion of trapped excitation energy into electron transport ($\Psi_0/(1 - \Psi_0)$) and the quantum efficiency of light trapping ($\phi_{P_0}/(1 - \phi_{P_0})$). Under high-shading conditions, the PI_{ABS} is expected to decrease, in comparison to shading conditions, due to light stress. In this study, the PI_{ABS} and its parameters were significantly ($p < 0.05$) lower outside than under the shading. These findings are consistent with previous studies (Mengarda *et al.*, 2012; Yao *et al.*, 2017) that found that the PI_{ABS} is higher under shading conditions than under high shading conditions. In contrast, plant group B after 120 duLN the outside, unshaded plants had a higher value than the plants under the grey and white netting.

5.3 The effect of different laminated shade netting on the redox state of PSI through the 820 nm modulated reflection curve and associated parameters

Under stress conditions, the 820 nm modulated reflection signal exhibits a shallower basin, often appearing nearly linear. This indicates reduced PSI efficiency, as the basin depth reflects the extent of PSI oxidation and re-reduction dynamics. A well-functioning PSI shows a deeper basin, which indicates active electron flow.

In this study, shading appeared to improve the plant vitality of *Lessertia frutescens*, as seen in the higher oxidation and re-reduction rates of PSI in shaded plants compared to the outside, unshaded plants. However, plant group B showed an exception after 120 days under laminated shade netting, where the outside, unshaded plants exhibited higher PSI efficiency.

A similar trend was observed in a Welsh onion (*Allium fistulosum*) study where plants grown under 20% shade showed improved PSI efficiency (Liu *et al.*, 2021). This suggests that moderate shading can enhance PSI function by reducing photo-inhibitory stress.

To further assess PSI activity, key parameters such as the initial modulated reflection ratio (MR_0/MR_{min}), the fast oxidation phase (V_{ox}), and the slow re-reduction phase (V_{re-red}) were analysed. MR_0/MR_{min} and V_{ox} followed a similar trend, where the outside, unshaded plants of plant group A exhibited higher values than those under the laminated shade netting, gradually increasing over time. The same pattern was observed in plant group B after 60 days under laminated shade netting (duLN) however, after 120 days, the highest values were recorded in outside, unshaded plants. The re-reduction phase (V_{re-red}) of plant group A followed a similar pattern, with lower rates in outside, unshaded plants compared to shaded ones. In contrast, plant group B showed variations in V_{re-red} without a clear trend.

The variation seen in group B after 120 days under laminated shade netting (duLN) may be due to a gradual decline in light intensity over time, which could have reduced photo-inhibitory stress in exposed plants. This suggests that long-term acclimation to changing shading conditions plays a role in PSI efficiency and redox dynamics.

5.4 The effect of different laminated shade netting on the plant's photosynthetic efficiency based on absorption fluxes

The photosynthetic efficiency of *Lessertia frutescens* can be assessed through various absorption flux parameters. In this study, light absorption per reaction centre (ABS/RC), trapped energy flux per reaction centre (TR₀/RC), electron transport flux per reaction centre (ET₀/RC), dissipation energy per reaction centre (DI₀/RC), and the re-oxidation of electron acceptors per reaction centre (RE₀/RC) were evaluated (Figure 26 and 27).

Overall, ABS/RC, TR₀/RC, and DI₀/RC decreased under shade netting, except in plant group B after 120 days, where ABS/RC and TR₀/RC remained unchanged between shaded and outside, unshaded plants. The RE₀/RC values for both groups exhibited a decreasing trend under shading conditions. However, no significant ($p < 0.05$) differences were observed in plant group A after two months of treatment. After 120 days, a notable increase in RE₀/RC was detected in the shaded plants, suggesting a delayed response to acclimatisation. In contrast, ET₀/RC remained relatively stable across all treatments. No significant ($p < 0.05$) differences were observed between plants grown in full sunlight and those under laminated shade netting, indicating that electron transport efficiency was not substantially affected by the altered shading conditions (Table 19 - 20).

These findings align with previous research on shade-acclimated plants. Studies have shown that ABS/RC, TR₀/RC, and DI₀/RC generally decrease under shading, while ET₀/RC tends to increase (Yao *et al.*, 2017). For example, in soybean, the shade-tolerant cultivar L32 exhibited higher ABS/RC, TR₀/RC, and ET₀/RC values compared to the less shade-tolerant L29 across different shading treatments. Under reduced shading conditions, soybean plants adapted by redistributing the absorbed energy, increasing electron transfer efficiency (ET₀/RC), and reducing heat dissipation (DI/RC) to improve solar energy utilization. Although *Lessertia frutescens* exhibited a similar decline in ABS/RC, TR₀/RC, and DI₀/RC, the lack of a significant increase in ET₀/RC suggests species-specific differences in how excess energy is managed under shading conditions (Tmušić *et al.*, 2021).

5.5 The effect of different laminated shade netting on the plant's photosynthetic efficiency based on the cross-section

The photosynthetic efficiency of *Lessertia frutescens* can be evaluated by analysing cross-section parameters related to light absorption, electron transport, and energy dissipation. Previous studies have shown that shaded plants exhibit lower electron transport per cross-section (ET/CS_m), absorption flux per cross-section (ABS/CS_m), and trapped energy flux per cross-section (TR/CS_m) while displaying higher dissipation of excess energy as heat (DI/CS_m) as a photoprotective strategy to minimize damage under reduced light availability (Kalaji *et al.*, 2012). Similarly, high-light stress has been associated with declines in ABS/CS_m , ET/CS_m , and DI/CS_m , further emphasizing the role of these parameters in photosynthetic acclimation (Faseela & Puthur, 2017).

In this study, plant group A exhibited an increase in ABS/CS_m , TR/CS_m , ET/CS_m , and re-oxidation per cross-section (RE/CS_m) under shading, suggesting enhanced photosynthetic efficiency and improved energy utilization. Conversely, plants grown under full sunlight displayed higher dissipated energy (DI/CS_m), indicating a greater reliance on non-photosynthetic quenching to mitigate excessive light exposure. For plant group B, after 60 days under laminated shade netting, the outside, unshaded plants and those under white laminated shade netting treatments resulted in lower ABS/CS_m and TR/CS_m , with the lowest energy dissipation observed under the white laminated shade netting. However, no significant ($p < 0.05$) differences were observed among the other treatments. After 120 days, only minor variations were detected, suggesting that the plants had reached a stable acclimation state, potentially due to cultivar-specific adaptations to high-light stress.

5.6 The effect of different shading conditions on the chlorophyll content

Chlorophyll plays a crucial role in photosynthesis by absorbing light; its variation is often a response to environmental changes. Under high light intensities, chlorophyll content typically decreases as part of a photoprotective mechanism, preventing excess energy absorption that could lead to the overproduction of reactive oxygen species (ROS). Conversely, under low shading conditions, chlorophyll content increases to maximize light harvesting from the limited available energy.

In this study, the chlorophyll content of plant group B remained unchanged despite the modifications in shading conditions caused by shade netting, whereas plant group A showed a clear response. The chlorophyll content of group A increased under shading, ensuring that the plants could still capture sufficient light for photosynthesis. This suggests that plant group A can acclimate to lower light environments, as adjustments in light intensity often trigger changes in the abundance of protein complexes within the thylakoid membrane (Timperio *et al.*, 2012).

Similar trends have been reported in previous studies. Umesh *et al.* (2022) found that plants grown under shading exhibited higher chlorophyll concentrations. Meanwhile, Mauro *et al.* (2011) observed a linear decrease in chlorophyll content in clover plants as shading levels increased. In the present study, chlorophyll content decreased as light intensity increased, with the lowest values recorded in outside, unshaded plants. This aligns with the well-documented protective strategy in which plants reduce chlorophyll levels under high light to mitigate potential photodamage.

CHAPTER 6 CONCLUSION

This study investigated the effects of different shade netting on the photosynthetic efficiency of *Lessertia frutescens* to optimize its cultivation. It analysed key photosynthetic parameters: the redox states of PSII and PSI, performance indexes, absorption fluxes, cross-section parameters, and chlorophyll content. These provide insight into the physiological responses of *Lessertia frutescens* under different coloured shading conditions. The findings contribute to understanding how controlled light environments influence photosynthetic efficiency and plant vitality.

The hypothesis was mainly supported by the hypothesis that the photosynthetic efficiency would be higher under netting than outside, unshaded plants. Plants under shading exhibited improved photosynthetic performance, as indicated by a higher maximum quantum yield (F_v/F_M), suggesting that shading mitigates photodamage and enhances PSII efficiency. The OJIP transient retained its characteristic shape across all shading treatments, though the outside, unshaded plants exhibited higher minimal fluorescence (F_0). Indicating potential PSII damage due to high light intensities. While green netting was hypothesised to promote the highest photosynthetic efficiency, the results did not show significant ($p < 0.05$) superiority over other shading treatments, implying that spectral composition alone does not fully dictate fluorescence results.

