Effects of CO_2 concentration
on photosynthesis, transpiration and production of greenhouse fruit vegetable crops

Promotor: Dr. ir. H. Challa
hoogleraar in de Tuinbouwplantenteelt
in het bijzonder de beschermde teelt

Stellingen

1 Verbreding en verbetering van de CO_2-toediening in kassen zal de produktie van de Nederlandse glastuinbouw met miljoenen guldens per jaar verhogen.

2 Stomata have been delegated the task of providing food while preventing thirst.
(Raschke, 1976).

3 Iedere 100 μmol mol^{-1} verhoging van de CO_2-concentratie in het trajekt van 200 tot 1200 μmol mol^{-1} leidt bij tomaat, komkommer en paprika planten tot 3 à 4% en bij aubergine tot circa 11% verlaging van de stomataire geleidbaarheid.
(Dit proefschrift).

4 In een niet geventileerde kas heeft CO_2-dosering slechts beperkte invloed op de verdamping van een gewas. (Dit proefschrift).

5 Het vóórkomen van een 'midday depression' bij een gewas in een kas is grotendeels te verklaren uit het dagelijks verloop van de omgevingsfaktoren. (Dit proefschrift).

6 Verhoging van de CO_2-concentratie veroorzaakt een verschuiving van allocatie van biomassa ten gunste van de vruchten, alleen indien CO_2-toediening een toename van het aantal vruchten aan de plant teweeg brengt. (Dit proefschrift).

7 Het effekt van de CO_2-concentratie op de produktie van vruchtgroentengewassen laat zich goed beschrijven door de vuistregel $X = 1.5 * (1000/C)^2$, met C de CO_2 concentratie in μmol mol^{-1} en X de relatieve verhoging van de produktie in % per 100 μmol mol^{-1} CO_2. (Dit proefschrift).

8 Pulsgewijs (of intermitterend) doseren heeft geen aantoonbare voordelen boven continu doseren. (Dit proefschrift).

9 Gezien de sterke fluctuatie van de CO_2-concentratie van de buitenlucht, zou optimalisatie van de CO_2-dosering gebaseerd moeten worden op momentane, lokale meting van de CO_2-concentratie buiten de kas.

10 Het is gewenst bij optimalisatie van de CO_2-dosering niet alleen economische, maar vooral ook ecologische doelstellingen na te streven; in de praktijk is dit te realiseren door aan de ecologische effekten economische konsekwenties te verbinden.

11 Als maatregelen ter beperking van het toenemend broeikaseffekt worden uitgesteld tot de modellen hieromtrent gelijkluidende resultaten geven, zullen de thans waarneembare tendensen tot veranderingen in het klimaat statistisch significant worden.

12 Het milieu zou ermee gediend zijn als de Landbouwuniversiteit aan landbouwkundige promovendi de titel 'Doctor in de Landbouw- en Milieuwetenschappen' pas verleende nadat zij ook in de milieuwetenschappen een proeve van bekwaamheid hadden afgelegd.

13 Het feit dat de Nefyto (Nederlandse Stichting voor Fytopharmacie) sterk pleit voor een Europese regeling van bestrijdingsmiddelengebruik (Platform, oktober 1993) is een teken van geringe fiducie in de besluitvaardigheid van de EG.

14 De toenemende commercialisering en daaruit voortvloeiende eigen profilering van onderzoeksinstanties en voorlichtinggevende organisaties verzwakt het landbouwkennissysteem en daarmee de ontwikkeling van de Nederlandse land- en tuinbouw.

15 Door de schaalvergroting in het basisonderwijs gaan minder kinderen lopend of fietsend naar school: gewenning van de schooljeugd aan het dagelijks gebruik van de auto is schadelijk voor de volksgezondheid en voor het verkeersbeeld in de toekomst.

Stellingen behorend bij het proefschrift
'Effects of CO_2 concentration on photosynthesis, transpiration and production of greenhouse fruit crops', door Elly M. Nederhoff. Wageningen, 25 oktober 1994.

Elly M. Nederhoff

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on photosynthesis, transpiration and production of greenhouse fruit vegetable crops

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Abstract

Nederhoff E.M., 1994. Effects of CO_2 concentration on photosynthesis, transpiration and production of greenhouse fruit vegetable crops. Dissertation. Agricultural University, Wageningen, The Netherlands. Summaries in English and Dutch. 213 p.

The effect of the CO_2 concentration of the greenhouse air (C) in the range 200 to 1100 µmol mol^{-1} was investigated in tomato (*Lycopersicon esculentum* Mill.), cucumber (*Cucumis sativus* L.), sweet pepper (*Capsicum annuum* L.) and eggplant (*Solanum melongena* L.), grown in greenhouses.

The effect of C on canopy net photosynthetic CO_2 assimilation rate (or photosynthesis, P) was expressed by a set of regression equations, relating P to PAR, C and LAI. A rule of thumb ('CO_2-rule') was derived, approximating the relative increase of P caused by additional CO_2 at a certain C. This CO_2-rule is: $X = (1000/C)^2 * 1.5$ (X in % per 100 µmol mol^{-1}, and C in µmol mol^{-1}). Two models for canopy photosynthesis were examined by comparing them with the experimental photosynthesis data. No 'midday depression' in P was observed.

The effects of C on leaf conductance (*g*) and on rate of crop transpiration (*E*) were investigated. An increase of 100 µmol mol^{-1} in C reduced *g* by about 3-4% in sweet pepper, tomato and cucumber and by about 11% in eggplant. The effect of C on *E* was analyzed by combining the regression equation for *g* with the Penman-Monteith equation for *E*. C had only a relatively small effect on *E*, owing to thermal and hydrological feedback effects. The decoupling of *g* and *E* was quantified. No time-dependent variation or 'midday depression' in *E* was observed, and no significant effect of C on average leaf temperature was established.

In five experiments, the effect of C on growth and production and on specific features were analyzed: light use efficiency was increased by about 10 to 15% per 100 µmol mol^{-1} increase in C; fruit set of sweet pepper was greatly increased by high C; allocation of biomass to fruits was increased by high C in sweet pepper and cucumber; specific leaf area (SLA) was reduced by 15 to 20% at 150 to 250 µmol mol^{-1} increase in C (except in cucumber); dry matter content (DMC) of vegetative organs slightly increased at high C (also not in cucumber); fruit production (dry weight) was most affected by C in sweet pepper; fresh weight fruit production per unit CO_2 was highest in cucumber; fruit quality was not influenced by C. High C promoted the 'short leaves syndrome' in tomato and 'leaf tip chlorosis' in eggplant, probably related to calcium and boron translocation, respectively. The observed effect of C on production was larger than expected on the basis of the CO_2-rule. Intermittent CO_2 supply (ICS) could under normal ventilation accomplish only a limited increase in average C, and hence a limited increase in production. No physiological advantages of ICS were revealed.

Key words: carbon dioxide, CO_2 concentration, conductance, cucumber, decoupling, eggplant, feedback, glasshouse, greenhouse, growth, photosynthesis, production, stomata, sweet pepper, tomato, transpiration.

Opgedragen aan mijn vader
en aan de nagedachtenis van mijn moeder

Voorwoord

Een proefschrift schrijven was voor mij al jaren een wens en in het najaar van 1990 kreeg ik de gelegenheid dit voornemen uit te voeren. Bij mijn onderzoek op het Proefstation in Naaldwijk aan licht, ventilatie, fotosynthese en groei was vanzelf 'CO_2' komen bovendrijven, en de verzamelde informatie leende zich goed voor een proefschrift. Er moesten nog een aantal experimenten worden verricht, maar daarvoor wist ik mij verzekerd van uitstekende assistentie in de persoon van Erik de Ruiter. Als promotor stelde ik me voor Prof. Challa in Wageningen, en gelukkig bleek hij bereid die taak op zich te nemen. Toen ook thuis de randvoorwaarden in orde waren, ben ik eind 1990 gaan schrijven: eerst artikelen voor wetenschappelijke tijdschriften en vervolgens de ontbrekende stukken om een proefschrift samen te stellen.

Met genoegen en voldoening kijk ik nu terug op de afgelopen vier jaar, waarin dit boekje tot stand is gekomen. Graag wil ik op deze plaats eenieder bedanken, zonder wie het nooit zou zijn geworden wat het nu is.

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Alle experimenten voor dit proefschrift zijn verricht op het Proefstation voor Tuinbouw onder Glas in Naaldwijk. Het bestuur, de direktie en het voormalig direktieteam (de heren Kooistra en van Oosten) van het Proefstation ben ik zeer erkentelijk dat ze mij tijd en gelegenheid hebben gegeven om te promoveren. Ook dank ik Gerard Welles (hoofd van de afdeling Teelt en Kasklimaat) en Sjaak Bakker (hoofd van de sektie Kasklimaat) voor het in mij gestelde vertrouwen.

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Het hier beschreven onderzoek is, zoals al het werk van het Proefstation, uitgevoerd met als doel de Nederlandse glastuinbouw verder te ontwikkelen. Ik hoop dat de glastuinbouw sterk en op een gezonde basis zal kontinueren. Mijn wens is dat de resultaten van mijn werk mogen bijdragen aan het optimaliseren van het telen onder glas.

Maasdijk, september 1994

Contents

ABSTRACT V

VOORWOORD VII

CONTENTS XI

ACCOUNT XV

APPENDICES XVII
- I Acronyms XVII
- II Symbols and parameters with values and units XVII
- III Unit conversions XX

1 GENERAL INTRODUCTION 1
- 1.1 FIELD OF RESEARCH 1
- 1.2 CO_2 ENRICHMENT IN PRACTICE 5
  - 1.2.1 Carbon dioxide properties and units 5
  - 1.2.2 History of CO_2 enrichment and related research 5
  - 1.2.3 Current methods of CO_2 enrichment 6
  - 1.2.4 Harmful gases 8
  - 1.2.5 Rate of CO_2 supply in greenhouses 9
  - 1.2.6 Control and strategy of CO_2 supply 10
- 1.3 DESCRIPTION AND ANALYSIS OF THE PROBLEM 12
  - 1.3.1 Problem description 12
  - 1.3.2 Problem analysis 13
- 1.4 RESEARCH QUESTIONS, OBJECTIVES AND APPROACH 15
  - 1.4.1 Restrictions to the research 15
  - 1.4.2 Research questions 16
  - 1.4.3 Objectives of the thesis 16
  - 1.4.4 Approach and structure of the thesis 17

2 EFFECTS OF CO_2 ON CANOPY NET PHOTOSYNTHESIS 19
- 2.1 INTRODUCTION 19
  - 2.1.1 Outline of the problem 19
  - 2.1.2 Objectives 21
  - 2.1.3 Approach and structure of the chapter 21
- 2.2 MATERIALS AND METHODS 22
  - 2.2.1 Crops, greenhouses and environmental factors 22
  - 2.2.2 Principle of the photosynthesis measurements 24
  - 2.2.3 Photosynthesis measurements 25
  - 2.2.4 Data processing 27
- 2.3 ACCURACY OF THE MEASUREMENTS 28
  - 2.3.1 Type of errors 28

2.3.2 Incidental errors in the measurement 28
2.3.3 Regular measuring error 29
2.3.4 Conclusion 31
2.4 REGRESSION MODEL AND CHARACTERISTICS OF CANOPY PHOTOSYNTHESIS 32
2.4.1 Regression model 32
2.4.2 Regression models fitted to the measurements 33
2.4.3 Characteristics of canopy photosynthesis 34
2.4.4 Remarks and conclusions 37
2.5 TIME-DEPENDENT VARIATION IN PHOTOSYNTHESIS 38
2.5.1 Midday depression 38
2.5.2 Approach 38
2.5.3 Results 38
2.6 A 'CO_2-RULE' DERIVED FROM THE MEASUREMENTS 41
2.6.1 Introduction 41
2.6.2 Approach 41
2.6.3 Results and discussion 43
2.7 TEST OF TWO EXISTING MODELS 44
2.7.1 Models, parameters and units 44
2.7.2 Modified Acock model 45
2.7.3 Modified Thornley model 46
2.7.4 Procedure 47
2.7.5 Results 49
2.7.6 Discussion 52
2.8 DISCUSSION 55

3 EFFECTS OF CO_2 ON LEAF CONDUCTANCE AND CANOPY TRANSPIRATION 57
3.1 INTRODUCTION 57
3.1.1 Outline of the problem 57
3.1.2 Stomata 59
3.1.3 Other conductances 59
3.1.4 Diffusion 60
3.1.5 Units of conductance 60
3.1.6 Stomatal action 60
3.1.7 Relation between conductance and transpiration 61
3.1.8 Decoupling factor 62
3.1.9 Leaf temperature 63
3.2 OBJECTIVES AND APPROACH 64
3.2.1 Objectives 64
3.2.2 Experimental approach 64
3.3 METHODOLOGY 66
3.3.1 Set-up of experiments 66
3.3.2 Measuring conductance 68
3.3.3 Measuring transpiration 68

3.3.4 Analysis of conductance measurements . . . 69
3.3.5 Analysis of transpiration measurements (Penman-Monteith model) . 70
3.3.6 Coupling between conductance and transpiration . . . 72
3.3.7 Data processing . . . 72
3.4 EFFECTS OF CO_2 ON LEAF CONDUCTANCE AND CANOPY TRANSPIRATION OF SWEET PEPPER . . . 74
3.4.1 Materials and methods . . . 74
3.4.2 Results . . . 76
3.4.3 Discussion . . . 81
3.5 EFFECTS OF CO_2 ON CONDUCTANCE AND TRANSPIRATION OF TOMATO AND CUCUMBER . . . 84
3.5.1 Materials and methods . . . 84
3.5.2 Results . . . 86
3.5.3 Discussion . . . 90
3.6 EFFECTS OF CO_2 ON LEAF CONDUCTANCE AND ESTIMATED CANOPY TRANSPIRATION OF EGGPLANT . . . 93
3.6.1 Materials and methods . . . 93
3.6.2 Results . . . 96
3.6.3 Discussion . . . 96
3.7 SYNTHESIS AND DISCUSSION . . . 102
3.7.1 Effects of CO_2 concentration on leaf conductance . . . 102
3.7.2 Calculated effect of CO_2 concentration on transpiration (decoupling) 102
3.7.3 Time-dependent variation in transpiration (midday depression) 105
3.7.4 Consequences of stomatal closure for leaf temperature . . . 106
3.7.5 Approach and results considered . . . 107

4 EFFECTS OF CO_2 CONCENTRATION ON GROWTH AND PRODUCTION . . . 109
4.1 INTRODUCTION . . . 109
4.1.1 Outline of the problem and approach for research . . . 109
4.1.2 Growth rate (GR) and light use efficiency (LUE) . . . 110
4.1.3 Fruit set . . . 110
4.1.4 Allocation of dry matter . . . 111
4.1.5 Fruit properties . . . 112
4.1.6 Morphological adaptations to high CO_2 . . . 112
4.1.7 Nutrient concentration in leaves . . . 113
4.1.8 Intermittent CO_2 supply (ICS) . . . 114
4.2 OBJECTIVES AND EXPERIMENTS . . . 115
4.2.1 Objectives . . . 115
4.2.2 Experiments . . . 115
4.3 EFFECTS OF CONTINUOUS AND INTERMITTENT CO_2 SUPPLY ON GROWTH AND PRODUCTION OF CUCUMBER . . . 116
4.3.1 Growth and production of cucumber at two CO_2 concentrations (cucumber experiment 1) . . . 116
4.3.2 Production of cucumber under continuous or intermittent CO_2 (cucumber experiment 2) . . . 120

4.4 EFFECTS OF CONTINUOUS AND INTERMITTENT CO_2 SUPPLY ON FRUIT SET AND YIELD OF SWEET PEPPER 124
4.4.1 Introduction 124
4.4.2 Materials and methods 124
4.4.3 Results 125
4.5 EFFECTS OF CO_2, PLANT DENSITY AND PRUNING ON LEAF DEFORMATION (SLS) AND FRUIT PRODUCTION OF TOMATO 130
4.5.1 Introduction 130
4.5.2 Materials and methods 132
4.5.3 Results 136
4.5.4 Discussion 137
4.6 EFFECTS OF CO_2 ON LEAF TIP CHLOROSIS (LTC) AND FRUIT PRODUCTION OF EGGPLANT 143
4.6.1 Introduction 143
4.6.2 Materials and methods 144
4.6.3 Results 146
4.6.4 Discussion 150
4.7 SYNTHESIS AND DISCUSSION 153
4.7.1 Structure 153
4.7.2 Growth and production of cucumber 155
4.7.3 Growth and production of sweet pepper 155
4.7.4 Growth and production of tomato 157
4.7.5 Hypothesis explaining species-specific features 159
4.7.6 Growth rate (GR) and light use efficiency (LUE) 161
4.7.7 Fruit set 162
4.7.8 Allocation of dry matter 163
4.7.9 Morphological characteristics 164
4.7.10 Fruit production 166
4.7.11 Fruit properties 168

5 FINAL DISCUSSION 171
5.1 RESULTS CONSIDERED 171
5.2 THE 'CO_2-RULE' 172
5.3 SIDE EFFECTS OF CO_2 ENRICHMENT 176
5.4 INTERMITTENT CO_2 SUPPLY (ICS) 178
5.5 EVALUATION OF THE APPROACH 180

RECOMMENDATIONS FOR PRACTICAL HORTICULTURE 183

SUMMARY 187

SAMENVATTING 193

REFERENCES 199

CURRICULUM VITAE 217

Account

The chapters 2, 3 and 4 are compilations of published articles, which were enhanced when necessary and reorganized to fit into the structure of the thesis.

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Appendices

Appendix I *Acronyms*

acronym	meaning	units
CER	carbon dioxide exchange rate	
DM	dry matter	--
CP	compensation point	
DMC	dry matter content	%
ICS	intermittent CO_2 supply	--
LAI	leaf area index, leaf area per area cropped ground area	$m^2\ m^{-2}$
LAR	leaf area ratio (leaf area per plant dry weight)	$cm^2\ g^{-1}$
LTC	leaf tip chlorosis	--
LUE	light use efficiency in relation to photosynthesis	$\mu g\ J^{-1}$
	or quantum yield	$mol\ mol^{-1}$
LUE	light use efficiency in relation to dry matter production	$g\ MJ^{-1}$
PAR	photosynthetically active radiation (400-700 nm), light energy flux	$W\ m^{-2}$
	or photosynthetically active radiation, photon flux	$\mu mol\ m^{-2}\ s^{-1}$
PRESS	predicted error sum of squares	--
RH	relative humidity	%
SLA	specific leaf area	$cm^2\ g^{-1}$
SLS	short leaf syndrome	--
VPD	vapour pressure deficit	kPa

Appendix II *Symbols and parameters with values and units*

symbol	meaning [value]	units
a	constant relating model and measurement	
a_A	constant in the Acock photosynthesis model [8.5E-5]	$m^3\ J^{-1}$
a_r	constant in CO_2-rule	--
b	constant relating model and measurement	
b_A	constant in the Acock photosynthesis model [2.1E-2]	$m^2\ s\ J^{-1}$
b_r	constant in CO_2-rule	$\%\ (100\ mol\ mol^{-1})^{-1}$
C	CO_2 concentration	$\mu mol\ mol^{-1}$
C′	CO_2 concentration after unit conversion	$g\ m^{-3}$
c_c	unit conversion for CO_2 [1.83E-3]	$g\ m^{-3}\ (\mu mol\ mol^{-1})^{-1}$
c_g	unit conversion [1000]	$g\ kg^{-1}$
c_t	unit conversion considering time [3600]	$s\ h^{-1}$
c_x	parameters in regression equation for conductance	--

continue Appendix II Symbols and parameter with values and units

D	vapour pressure deficit	kPa
D	diffusion coefficient	$m^2\ s^{-1}$
E	rate of transpiration of the crop	$g\ m^{-2}\ s^{-1}$
F_J	ratio of average PAR at leaf level relative to top	--
G_c	rate of CO_2 emission from the ground	$g\ m^{-2}\ h^{-1}$
g	conductance for water vapour	$mm\ s^{-1}$
h	average height of the greenhouse	m
I	incident PAR (400-700 nm), photon flux	$\mu mol\ s^{-1}m^{-2}$
I′	I derived from global radiation	$\mu mol\ s^{-1}m^{-2}$
J	incident PAR (400-700 nm), light energy flux	$W\ m^{-2}$
J_o	J on top of the canopy	$W\ m^{-2}$
K	extinction coefficient for light	--
K	coupling between conductance and transpiration	--
L	leaf area index (LAI)	$m^2\ m^{-2}$
L_c	rate of loss of CO_2 to the outside	$g\ m^{-2}\ h^{-1}$
m	transmission coefficient for light	--
N	concentration of N_2O	$\mu mol\ mol^{-1}$
N	number of moles	--
P	photosynthesis rate of canopy	$g\ h^{-1}m^{-2}$ (ground)
P_l	photosynthesis rate of leaves	$g\ s^{-1}m^{-2}$ (leaf area)
p_x	parameters in regression equation for photosynthesis	--
p	pressure	Pa
Q	global radiation	$W\ m^{-2}$
R_c	crop dark respiration rate	$g\ h^{-1}m^{-2}$ (ground)
R′	parameter for crop dark respiration per leaf area	$g\ s^{-1}m^{-2}$ (leaf area)
R	molar gas constant [8.31]	$J\ mol^{-1}\ K^{-1}$
r	resistance for water vapour	$s\ m^{-1}$
S	incident light flux (PAR), during previous week	$W\ m^{-2}$
S_o	S on top of the canopy	$W\ m^{-2}$
S_c	rate of supply of carbon dioxide	$g\ m^{-2}\ h^{-1}$
s	slope of saturated water vapour curve at air temperature	$kPa\ K^{-1}$
T	temperature	C
T	absolute temperature	K
t	time	s or h
t_x	parameters in regression of leaf temperature	
V	volume	m^3
u, v	wind speed	$m\ s^{-1}$
X	relative effect of the CO_2 concentration	% $(100\ \mu mol\ mol^{-1})^{-1}$
z	path length	m

continue appendix II Symbols and parameter with values and units

Greek		
α	initial light use efficiency	
	or light utilization or photosynthetic efficiency	$g\ J^{-1}$
	convertible to quantum yield	$mol\ mol^{-1}$
β	parameter for maximum photorespiration	$g\ m^{-2}\ s^{-1}$
γ	apparent psychrometric constant [0.067]	kPa K
ϵ	error	
ΔT	difference in temperature	K or C
θ	curvature in the photosynthesis-light curve [0.95]	---
ξ	air exchange rate	$m\ h^{-1}$
ξ'	air exchange factor	h^{-1}
λ	vaporization energy of water [2.45E6]	$J\ kg^{-1}$
ρ_c	gas density of CO_2 [1.83 at 20 °C and 101.3 kPa]	$kg\ m^{-3}$
$\rho * c_p$	volumetric heat capacity of air [1240]	$J\ m^{-3}\ K^{-1}$
τ	conductance to CO_2 transfer	$m\ s^{-1}$

subscripts	
A	belonging to the (modified) Acock model
a	of air (VPD, temperature) or of the ambient (CO_2 concentration)
b	of boundary layer (conductance)
c	of carbon dioxide
	or: at canopy level, i.e. incident (global radiation, PAR)
	or: of the canopy, of the crop (photosynthesis, respiration)
calc	calculated
ec	with estimated conductance (transpiration)
empty	without crop
g	gross
L	leaf, of leaves
ℓ	leaf, of leaves
ℓ-a	between leaf and air (VPD, temperature)
m	maximum
max	maximum
mean	mean, average
meas	measured (transpiration)
model	modelled
mc	with measured conductance (transpiration)
n	net (radiation, photosynthesis)
0	on top of the canopy (radiation)
p	according to the Penman-Monteith equation (transpiration)
r	radiation based (transpiration)
T	belonging to the (modified) Thornley model
t	at time t

Appendix III *Unit conversions*

a. Carbon dioxide

dimension	units
ratio of CO_2 to air, in volume:	1 vpm = 1 µl l^{-1} = 1 ml m^{-3} = 1 cm m^{-3} = 0.0001 % (volume)
ratio of CO_2 to air, in moles:	1 ppm = 1 µmol mol^{-1} = 1 mmol kmol^{-1}
ratio of CO_2 to air, in weight:	1 mg kg^{-1} = 0.0001 % (weight)
weight of CO_2 per volume of air:	1 mg m^{-3} = 1 µg l^{-1}
moles CO_2 per volume of air:	1 µmol m^{-3}
partial pressure:	1 Pa = 10 µbar

Conversion (at 20 °C and 101.3 kPa):

1 vpm = 1 ppm = 1.53 mg kg^{-1} = 1.83 mg m^{-3} = 41.6 µmol m^{-3} = 0.101 Pa

(after Lawlor, 1993)

b. Carbon dioxide flux (CO_2 exchange rate, photosynthesis rate)

1 g m^{-2} h^{-1} ≈ 6.3 µmol m^{-2} s^{-1} (at 20 °C and 101.3 kPa)

c. Conductance

1 mm s^{-1} ≈ 0.042 mol m^{-2} s^{-1} (at 20 °C and 101.3 kPa)

d. Photosynthetically Active Radiation

1 W m^{-2} PAR ≈ 4.5 µmol m^{-2} s^{-1} PAR
(McCree, 1972; Meek et al., 1972)

e. Global radiation → Photosynthetically Active Radiation

1 W m^{-2} global radiation ≈ 2 µmol m^{-2} s^{-1} PAR
(Thimijan & Heins, 1983; Meek et al., 1984)

f. Light Use Efficiency

1 µg J^{-1} ≈ 0.0052 mol mol^{-1}

1 General introduction

1.1 FIELD OF RESEARCH

Carbon is a principal element for life, as it comprises a major part of the dry matter in living organisms (about 50%, Levanon et al., 1986; about 30-40% in tomato plants, Ho, 1976). The carbon in the terrestrial food pyramid is acquired almost exclusively by green plants, taking up carbon dioxide gas (CO_2) from the surrounding air. CO_2 enters the stomatal cavities of the plant through the stomata in the leaf surface, and is assimilated to carbohydrates at the chloroplasts under the use of light energy. The rate of CO_2 assimilation (i.e. photosynthesis) is influenced by the amount of CO_2 present in the vicinity of the plant. Supply of additional CO_2 to the surrounding air usually accelerates the assimilation rate, and hence promotes the production of agricultural crops (Kimball, 1986). This effect is based on decrease of the CO_2 uptake limitation and suppression of the photorespiration. The effect of the CO_2 concentration (C) of the air on the production of greenhouse crops is generalized in **Fig. 1.1**.

Increase of C can be achieved relatively easily in a closed environment such as a greenhouse. The term greenhouse is used here for a structure covered with glass or plastic of considerable size (at least man's height), in which a crop can be grown; it protects the crop from external conditions and enables a grower to manipulate the crop's environment. C, as well as temperature and humidity of the air, are the main environmental conditions that are controlled in greenhouses.

CO_2 enrichment, i.e. supply of either pure CO_2 or CO_2-rich combustion gas, is a powerful tool to enhance the horticultural production. It has become common practice in the greenhouse industry (see section 1.2.2). Without enrichment, C may drop below the normal outside atmospheric concentration, which is about 350 $\mu mol\ mol^{-1}$ at present. Enrichment in a closed greenhouse aims at raising C up to twice or thrice this atmospheric concentration: recommended is 700 to 900 $\mu mol\ mol^{-1}$ (Mortensen, 1987). During ventilation, the achieved concentration is usually (far) lower, due to loss of a part of the CO_2 supplied by exchange of greenhouse air and outside air. The C range occurring in a well controlled greenhouse cultivation varies roughly from 200 to 1200 $\mu mol\ mol^{-1}$.

The favourable effect of high C is particularly valuable at high radiation, because the rate of photosynthesis increases with increasing radiation.

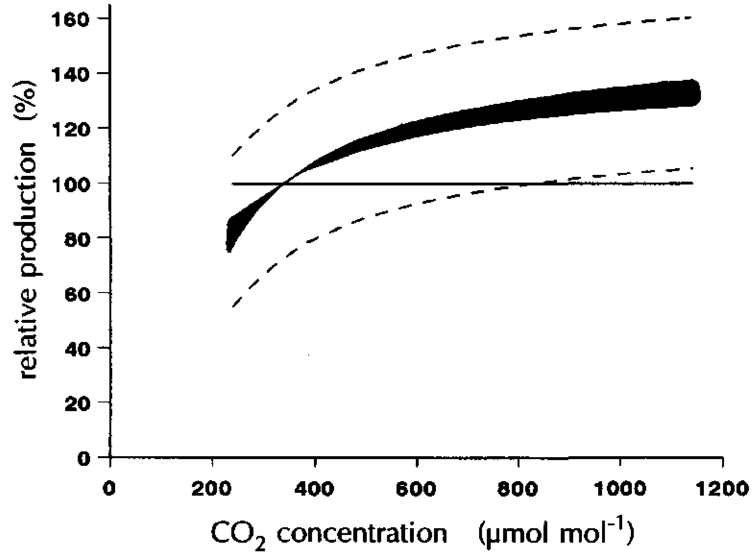

Fig. 1.1 *Relative production (%) of greenhouse fruit vegetable crops as function of the average CO_2 concentration (μmol mol^{-1}) during the cultivation, with the production at 340 μmol mol^{-1} as reference (100%).*

▬ *95% confidence interval of the mean of the observations*

- - - - - *95% confidence interval of the observations.*

After Nederhoff (1987), revised by H.W. de Ruiter. **Sources:** *Anonymous, 1984 (Grower 101(2): 30); Buitelaar & Nederhoff, 1991 (Groenten+Fruit/Glasgroenten 1(39): 32-33); Calvert & Slack, 1975 (J.Hort.Sci. 50: 61-71); Frydrych, 1984 (Acta Hort. 162: 271-278); Hand, 1989 (Grower 112(18): 19-21); Hand & Slack, 1988 (Grower 109(6): 27-34); Hartz et al., 1991 (J.Amer.Soc.Hort.Sci. 116: 970-973); Hayman & Talent, 1987 (Grower 107(7): 25-27); Heij & van Uffelen, 1984 (Acta Hort. 162: 29-35); Heins et al., 1984 (Acta Hort. 162: 21-28); Kimball, 1983 (Agronomy J. 75: 779-788); Kimball & Mitchell, 1979 (J.Amer.Soc.Hort.Sci. 104: 515-520); Nederhoff, 1988 (Acta Hort. 229: 341-348); Nilsen et al., 1983 (Sci.Hort. 20: 1-14); Peet & Willits, 1982 (HortScience 17: 948-949); Peet & Willits, 1984 (Sci.Hort. 24: 21-32); Slack, 1985 (Grower 103(2): 23-27); Slack & Hand, 1985 (J.Hort.Sci. 60: 507-516); Slack & Hand, 1986 (J.Hort.Sci. 61: 303-306); Slack et al., 1988 (J.Hort.Sci. 63: 119-129); Uffelen & Nederhoff, 1985 (G+F (41)22: 40-43); Uffelen & Nederhoff, 1986 (G+F 42(1): 28-29); Willits & Peet, 1989 (Agric.For.Meteor. 44: 275-293); Yelle et al., 1990 (J.Amer.Soc.Hort.Sci. 115: 52-57).*

A complication is that high radiation is usually accompanied by a high rate of air exchange for cooling and hence by a great loss of CO_2 from the greenhouse. This makes it difficult to achieve high C in combination with high light. From an environmental point of view, excessive emission of CO_2 to the atmosphere is unacceptable. From a grower's point of view, the costs for CO_2 supply must be compensated by the resulting monetary benefits, i.e. CO_2 enrichment needs to be economically optimized.

Besides the positive effects already mentioned, also negative effects of long-term high C may occur, which may reduce the performance of the crop later in the season. This implies that CO_2 supply needs to be optimized by physiological criteria as well, thus insuring the quality of the plants and hence the production in the long term. Not much is known yet about optimization with respect to long-term responses to C. Some aspects of this issue are to be investigated in the present study. A proper analysis of the problem is made in section 1.3.2, after a description of the current practice (section 1.2.3), of the growers' observations and of lacks in the present knowledge (section 1.3.1).

Some debate is going on in the Netherlands these days, about the desirability of further increasing the horticultural production, which is the result and even the purpose of CO_2 enrichment. Nevertheless, in spite of an overproduction of some fruit vegetables on macro scale at present, increase of production is considered absolutely necessary for individual growers to enhance their fruit production, in order to compensate for increasing production costs and decreasing market prices. A relatively new objective in agriculture is to reduce the inputs (energy, fertilizers, pesticides) and the amount of waste (materials, fertilizers, pesticides, carbon dioxide, nitrous oxide) per unit output, or vice versa, to increase the production relative to the input and the waste (increased efficiency of the production). Improving the strategy for CO_2 enrichment can serve to achieve both objectives: increase of production and of efficiency.

Carbon dioxide belongs to the 'greenhouse gases' that are held responsible for the global warming (IPCC, 1990). The present study, however, is restricted to the effects of C on greenhouse crops, from a horticultural point of view. When CO_2 is supplied into a greenhouse, a smaller or larger part of this gas is released into the atmosphere. This is considered a serious drawback, which must be minimized. This study aims at optimizing the CO_2 supply and thus minimizing the losses. In addition, it will provide the necessary information to another project in Naaldwijk, dedicated to energy use and CO_2 emission by horticultural enterprises in view of the environmental targets (Vermeulen & Van De Beek, 1993).

The crops investigated here are the four major greenhouse fruit vegetable crops: tomato (*Lycopersicon esculentum* Mill.), cucumber (*Cucumis sativus* L.), sweet pepper (*Capsicum annuum* L.) and eggplant (*Solanum melongena* L.). These crops are of great economic value: the figures for yield in the Netherlands over the year 1992, in million kg and in million Dutch guilders (with 1 Dutch guilder ≈ 0.5 U.S.Dollar), respectively, are: tomato 610 and 820;

cucumber 470 and 490; sweet pepper 195 and 580; eggplant 27 and 73. These products together represent 62 % of the turnover of fresh vegetables marketed at the Dutch auctions (LEI/CBS, 1993). As the value of vegetables and ornamentals produced in greenhouses in the Netherlands is over 6000 million guilders per year, it is obvious that small improvements, e.g. in CO_2 enrichment, may yield a few hundreds of million guilders on an annual basis.

The experimental work has been guided by the state of the art and by actual questions concerning CO_2 enrichment in the greenhouse horticultural industry in the Netherlands (which belongs to the most advanced in the world). Because of this demand for practical relevancy, the scope of the work is fairly wide, so that not all aspects are thoroughly investigated for all crops.

1.2 CO_2 ENRICHMENT IN PRACTICE

1.2.1 Carbon dioxide properties and units

Carbon dioxide (CO_2) is under normal conditions a colourless, acid, non-toxic gas, with boiling point -78.5 °C. The molecular weight is 44.01 g mol^{-1}. The gas density at normal atmospheric pressure (101.3 kPa) is 1.98 kg m^{-3} at 0 °C and 1.83 kg m^{-3} at 20 °C. The density relative to air is 1.53.

The amount of CO_2 in the air can be defined in different dimensions and expressed in different units (**Appendix III**). Unit conversion can be made by using the ideal gas equation:

$$p * V = N * R * T \qquad \textbf{eqn. 1.1}$$

where N is number of moles, V is volume (m^3), p = pressure (Pa), T is temperature (K) and R is molar gas constant (8.31 J mol^{-1} K^{-1}). In the present work, the CO_2 concentration of the air (C) is expressed in µmol mol^{-1}, which numerically equals ppm, vpm, µl l^{-1} etc. Usually µmol mol^{-1} is used in scientific literature, while ppm, vpm and µl l^{-1} are commonly used in the horticultural practice.

1.2.2 History of CO_2 enrichment and related research

One of the earliest steps on the path to CO_2 enrichment may have been the 'discovery' of photosynthesis, roughly between 1770 and 1845. The first modern experiments on effects of C on plants were done at the end of the last century and the beginning of this century (e.g. Brown & Escombe, 1902). In the twenties and thirties experiments were done in England on C effects on tomatoes in greenhouses (Owen et al., 1926) and on types of fuels as source of CO_2 (Bolas & Melville, 1935). Some of the early experiments (e.g. Brown & Escombe, 1902) were apparently disturbed by impurities in the gas supplied, whereas on the other hand, many early trials yielded results similar to those of recent work (overview by Wittwer, 1986).

The first practical application of CO_2 enrichment in greenhouses was reported already at the end of the 19th century in Germany and England. Later, in 1931, CO_2 was used on some scale in Germany (Wittwer, 1986). In the Netherlands, a few experiments in the twenties and thirties gave no convincing results, and consequently CO_2 control was not commercially practised in that time (review by Van Berkel, 1986). On the other hand, it has been common practice for many years to heavily mulch the soil with organic material for CO_2 enrichment.

The revolution came in 1961, when some growers in the Westland area (in the Netherlands) installed some simple kerosene (paraffin) burners for heating their lettuce (new fast growing varieties that were susceptible to frost injury). The burners released the flue gases in the greenhouse. Some growers who used their burners also by day saw a noticeable increase in growth of their lettuce plants (Steiner, 1962). This was ascribed to a higher temperature during day time and to elevated C. Also in other countries, the benefits of CO_2 enrichment were re-established only after 1961 (Wittwer, 1986; Bauerle et al, 1986).

Thanks to the work of Gaastra (1959) the growers' observations were quickly recognized and understood, which has certainly stimulated the fast application and development of techniques. Also in other countries at the end of the fifties and early sixties, carbon dioxide enrichment received renewed attention from researchers. In 1962 on the 16th International Horticultural Congress Meetings in Brussels, numerous papers about CO_2 were published by several European researchers, followed by articles in trade journals and scientific journals. The first experiments on CO_2 at the Glasshouse Crops Research Station in Naaldwijk took place in 1962, with a number of vegetables and flowers (Van Berkel & Van Winden, 1963).

Meanwhile, the practical application of CO_2 enrichment increased rapidly. First the simple kerosene burners or propane burners with automated control were used and later a new automated kerosene burner was introduced (Van Berkel, 1986). In 1970, natural gas (low-sulphur) became available for the growers in the Westland area, a fuel that proved to be perfectly suitable for CO_2 supply. Growers switched over to natural gas burners, either small burners inside the greenhouse, or one large burner with a central heating system. Growers with a central heater started extracting flue gas from the chimney and inserting it into the greenhouse. After 1979, an infra red gas analyzer for CO_2 became commercially available. The installation of this sensor in many greenhouses marked a revolution in the CO_2 enrichment history.

Since the eighties, CO_2 enrichment is a standard measure in most greenhouses. It was estimated in 1979 that 80% (cucumber) to 95 % (sweet pepper) of the greenhouse area with a central heating system was supplied with flue gas CO_2, as well as the majority of the chrysanthemum, rose and lily producing greenhouses (Van Berkel, 1986).

1.2.3 Current methods of CO_2 enrichment

C in the (semi-)closed environment of a greenhouse frequently drops below the ambient level, whenever the photosynthesis is larger than the CO_2 influx by supply and air refreshment. With such so-called CO_2 depletion, C is sometimes as low as 150 µmol mol^{-1} (Heij & De Lint, 1984; Drakes, 1984). CO_2

depletion is very unfavourable for plant growth and production (**Fig. 1.1**). The principal objective of CO_2 enrichment is therefore preventing depletion (Slack & Hand, 1986). Elevation of C considerably beyond the outside concentration is accomplished in general only at limited ventilation.

Carbon dioxide can be obtained in various ways: as pure (liquid) carbon dioxide gas from refillable steel bottles or from a bulk storage vessel (Nederhoff, 1994b); or by combustion of an appropriate fossil fuel (Hand, 1982), either with small burners inside the greenhouse or with one large burner belonging to a central heating system, optionally connected with a heat storage facility (Nederhoff, 1994b). Liquid CO_2 is normally free of impurities and is therefore the safest source. It is easily transportable and distributable (low volume, as it is 100% CO_2). Moreover, its availability is not related to production of heat. Hence pure CO_2 is considered as the ideal method for enrichment (Hand, 1982; Mortensen, 1987). The price of the commercially available pure CO_2 cannot compete usually with that of CO_2 obtained by combustion of natural gas. The feasibility of a CO_2 source or system depends on economic factors.

CO_2 from burners in the greenhouse

Combustion of fossil fuel is the oldest and still most common method for CO_2 enrichment. The least complicated method is using small burners inside the greenhouse. Such burners release heat and flue gases together directly in the greenhouse (although there are some types with a chimney). A major disadvantage is that they are used primarily for heating, whereas C is considered of minor importance. Hence, in winter the concentrations of CO_2 and of concomitant noxious gases frequently become extremely high, while on warmer days no CO_2 is supplied at all. Secondly, most small burners can operate only on full capacity, which makes the control very inaccurate (on/off) and the distribution of CO_2 far from homogeneous (Nederhoff, 1994b). Thirdly, it is virtually impossible to monitor for incomplete combustion, as can be done with a central burner.

Central burner and heat storage

The alternative method for flue gas CO_2 supply is by using a large burner connected to a central hot-water pipe heating system. The flue gases of such a burner can be made available for CO_2 enrichment. Combustion produces, besides CO_2, also a substantial amount of heat: 1 m^3 natural gas (at 20 °C and 101.3 kPa atmospheric pressure) yields about 35 MJ heat (gross calorific value) and 1.8 kg CO_2 (Nederlandse Gasunie, 1988). If CO_2 is demanded while heating is not necessary, e.g. on warmer days, the heat can be stored in a day-to-night heat storage facility and brought into the greenhouse at night. The central heating system enables enrichment during far more hours in spring and autumn than can be achieved with small burners in the greenhouse, because CO_2 and heat are produced outside the greenhouse and can be introduced separately into the greenhouse. The presence of a heat storage facility enables enrichment in summer, provided the nights are cool enough to need heating.

The storage system is usually a well insulated hot water tank with a volume between 30 and 130 m^3 per ha greenhouse. When the water temperature of a medium size heat storage tank (75 m^3) is increased by 60 °C, CO_2 can be supplied during 10 hours per day at a rate of about 12 g m^{-2} h^{-1} (Nederhoff, 1994b), which is almost three times the 'standard' supply rate (section 1.2.5). In the Netherlands approximately 1000 ha of greenhouse area, mostly used for fruit vegetable production, is equipped with a heat storage facility.

CO_2 distribution system

CO_2 enrichment from a central boiler requires an adequate CO_2 transport and distribution system of sufficient capacity (Nederhoff, 1994b). The volume of flue gases to be transported depends on the desired CO_2 supply, on the CO_2 content and the temperature of the flue gas. Each 1 m^3 natural gas combusted with the required amount of air (8.5 m^3) produces almost 10 m^3 flue gas (0 °C and normal atmospheric pressure) with about 10 % CO_2 (Nederlandse Gasunie, 1988). A centrifugal fan extracts the flue gas from the stack and dilutes it with air if necessary. The gas stream is blown into a main transport duct to the greenhouse, where it is distributed through a net of perforated, polyethylene lay-flat ducts of 50 mm diameter.

1.2.4 Harmful gases

The composition of flue gas is determined by the fuel used and by the technical properties of the burner. Combustion gases of sulphur-free natural gas, as used in the Netherlands, consist of CO_2, H_2O and inevitably also some NO_x. With incomplete combustion, e.g. due to insufficient air supply, other gases may be released as well, such as lethal carbon monoxide (CO), phytotoxic ethylene (C_2H_4) and other unsaturated hydrocarbons. The risk that supply of flue gas CO_2 causes harmful concentrations of noxious gases in the greenhouse can be minimized by some simple measures:

- limiting the supply by maximizing the CO_2 set point (e.g. 1200 µmol mol^{-1})
- using burners that satisfy the modern regulations for low NO_x production (in last years the emission of NO_x is reduced rigorously by technical improvements on the burners, owing to new legislation; Kooiman, 1990)

- stopping the insertion of flue gases in case of incomplete combustion (a carbon monoxide device is often used to switch off the enrichment as soon as excessive CO is detected in the flue gas).

1.2.5 Rate of CO_2 supply in greenhouses

The amount of CO_2 to be supplied varies substantially. Under given, static conditions, the required CO_2 supply rate to achieve a desired C, or the achievable C at a certain supply rate, can be calculated using the mass-balance of CO_2. The rate of CO_2 supply (S_c) equals the rate of photosynthesis (P) plus the rate of CO_2 loss by ventilation (L_c). CO_2 emission from the soil is neglected. In a simplified approximation (steady state assumed), L_c depends on the air exchange rate (ξ in $m^3\ m^{-2}\ h^{-1}$) and on the difference in CO_2 concentration between greenhouse air (C) and ambient air (C_a). Thus S_c is approximated by:

$$S_c = \xi * (C - C_a) * \rho_c + P \qquad \textbf{eqn. 1.2}$$

with S_c (supply rate) and P (canopy photosynthesis rate) in g $m^{-2}\ h^{-1}$ and ρ_c the density of CO_2 gas (1.83 kg m^{-3} at 101.3 kPa atmospheric pressure and 20 °C). Whenever C equals C_a, S_c must only compensate P (about 1 to 5 g $m^{-2}\ h^{-1}$), independently of ξ. In other cases, ξ can be calculated with an appropriate model for ventilation (Bot, 1983; Nederhoff et al., 1985; De Jong, 1990; Fernandez & Bailey, 1992). To give an order of magnitude: according to Bot (1983), ξ is about 20 m h^{-1} at window opening 20% and wind speed 4 m s^{-1}. When C is 700 µmol mol^{-1}, L_c is around 13 g $m^{-2}\ h^{-1}$ and S_c should be 14-18 g $m^{-2}\ h^{-1}$. With higher ventilation rates or higher target C, the required supply rate would be even higher, but this is only hypothetical.

A generally recommended standard (minimum) supply rate is to supply the flue gas of 25 $m^3\ ha^{-1}\ h^{-1}$ natural gas, equivalent to 4.5 g $m^{-2}\ h^{-1}$ CO_2 (Van Berkel & Verveer, 1984, in accordance with Hand, 1984). This rate is usually sufficient to maintain a high C (about 1000 µmol mol^{-1}) in a closed greenhouse and to prevent CO_2 depletion in a ventilated greenhouse. Often the supply rate is fixed or maximized on this 4.5 g $m^{-2}\ h^{-1}$ for practical or economic reasons.

Excessive supply of CO_2 is undesired. At higher C, the CO_2 waste by leakage and ventilation is considerable. Rapid supply while ventilators are closed may lead to extremely high C, which, in the best case, does not contribute to the photosynthesis, and may even be unfavourable. C in the order of several thousands µmol mol^{-1}, which frequently occurs in cold winters in greenhouses with hot air heaters, may cause severe injury to crops (Holländer & Krug, 1991). Increased supply of flue gas increases the risk of reaching threshold levels of harmful gases.

1.2.6 Control and strategy of CO_2 supply

CO_2 is more or less scarce and expensive, and CO_2 supply may have negative side effects (section 1.3). So deciding on CO_2 supply requires great care. Decision making, also CO_2 management, involves various time horizons: short-term (control), medium-term (strategy) and long-term (investments). CO_2 control in a strict sense may be considered essentially as set point realization. It comprises instantaneous actions, such as opening valves, activating burners, starting fans or electro motors etc., for supply of pure CO_2 or of flue gas. This control is based on comparing the actual, measured C with a target value (set point). C is mostly measured with an IRGA (Infra Red Gas Analyzer) of a relatively inexpensive type (different manufacturers, overview in Pallas, 1986). Instantaneous CO_2 control is usually delegated to a CO_2 controller, either an IRGA that can switch the CO_2 supply on/off, or a climate control computer.

A CO_2 strategy is defined here as a (computerized) procedure for CO_2 enrichment with a time horizon of days or weeks, that generates the set points for a CO_2 controller. A computerized strategy uses a number of settings (parameters in the strategy-algorithm) that can be set by the grower. Two simple strategies are supply at a constant rate and supply to a constant level (set point).

A strategy commonly applied in commercially available computers (**Fig. 1.2**) calculates the instantaneous set point in dependence of the heat demand and the ventilation rate (usually relative window opening). It uses three CO_2 set points: high, low and minimum. High C (e.g. set at 800 $\mu mol\ mol^{-1}$) is taken as set point as long as heating is required; low C (e.g. 500 $\mu mol\ mol^{-1}$) is the set point when there is no heat demand and no or little ventilation (this ventilation level is set); minimum C (e.g. set at 350 $\mu mol\ mol^{-1}$) is aimed at when the greenhouse is ventilated to a certain extent (also set).

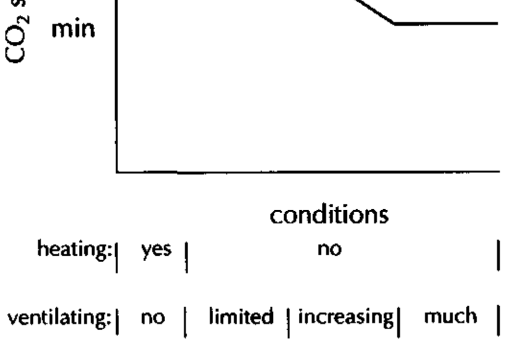

Fig. 1.2 *Example of a commonly applied strategy for CO_2 supply, with set point dependent on the conditions (PTG, 1992).*

An alternative CO_2 strategy is known as 'pulsed' or 'intermittent' CO_2 supply (Clough & Peet, 1981; Enoch, 1984; Mortensen, 1984). The principle is supplying CO_2 periodically in short pulses, while the ventilation windows are temporary closed. When the air temperature rises to a certain level, the ventilators must be opened and C inevitably declines. This would theoretically result in a more efficient utilization of the carbon dioxide gas. Moreover, positive effects of high C would be accomplished, while negative side effects (long-term adaptation to high C) would be prevented.

All currently used, commercially available strategies, simple or more sophisticated, require a number of settings (such as CO_2 set points, or in the example described above, ventilation levels). There are virtually no recommendations and no methods on a theoretical basis to estimate the optimal value of the settings. Therefore, attempts are made to design alternative control strategies, based on quantitative knowledge of the processes involved, represented by simulation models. By comparing estimated costs and benefits, the economically optimal C can be calculated. The processes considered are photosynthesis, associated production and CO_2 losses by air-exchange, and the constraints are the actual conditions and prices of CO_2 and of the product (Challa & Schapendonk, 1986; Seginer et al., 1986; Nederhoff, 1988). It must be emphasized that such strategies deal with instantaneous optimization of the CO_2 supply, ignoring the long-term aspects.

1.3 DESCRIPTION AND ANALYSIS OF THE PROBLEM

1.3.1 Problem description

After the wide-spread introduction of CO_2 enrichment in the eighties, it was generally experienced that CO_2 enrichment indeed brought about the well-known increase of production (**Fig. 1.1**). However, there were also clear indications for undesired side effects of high C. For example, high C was sometimes accompanied by markedly reduced air humidity, indicating an (undesirable) reduction of crop transpiration (De Koning, 1989). This could hamper the uptake of nutrients into the crop: e.g. high C aggravated chlorosis of leaves in eggplant (Buitelaar, 1991). Sometimes damage or adaptation was observed after long-term CO_2 enrichment: in summer, high C promoted leaf deformation in tomato on a wide scale (Van Velden, 1990). The affected tomato cultures had a high production in summer, but performed less well in autumn. In the past, severe leaf damage used to occur in greenhouse crops after CO_2 enrichment (mostly with flue gases, containing harmful gases, section 1.2.4).

The various effects of C on plants have different response times. The positive effect of C on photosynthesis is instantaneous; the effects on the associated growth and fruit production come to expression with some delay; possible adaptations of plants to high C are observed mostly after some weeks of high C; reduction of transpiration, due to high C occurs almost instantaneously, while the resulting nutrient deficiency may appear later and can have a long-lasting impact.

Many publications about CO_2 effects on crop growth and production have appeared since the beginning of this century (section 1.2.2). However, the understanding of the integrated effects of C in practical cultivations is still insufficient. Many questions are still actual, for example concerning effect of C on transpiration and the impact of this on the cultivation; strategies for optimal CO_2 supply in relation to instantaneous conditions; pros and cons of intermittent CO_2 supply as compared to supply to a constant C; effect of C on harvest index and fruit dry matter content.

Another problem in relation to CO_2 enrichment, is the increased emission of carbon dioxide gas by leakage or ventilation. The amount of CO_2 emitted depends on the air exchange rate and on the CO_2 concentration (section 1.2.5). Wasting CO_2 gas (or wasting energy) is undesirable from an economic point of view, and is no longer acceptable in view of the consequences for the environment.

It will be clear that proper application of CO_2 supply is a complicated matter, because positive and negative effects, short-term and long-term effects play a role. This means that CO_2 supply should be considered as an optimization problem, taking into account all relevant aspects. It must be emphasized that these questions refer to practical problems, so can be studied the best under practical conditions.

The grower decides about CO_2 supply by choosing the actual settings of the control device or control computer for CO_2 enrichment (section 1.2.6). Growers generally aim at maximizing the annual financial outcome (yield minus costs), which requires a high production at relatively low costs on an annual basis. So from this point of view, the policy regarding CO_2 enrichment is to maximize the growth and production in the longer term (for the duration of a cultivation), whereby the costs must be compensated by the revenues. This approach contributes to careful use of CO_2, but it does not explicitly account for reduction of the CO_2 emission to the atmosphere. The targets of reducing CO_2 emission can be incorporated explicitly in a new generation of climate control and optimization systems that is being developed (Bakker et al., 1994).

1.3.2 Problem analysis

In order to specify the problem, some important considerations in relation to CO_2 enrichment are stated here and explicated below:

1. high C enhances the rate of net CO_2 assimilation, growth and production
2. high C may cause (negative) side effects on plants
3. extremely high C causes severe injury to plants
4. noxious gases in combustion gases are detrimental for plants
5. CO_2 enrichment is accompanied by costs (investments and running cost)
6. emission of 'greenhouse gases' to the environment must be reduced.

Ad 1. High C generally increases the rate of net CO_2 assimilation, (photosynthesis) i.e. dry matter production, which results ultimately in the well-known increase in fruit production. **Fig. 1.1** surveys the results of a number of experiments with greenhouse crops, reported in the literature. The small band in the figure shows that the general trend of the CO_2 effect on production forms a saturation curve, with a similar shape as the photosynthesis-CO_2 relationship. The wide band demonstrates that the variation in the CO_2 effect on production is considerable. According to Kimball (1986), the estimated relative effect of a doubling of the atmospheric CO_2 concentration on production varies between +11% and +32% for the fruit vegetable crops. Also within one crop (tomato or cucumber) a wide variation in results can be found, while reports about sweet pepper and eggplant are scarce (Kimball, 1986).

The large variability is plausible, as fruit production is the ultimate result of many underlying processes of which many are sensitive to the conditions, for instance net photosynthesis, flowering, fruit set, dry matter allocation and formation of fresh biomass. Moreover, the production might be affected negatively by deficiency of water or minerals, by pests, diseases, physiological disorders and by side effects of high C (see below). Also species-specific responses to C, as well as particular conditions in the experiments reported, may contribute to the large variability in results.

Ad 2. Three types of (negative) side effects of high C can be distinguished:
a. reduced stomatal opening (Raschke, 1975), and hence reduced rate of transpiration. Reduced transpiration may increase the leaf temperature and the supply to the leaves of some nutrients, such as calcium and boron, that are translocated almost exclusively by the transpiration flow through the xylem (Mengel & Kirkby, 1978). Reduction of stomatal opening occurs instantaneously and is reversible, whereas the resulting problems appear later and are irreversible. Not much is known about the occurrence and the extent of this problem.
b. morphological adaptations after long-term exposure to high C. The most frequently reported symptom is increased leaf thickness (reduced SLA), sometimes reduced leaf area and in addition severe curling of leaves (e.g. Madsen, 1976). Not much is known about the consequences of reduced active leaf area on fruit production, and about the response of a canopy with reduced leaf area to C. It is also not well understood whether C affects other morphological parameters, such as harvest index (dry matter allocation) and fruit dry matter content.
c. reduced photosynthetic efficiency following prolonged exposure to high C (e.g. Von Caemmerer & Farquhar, 1984; Yelle et al., 1989a,b).

Ad 3. Plant injury due to high C is evident only in case of extremely high concentrations (e.g. 10000 μmol mol^{-1} CO_2, Holländer & Krug, 1991). As these detrimental effects have been acknowledged for many years already, growers try to avoid such extremes. So C normally applied in practice and considered in the current study, is far lower than the estimated threshold levels of C.

Ad 4. Critical concentrations of noxious gases may prevail and may be extremely detrimental (e.g. in case of ethylene). Therefore accumulation of noxious gases must be avoided. This can easily be achieved with the present technology, which is applied on a wide scale (section 1.2.3).

1.4 RESEARCH QUESTIONS, OBJECTIVES AND APPROACH

1.4.1 Restrictions to the research

The ultimate objective of this study is to contribute to optimization of the CO_2 supply according to economical and ecological criteria. Optimization requires a proper definition of the targets, and a quantitative description of the system involved, with its relations to the environmental conditions. This implies that all topics (points 1-6) are relevant for CO_2 optimization. Points 1-4 are important as they represent the processes that influence the horticultural production. Point 5, the economical consequences of CO_2 supply, is crucial for horticultural enterprises. Point 6, preservation of the natural environment, is a principal concern of the present-day society.

As it was necessary to restrict this study, it was decided to consider just the physiological aspects of CO_2 enrichment. Hence, points 5 and 6, which have been the incentives for starting the present study, are dealt with in other studies in Naaldwijk (economic aspects of CO_2 enrichment, Vermeulen, 1992; and targets for CO_2 emission to the atmosphere, Vermeulen & Van De Beek, 1993). Also point 2c is part of another study presently undertaken (Heuvelink, 1994, pers. comm.). Points 3 and 4 are sufficiently understood for the practical purpose. Therefore this study focuses on points 1 (stimulating effects of CO_2) and 2a and 2b (negative side effects), which comprise the essential effects of CO_2 enrichment on plants.

The study is also restricted with respect to the crop species. Investigated are the four main species in the group fruit vegetable crops in greenhouses, being tomato (*Lycopersicon esculentum* Mill.), cucumber (*Cucumis sativus* L.), sweet pepper (*Capsicum annuum* L.) and eggplant (*Solanum melongena* L.). In these crops, the experience and knowledge about CO_2 effects is most advanced and CO_2 enrichment is commonly applied. They have a great economic importance (section 1.1). The crops are more or less similar with respect to growth form and cultivation method: they can be grown year round, with planting in December and ending in November, while also autumn cultivation exists, with planting in summer and harvesting until December. To exclude the complication that C would influence the size of the plant stand in an early phase already, the C treatments were applied only to full-grown plants.

It should be remarked that all questions raised concerning effects of C are related to fruit production, rather than fruit quality. Indeed it was observed by growers and reported in the literature (Slack et al., 1988) that C does not markedly affect the various aspects of fruit quality, other than fruit size.

Nevertheless, because fruit quality is extraordinary important in the Dutch horticulture, also fruit quality aspects were observed in relation to C.

1.4.2 Research questions

Distinguished are main effects and side effects of C on greenhouse crops. With main effects, processes are involved that determine the production, such as photosynthesis, flowering and fruit set, dry matter allocation and formation of fresh biomass. The most import main effect is a stimulation of the photosynthesis. The main effect of C can also be called quantitative effect or production-determining effect or even production-stimulating effect. Side effects are for example effects on stomatal opening, transpiration and nutrient translocation and morphological properties. These might lead to reduction of production and are called production-reducing effects or also qualitative effects.

Not much is known about the integrated results of various counteracting effects of C on plants (production-determining and -reducing effects). Important is also the time aspect, because high C might have a positive effect in the short term in the form of increased photosynthesis, but might work out negatively in the long term. The long-term result is the ultimate cumulative fruit production. Based on the problem description and analysis, with the restrictions taken into account, the following questions for research are formulated:

I. What are the main effects of C in crops under realistic greenhouse conditions:
a. on rate of canopy net photosynthesis and dry matter gain (quantitative short-term effect)?
b. on cumulative fruit production (quantitative long-term effect) ?
II. If there is a discrepancy in the response of photosynthesis and of fruit production to C, can this be explained by side effects, either:
a. reduced stomatal conductance and consequently reduced transpiration (qualitative medium-term) ?
b. adaptations to long-term exposure to high C, such as adapted morphological parameters (qualitative long-term) ?

1.4.3 Objectives of the thesis

The present study aims at analysis and quantification of the main effects and side effects of the CO_2 concentration (C, 200 - 1100 µmol mol^{-1}) on growth and fruit production of tomato, cucumber, sweet pepper and eggplant crops in long-term, semi-commercial cultivations in greenhouses. The outcome of this study must provide a scientific basis for a policy of optimal CO_2 supply, which can take advantage of the positive effects, while minimizing the negative consequences of CO_2 enrichment.

1.4.4 Approach and structure of the thesis

To answer the research questions formulated above, it was considered essential to collect information on the effects of CO_2 on crop performance in greenhouses of at least semi-practical size, in a medium-long term (months, also during summer) and under 'normal' greenhouse conditions. Research on environmental conditions requires a great number of identical, and independently controllable, greenhouse compartments. All compartments must be fully computerized regarding climate control and irrigation equipment, in order to be comparable to practical horticulture. Some greenhouse complexes at the Glasshouse Crops Research Station (Naaldwijk, The Netherlands) that meet these high requirements were used for the present work. The intension was to investigate the processes on the scale of a whole canopy, which has consequences for the methods of data acquisition and interpretation.

Before discussing the integrated effects of C on plants, first the effects of C on canopy photosynthesis (chapter 2) and transpiration (chapter 3) are studied. The effects of C on the rate of net CO_2 assimilation were investigated by measurements in whole crops in large greenhouses. The results are compared to simulation models and used to fit a regression model and to derive a rule of thumb ('CO_2-rule'). The effect of C on stomatal conductance and canopy transpiration is examined to determine whether high C has a significant effect on leaf temperature and leaf nutrient status.

Chapter 4 deals with the integrated effects of C, for instance on vegetative growth, fruit set, allocation of dry matter, morphological properties and reproductive production. It is tested here whether the CO_2-rule applies also to fruit production. In two trials described in that chapter, some treatments with intermittent CO_2 supply are compared with constant C treatments, with respect to effects on growth and production. Also some particular features are described. Each feature was investigated in the species that was expected to respond most clearly to C in this respect.

Chapter 5 reviews to what extent the newly obtained information contributes to the existing knowledge. Some aspects are reconsidered: the validity of the CO_2-rule, the side effects of CO_2 enrichment and the approach. The thesis ends up with recommendations for practical horticulture.

2 Effects of CO_2 on canopy net photosynthesis

2.1 INTRODUCTION

2.1.1 Outline of the problem

This thesis aims at analysis and quantification of the effects of the CO_2 concentration (C) of the greenhouse air on growth and production of greenhouse fruit vegetable crops (section 1.4.3). C primarily affects the net CO_2 assimilation rate, shortly called photosynthesis (P), while P, in turn, largely determines growth and production. Therefore, the effect of C on P is investigated first, both by empirical research and modelling. It is briefly outlined here why there is still a need for experiments and models on photosynthesis, in spite of the many years of photosynthesis research.

Measurements on photosynthesis generally have been done on single leaves in a cuvette or on a few plants in a chamber. An important shortcoming of such measurements is that some important conditions (e.g. light interception, micro climate, crop size and stage) do not sufficiently resemble those of full-grown, producing crops, where the research questions (section 1.4.2) refer to. Photosynthesis measurements in somewhat larger plant stands in greenhouses were performed by a few authors only (Hand, 1973; Dayan et al., 1985; Matthews et al., 1987; Hand et al., 1992, 1993a). These experiments were mostly restricted to one crop, short measuring periods, and were often carried out in a single greenhouse, mostly only at ambient CO_2 concentration. Hence, it was felt that the effect of C on P of greenhouse fruit vegetable crops was not adequately described to form the basis of this study. Moreover, no data sets were available for parametrisation and validation (verification) of models. For instance, an explanatory model for canopy photosynthesis of greenhouse crops (Gijzen, 1992) was to be validated by comparison with measured data, but such data sets were not available.

A wide variety of models can be found in the literature, ranging from a very simple to a very sophisticated explanatory model containing numerous state variables. There are great differences among models, also with respect to accuracy, universality, complexity etc. It depends on the purpose, which model is most appropriate.

In order to select a model, the purposes of the modelling must be clear. A model is needed here in the first place as a research tool, i.e. as a method to describe and generalize the results of the measurements concerning the effects of C on P. This modelling can be seen as an extension of the empirical research. Secondly, a particular aim is to derive a very simple type of model, a 'CO_2-rule' that might serve as a rule of thumb for roughly estimating the effect of C on P, in a fast and easy way. This purpose will be explicated further in section 2.6. The third objective is to obtain a model, or to test an existing model, which ultimately can be implemented in a computerized system for CO_2 control or decision support.

For the first purpose, a model as a research tool, the absolute level of predicted P is less important than the effect of C on P. The second purpose requires a very simple model, with a minimal number of mathematical operations, while the accuracy may be less. The third application requires a reasonable absolute accuracy and a good prediction of the effects of all relevant factors. The complexity of the model is less important.

Many photosynthesis models have been published, but not all models are appropriate for whole canopies, not all account for the effect of C, not all are properly documented, not many are validated and only a few are tested for greenhouse crops. For the second purpose, no suitable models can be found in the literature. For the third purpose, two well-known models are available that might suit: the 'Acock model' (Acock et al., 1976; 1978; Acock, 1991) and the 'Thornley model' (Thornley, 1976; Thornley et al., 1992). These models do account for the effect of C on P of a whole canopy; they are compact, easy-to-use and well-documented. The Acock model was applied for simulation of tomato growth, for instance by Tchamitchian & Longuenesse (1991), and implemented in an optimization routine by Jones et al. (1990). The Thornley model was applied for cucumber by Hand et al. (1992). Also the model of Gijzen (1992) is intended for climate control and decision support. This model will be discussed elsewhere (Gijzen et al., 1995).

In relation to canopy photosynthesis models, it can be questioned whether P is simply a function of instantaneous conditions (PAR, C, temperature, air humidity, water status) and plant parameters (LAI). There are indications for other influences as well. First there may be a time-dependent variation within a day. This can be a temporary dip, a 'midday depression' (Meidner & Mansfield, 1968), or a slow decline during the course of the day (Ito, 1971). This might be based on assimilate accumulation, on light, water relations or temperature, but it was also suggested that there is a type of endogenous mechanism, more or less independent of external conditions (Meidner &

Mansfield, 1968). The occurrence of a time-dependent variation in the measurements is examined here.

Secondly, the photosynthetic system may adapt to high C. It is often reported that the photosynthetic efficiency per unit leaf area (CO_2 exchange rate, CER) declines after long-time exposure (weeks) to high C (Hurd, 1968; Von Caemmerer & Farquhar, 1984; Sage et al., 1989; Yelle et al., 1989a,b; Dugal et al., 1990, Bunce, 1992). This topic is not investigated here: it is a main topic in modern photosynthesis research and it will be investigated on the scale of greenhouses in another study (Heuvelink, 1994, pers. comm.).

2.1.2 Objectives

The first objective of the present work is to obtain reliable data sets on the effects of C on the net photosynthetic CO_2 assimilation rate (P) of stands of greenhouse cucumber, sweet pepper and tomato under 'practical' conditions and a realistic range of CO_2 concentration (about 200 to 1100 μmol mol^{-1}). The data sets, intended for modelling, should contain the photosynthetic rate in combination with environmental conditions. The second objective is to derive a rule of thumb for CO_2 ('CO_2-rule'), describing the effects of C on P in global terms. The third objective is to verify existing models (Acock et al. 1978; Thornley, 1976) regarding the sensitivity to prevailing C. The research is restricted to the instantaneous response of P to C. It is considered whether a time-dependent variation (e.g. midday depression) in canopy photosynthesis occurs in the measurements.

2.1.3 Approach and structure of the chapter

Canopy photosynthesis was investigated in six semi-commercial cultivations under practical conditions, mostly with parallel measurements in several similar greenhouse compartments with various CO_2 concentrations. Before the data were used, they were subjected to a detailed error analysis (section 2.3) and it was checked whether a time-dependent variation within a day existed in the measurements (section 2.5). The results are presented and generalized by a regression equation, fitted to each data set. These regression models, meant just as a research tool, are used to estimate the main photosynthesis characteristics and to compare the results of the various crops (section 2.4). The regression equations are used also to derive a rule of thumb and it is discussed if one generic rule applies to all experiments (section 2.6). Finally, the data sets and the derived regression models are compared to two existing models (section 2.7), using the measured instantaneous conditions and measured leaf area index as input.

2.2 MATERIALS AND METHODS

2.2.1 Crops, greenhouses and environmental factors

Experiments were done with cucumber in autumn and spring, sweet pepper in spring and autumn and tomato in spring. Information about the six cultures and conditions is given in **Table 2.1**. Expt 1 was described previously (Nederhoff et al., 1988), while here only the data of that work are used.

Expt 1 differed from expts 2-6 with respect to the facilities. The main difference was the CO_2 supply method: in expt 1, CO_2 was given in pulses, resulting in a saw-tooth C pattern. In all other experiments, CO_2 was supplied continuously through mass-flow controllers, to maintain a constant C. Sweet pepper (expt 3) was planted and investigated in spring. This crop was maintained until the autumn (long season cultivation) and investigated again until October (expt 4). This old sweet pepper crop was compared to a young, newly planted sweet pepper crop in autumn (expt 5).

Expt 2 to 6 were made in a north-south oriented Venlo-type glasshouse complex, with normal pipe heating and with ventilation windows in the roof. The compartments had 192 m^2 ground area and 576 m^3 volume. The plants were grown on rockwool wrapped in plastic. Several times a day, the plants were supplied with a standard nutrient solution (Sonneveld & De Kreij, 1987) and the surplus was drained out. As the soil had not been used as a growing medium for years and was covered with white polyethylene film, emission of CO_2 from the soil was considered negligible.

The crops were grown under about 500 $\mu mol\ mol^{-1}$ at no or little ventilation, and at about outside concentration during ventilation. In this way, it was attempted to avoid differences between compartments and, moreover, to avoid serious negative adaptation to high C.

A micro-computer connected to a VAX-network (Bakker et al., 1988) was used to control the heating, ventilation, CO_2 supply, nutrient solution supply and the photosynthesis measurements. Climate control parameters were set according to standard commercial practice. The air temperature was monitored with calibrated, screened and aspirated PT-100 sensors, in the middle of each compartment, 1.5 m above the ground. Temperature and radiation were recorded once a minute, C once per 5 min. The achieved environmental conditions are summarized in **Table 2.1**. Photosynthetic active radiation (PAR, i.e. light flux, 400 - 700 nm) was measured with an energy response meter (TFDL, Wageningen, the Netherlands), located outside the greenhouse on a meteorological tower at the Research Station.

Table 2.1. *Information on cultivation and environmental conditions in the six experiments with canopy photosynthesis measurements*

experiment no	1 [a]	2	3	4[b]	5	6
crop	cucumber	cucumber	pepper	pepper	pepper	tomato
season	autumn	spring	spring	aut., old [b]	aut., yng	spring
cultivar	Corona	Lucinde	Delphine	Delphine	Rumba	Blizzard
planting date	13.08.87	18.12.87	18.12.87	18.12.87	13.07.88	19.12.88
plant density [d]	1.49/1.92	1.43/1.79	2.52/3.12	2.52/3.12	2.52/3.12	1.85/2.08
compartments	3, 4, 7 [a]	6, 8	4	4	6, 8	6, 8
cropped area (m^2)	43 [a]	154	154	154	154	170
start measurem. [c]	25.8/237	22.3/82	23.3/82	4.8/217	4.8/217	8.2/39
end measurem. [c]	20.10/293	13.5/134	24.6/176	16.10/296	20.11/324	2.5/122
nmbr of observ.	1218	348	556	438	1243	1568
LAI (at day) [d]	3.0 (246)	2.3 (48)	0.9 (48)	4.5 (165)	1.9 (223)	1.6 (61)
LAI (at day) [d]	3.5 (262)	3.3 (82)	4.5 (164)	6.9 (223)	---	2.4 (102)
LAI (at day) [d]	3.4 (295)	4.1 (136)	5.1 (176)	6.0 (307)	2.8 (333)	3.2 (128)
average LAI	3.4	3.6	2.8	6.7	2.7	2.2
global rad. (W/m^2)[e]	5/189/678	9/136/618	9/166/584	6/100/485	6/111/649	1/150/654
PAR (µmol s^{-1} m^{-2}) [e]	3/198/639	6/153/599	6/184/581	3/108/425	3/117/630	1/159/573
air temp. (°C) [e]	18/25/31	19/23/27	18/25/30	19/24/30	14/24/34	12/18/25
CO$_2$ (µmol mol^{-1})[e]	128/ 544/ / 1932	297/ 504/ / 996	284/ 530/ / 1483	296/ 454/ / 1186	292/ 440/ / 956	109/ 388/ / 945

[a] *expt 1 was carried out in another greenhouse complex (Nederhoff et al., 1988)*

[b] *same crop as in expt 3, but investigated some months later*

[c] *date / day number*

[d] *plant density (plants/m^2) is (1) total number of plants related to total ground area and (2) total number of plants related to cropped area, excluding main path*
LAI is leaf area per unit cropped ground area (main path excluded)

[e] *minimum, mean and maximum values obtained during the measurements. Global radiation outside, PAR at crop level, temperature and CO$_2$ inside greenhouse*

When necessary, an approximated conversion from energy flux to quantum flux was made (1 W m^{-2} PAR = 4.5 µmol s^{-1} m^{-2}; McCree, 1972; Meek et al., 1984). PAR was not measured inside the greenhouse, because shading by greenhouse construction elements would seriously complicate the light measurements. PAR at crop level was estimated by multiplying the measured outside PAR by a calculated instantaneous light transmission coefficient of the greenhouse cover. For this reason, a coefficient for overcast sky conditions was determined by using two identical PAR meters, one inside and one outside the greenhouse, yielding a value of almost 70%. The actual instantaneous transmission was calculated according to Gijzen (1992) with a model of Bot (1983). Input to this model were the measured light transmission coefficient under overcast sky, the calculated sun position and the calculated ratio between diffuse and direct light (Spitters et al., 1986).

Radiation increases the greenhouse air temperature and thus promotes the automatic ventilation, so it was generally difficult to achieve high C in combination with high radiation. Hence such conditions were scarce in some data sets (**Fig. 2.1**). As the measurements were stopped at high ventilation that usually goes together with high radiation, incident PAR did not exceed 700 μmol s^{-1} m^{-2}.

2.2.2 Principle of the photosynthesis measurements

The method applied for measuring canopy photosynthesis was to use whole greenhouse compartments as semi-open systems, and to approximate the air exchange rate on-line with nitrous oxide (N_2O) as tracer gas (after Lake, 1966). N_2O has the same molecular weight as CO_2, is not phytotoxic, is virtually absent in the outside air, is not absorbed by the plants and is easy to measure with an infra-red gas analyzer. CO_2 was injected into the greenhouse with a measured rate to maintain a set point. The CO_2 balance of the greenhouse air, in which the canopy photosynthesis (P) is the only unknown term, was calculated as:

$$dC(t) / dt = (G_c + S_c - P - L_c) / (h * \rho_c) \qquad \textbf{eqn. 2.1}$$

where:

$C(t)$ = CO_2 concentration (μmol mol^{-1}) at time t
G_c = rate of CO_2 emission from the ground (ignored, see above)
P = rate of net CO_2 uptake (g m^{-2} h^{-1})
S_c = rate of CO_2 supply (g m^{-2} h^{-1})
L_c = rate of loss of CO_2 to the outside (g m^{-2} h^{-1})
h = average height of the greenhouse (3 m)
ρ_c = density of CO_2 (1.83 kg m^{-3} at 20 °C and 101.3 kPa).
G_c, P, S_c and L_c on a ground area basis (1 g m^{-2} h^{-1} CO_2 = 6.3 μmol m^{-2} s^{-1}).

L_c was calculated as follows:

$$L_c = \xi' * h * \rho_c * [C(t) - C_a(t)] \qquad \textbf{eqn. 2.2}$$

where:

$C_a(t)$ = ambient (outside) CO_2 concentration (μmol mol^{-1}) at time t
ξ' = air exchange factor (h^{-1})

This ξ' was determined with the decay rate method (Goedhart et al., 1984): after the N_2O injection was stopped, the concentration declined and ξ' was calculated from the N_2O concentration at time t (N(t) in µmol mol^{-1}) by:

$$\xi' = [\ln(N(t_2)) - \ln(N(t_1))] / \Delta t \qquad \textbf{eqn. 2.3}$$

2.2.3 Photosynthesis measurements

Nitrous oxide was injected from high-pressure bottles up to a peak concentration of about 100 µmol mol^{-1} if vents were closed and up to about 300 µmol mol^{-1} at relatively high ventilation. In the first 10-15 min after injection, the distribution of N_2O was considered insufficiently homogeneous, so these measurements were discarded by an automatic procedure. The measurements were stopped when the window opening or the wind speed became too high (i.e. if the air exchange rate approached 3.5 h^{-1}) to avoid excessive tracer gas usage and inaccurate measurements.

The carbon dioxide concentration of the greenhouse air was kept on its set point by controlling the supply of pure CO_2, using feedback control. The injection rate was set between 6 and 12 g m^{-2} h^{-1}, and was recorded by calibrated thermal mass flow controllers (model 5851, Brooks, Veenendaal, The Netherlands).

The CO_2 and N_2O supply were controlled independently by computer-steered solenoid valves, in each greenhouse compartment separately. The gases were led to the compartment and injected there into the air stream of a centrifugal fan, connected to a network of plastic ducts on the ground. The CO_2 valves were opened automatically every 5 min for an interval, proportional to the difference between the actual and the desired C.

The sampling system in each greenhouse compartment consisted of a network of 6 mm nylon tubes, with eight inlet points connected to one central dust filter and one main tube towards the analyzer. The inlets were located in the upper half of the canopy, spread over the greenhouse compartment. Outside air was sampled through an inlet mounted on a location, where it was not directly influenced by CO_2 emission from the greenhouse. A computer-controlled multiplexer switched the sampling every minute between the four compartments and the outside, yielding a measuring cycle of 5 min. The sampled air was pumped forward to the gas analyzers. A second pump in a shunt continuously pumped air from the four sampling networks that were not analyzed, in order to reduce the lag time.

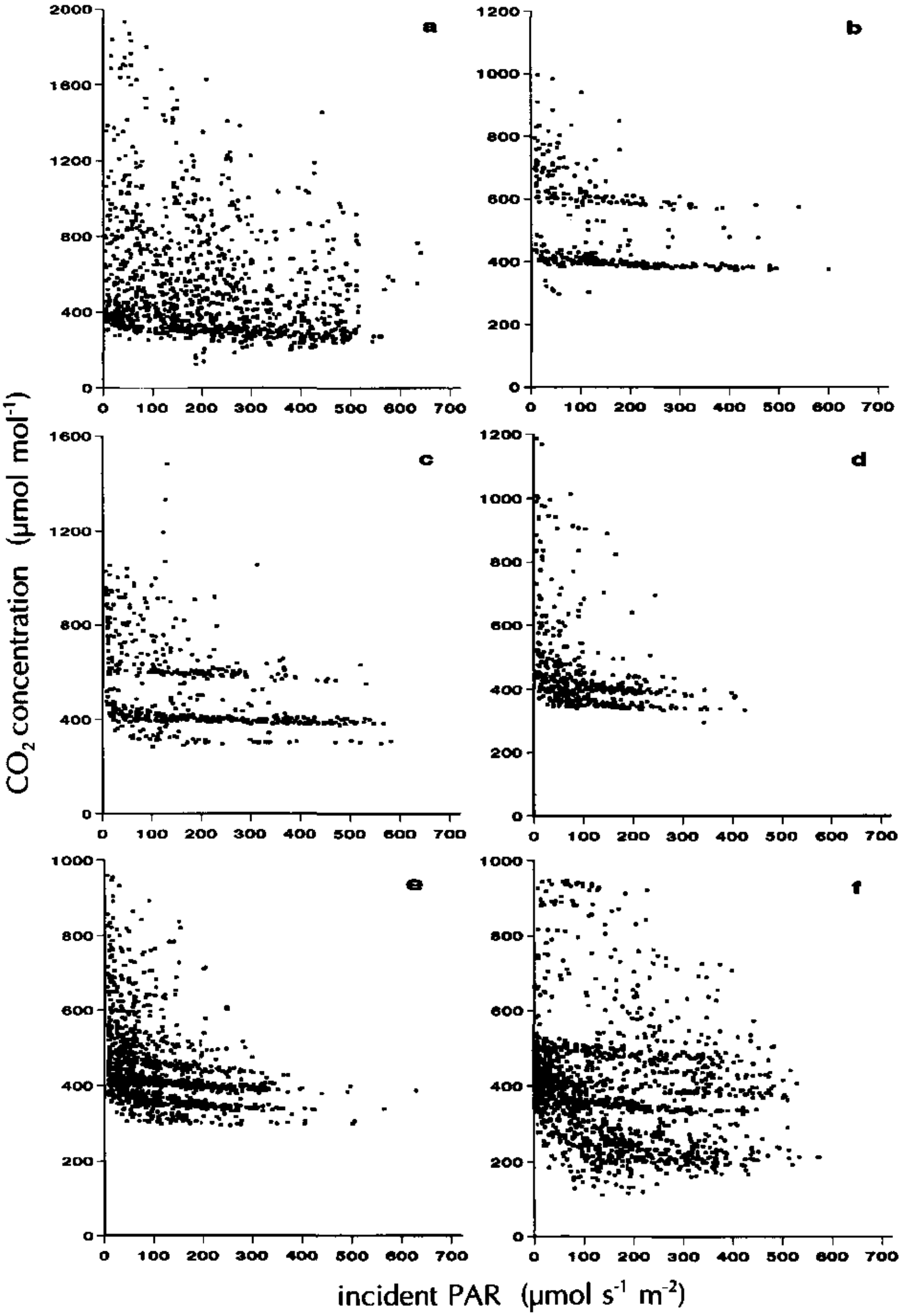

Fig. 2.1 *Incident PAR (µmol s^{-1} m^{-2}) and concomitant CO_2 concentration (µmol mol^{-1}) in the photosynthesis measurements.* **a - f** *are the six experiments as in* ***Table 2.1.***

Two infra-red gas analyzers (type G, Hartmann and Braun, Frankfurt, Germany), were used to measure the concentration of CO_2 and N_2O. The ranges were 0-300 for N_2O and 0-1000 and 0-2000 µmol mol^{-1} for CO_2. Both analyzers were calibrated at least once a week with pure nitrogen for zero calibration and with certificated calibration gases for span calibration. As the CO_2 analyzer was slightly sensitive to the N_2O gas, and vice versa, the measurements were corrected for this cross-sensitivity. This was established regularly with calibration gases.

2.2.4 Data processing

The measurements were controlled and recorded by the micro computer that was also used for environmental control. The data were transferred over the network to a VAX computer. Every night, the data of the previous 24 hours were processed automatically with a FORTRAN routine. Further analysis was done with the statistical package GENSTAT (Payne & Lane, 1987).

Some form of averaging was required to reduce the number of data (over 30.000 rough data records) and to filter out certain type of errors. An important error was caused by supply of CO_2 (S_c in eqn. 2.1): a high S_c in one 5-min interval could result in a negative value of calculated P in that particular interval, followed by a high calculated P in the next interval. To reduce this type of measuring errors, the calculations on the CO_2 balance (eqn. 2.1-2.3) were performed over three consecutive 5-min measuring cycles. The rate of change in C (dC(t) / dt) was calculated as the difference in C at the start and the end of this 15-min interval, divided by the time. For C(t) the average CO_2 concentration in this interval was taken, which was allowed because C was kept fairly constant. The air exchange factor in this period was calculated with linear regression on the logarithms of the measured N_2O concentration (eqn. 2.3). Before further analysis, the data records were averaged over 30 min intervals.

The choice of the length of the smoothed interval is based on some considerations. Smoothing over longer time intervals generally leads to loss of information, while the use of shorter intervals would increase the inaccuracy in observed P. An interval length of 30 min was considered a reasonable compromise for stable as well as unstable conditions (i.e. with constant or changing light and vents), regarding the time constants of the CO_2 supply and control system.

The data sets obtained consisted of 348 to 1568 data records, containing 30 min average values of measured canopy photosynthesis, PAR and C.

2.3 ACCURACY OF THE MEASUREMENTS

taken from: E.M. Nederhoff & J.G. Vegter, 1994a, Annals of Botany 73: 353-361.

2.3.1 Type of errors

As the photosynthesis measurements were performed automatically and continuously during day and night, some types of incidental errors in the measurements could not always be avoided. Apart from those errors, there was also the regular inaccuracy in the measurements. It is first explained how the incidental errors occurred and how they were discarded from the data sets. Then the regular measuring error is estimated.

2.3.2 Incidental errors in the measurement

To investigate the presence and size of errors, the photosynthesis measurements were done in the greenhouse compartments when they were without crop (P_{empty}). These 'zero-measurements' could reveal only the incidental errors and an off-set (deviation from 0) in the measurements. P_{empty} varied from 0 to about -0.3 g m^{-2} h^{-1}, which is considered too large an error compared to photosynthesis at low light conditions. Three conditions were detected as possible sources of error.

Determination of P was sometimes obviously incorrect for about 5 min after the ventilation rate had changed drastically. Secondly the measurements could be disturbed by human activity (opening of doors). These activities had to be recorded, but this was overlooked sometimes during the long-term measurements. In the third place, air was not only exchanged with the outside, but also with the corridor, by leakage through the inner glasshouse walls. To maintain the same CO_2 and N_2O concentration in the corridor as in the outside air, the vents of the corridor had to be open, but they may occasionally have been closed.

Before analysis, all data were checked for such incidental errors. Periods of disturbance were detected from an anomalous course of observed P, contrary to the course of PAR and C, or from exceptional measurements at night, when normally a fairly stable respiration rate was observed. If the measurements were obviously disturbed or suspected, the data for that period (some hours to some days) were rigorously discarded. The ultimate numbers of reliable data used in the analysis, are given per experiment in **Table 2.1**.

2.3.3 Regular measuring error

Error analysis

Apart from incidental errors, all observations contain an uncertainty i.e. inaccuracy or error. The error in calculated P depends on the errors in the underlying measurements. Combining and rearranging the equations used for the calculation of P (eqn. 2.1-2.3) yields:

$$P = S_c - a_p * \{(C2 - C1) + (0.5 * C1 + 0.5 * C2 - C_a) * [\ln(N1) - \ln(N2)]\}$$

eqn. 2.4

where C1 = CO_2 concentration at time t1 and C2 at time t2; C_a = outside CO_2 concentration, N1 = N_2O concentration at time t1 and N2 at t2; S_c = CO_2 supply rate and $a_p = h * \rho_c / (t2 - t1)$. Each of the measurements used in eqn. 2.4 (C1, C2, C_a, N1, N2, S_c) contains an error (ϵ). Thus C1 must be replaced by C1 + ϵ_{C1} and P should be P + ϵ_P, etc. in eqn. 2.4. The errors in these measurements (ϵ_{C1}, ϵ_{C2}, ϵ_{Ca}, ϵ_{N1}, ϵ_{N2} and ϵ_{Sc}) are 'partial measure errors'. The properties of the resulting overall error (ϵ_P) will be derived from those of the partial errors.

It is likely that the errors in the measurements made by one sensor (e.g. the CO_2 analyzer) are correlated, at least to a large extent. Hence, the relative errors in C1, C2 and C_a are assumed equal and the same holds for N1 and N2. It is not probable, although possible, that the measurements of different devices are correlated. In case of complete correlation between measurements of one analyzer, only three measuring errors play a role: ϵ_C, ϵ_N and ϵ_{Sc}.

The two gas analyzers were frequently calibrated, (see above), permitting to assume low errors in the CO_2 and N_2O measurements. Arbitrarily, the relative standard errors in these measurements are taken 5%. The error in S_c depended on the accuracy of the mass-flow controllers. Based on their specifications, this relative standard error can be assumed a few percent, but also for this measurement a 5% relative standard error was assumed.

First order error analysis

When the measuring errors are not too large, a good indication of the standard error can be obtained by first order error analysis (e.g. Lindgren, 1968). The standard error in P (ϵ_P) is found by:

$$\epsilon_P = \sum_i b_i * \epsilon_i$$

eqn. 2.5

where ϵ_i (with i = 1 to n) replaces the partial errors (ϵ_{C1}, ϵ_{C2}, ϵ_{Ca}, ϵ_{N1}, ϵ_{N2}, ϵ_{Sc} or ϵ_C, ϵ_N, ϵ_{Sc}). Each coefficient b_i is the first partial derivative of eqn. 2.4 to that i^{th} element. By analysis of eqn. 2.4 (and by a numerical method as well), it was determined that a relative error ϵ_C in the CO_2 measurements resulted in a relative error in observed P of magnitude $|L_c/P| * \epsilon_C$. Notice that eqn. 2.4 contains L_c in a concealed form. An error in N1 and N2 (as long as the relative errors were equal) did not have any effect on ϵ_P (eqn. 2.3). A relative error of ϵ_{Sc} in the CO_2 supply rate caused a relative error $|S_c / P| * \epsilon_{Sc}$ in P.

Eqn. 2.4 and 2.5 were applied to the data sets, under the assumption that the errors in the measurements of one analyzer were correlated. The ratios $|L_c/ P|$ and $|S_c/ P|$ were determined for all observations. **Table 2.2** presents for each data set the 95% quantile of these ratios. For example, the 95% quantile of $|L_c/P|$ is 1.1 in expt 2, meaning that the value of this ratio is below 1.1 in 95% of the observations. Consequently, a relative standard error of 5% in C causes less than 5.5% relative standard error in P, in 95% of the observations in expt 2. Fully correlated relative errors in N1 and N2 have no influence on P (eqn. 2.3).

The overall relative standard error in P caused by the different sources of error can now be found as the root of the sum of squares of the partial relative standard errors, taking into account the factors $|L_c/ P|$ and $|S_c/ P|$. In all experiments, the resulting estimated overall relative standard error appears to be in the order of ±10%, at least if the majority of the observations is considered (Table 2.2) and under the assumption that the errors of different devices are not correlated. The use of 95% quantiles implies that in 5% of the observations, the estimated partial error is larger than the values presented.

Table 2.2. *Estimated relative standard error (in %) in observed canopy photosynthesis rate (P), and 95% quantiles of some ratios used for error analysis. Values of $P < 0.1$ were ignored in the calculation of the quantiles.*

experiment:	2	3	4	5	6
95%-quantile of $\mid L_c / P \mid$	1.1	1.2	1.3	1.2	2.1
95%-quantile of $\mid S_c / P \mid$	1.2	1.7	1.5	1.6	1.0
relative standard error (%)	8.1	10.4	9.9	10.0	11.6

Error analysis on artificial data

The same approach with eqn. 2.4 and 2.5 was applied to an artificial data set, consisting of various fictive but realistic combinations of S_c, C1, N1 and N2, with C_a = 350 μmol mol^{-1} and with C2 = C1 (constant CO_2 level). The conditions chosen covered the ranges present in the experimental data sets. If the measurements of one analyzer are assumed completely correlated, and if the partial standard errors are taken 5%, the relative standard error ϵ_P is 5% as well. If the partial errors would be independent (which is not realistic), the relative standard error ϵ_P would be larger. Usually, ϵ_P would be below 10%, except if low photosynthesis rates are low ($P < 0.5$ g m^{-2} h^{-1}) or if high CO_2 supply rates are combined with high CO_2 exchange with the outside.

2.3.4 Conclusion

Based on the results of both the theoretical error analysis and the error analysis on artificial data, it is concluded that the estimated relative standard error in observed photosynthesis rate (P) is in the order of 10%, ignoring some large relative errors occurring under particular conditions. Very high relative errors are possible, particularly at high C in combination with high ventilation rates, and secondly at low P. The first condition is rare in the measurements, the second condition, low P, occurs very frequently, e.g. at the beginning and ending of a day. The relatively good accuracy of the majority of data permits to use the data for interpretation and modelling. On the other hand, the possible large relative errors at low P require attention at the analysis and interpretation.

2.4 REGRESSION MODEL AND CHARACTERISTICS OF CANOPY PHOTOSYNTHESIS

taken from: E.M. Nederhoff & J.G. Vegter, 1994a, Annals of Botany 73: 353-361.