Although both plant groups A and B belong to *Lessertia frutescens*, their acclimation strategies appear to differ. Group A exhibited higher vitality under shaded conditions, as indicated by increased PSII efficiency, chlorophyll content, and improved PSI function. In contrast, plant group B showed less predictable responses, particularly after prolonged exposure to laminated shade netting. The decline in PSI efficiency and performance index values in plant group B after 120 days suggests that extended shading did not enhance plant vitality. These findings highlight the importance of considering intraspecific variation when optimizing shading strategies for cultivation, as species-wide generalizations may overlook critical physiological differences.

The performance indices (PI_{TOTAL}) were generally higher under shade netting, indicating improved plant vitality in altered-light environments. Although some variation was observed among different plant groups, the general trend suggested that moderate shading enhanced photosynthetic function. The redox state of PSI also demonstrated higher oxidation and re-reduction rates in plants under shade netting, reinforcing the conclusion that shading mitigates photoinhibitory stress.

The analysis of absorption fluxes revealed that ABS/RC , TR_0/RC , and DI_0/RC generally decreased under shading, with no significant ($p < 0.05$) impact on electron transport efficiency (ET_0/RC). This suggests that *Lessertia frutescens* effectively acclimates to shaded environments without compromising its photosynthetic electron transport processes. Similarly, cross-section parameters indicated that shaded plants exhibited increased ABS/CS_m , TR/CS_m , and ET/CS_m . In contrast, outdoor unshaded plants displayed higher dissipation energy (DI/CS_m) as a photoprotective strategy against excess light exposure.

Chlorophyll content analysis further supported these findings, as shaded plants maintained higher chlorophyll levels, optimising light capture for photosynthesis. In contrast, outdoor unshaded plants exhibited reduced chlorophyll content, likely as a photoprotective adaptation to high irradiance conditions. While the response varied between plant groups, the general trend aligned with previous studies indicating that shading facilitates chlorophyll retention in shade-adapted species.

Overall, this study demonstrates that *Lessertia frutescens* benefits from moderate shading, which enhances photosynthetic efficiency by reducing photodamage and optimising energy utilisation. However, the hypothesis that green netting would provide the most significant benefit was not supported, suggesting that other environmental factors and species-specific responses play a role in determining optimal shading conditions.

CHAPTER 7 RECOMMENDATIONS

This study demonstrated that different populations of *Lessertia frutescens* exhibit distinct photosynthetic responses. Therefore, it is recommended that seeds from various known geographical locations be collected and tested under various environmental conditions. Additionally, medicinally relevant metabolites should be thoroughly investigated and analysed under varying conditions to assess their response. Future studies should include more comprehensive data on the specific wavelengths transmitted through shading nets and under ultraviolet (UV) irradiation. It is also recommended that a more detailed description of the different morphological forms of *Lessertia frutescens* be provided, accompanied by a distribution map to illustrate its geographical range better.

REFERENCE LIST

- Aboyade, O.M., Styger, G., Gibson, D. & Hughes, G. 2014. *Sutherlandia frutescens*: The meeting of science and traditional knowledge. *Journal of Alternative and Complementary Medicine*, 20(2):71–76.
- Ahemd, H.A., Al-Faraj, A.A. & Abdel-Ghany, A.M. 2016. Shading greenhouses to improve the microclimate, energy and water saving in hot regions: A review. *Scientia Horticulturae*, 201:36–45.
- Bence, A., Worthen, D., Adams, V. & Crooks, P. 2002. The antiproliferative and immunotoxic effects of L-canavanine and L-canaline. *Anticancer Drugs*, 13(3):313-320.
- Bjorkman, O. & Demmig, B. 1987. Photon yield of O₂ evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. *Planta*, 170:489–504.
- Blanke, M.M. 2009. The Structure of Coloured Hail Nets Affects Light Transmission, Light Spectrum, Phytochrome and Apple Fruit Colouration. *Acta Horticulturae*, 817:177-184.
- Colling, J., Stander, M.A. & Makunga, N.P. 2010. Nitrogen supply and abiotic stress influence canavanine synthesis and the productivity of in vitro regenerated *Sutherlandia frutescens* microshoots. *Journal of Plant Physiology*, 167(17):1521–1524.
- Crooks, P. & Rosenthal, G. 1996. Use of L-canavanine as a chemotherapeutic agent for the treatment of pancreatic cancer. *Pharmaceutical Sciences Faculty Patents*, 107. https://uknowledge.uky.edu/ps_patents/107.
- Dekmak, M.Y., Mäusle, S.M., Brandhorst, J., Simon, P.S. & Dau, H. 2023. Tracking the first electron transfer step at the donor side of oxygen-evolving photosystem II by time-resolved infrared spectroscopy. *Photosynthesis Research*, <https://doi.org/10.1007/s11120-023-01057-3>.
- Faseela, P. & Puthur, J.T. 2017. Chlorophyll a fluorescence changes in response to short- and long-term high light stress in rice seedlings. *Indian Journal of Plant Physiology*, 22(1):30–33.
- Fernandes, A.C., Cromarty, A.D., Albrecht, C. & Jansen Van Rensburg, C.E. 2004. The antioxidant potential of *Sutherlandia frutescens*. *Journal of Ethnopharmacology*, 95(1):1–5.
- Goldblatt, L. & Manning, J.C. 2003. *Lessertia frutescens*. SANBI.

- Goltsev, V.N., Kalaji, H.M., Paunov, M., Baba, M., Horaczek, T., Mojski, J., Kociel, H. & Allakhverdiev, S.I. 2016. Variable fluorescence and its use for assessing physiological condition of plant photosynthetic apparatus. *Plant Physiology*, 63(6):869–893.
- Hansatech Instruments. s.a. <https://ppsystems.com/wp-content/uploads/M-PEA-NEW.pdf> Date of access: 20 Aug. 2024.
- Hashim, M., Ahmad, B., Drouet, S., Hano, C., Abbasi, B.H. & Anjum, S. 2021. Comparative effects of different light sources on the production of key secondary metabolites in plants in vitro cultures. *Plants*, 10,1521.
- Hogewoning, S.W., Trouwborst, G., Maljaars, H., Poorter, H., van Ieperen, W. & Harbinson, J. 2010. Blue light dose-responses of leaf photosynthesis, morphology, and chemical composition of *Cucumis sativus* grown under different combinations of red and blue light. *Journal of experimental botany*, 61(11):3107–3117.
- Hopkins, W.G. & Hüner, N.P.A. 2008. *Introduction to plant physiology*. 4th ed. Hoboken: John Wiley & Sons, Inc.
- Huang, C.J., Wei, G., Jie, Y.C., Xu, J.J., Anjum, S.A. & Tanveer, M. 2016. Effect of shade on plant traits, gas exchange and chlorophyll content in four ramie cultivars. *Photosynthetica*, 54(3):390–395.
- Ilić, Z.S. & Fallik, E. 2017. Light quality manipulation improves vegetable quality at harvest and postharvest: A review. *Environmental and Experimental Botany*, 139: 79-90.
- Ilić, Z.S., Milenković, L., Šunić, L. & Fallik, E. 2015. Effect of coloured shade-nets on plant leaf parameters and tomato fruit quality. *Journal of the Science of Food and Agriculture*, 95(13):2660–2667.
- Ilić, Z.S., Milenković, L., Šunić, L., Barać, S., Cvetković, D., Stanojević, L. Kevrešan, Ž & Mastilović, J. 2019. Bioactive constituents of red and green lettuce grown under colour shade nets. *Emirates Journal of Food and Agriculture*, 31(12):937–944.
- Johnstone, D., Moore, G., Tausz, M. & Nicolas, M. 2013. The measurement of plant vitality in landscape trees. *Arboricultural Journal*, 35(1):18–27.
- Kalaji, H.M., Carpentier, R., Allakhverdiev, S.I. & Bosa, K. 2012. Fluorescence parameters as early indicators of light stress in barley. *Journal of Photochemistry and Photobiology B: Biology*, 112:1–6.

- Kalaji, H.M., Jajoo, A., Oukarroum, A., Brestic, M., Zivcak, M., Samborska, I.A., Cetner, M.D., Łukasik, I., Goltsev, V. & Ladle, R.J. 2016. Chlorophyll *a* fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. *Acta Physiologiae Plantarum*, 38(4).
- Kalaji, M.H., Goltsev, V.N., Zuk-Golaszewska, K., Zivcak, M. & Brestic, M. 2017. *Chlorophyll Fluorescence Understanding Crop Performance-Basics and Applications*. London: CRC Press.
- Kautsky, H. & Hirsch, A. 1931. Neue Versuche zur Kohlensäureassimilation. *Naturwissenschaften*, 19.
- Khan, I., Zada, A., Jia, T. & Hu, X. 2023. Effect of the Enhanced Production of Chlorophyll *b* on the Light Acclimation of Tomato. *International Journal of Molecular Sciences*, 24(4).
- Korth, S. 2021. *Sutherlandia frutescens: A Woody Perennial that Packs a Medicinal Punch*. <https://conservancy.umn.edu/bitstream/handle/11299/225167/Sutherlandia%20frutescens%20%20by%20Sarah%20Korth.pdf?sequence=1> Date of access: 05 Apr. 2023.
- Kume, A., Akitsu, T. & Nasahara, K.N. 2018. Why is chlorophyll *b* only used in light-harvesting systems? *Journal of Plant Research*, 131(6):961–972.
- Li, Y., He, N., Hou, J., Xu, L., Liu, C., Zhang, J., Wang, Q., Zhang, X. & Wu, X. 2018. Factors influencing leaf chlorophyll content in natural forests at the biome scale. *Frontiers in Ecology and Evolution*, 6:64.
- Liu, J., Guo, Y.Y., Bai, Y.W., Camberato, J.J., Xue, J.Q. & Zhang, R.H. 2018. Effects of Drought Stress on the Photosynthesis in Maize. *Russian Journal of Plant Physiology*, 65(6):849–856.
- Liu, X., Gao, S., Liu, Y., Cao, B., Chen, Z. & Xu, K. 2021. Alterations in leaf photosynthetic electron transport in Welsh onion (*Allium fistulosum* L.) under different light intensity and soil water conditions. *Plant group Biology*, 23(1):83–90.
- Lobos, G.A., Retamales, J.B., Del Pozo, A., Hancock, J.F. & Flore, J.A. 2009. *Physiological Response of Vaccinium corymbosum “Elliott” to Shading Nets in Michigan*.
- Lobos, G.A., Retamales, J.B., Hancock, J.F., Flore, J.A., Cobo, N. & del Pozo, A. 2012. Spectral irradiance, gas exchange characteristics and leaf traits of *Vaccinium corymbosum* L. “Elliott” grown under photo-selective nets. *Environmental and Experimental Botany*, 75:142–149.
- Manja, K. & Aoun, M. 2019. The use of nets for tree fruit crops and their impact on the production: A review. *Scientia Horticulture*, 246:110-122.

- Mauro, R.P., Occhipinti, A., Longo, A.M.G. & Mauromicale, G. 2011. Effects of Shading on Chlorophyll Content, Chlorophyll Fluorescence and Photosynthesis of Subterranean Clover. *Journal of Agronomy and Crop Science*, 197(1):57–66.
- Mengarda, L.H.G., Milanez, C.R.D., Silva, D.M., Aguilar, M.A.G. & Cuzzuol, G.R.F. 2012. Morphological and physiological adjustments of Brazilwood (*Caesalpinia echinata* Lam.) to direct solar radiation. *Brazilian society of plant physiology*, 24(3):161–172.
- Milenkovic, L., Ilic, Z.S., Đurovka, M., Kapoulas, N., Mirecki, N. & Fallik, E. 2012. Yield and pepper quality as affected by light intensity using colour shade nets. *Agriculture and forestry*, 58(1):19–33. https://www.agricultforest.ac.me/paper.php?journal_id=161&id=2085 Date of access: 12 Nov. 2024.
- Milenković, L., Stanojević, J., Cvetković, D., Stanojević, L., Lalević, D., Šunić, L., Fallik, E. & Ilić, Z.S. 2019. New technology in basil production with high essential oil yield and quality. *Industrial Crops and Products*, 140.
- Mncwangi, N., Viljoen, A., Mulaudzi, N. & Fouche, G. 2022. *Lessertia frutescens*. In: *The South African Herbal Pharmacopoeia: Monographs of Medicinal and Aromatic Plants*. Pretoria:Elsevier. pp. 321–344.
- Moche, D. 1998. A Biosystematic Study of the Genus *Sutherlandia*. Rand Afrikaans University. <https://docslib.org/doc/1716265/a-biosystematic-study-of-the-genus-sutherlandia-br-r-fabaceae> Date of access: 30 Mar. 2023.
- Moualeu-Ngangue, D.P., Chen, T.W. & Stützel, H. 2017. A new method to estimate photosynthetic parameters through net assimilation rate–intercellular space CO₂ concentration (A–C_i) curve and chlorophyll fluorescence measurements. *New Phytologist*, 213(3):1543–1554.
- Murchie, E.H. & Lawson, T. 2013. Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. *Journal of experimental botany*, 64(13):3983–3998.
- Opti-Sciences, Inc. s.a. *CCM-300 Chlorophyll Content meter for small leaves and difficult samples Operation manual*. www.optisci.com.
- Ortega, A. 2003. A new role for GABA: Inhibition of tumour cell migration. *Trends in Pharmacological Sciences*, 24(4):151–154.

- Paradiso, R. & Proietti, S. 2022. Light-Quality Manipulation to Control Plant Growth and Photomorphogenesis in Greenhouse Horticulture: The State of the Art and the Opportunities of Modern LED Systems. *Journal of Plant Growth Regulation*, 41(2):742–780.
- SANBI. s.a. *Ecosystem Services*. <https://www.sanbi.org/ecosystem-services/> Date of access: 31 Mar. 2022.
- Schansker, G., Srivastava, A., Govindjee & Strasser, R.J. 2003. Characterization of the 820-nm transmission signal paralleling the chlorophyll a fluorescence rise (OJIP) in pea leaves. *Functional Plant group Biology*, 30(7):785–796.
- Shevela, D., Björn, L.O. & Govindjee. 2019. *Photosynthesis: Solar energy for life*. Singapore: *World Scientific*. <http://dx.doi.org/10.1142/10522> Date of Access: 11 Feb. 2024.
- Shu, P., Gong, X., Du, Y., Han, Y., Jin, S., Wang, Z., Qian, P. & Li, X. 2024. Effects of Simulated Acid Rain on Photosynthesis in *Pinus massoniana* and *Cunninghamia lanceolata* in Terms of Prompt Fluorescence, Delayed Fluorescence, and Modulated Reflection at 820 nm. *Plants*, 13(5).
- Strasser, R.J., Tsimilli-Michael, M., Qiang, S. & Goltsev, V. 2010. Simultaneous in vivo recording of prompt and delayed fluorescence and 820-nm reflection changes during drying and after rehydration of the resurrection plant *Haberlea rhodopensis*. *Biochimica et Biophysica Acta – Bioenergetics*, 1797:1313–1326.
- Strasser, R.J., Srivastava, A. & Govindjee, G. 1995. Polyphasic chlorophyll a fluorescence transient in plants and cyanobacteria. *Photochemistry and Photobiology*, 61(1):32–42.
- Strasser, R.J., Tsimilli-Michael, M., Dangre, D., Rai, M. 2007. Biophysical Phenomics Reveals Functional Building Blocks of Plants Systems Biology: a Case Study for the Evaluation of the Impact of Mycorrhization with *Piriformospora indica*. *Soil Biology*, 11:319–341.
- Strasser, R.J., Tsimilli-Michael, M. & Srivastava, A. 2004. Analysis of the Chlorophyll a Fluorescence Transient, 321–362.
- Strasser, R.J., Srivastava, A. & Tsimilli-Michael, M. 2002. The fluorescence transient as a tool to characterize and screen photosynthetic samples. *Probing photosynthesis*, 445–483.
- Swaffer, D., Ang, C., Desai, P., Rosenthal, D., Thomas, D., Crooks, P. & John, W. 1995. Combination therapy with 5-fluorouracil and L-canavanine: in vitro and in vivo studies. *Anticancer Drugs*, 6(4):586–593.