2.4.1 Regression model

The photosynthesis data were analyzed by fitting a multiple regression equation to the data of each experiment. This was not an attempt to develop a new photosynthesis model, but a method to synthesize the experimental results.

For the dependence of P both on light and on C, a double rectangular hyperbolic relation was chosen (Thornley & Johnson, 1990). Adding a term for temperature appeared not to be useful, because of the high correlation between temperature and PAR. The leaf area index (LAI) was considered in two ways: a saturation term [^{e}log(LAI)] multiplicative to the terms for light and C accounted for the light interception effect. LAI was considered to also represent the amount of biomass, which determines the maintenance respiration. This effect was expressed by an additive linear term [$p_6 * \mathrm{LAI}$]. In the model fitting procedure, however, it appeared that with most data sets, the regression equation performed significantly better if the term ^{e}log(LAI) was omitted. Also some other variants of the proposed model were tested, but the best fit was found by the model:

$$P = p_1 + p_2 * [\, I / (p_3 + I)] * [C / (p_4 + C)] + p_5 * L \qquad \textbf{eqn. 2.6,}$$

where p_n is a parameter, I is incident PAR at crop level (in µmol s^{-1} m^{-2}), C the CO_2 concentration (in µmol mol^{-1}) and L the leaf area index (LAI in m^2 m^{-2}) as measured regularly during the cropping period (**Table 2.1**).

The common photosynthesis characteristics could not be obtained directly from the fitted equation, but were calculated, as follows:

- maximum canopy photosynthesis rate (P_{max}) as P under high PAR and high C;
- light compensation point as PAR where P is zero;
- light use efficiency (LUE, light utilization efficiency, photosynthetic efficiency or photochemical efficiency, or if expressed in mol CO_2 per mol photons also called quantum yield) as initial slope of the photosynthesis-light curve;
- CO_2 compensation point as the CO_2 concentration where P is zero;
- CO_2 use efficiency (or CO_2 utilization efficiency or carboxylation efficiency or mesophyll conductance) as P per unit CO_2 at CO_2 compensation point;
- relative effect of a CO_2 step from 350 to 700 µmol mol^{-1} on P.

Most calculations were made at two levels of C or at two levels of light. L was set to the average value of the measured LAI.

Table 2.3. *Parameters p_1 to p_5 of the multiple regression equation* $P = p_1 + p_2 * [I / (p_3 + I)] * [C / (p_4 + C)] + p_5 * L$ *(eqn. 2.6) fitted to the canopy photosynthesis data of the six experiments (see* ***Table 2.1****), with P = rate of net canopy photosynthesis (g m^{-2} h^{-1}); I = incident PAR (µmol s^{-1} m^{-2}); C = CO_2 concentration (µmol mol^{-1}) and L = LAI. Between brackets the standard error.*
p_1 & p_5 and p_2 & p_3 and sometimes p_2 & p_4 were highly correlated ($r > 0.5$).

parameter	p_1	p_2	p_3	p_4	p_5	r^2
1. cucumber	-1.39 (0.2)	8.3 (0.4)	268 (21)	281 (19)	0.23 (0.07)	82.5
2. cucumber	1.33 (0.3)	17.1 (2.6)	771 (125)	262 (57)	-0.54 (0.08)	90.9
3. pepper	-0.51 (0.1)	25.8 (5)	1202 (233)	464 (72)	-0.08 (0.02)	89.5
4. pepper	1.15 (0.4)	36.8 (14)	1929 (770)	205 (63)	-0.26 (0.06)	87.0
5. pepper	-2.48 (0.1)	9.3 (0.8)	573 (55)	119 (24)	0.71 (0.04)	87.6
6. tomato	-0.78 (0.0)	11.9 (0.6)	577 (42)	221 (14)	0.18 (0.02)	89.2

2.4.2 Regression models fitted to the measurements

Table 2.3 presents the fitted parameter values; **Table 2.4** gives some photosynthesis characteristics calculated with this model, and **Fig. 2.2** the resulting curves. The regression model explained 83 to 91% of the variance in the data (r^2 in **Table 2.3**). Parameter p_5, describing an effect of LAI, is rather variable (positive and negative). This is explicable, because this term accounts for the maintenance respiration with LAI representing the amount of biomass. As P is affected negatively by much biomass, p_5 is negative in crops with a considerably increased LAI.

The values found for some of the characteristics (**Table 2.4**) may slightly depend on the type of regression model (eqn. 2.6) applied in the analysis (Peat, 1970). It should be remarked that in expt 3 four data ($P > 4.5$ g m^{-2} h^{-1}) were ignored in the fitting procedure, as they severely reduced the fit. These points were extreme compared to previous and subsequent measurements.

The six experiments yielded rather different results (**Table 2.3** and **2.4** and **Fig. 2.2**), but it seems that a large variability is in the nature of photosynthesis, already at the level of a leaf, and so certainly also on the level of a canopy (Acock et al., 1976; Jones et al., 1991a; Hand et al., 1993a). A part of the variation found in the literature may be due to the fact that in greenhouses C is more variable than in the open field. In the following, the results are compared with the literature and related to the prevailing conditions.

Table 2.4. *Values of some canopy photosynthesis characteristics calculated with the fitted regression equations (**Table 2.3**); between brackets the standard error; C.P. = compensation point; LUE = light use efficiency.*

experiment:	1	2	3	4	5	6
n	1218	348	556	438	1243	1568
average LAI	3.4	3.6	2.8	6.7	2.7	2.2
P_{max} [a]	4.8	8.0	8.6	--	5.2	6.5
(g m^{-2} h^{-1} CO_2)	(0.1)	(0.6)	(0.7)	--	(0.3)	(0.2)
P_{max} / LAI	1.4	2.2	3.1	--	1.9	2.9
(g m^{-2} h^{-1}) [a]	(0.04)	(0.2)	(0.3)	--	(0.1)	(0.1)
light C.P. [b]	40 / 27	53 / 37	86 / 53	54 / 40	51 / 42	32/ 24
(µmol s^{-1} m^{-2})	(2) / (1)	(3) / (2)	(3) / (2)	(2) / (2)	(1) / (1)	(1) / (1)
LUE [b] (mol mol^{-1})	0.10/0.15	0.08/0.11	0.06/0.09	0.07/0.10	0.07/0.09	0.08/0.10
standard error (0.001 *)	(6) / (7)	(5) / (7)	(3) / (4)	(4) / (7)	(3) / 3)	(2) / (3)
CO_2 C.P. [c]	37 / 27	29 / 16	54 / 27	20 / 9	19 / 11	18 / 11
(µmol mol^{-1})	(3) / (2)	(4) / (2)	(4) / (2)	(4) / (2)	(3) / (2)	(1) / (0.7)
CO_2 use effic. [c,d]	7.6 / 10.9	10 / 19	6.5 / 14	15 / 36	13 / 23	11 / 18
(m h^{-1})	(0.2) / (0.3)	(1) / (2)	(0.3) / (1)	(2) / (6)	(1) / (2)	(0.3) / (1)
CO_2 effect (%) [c]	36 / 34	33 / 30	53 / 46	26 / 24	18 / 16	27 / 26
350 →700 µmol mol^{-1}	(2) / (1)	(5) / (5)	(5) / (4)	(6) / (6)	(3) / (3)	(1) / (1)

[a] *incident PAR 1350 µmol s^{-1} m^{-2} (300 W m^{-2}) and CO_2 1000 µmol mol^{-1}*

[b] *two CO_2 levels: 350 and 1000 µmol mol^{-1}*

[c] *two levels of PAR: 450 and 1350 µmol s^{-1} m^{-2} (100 and 300 W m^{-2}) at crop level*

[d] *CO_2 concentration increased from compensation point to 10 µmol mol^{-1} more*

2.4.3 Characteristics of canopy photosynthesis

Light saturation and maximum photosynthesis

In none of the six experiments was the rate of photosynthesis saturated at the prevailing light conditions (700 µmol s^{-1} m^{-2}). This is in accordance with other findings: most of the surveyed photosynthesis curves showed no light saturation or only at high PAR (Hand et al., 1993a). These authors observed that the photosynthesis rate of an eggplant canopy approached saturation at 900 µmol s^{-1} m^{-2} PAR, whereas under high C this occurred at even more light. Warren Wilson et al. (1992) observed light saturation only at 2500 µmol s^{-1} m^{-2}.

The calculated values for P_{max} (**Table 2.4**) varied from 5 to 9 g m^{-2} h^{-1} CO_2 uptake per ground area, which compares with a value of 9 g m^{-2} h^{-1} measured by Warren Wilson et al. (1992). The ratio P_{max}/L was between 1.5 and 3 g m^{-2} h^{-1}; it was highest for the crops with about the lowest LAI (expt 3 and 6).

This can be explained by a relatively high light interception of all leaf layers, i.e. a relatively low mutual shading. Canopy P_{max}/L is lower than P_{max} of single tomato leaves (e.g. Jones et al., 1991a), which is also explained by the effect of mutual shading of leaf layers in a full-grown canopy.

Light related characteristics

The calculated light compensation points (**Table 2.4**) were between 32 and 86 µmol s^{-1} m^{-2} PAR at low C. The review by Warren Wilson et al. (1992) showed values within the range 45 to 100 (extremes of 180) µmol s^{-1} m^{-2}. The light compensation point normally decreases with increasing C (Warren Wilson et al., 1992; Hand et al., 1993a), as also seen in our calculated data.

The calculated initial light use efficiency (LUE) of the canopy at low C was 11 to 18 µg J^{-1} (CO_2 uptake per unit incident PAR-energy), i.e. 0.06 to 0.10 mol mol^{-1} (mol CO_2 per mol intercepted PAR-photons, **Table 2.4**). At the light compensation points, the LUE was slightly lower (data not presented). These values are of the order of magnitude reported for canopies (Acock et al., 1976; Hand et al., 1993a) and somewhat higher than for leaves (Farquhar et al., 1980). Goudriaan et al. (1985) cited that the measured LUE rarely exceeds 17 µg J^{-1} (0.09 mol mol^{-1}), while from theoretical considerations the maximum leaf LUE is approximately 25 (0.13 mol mol^{-1}). So in expt 1 at high C our calculated LUE is unrealistic. A higher LUE at higher C (**Table 2.4**) is consistent with the literature (Farquhar et al., 1980; Hand et al., 1993a).

The conversion efficiency for light at 100 W m^{-2} and low C ranged from 0.015 to 0.05 mol mol^{-1} (data not presented), which compares with values reported by Warren Wilson et al. (1992) and Hand et al. (1993a).

CO_2 related characteristics

The CO_2 compensation point (**Table 2.4**) was 18 to 53 µmol mol^{-1} at 100 W m^{-2} and 8 to 27 at high light. Acock et al. (1976) reported CO_2 compensation of a tomato canopy of 63 µmol mol^{-1}. Our values compare very well with data determined by Farquhar et al. (1980), although these were for leaves. Somewhat higher values were reported for a single leaf (Goudriaan et al., 1985) and for a single plant in a cuvette (Nilwik, 1980).

The CO_2 use efficiency at the CO_2 compensation point was between 6.5 and 15 m h^{-1} (g m^{-2} h^{-1} CO_2 exchange per g m^{-3} CO_2) at low light and it was higher at high light. The CO_2 use efficiency was highest in expt 4, explicable by the high LAI. The observed range compares with the value derived by Goudriaan et al. (1985) and it is slightly higher than the value estimated by Jones et al. (1991a).

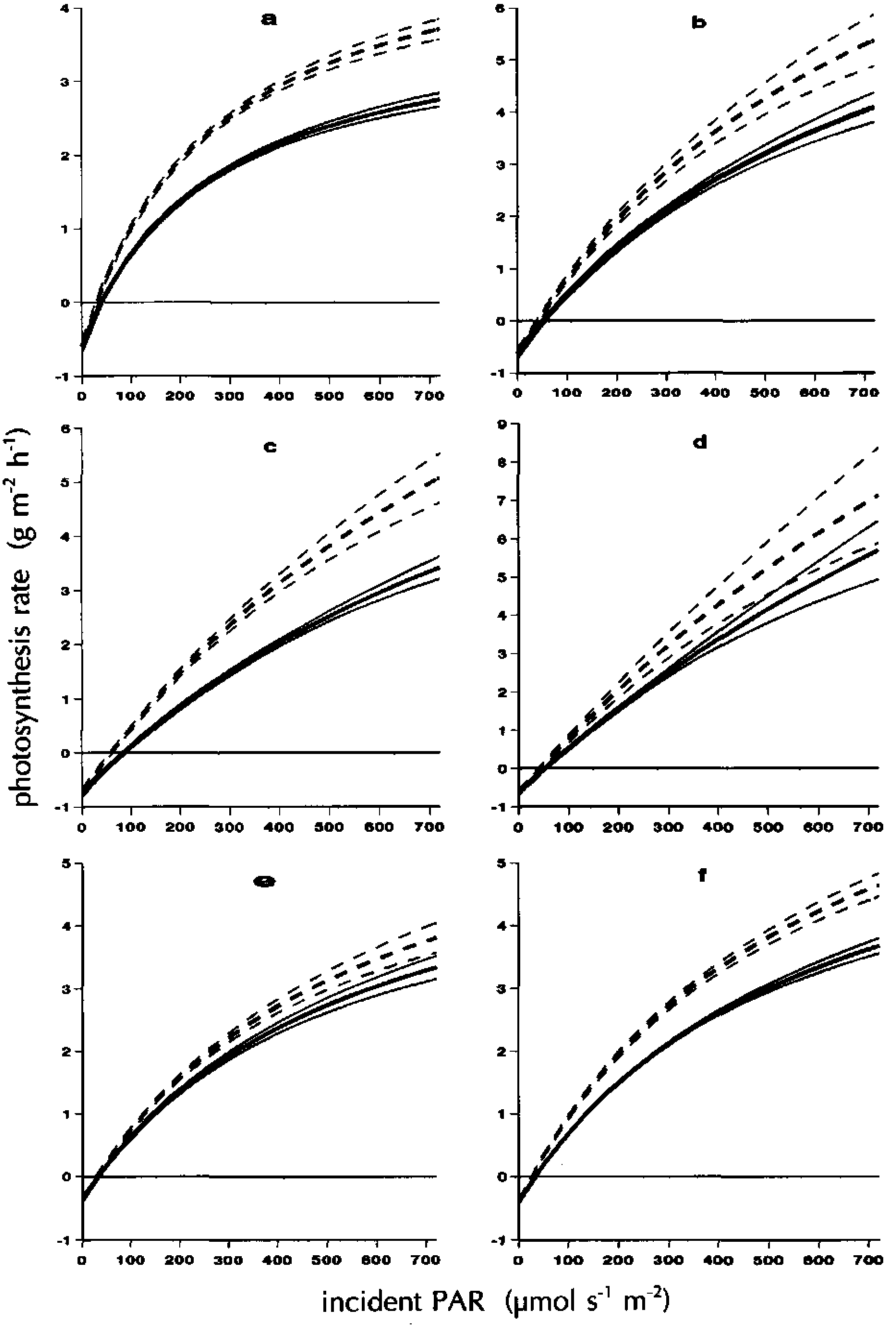

Fig. 2.2. *Net canopy photosynthesis rate (P in g m^{-2} h^{-1} CO_2 uptake related to ground area) calculated with the fitted regression model* (**Table 2.3**) *as function of incident PAR (µmol s^{-1} m^{-2}), with for L the average measured LAI* (**Table 2.1**).
Calculated for 350 µmol mol^{-1} CO_2 (———) and 700 µmol mol^{-1} CO_2 (– – – –). Thin lines indicate 95% confidence limits. **a - f** *are the six experiments as in* **Table 2.1**.

The effect of C on P is shown in **Table 2.4** and in **Fig. 2.2**. Note the strong response to C and light in expt 3 and the relatively weak responses in expt 5, both young sweet pepper crops. Also P_{max} and P_{max} / L were higher in expt 3 than in expt 5, although the calculations were made with equal light and about the same LAI. In expt 5, both LAI and average light were low (**Table 2.1**), probably explaining the overall low photosynthesis rates. A low LAI is responsible for a low response to light (e.g. Thornley & Johnson, 1990), while unfavourable light conditions cause that P is low and the response of P to C less pronounced (e.g. Gaastra, 1959).

In addition, some seasonal acclimation might play a role, although this cannot be proven. It was observed that the specific leaf area was markedly greater in expt 3 than in expt 5. The thinner leaves in expt 3 can be regarded as 'winter leaves' and those in expt 5 as 'summer leaves'. Ludwig & Withers (1978) observed different responses of summer and winter leaf photosynthesis to light and C, associated with different rates of photorespiration.

2.4.4 Remarks and conclusions

The large variability in calculated photosynthesis curves (**Fig. 2.2**) and characteristics (**Table 2.4**) - although not larger than in the literature - is attributed to several factors:

a. correlation between factors: a small photosynthetic apparatus (low LAI) decisively reduces the response of the canopy to light and reduces P_{max}; low light conditions cause that the effect of C is less clearly observable.
b. limited ranges of C and light conditions in the measurements (high light in combination with high C were rare in some experiments, **Fig. 2.1**);
c. measuring errors (estimated 10% and occasionally higher);
d. errors in the estimation of incident PAR quantum flux (the light transmission coefficient of the greenhouse cover, the unit conversion);
e. fast variations in PAR and C, while 30-min average values were used (inducing overestimation of calculated P, due to the non-linearity of P)
f. seasonal variations or adaptations to climatic conditions;
g. factors influencing photosynthesis that were not accounted for in the regression equation, such as plant age, size, plant condition, sink strength of fruits, nutrition, water status, air temperature and humidity;
h. genetic differences between species.

Points **a** to **d** are relevant, as follows from the discussion above. Point **e** is of minor importance. Point **f** might perhaps play a role, e.g. as discussed with respect to the differences between expt 3 and 5. Also point **g** and **h** might be true, but the present data material cannot provide evidence for particular effects.

2.5 TIME-DEPENDENT VARIATION IN PHOTOSYNTHESIS

2.5.1 Midday depression

Since a long time, it is frequently reported in the literature that canopy photosynthesis may show a depression in the course of the day [see for instance Meidner & Mansfield (1968), quoting a publication of 1921 and Madsen (1976), referring to a publication of 1938]. Such a dip is called 'midday depression' or more particular 'midday closure of stomata' (Meidner & Mansfield, 1968). Some authors reported a lower P in the afternoon than in the morning hours under similar light conditions (hysteresis, e.g. Ito, 1971). Some authors (e.g. Meidner & Mansfield, 1968) suggested that the depression is partly caused by an endogenous mechanism. On the other hand, it is often discussed which conditions induced this depression, for instance high temperatures (Heath & Orchard, 1957), water stress (Kitano & Eguchi, 1993), inhibition by excessive light (Correia et al., 1990) or saturation with starch (Ito, 1971; Madsen, 1976).

2.5.2 Approach

It was investigated if there was a time-dependent variation in measured P, irrespective of the main environmental conditions. This was done by comparison of measured P (P_{meas}) to a 'standard' value of P (P_{model}), and to relate the relative difference [(P_{meas} - P_{model}) / P_{meas}] to the time of the day. P_{model} must be obtained by a photosynthesis model that accounts exclusively for instantaneous effects on P, for instance the model of Acock et al. (1976) or of Thornley (1976). It is preferred to determine P_{model} with the fitted regression equation (**Table 2.3**), because these were direct results of the present work and they were also solely based on instantaneous effects. In case of a midday depression, P_{meas} would be lower than 'normal', so the model would overestimate the canopy photosynthesis, and the relative difference would be negative in the afternoon. A regression line (linear or polynomial) fitted to the data, would show a negative trend.

2.5.3 Results

Fig. 2.3 shows the curves of [(P_{meas} - P_{model}) / P_{meas}] versus time of the day. First, it can be seen from **Fig. 2.3a** that expt 1 has the largest variance in the data, which is in accordance with the lowest value for r^2 (percentage of variance explained, in **Table 2.3**). This plot shows a number of deeply negative values. In this particular experiment, the overestimation of P could be ascribed

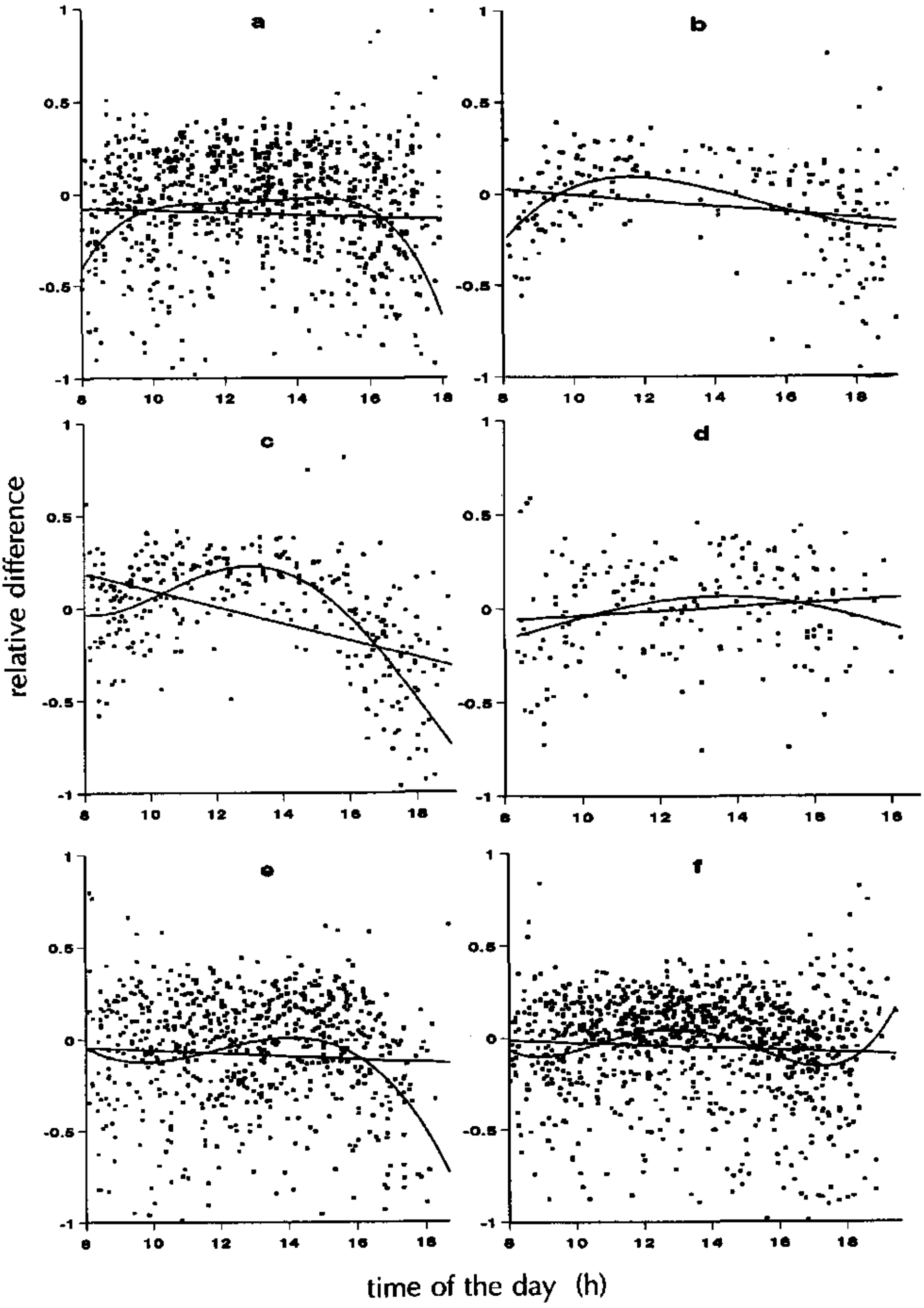

Fig. 2.3 *Relative difference in canopy photosynthesis as measured and estimated with the regression equations (**Table 2.3**) [$(P_{meas} - P_{model}) / P_{meas}$] versus time of the day. Some extreme values are outside the plots.* **a** - **f** *are the experiments as in* **Table 2.1**.

to inaccurate measurements, due to not constant CO_2 concentration (Nederhoff et al., 1988). In all other experiments CO_2 was supplied to a constant level.

Generally, the lines plotted in the figures show no systematic decline during the course of the day. The polynomial curve fitted is often negative after 16:00, but it is also often negative in the early morning hours. This indicates that other aspects (for instance the calculation of light interception at lower sun altitudes) play a role, rather than a midday depression.

It must be emphasized that no photosynthesis measurements were available of very sunny summer days. So the measurements do not provide evidence for the non-existence of a midday depression in general, but it can be concluded that a midday depression did not have a significant influence on the present photosynthesis measurements. This conclusion is in accordance with results of Hand et al. (1992 and 1993a) and Heuvelink (1995a). The latter author also did not observe hysteresis in canopy photosynthesis of tomato in greenhouses on sunny summer days under high C.

2.6 A 'CO_2-RULE' DERIVED FROM THE MEASUREMENTS

2.6.1 Introduction

One of the purposes of the work on photosynthesis is to establish a 'CO_2-rule', i.e. a rule of thumb describing in global terms the effects of C on production. This is intended to be applied by growers and advisers, for obtaining a sound estimation of the expected effect of CO_2 enrichment. It can be a helpful tool in economic studies and global explorations concerning CO_2 enrichment. It is assumed preliminary that the CO_2-rule can be derived from analysis of the effect of C on canopy photosynthesis, and that this relation can be transformed to a relation for the effect of C on growth and production (in chapters 4 and 5).

The CO_2-rule is intended to be a similar tool as the 'light-rule', a rule of thumb for light utilization. The light-rule, stating: '1% more light provides 1% more production', was introduced in the seventies (Verhaegh, 1980) and is generally applied in the Dutch greenhouse horticulture since then. This simple linear relationship without light saturation provides a reasonable estimation of the effect of a difference in light on fruit production. It is considered to be valid in the longer term, i.e. for the duration of a tomato cultivation (Buitelaar, 1984; Challa, 1984). A similar linear dependence was reported by other authors (e.g. Cockshull et al., 1992).

For the effect of C on P or on growth or production, such a simple description does not exist, but there is certainly a need for it in the horticultural practice. A rule of thumb must, by definition, be a simple expression, allowing a quick approximation, preferably without using a calculator. This conflicts with the fact that the response curve is a saturation type of curve with a (negative) intercept (**Fig. 1.1** and **2.2**). Gifford (1980) proposed the empirical relationship: $G = G_0 * (1 + \beta * \ln (C / C_0))$, with G the net growth and with the subscript 0 indicating a reference CO_2 concentration. This equation was referred to as 'the simplest one-parameter equation that also shows diminishing returns with increasing CO_2' (Goudriaan & De Ruiter, 1983). However, this equation is still too complex for a rule of thumb.

2.6.2 Approach

In search of a simple description of the effect of C on P, it was decided to focus on the relative effect (X in %) of an increase of 100 μmol mol^{-1} CO_2 at given C. The relative effect declines exponentially with increasing C and tends to zero. A CO_2 concentration of 1000 μmol mol^{-1} was (preliminary) chosen as reference.

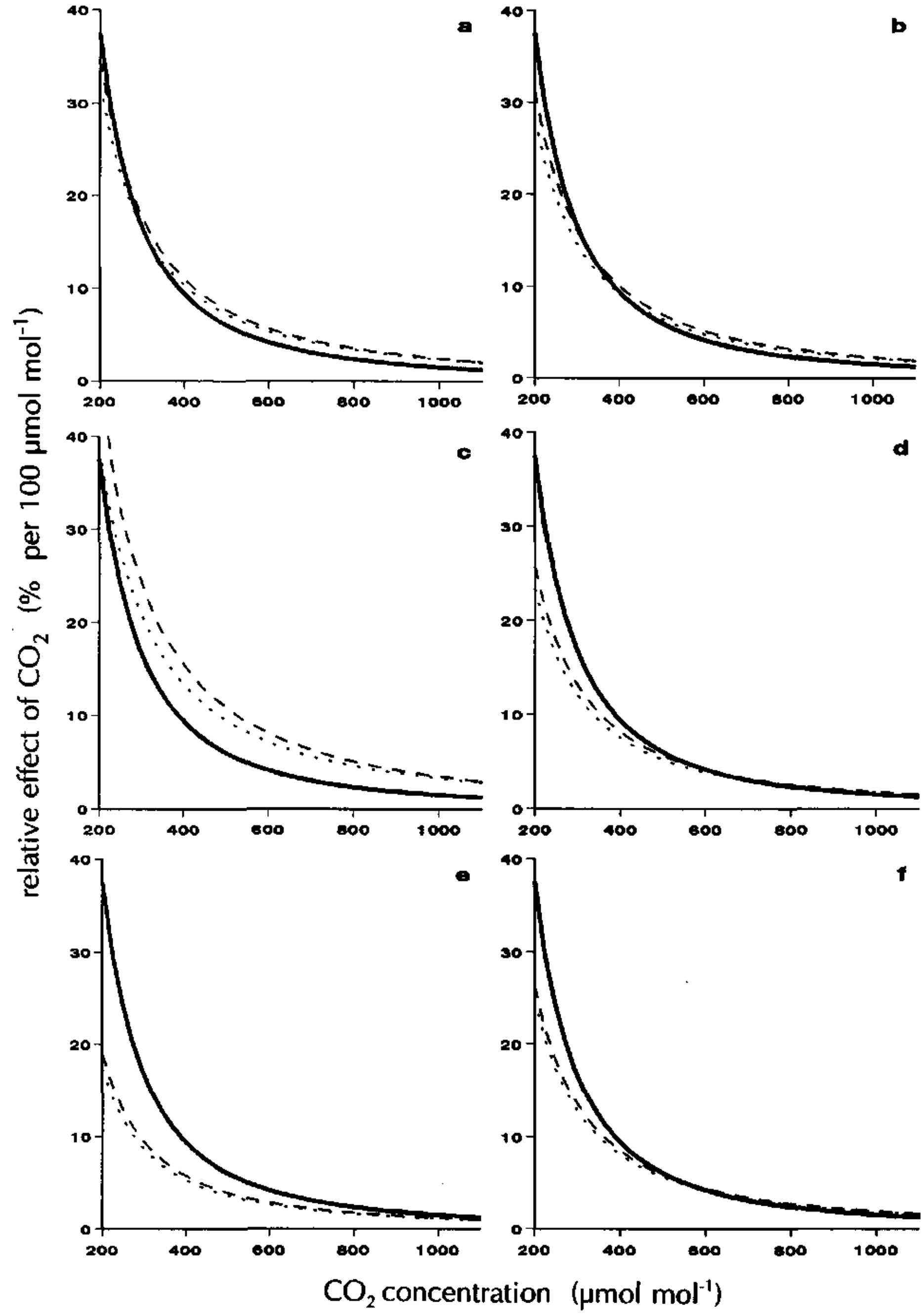

Fig. 2.4 *Comparison of the 'CO$_2$-rule' and the fitted regression equations* (**Table 2.3**) *regarding calculated relative effect of CO$_2$ concentration on photosynthesis (in % per 100 µmol mol^{-1} CO$_2$).* **a - f** *are the six experiments as in* **Table 2.1**

——— *CO$_2$-rule [$X = (1000 / C)^2 * 1.5$], eqn. 2.7b*

.... *fitted regression equations with incident PAR = 350 µmol s^{-1} m^{-2}*

– – – – *fitted regression equations with incident PAR = 1400 µmol s^{-1} m^{-2}.*

For describing the saturating decline, a statement of the form '1000/C' was proposed. To approximate the exponential relationship, the ratio 1000/C was quadrated. It was investigated if the relative effect of 100 μmol mol^{-1} increase in CO_2 at a certain C could be described by an expression of the form:

$$X = a_r * (1000/C)^2 + b_r \qquad \textbf{eqn. 2.7a}$$

The approach was to adjust this model to make it fit to the regression equations obtained in the six photosynthesis experiments (**Table 2.3**). The issue was not to find the best fit, but to find one equation with an acceptable fit to all six experiments. The parameters a_r and b_r should preferably get an easy-to-remember value.

2.6.3 Results and discussion

After testing various parameter settings, it was found that a value of 1.5 for parameter a_r gave acceptable results in general, while parameter b_r could be ignored. So the resulting 'CO_2-rule' proposed here, is:

$$X = (1000/C)^2 * 1.5 \qquad \textbf{eqn. 2.7b,}$$

with 1000 and C expressed in μmol mol^{-1}, and with the relative effect (X) in %.

In view of the issue, the statistical significance of the fitted parameters a_r and b_r of eqn. 2.7a is not essential. Instead, this CO_2-rule (with b_r = 1.5) is compared to the regression equations, which were fitted to the data sets (**Table 2.3**). In expt 4, 5 and 6, the CO_2-rule overestimates the effect (**Fig. 2.4**). Parameter b_r should be around 1.3 to fit to these experiments. In section 2.4.3, it was already discussed that expt 5 showed a weak response, due to low LAI and low light. It is arbitrarily decided that in general the proposed CO_2-rule with b_r = 1.5, gives satisfactory results when compared to the empirically established regression models. So the CO_2-rule may be used as a rule of thumb to roughly estimate the effect of C on canopy photosynthesis of tomato, cucumber and sweet pepper in greenhouses under normal conditions.

2.7 TEST OF TWO EXISTING MODELS

E.M. Nederhoff & J.G. Vegter, 1994b, Annals of Botany 73: 421-427.

2.7.1 Models, parameters and units

Two well-known models were selected that might be useable for computer control or decision support systems concerning CO_2 enrichment (section 2.1.1): the 'Acock model' (Acock et al., 1976; 1978; Acock, 1991) and the 'Thornley model' (Thornley, 1976; Thornley et al., 1992). These models are compact, easy to use, well-documented and applied by several other authors. These models were to be tested by comparison with the data sets.

The two models have the main parameters in common, which are listed below. To distinguish between the two, a subscript A (for Acock) or T (for Thornley) is added when necessary. Further a subscript ℓ (for leaf) or c (for canopy) is sometimes added. To enable comparison of the modelling results, some unit conversion is applied, e.g. with respect to C and light use efficiency. The photosynthesis and respiration rates are purposely expressed in different units for leaves and for canopies.

The common parameters are:
P_ℓ = leaf photosynthesis rate (g s^{-1} m^{-2} leaf area)
P_c = canopy photosynthesis rate (g h^{-1} m^{-2} (cropped) ground area)
R_c = rate of crop dark respiration of the canopy (g h^{-1} m^{-2} ground area)
R' = parameter for crop dark respiration rate, related to leaf area (g s^{-1} m^{-2} leaf area)
α = initial light use efficiency (light utilization or photosynthetic efficiency) (g J^{-1}) convertible to quantum yield (1g $J^{-1} \approx 0.0052$ mol CO_2 per mol PAR)
C = CO_2 concentration (µmol mol^{-1})
C' = CO_2 concentration after unit conversion (g m^{-3})
where: $C' = c_c * C$; with $c_c = 1.83E\text{-}3$ g m^{-3}
τ = conductance to CO_2 transfer (m s^{-1})
J = flux of incident photosynthetically active radiation (400-700 nm, W m^{-2})
L = leaf area index (LAI in m^2 m^{-2})

The subscripts used are:
A = Acock model   T = Thornley model
g = gross   n = net
c = canopy ℓ = leaf
0 = at top of canopy

2.7.2 Modified Acock model

The model for canopy photosynthesis of Acock (Acock, 1991) is similar to the leaf photosynthesis model in earlier work (Acock et al., 1976; 1978). These authors considered this early model a satisfactory empirical description of the response to light and C of a crop grown in rows (Acock et al., 1976). The addition in the newer model (Acock, 1991) is that it accounts for the fact that the parameters R and τ of the leaves change with depth in the canopy. They are assumed dependent on the average light received by the leaves in the previous week. The gross photosynthesis rate is calculated as:

$$P_{c\,g,A} = \alpha_{c,A} * J_0 * \tau_{c,A} * C' * 3600 \;/\; (\alpha_{c,A} * J_0 + \tau_{c,A} * C') \qquad \textbf{eqn. 2.8.}$$

Here J_0 (PAR at the top of the canopy) rather than J_ℓ (average PAR at leaf level) should be used (Acock, 1992, pers. comm.). The light extinction in the canopy is calculated with the classic light extinction formula (Monsi & Saeki, 1953) and is incorporated in the calculation of the CO_2 conductance ($\tau_{c,A}$):

$$\begin{aligned}\tau_{c,A} = a_A / (b_A * K) * \ln\{\,[\,b_A * S_0 * K + (1 - m)\,]\,/ \\ [\,b_A * S_0 * K * \exp(-K * L) + (1 - m)\,]\,\}\end{aligned} \qquad \textbf{eqn. 2.9,}$$

where:

K = extinction coefficient for light (-)

m = leaf transmission coefficient for light

S = average PAR during previous week (W m^{-2}), S_0 is at top of the canopy

a_A = constant (8.5E-5 $m^3\ J^{-1}$; Acock et al., 1978; Acock, 1991)

b_A = constant (2.1E-2 $m^2\ s\ J^{-1}$; Acock et al., 1978; Acock, 1991)

Canopy light use efficiency ($\alpha_{c,A}$) is derived from leaf light use efficiency ($\alpha_{\ell,A}$):

$$\alpha_{c,A} = \alpha_{\ell,A} * [1 - \exp(-K * L) / (1 - m)] \qquad \textbf{eqn. 2.10.}$$

To obtain the canopy net photosynthesis rate ($P_{c\,n,A}$) the gross photosynthesis ($P_{c\,g,A}$) is reduced with a canopy respiration rate ($R_{c,A}$), all in g $h^{-1}m^{-2}$ ground, according to:

$$P_{c\,n,A} = P_{c\,g,A} - R_{c,A} \qquad \textbf{eqn. 2.11.}$$

Modification. Acock (1991) distinguished photorespiration (calculated from CO_2 and O_2 concentration at the chloroplast and from CO_2 and O_2 conductance) and maintenance respiration (dependent on turnover costs of various leaf components). Growth respiration was not considered part of the photosynthesis process. We prefer not to include parameters of which the values are not available, like the mentioned O_2 parameters. Therefore, crop respiration is approximated here (in accordance with Acock et al., 1978) as follows:

$$R_{c,A} = R'_A * L * 3600 \qquad \textbf{eqn. 2.12,}$$

This equation accounts for the increasing crop respiration when the crop grows during the season. This approach is simple and straightforward. It does not account explicitly for a variation in R of leaves of different depths in the canopy. Note that $R_{c,A}$ is expressed in g h^{-1} m^{-2} ground area and R'_A in g s^{-1} m^{-2} leaf area. Here $R_{c,A}$ and R'_A include the respiration of the whole crop, whereas Acock et al. (1978) used a leaf respiration term, containing just the leaf component of dark respiration, divided by LAI.

2.7.3 Modified Thornley model

In the model of Thornley (Thornley, 1976; Thornley et al., 1992) the leaf photosynthesis rate ($P_{\ell,T}$) is simulated by a non-rectangular hyperbola. It is written explicitly by:

$$\begin{aligned} P_{\ell,T} = 1 / (2 * \theta) * \{ & \alpha_{\ell,T} * J_\ell + P_{\ell\, m,T} - \\ & \mathrm{SQRT}((\alpha_{\ell,T} * J_\ell + P_{\ell\, m,T})^2 - 4 * \theta * \alpha_{\ell,T} * J_\ell * P_{\ell\, m,T})\} \end{aligned} \qquad \textbf{eqn. 2.13}$$

where:

θ = parameter for curvature of light response curve (0.95)

$P_{\ell\, m,T}$ = light saturated value of leaf photosynthesis rate (g s^{-1} m^{-2} leaf area)

Both $P_{\ell\, m,T}$ and $\alpha_{\ell,T}$ (initial leaf light use efficiency) depend on the CO_2 concentration (C′ in g m^{-3}), according:

$$P_{\ell\, m,T} = \tau_{\ell,T} * C' \qquad \textbf{eqn. 2.14}$$

and

$$\alpha_{\ell,T} = \alpha_{\ell\, m,T} * (1 - (\beta / P_{\ell\, m,T})) \quad \textbf{eqn. 2.15}$$

with:

$\alpha_{\ell\, m,T}$ = leaf light use efficiency at high C (g J^{-1})

β = parameter for maximum photorespiration (g m^{-2} s^{-1})

Canopy net photosynthesis rate ($P_{c\,n,T}$ in g h^{-1} m^{-2}) is calculated as:

$$P_{c\,n,T} = L * P_{\ell,T} * 3600 - R_{c,T} \qquad \textbf{eqn. 2.16}$$

with

$$R_{c,T} = R_{c\,m,T} * [1 - \mathrm{EXP}(-L * R'_T * 3600 / R_{c\,m,T}) \qquad \textbf{eqn. 2.17}$$

with $R_{c\,m,T}$ = maximum crop dark respiration (0.72 g h^{-1} m^{-2}). Again R'_T is expressed in g s^{-1} m^{-2} leaf area and P_c and R_c in g h^{-1} m^{-2} cropped ground area.

Modification. To estimate the light interception, Thornley et al. (1992) assumed a standard overcast sky light distribution, a uniform distribution of light over the total leaf area and black canopy rows. Five components of PAR in different directions were estimated, based on the row dimensions. We think, however, that it is undesirable for real applications, to have to use accurate estimations of the (changing) canopy sizes and to estimate five PAR components. Therefore, although we are aware of the importance of the light interception part in the model, we simplify this part by replacing the extensive calculations and complex assumptions by one simple equation. Our modification assumes that J_ℓ is approximated by giving it a fixed fraction (F_J) of J_0. F_J is defined as the ratio J_ℓ / J_0 with $0 < F_J < 1$, and with J_ℓ the average PAR at leaf level and J_0 PAR on top of the canopy (W m^{-2}). Thus:

$$J_\ell = J_0 * F_J \qquad \textbf{eqn. 2.18}$$

F_J must be derived from radiation measurements inside the canopy or must be fitted, as done in this work.

2.7.4 Procedure

The canopy net photosynthesis rate was calculated with the models, using the 30-min recordings of J_0, S_0, C and the observed LAI as input. J_0 was obtained from outside measured PAR, multiplied by the calculated instantaneous transmission factor of the greenhouse cover (section 2.2.1). S_0 was obtained from the PAR recordings by the meteorology service on the Research Station in Naaldwijk, also multiplied by the calculated transmission. C was measured in the experiments. L was obtained by interpolating LAI measured periodically.

All modelling and calculating was done with the statistical package GENSTAT (Payne & Lane, 1987). The goodness of fit between model calculations and measurements was expressed by the intercept (*a*), slope (*b*) and percentage of variance explained (r^2) of the relation $P_{model} = a + b * P_{meas}$ and by the predicted error sum of squares (PRESS), which was calculated as $\sum (P_{model} - P_{meas})^2 / (n - 1)$. Further in run ii the residuals were examined by plotting ($P_{meas} - P_{model}$) and ($P_{meas} - P_{model} / P_{meas}$) versus C and versus PAR (plots are not presented).

Both models were tested with all six data sets in various ways:

run i. both models with the originally published ('standard') parameter values;
run ii. both models with relevant parameters tuned to each particular data set;
run iii. modified Acock model tuned to one data set (expt 6, tomato) and applied to all data sets;
run iv. modified Thornley model with only the light interception (parameter F_J) fitted to each data set and the other parameters standard.

In run i, the 'standard' parameter values were those reported in the original papers (Acock et al., 1976 (expt no. 4); Acock et al., 1978 and Thornley et al., 1992) as mentioned in **Table 2.5** and **2.7**, respectively. In run ii, the main adjustable parameters were fitted using a GENSTAT fitting routine, to obtain the best agreement between the model and the measured photosynthesis data.

Acock et al. (1976) regarded α, R and τ as adjustable. Parameter m has only a very weak influence on the calculations, so this was assigned a fixed value (0.10, which is about the average of the 'standard' values). Also b_A was kept at its standard value, in accordance with Acock (1991). K is theoretically of great importance, although varying the value of K between 0.6 and 0.8 caused only little difference in the fitting results. Nevertheless, K was estimated as well. With two data sets (expt 4 and 5), however, the fitting routine did not succeed in estimating all four relevant parameters in one run. Therefore, K was given a certain value in the range from 0.10 to 2.00 (increased by 0.02 in every new calculation) and at each value of K, the parameters $\alpha_{\ell,\ A}$, R'_A and a_A were fitted (a_A determines $\tau_{c,\ A}$). The combination of parameters with the highest r^2 and the lowest PRESS was considered the most adequate (only in expt 4 and 5).

The background of run iii with the Acock model was that perhaps some main circumstances (greenhouse, cultivation method) were principally different in our experiments and in the experiments that Acock used for the modelling. This might have a significant effect. Hence, we tested if adjusting the model to one of our data sets would improve the fit to the other data sets.

Thornley et al. (1992) regarded all parameters as adjustable, of which α and τ were relatively important. In run ii, the parameters $\alpha_{\ell m,T}$, β, $\tau_{\ell,T}$, R'_T and F_J were fitted, while θ and $R_{c\ m,\ T}$ were kept at their standard values. In the modified Thornley model, F_J (the ratio J_ℓ / J_0, eqn. 2.18) was introduced to replace the light interception calculation. F_J is an essential parameter with a dominant influence on the calculation, but its value was not known. In addition, F_J was fully correlated with $\alpha_{\ell\ m,T}$ and hence an auxiliary parameter ($\alpha'_{\ell m,T} = \alpha_{\ell\ m,T} * F_J$) had to be used in run ii. Then F_J was fitted separately in run iv, while all other parameters were kept standard. Subsequently $\alpha_{\ell\ m,\ T}$ could be determined from $\alpha'_{\ell\ m,T} / F_J$ (results in **Table 2.8**). A final test as in run iii with the Acock model was not possible with the Thornley model, because the light interception factor (F_J) was always unknown. Instead, run iv demonstrated how the Thornley model performed if only F_J was adjusted to the measurements.

Finally the response of both models to the prevailing C was examined by comparing the photosynthesis - CO_2 curves of the two tuned, modified models and of the regression equations derived from the measurements (section 2.4). The regression model was considered as reference, as it reflected the results of representative measurements. Because only the response to C is studied here, the other conditions were assumed constant, as indicated at **Fig. 2.5**.

2.7.5 Results

Modified Acock model

Table 2.5 presents the standard and fitted values of the modified Acock model and **Table 2.6** the goodness of fit of the various runs. The simulations with the standard parameter set ST1 (sweet pepper, Acock et al., 1976) mostly underestimated the photosynthesis rate at high photosynthesis (**Table 2.6**, run i). With the parameter set ST2 (tomato, Acock et al., 1978) the fit was equal or worse in all cases (data not presented).

In run ii, which involved parameter fitting to each experiment separately, the agreement between calculations and measurements was the best, as could be expected. The PRESS was decreased to 0.1 - 0.2 and of the relation $P_{model} = a + b * P_{measured}$, *b* was improved. No correlation was found between the residuals and C nor between residuals and PAR.

In run iii, with the model adjusted to expt 6 (tomato) and applied to the other data sets, the PRESS and the r^2 were more or less comparable to those of run i, and worse than of run ii (**Table 2.6**). This demonstrates that the fit does not depend on certain conditions (greenhouse, cultivation method), typical for our series of measurements.

Modified Thornley model

The results are given in **Table 2.7** and **2.8**. The modified Thornley model with standard parameters (run i) largely overestimated the measured data of all experiments (data not shown), because the light extinction calculation was omitted and not replaced by an alternative estimation (in run i, F_l was taken 1).

In run ii, with all parameters fitted to each experimental data set, the fit and the parameter values were reasonable, except in expt 3 (discussed below). Also with this model the residuals were not related to C or PAR. In run iv, with only parameter F_l fitted to the data sets, the performance was in most cases acceptable as well, mostly comparable to that in run ii (**Table 2.8**). This demonstrates the dominant influence of the light interception in this model.

Models' sensitivity to CO_2 compared

Fig. 2.5 shows the comparison of the three photosynthesis-CO_2 curves. In expt 2 (Fig. 2.5b), the three curves were about equal; in expt 4 (Fig. 2.5d), the 95% confidence interval of the regression model was rather wide; in the other experiments, the curves partly overlapped and partly were distinct. In expt 1, 5 and 6 the model calculations came out higher than the regression line. It should be noted that with other values for PAR and LAI taken, the results would have been slightly different.

Table 2.5. *Parameters of the modified Acock model. 'Standard' parameter sets from Acock et al. (1976 and 1978, resp.) and 1 to 6 the sets of parameter values (with standard error) fitted in run ii to the six experiments.* b_A*=2.1 E-2* $m^2\ s\ J^{-1}$*,* m*=0.10.*

data set	n	$\alpha_{L,A}$[a] μg J^{-1}	a_A μm^{-3} J^{-1}	R'_A μg s^{-1} m^{-2}	K -
Standard-1	---	13.7	85	38	0.82
Standard-2	---	10.1	85	14	0.52
1. cucumber	1218	22.1 (1.7)	43 (1.4)	32 (2.4)	0.61 (0.1)
2. cucumber	348	17.8 (0.9)	85 [b]	47 (3.4)	1.43 (0.4)
3. pepper	556	16.0 (0.7)	77 (5.2)	73 (3.9)	1.45 (0.2)
4. pepper	438	18.9 (0.6)	85 [b]	27 (1.6)	0.60 [c]
5. pepper	1243	22.7 (0.5)	85 [b]	46 (1.8)	0.42 [c]
6. tomato	1568	21.1 (0.7)	89 (2.2)	57 (2.2)	0.94 (0.0)

[a] *convertible to quantum yield with 1 g* J^{-1} *≈ 0.0052 mol* mol^{-1}

[b] *value assumed*

[c] *best fitted value determined by procedure described in text*

Table 2.6. *Goodness of fit of the modified Acock model to the data of the six experiments in run i, ii and iii. PRESS (predicted error sum of squares) is* $(P_{model} - P_{measured})^2/(n-1)$ *in* $(g\ m^{-2}\ h^{-1})^2$*; a is the intercept, b the slope and* r^2 *the percentage of variance explained of the relation* $P_{model} = a + b * P_{measured}$*.*

run	**run i**				**run ii**				**run iii**			
	with parameters				with fitted parameters				with parameters as fitted to			
expt	standard (Tbl 2.5, ST1)				(Tbl 2.5, data sets 1-6)				tomato (Tbl 2.5, data set 6)			
	PRESS	a	b	r^2	PRESS	a	b	r^2	PRESS	a	b	r^2
1.	0.32	0.06	0.80	74.8	0.22	0.26	0.80	80.4	0.47	0.09	1.12	77.5
2.	0.24	0.06	0.73	87.9	0.16	0.12	0.87	88.1	0.21	0.11	1.01	87.5
3.	0.25	0.35	0.73	84.4	0.20	0.16	0.84	85.7	0.55	0.53	1.02	84.8
4.	0.51	-0.34	0.63	84.5	0.15	0.12	0.81	83.3	0.53	-0.53	0.86	83.3
5.	0.11	0.12	0.73	81.2	0.09	0.12	0.79	82.8	0.18	0.19	1.02	80.2
6.	0.26	0.07	0.65	88.8	0.11	0.11	0.89	89.3	(0.11)	(0.11)	(0.89)	(89.3)

Table 2.7. *Parameters of the modified Thornley model. 'Standard' parameters are from Thornley et al. (1992). 1 to 6 are the sets of parameter values (with s.e.) fitted in run ii to the six experiments. $\theta = 0.95$, $R_{c\,m,\,T} = 0.72\ g\ h^{-1}\ m^{-2}$.*

data set	n	$\alpha_{l\,m,T}$ [a] µg J^{-1}	F_J --	β µg $m^{-2}\,s^{-1}$	$\tau_{l,\,T}$ 10^{-4} m s^{-1}	R'_T µg $s^{-1}\,m^{-2}$
'Standard'	--	17.0	--	400	15.0	50
1. cucumber	1218	16.2 (0.4)	0.26 (0.002)	31 (8)	3.0 (0.04)	23 (.3)
2. cucumber	348	15.0 (1.1)	0.28 (0.004)	58 (24)	4.6 (0.3)	58 (9)
3a. pepper	556	17.6 (0.8)	0.28 (0.005)	400 [b]	15.0 (1.3)	64 (10)
3b. as 3a [c]	556	20.5 (0.8)	0.19 (0.003)	56 [b]	5.8 (0.5)	66 (12)
4. pepper	438	16.2 (1.1)	0.19 (0.002)	64 (13)	3.0 (0.3)	52 (8)
5. pepper	1243	14.1 (0.6)	0.35 (0.002)	42 (13)	4.8 (0.1)	72 (4)
6. tomato	1568	14.8 (0.2)	0.50 (0.004)	111 (5)	7.0 (0.1)	46 (4)

[a] *and* [b] *as at Table 2.5*

[c] *same data set as at expt 3a, but with other preset value for β*

Table 2.8. *Goodness of fit of the modified Thornley model to the data of the six experiments in run ii and iv. PRESS (predicted error sum of squares) is $(P_{model} - P_{measured})^2/(n-1)$ in $(g\ m^{-2}\ h^{-1})^2$; a is the intercept, b the slope and r^2 the percentage of variance explained of the relation $P_{model} = a + b * P_{measured}$.*

expt	**run ii** with all parameters fitted, includ. F_J (Tbl 2.7, data set 1-6)				**run iv** with F_J fitted (see Tbl 2.7) and other parameters standard			
	PRESS	a	b	r^2	PRESS	a	b	r^2
1.	0.21	0.24	0.81	80.7	0.36	-0.07	0.93	74.4
2.	0.17	0.13	0.87	87.0	0.19	0.11	0.88	85.8
3a.	0.35	0.22	0.73	74.1	0.35	0.26	0.71	74.0
3b. [c]	0.37	0.24	0.71	72.9	0.38	0.25	0.71	71.7
4.	0.12	0.09	0.86	86.1	0.13	0.07	0.87	85.8
5.	0.07	0.07	0.88	87.7	0.08	0.10	0.84	86.1
6.	0.13	0.11	0.88	86.8	0.21	-0.04	0.93	81.7

[c] *same experiment as at expt 3a, but with other preset value for β*

2.7.6 Discussion

Fitted parameters

Parameter fitting leads by definition to a good fit, unless the structure of the model is incorrect. The results, both with the Acock model and the Thornley model, and the fact that the residuals of run ii were not related to PAR and C demonstrate that basically the models were correct and adequate to fit our canopy photosynthesis measurements. We discuss here whether the fitted values of our parameters were sensible.

The important parameter for leaf light use efficiency (α_l) came out on 16 - 23 µg J^{-1} (or 0.08 - 0.12 mol mol^{-1}) in the Acock model **(Table 2.5)**. Some of these values were higher than usually observed as maximum values for leaf initial light use efficiency (0.017 µg J^{-1} $\approx$ 0.09 mol mol^{-1}), although the theoretical maximum is far higher (Farquhar et al., 1980; Goudriaan et al., 1985). Acock et al. (1976) reported a range from 5 to 25 µg J^{-1} for α of the whole canopy. It is concluded that the Acock model fitted to our data only if unusually high values of the light use efficiency were accepted.

The values of α fitted by the Thornley model were 14 - 17.6 µg J^{-1} (or 0.07 - 0.09 mol mol^{-1}), and an extreme value of 20.5 µg J^{-1} (or 0.11 mol mol^{-1}) in expt 3 (**Table 2.7**). These values were not much different from those derived from our own measurements (section 2.4.3) and those reported in the literature (see 'standard' values in **Table 2.5** and **2.7**).

The values of K fitted by the Acock model were in two cases higher than commonly reported (K > 1.0). Monsi & Saeki (1953), however, determined values up to 2, the higher values particularly in canopies with horizontal leaves in a mosaic-like leaf distribution. The cucumber spring crop (expt 2) was such a crop.

The values of a_A (determining CO_2 conductance, $\tau_{c,A}$) in the Acock model were about equal to, or lower than, the standard values (**Table 2.5**). Also $\tau_{l,T}$ in the Thornley model came out lower than the standard values (**Table 2.7**), except in expt 3a, owing to correlation with β (see below). The fairly low τ_l might be explained by the moderate light intensities and the relatively high C in our experimental data. Both conditions significantly reduce the leaf conductance by stomata closure (Raschke, 1975; chapter 3).

The fitted values for crop dark respiration (R' in **Table 2.5** and **2.7**) ranged from 23 to 73 µg s^{-1} m^{-2} leaf area. The 'standard' values were 14, 38 and 50 µg s^{-1} m^{-2} leaf area, but these values were supposed to involve just the leaf component of dark respiration. In Acock et al. (1976) the values for dark respiration of the crop varied from 75 to 200 µg s^{-1} m^{-2} (probably m^2 ground area). So the fitted values and the variation in values agree with published data.

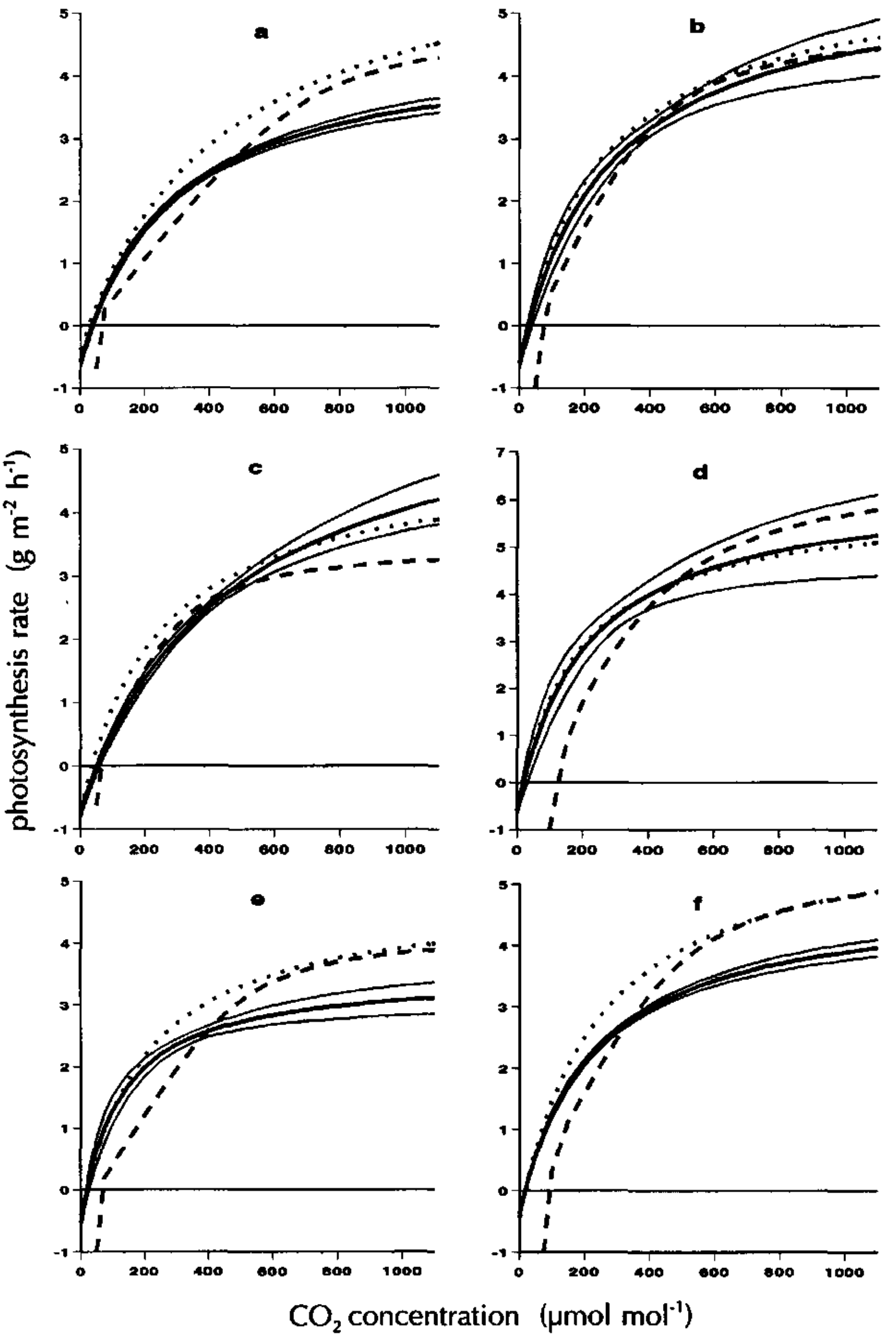

Fig. 2.5 *Sensitivity of photosynthesis models to prevailing CO$_2$ concentration. Canopy net photosynthesis rate (P in g h^{-1} m^{-2} ground area) as function of CO$_2$ concentration (µmol mol^{-1}), calculated for 100 W m^{-2} incident PAR, and for observed LAI (**Table 2.1**). **a - f** are the six experiments as in **Table 2.1**.*

——— *regression model fitted to measurements, with 95% confidence limits;*
.... *modified Acock model with fitted parameters (**Tables 2.5** and **2.6ii**);*
– – – – *modified Thornley model with fitted parameters (**Tables 2.7** and **2.8ii**)*

The fitted values for parameter β (photorespiration) in the modified Thornley model were far lower than the reported value. Because in expt 3, the fitting of the four selected parameters could not be achieved, β was preset. With the standard value (β = 400 $\mu g\ m^{-2}\ s^{-1}$), $\tau_{\ell,T}$ came out far lower than in the other experiments. If β was chosen in the same order of magnitude as the other fitted values (56 $\mu g\ m^{-2}\ s^{-1}$), also the value of $\tau_{\ell,T}$ was in agreement with the other values of $\tau_{\ell,T}$ (**Table 2.7**, expt 3b). So further (e.g. in **Fig. 2.5**) data set 3b was used for this experiment.

Responses to CO_2 and light

The responses to C are shown in **Fig. 2.5**. The curves of the regression model were explained by the conditions during the measurements (section 2.4). For example, the apparently weak response of P to C in expt 5 (**Fig. 2.5e**), was explained by poor light conditions during the measurements. At low light, the effect of C is less pronounced (Gaastra, 1959). It is remarkable, but not explicable, that the modified Acock model and the modified Thornley model both had a higher maximum photosynthesis rate and a stronger response to light and C than the actual crop in expt 1, 5 and 6.

In the CO_2 range below 500 $\mu mol\ mol^{-1}$, the Thornley model responded stronger to C than the Acock model and than the regression equation (**Fig. 2.5**). In contrast to the Acock model, the Thornley model accounts for the C effect on α (eqn. 2.14 and 2.15). Moreover, the Thornley model uses a linear dependency of $P_{\ell\ m,T}$ on C (eqn. 2.14), while a weaker relation would be more realistic. A shortcoming of the Acock model is that it does not account for the effect of C on the light use efficiency α (only on the gross photosynthesis $P_{c\ g,A}$). The fitting routine established only one value for α for the whole data set. For a test, α in the Acock model was calculated per data record, in dependence of measured C, using the theory of Goudriaan et al. (1985). However, this enhancement did not improve the performance of the modified Acock model.

Finally, it is concluded from Fig. 2.5 and from the fact that the residuals of run ii were not correlated with C and PAR, that the modified Thornley model performed moderately, and the modified Acock model reasonably.

2.8 DISCUSSION

Here the approach for photosynthesis research is evaluated, particularly with respect to the measurements, as these comprised the greater part of the work. Because it was emphasized that the measurements had to be done on a large scale (whole greenhouses), rather than on laboratory scale, it is reconsidered what the reason was for this approach and if it has been worthwhile. Further, the reliability of the obtained results is evaluated.

Generally, after a model has been developed, it needs to be evaluated, possibly by comparison with reality (i.e. validated). The reality of a large greenhouse production system cannot be imitated by a small cuvette, because of the following differences. In cuvettes mostly leaves or seedlings are investigated during a short observation period, whereas in greenhouses large crops can be investigated for a longer period, including the harvest period. The conditions in a small-scale set-up are normally constant; often artificial light is used with a light flux far below that of the natural light. In greenhouses, however, the natural light conditions occur, with large fluctuations and high amplitudes. In a cuvette, the leaves are directly lit, whereas in a greenhouse crop there is a great temporal and spatial variation in light interception. This implies that a model of leaf photosynthesis must be combined with a model for light distribution, to calculate the crop photosynthesis. Respiration, which has a great impact on net photosynthesis, comprises more components in a canopy than in a single leaf.

The aspects mentioned are all implicitly incorporated in large-scale experiments, where net canopy CO_2 uptake is measured in combination with the relevant explaining conditions. The argumentation for this approach is that the obtained data can directly be used for validation of a model, while the model can be implemented in practical applications without further enhancements (e.g the model of Gijzen, 1992; Gijzen et al., 1995). The data were also used to derive the so-called CO_2-rule, describing the effects of CO_2 on photosynthesis. Because this rule is derived from measurements in semi-commercial cultivations, it is directly applicable to practical situations. Without measurements in greenhouses, such results could not be accomplished directly.

Important for the merits of the obtained data sets is their reliability. The accuracy of the photosynthesis measurements was estimated by error analysis (section 2.3). This showed that the majority of the data is reasonably accurate (± 10%) and about 5% of the data contain a large inaccuracy. However, **Fig. 2.3** reveals a large variance, indicating that many observations (P_{meas}) have a large relative discrepancy to the 'standard' canopy photosynthesis (P_{model}) under the conditions recorded. This is not necessarily caused by to inaccuracy in the photosynthesis measurements, but can be ascribed partly to errors and inaccuracy in the measurements of the environmental conditions (input to the regression equation that yielded P_{model} in Fig. 2.3).

For instance, the light received by the canopy was approximated from measurements outside. This was done on purpose, because light measurements inside the greenhouse always give serious complications by local shading of the sensor. An estimation of the instantaneous light transmission coefficient was calculated on the basis of sun altitude and estimated ratio of direct and diffuse light (section 2.2.1). This estimation contributes to the inaccuracy. As P responds to quantum flux, while energy flux was measured, this conversion increased the inaccuracy. The measurements of the CO_2 concentration might have an inaccuracy of about 5%, in spite of frequent calibration. Air temperature measurements are estimated to be fairly accurate (± 0.2 °C), which error is hardly of importance for the photosynthesis calculation.

The variance in Fig. 2.3 is considered an inevitable consequence of measuring in semi-practical greenhouses. The spreading in the data is compensated for by the great number of observations, and hence the data sets were considered suitable for interpretation and modelling. The percentage of variance explained by the regression equation, may be increased by a more refined analysis.

It is concluded that the data sets are valuable, as they are complementary to other, more refined measurements in laboratory experiments. They provide information on the functioning of actual crops under representative conditions. It is accepted that measurements on this scale are less accurate than possible in laboratory experiments.

3 Effects of CO_2 on leaf conductance and canopy transpiration

3.1 INTRODUCTION

3.1.1 Outline of the problem

Besides the positive effects of a high CO_2 concentration (C) on plants mainly via the photosynthesis (chapter 2), also negative side effects of high C are known (section 1.3). Some side effects might be caused by a reduction of the stomatal conductance (g) and hence of the canopy transpiration (E) under elevated C.

High C may improve the water use efficiency by increased photosynthesis and reduced transpiration (Kimball & Idso, 1983; Morison & Gifford, 1984; Radoglou et al., 1992; Bowes, 1993), which is favourable in case of scarcity of good water. In other cases, however, reduced transpiration may be more or less disadvantageous: it may increase the leaf temperature, particularly under high radiation, and in the long term, it may induce local mineral deficiencies of those nutrients that depend for translocation on the transpiration flow (Mengel & Kirkby, 1978). Excessive leaf temperatures and insufficient nutrient translocation are unfavourable for plant growth and fruit production.

Stomata respond to C of the surrounding air. A CO_2 concentration of several thousands µmol mol^{-1} can disorganize the stomata, so that they are widely open, even at night (Holländer & Krug, 1992). For the range under consideration (about 200 - 1100 µmol mol^{-1}), it is often observed that increased C decreases the stomatal opening (Freudenberg, 1940; Heath, 1948; Kuiper, 1961; Raschke, 1975; Burrows & Milthorpe, 1976; Morison, 1987). There is no common opinion about the effect of C on E. It is sometimes reported that high C reduces the transpiration of greenhouse crops (Mortensen, 1987). Such an effect was indeed established on the scale of leaves and plants in controlled environment chambers (Janes, 1970), or in 'mini-greenhouses' (Shaer & Van Bavel, 1987). In other experiments on transpiration, however, no significant C effect was observed (Stanghellini, 1987; Jolliet & Bailey, 1992). These latter experiments were done in large greenhouses.

C does not directly affect *E*, other than through the stomata. About the relation between *g* and *E*, two views can be found, a physiological one: "transpiration is considerably controlled by stomata" (e.g. Raschke, 1975) and a physical one: "transpiration is determined by environmental factors, mainly radiation and vapour pressure deficit" (e.g. Penman, 1948; Stanghellini, 1987).

Jarvis & McNaughton (1986) investigated conductance and transpiration in systems of different scale (single pore, leaf, plant, canopy, region) and concluded that "one cannot naively extrapolate from one scale to the next". These authors proved that the scale of the system determines to what extent the conductance and transpiration are related, which can be expressed by a so-called decoupling factor. Decoupling of conductance and transpiration is owing to feedback effects (Burrows & Milthorpe, 1976; Jarvis, 1985; Jarvis & McNaughton, 1986), which can be particularly strong in greenhouses (Aubinet et al., 1989). Therefore the feedbacks needed to be considered here. The principle is exposed in **Fig. 3.1** (section 3.1.7).

Another point of discussion is whether stomata adapt to long-term high C, like the photosynthetic efficiency appears to do (e.g. Von Caemmerer & Farquhar, 1984; Yelle et al., 1989a,b). Imai & Murata (1978) observed such an adaptation of stomata, but Jones and Mansfield (1970) found that the stomatal behaviour generally did not adapt to long-term CO_2 conditions. Hicklenton & Jolliffe (1980) reported that the stomatal resistance did not adapt to C in the early stage of leaf development, whereas a number of other features related to leaf photosynthesis did adapt. Radoglou & Jarvis (1992) observed that the stomatal density and stomatal index are not affected by C; Peñuelas & Matamala (1990) found that the stomatal index has not changed in response to the raise of the CO_2 concentration in the atmosphere in the last centuries. A good understanding of the occurrence of stomatal adaptation in greenhouse cultures is required.

Finally, it can be questioned if there is a time-dependent variation in transpiration. It has been reported that crops might have a 'midday depression' or declining photosynthetic and transpirational activity during the course of the day, as mentioned for photosynthesis in section 2.1.1 and 2.5. Also this aspect needs to be considered under realistic greenhouse conditions. Before the objectives are formulated in section 3.2.1, first some basic features about conductance and transpiration are exposed.

3.1.2 Stomata

Stomata are pores with their adjacent guard cells, in the epidermis of leaves, through which CO_2 enters and water vapour leaves the leaf interior. Stomata are roughly 20 µm long and the pore area (pore density multiplied by maximum pore opening) lies in the order of 0.5 and 1.2% of the leaf area (Ticha, 1982). The number of stomata is about 1 per 5 cells (stomatal index) or 100 - 450 stomata per mm^2 (stomatal density) in the lower side of a fully expanded leaf of a greenhouse fruit vegetable crop. The number of stomata is significantly higher in the lower (abaxial) leaf side than on the upper (adaxial) leaf side in most plant species (Gay & Hurd, 1975; Ticha, 1982; Woodward & Bazzaz, 1988).

The rate of gas exchange between leaf and environment is not primarily dependent on size, index or density, but on the average degree of opening of the stomata. The stomata are continuously in motion and do not all have the same relative opening at a certain moment. Opening and closing of a stoma is controlled by variations in osmotic pressure in the two guard cells, which is mediated by the quickly varying concentration of K^+ in these guard cells, which is influenced by two internal feedback loops, a CO_2 and a H_2O loop (Raschke, 1975). These are affected by internal and external conditions (see below). The stomata operate according to a refined strategy, optimizing either the transpiration or the CO_2 assimilation, while achieving a second-best solution for the other process. So CO_2 assimilation and transpiration are influenced by stomatal opening, but in turn, these processes have a (direct or indirect) effect on the stomatal opening (Farquhar et al., 1978; Jarvis & Morison, 1981).

3.1.3 Other conductances

The stomata form one of the barriers for gases in the pathway from ambient air to the interior of plant leaves. Other internal barriers, in series with the stomata, are formed by the cell walls and the substomatal cavity. The resistances of these barriers are negligible (Burrows & Milthorpe, 1976; Monteith, 1973). Parallel with the path through the stomata is the path through the cuticle. However, the cuticle resistance is so large, that this path usually can be neglected compared to conduction through the stomata. Therefore, the stomatal conductance is often considered equal to leaf conductance (the combination of stomatal and cuticle conductance), which can be measured with a porometer. An important external barrier between leaf and surrounding air is formed by the boundary layer. The boundary layer conductance is of the same order of magnitude as the leaf conductance and may have an important impact on the gas exchange. Hence, the boundary layer conductance must be carefully considered (section 3.3.5).

3.1.4 Diffusion

Transition of gases through a stoma is a diffusion process. According to the gas diffusion theory, the flow rate (F in $m^3\ m^{-2}\ s^{-1}$) of a gas through a medium is described by $F = -D * dC/dz$ with C the gas concentration (µmol mol^{-1}), z the path length (m) between two points x and y and D the diffusion coefficient (D_{H2O}=2.19E-5 $*(T/273)^{1.8}$ and D_{CO2}=1.40E-5 $*(T/273)^{1.8}$ $m^2\ s^{-1}$). For a finite length z, this is approximately equal to:

$$F = g * (C_x - C_y) \qquad \textbf{eqn. 3.1}$$

with $g = 1/r = D/z$. g is conductance ($m\ s^{-1}$) and r is resistance ($s\ m^{-1}$) to gas transfer. So stomatal conductance can be defined as the proportionality parameter relating the water vapour flux through the pore, to the driving force (Burrows & Milthorpe, 1976 and Jarvis & Morison, 1981).

In the same medium, the conductances for CO_2 and for H_2O are different, because of different diffusion coefficients, depending on the molecular weights of the gases. It is generally adopted that the leaf conductances of CO_2 and H_2O are related as $g_{l,\ CO2} = 0.6 * g_{l,\ H2O}$, and the boundary layer conductances as $g_{b,\ CO2} = 0.7 * g_{b,\ H2O}$ (Burrows & Milthorpe, 1976; Goudriaan & Van Laar, 1978).

3.1.5 Units of conductance

In studies on transpiration, the stomatal opening is preferably expressed as conductance or conductivity, rather than as resistance, because resistance can give a misleading impression. At wide stomatal opening, the resistance (with a hyperbolic curve if plotted against stomatal opening) shows only very small changes, whereas changes in conductance (related linearly to stomatal opening) are still large (Burrows & Milthorpe, 1976; Hall et al., 1976). The units used are $m\ s^{-1}$ or $mm\ s^{-1}$ (mm^3 water vapour transfer per mm^2 leaf area per second). Alternatively, the unit $mol\ m^{-2}\ s^{-1}$ is sometimes used (conversion in **Appendix III**).

3.1.6 Stomatal action

Stomatal conductance at a particular time is determined by various conditions, like incident quantum flux, humidity of the ambient air, leaf temperature, ambient carbon dioxide concentration and mineral nutrition. Also internal plant parameters play a role, like leaf water potential, leaf age, leaf ABA content (influenced by previous water stress) and mineral composition (Burrows & Milthorpe, 1976). Drought stress leads to stomata closure. Plants that have experienced (drought) stress are more sensitive to CO_2 (Lenz & Huch, 1986), which is probably caused by a higher content of ABA (Raschke, 1975; Davies et al., 1981; Willmer et al., 1988). Also certain cultivation measures may have an influence, e.g. high fruit load may increase the stomatal conductance (Hall & Milthorpe, 1978).

Stomata tend to close in response to increasing C (section 3.1.1). In addition, several interactions are known between C and other factors with respect to stomatal conductance (Morison, 1987). A concept of a response pattern of *g* to C is that the stomata tend to conserve a constant internal CO_2 concentration (C_i), or (at varying ambient CO_2, C_a) a constant ratio C_i/C_a (Goudriaan & Van Laar, 1978; Bell, 1982). This explains the large stomatal aperture at more light, i.e. at higher photosynthesis rate (higher F in eqn. 3.1). Also the partial stomata closure at high C is in line with this concept.

3.1.7 Relation between conductance and transpiration

It seems logical that reduction of stomatal conductance causes a reduction in transpiration. However, conductance and transpiration are decoupled to some extent, particularly in greenhouses (Burrows & Milthorpe, 1976; Jarvis, 1985; Jarvis & McNaughton, 1986). This is explained in **Fig. 3.1**, depicting the course of events following a stepwise increase in C. High C causes partial stomatal closure (reduced *g*) and thus initially reduces *E* of leaves. Reduction of the transpiration slightly increases the leaf temperature and decreases the absolute humidity (i.e. increases the vapour pressure deficit, VPD-air), first in the boundary layer and later also in the surrounding air. Increased leaf temperature and reduced absolute humidity imply an increase of the vapour pressure deficit between the stomatal cavities and the air (VPD-leaf-air). An increase in VPD reduces *g* (Bakker, 1991a), thus enforcing the effect of C on *g*. On the other hand, increased VPD increases *E* through a larger driving force, thus counteracting the effect of stomata closure

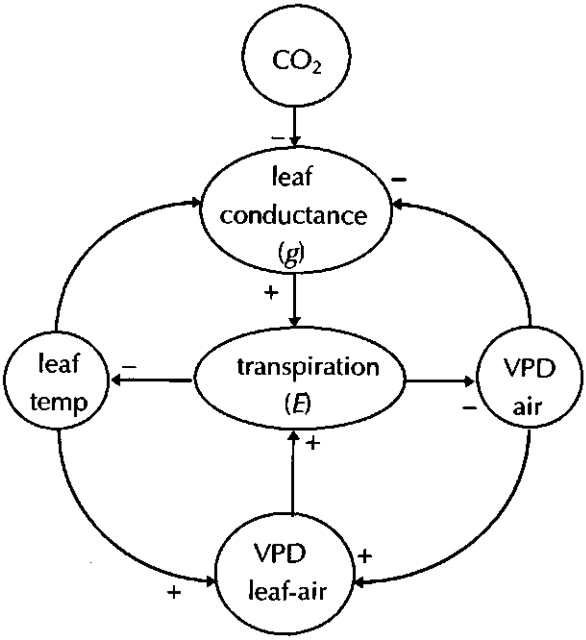

Fig. 3.1 *Schematic representation of the thermal and hydrologic feedback loops influencing leaf conductance and canopy transpiration of plants in a greenhouse.* ***symbols:*** *+ synergistic; - antagonistic effect.*

through C on *E*. The phenomenon of adapting leaf temperature with its consequences is called 'thermal feedback' and the adapting air humidity with its consequences is 'hydrologic feedback'.

In an open field cultivation, only the air humidity inside the canopy will adapt, while in a protected cultivation also the humidity of the greenhouse air will be affected. This explains that the gain (i.e. the strength) of the feedback loops is usually stronger in a greenhouse than in the open field. The gain depends on the conditions, like ventilation rate and size of the crop. The hydrologic feedback is stronger in a greenhouse with less ventilation, weaker in a well ventilated greenhouses and negligible in the open field. The thermal and hydrologic feedbacks are more important with larger LAI (Aubinet et al., 1989). Hence, it is not surprising that different results were reported (section 3.1.1) regarding effects of C on transpiration of greenhouse crops.

3.1.8 Decoupling factor

Jarvis & McNaughton (1986) defined a decoupling factor (**Ω**) to describe how the saturation deficit at the leaf surface near the pores is linked to that of the air outside the boundary layer. This same decoupling factor **Ω** was used to relate changes in conductance and transpiration. **Ω** indicates then the extent of control of one entity by the other, e.g.:

$$dE / E = (1-\Omega) * dg_s / g_s \qquad \textbf{eqn. 3.2}$$

Ω is dimensionless with values between 0 (full coupling) and 1 (full decoupling). A value of **Ω** close to unity indicates that the transpiration is insensitive to changes in stomatal conductance. As described above, the coupling between stomata and transpiration is weak (**Ω** is high) in a greenhouse-canopy system, which must be ascribed to the feedback. This can be illustrated by comparing different systems: single pore, leaf, canopy.

At one single pore, without a boundary layer above it, the external conditions are not influenced by the small amount of water vapour transpired through this pore into the space, so the conditions near the pore and in the surrounding air are strongly linked. Hence **Ω** is close to 0 and the water vapour flux under given conditions is fully controlled by the stomatal opening. If a leaf with a boundary layer is considered, with numerous pores, the humidity near the leaf surface is influenced by the transpiration rate through these pores. The conditions in the boundary layer are less linked to that in the surrounding air (**Ω** between 0 and 1) and the transpiration is not only dependent on the pore opening. In a whole canopy, the conditions near the pores and in the surrounding air are usually weakly coupled (**Ω** close to 1). This holds particularly at a high boundary layer resistance, i.e. low air velocity. The impact of stomata on transpiration is small under these circumstances. Because in greenhouses the air velocity is usually low, the boundary layer effect is strong and conductance and transpiration are largely decoupled. In addition, the absolute humidity is adapted, not only in the boundary layer, but even in the surrounding air of a closed greenhouse.

3.1.9 Leaf temperature

The temperature of a leaf depends on in- en effluxes of energy, in the form of short- and long-wave radiation, convection, evapotranspiration (or condensation) and a storage flux (photosynthesis and thermal energy). Under normal Dutch greenhouse conditions, at day time, the leaf temperature is usually higher than the average temperature of the surrounding greenhouse air. The main energy efflux is caused by evaporation (Stanghellini, 1987). This can be regarded as 'leaf cooling by transpiration'. The possible influence of elevated C on this energy balance consists of a reduction of the leaf transpiration (by partial stomata closure) and consequently an increase in leaf temperature.

3.2 OBJECTIVES AND APPROACH

3.2.1 Objectives

The work in this chapter aims at quantifying the effects of the CO_2 concentration (200 - 1100 μmol mol^{-1}) on stomatal conductance, canopy transpiration and leaf temperature of the fruit vegetable crops tomato, sweet pepper, cucumber and eggplant in greenhouses. This must enable evaluating relations of these features with possible negative side effects of CO_2 enrichment (increased leaf temperature and in chapter 4 nutritional aspects). Also a possible adaptation of the stomatal response to long-term CO_2 enrichment had to be examined, as well as the occurrence of a midday depression. Because the effect of C on transpiration may appear so differently when observed on different scales, the investigations were done in greenhouses of a considerable size and under realistic conditions, to allow transfer of the conclusions to practical horticulture.

3.2.2 Experimental approach

To investigate the effects of C on leaf conductance and transpiration, these entities were measured in full-grown crops in large greenhouses under different, controlled CO_2 concentrations between about 200 and 1100 μmol mol^{-1}. For leaf conductance, a regression model was fitted to the measurements, which describes the dependence of *g* on radiation, air humidity, CO_2 concentration and optionally leaf temperature.

With transpiration, the effect of C is primarily an effect of stomata closure (i.e. of *g*) and secondly of adapted VPD-air and leaf temperature. According to Jarvis (1981), variations in transpiration rate can be estimated adequately by the Penman-Monteith combination equation (P-M model, Monteith, 1965, section 3.3.5), implemented with leaf conductance measurements from a diffusion porometer. So it is assumed that the effect of C on *E* can be approximated by the P-M model for the effects of *g* on *E*, combined with the regression equation for the effect of C on *g*, with the adapted VPD taken into account by using the actual (measured) VPD values as input data. It was tested if this approach yielded a satisfactory description of canopy transpiration, by comparing measured and calculated transpiration at various C.

The P-M model was preferred above other models, because it is a universal, well-established, well-documented and yet a simple model. In a comparison of measurements and models, a simplified Penman model gave good predictions on average (Jolliet & Bailey, 1992). However, the individual variations were large, because that version of the model did not account for stomatal conductance. The extensive Penman-Monteith combination equation, however, which is used here, incorporates the stomatal conductance. In work of Zhang & Lemeur (1992), the P-M model results were close to measured evapotranspiration.

To describe the effect of C on *E*, it is necessary to know what the effect is of a change in *g* on *E*. It is beyond the scope of this study to perform detailed measurements on leaf conductance, leaf temperature, transpiration, air temperature, humidity and turbulence, with their spatial variation, as well as of the boundary layer and the feedback loops in the crop-greenhouse system. It was considered sufficient to obtain a global description of the relation of *g* and *E*. Hence, the relation between *g* and *E* was investigated on the basis of calculations with the regression equation for *g* and the P-M model for *E*, after validation of these calculations with the measurements.

3.3 METHODOLOGY

3.3.1 Set-up of experiments

Conductance and transpiration were measured in four experiments, where the CO_2 concentration was varied. All experiments were performed in Venlo-type glasshouses, either in one greenhouse compartment (tomato), or in two (sweet pepper and eggplant) or in three (cucumber). The compartments were large enough (over 150 m^2) to avoid edge effects in the measurements and to be comparable with commercial greenhouses, for instance regarding hydrologic feedback effects. Each compartment was independently controllable with respect to environmental conditions and irrigation. Climate control, irrigation and data acquisition were made by a micro computer connected to a VAX network (Bakker et al., 1988).

The greenhouses were provided with a pipe heating system and with ventilation windows in the roof. Both were used exclusively for temperature control. In contrast to artificially controlled chambers, it is difficult in large greenhouses to maintain a desired humidity. Although some humidity control is possibly by means of heating and ventilating, it was preferred in all experiments not to control, but only to record the air humidity. Temperature and humidity were measured by calibrated, shielded and aspirated PT-100 psychrometers (dry and wet bulb temperatures), about 1.5 m above the ground. The data of three sensors per compartment were averaged and used for the analysis. More specific information on climatic conditions is given per experiment.

During most measurements, no severe heating was necessary. Nevertheless it must be considered how the heating pipe temperature should be taken into account in the analysis. It has been suggested that warm heating pipes promote the crop transpiration (De Graaf & Van Den Ende, 1981). However, this is no particular heating pipe effect (i.e. no direct radiative effect of the heating pipes) but an effect of increased temperature and decreased air humidity on transpiration (Jolliet & Bailey, 1992). Also Stanghellini (1987) found that a particular effect of the heating pipes on transpiration was relatively negligible, provided the resulting actual air temperature and humidity were taken into account. Hence temperature and humidity of the greenhouse air were considered as factors in a multifactor analysis and heating pipe effects were not taken into account as a distinct factor.

All cultivation measures were made in a standard way, i.e. according to the current guidelines for the commercial practice. Certain conditions (drought stress, fruit load) might have an impact on the stomatal behaviour (section 3.1.6). To prevent drought stress, much care was paid to optimize the water supply. The plants, all grown on rockwool, were frequently irrigated, from 5 times per day in spring up to 35 times per day in summer. The plants were irrigated with a standard nutrient solution, different for each crop (Sonneveld & Straver, 1989). The required amount and frequency of irrigation was calculated with a simple transpiration model (De Graaf, 1988). Always an oversupply was given, mostly of about 30%, and the excess was drained out. The amount of drain water was measured and the signal was used for feedback control of the irrigation.

Pure CO_2 was supplied through a distribution network, consisting of perforated polyethylene tubes, 3.2 m apart on the ground, below the plants. The CO_2 supply was automatically controlled, according to a certain strategy (described per experiment).

VPD-leaf-air was derived from the measured dry and wet bulb air temperature and measured leaf temperature, assuming saturated vapour pressure in the stomatal cavities. Leaf temperature was measured during the leaf conductance measurements with a thermocouple in the porometer. In a test with measurements on a great number of leaves, this sensor gave on average equal values as a separate, hand-hold thermocouple attached to the leaves.

Photosynthetically Active Radiation (PAR, or light flux, waveband 400 - 700 nm) was used for analysis of leaf conductance, while global radiation was the explaining factor for transpiration, in accordance with the theory (Raschke, 1975; Jarvis, 1981). PAR at leaf level was measured with a quantum-sensor (LI-190s-1, LI-COR, Lincoln, Nebraska) mounted on the porometer. Hence, these PAR measurements were done only during the conductance measurements. Global radiation outside was measured continuously with a solarimeter (type CM11, Kipp, Delft, the Netherlands) on a meteorologic tower. When required, an approximation of PAR was derived from these measurements by unit conversion (**Appendix III**).

The transmission of the glasshouse cover for diffuse light was determined occasionally under an overcast sky, using two identical energy-response PAR meters (TFDL, Wageningen, The Netherlands). One was placed outside and one inside the greenhouse above the canopy. This coefficient was found to be 55% in the glasshouse used for the eggplant experiment and 65 to 70% in the other experiments. The actual transmission of the glasshouse cover, however, may fluctuate considerably, particularly when overcast and sunny weather conditions alternate. Except in sweet pepper, the instantaneous transmission was computed according to Gijzen (1992) using the transmission model of Bot (1983), the measured transmission coefficient for diffuse light, the calculated sun position and the calculated ratio between diffuse and direct radiation (Spitters et al., 1986).

The measuring cycle was 1 min for global radiation, temperatures and transpiration, and 4 min for CO_2. These data were averaged over the time interval required for one series of conductance measurements (8 to 20 min).

3.3.2 Measuring conductance

Leaf conductance for water vapour was measured with a null-balance steady state (continuous flow) porometer (model 1600, Li-Cor, Nebraska, USA), attached to a single leaf. About 20 to 60 seconds after a leaf was enclosed, the reading was transferred to a portable micro-computer.

Mostly 10 (sometimes 20) leaves were sampled in one series, to obtain one average value. This took about 8 (or 16) min. The leaves were selected at random, so usually in subsequent cycles other leaves were sampled. Stomatal aperture declines with ageing of leaves (Field, 1987; Willmer et al., 1988) and stomata of older leaves respond less sensitive to C than those of younger leaves (Lenz & Huch, 1986). To standardize for leaf age, always full-grown, but still fairly young leaves were sampled on a standardized position (different per experiment). Conductance was always measured on the underside of leaves only, because by far the greatest number of stomata occur on this side in most commonly applied species (Ticha, 1982). Because conductance was measured comparatively under low and high C, it was sufficient to measure only the lower leaf side.

3.3.3 Measuring transpiration

Transpiration was measured (except in eggplant) by weighing lysimeters, consisting of high accuracy balances (type ED-60-T, Berkel-Patent, Rotterdam, the Netherlands). The capacity was 90 kg and the inaccuracy about 10 g (resolution 2 g, reproductivity 6-8 g). There were three lysimeters per compartment, one on the east side and two on the west side of the double plant rows under the gutter. Basically the results of the three lysimeters per compartment were averaged before data analysis (details described per experiment). Each lysimeter, located in the middle of a crop row, carried a rockwool slab with two or three plants (different per experiment). These plants received the same treatment, including water supply, as all other plants in the compartment.

The lysimeters were connected to the VAX computer and were monitored every minute. A decrease in the weight of the whole system (plants, rockwool and drain collector) was a direct indication of transpiration. Surplus water drained out of the system and was weighed separately. The calculation of transpiration was corrected for water deliveries and for drain. The lysimeters were sampled every min, and the readings were smoothed over intervals of 8 to 20 min.

3.3.4 Analysis of conductance measurements

The instantaneous measurements of *g* were analyzed with regression, to account for the effects of various factors. Based on the literature (section 3.1.6) the following factors were considered most relevant: incident PAR (light flux at crop level, I_c), VPD-leaf-air ($D_{\ell\text{-}a}$), CO_2 concentration (C) and optionally leaf temperature (T_ℓ). Other factors were assumed equal in the crops to be compared, or negligible. An equation of Burrows & Milthorpe (1976) was used to relate *g* to PAR:

$$g = c_1' * (1 - c_2 * \mathrm{EXP}(-c_3 * I_c)) \qquad \textbf{eqn. 3.3.}$$

For the effect of VPD-leaf-air on *g* the equation was: $g = c_1'' * \mathrm{EXP}(-c_4 * D_{\ell\text{-}a})$ (Bakker, 1991a). For the response of *g* to C the same exponential curve was chosen, because *g* responds similarly to VPD and to C (Burrows & Milthorpe, 1976), thus: $g = c_1''' * \mathrm{EXP}(-c_5 * C)$. The effect of leaf temperature (T_ℓ) on stomatal conductance can be described by $g = c_1'''' / (1 + c_6 * (T_\ell - c_7)^2)$ (Stanghellini, 1987).