- The Calvin cycle: How the products of light reactions, ATP and NADPH, are used to fix carbon into sugars in the second stage of photosynthesis.* s.a. Khan academy. <https://www.khanacademy.org/science/ap-biology/cellular-energetics/photosynthesis/a/calvin-cycle> Date of access: 21 Nov. 2024.
- Thoma, F., Somborn-Schulz, A., Schlehuber, D., Keuter, V. & Deerberg, G. 2020. Effects of light on secondary metabolites in selected leafy greens: A review. *Front. Plant Sci*, 11:497. doi: 10.3389/fpls.2020.00497.
- Timperio, A.M., Gevi, F., Ceci, L.R. & Zolla, L. 2012. Acclimation to intense light implies changes at the level of trimeric subunits involved in the structural organization of the main light-harvesting complex of photosystem II (LHCII) and their isoforms. *Plant Physiology and Biochemistry*, 50:8–14.
- Tmušić, N., Ilić, Z.S., Milenković, L., Šunić, L., Lalević, D., Kevrešan, Ž., Mastilović, J., Stanojević, L., & Cvetković, D. 2021. Shading of medical plants affects the phytochemical quality of herbal extracts. *Horticulturae*, 7(11). <https://www.mdpi.com/2311-7524/7/11/437>
- Tsimilli-Michael, M. & Strasser, R.J. 2008. In vivo assessment of stress impact on plant's vitality: Applications in detecting and evaluating the beneficial role of mycorrhization on host plants. In: *Mycorrhiza: State of the Art, Genetics and Molecular Biology, Eco-Function, Biotechnology, Eco-Physiology, Structure and Systematics (Third Edition)*. Springer-Verlag Berlin Heidelberg. pp. 679–703.
- Umesh, C.V., Jamsheer, A.M. & Prasad, M.A. 2018. The role of flavonoids in drug discovery-review on potential applications. *Research journal of life science, bioinformatics, pharmaceutical and chemical sciences*, 4(1):70-77. DOI - 10.26479/2018.0401.06
- Umesh, M.R., Angadi, S., Begna, S., Gowda, P. & Prasad, P.V.V. 2022. Shade tolerance response of legumes in terms of biomass accumulation, leaf photosynthesis, and chlorophyll pigment under reduced sunlight. *Crop Science*, 63(1):278–292.
- Wang, T. yang, Li, Q. & Bi, K. shun. 2018. Bioactive flavonoids in medicinal plants: Structure, activity and biological fate. *Asian Journal of Pharmaceutical Sciences*, 13(1):12–23.
- van Wyk, B.E. & Albrecht, C. 2008. A review of the taxonomy, ethnobotany, chemistry and pharmacology of *Sutherlandia frutescens* (Fabaceae). *Journal of Ethnopharmacology*, 119: 620–629. doi:10.1016/j.jep.2008.08.003.

- Xu, Y. 2016. Seven dimensions of light in regulating plant growth. *Acta Horticulturae*, 1134:56(445–452).
- Yang, Q., Pan, J., Shen, G. & Guo, B. 2019. Yellow light promotes the growth and accumulation of bioactive flavonoids in *Epimedium pseudowushanense*. *Journal of Photochemistry and Photobiology B: Biology*, 197.
- Yao, X., Li, C., Li, S., Zhu, Q., Zhang, H., Wang, H., Yu, C. Martin, S. K. & Xie, F. 2017. Effect of shade on leaf photosynthetic capacity, light-intercepting, electron transfer and energy distribution of soybeans. *Plant Growth Regulation*, 83(3):409–416.
- Zonyane, S., Chen, L., Xu, M.J., Gong, Z.N., Xu, S. & Makunga, N.P. 2019. Geographic-based metabolomic variation and toxicity analysis of *Sutherlandia frutescens* L. R.Br. – An emerging medicinal crop in South Africa. *Industrial Crops and Products*, 133:414–423.
- Zonyane, S., Fawole, O.A., Grange, C. la, Stander, M.A., Opara, U.L. & Makunga, N.P. 2020. The implication of chemotypic variation on the antioxidant and anti-cancer activities of *Sutherlandia frutescens* (L.) R.Br. (fabaceae) from different geographic locations. *Antioxidants*, 9(2).

APPENDIX:

7.1 Light intensities

Table 21: The statistical analysis of the light intensities under the different netting, with L1= outside, L2=Grey, L3= White, L4 = Green, on different times of the day, 10:00, 12:00, 15:00.

	L1 10:00	L1 12:00	L1 15:00	L2 10:00	L2 12:00	L2 15:00	L3 10:00	L3 12:00	L3 15:00	L4 10:00	L4 12:00	L4 15:00
L1 10:00												
L1 12:00	0,056											
L1 15:00	1,000	0,183										
L2 10:00	<0,001	<0,001	<0,001									
L2 12:00	0,002	<0,001	0,001	0,340								
L2 15:00	<0,001	<0,001	<0,001	1,000	0,280							
L3 10:00	<0,001	<0,001	<0,001	0,999	0,084	1,000						
L3 12:00	0,001	<0,001	<0,001	0,429	1,000	0,357	0,103					
L3 15:00	<0,001	<0,001	<0,001	1,000	0,600	1,000	0,973	0,620				
L4 10:00	<0,001	<0,001	<0,001	1,000	0,400	1,000	0,999	0,461	1,000			
L4 12:00	0,002	<0,001	<0,001	0,416	1,000	0,345	0,099	1,000	0,652	0,412		
L4 15:00	<0,001	<0,001	<0,001	0,076	<0,001	0,098	0,344	<0,001	0,033	0,070	<0,001	

7.2 Chlorophyll content:

Table 22: The statistical analysis of the chlorophyll content of *Lessertia frutescens* under different shading conditions. The first letter refers to the plant, the number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The number refers to the days that the plants were under laminated shade netting.

	AL1 60	AL1 120	AL2 60	AL2 120	AL3 60	AL3 120	AL4 60	AL4 120	BL1 60	BL1 120	BL2 60	BL2 120	BL3 60	BL3 120	BL4 60	BL4 120
AL1 60																
AL1 120	0,381															
AL2 60	<0,001	0,409														
AL2 120	<0,001	0,877	1,000													
AL3 60	<0,001	0,018	0,998	0,792												
AL3 120	<0,001	0,001	0,831	0,259	1,000											
AL4 60	<0,001	0,264	1,000	1,000	0,999	0,868										
AL4 120	<0,001	<0,001	0,586	0,105	0,999	1,000	0,615									
BL1 60	0,278	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001								
BL1 120	0,007	0,992	1,000	1,000	0,750	0,285	0,998	0,137	<0,001							
BL2 60	0,460	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	1,000	<0,001						
BL2 120	0,006	0,999	0,950	1,000	0,170	0,016	0,864	0,004	<0,001	1,000	<0,001					
BL3 60	0,610	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	1,000	<0,001	1,000	<0,001				
BL3 120	0,008	1,000	0,813	0,998	0,052	0,003	0,631	0,001	<0,001	1,000	<0,001	1,000	<0,001			
BL4 60	0,999	0,936	<0,001	0,003	<0,001	<0,001	<0,001	<0,001	0,002	0,076	0,002	0,072	0,004	0,100		
BL4 120	0,005	0,999	0,964	1,000	0,199	0,021	0,893	0,005	<0,001	1,000	<0,001	1,000	<0,001	1,000	0,056	

7.3 OJIP

7.3.1 Plant group A

Table 23: The statistical analysis of the O inclination point of *Lessertia frutescens*, plant group A, under different shading conditions. The number after the L refers to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The last number refers to the number of days that the plants were under laminated shade netting.

O point	L1 60	L2 60	L3 60	L4 60	L1 120	L2 120	L3 120	L4 120
L1 60								
L2 60	1.000							
L3 60	1.000	1.000						
L4 60	1.000	1.000	1.000					
L1 120	0.988	1.000	1.000	1.000				
L2 120	0.685	0.998	0.999	1.000	1.000			
L3 120	0.712	0.999	1.000	1.000	1.000	1.000		
L4 120	0.884	1.000	1.000	1.000	1.000	1.000	1.000	

Table 24: The statistical analysis of the K inclination point of *Lessertia frutescens*, plant group A, under different shading conditions. The number after the L refers to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The last number refers to the number of days that the plants were under laminated shade netting.

K point	L1 60	L2 60	L3 60	L4 60	L1 120	L2 120	L3 120	L4 120
L1 60								
L2 60	0.326							
L3 60	0.263	1.000						
L4 60	0.158	1.000	1.000					
L1 120	0.237	1.000	1.000	1.000				
L2 120	<0.001	0.611	0.753	0.930	1.000			
L3 120	<0.001	0.781	0.886	0.980	1.000	1.000		
L4 120	0.002	0.986	0.996	1.000	1.000	1.000	1.000	

Table 25: The statistical analysis of the J inclination point of *Lessertia frutescens*, plant group A, under different shading conditions. The number after the L refers to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The last number refers to the number of days that the plants were under laminated shade netting.

J point	L1 60	L2 60	L3 60	L4 60	L1 120	L2 120	L3 120	L4 120
L1 60								
L2 60	<0.001							
L3 60	0.001	1.000						
L4 60	0.000	1.000	1.000					
L1 120	0.061	1.000	1.000	1.000				
L2 120	<0.001	0.364	0.201	0.636	0.259			
L3 120	<0.001	0.706	0.491	0.906	0.509	1.000		
L4 120	<0.001	0.973	0.895	0.998	0.844	1.000	1.000	

Table 26: The statistical analysis of the I inclination point of *Lessertia frutescens*, plant group A, under different shading conditions. The number after the L refers to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The last number refers to the number of days that the plants were under laminated shade netting.

I point	L1 60	L2 60	L3 60	L4 60	L1 120	L2 120	L3 120	L4 120
L1 60								
L2 60	1.000							
L3 60	1.000	0.218						
L4 60	0.999	0.003	1.000					
L1 120	1.000	0.512	1.000	1.000				
L2 120	0.339	<0.001	0.404	0.999	0.969			
L3 120	0.914	<0.001	0.993	1.000	1.000	1.000		
L4 120	1.000	0.092	1.000	1.000	1.000	0.753	1.000	

Table 27: The statistical analysis of the P inclination point of *Lessertia frutescens*, plant group A, under different shading conditions. The number after the L refers to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The last number refers to the number of days that the plants were under laminated shade netting.

P point	L1 60	L2 60	L3 60	L4 60	L1 120	L2 120	L3 120	L4 120
L1 60								
L2 60	<0.001							
L3 60	<0.001	0.611						
L4 60	<0.001	<0.001	0.090					
L1 120	<0.001	<0.001	<0.001	0.104				
L2 120	<0.001	0.172	1.000	0.374	<0.001			
L3 120	<0.001	0.076	1.000	0.597	<0.001	1.000		
L4 120	<0.001	0.023	1.000	0.930	<0.001	1.000	1.000	

7.3.2 Plant group B

Table 28: The statistical analysis of the O inclination point of *Lessertia frutescens*, plant group B, under different shading conditions. The number after the L refers to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The last number refers to the number of days that the plants were under laminated shade netting.

O point	L1 60	L2 60	L3 60	L4 60	L1 120	L2 120	L3 120	L4 120
L1 60								
L2 60	1.000							
L3 60	1.000	1.000						
L4 60	1.000	1.000	1.000					
L1 120	1.000	1.000	1.000	1.000				
L2 120	1.000	1.000	1.000	1.000	1.000			
L3 120	1.000	1.000	1.000	1.000	1.000	1.000		
L4 120	1.000	1.000	1.000	1.000	1.000	1.000	1.000	

Table 29: The statistical analysis of the K inclination point of *Lessertia frutescens*, plant group B, under different shading conditions. The number after the L refers to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The last number refers to the number of days that the plants were under laminated shade netting.

K point	L1 60	L2 60	L3 60	L4 60	L1 120	L2 120	L3 120	L4 120
L1 60								
L2 60	0.999							
L3 60	0.919	1.000						
L4 60	0.990	1.000	1.000					
L1 120	0.932	1.000	1.000	1.000				
L2 120	1.000	1.000	1.000	1.000	1.000			
L3 120	1.000	1.000	0.992	1.000	0.998	1.000		
L4 120	0.969	1.000	1.000	1.000	1.000	1.000	0.999	

Table 30: The statistical analysis of the J inclination point of *Lessertia frutescens*, plant group B, under different shading conditions. The number after the L refers to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The last number refers to the number of days that the plants were under laminated shade netting.

J point	L1 60	L2 60	L3 60	L4 60	L1 120	L2 120	L3 120	L4 120
L1 60								
L2 60	0.983							
L3 60	0.336	0.999						
L4 60	0.766	1.000	1.000					
L1 120	0.254	0.992	1.000	1.000				
L2 120	1.000	1.000	0.916	1.000	0.864			
L3 120	1.000	1.000	0.419	0.958	0.461	1.000		
L4 120	0.795	1.000	1.000	1.000	1.000	1.000	0.977	

Table 31: The statistical analysis of the I inclination point of *Lessertia frutescens*, plant group B, under different shading conditions. The number after the L refers to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The last number refers to the number of days that the plants were under laminated shade netting.

I point	L1 60	L2 60	L3 60	L4 60	L1 120	L2 120	L3 120	L4 120
L1 60								
L2 60	<0.001							
L3 60	1.000	<0.001						
L4 60	0.011	1.000	0.003					
L1 120	1.000	<0.001	1.000	0.002				
L2 120	0.017	1.000	0.009	1.000	0.003			
L3 120	0.895	0.016	1.000	0.677	0.883	0.817		
L4 120	0.550	0.260	0.928	0.996	0.446	0.999	1.000	

Table 32: The statistical analysis of the P inclination point of *Lessertia frutescens*, plant group B, under different shading conditions. The number after the L refers to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The last number refers to the number of days that the plants were under laminated shade netting.

P point	L1 60	L2 60	L3 60	L4 60	L1 120	L2 120	L3 120	L4 120
L1 60								
L2 60	<0.001							
L3 60	1.000	<0.001						
L4 60	<0.001	0.990	<0.001					
L1 120	0.117	1.000	0.166	0.888				
L2 120	<0.001	0.425	<0.001	1.000	0.316			
L3 120	<0.001	1.000	<0.001	1.000	0.967	1.000		
L4 120	<0.001	0.556	<0.001	1.000	0.397	1.000	1.000	

7.3.3 The maximum quantum yield of PSII (F_v/F_m)

Table 33: The statistical analysis of the maximum quantum yield of PSII (F_v/F_m) of *Lessertia frutescens* under different shading conditions. The first letter refers to the plant, the number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The number refers to the days that the plants were under laminated shade netting.

	AL1 60	AL1 120	AL2 60	AL2 120	AL3 60	AL3 120	AL4 60	AL4 120	BL1 60	BL1 120	BL2 60	BL2 120	BL3 60	BL3 120	BL4 60	BL4 120
AL1 60																
AL1 120	<0,001															
AL2 60	<0,001	0,777														
AL2 120	<0,001	<0,001	0,010													
AL3 60	<0,001	0,999	0,994	<0,001												
AL3 120	<0,001	<0,001	0,011	1,000	<0,001											
AL4 60	<0,001	1,000	0,571	<0,001	1,000	<0,001										
AL4 120	<0,001	0,014	0,533	0,992	0,019	0,994	<0,001									
BL1 60	1,000	0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001								
BL1 120	<0,001	0,963	1,000	0,778	1,000	0,794	0,973	0,998	<0,001							
BL2 60	<0,001	0,096	0,960	0,823	0,221	0,848	0,013	1,000	<0,001	1,000						
BL2 120	<0,001	0,811	1,000	0,026	0,997	0,032	0,667	0,673	<0,001	1,000	0,982					
BL3 60	<0,001	1,000	0,974	<0,001	1,000	<0,001	1,000	0,014	<0,001	0,999	0,162	0,983				
BL3 120	<0,001	1,000	0,855	<0,001	1,000	<0,001	1,000	0,003	<0,001	0,994	0,059	0,896	1,000			
BL4 60	<0,001	0,101	0,963	0,839	0,238	0,862	0,015	1,000	<0,001	1,000	1,000	0,982	0,174	0,065		
BL4 120	<0,001	0,007	0,347	1,000	0,010	1,000	<0,001	1,000	<0,001	0,987	1,000	0,478	0,007	0,002	1,000	

7.3.4 The total performance index (PI_{TOTAL})

Table 34: The statistical analysis of the total performance index (PI_{TOTAL}) of *Lessertia frutescens* under different shading conditions. The first letter refers to the plant, the number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The number refers to the days that the plants were under laminated shade netting.

	AL1 60	AL1 120	AL2 60	AL2 120	AL3 60	AL3 120	AL4 60	AL4 120	BL1 60	BL1 120	BL2 60	BL2 120	BL3 60	BL3 120	BL4 60
AL1 60															
AL1 120	0,999														
AL2 60	0,004	0,378													
AL2 120	<0,001	<0,001	<0,001												
AL3 60	<0,001	0,075	1,000	<0,001											
AL3 120	<0,001	<0,001	0,002	0,639	0,077										
AL4 60	0,007	0,436	1,000	<0,001	1,000	0,002									
AL4 120	<0,001	0,001	0,493	0,006	0,980	0,931	0,522								
BL1 60	0,991	1,000	0,940	<0,001	0,642	0,002	0,954	0,109							
BL1 120	0,005	0,167	0,995	0,455	1,000	0,998	0,994	1,000	0,605						
BL2 60	0,748	1,000	0,480	<0,001	0,061	<0,001	0,571	<0,001	1,000	0,272					
BL2 120	0,079	0,891	1,000	<0,001	0,918	<0,001	1,000	0,098	0,999	0,901	0,987				
BL3 60	0,728	1,000	0,512	<0,001	0,070	<0,001	0,602	<0,001	1,000	0,287	1,000	0,988			
BL3 120	0,043	0,789	1,000	<0,001	0,979	<0,001	1,000	0,195	0,996	0,952	0,938	1,000	0,956		
BL4 60	0,028	0,695	1,000	<0,001	0,996	0,001	1,000	0,330	0,990	0,977	0,872	1,000	0,889	1,000	
BL4 120	<0,001	0,023	0,977	0,001	1,000	0,510	0,974	1,000	0,386	1,000	0,016	0,623	0,018	0,795	0,915

Table 35: The statistical analysis of the efficiency of light capturing (RC/ABS) of *Lessertia frutescens* under different shading conditions. The first letter refers to the plant, the number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The number refers to the days that the plants were under laminated shade netting.