Some correlation between certain factors was inevitable (e.g. a high radiation is generally accompanied by high temperature, high VPD-air and low C), but it was checked that fairly wide ranges of combinations of conditions were present in the measurements and that C was not correlated to VPD (Tables 3.1, 3.2, 3.5, 3.6, 3.9, 3.10). Also some interactions between factors might exist, with respect to stomatal response. Thus the regression equations for the effects of various factors were combined by multiplication (Stanghellini, 1987), yielding the following regression equation for four factors:

$$g = c_1 * (1 - c_2 * \mathrm{EXP}(-c_3 * I_c)) * \mathrm{EXP}(-c_4 * D_{\ell\text{-}a}) * \mathrm{EXP}(-c_5 * C) / (1 + c_6 * (T_\ell - c_7)^2) \qquad \textbf{eqn. 3.4.}$$

The parameters c_1 to c_7 were fitted to the data. Parameter c_1 represents the saturation value of conductance, c_5 describes the CO_2 influence and c_7 is the leaf temperature at which *g* is maximal. The regression analysis was built up stepwise, by fitting first just the PAR term (eqn. 3.3), then adding the other terms one after each other and ending up with the full equation (eqn. 3.4).

The multiplicative structure of eqn. 3.4 allowed the expression of the C effect in one simple relative figure, derived from parameter c_5. The relative decrease in conductance was expressed in % per 100 μmol mol^{-1} increase of C. The results are valid at any level of radiation, VPD-leaf-air and C, in the ranges achieved in the measurements (presented per experiment), and under the condition that other factors than C remained constant.

3.3.5 Analysis of transpiration measurements (Penman-Monteith model)

The impact of radiation on transpiration was examined with the simple linear equation (yielding E_r):

$$E_r = c_8 * 1000 * Q_n / \lambda \qquad \textbf{eqn. 3.5}$$

with λ the vaporization energy of water (2.45E6 J kg^{-1}) and with a factor for unit conversion (1000 g kg^{-1}). Q_n was net (absorbed) radiation, obtained from global radiation measured outside, corrected for transmission through the greenhouse cover (estimated per experiment), reflection by leaves and transmission through the row crop. The sizes of these effects are highly variable. The amount of light absorbed by the crop (relative to the incoming light) depends on the row dimensions, the sun altitude and the sun position, relative to the orientation of the rows (Gijzen & Goudriaan, 1989; Hand et al., 1993b). For a plain approximation, it was assumed that reflection on the crop and transmission through the crop together were 30% (after Monteith & Unsworth, 1990). Thus Q_n was $0.7 * Q_c$. The contribution of long wave radiation in the energy balance was assumed negligible.

As proposed by Jarvis (1981), the transpiration of the canopy can be described as a function of radiation, air humidity and estimated leaf conductance, with the use of the Penman-Monteith (P-M) model (Monteith, 1965; Jarvis, 1981 and Jarvis et al., 1981), as follows:

$$E_p = (s * Q_n + \rho * c_p * D_a * g_b) / (\lambda * (s + \gamma * (1 + g_b / g))) \qquad \textbf{eqn. 3.6,}$$

where $\rho * c_p$ is the volumetric heat capacity of air (1240 J m^{-3} K^{-1}), λ the latent heat of vaporization of water (2.45E6 J kg^{-1}), γ the apparent psychrometric constant (0.067 kPa K^{-1}), s the slope of the saturated water vapour pressure curve at air temperature (ranging from 0.145 at 20 °C to 0.244 kPa K^{-1} at 30 °C) and g_b the boundary layer conductance. Q_n was approximated as above. The P-M model requires VPD-air as input, under the assumption that the temperatures of leaf and air do not differ too much (the estimated difference was usually less than 2 ^oC in the measurements).

The parameter g_b requires special attention. g_b represents the conductance of the air between the leaf surface and the greenhouse atmosphere at a certain reference height. It actually includes the conductances for diffusion out of the boundary layer and from inside-the-canopy to outside-the-canopy. Several descriptions can be found in the literature for the various conductances (e.g. boundary layer conductance, external conductance, aerodynamic conductance) and it is not always clear which conductance is meant exactly. The conductances depend on wind speed and leaf properties. Not much is known about wind speed inside greenhouses, particularly not about variation in turbulence inside the canopy under various window openings and variable outdoor wind speed etc.

It was decided to work with one value for g_b for all four crops in all situations, in spite of different average leaf sizes and variation in wind speed. This seemed the best approach because of uncertainty about spatial variation in conditions and uncertainty about turbulence. In addition, Stanghellini (1987) concluded that the fluctuations in external resistance are extremely small compared to the large fluctuations in internal (i.e. stomatal) resistance.

In order to approximate a value for g_b, an average was sought of indications in the literature. In work with a synthetic leaf in a canopy, Stanghellini (1985) found an external resistance of 10 mm s^{-1}. Zhang & Lemeur (1992) established diurnal averages of aerodynamic conductance between 7 and 12 mm s^{-1}. g_b may also be approximate from estimated leaf boundary layer conductance. It is possible to measure g_b, as proposed by Kitano & Eguchi (1989), but this is a huge undertaking. There are some simple approximations available, e.g. as proposed by Burrows & Milthorpe (1976): $g_b = (u/w)^{0.5}/a_b$, where u is wind speed in cm s^{-1}, w the diameter of a leaf in cm and a_b a value between 1.3 (outdoor and growth chambers) and 3.4 (laminar flow conditions), yielding g_b in cm s^{-1}. For an approximation, it is assumed that $u = 50$ cm s^{-1} (partial ventilation), $w = 15$ cm, $a_b = 2$. This yields $g_b = 0.9$ cm s^{-1}. In an extensive experiment, Kitano & Eguchi (1990) observed values between 3 and 12 mm s^{-1} for leaf boundary layer conductance. Based on these and other indications (Monteith & Unsworth, 1990; Aubinet et al., 1989) a value of 10 mm s^{-1} was adopted for g_b in eqn. 3.6.

The P-M model has some other complications. First the leaf area index is assumed to play no role ('single leaf' or 'big leaf' model, Monteith, 1963), at least when LAI is reasonably large (Monteith, 1965). Secondly, g in the P-M equation represents the canopy conductance. This is a somewhat abstract parameter, which can theoretically be obtained by integration of leaf conductance multiplied by area of individual leaves, weighted for their contribution to the total transpiration (Jarvis, 1981). In the present experiment, however, g was measured in the top of the crop, and it was higher than the average conductance of all leaves in the canopy, owing to higher light intensity at the top. This may lead to an overestimation of the canopy transpiration.

The reason for performing the leaf conductance measurements only on young, mature leaves in the top of the plants, though, was that spatial variation of other factors was excluded in this way (the variability in leaf age, irradiance, humidity), thus enabling to establish the effects of C on leaf conductance. This approach was considered acceptable for two reasons. The top of the plants attribute by far the largest part of the canopy transpiration (Hurd, 1969; De Graaf, 1991), so largely determine the canopy conductance. Secondly, only the relative effect of C on canopy transpiration was regarded.

3.3.6 Coupling between conductance and transpiration

Quantification of the effect of C on transpiration, required a good understanding of the relation between conductance and transpiration (section 3.2.2). This was studied by calculating the coupling factor K, using a fictive data set. K expresses the relation between a change in g and a concomitant change in E, as follows:

$$K = [(E_2-E_1) / E_{mean}] \; / \; [(g_2-g_1) / g_{mean}] \qquad \textbf{eqn. 3.7}$$

g was calculated with the regression equation and E with the P-M model. The subscripts 1 and 2 refer to two situations to be compared. K (after Aubinet et al., 1989) is comparable to 1 - Ω (Ω of Jarvis & McNaughton, 1986; eqn. 3.2). K was established for several steps of C and for several assumed conditions (air temperature, global radiation and VPD). VPD-leaf-air was assumed equal to VPD-air, after it was checked that the effect of this simplification on K was negligible.

3.3.7 Data processing

The conductance measurements of several days were pooled and analyzed as one data set per experiment (some exceptions are mentioned). Pooling was necessary to obtain data sets large enough for regression analysis. Because leaf conductivity was always measured on young, mature leaves in the top of the plants, the data can be assumed independent of plant age, day number etc. No systematic effects of day number on conductance were found (an exceptional day in eggplant is mentioned). If more than one greenhouse compartment was used for the measurements, it was checked that no differences existed in stomatal and transpirational behaviour under equal conditions at the same time, between the different plant stands.

Rate of transpiration was recorded continuously during the whole cultivation period with 1-min readings, and the data were averaged over the intervals of leaf conductance measurements (8 to 20 min). Also the transpiration data did not reveal a systematic day effect over a relatively short period (weeks), when plant size did not markedly change. Therefore also the transpiration data were pooled over a certain period.

All data analysis was done using the statistical package GENSTAT (Payne & Lane, 1987).

3.4 EFFECTS OF CO_2 ON LEAF CONDUCTANCE AND CANOPY TRANSPIRATION OF SWEET PEPPER

E.M. Nederhoff, A.A Rijsdijk and R. de Graaf, 1992, Scientia Horticulturae 52: 283-301.

3.4.1 Materials and methods

Crop, greenhouse and environment

Sweet peppers (*Capsicum annuum* L., cv. Mazurka) were sown on 6 October and planted on 7 December 1989. Plants were grown on rockwool slabs and received standard nutrition (Sonneveld & Straver, 1989). There were four rows of pepper plants per 3.20 m, at a density of 3.1 plants per m^2 and two stems per plant. Plant length was 1.75 m and LAI was around 2 on 25 April. This increased to length of 2 m and LAI of around 4 by the end of June.

Red fruits were harvested regularly, with care taken to avoid excessive fruit removal, because this might lead to appreciable stomatal closure (Hall & Milthorpe, 1978). The fruit production followed a normal pattern with about 1 kg m^{-2} harvested by 25 April and around 6 kg m^{-2} by 26 June.

The experiment was performed in two compartments (12 m x 12.8 m) of a north-south orientated Venlo-type glasshouse with a ridge height of 3.9 m. Measurements of transmissivity of the glasshouse cover came out on 70%, under overcast sky as well as under direct sun shine at high sun altitude. In this experiment, the transmission of the glasshouse cover was assumed 70% under all circumstances.

Heating temperature set points were 25/15 °C (day/night) and ventilation started at 26/16 °C. A so called 'minimum ventilation' of 5 to 15% of the maximum opening was applied from May onward, particularly effective under low light conditions. This is a measure to stimulate transpiration and in this way promote root growth. Irrigation was controlled by feed-forward (on-line calculation of transpiration with a model of De Graaf, 1988) and feed-back (weighing of drain water). In summer the crop was irrigated 10 to 30 times per day with a surplus of 33% of the calculated water demand.

Pure CO_2 was supplied computer-controlled through a network on the soil. C was monitored with an infra-red gas analyzer (URAS type 3G, Hartmann and Braun, Frankfurt, Germany), which was calibrated with calibration gases before every measuring day. Air was sampled continuously on eight places in each compartment, at about 1.5 m height.

Normally, except on measuring days, CO_2 set points were equal in both compartments and according to the practical regime: 450 - 550 with little or no ventilation and around 350 μmol mol^{-1} with much ventilation. On measuring days (except on day 173) the CO_2 concentrations were different in the two compartments. High and low C were alternated per measuring day over the two compartments, to prevent differences in growth between the two crops.

Conductance and transpiration measurements

The measurements were conducted on 25 April, 2 and 16 May, 13, 22, 27 and 29 June 1990 (day numbers 115, 122, 136, 164, 173, 178, 180), between 7:00 and 21:00 h.

Leaf conductance for water vapour was measured with the Li-Cor-1600 diffusion porometer. The porometer was attached to the lower side of mature, green, healthy leaves, exposed to full light, in the upper 0.4 m of the crop. A series of 20 readings was made, which lasted about 15 min. The next series of 20 was sampled in the other location (these data are not used here). The measuring cycle was between 60 to 90 min. The environmental conditions were averaged over the measuring interval.

The rate of canopy transpiration was measured on a 1-min basis, with three weighing lysimeters. The three data were averaged before analysis. Each lysimeter carried a rockwool slab with three plants, representing 0.97 m^2 ground area.

Data analysis

Leaf conductance was analyzed with the regression model (eqn. 3.3 and 3.4) and transpiration with eqn. 3.5 and 3.6). The conductance data of all seven measuring days were pooled and analyzed in one data set (n=116). Transpiration was recorded continuously on a 1-min basis during the whole cultivation. The following data sets were assembled:

- transpiration data collected during the conductance measurements (intervals of ca. 15 min, n=116)
- transpiration data collected during the seven measuring days (10 min averages of periods 7.00 - 21.00 h; n=1176)
- transpiration data of measuring days in April/May (n=504)
- transpiration data of measuring days in June (n=672).

The latter two data sets are obtained by splitting the large data set. This was done because the crop gradually changed during the season, e.g. LAI increased.

Transpiration rates that were measured (E_{meas}) were compared to those derived with the P-M model (E_p). E_p was based either on measured values of g (denoted as $E_{p,mc}$, n=116) or on values of g estimated with the fitted regression equation (eqn. 3.4d, **Table 3.3**). These latter are denoted as $E_{p,ec}$ (n=1176, 504 and 672).

Table 3.1 *Ranges of leaf conductance, canopy transpiration and environmental conditions, in two (partly overlapping) data sets of measurements in sweet pepper.*

			n = 116			n = 1176		
			min.	mean	max.	min.	mean	max.
day	day	--	115	153	180	115	153	180
g	conductance	mm s^{-1}	0.3	10.3	27.4	--	--	--
E_{meas}	transpiration	$\text{g m}^{-2}\text{ s}^{-1}$	0.007	0.07	0.16	0.001	0.07	0.16
Q	glob. radiation	W m^{-2}	12	370	970	0	366	1005
T_a	air temp.	°C	18.8	26.1	29.8	--	--	--
T_l	leaf temp.	°C	19.5	26.9	31.4	18.0	26.4	30.2
D_a	VPD-air	kPa	0.25	0.99	2.05	0.03	1.02	2.30
C	CO_2 conc.	$\mu\text{mol mol}^{-1}$	308	595	1215	292	598	1716

Table 3.2 *Correlation coefficients of leaf conductance, crop transpiration and environmental conditions in the sweet pepper experiment.* $r_{(P=0.01,\ n=116)} = [0.22]$.

		1	2	3	4	5	6	7	8	9	10
1	conductance	1.00									
2	transpiration	0.74	1.00								
3	day number	0.05	-0.19	1.00							
4	compartment	-0.11	-0.04	-0.01	1.00						
5	time	-0.18	-0.04	0.08	0.01	1.00					
6	PAR	0.70	0.96	-0.34	0.00	-0.05	1.00				
7	air temp.	0.53	0.68	0.16	0.08	0.10	0.62	1.00			
8	leaf temp.	0.49	0.71	0.11	0.00	0.16	0.65	0.95	1.00		
9	VPD-leaf-air	0.03	0.56	-0.36	0.29	0.31	0.58	0.45	0.52	1.00	
10	CO_2	-0.58	-0.45	0.13	0.11	-0.06	-0.42	-0.31	-0.29	0.08	1.00

3.4.2 Results

Time courses

With equal environmental conditions in the two compartments (day 173), g and E of the two crops did not differ significantly (**Fig. 3.2**). This provided evidence that differences in g, observed on other measuring days, could be ascribed to the conditions rather than to crop effects. On day 115 (**Fig. 3.3**), with low C at about 350 $\mu\text{mol mol}^{-1}$ and high C between 600 and 1100 $\mu\text{mol mol}^{-1}$, conductance was far lower (more than 50% reduction) and transpiration was a little lower (approx. 10% reduction) in the compartment with high C. VPD-air was far higher and leaf temperature in the afternoon slightly higher in the compartment with high C.

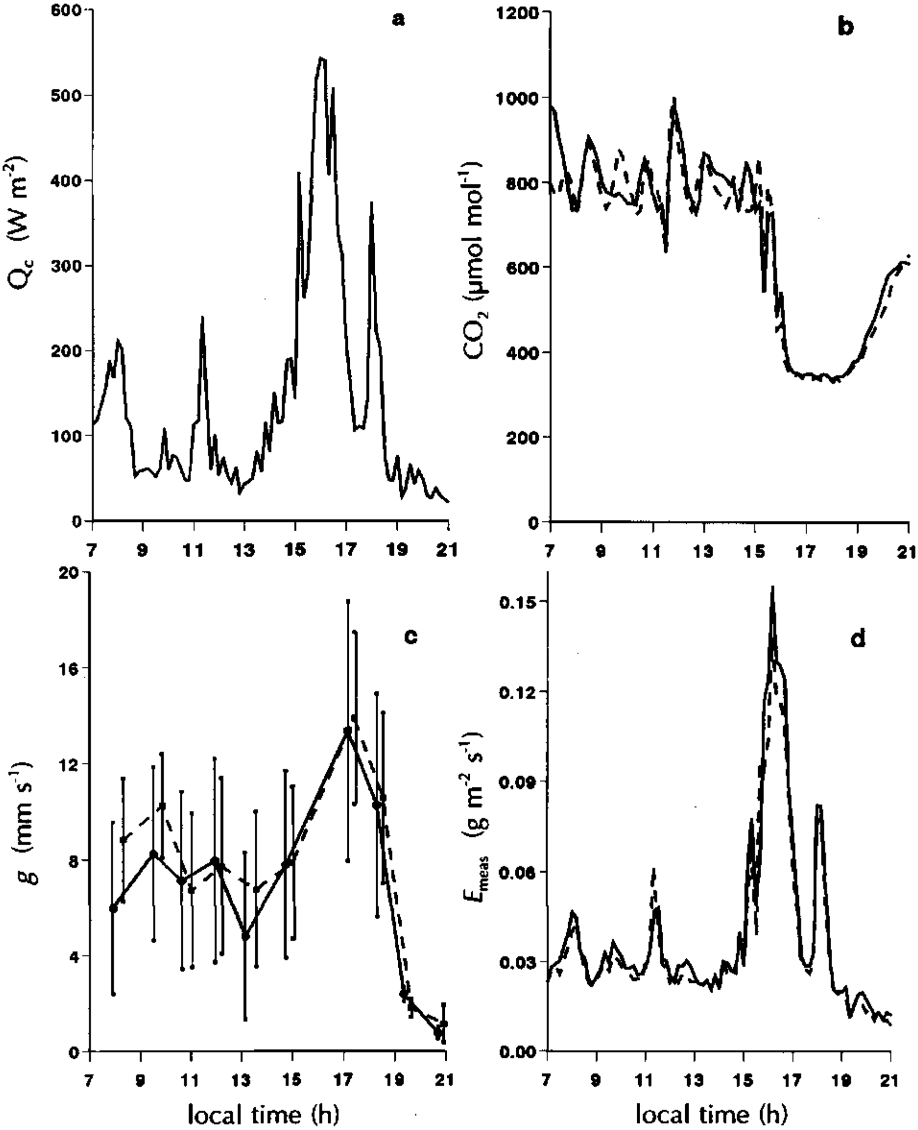

Fig. 3.2 *Measurements of 22 June 1990 (day 173) on sweet pepper in two glasshouse compartments with equal environmental conditions.*

a. Q_c = *incident global radiation (W m^{-2})*

b. CO_2 = *CO_2 concentrations (µmol mol^{-1})*

c. g = *leaf conductance (mm s^{-1}) at top of the crop in full light* [1]

d. E_{meas} = *measured rate of transpiration (g m^{-2} s^{-1})*

[1] *error bars indicate the standard deviation of the measurements (n=20).*

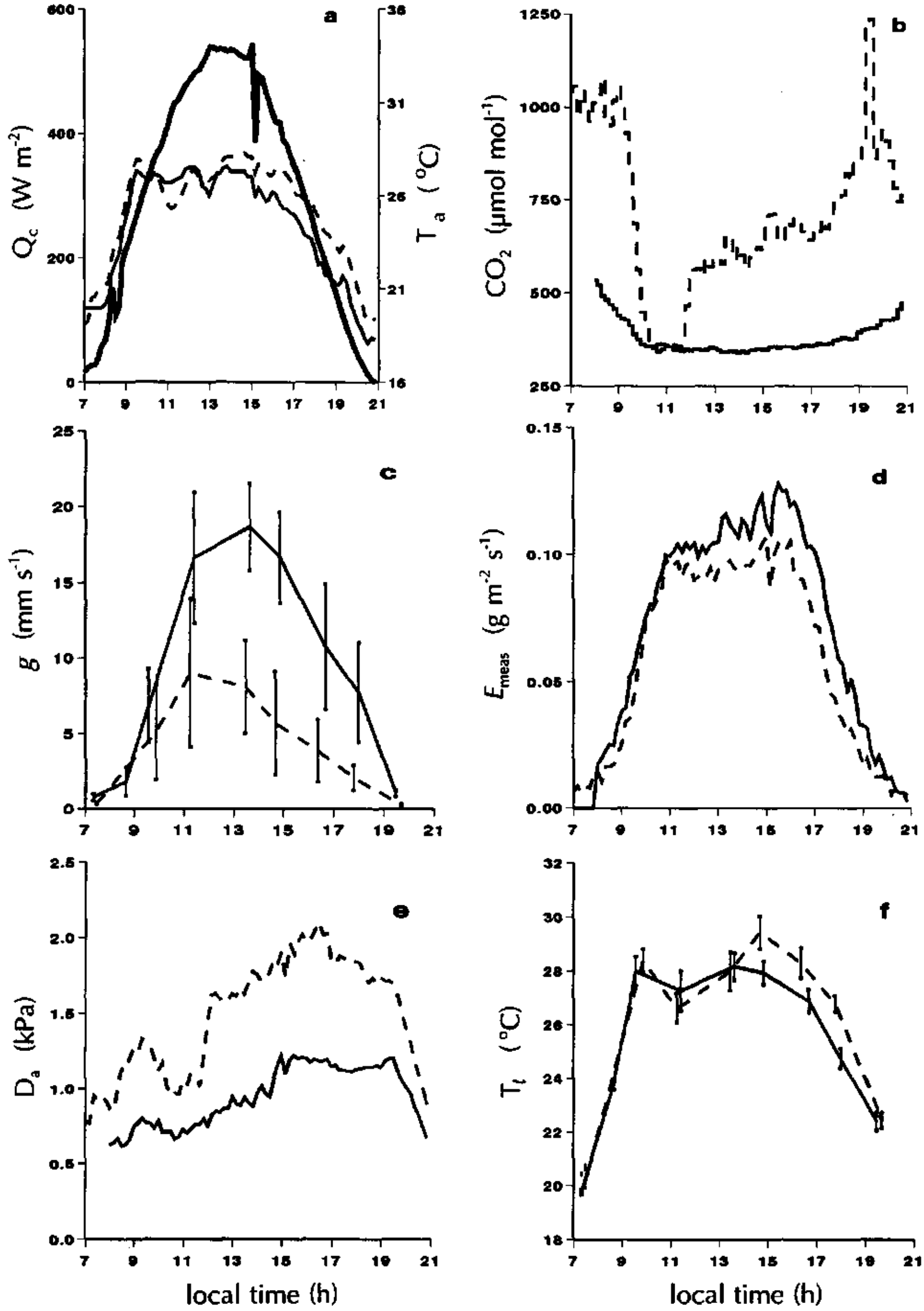

Fig. 3.3 *Measurements of 25 April 1990 (day 115) on sweet pepper in glasshouse compartments with low CO_2 (———) and high CO_2 (– – – –)*

a. *T_a = air temperatures (°C); Q_c = incident global radiation (W m^{-2}) (———);*

e. *D_a = vapour pressure deficits of greenhouse air (kPa)*

f. *T_l = temperature of the leaves (°C)* [1]

b, c, d, [1] *as in* ***Fig. 3.2***

Conductance

Table 3.1 shows the ranges of conditions and **Table 3.2** the linear correlation between the variables. Conductance is correlated positively to PAR, air temperature and leaf temperature and negatively to C, and seems not correlated to VPD-leaf-air. This is probably caused by correlation between VPD and temperatures. VPD and CO_2 are not correlated.

In **Fig. 3.4**, measured *g* is plotted versus PAR and the exponential curve (eqn. 3.3) is fitted to the data, yielding r^2=49% (**Table 3.3**). The relative effect of C, according to eqn. 3.4d and 3.4f, came out on 3.1% decrease in *g* per 100 µmol mol^{-1} increase in C (parameter c_5 in Table 3.3). Using eqn. 3.4d the measured effects of PAR, VPD and CO_2 on *g* are demonstrated in **Fig. 3.7**.

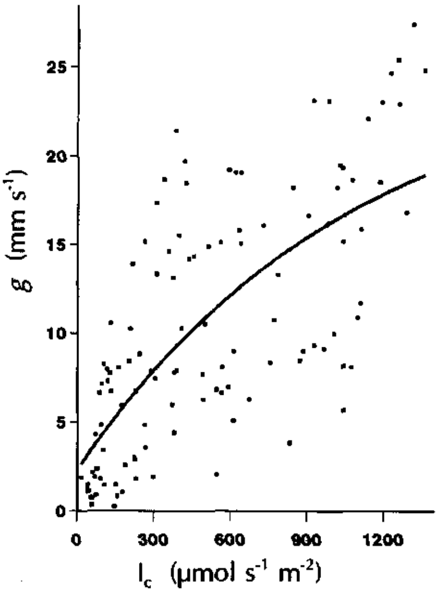

Fig. 3.4 *Measured conductance (g in mm s^{-1}) of sweet pepper versus incident PAR (I_c in µmol s^{-1} m^{-2}), with an exponential curve fitted (eqn. 3.3,* ***Table 3.3****, r^2 = 49%).*

Table 3.3 *Fitted values of parameters c_1 to c_7 of the regression equations, relating measured leaf conductance (g in mm s^{-1}) of sweet pepper to the conditions:*
$$g = c_1 * (1 - c_2 * EXP(-c_3 * I_c)) * EXP(-c_4 * D_{l-a}) * EXP(-c_5 * C) \,/\, (1 + c_6 * (T_l - c_7)^2) \quad \text{(eqn. 3.4)}$$
with I_c, D_{l-a}, C and T_l as in Appendix II. Day number 115 - 180 (1990), n=116.

eqn.	c_1	c_2	c_3	c_4	c_5	c_6	c_7	r^2
3.3	25.6	0.91^a	$0.92E-3^b$	--	--	--	--	49.0
3.4a	25.6^c	1.02^a	$2.28E-3^a$	0.31^a	--	--	--	62.0
3.4b	25.6^c	0.93^a	$2.81E-3^a$	--	$9.42E-4^a$	--	--	58.9
3.4c	25.6^c	0.91^a	$1.03E-3^a$	--	--	$1.27E-2^a$	26.7^a	50.4
3.4d	25.6^c	1.01^a	$2.64E-3^a$	0.23^a	$3.11E-4^b$	--	--	62.3
3.4e	25.6^c	0.88^a	$2.87E-3^a$	--	$9.08E-4^a$	1.21E-2	27.9^a	59.3
3.4f	25.6^c	0.98^a	$2.76E-3^a$	0.23^a	$3.15E-4^b$	0.65E-2	28.9^a	62.2

[a] *significant at 1%* [b] *significant at 5%* [c] *set a priori*

Transpiration

Measured transpiration rates are plotted versus global radiation (**Fig. 3.5**). Eqn. **3.5** fitted to the data yielded: $E_r = 0.83 * \lambda^{-1} * 1000 * Q_n$ (with $Q_n = Q_c * 0.7$ and n=116). The value 0.83, being parameter c_8, equals the ratio $\lambda E/Q_n$. It indicates that on average 83% of the absorbed energy was converted to latent heat. With the large data set (n = 1176) the same value for c_8 is found. The outcome of c_8 is influenced by the estimation of Q_n. Monteith (1965) showed that the ratio $\lambda E/Q_n$ varied strongly and increased with increase of the stomatal conductance. The high values of r^2 (91 and 89% respectively), confirm that transpiration is explained to a large extent by radiation, as generally reported.

Calculated transpiration rates (E_p) were compared to measured rates (E_{meas}), by fitting the linear: $E_p = a + b * E_{meas}$. **Table 3.4** presents the intercept (a), slope (b) and percentage of variance explained (r^2) of these linears. E_{meas} was in reasonable agreement, not only with $E_{p,mc}$, but also with $E_{p,ec}$ (**Fig. 3.6**).

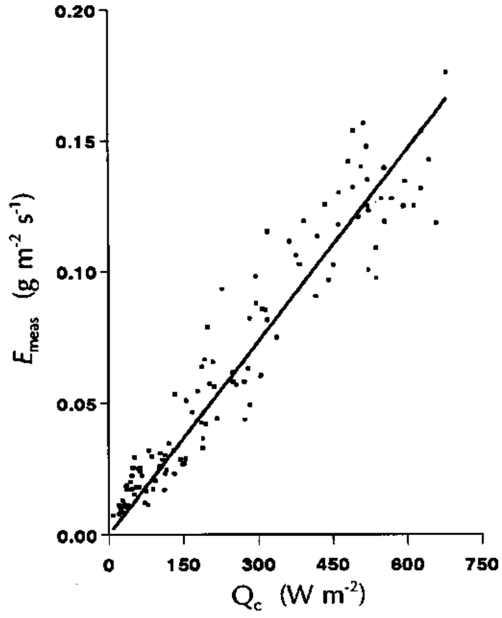

Fig. 3.5 *Measured rate of transpiration (E_{meas} in g m^{-2} s^{-1}) of sweet pepper versus incident global radiation (Q_c in W m^{-2}), with eqn. 3.5 fitted to the data (n=116, r^2=91%).*

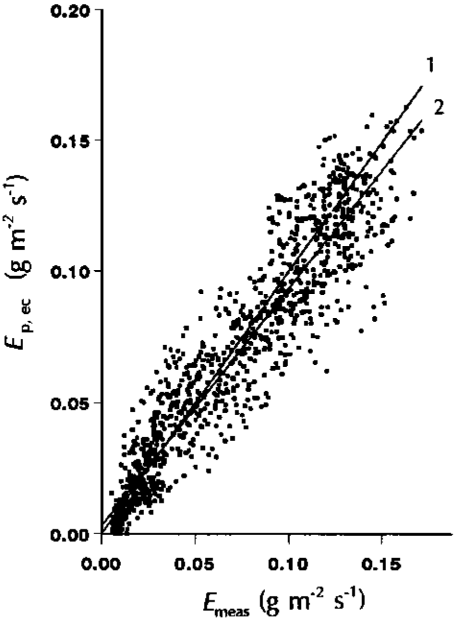

Fig. 3.6 *Rate of transpiration (in g m^{-2} s^{-1}) according to Penman-Monteith ($E_{p,ec}$, eqn. 3.6, with leaf conductance estimated with eqn. 3.4d) versus measured transpiration (E_{meas}) of sweet pepper. 1 line of unity; 2 fitted line (**Table 3.4**, n=1176, r^2=89%).*

Table 3.4 *Comparison of calculated (E_p) and measured (E_{meas}) rates of crop transpiration of sweet pepper (in g m^{-2} s^{-1}), by fitting the relation $E_p = a + b * E_{meas}$. $E_{p,mc}$ was computed based on measured values of g, and $E_{p,ec}$ based on estimated values of g (using* ***eqn. 3.4d, Table 3.3****). The four data sets are partly overlapping.*

			g measured			*g* estimated		
	day no	n	a	b	r^2	a	b	r^2
$E_{p,mc}$	115-180	116	-.001	.93	93	.004	.90	91
$E_{p,ec}$	115-136	504	--	--	--	.009	.91	89
$E_{p,ec}$	164-180	672	--	--	--	.002	.84	91
$E_{p,ec}$	115-180	1176	--	--	--	.003	.90	89

3.4.3 Discussion

Time courses

On day 115 (**Fig. 3.3**), leaf conductance was considerably lower, while VPD-air and leaf temperature and thus VPD-leaf-air were higher at high C. The large difference in *g* is a consequence of the difference in C, but strongly enforced by different air humidities. High VPD decreases leaf conductance and increases transpiration. Hence the differences in conductance were large and the difference in transpiration small. These observed changes perfectly illustrate the thermal and hydrologic negative feedback loops of the greenhouse-crop system (section 3.1.7 and **Fig. 3.1**). These feedback effects were stronger on this day than on most other days, because of a relatively low rate of ventilation (section 3.1.7).

In the compartment with high C, the supply was stopped and ventilation was slightly increased between 10:00 and 12:00 h, which had consequences for CO_2, VPD-air and leaf temperature. The conductances did not become equal in this time interval, explained only partly by persisting difference in VPD-air. Probably also a slow stomatal response played a role. Raschke (1972) observed that the stomatal reaction started a few seconds after a change in C; the half-time of closing was less than 2 min, whereas stomatal opening took up to an hour. Also Kuiper (1961) found a faster closing (5 min) than opening (25 min) of stomata in response to light.

Conductance

It follows from **Table 3.3** how leaf conductance is influenced by PAR, VPD, CO_2 and leaf temperature. Eqn. 3.4a-f were fitted to the data, with a fixed value for c_1 (c_1 = 25.6, as obtained with eqn. 3.3). If c_1 was not fixed, it varied too much, probably due to the small number of data relative to the number of parameters to be fitted. Eqn. 3.3 explained 49% of the variance of the leaf conductance data. If a term for C was added, r^2 became 59% (eqn. 3.4b), indicating the great impact of C. However, this term probably incorporates also effects of other factors. Therefore first the effects of PAR and VPD were calculated and then the additional effect of C (eqn. 3.4a and 3.4d). The difference in r^2 between 3.4a and 3.4d seems small, but is significant with $P<0.10$. With PAR and leaf temperature as explaining factors, r^2 was 50.4% (eqn. 3.4c) and adding the term for C increased r^2 to 59% (eqn. 3.4e). Eqn. 3.4f, with all factors, gave a better fit than eqn. 3.4e, but not better than eqn. 3.4d. This indicates that leaf temperature did not contribute to the explanation of the variance, if PAR, VPD and CO_2 were already accounted for. Thus eqn. 3.4d was found the most appropriate to illustrate the effect of C.

The effect of the environmental factors on conductance is demonstrated in **Fig. 3.7**, where *g* is computed with eqn. 3.4d (**Table 3.3**) for different environmental conditions. The effect of C in the range 200 - 1100 μmol mol^{-1} was smaller than the effect of PAR in the range 0 - 1500 μmol s^{-1} m^{-2} and of VPD-leaf-air in the range 0.5 - 2.0 kPa.

The relative C effect was about 3.1% per 100 mol mol^{-1} CO_2, according to eqn. 3.4d and eqn. 3.4f, at any level of CO_2, VPD-leaf-air and PAR. So elevation of C from 350 to 700 μmol mol^{-1}, as commonly applied, reduced *g* roughly by 10% and a C increase from 300 to 1000 μmol mol^{-1} reduced *g* by about 20%, at least at constant VPD-leaf-air. With eqn. 3.4b and 3.4e, the calculated CO_2 effect seemed higher, but c_5 in this equation does not represent the CO_2 effect only, as the effect of VPD was not taken into account here.

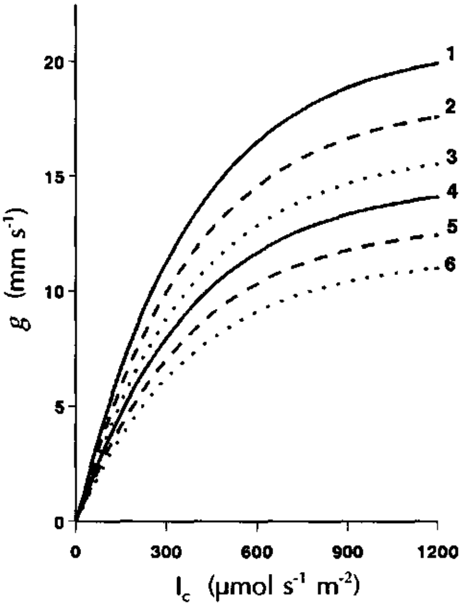

Fig. 3.7 *Calculated conductance (g in mm s^{-1}, eqn. 3.4d,* ***Table 3.3****) of sweet pepper as function of incident PAR (I_c in μmol $s^{-1}m^{-2}$), with chosen values for CO_2 (μmol mol^{-1}) and VPD (kPa).*

line	*1*	*2*	*3*	*4*	*5*	*6*
CO_2	*300*	*700*	*1000*	*300*	*700*	*1000*
VPD	*0.5*	*0.5*	*0.5*	*2.0*	*2.0*	*2.0*

Conductance, transpiration and CO_2 effects

As shown in **Fig. 3.6**, the calculated transpiration rate (E_p) was, on average, reasonably in agreement with the measured transpiration rate (E_{meas}). It is remarkable that E_p was slightly lower than E_{meas}, while an overestimation could be expected (section 3.3.5). It is concluded from **Table 3.4** and **Fig. 3.6** that the P-M model gives satisfactory results.

The direct relation between measured conductance and transpiration was fairly small, as r^2 was 51% (n=116, figures not shown). This was expected, because other factors (e.g. VPD) played a role. The approach chosen (section 3.2.2) was to combine the regression model for *g* with the P-M model for *E*.

With these two equations combined, an approximation was made of the reduction in *E*, caused by 10% reduction in *g*. It was observed that the P-M equation (eqn. 3.6) was fairly insensitive to changes in *g*, at least if *g* was high and if the boundary layer conductance (g_b) was assumed at 10 mm s^{-1}. A decrease in conductance of 10% decreased the calculated transpiration by only 1% at high conductance of 25 mm s^{-1}; by 2.2% at 10 mm s^{-1}; by 7.4% at 1 mm s^{-1} and by 9.7% at very low conductance of 0.1 mm s^{-1}. With a larger boundary layer conductance, a greater effect of *g* (and thus of C) on *E* would be found. If feedback effects were taken into account (i.e. enhanced VPD, particularly at low ventilation) the decrease in *g* would be larger and the decrease in *E* would be smaller. The conclusion is that the (direct and indirect) effects of C on stomata are considerable, whereas the consequences for canopy transpiration are dampened, as demonstrated in **Fig. 3.3**.

3.5 EFFECTS OF CO_2 ON CONDUCTANCE AND TRANSPIRATION OF TOMATO AND CUCUMBER

E.M. Nederhoff & R. de Graaf, 1993, Journal of Horticultural Science 68: 925-937.

3.5.1 Materials and methods

Crops, greenhouse and environment

Cucumbers (*Cucumis sativus* L., cv. Jessica) were sown on 1 August 1990 planted on 23 August on rockwool, at a density of 1.56 plants per m^2. The plants were topped mid September, and two side shoots were allowed to grow along the wire (2.2 m height). Three compartments were used for cucumber, which enabled simultaneous comparison of different C. The measurements were made between 11 September and 30 October 1990.

Tomatoes (*Lycopersicon esculentum* Mill., cv. Calypso) were sown on 26 November 1990, and placed in the greenhouse on 4 January and planted on the rockwool slabs on 18 January 1991. The density was 2.08 plants per m^2. The plants were not topped, but the top was led over the wire to grow downwards. All side shoots were pinched out at appearance. The measurements on tomato were done only in one compartment, between 19 February and 3 July 1991.

The mature fruits were harvested two (cucumber) and three (tomato) times per week. The cultivation technique, as well as the growth and production pattern were similar to common commercial practice in the Netherlands.

The greenhouse was the same computer-controlled, Venlo-type glasshouse complex as used for sweet pepper, with compartments of 12 m x 12.8 m. Also plant nutrition, irrigation, supply of pure CO_2 and C measuring were similar (section 3.4.1).

The CO_2 enrichment strategy during the cultivation was according to commercial practice, i.e. with a ventilation depending set point. Generally the concentration varied between 350 and 550 $\mu mol\ mol^{-1}$. On measurements days, however, CO_2 set points in the range 300 to 1200 $\mu mol\ mol^{-1}$ were maintained, often with two different concentrations in the two compartments or with different concentrations in the morning and the afternoon.

Global radiation was measured outside and multiplied with the calculated transmission of the glasshouse cover (section 3.3.1). Photosynthetically Active Radiation was measured with the PAR meter on the porometer. In the tomato experiment, however, these PAR measurements were not reliable, because of shading by upper leaves. So incident PAR was estimated here (called I′) from global radiation, multiplied with the calculated transmission factor and with a unit conversion factor (**Appendix III**).

Table 3.5 *Ranges of leaf conductance, canopy transpiration and environmental conditions, obtained in the experiments with cucumber and tomato.*

		cucumber in autumn n=216			tomato in February n=131			tomato in April n=55		
		min.	mean	max.	min.	mean	max.	min.	mean	max.
day	--	254	275	298	50	53	57	113	116	119
g	mm s^{-1}	2.2	12.0	22.4	8.4	13.6	21.3	12.7	16.6	21.3
E_{meas}	g m^{-2} s^{-1}	0.007	0.03	0.085	0.014	0.032	0.061	0.025	0.088	0.133
Q	W m^{-2}	3	266	721	28	280	498	190	619	925
I_c	μmol s^{-1} m^{-2}	7	219	991	(31)	(279)	(892)	(74)	(293)	(870)
T_a	°C	19.3	23.6	27.6	18.1	19.9	22.1	20.2	22.1	23.2
T_l	°C	19.9	24.3	29.6	19.1	21.4	23.3	21.1	23.4	25.3
D_a	kPa	0.29	0.88	2.05	0.32	0.54	0.84	0.13	0.60	1.03
$D_{l\text{-}a}$	kPa	0.32	0.92	2.23	0.35	0.59	0.95	0.14	0.65	1.08
C	μmol mol^{-1}	233	578	1667	304	605	1314	306	451	889

Table 3.6 *Correlation coefficients of leaf conductance, canopy transpiration and environmental conditions (symbols explained in Appendix II)*
a. *cucumber; n=216;* $r_{(P = 0.01,\ n = 216)} = [\,0.16\,]$
b. *tomato; n=186;* $r_{(P = 0.01,\ n = 186)} = [\,0.17\,]$.

a.	*g*	E_m	Q	I_c	I'_c	T_a	T_l	D_a	$D_{l\text{-}a}$	C
g	1.00									
E_{meas}	0.57	1.00								
Q	0.57	0.81	1.00							
I_c	0.59	0.65	0.71	1.00						
I'_c	0.54	0.80	0.99	0.71	1.00					
T_a	0.60	0.62	0.62	0.49	0.62	1.00				
T_l	0.49	0.58	0.59	0.61	0.61	0.83	1.00			
D_a	0.02	0.55	0.46	0.27	0.47	0.51	0.38	1.00		
$D_{l\text{-}a}$	0.02	0.58	0.49	0.35	0.50	0.50	0.48	0.98	1.00	
CO_2	-0.26	-0.18	-0.07	0.03	-0.06	0.05	0.12	0.13	0.14	1.00

b.	*g*	E_m	Q	I_c	I'_c	T_a	T_l	D_a	$D_{l\text{-}a}$	C
g	1.00									
E_{meas}	0.57	1.00								
Q	0.57	0.92	1.00							
I_c	0.54	0.36	0.49	1.00						
I'_c	0.56	0.94	0.99	0.44	1.00					
T_a	0.65	0.88	0.85	0.36	0.86	1.00				
T_l	0.60	0.85	0.89	0.55	0.88	0.89	1.00			
D_a	0.24	0.58	0.59	0.44	0.55	0.56	0.58	1.00		
$D_{l\text{-}a}$	0.23	0.56	0.59	0.51	0.55	0.51	0.62	0.98	1.00	
CO_2	-0.48	-0.35	-0.33	-0.21	-0.32	-0.30	-0.31	-0.09	-0.10	1.00

Conductance and transpiration measurements

The conductance measurements were performed on days 254, 255, 262, 271, 275, 288, 289, 298 (September/October) of 1990 in three compartments with cucumber and on days 50, 51, 52, 53, 56 and 57 (February) and 113, 114, 115, 116, 119 (April) of 1991 in one compartment with tomato. The transpiration rates were measured continuously, but only the data of the periods when conductance was measured, were considered in the data analysis.

The measurements with the Li-cor-1600 porometer were made usually between 9:00 or 10:00 and 16:00 or 17:00 h, in a continuous cycle. Each series of measurement, consisting of 10 readings on different leaves, lasted 8 to 10 min and yielded one average value. Conductance was always measured on the underside of full-grown, green and healthy leaves in the top of the canopy and in full light. The data were pooled into one data set (n=216) for cucumber and one (n=186) for tomato.

Transpiration was measured by the three lysimeters per compartment. However, in cucumber only the lysimeter measurements of the east side were used for analysis and in tomato only the averaged values of the two lysimeters of the west side of the double crop rows under the gutter. The leaf conductance measurements were restricted to the same location as the transpiration measurements (east and west, respectively). Each lysimeter carried two plants, representing 1.28 m^2 in the cucumber crop and 0.96 m^2 in tomato.

3.5.2 Results

CO$_2$ effects on leaf conductance

The ranges of leaf conductance and environmental data, obtained in the measurements, are presented in **Table 3.5. Table 3.6** shows that *g* is correlated to almost all factors, except VPD, in cucumber. There is a significant negative correlation between C and *g* in both crops. In cucumber, simultaneous measurements were conducted at different C. As an example, one day is presented with alternating C in two compartments (**Fig. 3.8**). High C reduced *g*; *E* was not significantly affected, although a tendency to reduction can be seen.

Measured *g* was plotted versus incident PAR (**Fig. 3.9**). With tomato, the data of February and April were distinguished by using different symbols and fitting different linear regression lines (Fig. 3.9b). Regression analysis with eqn. 3.4 on the data of each period separately did not give satisfactory results, probably because the ranges of conditions were too small. Therefore the data of the two periods were combined, on the assumption that the response was not dependent on day number or canopy age. This was reasonable, as in all cases the same type of leaves (young, almost full-grown leaves in the upper canopy part) were sampled.