	AL1 60	AL1 120	AL2 60	AL2 120	AL3 60	AL3 120	AL4 60	AL4 120	BL1 60	BL1 120	BL2 60	BL2 120	BL3 60	BL3 120	BL4 60
AL1 60															
AL1 120	<0,001														
AL2 60	1,000	<0,001													
AL2 120	<0,001	<0,001	<0,001												
AL3 60	0,999	<0,001	1,000	<0,001											
AL3 120	<0,001	<0,001	<0,001	1,000	<0,001										
AL4 60	1,000	<0,001	1,000	<0,001	1,000	<0,001									
AL4 120	<0,001	0,001	<0,001	0,987	<0,001	1,000	<0,001								
BL1 60	1,000	<0,001	1,000	<0,001	1,000	<0,001	1,000	<0,001							
BL1 120	1,000	<0,001	1,000	<0,001	1,000	<0,001	1,000	<0,001	1,000						
BL2 60	1,000	<0,001	1,000	<0,001	1,000	<0,001	1,000	<0,001	1,000	1,000					
BL2 120	<0,001	0,909	<0,001	0,001	<0,001	0,007	<0,001	0,627	<0,001	<0,001	<0,001				
BL3 60	1,000	<0,001	1,000	<0,001	1,000	<0,001	1,000	<0,001	1,000	1,000	1,000	<0,001			
BL3 120	<0,001	1,000	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	0,972	<0,001		
BL4 60	1,000	<0,001	1,000	<0,001	1,000	<0,001	1,000	<0,001	1,000	1,000	1,000	<0,001	1,000	<0,001	
BL4 120	<0,001	<0,001	<0,001	1,000	<0,001	1,000	<0,001	1,000	<0,001	<0,001	<0,001	0,377	<0,001	<0,001	<0,001

Table 36: The statistical analysis of the quantum efficiency of light trapping ($\phi_{Po}/(1 - \phi_{Po})$) of *Lessertia frutescens* under different shading conditions. The first letter refers to the plant, the number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The number refers to the days that the plants were under laminated shade netting.

	AL1 60	AL1 120	AL2 60	AL2 120	AL3 60	AL3 120	AL4 60	AL4 120	BL1 60	BL1 120	BL2 60	BL2 120	BL3 60	BL3 120	BL4 60
AL1 60															
AL1 120	<0,001														
AL2 60	<0,001	<0,001													
AL2 120	<0,001	<0,001	<0,001												
AL3 60	<0,001	<0,001	0,999	<0,001											
AL3 120	<0,001	<0,001	<0,001	1,000	<0,001										
AL4 60	<0,001	<0,001	0,275	<0,001	1,000	<0,001									
AL4 120	<0,001	<0,001	<0,001	0,998	<0,001	1,000	<0,001								
BL1 60	1,000	<0,001	<0,001	<0,001	<0,001	<0,001	0,008	<0,001							
BL1 120	<0,001	<0,001	0,938	<0,001	1,000	<0,001	1,000	<0,001	0,062						
BL2 60	<0,001	<0,001	1,000	<0,001	0,996	<0,001	0,188	<0,001	<0,001	0,896					
BL2 120	<0,001	0,647	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001				
BL3 60	<0,001	<0,001	0,987	<0,001	1,000	<0,001	1,000	<0,001	0,001	1,000	0,966	<0,001			
BL3 120	<0,001	1,000	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	1,000	<0,001		
BL4 60	<0,001	<0,001	0,985	<0,001	0,083	<0,001	<0,001	<0,001	<0,001	0,081	0,995	<0,001	0,039	<0,001	
BL4 120	<0,001	0,004	<0,001	<0,001	<0,001	0,008	<0,001	0,080	<0,001	<0,001	<0,001	0,986	<0,001	0,206	<0,001

Table 37: The statistical analysis of the conversion of trapped excited energy into electron transport ($\Psi_0/(1 - \Psi_0)$) of *Lessertia frutescens* under different shading conditions. The first letter refers to the plant, the number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The number refers to the days that the plants were under laminated shade netting.

	AL1 60	AL1 120	AL2 60	AL2 120	AL3 60	AL3 120	AL4 60	AL4 120	BL1 60	BL1 120	BL2 60	BL2 120	BL3 60	BL3 120	BL4 60
AL1 60															
AL1 120	<0,001														
AL2 60	1,000	<0,001													
AL2 120	<0,001	<0,001	<0,001												
AL3 60	1,000	<0,001	1,000	<0,001											
AL3 120	<0,001	<0,001	<0,001	1,000	<0,001										
AL4 60	1,000	<0,001	1,000	<0,001	1,000	<0,001									
AL4 120	<0,001	<0,001	<0,001	0,423	<0,001	1,000	<0,001								
BL1 60	1,000	<0,001	1,000	<0,001	1,000	<0,001	1,000	<0,001							
BL1 120	1,000	<0,001	1,000	<0,001	1,000	<0,001	1,000	<0,001	1,000						
BL2 60	1,000	<0,001	1,000	<0,001	1,000	<0,001	1,000	<0,001	1,000	1,000					
BL2 120	<0,001	1,000	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001				
BL3 60	1,000	<0,001	1,000	<0,001	1,000	<0,001	1,000	<0,001	1,000	1,000	1,000	<0,001			
BL3 120	<0,001	1,000	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	1,000	<0,001		
BL4 60	1,000	<0,001	1,000	<0,001	1,000	<0,001	1,000	<0,001	1,000	1,000	1,000	<0,001	1,000	<0,001	
BL4 120	<0,001	0,008	<0,001	<0,001	<0,001	0,002	<0,001	0,430	<0,001	<0,001	<0,001	0,598	<0,001	0,004	<0,001

Table 38: The statistical analysis of the performance index based on absorption (PI_{ABS}) of *Lessertia frutescens* under different shading conditions. The first letter refers to the plant, the number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The number refers to the days that the plants were under laminated shade netting.

	AL1 60	AL1 120	AL2 60	AL2 120	AL3 60	AL3 120	AL4 60	AL4 120	BL1 60	BL1 120	BL2 60	BL2 120	BL3 60	BL3 120	BL4 60
AL1 60															
AL1 120	0,525														
AL2 60	<0,001	0,043													
AL2 120	<0,001	<0,001	<0,001												
AL3 60	<0,001	0,504	1,000	<0,001											
AL3 120	<0,001	<0,001	0,010	1,000	<0,001										
AL4 60	<0,001	0,985	0,916	<0,001	1,000	<0,001									
AL4 120	<0,001	<0,001	0,520	0,715	0,026	1,000	<0,001								
BL1 60	1,000	0,997	0,001	<0,001	0,020	<0,001	0,169	<0,001							
BL1 120	0,025	1,000	0,998	<0,001	1,000	0,001	1,000	0,046	0,557						
BL2 60	0,004	1,000	0,349	<0,001	0,992	<0,001	1,000	<0,001	0,470	1,000					
BL2 120	0,003	1,000	0,718	<0,001	1,000	<0,001	1,000	<0,001	0,408	1,000	1,000				
BL3 60	0,036	1,000	0,090	<0,001	0,822	<0,001	1,000	<0,001	0,815	1,000	1,000	1,000			
BL3 120	0,143	1,000	0,020	<0,001	0,462	<0,001	0,993	<0,001	0,959	1,000	1,000	1,000	1,000		
BL4 60	<0,001	0,405	1,000	<0,001	1,000	<0,001	1,000	0,054	0,014	1,000	0,976	0,999	0,721	0,354	
BL4 120	<0,001	0,079	1,000	<0,001	1,000	0,039	0,963	0,721	0,002	0,999	0,524	0,829	0,181	0,051	1,000

Table 39: The statistical analysis of the probability to reduce end electron acceptors ($\delta_0/(1 - \delta_0)$) of *Lessertia frutescens* under different shading conditions. The first letter refers to the plant, the number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The number refers to the days that the plants were under laminated shade netting.

	AL1 60	AL1 120	AL2 60	AL2 120	AL3 60	AL3 120	AL4 60	AL4 120	BL1 60	BL1 120	BL2 60	BL2 120	BL3 60	BL3 120	BL4 60
AL1 60															
AL1 120	<0,001														
AL2 60	1,000	<0,001													
AL2 120	<0,001	<0,001	<0,001												
AL3 60	1,000	<0,001	1,000	<0,001											
AL3 120	<0,001	<0,001	<0,001	0,015	<0,001										
AL4 60	1,000	<0,001	1,000	<0,001	1,000	<0,001									
AL4 120	<0,001	<0,001	<0,001	<0,001	<0,001	0,743	<0,001								
BL1 60	1,000	0,024	1,000	<0,001	1,000	<0,001	1,000	<0,001							
BL1 120	1,000	0,001	1,000	<0,001	1,000	<0,001	1,000	<0,001	1,000						
BL2 60	1,000	<0,001	1,000	<0,001	1,000	<0,001	1,000	<0,001	1,000	1,000					
BL2 120	<0,001	0,044	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001				
BL3 60	1,000	<0,001	1,000	<0,001	1,000	<0,001	1,000	<0,001	1,000	1,000	1,000	<0,001			
BL3 120	<0,001	0,010	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	1,000	<0,001		
BL4 60	1,000	<0,001	1,000	<0,001	1,000	<0,001	1,000	<0,001	1,000	1,000	1,000	<0,001	1,000	<0,001	
BL4 120	<0,001	<0,001	<0,001	<0,001	<0,001	0,035	<0,001	1,000	<0,001	<0,001	<0,001	0,042	<0,001	0,172	<0,001

7.4 Per cross section

7.4.1 Plant group A

Table 40: The statistical analysis of the light absorption per cross section (ABS/CS_m) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days).

ABS/CS	L1 60	L1 120	L2 60	L2 120	L3 60	L3 120	L4 60	L4 120
L1 60								
L1 120	0.999							
L2 60	<0.001	<0.001						
L2 120	0.035	<0.001	0.274					
L3 60	0.014	<0.001	0.736	1.000				
L3 120	0.057	<0.001	0.320	1.000	1.000			
L4 60	0.956	0.212	<0.001	0.434	0.215	0.592		
L4 120	0.127	0.001	0.165	1.000	1.000	1.000	0.727	

Table 41: The statistical analysis of the heat dissipation per cross section (DI₀/CS_m) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days).

DI/CS	L1 60	L1 120	L2 60	L2 120	L3 60	L3 120	L4 60	L4 120
L1 60								
L1 120	<0.001							
L2 60	0.001	0.023						
L2 120	<0.001	1.000	<0.001					
L3 60	0.001	0.053	1.000	<0.001				
L3 120	<0.001	1.000	<0.001	1.000	<0.001			
L4 60	<0.001	0.606	0.922	0.001	0.987	0.001		
L4 120	<0.001	1.000	<0.001	0.999	<0.001	0.999	0.173	

Table 42: The statistical analysis of the excitation energy flux trapping per cross section (TR_0/CS_m) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days).

TR/CS	L1 60	L1 120	L2 60	L2 120	L3 60	L3 120	L4 60	L4 120
L1 60								
L1 120	1.000							
L2 60	<0.001	<0.001						
L2 120	<0.001	<0.001	0.987					
L3 60	<0.001	<0.001	0.663	1.000				
L3 120	<0.001	<0.001	0.983	1.000	1.000			
L4 60	0.145	0.385	<0.001	0.016	0.265	0.035		
L4 120	<0.001	<0.001	0.758	1.000	1.000	1.000	0.110	

Table 43: The statistical analysis of the electron flux transported per cross section (ET_0/CS_m) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days).

ET/CS	L1 60	L1 120	L2 60	L2 120	L3 60	L3 120	L4 60	L4 120
L1 60								
L1 120	0.588							
L2 60	<0.001	<0.001						
L2 120	<0.001	<0.001	1.000					
L3 60	<0.001	<0.001	0.205	0.326				
L3 120	<0.001	<0.001	1.000	1.000	0.677			
L4 60	<0.001	0.004	<0.001	<0.001	0.231	<0.001		
L4 120	<0.001	<0.001	0.954	0.987	0.999	1.000	0.009	

Table 44: The statistical analysis of the re-oxidation of electron acceptors per cross section (RE_0/CS_m) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days).

RE/CS	L1 60	L1 120	L2 60	L2 120	L3 60	L3 120	L4 60	L4 120
L1 60								
L1 120	1.000							
L2 60	<0.001	<0.001						
L2 120	<0.001	<0.001	0.061					
L3 60	<0.001	<0.001	1.000	0.204				
L3 120	<0.001	<0.001	0.807	0.986	0.993			
L4 60	<0.001	0.002	0.839	<0.001	0.451	0.008		
L4 120	<0.001	<0.001	1.000	0.013	1.000	0.582	0.985	

7.4.2 Plant group B

Table 45: The statistical analysis of the light absorption per cross section (ABS/CS_m) of *Lessertia frutescens*, Plant group B, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days).

ABS/CS	L1 60	L1 120	L2 60	L2 120	L3 60	L3 120	L4 60	L4 120
L1 60								
L1 120	0.124							
L2 60	0.002	1.000						
L2 120	<0.001	0.946	0.963					
L3 60	0.998	0.564	<0.001	<0.001				
L3 120	<0.001	1.000	1.000	1.000	<0.001			
L4 60	0.001	1.000	1.000	0.999	<0.001	1.000		
L4 120	<0.001	0.957	0.991	1.000	<0.001	1.000	0.999	

Table 46: The statistical analysis of the heat dissipation per cross section (DI_0/CS_m) of *Lessertia frutescens*, Plant group B, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days).

DI/CS	L1 60	L1 120	L2 60	L2 120	L3 60	L3 120	L4 60	L4 120
L1 60								
L1 120	0.674							
L2 60	0.102	1.000						
L2 120	0.991	0.969	0.408					
L3 60	0.005	1.000	0.994	0.004				
L3 120	1.000	0.652	0.014	1.000	<0.001			
L4 60	0.175	1.000	1.000	0.516	0.965	0.045		
L4 120	0.143	1.000	1.000	0.422	0.983	0.029	1.000	

Table 47: The statistical analysis of the excitation energy flux trapping per cross section (TR_0/CS_m) of *Lessertia frutescens*, Plant group B, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days).

TR/CS	L1 60	L1 120	L2 60	L2 120	L3 60	L3 120	L4 60	L4 120
L1 60								
L1 120	0.006							
L2 60	<0.001	1.000						
L2 120	<0.001	0.984	0.999					
L3 60	0.689	0.595	<0.001	<0.001				
L3 120	<0.001	1.000	1.000	0.996	<0.001			
L4 60	<0.001	1.000	1.000	1.000	<0.001	1.000		
L4 120	<0.001	0.913	0.984	1.000	<0.001	0.914	0.997	

Table 48: The statistical analysis of the electron flux transported per cross section (ET_0/CS_m) of *Lessertia frutescens*, Plant group B, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days).

ET/CS	L1 60	L1 120	L2 60	L2 120	L3 60	L3 120	L4 60	L4 120
L1 60								
L1 120	<0.001							
L2 60	1.000	<0.001						
L2 120	<0.001	1.000	<0.001					
L3 60	1.000	<0.001	1.000	<0.001				
L3 120	<0.001	1.000	<0.001	0.885	<0.001			
L4 60	0.955	<0.001	0.904	<0.001	0.295	<0.001		
L4 120	<0.001	0.996	<0.001	0.989	<0.001	0.076	<0.001	

Table 49: The statistical analysis of the re-oxidation of electron acceptors per cross section (RE_0/CS_m) of *Lessertia frutescens*, Plant group B, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days).

RE/CS	L1 60	L1 120	L2 60	L2 120	L3 60	L3 120	L4 60	L4 120
L1 60								
L1 120	0.070							
L2 60	1.000	0.013						
L2 120	0.155	0.994	0.026					
L3 60	1.000	0.001	0.999	<0.001				
L3 120	0.005	1.000	<0.001	0.973	<0.001			
L4 60	0.822	0.575	0.692	0.975	0.071	0.124		
L4 120	0.001	1.000	<0.001	0.791	<0.001	1.000	0.053	

7.5 Per reaction center

7.5.1 Plant group A

Table 50: The statistical analysis of the light absorption per reaction centre (ABS/RC) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days).

ABS/RC	L1 60	L1 120	L2 60	L2 120	L3 60	L3 120	L4 60	L4 120
L1 60								
L1 120	<0.001							
L2 60	<0.001	1.000						
L2 120	<0.001	0.020	<0.001					
L3 60	<0.001	1.000	1.000	<0.001				
L3 120	<0.001	0.087	<0.001	1.000	0.011			
L4 60	<0.001	1.000	1.000	<0.001	1.000	0.001		
L4 120	<0.001	0.517	0.012	0.953	0.210	1.000	0.139	

Table 51: The statistical analysis of the heat dissipation per reaction centre (DI₀/RC) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days).

DI/RC	L1 60	L1 120	L2 60	L2 120	L3 60	L3 120	L4 60	L4 120
L1 60								
L1 120	<0.001							
L2 60	<0.001	1.000						
L2 120	<0.001	0.010	0.001					
L3 60	<0.001	1.000	1.000	0.001				
L3 120	<0.001	0.024	0.003	1.000	0.007			
L4 60	<0.001	1.000	1.000	<0.001	1.000	<0.001		
L4 120	<0.001	0.248	0.149	0.993	0.163	1.000	0.041	

Table 52: The statistical analysis of the excitation energy flux trapping per reaction centre (TR₀/RC) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days).