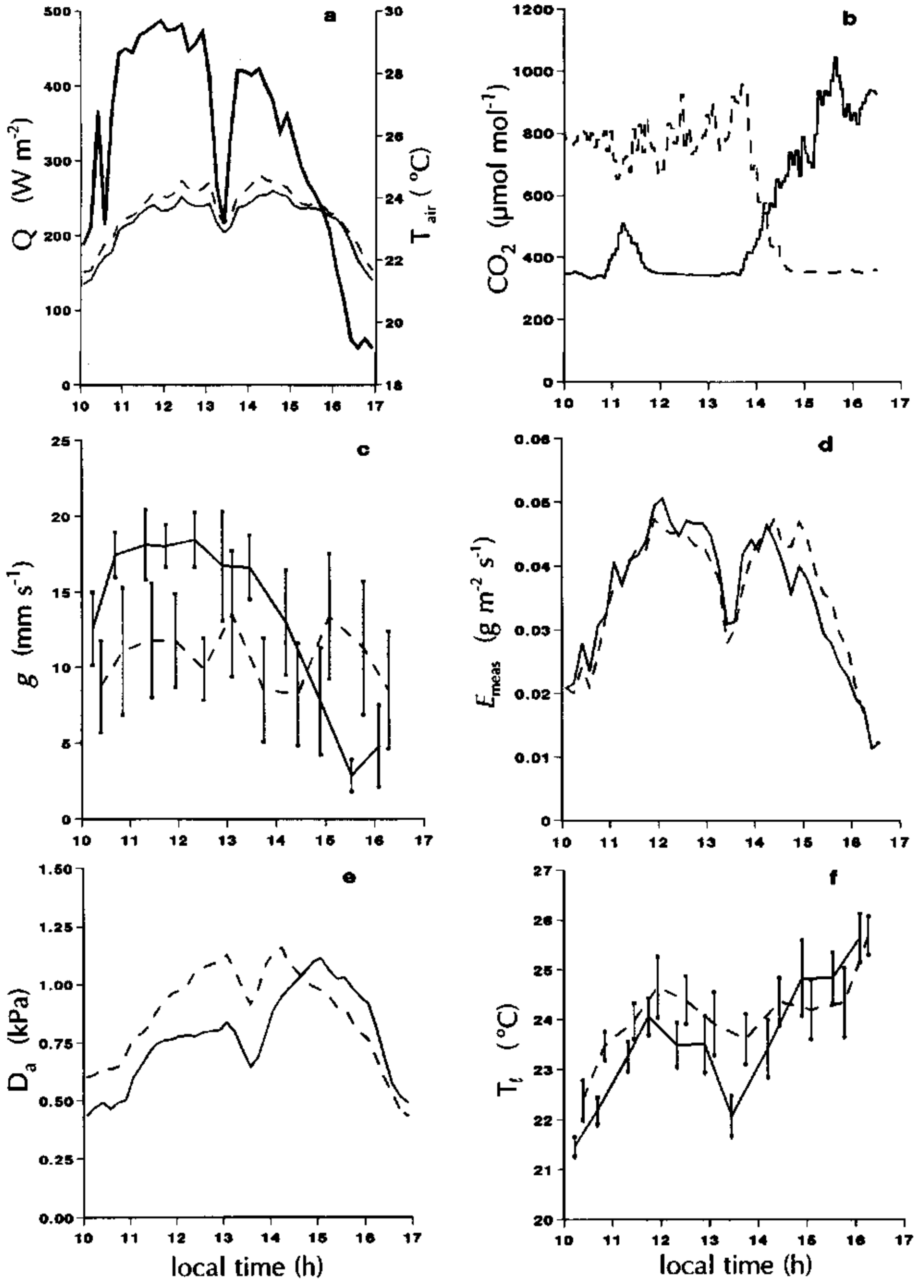

Fig. 3.8 *Time courses of measurements on 16 October 1990 (day 289) in two compartments with cucumber.*
a. *T_{air} = air temperature (°C); Q = global radiation outside (W m^{-2} ——)*
b, c, d, e, f, ^{1} *as in Fig. 3.2 and 3.3.*

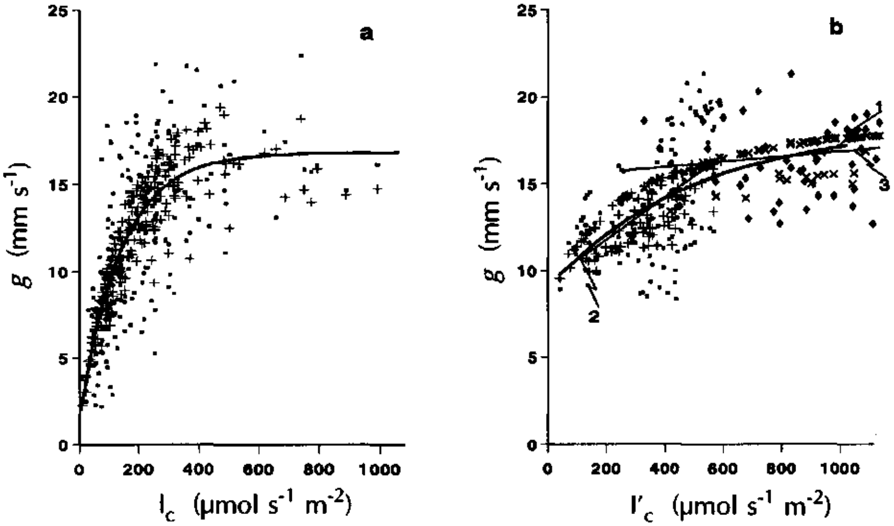

Fig. 3.9 *Leaf conductance (g in mm s^{-1}) versus incident PAR (I_c in µmol m^{-2} s^{-1})*
a. *cucumber, measured (■) and calculated with eqn. 3.4i,* ***Table 3.7*** *(+) and regression line (eqn. 3.4g, r^2=52%, n=216)*
b. *tomato, data measured in February (■), measured in April (◆), calculated with eqn. 3.4m for February (+) and for April (X).*
1 = regression line for all data (eqn. 3.4k, r^2=34%, n=186)
*2 = line for data of February (g = 9.4 + 0.012 * PAR, r^2=30%, n=131)*
*3 = line for data of April (g = 15.4 + 0.0015 * PAR, n.s., n=55).*

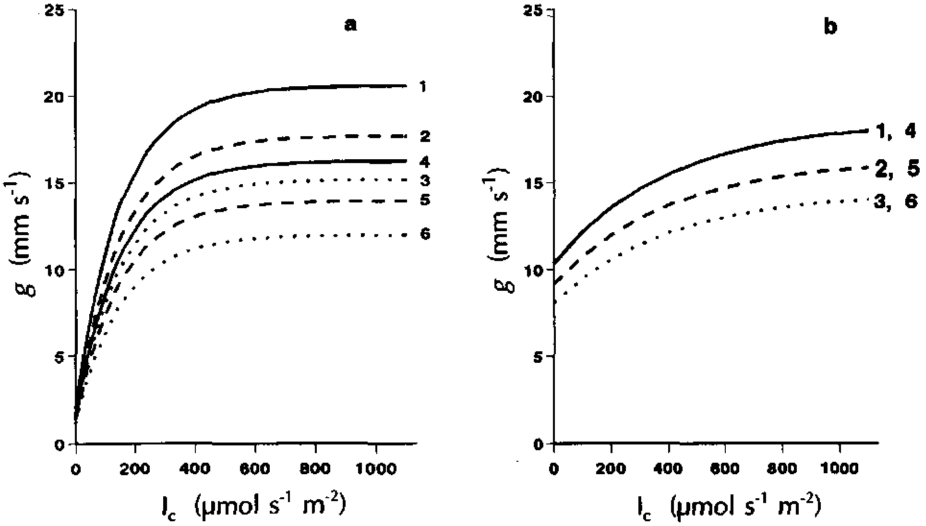

Fig. 3.10 *Leaf conductance (g in mm s^{-1}) calculated with regression eqns (**Table 3.7**) versus incident PAR (I_c in µmol s^{-1} m^{-2}) with chosen values of VPD-leaf-air (kPa) and CO_2 (µmol mol^{-1}).*
a. *cucumber (eqn. 3.4i)*
b. *tomato (eqn. 3.4m).*

legend:

line	1	2	3	4	5	6
CO_2	300	700	1000	300	700	1000
VPD	0.5	0.5	0.5	2.0	2.0	2.0

Table 3.7 *Fitted values of parameters c_1 to c_7 of the regression equations, relating leaf conductance (g in mm s^{-1}) to environmental conditions:*
$g = c_1 * (1 - c_2 * EXP(-c_3 * I_c)) * EXP(-c_4 * D_{l\text{-}a}) * EXP(-c_5 * C)/(1 + c_6 * (T_l - c_7)^2)$
a. *cucumber (n=216)* **b.** *tomato (n=186).*

eqn.	c_1	c_2	c_3	c_4	c_5	c_6	c_7	r^2
a. cucumber								
3.4g	16.8^a	0.91^a	$7.2E\text{-}3^a$	--	--	--	--	52
3.4h	21.2^a	0.90^a	$6.1E\text{-}3^a$	0.20^a	--	--	--	55
3.4i	25.0^a	0.91^a	$6.5E\text{-}3^a$	0.16^a	$3.8E\text{-}4^a$	--	--	61
3.4j	31.8^a	0.89^a	$8.9E\text{-}3^a$	0.24^a	$4.3E\text{-}4^a$	$6.5E\text{-}3^b$	31.1^a	66
b. tomato								
3.4k	18.4^a	0.51^a	$2.0E\text{-}3^a$	--	--	--	--	34
3.4l	22.0^a	0.56^a	$2.0E\text{-}3^a$	0.23^b	--	--	--	35
3.4m	20.3^a	0.44^a	$2.5E\text{-}3^a$	--	$3.1E\text{-}4^a$	--	--	44
3.4n	22.7^a	0.48^a	$2.4E\text{-}3^a$	0.15^a	$3.0E\text{-}4^a$	--	--	44

[a] *significant at 1%* [b] *significant at 5%*

Stepwise regression was applied and the results are presented in **Table 3.7**. The significance of the difference in r^2 (percentage of variance explained) of the different equations was tested with an F-test. If only PAR was fitted, r^2 was 52% in cucumber and 34% in tomato. Addition of any other term increased r^2 significantly ($P<0.01$). VPD-leaf-air had only a small impact, whereas the contribution of the CO_2 term was considerable.

The relative effect of C on *g* was calculated with the fitted equations, containing a C term (eqn. 3.4i,j and m,n **Table 3.7**). According to these equations, each 100 μmol mol^{-1} increase in CO_2, decreased *g* by about 4% in cucumber and by about 3% in tomato. The effects of the relevant factors on *g*, as determined with eqn. 3.4i and 3.4m (Table 3.7) for cucumber and tomato, respectively, are shown in **Fig. 3.10**.

Canopy transpiration and relation to leaf conductance

The ranges of transpiration and environmental factors are shown in **Table 3.5**. From the correlation matrix (**Table 3.6**) it is evident that E_{meas} was correlated with all factors mentioned; negatively correlated with CO_2 and positively correlated with VPD-air. In **Fig. 3.11**, E_{meas} is compared to E_p, which is calculated with the P-M equation, from measured *g*, VPD-air and estimated absorbed global radiation. In cucumber, the P-M equation overestimated the transpiration rate. In tomato, the calculated values tended to be too high in the lower ranges and too low in the higher ranges of transpiration.

Table 3.8 *Coupling factors ($K = [(E_2-E_1) / E_{mean}] / [(g_2-g_1) / g_{mean}]$) relating changes in leaf conductance (g in mm s^{-1}) and transpiration rate (E in g m^{-2} s^{-1}), caused by CO_2 steps, at different levels of radiation outside (Q in W m^{-2}) and VPD (D_a in kPa). CO_2 concentration (in µmol mol^{-1}) indicated by the subscript. g calculated with* ***eqn. 3.4i*** *(**Table** 3.7) and E with* ***eqn. 3.6*** *(Penman-Monteith), with air temperature 25 °C and 65% transmission of the glasshouse.*

Q	D_a	g_{300}	g_{1200}	E_{100}	E_{1200}	$K_{300\text{-}400}$	$K_{1100\text{-}1200}$	$K_{300\text{-}1200}$
50	0.25	0.86	0.61	0.009	0.008	0.24	0.30	0.27
50	2.00	0.65	0.46	0.033	0.030	0.29	0.36	0.33
250	0.25	1.91	1.35	0.035	0.034	0.12	0.16	0.14
250	2.00	1.44	1.02	0.063	0.060	0.16	0.20	0.18
1000	0.25	2.14	1.52	0.130	0.124	0.11	0.14	0.13
1000	2.00	1.62	1.15	0.155	0.147	0.14	0.18	0.16

The effect of C on *E* was studied by calculating the *K*-values, describing the relation between calculated changes in *g* and *E* (eqn. 3.7). The results are given in **Table 3.8**. According to eqn. 3.4i (for cucumber), an increase of C from 300 to 1200 µmol mol^{-1} reduced *g* by 34% (and according to eqn. 3.4m for tomato by 28%), whereas *E* was reduced far less. Under all conditions chosen, *K* was less than 0.4 (for tomato less than 0.22). Under higher radiation *K* was less than 0.20 (for tomato less than 0.18). A *K*-value of 0.2 implies that a 34% reduction in *g* was accompanied by less than 7% reduction in *E*.

3.5.3 Discussion

CO_2 effects on leaf conductance

It is evident that C has an effect on *g*, as follows from the time courses (**Fig. 3.8**), the correlation matrix (**Table 3.6**) and the regression equations (**Table 3.7**). In the correlation matrix, it is remarkable, that between VPD and *g*, no significant correlation was found in cucumber, while in tomato, an unexpected positive correlation was found. This is probably owing to the relatively small range of VPD-air in the tomato experiment. The measured leaf conductance both of cucumber and tomato plotted versus PAR (**Fig. 3.9**) show the expected saturation pattern (Burrows & Milthorpe, 1976). The scatter in Fig. 3.9 was expected, because VPD and CO_2 are not taken into account here. Further, in tomato, incident PAR was calculated from radiation measured outside, which did not account for a difference in radiation between east and west side of a row. The fitted regression lines are shown in **Fig. 3.10**. The relative CO_2 effect (3 and 4% per 100 µmol mol^{-1} increase in CO_2), is in the same order of magnitude as that of greenhouse grown sweet pepper (3% per 100 µmol mol^{-1} CO_2, section 3.4.3).

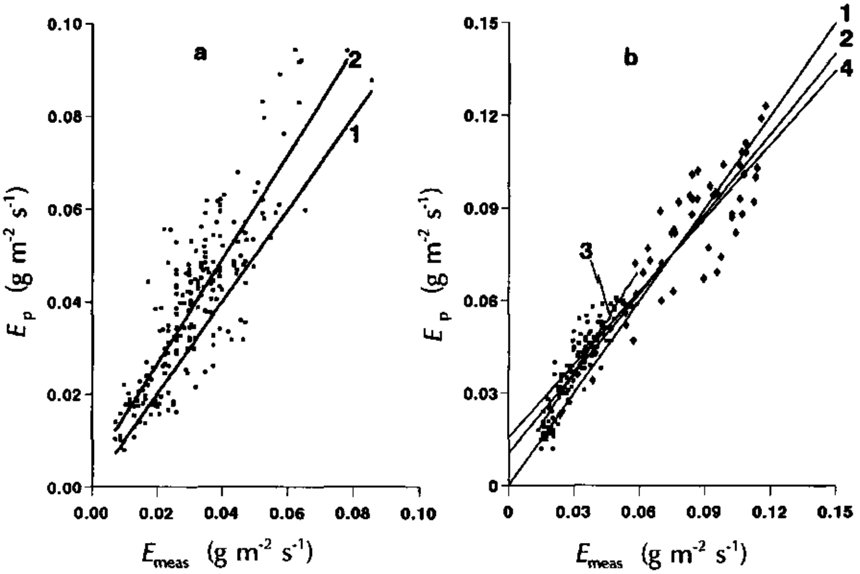

Fig. 3.11 *Calculated transpiration rate (E_p in g m^{-2} s^{-1}) versus measured transpiration (E_{meas}). E_p was obtained with the Penman-Monteith equation, using leaf conductance measured in the top leaves.*

a. *cucumber*
1 = line of unity
*2 = linear regression line (E_p = 0.004 + 1.13 * E_m, r^2=74%, n=215)*

b. *tomato, with data of February (■)* and April (◆) *distinguished*
1 = line of unity
*2 = linear for all data (E_p = 0.011 + 0.86 * E_m, r^2=91%, n=186)*
*3 = linear for data of February (E_p = 0.002 + 1.14 * E_m, r^2=83%, n=131)*
*4 = linear for data of April (E_p = 0.016 + 0.79 * E_m, r^2=77%, n=55).*

Canopy transpiration rate

In the comparison of measured and calculated canopy transpiration rates (**Fig. 3.11**), most of the scatter is probably caused by inaccuracy in the estimation of incident global radiation. It is improbable that variations in temperature of the heating pipes caused much scattering, as this effect is usually small (Stanghellini, 1987; Jolliet & Bailey, 1992; section 3.3.1).

The fitted lines in **Fig. 3.11** deviate from unity, which might be explained by the fact that the conductance of leaves in the top of the canopy was used in the calculations, rather than the 'canopy conductance', which would be lower (section 3.3.5). Although this simplification undoubtedly influenced the calculation of E_p, the agreement between E_p and E_{meas} was still reasonable and considered satisfactory (Fig. 3.11). Hence, it is concluded that the P-M equation implemented with the measured conductance of top leaves, can be used adequately to calculate the relative differences in canopy transpiration, e.g. caused by different C.

The calculated coupling factor *K*, relating *g* to *E* (**Table 3.8**) is in the order of magnitude as those calculated by Aubinet et al. (1989), using the data of Shaer & Van Bavel (1987). It is concluded that in cucumber and tomato under near-commercial conditions, the effect of C on stomata is significant, whereas the effect on transpiration is small and usually negligible, except under very low radiation.

3.6 EFFECTS OF CO_2 ON LEAF CONDUCTANCE AND ESTIMATED CANOPY TRANSPIRATION OF EGGPLANT

E.M. Nederhoff, 1992, Journal of Horticultural Science 67: 795-803.

3.6.1 Materials and methods

Crop, greenhouse and environment

Eggplant (*Solanum melongena* L., cv. Cosmos) was sown on 20 October 1990 and planted on 10 December on rockwool slabs. The experiment lasted until 18 July 1991. Plant density was 2.5 plants per m^2, with two stems per plant, in two double rows of plants per 3.2 m glasshouse bay.

Fruit setting was stimulated by spraying the flowers once per five days with Tomatotone (spraying solution of 0.02 volume% 4-chlorophenoxy acetic acid, a synthetic auxin, Van Ravestijn, 1983). Crop management, e.g. spraying, leaf and fruit thinning (if not part of a treatment) and pest and disease control were performed according to normal commercial practice.

The measurements were made in a Venlo-type glasshouse complex, with ridge height of 3.75 m and with a north-south orientation. The transmission of the glasshouse cover for diffuse global radiation was about 55%. In four compartments (16 m x 16 m) an experiment with eggplant was carried out (section 4.6), and in two of these four compartments leaf conductance was measured. Control of environmental factors and irrigation, plant nutrition, CO_2 supply and data acquisition were similar to those in the other experiments (sections 3.4.1 and 3.5.1). Differences were that the transpiration could not be measured and that there was another heating system and another CO_2 measuring device.

The greenhouse compartments were heated using eight pipes of 22 mm diameter per 3.2 m bay, at 0.4 to 0.8 m above the soil. Heating temperature set points were maintained at around 24/24 °C (day/night) in the first three weeks after planting, gradually decreased to 22/19 °C in January and February and maintained at 20/18 °C from March until the end of the experiment. Until the end of February an energy saving screen was closed at night. Ventilation started in March and was set at 25 °C. From March onward at low irradiance (< 100 $W\ m^{-2}$ global radiation outside) a minimum heating pipe temperature of 50 °C was used. This is a practical method to harden the plants by stimulating the transpiration. Temperature and air humidity were measured by screened and aspirated PT-100 psychrometers, one in each compartment at about 1.5 m above the ground.

The CO_2 monitor was an infra-red gas analyzer, type Ultramat (Siemens, Munich, Germany), which was zero-calibrated automatically every 24 h and calibrated with standard gas mixtures every six weeks. The four glasshouse compartments were connected to one CO_2 analyzer by a multiplexer, taking four samples in four minutes. Each compartment had one sampler duct with two inlets on 1.5 m from the ground. The distribution of CO_2 through a network on the ground, was examined several times and was found to be homogeneous.

From planting until week 4, the concentration was kept equal in the four compartments at 400 to 600 µmol mol^{-1}. In week 5 the C treatments were started: high C was set 750 µmol mol^{-1} from week 6 to 29 and low C 450 µmol mol^{-1} from week 5 to 11 and 350 µmol mol^{-1} from week 12 to 29. The achieved concentrations, averaged over week 6 to 29 were 413 and 688 µmol mol^{-1} respectively (details in section 4.6).

Leaf conductance measurements

Leaf conductance measurements were performed in the two compartments, on 5 and 7 February, 5 and 6 March, 11 and 12 April, 28 and 29 May 1991 (day of the year 36, 38, 64, 65, 101, 102, 148, 149), mostly from 10:00 to 16:00 h. On days 36, 64, 102 and 149, C was kept equal in the two compartments, to check if the stomatal behaviour adapted to long-term C. On all other days of the season, C was maintained low and high according to the CO_2 experiment, during the photoperiod (obtained conditions in **Table 3.9**).

Leaf conductance was measured (section 3.3.2) with the Li-Cor-1600 diffusion porometer on the lower surface of young, but mature, green and healthy leaves, that were exposed to full light or sunshine, located in the upper 0.40 m of the third plant of all rows. First 10 leaves were measured in one compartment, which took about 15 min, and subsequently 10 leaves in the second compartment were measured, etc., the two compartments in a continuous cycle. Leaf temperature and PAR were measured instantaneously with a thermocouple and a LI-190S-1 sensor, respectively, in the porometer. The 10 leaf conductance data and the relevant environmental conditions recorded in the measuring interval were averaged, to obtain one data record per interval. In this way, 8 to 14 data points (means of 10 measurements) were obtained per measuring day, per compartment. No systematic differences in stomatal response between the compartments were observed, so the data of the two compartments and of all eight measuring days were pooled into one data set (n=156).

In the greenhouse used, no transpiration measurements could be carried out. Therefore, the canopy transpiration rate was approximated, using the Penman-Monteith equation (eqn. 3.6, section 3.3.5). The transpiration had to be calculated for the whole season, whereas the required values of leaf conductance were available only on the eight measuring days. Therefore, leaf conductance was approximated with the fitted regression equation (eqn. 3.4p, below) from measured hourly environmental data. For this estimation, $D_{l\text{-}a}$ was assumed equal to D_a. Incident PAR (I_c) was approximated from outside global radiation, assuming 55% transmission of the glasshouse cover and adding a unit conversion factor (**Appendix III**).

Table 3.9 *Ranges of leaf conductance, canopy transpiration and environmental conditions in eggplant experiment. Long term data cover 5 February - 18 July 1991.*

		conductance data set comp. 6 & 8 (n=156)			long term data, comp. 6 (n=1640)			long term data, comp. 8 (n=1640)		
		min.	mean	max.	min.	mean	max.	min.	mean	max
day	--	36	95	149	36	118	199	36	118	199
g	mm s^{-1}	1.7	8.5	17.1	2.7 [a]	21.0	35.6	2.4	15.2	3.5
Q	W m^{-2}	48	404	850	3	335	904	3	335	904
I_c	μmol s^{-1} m^{-2}	59	294	806	--	--	--	--	--	--
T_a	°C	17.3	25.2	30.2	18.7	24.5	33.0	19.0	24.6	33.3
T_l	°C	20.2	25.2	31.1	--	--	--	--	--	--
D_a	kPa	(0)	0.9	2.2	0.2	0.7	2.3	0.1	0.9	2.4
$D_{l\text{-}a}$	kPa	(0)	0.9	2.4	--	--	--	--	--	--
C	μmol mol^{-1}	323	547	1177	163	413	915	204	688	1234

[a] *calculated from measured environmental conditions*

Table 3.10 *Correlation coefficients (r) for the measured climatic conditions and leaf conductance in the eggplant (n=156).* $r_{(P = 0.01,\, n = 156)} = [0.19]$.

		1	2	3	4	5	6	7	8
time	1	1.00							
glob. rad. outside	2	0.27	1.00						
incident PAR	3	0.28	0.85	1.00					
air temperature	4	0.28	0.90	0.76	1.00				
leaf temperature	5	0.28	0.89	0.75	0.96	1.00			
VPD-air	6	0.21	0.42	0.26	0.39	0.48	1.00		
CO_2	7	-0.01	-0.63	-0.67	-0.57	-0.55	-0.06	1.00	
leaf conductance	8	0.24	0.69	0.77	0.67	0.58	-0.15	-0.78	1.00

3.6.2 Results

On four measuring days the climatic conditions (particularly C) were kept equal in the two compartments, sometimes low (around 350 μmol mol^{-1}) and sometimes higher (700 - 800 μmol mol^{-1}). On these days the measured leaf conductance was not significantly different in both compartments. As an example, the last measuring day is presented in **Fig. 3.12**. This indicated that there was no effect of crop nor of compartment and no adaptation of stomatal behaviour to long-term CO_2 enrichment.

At contrasting C, as normally maintained throughout the cultivation, leaf conductance was always lower at higher C, in most cases significantly so. A typical day is presented in **Fig. 3.13**. The multiple regression equation fitted to the pooled data gave the following result:

$$\boldsymbol{g = 24.5 * (1 - 0.85 * EXP(-4.69E\text{-}3 * I_c)) * EXP(-0.19 * D_{l\,-a}) * EXP(-1.07E\text{-}3 * C)}$$

(eqn. 3.4p) with r^2=83.2. The observed effects of PAR, VPD-leaf-air and C on leaf conductance are visualized by lines computed with this equation (**Fig. 3.14**). Parameter c_5 (-1.07E-3) represents the relative C effect. It indicates about 11% decrease in *g* per 100 μmol mol^{-1} increase in C.

In **Fig. 3.13**, the transpiration rate calculated with the P-M equation (eqn. 3.6) from measured radiation, VPD-air and leaf conductance, responded not very pronounced to C. VPD-air was clearly higher at high C, and leaf temperature tended to be higher. The calculated transpiration, accumulated between 1 January and 18 July was 4% lower at high C than at low C (**Fig. 3.15**). In shorter intervals, however, greater differences occurred, e.g. 15% over a 5-weeks period between 12 February (start of CO_2 enrichment) and 18 March (start of leaf analysis, section 4.6). For the boundary layer conductance g_b, a value of 10 mm s^{-1} was assumed (section 3.3.5). If 20 mm s^{-1} was used instead, the calculated reduction in transpiration at high C compared to low C would be 6.6% over the whole season and 18% over the 5-weeks period in spring.

3.6.3 Discussion

Leaf conductance and long-term CO_2 effect

Elevation of C clearly reduced the leaf conductance, as can be seen from the clear negative correlation of *g* and C (**Table 3.10**), and from the regression equation (above) and the diurnal courses (**Fig. 3.13**). To test the impact of the various factors, the regression equation was applied stepwise. First only PAR was taken into account, which gave r^2 of 65% (percentage of the variance accounted for). With PAR and VPD-leaf-air as factors, r^2 was 76% and with PAR, VPD-leaf-air and C as factors (eqn. 3.4p), r^2 increased to 83%. This proves the highly significant effect of C on leaf conductance ($P<0.001$).

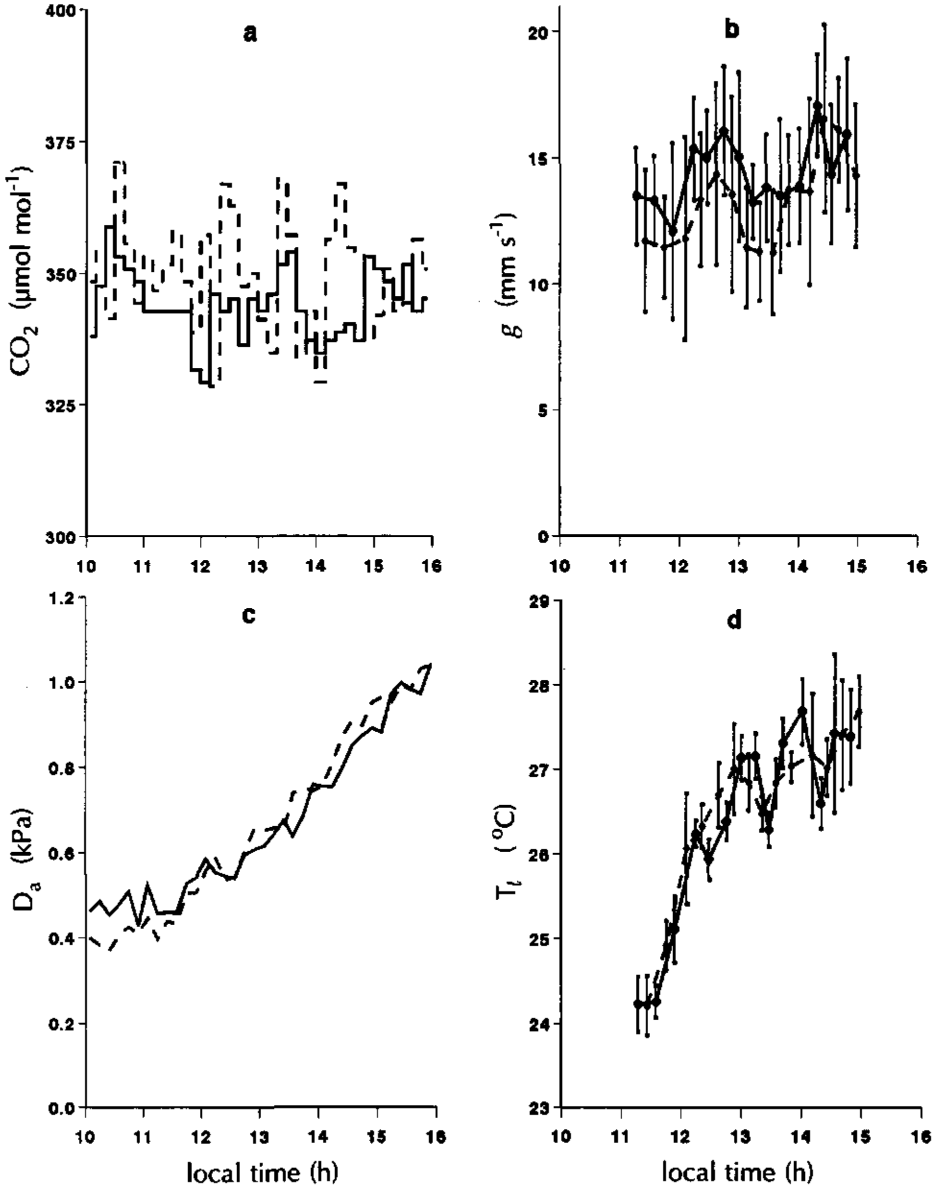

Fig. 3.12 *Measurements on 29 May 1991 (day 149) on eggplant in two glasshouse compartments under equal environmental conditions:*

a. *CO_2 = CO_2 concentrations (µmol mol^{-1})*
b. *g = leaf conductance (mm s^{-1}) at top of the crop [1]*
c. *D_a = vapour pressure deficit of glasshouse air (kPa)*
d. *T_l = temperature of the leaves (°C) [1]*
[1] error bars indicate the standard deviation of the measurements (n=10).

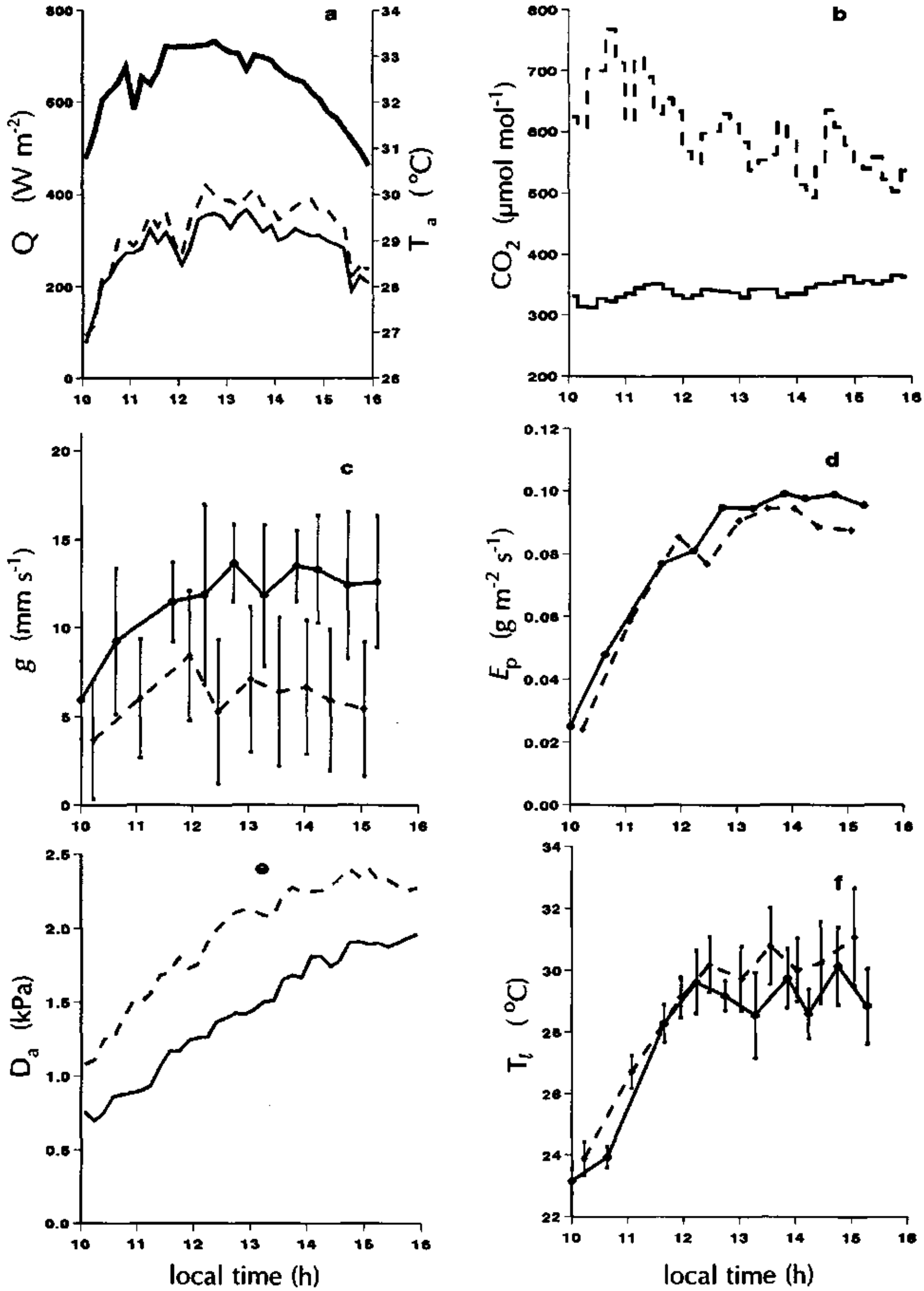

Fig. 3.13 *Measurements on 11 April 1991 (day 101) on eggplant in two glasshouse compartments, one with low CO_2 (———) and one with high CO_2 (– – – – –).*
a. *T_a = air temperatures (°C), Q = global radiation outside ($W m^{-2}$ ▬▬);*
d. *E_p = calculated rate of crop transpiration ($g m^{-2} s^{-1}$)*
b, c, e, f, ^{1} *as in Fig. 3.2 and 3.3*

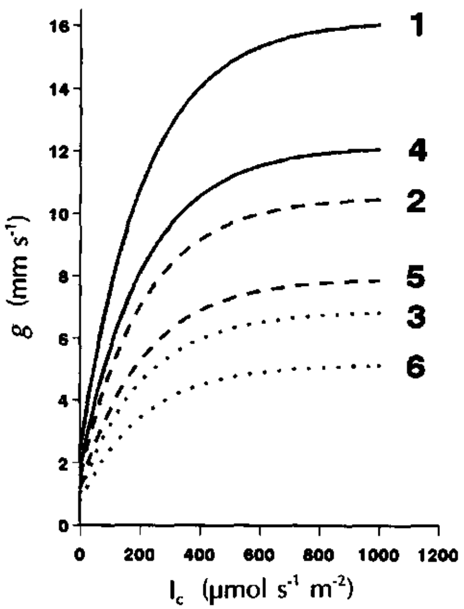

Fig. 3.14 *Leaf conductance (g in mm s^{-1}, calculated with the fitted regression equation eqn. 3.4p) of eggplant versus incident PAR (I_c in μmol m^{-2} s^{-1}), with chosen values for CO_2 (μmol mol^{-1}) and VPD (kPa).*

line	1	2	3	4	5	6
CO_2	300	700	1000	300	700	1000
VPD	0.5	0.5	0.5	2.0	2.0	2.0

The relative effect of C on stomatal opening, established with eqn. 3.4p, is about 11% decrease in conductance per 100 μmol mol^{-1} increase in C (**Fig. 3.14**). This is about three times stronger than that established for sweet pepper, tomato and cucumber (about 3 - 4% per 100 μmol mol^{-1}, sections 3.4.3 and 3.5.3). This confirms the observation of De Koning (1989) that eggplant is exceptionally sensitive to C. It is not understood why the stomatal response to C is much stronger in eggplant than in other crops. It could have the same (plant specific) background as the sensitive stomatal response of eggplant to water conditions, reported by Behboudian (1977), Daunay et al. (1986a,b) and Srinivasa Rao & Bhatt (1990). These authors also, had no explanation for the exceptional behaviour of eggplant. The sensitive stomatal response to C can be a consequence of the growing conditions, because in glasshouse culture in the Netherlands, eggplant is grown at higher temperature and higher air humidity than other fruit vegetable crops.

Root growth can be suboptimal as a consequence of high humidity (Swalls & O'Leary, 1975; Gislerød & Nelson, 1989) or to high fruit load (Hurd et al., 1979; Quast, 1977). If such a crop is exposed to high radiation, the stomata will partly close through the internal water feedback to prevent water shortage. Plants that have been exposed to drought stress have a higher ABA content and are more sensitive to C than well watered plants (Raschke, 1975; Willmer et al., 1988; Eamus & Narayan, 1989).

Theoretically, the strong stomatal response might be due to a high C during the cultivation, because high C might sometimes reduce the root growth (Peet & Kroen, 1991), although also the opposite is reported. However, C was maintained high in only one compartment and low in the other. If the strong stomatal response was due to high C, than the stomatal response would be different in the two compartments. This was not the case, as was seen on the days with equal C in the two compartments (**Fig. 3.12**).

The measurements of 7 February (data not presented) were the only ones that did not exhibit a lower leaf conductance at higher C, as in **Fig. 3.13**. On this day with low radiation (max. 100 W m^{-2} global radiation outside), leaf conductance was low as well (max. 4 mm s^{-1}) and could not be distinguished significantly in the two compartments, despite different C. A possible explanation is that the young plants did not respond to C, because no sensitivity to C had developed yet. However, this is not supported by the observations in young tomato plants. It might better be explained by a low stomatal response to C under low light (Morison, 1987). Most probable, however, is that the resolution of the conductance measurements was too low, compared to the low level of conductivity.

Transpiration

The fact that the approximated transpiration rate responded far less than conductance to C, also in this experiment with eggplant (**Fig. 3.15**), is based on the decoupling of conductance and transpiration, caused by the boundary layer effect and the feedback loops (section 3.1.7). The average relative humidity measured in this experiment was lower in the compartments with high C (in summer 5 - 10% lower relative humidity at equal temperatures). Further, at high C, the windows were opened wider (sometimes 10 - 20%) by the control computer, to achieve the temperature set points. This is explainable, as a reduced transpiration implies less evaporative leaf cooling.

The cumulative difference in calculated transpiration was 4% over the whole season and 15% over a certain period in spring (**Fig. 3.15**). This variation must be ascribed to changes in circumstances. The boundary layer conductance and the gains of the feedback loops are strongly dependent on the rate of air exchange with the outside and on the dimensions of the canopy: a small crop with little transpiration can hardly affect the VPD of the greenhouse air (hydrological feedback small); without ventilation the feedback effects are most pronounced. Between about day 50 and 75, the hydrologic feedback was weak, because the transpiration was still small and the ventilation was already started. A weak feedback implies that g and E are more coupled, so that a reduction in g (through high C) resulted in a marked reduction in E. Later in the season the feedback declined by increased ventilation and the effect of C on E decreased.

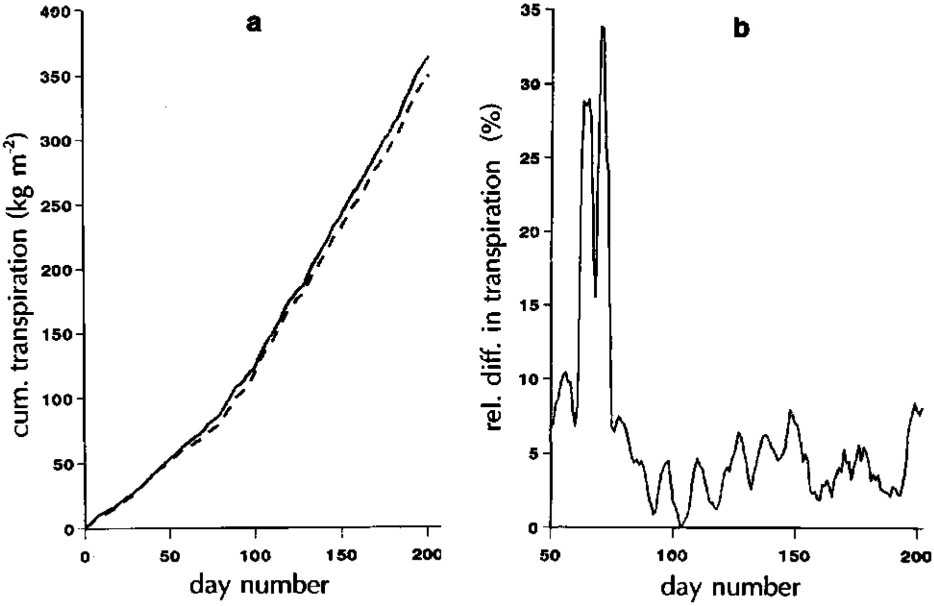

Fig. 3.15 a. *Cumulative transpiration (kg m^{-2}) of eggplant at low (——) and high (– – – –) CO_2 concentration as calculated with the Penman-Monteith equation, using eqn. 3.4p for leaf conductance.*

b. *Relative difference (%) in calculated transpiration between the crops under low and high CO_2 concentration (smoothed per week).*

In the P-M equation, parameter *g* actually indicates the canopy conductance, whereas here *g* of the upper leaves was implemented. In the other experiments (sections 3.4 and 3.5), the estimations by the P-M model with *g* of the upper leaves were reasonably in agreement with measured canopy transpiration rates. Some arguments for the applied approach were that the upper plant segment comprised by far the largest fraction of the total canopy transpiration (De Graaf, 1991). The transpiration estimations were used to explain the incidence of leaf tip chlorosis (section 4.6), which usually arises in the upper plant part. In addition, for considering relative differences in conductance, the upper leaves can be used without any problem.

3.7 SYNTHESIS AND DISCUSSION

3.7.1 Effects of CO_2 concentration on leaf conductance

In the present study, the stomatal response of sweet pepper, tomato, cucumber and eggplant was investigated under about equal ranges of conditions (although specific demands for temperature and nutrition were taken into account). The varieties used and the conditions applied were comparable to those in the commercial Dutch greenhouse industry. It is evident from the measurements (section 3.4 - 3.6) that leaf conductance strongly depends on radiation, but is also markedly influenced by C.

In two similar compartments, one with low and one with high C, the measured VPD-air was clearly higher at higher C, caused by a slight reduction in transpiration. Leaf conductance was lower, caused primarily by higher C (direct effect of C) and amplified by increased VPD-leaf-air (indirect effect of C, feedback). It must be emphasized that there was no humidity control. The overall change in *g*, brought about by direct and indirect C effects, can be called 'the apparent effects of CO_2 on leaf conductance'. In real greenhouse conditions, this apparent C effect can be considerable (**Fig. 3.3, 3.8** and **3.13**).

The real effect exclusively of C on *g* (derived from the regression equation accounting for PAR, VPD-leaf-air and C) was far smaller than this apparent effect: for sweet pepper, tomato and cucumber about 3 - 4% decrease in leaf conductance per 100 $\mu mol\ mol^{-1}$ increase in C and for eggplant about 11% per 100 $\mu mol\ mol^{-1}$ (in the range of about 200 to 1100 $\mu mol\ mol^{-1}$). **Fig. 3.16** demonstrates that the stomata of sweet pepper, tomato and cucumber are equally sensitive to C, whereas the stomata of eggplant responded more than three times stronger. It can be remarked that an exceptional stomatal response of eggplant in relation to drought stress was reported in the literature, but no physiological explanation was proposed (Behboudian, 1977; Daunay et al., 1986a,b; Srinivasa Rao & Bhatt, 1990).

No acclimation of the stomatal response to C could be established, when two plant stands of eggplant were compared, one continuously grown under low and one under high C. This confirms the results of Jones & Mansfield (1970) and Hicklenton & Jolliffe (1980).

3.7.2 Calculated effect of CO_2 concentration on transpiration (decoupling)

The impact of leaf conductance on canopy transpiration may be characterized by the coupling factor, *K* (Aubinet et al., 1989; eqn. 3.7). *K* is

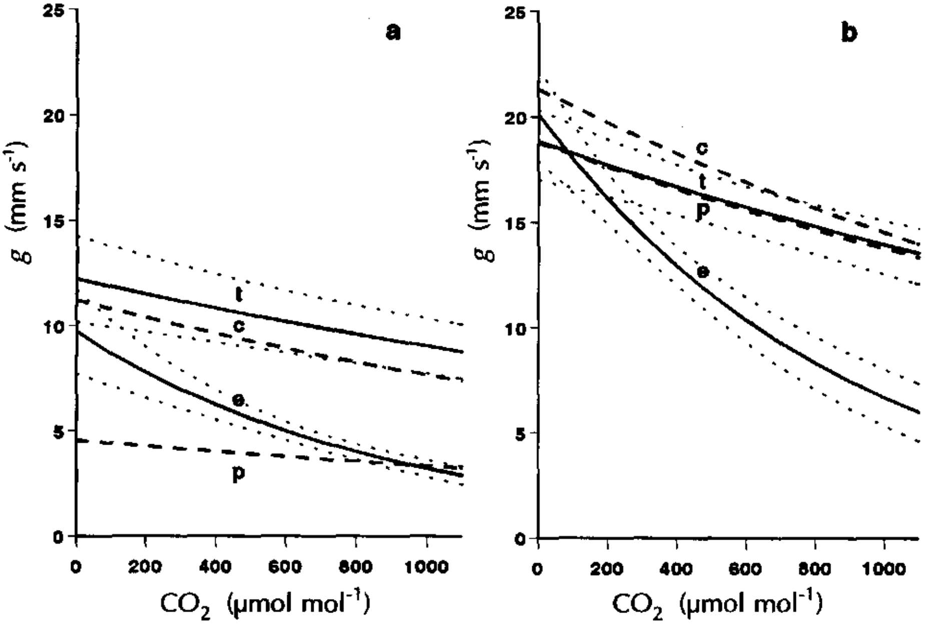

Fig. 3.16 *Calculated leaf conductance (g in mm s^{-1}) versus the CO_2 concentration (µmol mol^{-1}) for the four crops: t = tomato (eqn. 3.4n), c = cucumber (eqn. 3.4i), p = sweet pepper (eqn. 3.4d), e = eggplant (eqn. 3.4p). For tomato and eggplant the 95% confidence interval is given (.......). $VPD_{leaf\text{-}air}$ = 1 kPa.*

a. *with incident PAR = 100 µmol m^{-2} s^{-1}*

b. *with incident PAR = 1000 µmol m^{-2} s^{-1}.*

comparable to 1-Ω (Jarvis & McNaughton, 1986, eqn. 3.2), but *K* explicitly includes the feedback caused by adapted humidity of the greenhouse air. Some examples of calculated *K* were given in section 3.4.3 and 3.5.3 and **Table 3.8.** To demonstrate the limited effect of C on *E* in a more general form, *g*, *E* and *K* are calculated for several conditions. The environmental conditions are assumed constant, so the feedback effects are ignored. Conductance is calculated with a fitted regression equation for leaf conductance (eqn. 3.4i, for cucumber) and the concomitant value of transpiration with the Penman-Monteith model (eqn. 3.6), with g_b = 10 mm s^{-1}. E_p and K_{10} are presented in **Fig. 3.17** as function of *g*. To show the impact of g_b, *K* is also calculated for g_b = 20 mm s^{-1} (K_{20}). It appeared that *K* is independent of radiation and VPD, but is slightly dependent on temperature.

When the hydrologic and thermal feedbacks were taken into account, so the environmental conditions were not assumed constant, *g* would decrease more and *E* less, so *K* would be lower than in **Table 3.8** and **Fig. 3.17**. This occurs particularly at low ventilation, which is usually associated with low radiation and thus low *g*.

Considering that *K* in Fig. 3.17 is fairly overestimated at low *g*, it can be concluded that *K* (particularly K_{10}) is usually less than 0.5 and at higher radiation even less than 0.2. This implies that the decrease in canopy transpiration, caused by moderate CO_2 enrichment is in the order of some percents only, as the decrease in leaf conductance is 3-4% per 100 µmol mol^{-1} in most crops. This agrees with the measurements (**Fig. 3.3, 3.8** and **3.13**) and with the work of Stanghellini (1987) and of Jolliet & Bailey (1992), who found no significant effect of C on transpiration. On the contrary, Shaer & Van Bavel (1987) reported a 15-20% reduction in *E* for an increase in C of 330 to 1000 µmol mol^{-1}. However, their measurements were conducted on small plants in chambers, cooled with fans. In our full-grown canopies in large greenhouses with natural ventilation (i.e. low turbulence), the aerodynamic resistances will have been greater and the hydrologic feedback effect (adapted humidity of the greenhouse air) more pronounced. Both effects reduce the impact of C on *E*.

K is higher at low *g*, but decreases rapidly when *g* increases (**Fig. 3.17**). At low *g* (i.e. at low radiation), a considerable reduction of transpiration is not relevant with respect to leaf burning. When the low light conditions last more than several days, it might be harmful from the viewpoint of reduced nutrient transport, particularly in crops with a sensitive stomatal response to C (eggplant). Possible negative side effects of high C in eggplant are dealt with in section 4.6.

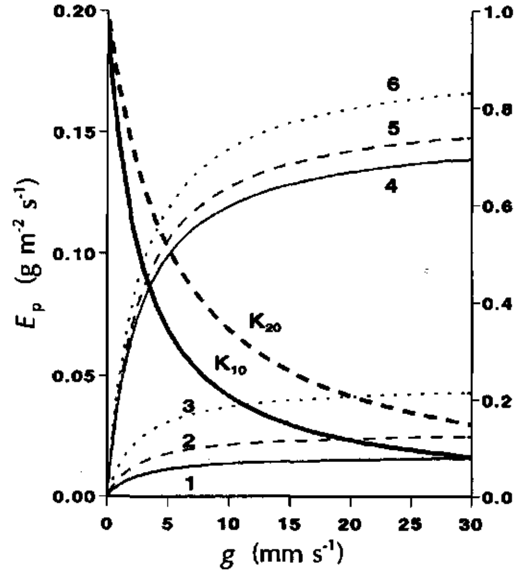

Fig. 3.17 *Calculated transpiration rate (E_p in g m^{-2} s^{-1}) and coupling factors (K, dimensionless) versus leaf conductance (g in mm s^{-1}), for various environmental conditions. K_{10} (-----) for g_b = 10 mm s^{-1} and K_{20} (- - - -) for g_b = 20 mm s^{-1}.*

3.7.3 Time-dependent variation in transpiration (midday depression)

Analogue to with photosynthesis (section 2.5), a midday closure of stomata (Meidner & Mansfield, 1968) might give a dip in the transpiration of a canopy. It was examined whether a midday depression, or more general a time-dependent variation, occurred in the observed transpiration. As with photosynthesis, this is done by comparing the measurements to a standard vale, obtained with a model that accounts for instantaneous effects of environmental factors, and not for an effect of time. For the three available data sets, the relative error (E_{meas} - E_{model}) / E_{model} is plotted versus time of the day (**Fig. 3.18**), similarly to Fig. 2.3 for photosynthesis. Fig. 3.18 shows no clear tendency or pattern that may indicate a time-dependent variation. The measurements were done only sometimes under real summer conditions. Notice that the light interception was only roughly approximated. It was neglected in the calculation, that the relative light interception is lower around noon than in the other hours (due to light falling on the path in the N-S oriented row-crop, Hand et al., 1993b). If this effect was considered, the curves would be higher around noon.

Finally, the absence of an endogenous depression do not mean that there is no decline or depression in the diurnal course. It may occur due to external conditions, e.g. deteriorated water supply (Kitano & Eguchi, 1993) etc. Recently, it was analyzed that canopy transpiration might change during the course of the day, through certain conditions that affect the boundary layer (Collatz et al., 1991).

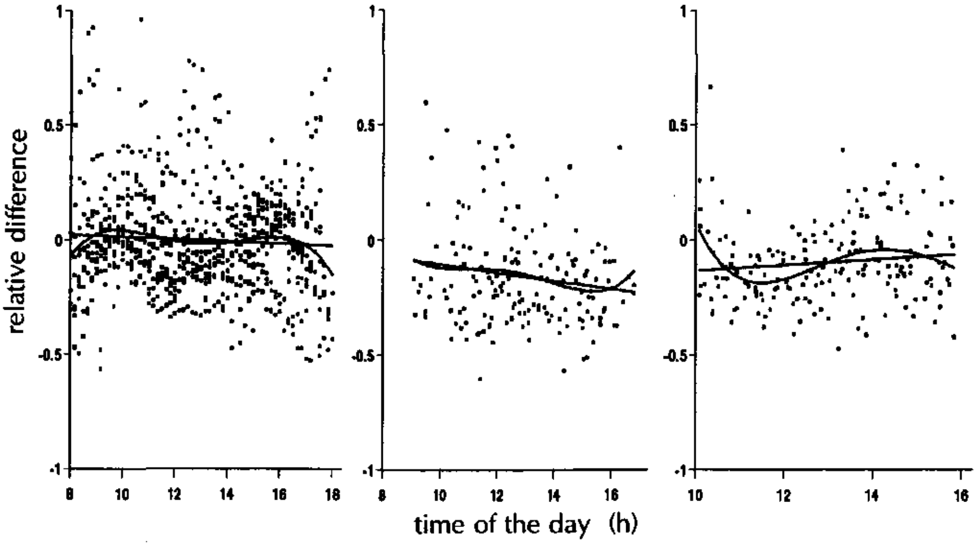

Fig. 3.18 *Relative difference in canopy transpiration as measured and calculated with the Penman-Monteith equation* [(E_{meas} - E_{model}) / E_{model}] *versus time of the day.*
a. *sweet pepper* **b.** *cucumber* **c.** *tomato*

3.7.4 Consequences of stomatal closure for leaf temperature

Partial stomata closure increase the resistance for diffusion of gases through the stomatal pore. The increased resistance for CO_2 diffusion to the stomatal cavities is largely compensated for by a strong positive effect of increased CO_2 concentration on CO_2 diffusion (eqn. 3.1). So the net result of elevated C is an increased CO_2 uptake (chapter 2).

Water vapour diffusion, however, is reduced, although this reduction is damped. So CO_2 enrichment reduces the efflux of latent heat and thus basically increases the leaf temperature (section 3.1.9). It is one of the aims of the present work to quantify the effect of C on the leaf temperature. Leaf temperature was measured by a build-in thermocouple in the porometer, which was calibrated previously by comparison with a separate, hand-held thermocouple on a great number of leaves. The available leaf temperature measurements, obtained in the conductance measurements, are analyzed here.

The observed leaf temperature was compared to the actual air temperature. The temperature difference between leaf and air ($\Delta T_{\ell\text{-}a}$) varied roughly between -1 and +2 °C, with a few extremes up to +3.5 °C. These results are comparable to ranges reported in the literature (Stanghellini, 1987; Collatz et al., 1991; Zhang & Lemeur; 1992; Grant & Baldocchi, 1992). However, the observation of $\Delta T_{\ell\text{-}a}$ was not accurate, because T_a was an average value of some sensors, all located on some distance of the leaves. An accurate investigation of $\Delta T_{\ell\text{-}a}$ itself, requires a refined method of measuring and analysis.

The measurements were considered sufficiently accurate, however, for the purpose of examining the effect of C on average leaf temperature. Therefore, the measurements of T_ℓ were exposed to a multiple regression equation, which accounts for the main factors: global radiation (Q_c), CO_2 concentration (C), dry bulb air temperature (T_a) and VPD of the air (D_a), as follows:

$$T_\ell = t_1 + t_2 * Q_c + t_3 * C + t_4 * T_a + t_5 * D_a \qquad \textbf{eqn. 3.8}$$

This equation was fitted to the data sets of sweet pepper, cucumber, tomato and eggplant (**Table 3.11**). For tomato and cucumber the large data sets were taken, including measurements from the east and west side of the rows.

Table 3.11 *Effects of some environmental factors on leaf temperature (in °C): fitted parameters t_1 to t_5 of the equation:* $\boldsymbol{T_\ell = t_1 + t_2 * Q_c + t_3 * C + t_4 * T_l + t_5 * D_a}$

	n	t_1	t_2	t_3	t_4	t_5	r^2
sweet pepper	116	-0.89	79E-5[a]	18E-5	1.04[a]	0.23	90.3
cucumber	299	3.50[a]	24E-4[a]	52E-5[a]	0.85[a]	-0.12[a]	75.7
tomato	373	7.36[a]	31E-4[a]	-6E-6	0.66[a]	0.03	83.9
eggplant	156	-3.64[a]	25E-5[a]	-75E-5[b]	0.83[a]	083[a]	90.9

[a] *significant at 1%* [b] *significant at 5%*

The percentage of variance explained (r^2) is high. The effect of C on T_ℓ expressed by parameter t_3, is statistically insignificant in two experiments, and in all experiments, the best estimates for t_3 are extremely low (**Table 3.11**). Because the air temperature has the main influence, the same regression analysis was also applied on $\Delta T_{\ell\text{-}a}$. This yielded a low r^2, but the results were similar: the influence of C on $\Delta T_{\ell\text{-}a}$ was negligible. So virtually no direct effect of C on leaf temperature was observed. In **Fig. 3.3, 3.8** and **3.13**, however, T_ℓ was higher at high C than at low C. This can be ascribed to increased air temperature and air VPD-air, as shown in the figures.

The conclusion is that the direct effect of C (in the range 200 - 1100 µmol mol^{-1}) on leaf temperature of a well watered greenhouse crop under normal conditions is negligible. This agrees with the findings of Stanghellini (1987). There might be an indirect effect, however, through adapted air temperature and humidity.

3.7.5 Approach and results considered

The experiments of the present chapter were undertaken, because it was not clear whether CO_2 enrichment markedly reduces the transpiration of greenhouse crops, as the literature was not consistent in this matter. The possible effect of C on transpiration is important because of the possible negative consequences, such as increased leaf temperature and reduced nutrient translocation. The value of this work is that the processes were studied under 'natural' greenhouse conditions, which makes that results and conclusions are valid for practical greenhouse horticulture.

The present work yielded a quantitative description of the effect of C on *g*. It was demonstrated that the apparent effect of C on *g* is based on a direct and an indirect effect of C. The indirect effect was caused by thermal and hydrological feedback.

The effect of C on *E* was studied by calculations with fictive data sets, using a derived regression equation for the effect of C on *g*, and using the Penman-Monteith equation for the effect of *g* on *E*. This yielded a generalized, qualitative insight in the effects of C on *E*. On the basis of these calculations and the described observations, it was concluded that C will normally not have a dramatic effect on *E* and that the consequences will be mostly not very serious. The effect of C on leaf temperature (a possible negative side effect) appeared to be very small. Reduced nutrition translocation (another possible negative side effect) will probably be serious only in eggplant or only under extreme conditions (chapter 4). These global conclusions were sufficiently to decide that a further, more thorough analysis of the transpiration processes was not necessary in view of the purpose of the study: evaluating the relation of partial stomata closure and possible negative side effects of high C.

4 Effects of CO_2 concentration on growth and production

4.1 INTRODUCTION

4.1.1 Outline of the problem and approach for research

Fruit production is the ultimate result of many processes and interferences. In section 1.4.2, a distinction was made between production-determining effects (main effects) and production-reducing effects (side effects) of the CO_2 concentration (C). The main production-determining effect of C (on canopy photosynthesis) was studied in chapter 2, and one side effect (partial stomata closure) in chapter 3. Another important side effect, viz. morphological adaptation to high C, is discussed in the present chapter. It also comprises an integration of the results of the previous chapters, as the overall effect of C on production is studied by analyzing the relevant underlying processes and considering the side effects of C. Thus this chapter deals with effects of C on:

1. dry matter (DM) production and growth rate
2. fruit set
3. allocation of DM to harvested organs (harvest index)
4. forming of fresh biomass, i.e dry matter content (DMC) of fruits
5. crop morphology, mainly leaf area and specific leaf area (SLA)
6. leaf nutrient concentration

Points 1 to 4 are production-determining effects and points 5 and 6 involve production-reducing side effects.

The present knowledge about effects of C on production-determining and production-reducing processes is briefly reviewed below. Also the concept of intermittent CO_2 supply (ICS) is explicated. At each topic, the approach for research is described. A topic is investigated sometimes only in one experiment, in the crop where it was expected to give the most pronounced response to C. Some results are only briefly presented in the next sections, and worked out in detail in the synthesis (section 4.7).

4.1.2 Growth rate (GR) and light use efficiency (LUE)

The basis of the production process is the canopy photosynthesis (P, chapter 2). After accounting for respiration, an amount of assimilates remains available for accumulation of the biomass, i.e. for growth. The absolute growth rate (GR) is the increment in total plant dry weight per day, mostly per m^2. P and hence GR are strongly dependent on light and C. To enable a comparison between various cultivations with respect to response to C, the effect of light is eliminated by dividing the increment in total dry weight by the intercepted light integral. This yields the average light use efficiency (LUE in g MJ^{-1}).

The approach in the present study was to determine GR and LUE and to relate them to the prevailing C (section 4.3.1 and 4.7.6). Only GR of above ground biomass was considered. The intercepted light integral was estimated from the daily integrals of global radiation measured outside, multiplied by the light transmission coefficient of the particular greenhouse (about 0.7), multiplied by the share of PAR in the global radiation (assumed 0.5) and multiplied by the interception factor ($1- e^{-k * LAI}$, with k = 0.7, Monsi & Saeki, 1953).

4.1.3 Fruit set

The number of fruits obtained on a plant depends on flowering and fruit set. Flowering usually does not limit the production in the crops investigated: many crops, except tomato, have inflorescences in abundance compared to the number of fruits that are ultimately set and produced (cucumber: Drews, 1979; Marcelis, 1992; sweet pepper: Rylski, 1986). Fruit set may limit the production. Fruit set and growth of young fruits may be hampered by limitation of assimilate availability (Calvert & Slack, 1975; Acock & Pasternak, 1986), which also depends on C. Slack (1986) reviewed some experiments where CO_2 supply increased fruit set of tomato under poor light conditions. Imazu et al. (1967) observed increased fruit set in eggplant under high C.

The effect of C on fruit set was investigated in an autumn cultivation of sweet pepper, because in such a cultivation (with planting date in the Netherlands from end of June to August), fruit set and yield can be relatively poor, compared to that of a spring crop. The idea was that under dull weather conditions in this season, the CO_2 concentration may be low, caused by insufficient air exchange to supply the CO_2 necessary for a full-grown crop. It was investigated (section 4.4) whether the number of fruits and total fruit weight production of autumn sweet pepper are improved by CO_2 enrichment.

4.1.4 Allocation of dry matter

The figures for DM allocation to fruits show a large variation. Sometimes figures in the order of 90 to 95% are mentioned for tomato, sweet pepper and eggplant (Claussen, 1976; Hall, 1977; Hurd et al., 1979; Nielsen & Veierskov, 1988), whereas Bhatt & Srinivasa Rao (1989) gave a figure as low as 35% in sweet pepper. In an extended study on tomato, Heuvelink (1995b) observed that after about 100 days 54-60% of the DM was distributed to the fruits in a spring cultivation. In a late autumn planting, it was far less due to poor fruit set. The share of the fruits in the total cumulative DM increases as the cultivation progresses (Rijsdijk et al., 1993a). These authors found the following values for fruit partitioning in almost year-round cultivations: sweet pepper 61-70, tomato 70-72, eggplant 72, and for cucumber in shorter cultivations 67-75%. Marcelis (1992) found for cucumber in the reproductive stage 67%. Comparable figures were reported for tomato over a long season, e.g. 72% (Hurd et al., 1979; De Koning, 1993) and 69% (Cockshull et al., 1992).

The allocation of DM depends on many factors, e.g. plant spacing (Widders & Price, 1989; Papadopoulos & Ormrod, 1990), light (Cockshull et al., 1992; Marcelis, 1993b), temperature (Liebig, 1978; Marcelis, 1993a), fruit load (Marcelis, 1993a), but not much is known about an effect of CO_2 (Acock & Pasternak, 1986; Farrar & Williams, 1991). An increased allocation to the reproductive organs at high C was observed in soybean (Clough & Peet, 1981).

High C stimulates P, which is a direct effect of C on source. It is demonstrated by several authors that source does not influence the DM allocation, but that sink strength appears to regulate the DM allocation (Gifford & Evans, 1981; Schapendonk & Brouwer, 1984; Farrar & Williams, 1991; Marcelis, 1993a,b). Therefore no direct effect of C on DM allocation was expected.

The hypothesis tested here is that C has no direct effect, but may have indirect effects on DM allocation via an influence of C on the number of fruits (see previous section) and thus on sink strength. The approach was to manipulate both sink and source (by fruit pruning and CO_2 supply, respectively) and to determine the relative contribution of the plant segments (i.e. leaves, stems, fruits) in the total above-ground plant dry weight gain. It was examined whether C affects the DM allocation in normally cultivated and in sink-limited plants (section 4.3.1 and 4.7.8).

4.1.5 Fruit properties

Fruit dry matter content

CO_2 enrichment enhances production in virtually all crops, owing to enhanced carbon assimilation. Increase of fruit production is achieved either by increase of the number of fruits, or by increased average fruit weight, or by both. It depends on the fruit load, influenced by fruit setting and fruit pruning, which of these effects is most pronounced. It is important in this respect to distinguish between fresh weight and dry weight fruit production. It is theoretically possible that a higher fruit DM production in combination with a higher fruit DMC results in a reduced fresh weight fruit production. It is generally accepted that C does not affect fruit DMC (Idso & Kimball, 1988; Slack et al., 1988; Ho & Grimbly, 1990).

The approach in the present experiments was to record total fresh fruit weight, average fruit weight and fruit DMC at every harvest, and to relate the results to C.

Fruit quality

The quality of the marketed products is extremely important in present-day horticulture. Little is known about the effects of CO_2 enrichment during cultivation on the quality of fruits. Some properties of tomato fruits have been investigated in CO_2 experiments, and mostly no effect at all or only small, statistically insignificant effects were found. Davies & Winsor (1967) observed no difference in reducing sugar and total solids content in tomato fruits of CO_2 enriched and non-enriched plants. Kretchman & Bauerle (1971) reported a small decrease in percentage off-colour fruits and fruits with cracks under high C, and a small increase in percentage first class tomato fruits. The same authors saw no significant differences in fruit size, puffiness (hollowness), shape and average number of defects per tomato fruit. Nilsen et al. (1983) observed no significant difference in tomato fruit quality under two CO_2 concentrations. Slack et al. (1988) found no effect of C on concentration of reducing sugar and firmness (compressibility) of tomato fruits. In the present work, the most relevant quality characteristics of fruits were considered in relation to C.

4.1.6 Morphological adaptations to high CO_2

Various effects of long-term high C on crop morphology have been reported, mainly with respect to leaf morphology. In young crops, high C may increase leaf area as a direct result of increased photosynthesis, e.g. in young cucumber plants (Aoki & Yabuki, 1977). In soybean a clear decrease of leaf area under high C was observed (Acock & Pasternak, 1986). The leaf area depends on the leaf weight per plant and on the SLA (in cm^2 g^{-1} dry weight). Many authors observed under high C a considerable increased leaf dry weight, accompanied by a slightly increased leaf area, implying a reduced SLA (Madsen, 1976; Clough & Peet, 1981; Frydrych, 1984; Jolliffe & Ehret, 1984; Goudriaan & Bijlsma, 1987).

Morphological adaptations in the form of reduced SLA appear to be extremely serious in tomato, particularly in summer (Van Velden, 1990). The tomato leaves are then short, thick, stiff, curled and somewhat crisp, grey-green to purple coloured, sometimes with necrotic spots and leaf tips (**Fig. 4.4**). Growers describe the phenomenon as 'short leaves' and as this is a good characterization, the term 'short leaves syndrome' (SLS) is used here.

It was investigated whether SLS or reduced SLA in tomato is associated with a long-lasting surplus of assimilates, i.e. a low sink/source ratio. In an experiment (section 4.5), an attempt was made to induce SLS and to compare the effect of C on production in a sound crop and in an SLS affected crop. The approach was to manipulate the sink/source ratio by leaf pruning, fruit pruning, plant density and CO_2 supply.

4.1.7 Nutrient concentration in leaves

Another possible side effect of CO_2 enrichment is reduction of the nutrient concentration in plants. Two mechanisms may play a role. In the first place, high C causes a reduction in stomatal opening and hence a (small) reduction in rate of transpiration (section 3.6.2), with a reduced translocation of nutrients as possible consequence. Internal mechanisms to compensate for reduced passive nutrient transport are redistribution of nutrients via the phloem and increased, selective uptake of necessary elements. As far as known, the elements calcium and boron lack both mechanisms (Mengel & Kirkby, 1978). So the availability of Ca and B for growing plant organs (leaves, buds, apex and fruits) is strongly related to the transpiration rate, although recent studies show that Ca is not solely dependent on passive mass flow (Atkinson, 1992).

Low transpiration may induce calcium deficiency (Bangerth, 1979; Adams, 1986; Ehret & Ho, 1986; Bakker & Sonneveld, 1988; Holder & Cockshull, 1990) or boron deficiency (Von Michael et al., 1969). Certain similarities were noticed between Ca and B, regarding their mobility and visible symptoms of deficiency (Marschner, 1986), and certain interactions between B and Ca were observed in eggplant (Jyothi & Shanmugavelu, 1985).

Secondly, high C stimulates the DM production, and hence CO_2 enrichment increases the requirement of nutrients. If the uptake of nutrients is not increased to the same extent as the assimilate production, the nutrient concentration in the DM decreases (Porter & Grodzinski, 1984; Eng et al., 1985; Kuehny et al., 1991), which is often called a 'diluting effect'. The combination of increased DM production and reduced Ca translocation owing to high C, may be expected to cause a fall in the concentrations of some nutrients, particularly in rapidly growing, non-transpiring organs. Indeed many authors observed at high C, a lower content of nutrients, mostly calcium, nitrogen and magnesium (Peet et al., 1986; Yelle et al., 1987; Peñuelas & Matamala, 1990; Peet & Kroen, 1991; Tripp et al., 1991a).

The effect of C on nutrient concentrations in plant organs was investigated with eggplant (section 4.6), because the stomatal response of eggplant is stronger than that of other crops (section 3.7.1). In addition, eggplant suffers sometimes from leaf tip chlorosis (**Fig. 4.6**), which appeared to be more severe under high C (Buitelaar, 1991).

It was investigated whether high C in eggplant induces leaf tip chlorosis (LTC) and whether this is associated with leaf boron deficiency. The effect of high C on fruit production was considered (section 4.6).

4.1.8 Intermittent CO_2 supply (ICS)

In the eighties, the concept of intermittent CO_2 supply (ICS) was proposed as a method, which enabled plants to take advantage of CO_2 enrichment, while preventing (negative) adaptations to long-term high C. ICS means that CO_2 is supplied in short periodical pulses (Clough & Peet, 1981; Enoch, 1984; Mortensen, 1984). More recently, Dugal et al. (1990) found positive effects of ICS on photosynthesis of young tomato seedlings. According to the work of Enoch (1984), Zipori et al. (1986) supplied CO_2 intermittently and also operated the ventilation windows intermittently, in order to increase the CO_2 use efficiency. Only while the ventilators were temporarily closed, CO_2 was to be supplied and when the windows had to be opened to prevent excessive temperatures, the supply was to be stopped.

In the present study, two experiments were conducted, where ICS was compared to CO_2 supply to a constant concentration (sections 4.3.2 and 4.4). However, ventilation was not done intermittently, because this would affect the average temperature and humidity, and would unnecessarily complicate the analysis of the results. Intermittent ventilation was considered no practical option, due to wearing off of the ventilation installation and moreover, because reduced ventilation may deteriorate the fruit quality: Slack et al. (1988) found that delaying the ventilation in favour of CO_2 enrichment reduced the fruit quality so much that the financial loss exceeded the saving in CO_2 and the increase in production, even in a temperate climate. So ventilation was done in both treatments as normally, i.e. on the basis of greenhouse air temperature.

4.2 OBJECTIVES AND EXPERIMENTS

4.2.1 Objectives

The objective of the present chapter is to analyze and quantify the effects of C on growth and production of greenhouse fruit vegetable crops under normal operating conditions, by investigating the effects of C on underlying processes, i.e. growth rate, fruit set, dry matter allocation, dry matter content, crop morphology and leaf nutrient content.

4.2.2 Experiments

The following experiments were carried out, which are presented in this chapter according to species:

- two experiments with cucumber, dealing with effects of C on growth and fruit production. In the second experiment, the effects of intermittent and constant level CO_2 enrichment were compared (section 4.3).
- an experiment on effects of C on fruit set of sweet pepper, and again on intermittent CO_2 supply (section 4.4).
- morphological adaptation to high C in tomato (SLS). It was investigated whether C has an effect on SLS and whether the effect of C on production is different in an SLS-affected crop and in a sound crop (section 4.5).
- an experiment on the occurrence of a form of leaf chlorisis (LTC) in eggplant in relation to C and leaf boron content (section 4.6).

In all experiments, the relevant growth and production parameters were recorded, to quantify the effects of C on underlying processes (section 4.7).

All experiments were done in medium-size, semi-commercial greenhouses under 'normal' greenhouse operating conditions, with sufficient capacity for CO_2 supply and with appropriate CO_2 control. A facility was present to scrub (filter) the greenhouse air, to maintain a low C.

The work is restricted to effects of C in the production phase, to avoid unnecessary complications in the analysis of the results. Seedlings and young, vegetative plants respond to C by forming more leaves (by weight and area) (Klapwijk & Wubben, 1984; Aoki & Yabuki, 1977). This would lead to a large photosynthetic apparatus in the CO_2 enriched crop at the start of the production phase. Therefore, the CO_2 treatments were started only when the (estimated) leaf area index exceeded 1. The time between planting and start of the CO_2 treatments was at least 2 weeks in cucumber and in summer planted sweet pepper, and at least 4 weeks in the tomato and eggplant experiment.

Observations were done only on above-ground organs. The omissions of root weight in the observations was assumed to induce no great errors. Marcelis (1992) observed that the root weight at the end of an experiment was only 2.6% of the final, accumulated plant dry weight produced.

4.3 EFFECTS OF CONTINUOUS AND INTERMITTENT CO_2 SUPPLY ON GROWTH AND PRODUCTION OF CUCUMBER

4.3.1 Growth and production of cucumber at two CO_2 concentrations

(cucumber experiment 1)

Objectives

High C increases the growth rate (GR) and light use efficiency (LUE, section 4.1.2). It is questioned whether C has an effect on dry matter allocation (section 4.1.4). Based on the literature, it is expected that source strength (availability of assimilates) has no effect per se on dry matter allocation, whereas sink strength (number of fruits) can have an effect.

An experiment was conducted to obtain information about the effects of C on GR and LUE, vegetative and generative (i.e. reproductive) production and on the allocation of assimilates in cucumber. Besides two levels of C, also two levels of fruit pruning were applied, to get a better insight into the effects of changes in source and in sink on dry matter allocation.

Experimental set-up and facilities

Cucumber plants (cv. Aramon) were planted on rockwool on 14 July 1993 in six Venlo-type greenhouse compartments, at a density of 1.35 plants per m^2. From 28 July onward, two levels of C were maintained: three compartments were controlled at 350 and three at 700 $\mu mol\ mol^{-1}$ by day. In each compartment, the plant stand was divided into two experimental plots, one plot had the normal fruit load, while in the other plot ⅔ of the inflorescences and young fruits were removed. The harvest started on 4 August and the experiment was ended on 19 October. On 24 August and 14 September some older leaves were pruned away to prevent fungus diseases. The removed leaves were weighed per plot.

Pure CO_2 was supplied automatically through distribution tubes on the soil. C was measured with calibrated infra-red gas analyzers (Ultramat, Siemens, Munich, Germany). Eight greenhouse compartments were connected to one analyzer by means of a multiplexer, which resulted in a measuring cycle of 8 min. In this experiment the CO_2 concentration, fruit production, fruit DMC and vegetative growth were observed. Three days a week fruits were harvested, counted and weighed; second class fruits were weighed. Occasionally the fruit DMC was determined on samples of whole fruits by weighing, drying (48 h at 80 °C in a forced ventilation oven) and weighing again. Vegetative growth was observed regularly (every 2 or 4 weeks) by harvesting 3 plants per experimental plot and determining the number of leaves and shoots, leaf area, fresh and dry weights of leaves and stems.

Table 4.1 *Main plant characteristics of cucumber expt 1, on two observation dates. wt = weight, av = average, fr = fruit.*

treatment:	unpruned		fruit-pruned		$LSD_{0.05}$
CO_2:	low	high	low	high	
(µmol mol^{-1})	(364)	(620)	(364)	(620)	
a. 26 August					
number of leaves (m^{-2})	57.1	56.6	84.5	87.7	13.2
number of shoots (m^{-2})	2.7	2.4	3.6	3.6	0.7
total shoot length (m m^{-2})	2.1	2.0	3.9	3.8	0.9
dry wt leaves (g m^{-2})	98.8	103.9	125.8	135.9	5.2
dry wt pruned leaves [a] (g m^{-2})	11.1	14.6	11.9	19.2	2.9
dry wt stems (g m^{-2})	67.5	72.1	92.8	109.0	5.9
DMC leaves (%)	12.7	13.1	12.4	12.8	0.5
DMC stems (%)	6.1	6.3	6.2	6.5	0.3
DMC fruits, 30 Aug (%)	3.9	4.0	3.9	3.8	0.25
av fruit wt (11-25 Aug) (g)	0.59	0.65	0.69	0.74	0.05
LAI	3.1	3.0	4.1	3.8	0.9
SLA (cm^2 g^{-1} dry weight)	315	289	323	283	53
LAR (cm^2 g^{-1} dry weight)	187	170	186	156	30
b. 19 October					
number of leaves (m^{-2})	110	109	122	129	29
number of shoots (m^{-2})	7.1	7.8	7.1	10.0	1.9
total shoot length (m m^{-2})	5.5	6.3	6.1	8.3	1.9
dry wt leaves (g m^{-2})	114	98	120	129	5.8
dry wt pruned leaves [b] (g m^{-2})	23.9	38.5	36.2	48.6	-
dry wt stems (g m^{-2})	103	104	113	146	10.5
DMC leaves (%)	14.3	13.9	14.2	13.7	0.3
DMC stems (%)	6.9	7.6	6.9	7.5	1.0
DMC fruits, 14 Oct (%)	3.7	3.5	3.5	3.4	0.4
av fr wt (20 Sep-21 Oct) (g)	0.43	0.46	0.45	0.44	0.06
LAI	3.3	3.0	3.7	3.9	0.2
SLA (cm^2 g^{-1} dry weight)	293	303	304	298	9.3
LAR (cm^2 g^{-1} dry weight)	153	147	157	140	10.6

[a] *leaf pruning of 24 August* [b] *leaf pruning of 14 September*

Results

The achieved CO_2 concentrations were on average 364 and 620 µmol mol^{-1} for low and high C, respectively, over the period 28 July - 19 October (**Fig. 4.1a**). On 12 August, four weeks after planting and two weeks after the start of CO_2 supply, the CO_2 enriched plants were clearly larger than

4.3.2 Production of cucumber under continuous or intermittent CO_2 (cucumber experiment 2)

Objective

The objective of this experiment is to test the practical value of intermittent CO_2 supply (ICS, section 4.1.8) in cucumber. Two treatments with intermittent CO_2 supply were compared with two constant CO_2 concentrations, with respect to their effects on cucumber fruit production and on CO_2 expenditure. In order to investigate just the effects of C, ventilation was done equally in all treatments, so ICS was not combined with intermittent ventilation, as proposed by Enoch (1984).

Experimental set-up

Four C treatments were applied in duplicate. The two constant level treatments had a set point of 300 and 450 µmol mol^{-1}, respectively. In the ICS treatments 8 min CO_2 supply was followed by a period of 82 or 172 min without enrichment. The CO_2 treatments are denoted as '300', '450', '8/90' and '8/180'. CO_2 was given only by day. C was controlled automatically by supply of pure CO_2 through a distribution network of plastic tubes, 3.2 m apart, on the soil. In the low C treatment, scrubbing (filtering) was applied. The scrubber consisted of a chemical filter (sodasorb, $NaOH + Ca(OH)_2$), provided with a ventilator system, placed in the greenhouse. The ventilator was switched on/off, depending on C (Heij & Van Uffelen, 1984). The amount of CO_2 supplied in 8 min was controlled by a solenoid valve, with the opening time (seconds per minute) proportional to the difference between the actual C and 500 µmol mol^{-1}. In this way, the CO_2 valve was opened the full 8 min per cycle during much ventilation, and proportionally shorter if ventilation was less. C was measured with commercially available infra-red gas analyzers (Siemens, Munich, Germany), which were calibrated frequently. Each meter measured one series of C treatments, with a measuring cycle of 8 min.

Crop and facilities

In eight compartments (9.6 m x 6 m) of a Venlo-type glasshouse complex, cucumber (cv. Lucinde) was planted in soil on 14 January 1986; the C treatments started on 29 January; the first harvest was on 27 February and the experiment was ended on 23 June. Plant density was 1.35 plants per m^2, with four rows per 3.2 m. Regularly some leaf pruning was applied, which was a normal measure in a dense canopy to prevent fungus diseases and to improve the quality of the fruits by increasing the irradiance on the fruits.

The environmental conditions maintained in the experimental greenhouse were similar to those in commercial greenhouses and were set equal in all compartments (except for CO_2). The environmental factors were controlled by a mini computer. Day temperature for heating was 23 and night temperature 20 °C initially. Ventilation started at 26 °C. The air temperature was measured every minute with a screened, aspired thermocouple in the middle of each compartment. The plants were irrigated twice a week and fertilized when necessary.

Observed were C and fruit production. In addition, in this experiment also the CO_2 expenditure was recorded as relative flow rate multiplied by the time in seconds that the CO_2 valve in a compartment was opened. The recordings were summed and then expressed in relative units.

Table 4.3 *Results of cucumber expt 2 with four CO_2 concentrations.*
av = average, cum = cumulative. '300' refers to correction for scrubbing (see text)*

CO_2 treatment	'300*'	'450'	'8/90'	'8/180'	$LSD_{0.05}$
av. CO_2 concentration (μmol mol^{-1})	318	459	379	355	28
relative CO_2 expenditure (%)	100	1180	360	190	200
relative cum. production (%)	100	129	110	108	2.1

Results and discussion

The C treatments always showed clear differences in achieved C (**Fig. 4.2a** and **Table 4.3**). The amount of carbon dioxide spent was recorded in relative figures (**Table 4.3**). Because at treatment '300' the scrubbers had filtered out carbon dioxide gas, a correction was necessary to make this treatment suitable as reference. It is obvious that without scrubbing, C in the treatment '300' would have been higher during a number of hours per day (indicated by '300*') and hence the production would have been higher. A correction was made, based on two assumptions: that the average C in the low C treatment would have been 20 μmol mol^{-1} higher if no scrubbing was applied (an even higher value would have been realistic too, see control treatment in section 4.4.3) and secondly that C has a linear effect on production in this small range. The thus corrected relative production figures are given in **Table 4.3**. The increase in production caused by ICS treatment '8/90' compared to '300*' was about ⅓ of the increase caused by continuously high C. About the same ratio was observed for CO_2 expenditure.

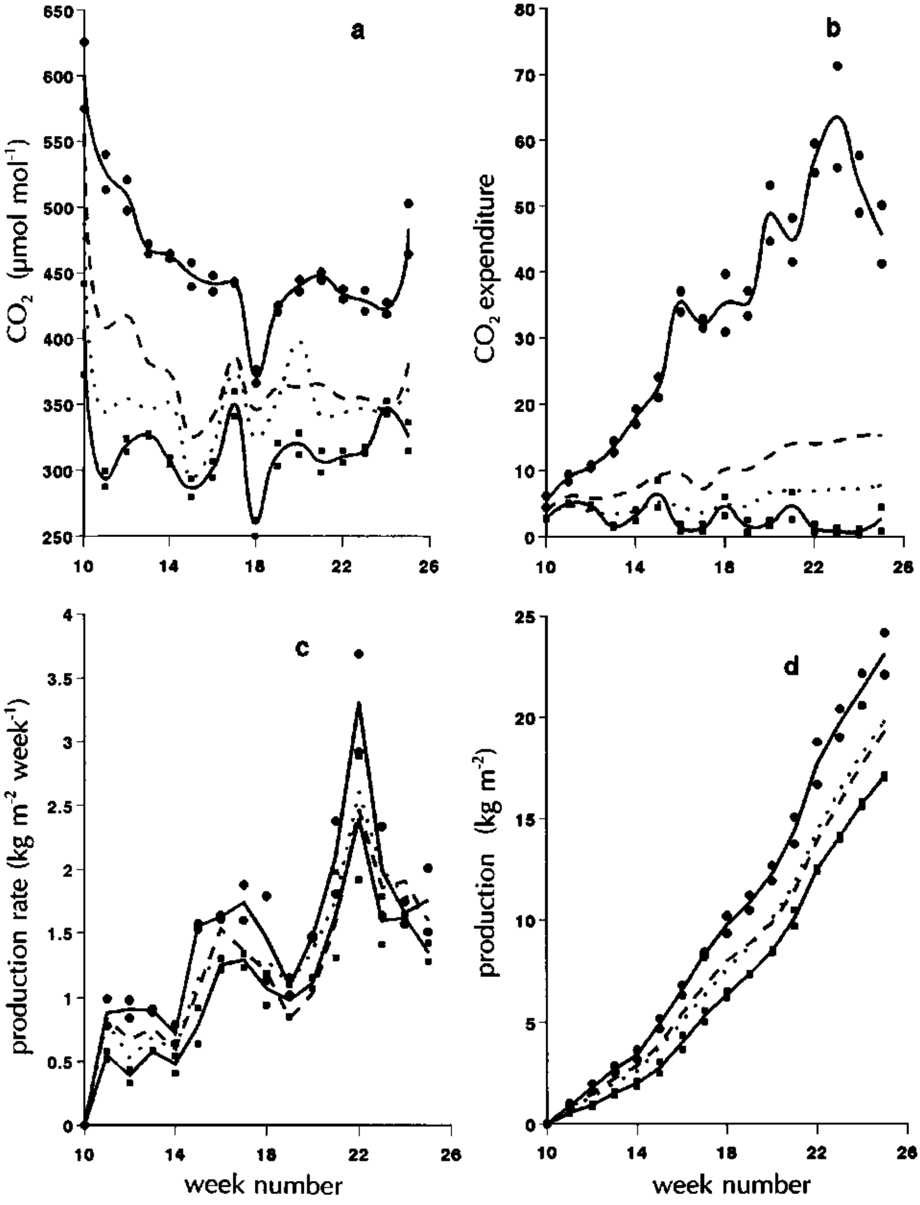

Fig. 4.2 *Results of cucumber expt 2. Points are individual observations and lines represent mean of two replicates. Results are not corrected for effect of scrubbing. CO$_2$ treatments: —■— '300'; —•— '450'; - - - - '8/90' ; . . . . '8/180'.*

a. *weekly averages of CO$_2$ concentration (µmol mol^{-1}, 10.00-16.00 h)*

b. *CO$_2$ expenditure in relative figures*

c. *rate of fruit production (kg m^{-2} week^{-1})*

d. *cumulative fruit production (kg m^{-2})*

The differences between the two ICS treatments were not statistically significant (**Table 4.3**), but treatment '8/180' seemed more efficient, because the CO_2 expenditure was 1/6 and the production 1/4 of that at high C. However, if treatment '300' was corrected by 30 µmol mol^{-1} (which was also realistic) the apparent advantage of treatment '8/180' disappeared.

These results imply that the effect of C is about proportional to the amount of C supplied, which findings agree with the literature: if 'partial' or 'fractional' enrichment was compared to constant level supply, the fruit production of cucumber was linearly related to C (Slack & Hand, 1985) or to the enrichment time (Willits & Peet, 1989).

4.4 EFFECTS OF CONTINUOUS AND INTERMITTENT CO_2 SUPPLY ON FRUIT SET AND YIELD OF SWEET PEPPER

E.M. Nederhoff & J.A.M. van Uffelen, 1988, Netherlands Journal of Agricultural Science 36: 209-217.

4.4.1 Introduction

In an autumn cultivation of sweet pepper (with planting date in the Netherlands from end of June to August), fruit set and yield can be relatively poor compared to those of a spring crop. Fruit set is especially problematic during periods of relatively warm, calm or dull weather, which are not unusual in autumn. In such periods, the air influx from outside is low, due to the calm conditions and CO_2 supply is sometimes sparse, e.g. in greenhouses with combustion gas enrichment and with limited heat storage.

The hypothesis is that the poor fruit set under such conditions is caused by limited assimilate availability (section 4.1.3), due to low C in the greenhouse air. The objective of the experiment was to investigate whether number of fruits and total fruit weight production of autumn sweet pepper are improved by CO_2 enrichment. In addition, the effects of intermittent CO_2 supply (ICS) and enrichment to a constant level were compared (section 4.1.8).

4.4.2 Materials and methods

Experimental set-up and facilities

The experiment was carried out in twelve compartments of a Venlo-type greenhouse of 9.6 m x 6 m each. Sweet pepper (cv. Bolero) was planted on 15 July 1985 in soil, at a density of 2.5 plants per m^2. Carbon dioxide enrichment started on 1 August. Harvesting started on 1 October and was finished on 19 November. The young plants were initially kept vegetative by weekly defloration/defruiting (removing all set flowers and young fruits). To examine the C effect on fruit set under various weather types (including unfavourable conditions, see section 4.1.3), fruit set was allowed for different groups of plants on different dates: 5, 13, 21 and 30 August (each compartment had the same four plots with fruit set dates in duplicate). As always in sweet pepper cultivation, the lateral shoots were topped in a young phase, to stop them growing. In all treatments a small and about equal amount of biomass was removed in this way.

The greenhouse climate was computer controlled. Except for C, the environmental conditions maintained in the experimental greenhouse were equal in all compartments. Set point for heating was 21 by day and 15 °C by night and ventilating started 2 °C above the heating set point. This relatively wide dead zone was applied in order to diminish (unnecessary) CO_2 losses. During the whole period, a small ventilation (minimum of 5 % of the window opening) was maintained continuously.

Carbon dioxide treatments

In the experiment the following six C treatments were applied in duplicate: continuous C of 250, 350 and 450 μmol mol^{-1} ('250', '350' and '450') and intermittent CO_2 supply (ICS) with one CO_2 pulse of 8 min per 40 min ('8/40') or per 104 min ('8/104') and a control treatment, without supply or filtering. C was controlled during day time only, by supply of pure CO_2 and in the treatment '250' by scrubbing (section 4.3.2). In the ICS treatments, the flux of CO_2 was adapted by hand to the average ventilation, so that in eight minutes the concentration rose to about 400 to 800 μmol mol^{-1} (depending on ventilation). C was measured with a commercially available CO_2 analyzer (Siemens, Munich, Germany), which was calibrated frequently. Eight compartments were measured by one device, resulting in a measuring cycle of eight min.

Observations

The relevant environmental conditions were recorded on a one min basis (only C once per 8 min). Observations on the crop were:

- fruit set: removed set flowers were counted on 13, 21 and 29 August
- yield: every week the ripe, red fruits were harvested, weighed and counted. At the end of the season (19 November) all mature fruits were harvested (mature green or red/green fruits separated from the red ones)
- vegetative plant parameters: on five dates two plants per compartment were sampled. Number of leaves, leaf area, fresh and dry weights of leaves and stems were recorded.