	L1 60	L1 120	L2 60	L2 120	L3 60	L3 120	L4 60	L4 120
L1 60								
L1 120	<0.001							
L2 60	<0.001	1.000						
L2 120	<0.001	0.037	<0.001					
L3 60	<0.001	1.000	0.997	<0.001				
L3 120	<0.001	0.174	<0.001	1.000	0.018			
L4 60	<0.001	1.000	1.000	<0.001	1.000	0.005		
L4 120	<0.001	0.695	0.005	0.929	0.275	1.000	0.249	

Table 53: The statistical analysis of the electron flux transported per reaction centre (ET₀/RC) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days).

	L1 60	L1 120	L2 60	L2 120	L3 60	L3 120	L4 60	L4 120
L1 60								
L1 120	0.056							
L2 60	0.994	<0.001						
L2 120	0.001	1.000	<0.001					
L3 60	1.000	0.101	0.086	<0.001				
L3 120	0.013	1.000	<0.001	1.000	0.008			
L4 60	0.995	0.270	0.020	0.002	1.000	0.056		
L4 120	0.153	0.999	<0.001	0.850	0.227	1.000	0.615	

Table 54: The statistical analysis of the re-oxidation of electron acceptors per reaction centre (RE_o/RC) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days).

RE/RC	L1 60	L1 120	L2 60	L2 120	L3 60	L3 120	L4 60	L4 120
L1 60								
L1 120	0.204							
L2 60	0.999	<0.001						
L2 120	1.000	0.001	1.000					
L3 60	0.897	<0.001	1.000	0.997				
L3 120	1.000	0.010	0.998	1.000	0.724			
L4 60	1.000	0.003	1.000	1.000	0.967	1.000		
L4 120	1.000	0.311	0.305	0.510	0.022	0.978	0.818	

7.5.2 Plant group B

Table 55: The statistical analysis of the light absorption per reaction centre (ABS/RC) of *Lessertia frutescens*, Plant group B, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days).

	L1 60	L1 120	L2 60	L2 120	L3 60	L3 120	L4 60	L4 120
L1 60								
L1 120	<0.001							
L2 60	<0.001	0.991						
L2 120	<0.001	0.708	0.999					
L3 60	<0.001	0.850	1.000	1.000				
L3 120	0.006	0.252	0.689	1.000	0.996			
L4 60	<0.001	1.000	1.000	0.896	0.983	0.243		
L4 120	<0.001	1.000	0.925	0.180	0.384	0.007	1.000	

Table 56: The statistical analysis of the heat dissipation per reaction centre (DI_0/RC) of *Lessertia frutescens*, Plant group B, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days).

DI/RC	L1 60	L1 120	L2 60	L2 120	L3 60	L3 120	L4 60	L4 120
L1 60								
L1 120	<0.001							
L2 60	<0.001	1.000						
L2 120	<0.001	0.995	0.997					
L3 60	<0.001	0.966	0.932	1.000				
L3 120	<0.001	0.683	0.333	0.993	1.000			
L4 60	<0.001	1.000	1.000	0.960	0.784	0.169		
L4 120	<0.001	1.000	0.991	0.324	0.118	0.005	1.000	

Table 57: The statistical analysis of the excitation energy flux trapping per reaction centre (TR_0/RC) of *Lessertia frutescens*, Plant group B, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days).

	L1 60	L1 120	L2 60	L2 120	L3 60	L3 120	L4 60	L4 120
L1 60								
L1 120	<0.001							
L2 60	0.001	0.926						
L2 120	0.020	0.473	1.000					
L3 60	0.004	0.795	1.000	1.000				
L3 120	0.108	0.160	0.856	1.000	0.988			
L4 60	<0.001	0.997	1.000	0.877	0.998	0.327		
L4 120	<0.001	1.000	0.879	0.164	0.604	0.013	1.000	

Table 58: The statistical analysis of the electron flux transported per reaction centre (ET₀/RC) of *Lessertia frutescens*, Plant group B, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days).

	L1 60	L1 120	L2 60	L2 120	L3 60	L3 120	L4 60	L4 120
L1 60								
L1 120	1.000							
L2 60	0.775	0.876						
L2 120	0.383	0.518	1.000					
L3 60	1.000	1.000	0.894	0.335				
L3 120	0.377	0.511	1.000	1.000	0.337			
L4 60	0.979	0.994	1.000	0.955	1.000	0.951		
L4 120	1.000	1.000	0.806	0.236	1.000	0.228	0.999	

Table 59: The statistical analysis of the re-oxidation of electron acceptors per reaction centre (RE₀/RC) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days).

RE/RC	L1 60	L1 120	L2 60	L2 120	L3 60	L3 120	L4 60	L4 120
L1 60								
L1 120	1.000							
L2 60	<0.001	0.001						
L2 120	0.957	1.000	<0.001					
L3 60	0.003	0.038	0.967	0.009				
L3 120	0.997	0.862	<0.001	0.001	<0.001			
L4 60	0.014	0.125	0.717	0.075	1.000	<0.001		
L4 120	0.987	1.000	<0.001	1.000	0.003	0.003	0.034	

7.6 The parameters of the 820 nm modulated reflection

7.6.1.1 Plant group A

Table 60: The statistical analysis of the re-oxidation of the MR_0/MR_{min} ratio of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days).

	L1 60	L1 120	L2 60	L2 120	L3 60	L3 120	L4 60	L4 120
L1 60								
L1 120	0,406							
L2 60	0,071	1,000						
L2 120	<0,001	<0,001	<0,001					
L3 60	<0,001	0,637	0,195	0,076				
L3 120	<0,001	<0,001	<0,001	0,238	<0,001			
L4 60	0,003	0,999	0,996	<0,001	0,962	<0,001		
L4 120	<0,001	0,184	0,009	0,601	1,000	<0,001	0,373	

Table 61: The statistical analysis of the fast phase (V_{ox}) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days).

Vox	L1 60	L1 120	L2 60	L2 120	L3 60	L3 120	L4 60	L4 120
L1 60								
L1 120	0,892							
L2 60	0,729	0,001						
L2 120	1,000	0,432	0,353					
L3 60	0,195	<0,001	0,999	0,016				
L3 120	0,869	0,003	1,000	0,609	0,989			
L4 60	0,667	0,001	1,000	0,281	1,000	1,000		
L4 120	0,832	0,002	1,000	0,531	0,995	1,000	1,000	

Table 62: The statistical analysis of the slow phase (V_{re-red}) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days).

Vre-red	L1 60	L1 120	L2 60	L2 120	L3 60	L3 120	L4 60	L4 120
L1 60								
L1 120	<0.001							
L2 60	0,711	<0.001						
L2 120	0,682	<0.001	1,000					
L3 60	0,014	<0.001	0,626	0,668				
L3 120	0,973	<0.001	1,000	1,000	0,124			
L4 60	0,840	<0.001	1,000	1,000	0,432	1,000		
L4 120	1,000	<0.001	0,294	0,260	<0.001	0,875	0,496	

7.6.1.2 Plant group B

Table 63: The statistical analysis of the re-oxidation of the MR_0/MR_{min} ratio of *Lessertia frutescens*, Plant group B, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days).

	L1 60	L1 120	L2 60	L2 120	L3 60	L3 120	L4 60	L4 120
L1 60								
L1 120	<0.001							
L2 60	0,001	<0.001						
L2 120	<0.001	0,536	<0.001					
L3 60	0,001	0,001	1,000	0,071				
L3 120	0,001	0,001	1,000	0,075	1,000			
L4 60	<0.001	0,645	0,001	1,000	0,090	0,094		
L4 120	<0.001	1,000	<0.001	0,731	<0.001	<0.001	0,866	

Table 64: The statistical analysis of the fast phase (V_{ox}) of *Lessertia frutescens*, Plant group B, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days).

Vox	L1 60	L1 120	L2 60	L2 120	L3 60	L3 120	L4 60	L4 120
L1 60								
L1 120	0,093							
L2 60	0,006	1,000						
L2 120	<0.001	1,000	0,954					
L3 60	0,920	0,813	0,248	0,003				
L3 120	0,454	0,996	0,938	0,142	1,000			
L4 60	<0.001	0,972	0,250	0,996	<0.001	0,007		
L4 120	<0.001	0,963	0,206	0,993	<0.001	0,006	1,000	

Table 65: The statistical analysis of the slow phase (V_{re-red}) of *Lessertia frutescens*, Plant group A, under different shading conditions. The number after the L to the shading condition, with L1= outside, L2=Grey, L3= White, L4 = Green. The second number refers to the days that the plants were under laminated shade netting (60 or 120 days).

Vre-red	L1 60	L1 120	L2 60	L2 120	L3 60	L3 120	L4 60	L4 120
L1 60								
L1 120	1,000							
L2 60	1,000	1,000						
L2 120	0,988	0,950	0,288					
L3 60	1,000	1,000	0,999	0,071				
L3 120	1,000	1,000	1,000	0,996	0,937			
L4 60	1,000	0,999	0,924	1,000	0,460	1,000		
L4 120	1,000	1,000	0,986	1,000	0,654	1,000	1,000	