4.4.3 Results

Table 4.4 presents the average, measured C during daylight hours. It was not possible to maintain the low set point properly at 250 μmol mol^{-1}, due to the high ventilation in the autumn period and the limited capacity of the CO_2 filtering installations. Unexpectedly, the average C in the control treatment was high (344 μmol mol^{-1}), especially in early August. This can be explained by CO2 release from the soil, limited uptake by the young crop and mainly by influx of CO_2 from the environment during ventilation. In this region, close to a large industrial area, the CO_2 concentration of the outside air is regularly high.

Table 4.4 *CO_2 concentration (μmol mol^{-1}) in the sweet pepper experiment, measured during daylight hours, averaged over two replicates.*

date	continuous CO_2			intermittent		control	LSD
	'250'	'350'	'450'	'8/40'	'8/104'		$P<0.05$
04-12 Aug	329	325	431	378	387	371	58.2
13-20 Aug	296	333	462	461	399	337	36.7
21-29 Aug	310	332	458	464	398	327	38.2
01-31 Aug	314	332	450	428	396	350	38.6
01-30 Sept	271	331	453	476	423	332	26.0
01-31 Oct	299	345	458	478	442	341	18.5
01-15 Nov	343	379	432	432	411	353	48.5
1 Aug-15 Nov	306	346	448	453	418	344	27.5

Table 4.5 *Vegetative plant parameters of sweet pepper, at 22 October in six CO_2 treatments, averaged over two plants per compartment and over two compartments. Leaf area in m^2 / plant; fresh weights (fre wt) and dry weights (dry wt) in g / plant; DMC in %; SLA and LAR in cm^2 g^{-1} dry weight.*

	continuous CO_2 (μmol mol^{-1})				intermittent		control	LSD[b]
C treatment	'250'	'350'	'450'	ANOVA[a]	'8/40'	'8/104'		$P<0.05$
average C	306	346	448		453	418	344	
# of leaves	204.8	173.3	159.5	l.s.	200.3	171.8	163.8	n.s.
leaf area	1.308	1.108	0.996	l.s.	1.134	1.091	0.948	n.s.
fre wt lvs	289.0	247.3	235.5	n.s.	259.0	239.9	216.8	n.s.
fre wt st	400.3	325.3	328.8	n.s.	396.0	340.3	324.5	n.s.
dry wt lvs	40.2	36.3	35.8	n.s.	39.8	34.3	32.9	n.s.
dry wt st	51.3	45.8	45.6	n.s.	55.3	44.5	46.2	n.s.
DMC leaves	13.9	14.7	15.2	l.s.	15.3	14.3	15.2	0.95
DMC stems	12.8	14.1	13.9	n.s.	13.9	13.1	14.2	n.s.
SLA	326.0	305.0	278.1	l.s.	285.7	318.6	288.3	20.4
LAR	143.7	135.2	122.3	l.s.	120.3	138.6	120.0	15.2

[a] *ANOVA: analysis of variance on the differences between continuous CO_2 levels: l.s. = linear component significant, n.s. = no significance at $P < 0.05$.*

[b] *least significant difference, calculated over all treatments.*

Table 4.6 *Fruit set of sweet pepper (number of removed fruits per m^2) at six CO_2 treatments, at three dates of defloration/defruiting, averaged over two compartments.*

	continuous CO_2			intermittent		control	LSD[b]
C treatment	'250'	'350'	'450'	'8/40'	'8/104'		$P<0.05$
average C	306	346	448	453	418	344	
non-cumulative							
13 Aug.	5.8	8.8	13.3	7.8	8.9	9.0	n.s.
21 Aug.	13.5	18.3	26.0	22.8	19.7	16.8	6.2
30 Aug.	8.0	11.6	15.9	17.8	12.9	9.8	n.s.
cumulative							
21 Aug.	19.3	27.2	39.4	30.6	28.6	25.8	10.5
30 Aug.	27.3	38.8	55.3	48.4	41.5	35.6	17.2

[b] *least significant difference, calculated over all treatments*

Table 4.7 **a.** *Cumulative fruit production of sweet pepper (number of red fruits per m^2) at six CO_2 treatments, averaged over two compartments.*
b. *average fruit weight (g)*

	continuous CO_2			intermittent		control	LSD[b]
C treatment	'250'	'350'	'450'	'8/40'	'8/104'		$P<0.05$
average C	306	346	448	453	418	344	
a. yield							
1 Oct[c]	0.8	3.4	4.2	1.8	1.9	1.6	n.s.
29 Oct [d]	7.4	10.5	14.5	12.9	9.5	8.9	4.3
19 Nov [d]	13.4	16.3	21.3	20.0	17.9	15.5	2.2
19 Nov all[e]	16.2	18.4	23.6	22.4	20.4	18.3	2.1
b. av fr wt							
1 Oct	210	189	207	180	216	189	38
29 Oct	183	175	177	171	189	182	13
19 Nov	173	168	171	165	177	175	12
19 Nov all [e]	166	165	168	163	173	169	11

[b] *least significant difference, calculated over all treatments*

[c] *only fruits from first fruit set treatment*

[d] *data from all fruit set treatments*

[e] *all mature fruits, including green and red/green*

The vegetative crop parameters observed on 22 October are shown in **Table 4.5**. Similar results were found on other sampling dates. The growth rate (rate of dry weight gain between two dates) is presented in section 4.7.3. Some calculated parameters show a significant CO_2 effect: leaf DMC was higher at higher C; SLA and leaf area ratio (LAR) were lower at higher C. In the continuous CO_2 treatments, number of leaves and leaf area were smaller at higher C. Some other tendencies to reduced vegetative growth at higher C were not significant, maybe owing to a large variability between individual plants and the small number of plants sampled.

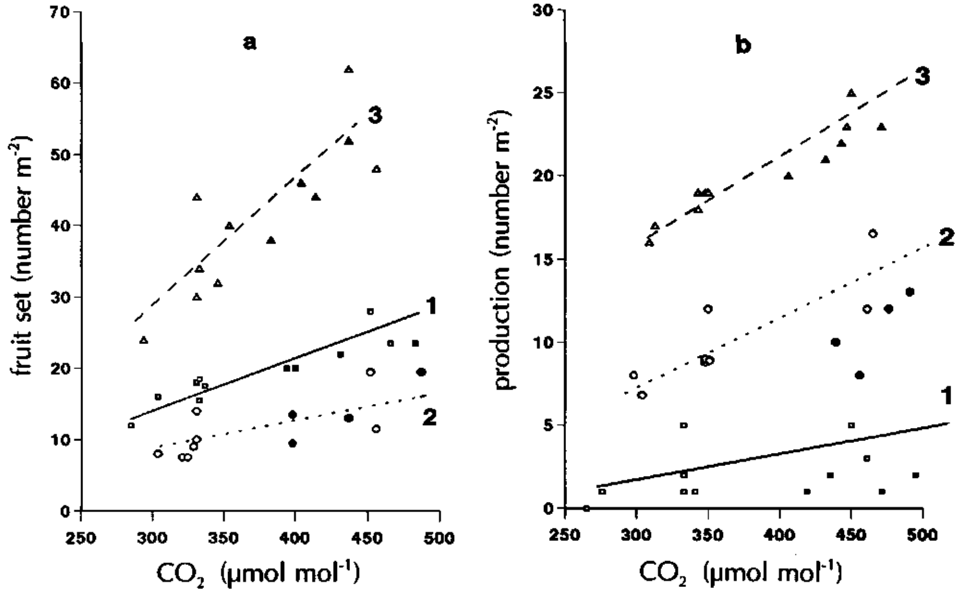

Fig. 4.3 *Results of sweet pepper versus average, measured CO_2 level (μmol mol^{-1}) in the preceding period (data per compartment). Linear regression (Y = a X + b) fitted through non-intermittent CO_2 treatments ('250', '350' & '450' and control).*

a. *Fruit set (number of fruits per m^2)*

1\. ——■-□—— *setting 14-21 Aug. vs. CO_2 of 13-20 Aug.; a=.07, b= -6.9, r=.62*

2\. ..... o . o ..... *setting 22-30 Aug. vs. CO_2 of 21-29 Aug.; a=.05, b= -5.0, r=.77*

3\. – – ▲ - △ – – *setting until 30 Aug. vs. CO_2 of 4-29 Aug.; a=.18, b= -24.9, r=.68*

b. *Fruit production (number of fruits per m^2)*

1\. ——■-□—— *at 1 Oct. vs. CO_2 in Sept.; a=.02, b= -3.7, r=.65*

2\. ..... o . o ..... *until 29 Oct. vs. CO_2 in Oct.; a=.05, b= -5.9, r=.86*

3\. – – ▲ - △ – – *until 19 Nov. vs. CO_2 1 Aug.- 15 Nov.; a=.05, b= 0.1, r=.96*

Open symbols: non-intermittent; closed symbols: intermittent CO_2 supply

Fruit set increased by elevated C (**Table 4.6**). The number of harvested fruits doubled with C increasing from 306 to 448 µmol mol^{-1}. The average fruit weights were not different (**Table 4.7**). The decreased vegetative growth and increased generative growth implied that more biomass was allocated to the fruits. In **Fig. 4.3a** the fruit set in each compartment is related to average C, measured in that compartment during the preceding period (mentioned at the figure). **Fig. 4.3b** shows the same for fruit harvest. According to these figures, the ICS treatments tended to perform slightly less than the non-intermittent treatments. The amount of second class fruits (1.5 to 2.7 % of the total yield, not included in the data) was not significantly related to C.

The ICS treatment '8/40' and the constant level treatment '450' had an comparable average C (**Table 4.4**). SLA, LAR and DMC of leaves and stems were not different between '8/40' and '450' (**Table 4.5**). The treatment with less frequent supply ('8/104') exhibited a lower leaf DMC and a higher SLA and LAR than '8/40' and '450', which is only partly explained by the lower average C (**Table 4.4**). It is not explainable why the control treatment (with about equal CO_2 concentration as the treatment '350'), had almost equal DMC, SLA and LAR as the treatments '450' and '8/40'. These aspects require further research.

4.5 EFFECTS OF CO_2, PLANT DENSITY AND PRUNING ON LEAF DEFORMATION (SLS) AND FRUIT PRODUCTION OF TOMATO

E.M. Nederhoff, A.N.M. de Koning & A.A. Rijsdijk, 1992, Journal of Hort. Science 67: 411-420.

4.5.1 Introduction

Morphological adaptations due to high C in the form of reduced leaf area (section 4.1.6) appear to be most serious in tomato. A producing tomato crop often shows an extremely low leaf area index in summer (De Koning, 1993) and growers experienced that CO_2 enrichment in summer reduced leaf sizes further and even caused a kind of leaf deformation in tomato (Van Velden, 1990). The leaves were short, thick, stiff, curled and somewhat crisp, grey-green to purple coloured, sometimes with necrotic spots and leaf tips (**Fig. 4.4**). Nevertheless, such a summer tomato crop is usually very productive, but later in the season the production may fall behind. The term 'short leaves syndrome' (SLS) was introduced for this phenomenon (section 4.1.6). SLS is undesirable, because apparently light interception, and hence crop photosynthesis and production are lower than in well foliated crops. Fruit quality may be affected negatively, since fruits are more exposed to solar radiation (Adegoroye & Jolliffe, 1987; Janse, 1988). Many (Dutch) tomato growers apply CO_2 enrichment in summer by using a heat storage tank, and the risk of severe SLS due to high C is regarded a major drawback of CO_2 enrichment.

Some authors (Madsen, 1976; Nagaoka et al., 1979; Tripp et al., 1991a,b) described a feature in tomato that resembles SLS. Nagaoka et al. (1979), for instance, described several morphological disturbances: leaves became thick, twisted and purple coloured at high light intensity combined with high C and at low night temperature (8 °C). These authors assumed that the disorder was caused by accumulation of assimilates. Frydrych (1984) induced leaf hypertrophy and reduced SLA by removing buds and fruits in tomato and sweet pepper. Van Gurp (1991) reported that fruit pruning reduced the length of leaves.

The factors that are believed to reduce SLA, and to induce SLS, are high radiation, high C and fruit pruning. These factors have in common that they all reduce the sink/source ratio. Therefore, it is proposed here as a working hypothesis that the phenomenon of reduced SLA can be considered as a symptom of overproduction of assimilates, i.e. of low sink/source ratio or sink limitation. Sink limitation means that the production of assimilates by the leaves exceeds the capacity of the sinks (mainly fruits) for receiving assimilates. So fruit production is sink-limited if there are insufficient fruits on the plant. Peet (1984) reported sink limited fruit production in soybean after partial defloration.

SLS has a negative effect on fruit production, both on the short and the medium term. In late spring and early summer, when SLS occurs and when the light conditions are favourable, fruit production is limited by sink limitation. On overcast days, or later in summer when the average light flux declines, the leaf area of an SLS-affected canopy is too small for an optimal light interception.

The objective of the present work is to investigate whether SLS (or reduced SLA) is associated with, or caused by, a long-lasting surplus of assimilates, i.e. a low sink/source ratio. Therefore the sink/source ratio was varied by applying two treatments of CO_2 and two treatments of night temperature (four climate combinations in triplicate), multifactorially combined with two plant densities and three pruning treatments (leaf pruning, fruit pruning and unpruned control). According to the working hypothesis, high CO_2, lower plant density (higher source) and fruit pruning (lower sink) would induce SLS and leaf pruning (lower source) would enlarge the area of new leaves. The effect of C on production was compared in a sound crop and an SLS affected crop.

Fig. 4.4 *Tomato plant from the experiment, showing the 'short leaves syndrome' (SLS).*

4.5.2 Materials and methods

Facilities, crop and conditions

The experiment was conducted in 12 compartments (9.6 m x 5.7 m) of a Venlo-type glasshouse with a maximum height of 3.3 m. Ventilation was by windows in the roof and heating with five heating pipes of 51 mm diameter, per 3.2 m. Measuring, control and data recording of environmental data was done by a microcomputer in a network (Bakker et al., 1988). Pure CO_2 from bulk storage was supplied through a network of nylon tubes on the ground, 3.2 m apart. C was measured every 4 min with an infra-red gas analyzer (type Ultramat, Siemens, Munich, Germany). The gas analyzers were automatically calibrated each day with a zero filter (drift) and every two weeks by hand using calibration gases (gain).

Tomato plants (cv. Calypso), were planted on 10 May 1990 on rockwool slabs in a recirculating system. A standard nutrient solution (Sonneveld & Straver, 1989) was supplied in sufficient amounts (10 - 30% oversupply). Plant density was 2.1 and 3.1 plants m^{-2}, with the rows 0.8 m apart.

Standard commercial practices were applied: pollination with an 'electric bee' three times a week; removing side shoots at appearance once a week. Six lower leaves per plant were removed on 31 May and another 2 leaves on 12 June, in all treatments. Some double trusses were diminished to normal proportions. The first harvest was on 25 June. The experiment was finished and the crop was harvested on 31 July 1990.

The average day totals of global radiation in May, June and July were 22.7, 16.9 and 20.9 MJ m^{-2} d^{-1} respectively, which was 9% higher than the average of the last 20 years. The measured environmental conditions in the glasshouse compartment can be seen from **Fig. 4.5**.

Treatments

The following treatments applied:

1. CO_2: low C (350 µmol mol^{-1}) and high C (550 µmol mol^{-1}) from sunrise to sunset. Until 13 June C was kept equal in all 12 compartments (on average 362 µmol mol^{-1}) to avoid variations in plant growth in the early phase. From 13 June until the end of the experiment, low C, maintained in 6 compartments, was on average 370, and high C in the other 6 compartments was on average 510 µmol mol^{-1} (**Fig. 4.5a**).

2. Night temperature: low (18 °C) and high (22 °C) night temperature from 20:00 to 5:00 h. Until 13 June heating temperatures were 21/20 °C (day/night) and ventilation temperatures 22/21 °C for all compartments. After mid June until the end of the experiment the set points for heating were 22 °C by day and 18

or 22 °C, for the low and high night temperature, respectively, by night. Ventilation temperatures were then 0.2 °C above heating temperatures. Minimum heating pipe temperature was 45 °C (day and night), with a proportional decrease with increasing solar radiation (-0.1 °C per 1 W m^{-2} above 100 W m^{-2}). Achieved temperatures are given in **Fig. 4.5b,c.**

3. Plant density: low (2.1) and high (3.1 plants m^{-2}) plant density. Each plot of plant density was 23 m^2 in area and consisted of six rows, of which only three rows were used for observations, one was a guard row near the wall and two were guard rows to the other plant density.

4. Pruning: leaf pruning (removing one third of leaves), fruit pruning (removing one third of fruits) and control (unpruned). In the leaf pruning treatment, each third leaf was removed, in addition to the leaf picking in all treatments. In the fruit pruning treatments each second, fifth, eighth and eleventh fruit of each truss was removed. In the control no additional leaf pruning or fruit pruning was applied.

The two levels of C and two night temperatures gave four combinations of climatic factors, which were applied in triplicate. In each of the 12 glasshouse compartments both plant densities were present, and within each density one row (3.8 m^2) was fruit-pruned, one row leaf-pruned and one row remained unpruned. This resulted in 72 experimental plots in total, each plot consisting of 8 or 12 plants (in the low and high density, respectively). The plants in the guard rows were not pruned. To minimize the consequences of mutual interference, the experimental plots were randomized.

Plant densities were maintained from planting until harvest; C and night temperature treatments started two weeks before the first harvest and the pruning treatments were both applied twice; 7 days before and 9 days after the first harvest.

Observations

The severity of SLS in all 72 plots was assessed visually on a relative scale between 1 and 5: a higher mark being recorded if leaves were shorter and more affected. Data of six observations (three observers and two occasions, 20 and 30 July) were averaged to one data set before statistical analysis.

At the end of the experiment (31 July) destructive measurements were done in all experimental plots. In each plot, four top segments with 12 leaves (> 1 cm) and one whole plant were harvested. The whole plant was split into a top segment and a lower part. The top segments had grown after the leaf pruning treatment had been applied. Leaf area of top leaves was measured with a planimeter (LI-3100, LI-COR, Lincoln, Nebraska). For all samples (five plant

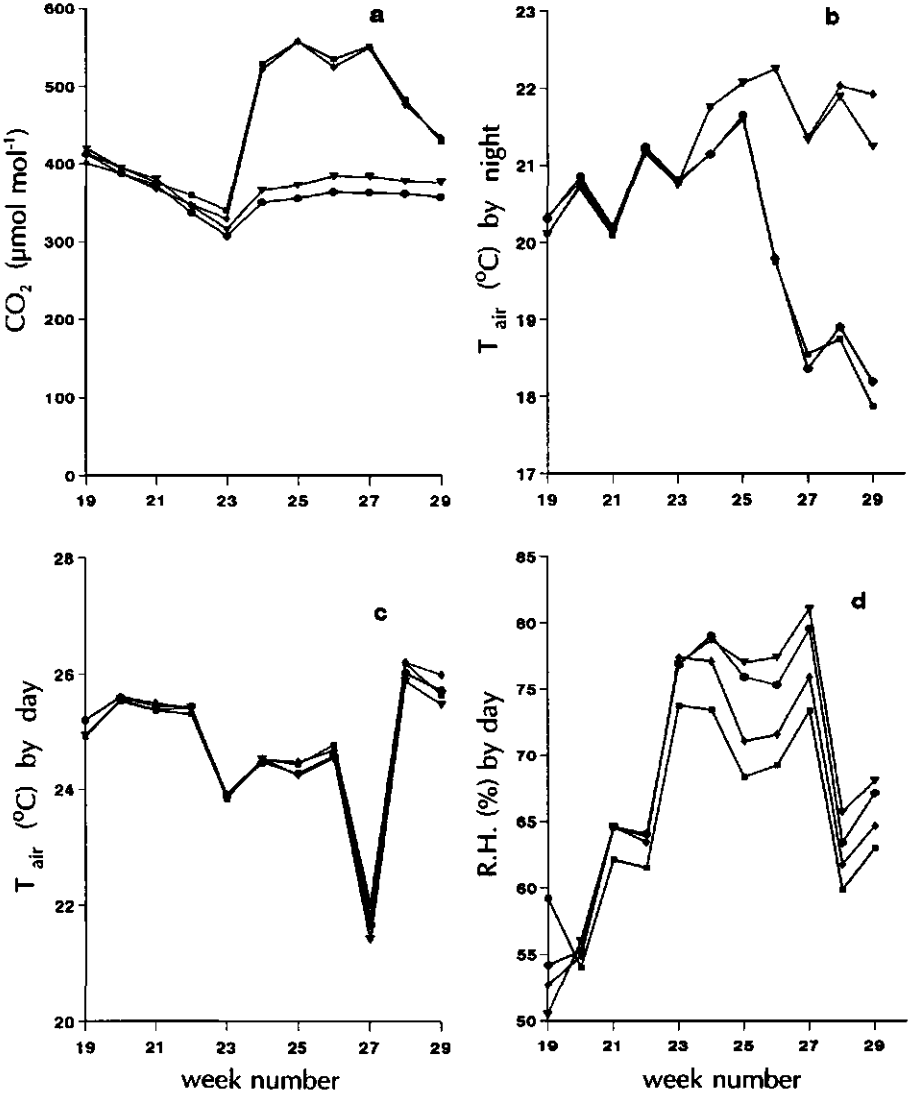

Fig. 4.5 *Environmental conditions in tomato experiment, measured in 12 experimental compartments, averaged per week over three compartments.*

a. *CO_2 concentration (µmol mol^{-1}, 10:00-16:00 h)*

b. *air temperature (°C) by night (22:00-4:00 h)*

c. *air temperature (°C) by day (10:00-16:00 h)*

d. *relative humidity (%) by day (10:00-16:00 h)*

——●—— *low CO_2, low night temp.;* ——▼—— *low CO_2, high night temp.;*
——■—— *high CO_2, low night temp;* —— ◆ —— *high CO_2, high night temp.*

tops and one lower plant part per plot) fresh and dry weights (dried 48 hours in a forced air oven at 80 °C) of leaves (including petioles) and stems were determined.

A rough approximation of total leaf area was made from measured SLA of top leaves and dry weight of total foliage. This was done instead of measuring total leaf area, which was virtually impossible because of the curliness of the older, by SLS affected leaves.

The effect of SLS on fruit production was studied by weighing and counting the harvested fruits three times a week from 25 June until 31 July. Fruits with blossom end rot were weighed and counted separately. Fruits that remained on the plants at the end of the experiment were measured as well. Fruit DMC was determined on five fruits per experimental plot from the harvest of 27 July.

Starch content of the leaves was determined in the fruit and leaf pruning treatments (48 plots). On 30 July, between 15:00 and 16:00 h, in each plot eight leaves were sampled, all being the 15th leaf from the top. Leaf 15 was an almost fully expanded leaf, grown during the treatment period. Leaves were dried (24 h at 100 °C) immediately after picking. The analysis was done using high performance liquid chromatography (Carbopack PA1, DIONEX, Breda, The Netherlands), after enzymatic hydrolysis, as described by Van De Woestijne & Van Elderen (1990).

Data analysis and notation

Main effects of C, night temperature, plant density and pruning were analyzed statistically with four factor ANOVA, using GENSTAT (Payne & Lane, 1987). The least significant difference was calculated with the Student's t-distribution (double-sided) with probability 95%.

Night temperature had no marked effects on relevant characteristics and no interactions with other factors. Also Tripp et al. (1991b) found no effect of night temperature on foliar deformation or foliar starch concentration. Therefore, in the current work, night temperature effects are not presented or discussed and results of other treatments are averaged over both temperatures treatments.

Table 4.9 summarizes the main effects of the factors C and plant density; **Table 4.10** of the pruning treatments and **Table 4.11** shows the results of the three factor analysis. No three-factor interactions existed and only some two-factor interactions were significant.

Effects are described below as a percentage increase (+) or decrease (-) relative to the response at the lowest level of the factor. The effects of the pruning treatments are given relative to the unpruned treatment.

4.5.3 Results

Leaf growth

The degree of SLS was assessed in three ways, as leaf area and SLA of 12 top leaves and as the visual score of degree of SLS. Leaf area and SLA were both inversely correlated with the degree of SLS, with a high correlation (**Table 4.8**). In fact were all vegetative characteristics mentioned (degree of SLS, SLA, leaf area, DMC of leaves and stem and leaf starch content) highly correlated ($P<0.01$).

SLS (**Tables 4.9a** and **4.10a**) was more severe at high C, at low plant density and with fruit pruning. Leaf area of top leaves (**Tables 4.9b** and **4.10b**) was influenced by C (-9%) and plant density (+29%). SLA of top leaves (**Tables 4.9c** and **4.10c**) was lower at high C (-14%) and it was higher at high plant density (+16%).

Stem DMC (**Tables 4.9d** and **4.10d**) was higher at elevated C (+5%) and lower at high density (-10%) and leaf pruning (-7%). The differences were small, but statistically significant. Differences in leaf DMC were less pronounced but similar in direction. Starch content of leaves (**Tables 4.9e** and **4.10e**) was influenced by C (+36%), plant density (-22%) and by pruning only in high plant density (**Table 4.11d**).

In summary, most characteristics related to SLS were significantly affected by C and plant density, and only weakly by pruning.

Fruit production

On 13 June, when the treatments were started, the plants carried almost 20 young fruits, with an estimated total fruit weight of 250 g/plant (fresh) or 15 g/plant (dry). Because those fruits were grown before the treatments were imposed, total harvest was not the most appropriate characteristic to evaluate the effects of C and pruning on fruit production. Therefore, the green fruits present on the plant at the end of the experiment, completely grown during the treatment period, were considered the best criterion (**Table 4.11e**). Harvested fruit weight and total fruit weight are presented only as main effects (**Tables 4.9f,g** and **4.10f,g**).

C had no effect on rate of appearance of leaves and trusses. Also fruit set was hardly different between low and high C, because virtually all flowers set to fruits under the summer light conditions, which can be seen from an almost equal number of fruits harvested (**Table 4.9f,i**). High C generally enhanced the production of fruit weight (**Table 4.9f,g**). The CO_2 effect was significant only in the treatment with least severe SLS (high-density/unpruned,**Table 4.11e**). In these crops, high C increased harvested fruit weight by 11%, and the green fruits on the plant by 31%, resulting in an 18% increase of total fruit weight.

Leaf pruning reduced total fruit weight by 13% (**Table 4.10g**), explainable by a reduced photosynthesis. Fruit pruning decreased the harvested weight by 23% (**Table 4.10g**), which was in accordance with the amount of fruits removed. However, the weight of fruits on the plants (**Table 4.11e**) exhibited no significant effects of pruning nor of C at low plant density.

The weight of fruits with blossom end rot was on average 0.4 kg m^{-2} and represented about 10% of the number of harvested fruits. The percentage of blossom end rot fruits (**Table 4.9h**) was not significantly affected by CO_2. The blossom end rot percentage was higher at low plant density, which were the plants with most severe SLS.

The average weight of good quality fruits (**Tables 4.9i** and **4.10i**) was 79 g, with small effects of density (-5%), leaf pruning (-9%) and fruit pruning (+5%). In the treatments with least SLS, average fruit weight was affected by C (+7%, data not shown). An increase of average fruit weight by elevated C is in accordance with literature (e.g. Acock & Pasternak, 1986). Fruit DMC (**Tables 4.9j** and **4.10j**) was slightly lower at high C than at low C and was highest in the fruit-pruned crops.

4.5.4 Discussion

Approximated sink/source ratios

For a better comparison of the treatments, the order of magnitude of the change in sink/source ratio, caused by each treatment was estimated, as follows. Sink, represented by the number of fruits m^{-2}, was affected by plant density (3.1 compared to 2.1 plant m^{-2} is +50%) and by fruit pruning (one third of fruits removed is -30% in mid June, which decreased to -20% later, because newly formed trusses were not pruned).

Source, being the daily crop photosynthesis, was influenced by C and by light interception, i.e. by LAI. This was affected by leaf pruning (one third of leaves removed, -30%), by plant density and by SLS. The initial difference in LAI between low and high density was 50% (2.1 versus 3.1 plants m^{-2}). Due to increasing SLS at wider planting, the difference in LAI increased to 55% in mid June and 65% later. Elevated C enhanced SLS, causing a 12% lower leaf area at the end of the experiment compared to low C. The combined effects of reduced leaf area and enhanced crop photosynthesis by CO_2 enrichment were roughly approximated with a simulation model (Challa, 1990).

Table 4.8 *Correlation coefficient (r) for the vegetative characteristics of tomato. n = 48 for leaf starch content and n = 72 for all other characteristics.* $r_{(P=0.01,\ n=48)} = [0.33]$ *and* $r_{(P=0.01,\ n=72)} = [0.27]$.

characteristic		1	2	3	4	5	6
degree of SLS	1	1.00					
leaf area of top leaves	2	-0.63	1.00				
specific leaf area (SLA)	3	-0.38	0.42	1.00			
DMC leaves	4	0.55	-0.64	-0.63	1.00		
DMC stems	5	0.36	-0.41	-0.73	0.86	1.00	
leaf starch content	6	0.61	-0.55	-0.30	0.55	0.32	1.00

Table 4.9 *Main effects of CO_2 concentration (low = 370, high = 510 μmol mol^{-1} on average) and plant density (low = 2.1, high = 3.1 plant/m^2) on some characteristics of tomato, observed at the end of the experiment (31 July).*
All data averaged over two night temperatures and three pruning treatments.

	characteristic	units	treatment	low	high	F.pr.
a.	degree of SLS	rel. [f]	CO_2	2.4	3.8	0.001
			plant density	3.4	2.8	0.003
b.	leaf area of top leaves [g]	m^2 p.p.	CO_2	0.174	0.158	0.156
			plant density	0.145	0.187	<0.001
c.	SLA of top leaves [g]	cm^2 g^{-1}	CO_2	159.5	137.5	0.003
			plant density	137.4	159.6	0.002
d.	DMC stems	%	CO_2	12.8	13.4	0.001
			plant density	13.8	12.4	<0.001
e.	starch content leaves	%	CO_2	2.83	3.84	0.034
			plant density	3.75	2.93	<0.001 [h]
f.	harvested fruit weight	kg m^{-2}	CO_2	6.86	7.34	0.003
			plant density	6.13	8.07	<0.001
g.	total fruit weight [i]	kg m^{-2}	CO_2	9.71	10.67	0.009
			plant density	8.60	11.80	<0.001
h.	blossom end rot fruits	%	CO_2	9.0	10.6	n.s.
			plant density	12.6	6.9	<0.001
i.	average weight of fruits	g	CO_2	77.6	80.7	0.206
			plant density	81.2	77.1	<0.001
j.	DMC fruits	%	CO_2	5.88	5.74	0.046
			plant density	5.80	5.82	n.s.

[f] *relative score: 1 = normally expanded leaves; 5 = extremely short leaves*

[g] *the top segment had not been exposed to the leaf pruning treatment*

[h] *interactions exist*

[i] *harvested fruits + fruits on the plant at 31 July*

Table 4.10 *Main effects of the pruning treatments on some crop characteristics of tomato, observed at the end of the experiment, averaged over all other treatments.*

characteristic	units	pruning treatment leaf	 fruit	 no	$P_{0.95}$	$LSD_{0.05}$
a. degree of SLS	rel.[f]	3.0	3.3	3.0	<0.001	0.18
b. leaf area of top leaves[g]	m^2 p.p.	0.158	0.171	0.169	0.184	0.015
c. SLA of top leaves[g]	$cm^2 g^{-1}$	158.3	140.6	146.7	0.028	13.0
d. DMC stems	%	12.4	13.6	13.3	<0.001	0.41
e. starch content leaves	%	3.54	3.13	-	0.007	0.30[h]
f. harvested fruit weight	$kg\ m^{-2}$	7.52	6.03	7.75	<0.001	0.37
g. total fruit weight[i]	$kg\ m^{-2}$	10.10	8.93	11.56	<0.001	0.67
h. blossom end rot fruits	%	8.6	10.0	10.8	0.100	2.0
i. average weight of fruits	g	73.2	84.2	80.0	<0.001	1.9
j. DMC fruits	%	5.69	5.91	5.83	<0.001	0.07

[f-i] *explained at Table 4.9*

Table 4.11 *Results of tomato, at two plant densities, three pruning treatments and two CO_2 levels (L = 370, H = 510 µmol mol^{-1} on average). Observations at the end of the experiment (31 July), averaged over two night temperatures.*

plant density:		low (2.1 pl. m^{-2})			high (3.1 pl. m^{-2})			$LSD_{0.05}$[j]
pruning:		leaf	**fruit**[k]	none	leaf	fruit	**none**[k]	
	CO_2							
a. degree of SLS[f]	L	2.5	3.1	2.7	2.1	2.2	1.8	
	H	4.0	4.2	3.9	3.5	3.8	3.5	.37/.57/.66
b. leaf area top[g]	L	0.15	0.16	0.15	0.17	0.22	0.19	
(m^2 p.p.)	H	0.13	0.13	0.14	0.17	0.17	0.20	.041/.028/.033
c. SLA top leaves[g]	L	149	145	149	181	160	173	
($cm^2 g^{-1}$)	H	142	116	124	160	142	141	26.0/25.5/25.2
d. leaf starch	L	3.2	3.2	-	2.9	2.1	-	
(%)	H	4.3	4.4	-	3.9	2.9	-	.56/.60/.94
e. fruits on plant	L	2.08	2.04	2.97	2.61	3.46	3.94	
($kg\ m^{-2}$)	H	2.40	2.19	3.14	3.27	3.92	5.18	1.1/1.1/1.1

[f-i] *explained at Table 4.9*

[j] *three values for $LSD_{(0.05)}$: first for comparing equal levels of CO_2 and plant density, second for equal CO_2 and third for all other cases.*

[k] *these treatments are compared in the discussion (section 4.7)*

Combining these various effects on sink and source resulted in the following estimates: smallest change in sink/source ratio by CO_2 treatment (initially 17% lower sink/source ratio at higher C declining to 10%); greatest change by fruit pruning (30 to 20% lower sink/source ratio) and intermediate change by higher plant density and by leaf pruning (about 20% higher sink/source ratio).

Short leaves (SLS)

CO_2 enrichment generally increases the production of assimilates, i.e. the source. In accordance with the working hypothesis, high C increased the degree of SLS and decreased the leaf area and SLA of top leaves (**Table 4.9a,b,c**). It also increased the DMC of leaves and stems (**Table 4.9d**), as well as leaf starch content (**Table 4.9e**). The high correlation between characteristics (**Table 4.8**) enables us now to define SLS by objective criteria, like reduced leaf area and reduced SLA, rather than by the subjective description of SLS, the original observation by growers. Decreased SLA at higher C is consistent with previous research (Madsen, 1976; Acock & Pasternak, 1986), and the same is true for increased DMC and leaf starch content at high C (Idso & Kimball, 1988; Acock & Pasternak, 1986; Tripp et al., 1991b). These characteristics may indicate a surplus of assimilates.

Lower plant density increases light interception and hence the source (photosynthesis on a per plant basis). So lower plant density increased SLS, as expected. Plant density had by far the largest effect on fruit weight, partly because the treatment was started at planting, whereas the other treatments were started five weeks later. As high C and low density had similar effects, in the plots with low-density/high-C the symptoms of SLS were most severe, leaf area and SLA were smallest and leaf starch content was highest, whereas at high-density/low-C the opposite was found (**Table 4.11**).

Pruning had only small effects on leaf weight and SLS. Tripp et al. (1991b) also found no effect of fruit removal on foliar deformation. An explanation for the relatively small effects of pruning might be that changes in sink/source ratio had a more local effect (Fisher, 1977; Ho & Hewitt, 1986), rather than an effect on the whole plant. This would imply that removal of leaves reduced growth of nearby fruits (lower average fruit weight, as observed, **Table 4.10i**), rather than of the apex. Also fruit removal can have its largest impact somewhat lower in the plant, without strong effects on the apex.

Leaf starch content of the 15th leaf from the top was highly correlated with SLS (**Table 4.8**), and similar results were found by De Kreij (1991, 1992). Tripp et al. (1991b) also reported an increase of leaf starch with increasing foliar deformation, but they concluded that no causal relationship existed. In high plant density, leaf starch was lower with fruit pruning than with leaf pruning

(**Table 4.11d**). This was not expected, but is in agreement with observations of Ammerlaan et al. (1986), who found that removal of tomato fruits caused accumulation of starch in older leaves and reduction of starch in younger leaves. In the present work, only young leaves (nearly full grown) were sampled. It is unexplained why the starch content presented here differed from that reported by Ammerlaan et al. (1986). Tripp et al. (1991b) observed no effect of fruit removal on foliar starch. Because of the variability in results, starch content is not considered an appropriate indicator of sink/source ratio.

Hypothesis for underlying mechanism of SLS

Nutrient deficiency might play a role with the incidence of SLS. Tripp et al. (1991a) observed that foliar deformation increased over the season, while foliar potassium (K) decreased in parallel. They observed in two out of eight cultivars a lower K content in crops with more deformation and concluded that K deficiency caused the phenomenon. This conclusion seems questionable, because a declining trend in K content in tomato leaves is commonly observed (Voogt, 1988).

There seem to be more indications that SLS is caused by deficiency of calcium in the apex. Crops with SLS frequently have symptoms of Ca deficiency, like yellow margins of young leaves and scorched leaf tips. Lower Ca uptake induced both severe SLS and blossom end rot in fruits in work of De Kreij (1991) and also in the present experiment blossom end rot seemed associated with SLS (**Table 4.9a,h**). Blossom end rot is caused by Ca deficiency (Ehret & Ho, 1986). In recent work, De Kreij (1992) could induce severe SLS by a low Ca supply. Ca deficiency in leaves can reduce leaf area of tomato (Holder & Cockshull, 1990; Bakker, 1991b). It is possible that Ca deficiency at leaf initiation or during early leaf growth, might cause an invisible injury, manifested later as reduced leaf size.

It is perceptible that a low sink/source ratio may lead to Ca deficiency in plant organs. Fruits are supplied with water to a large extent by the phloem sap (under some conditions 85 to 99%, Ho et al., 1987). The relative contribution of the phloem is larger if assimilate availability is greater, which is the case in a CO_2 enriched crop. Because phloem contains very little Ca (Bangerth, 1979; Adams, 1986), a high assimilation rate might induce Ca deficiency. The Ca containing xylem flows to the transpiring leaves and only small amounts reach the non-transpiring organs. This theory was postulated by Ho (Clover, 1991) for tomato fruits, but might also apply to the apex, assuming the apex to be a sink with a low transpiration, like fruits. Another way of putting it, is that high C increases the CO_2 assimilation, so that the available Ca is distributed over a larger amount of biomass (dilution effect, Porter & Grodzinski, 1984; Eng et al., 1985; Kuehny et al., 1991). The Ca content of the apex was not observed, as only the 15th leaf was sampled. No literature was found that could support this hypothesis (Ho, 1994, pers. comm.).

The relatively large effect of C on leaf growth might be explained by an additional effect of C on the plant Ca content. High C causes partial stomatal closure, and subsequently reduces crop transpiration (chapter 3), which can reduce the Ca translocation in the plant. The lower relative humidity measured in the compartments with high C (**Fig. 4.5d**) confirms that the transpiration was reduced. (In a semi-closed system as a greenhouse, a low air humidity is a direct consequence of low rate of crop transpiration). It is well known that particularly Ca deficiency can occur at reduced transpiration (Bangerth, 1979; Adams, 1986; Ehret & Ho, 1986; Holder & Cockshull, 1990; Bakker, 1991b) because this element is transported with the transpiration flow. The reduction of the Ca content was even more pronounces in the apex (Ehret & Ho, 1986) or in the youngest leaves (Armstrong & Kirkby, 1979), than in other organs at reduced transpiration.

Conclusions and recommendations

It is concluded that CO_2 enrichment is beneficial for fruit production, unless the crop suffers severely from SLS. With elevated C (around 510 μmol mol^{-1}) compared to low C (370 μmol mol^{-1}) the increase of fruit weight on the plants at the end of the experiment was 19% on average. It was insignificant in crops with severe SLS and 31% in the crop with the least SLS (high-density/unpruned).

As a method of avoiding short leaves, it can be recommended to increase the plant density or, in an early planted year-round crop, to maintain an extra shoot on the plants in spring. Further research is required to clarify the mechanism behind leaf deformation (SLS) in summer. It is particularly interesting to investigate the role of calcium in the incidence of SLS.

4.6 EFFECTS OF CO_2 ON LEAF TIP CHLOROSIS (LTC) AND FRUIT PRODUCTION OF EGGPLANT

E.M. Nederhoff & K. Buitelaar, 1992, Journal of Horticultural Science 67: 805-812.

4.6.1 Introduction

In glasshouse cultivation of eggplant, frequently leaf chlorosis occurs. It is considered a problem, because of possible reduction of production capacity. Two types of chlorosis may occur at the same time in the same canopy, but the two types have not clearly been distinguished until now. Most common is chlorosis caused by magnesium deficiency and characterized by irregular yellow spots in the interveinal mesophyll of older leaves (Mengel & Kirkby, 1978; Winsor & Adams, 1987). It prevails in bursts from spring until the end of the cultivation. The present work, however, deals with a form of chlorosis, that begins on the distal end of the leaf, near the main vein, both in the veins and the mesophyll. It is called here leaf tip chlorosis (LTC). It gradually covers the whole leaf and finally leads to loss of the leaf. Frequently a leaf with LTC has a break across the main vein, caused by brittleness. LTC usually arises in April/May in the upper half of the plants or in growing side shoots, and particularly in extremely large, young leaves (**Fig. 4.6**). LTC is stimulated by CO_2 enrichment (Buitelaar, 1991).

Until now, only magnesium was considered in relation to chlorosis in eggplant (e.g. De Kreij, 1989). However, LTC closely resembles the symptoms of boron deficiency (Shorrocks, undated; Roorda Van Eysinga & Smilde, 1981; Winsor & Adams, 1987). Symptoms similar to LTC in eggplant were observed in young sweet pepper leaves, where they were accompanied by leaf boron deficiency (Sonneveld, 1991).

The following hypothesis is proposed here to explain the observed stimulating effect of CO_2 on LTC. High CO_2 reduces stomatal conductance and hence plant transpiration, at least to some extent (section 3.6.2). In eggplant even a remarkably sensitive stomatal closure in response to CO_2 was measured (section 3.6.1). Reduced canopy transpiration may imply a reduction in translocation of the nutrients calcium and boron, which are transported almost exclusively by the xylem (Mengel and Kirkby, 1978). As those elements are relatively immobile (i.e. not redistributed), their availability for plant organs is strongly related to the rate of transpiration. Low transpiration induced B deficiency in the youngest leaves of tobacco (Von Michael et al., 1969). Another effect of elevated CO_2 is that it increases the dry matter production, and thus

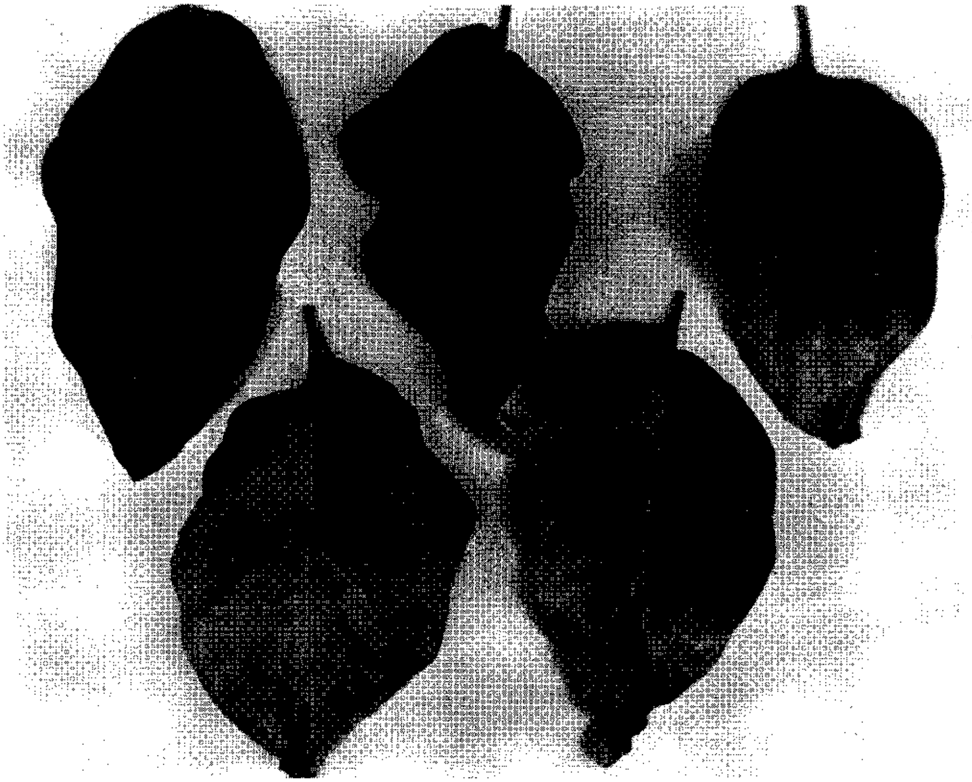

Fig. 4.6 *Subsequent stages of leaf tip chlorosis (LTC) in eggplant leaves, as observed in the experiment.*

further decreases the concentration of some nutrients in the dry matter ('diluting effect'). By these two processes, high C might induce B deficiency in fast growing, young leaves.

The aim of the present experiment was to investigate whether high C in eggplant induces leaf tip chlorosis and whether this is associated with leaf B deficiency. The effect of high C on fruit production was considered.

4.6.2 Materials and methods

The experiment was conducted in four identical compartments (16 m x 16m) of a computer-controlled, Venlo-type glasshouse complex. Two compartments received low C and two high C: the achieved average CO_2 concentrations were 413 and 663 $\mu mol\ mol^{-1}$, respectively (means over 2 compartments, over week 6 to 29, **Fig. 4.7b**). Pure CO_2 was supplied through a net work of perforated tubes on the ground, 3.2 m apart. C was monitored by an infra red gas analyzer, type Ultramat (Siemens, Munich, Germany), with a measuring cycle of 4 min.

Heating temperatures were 24/24 °C (day/night) in the first three weeks, gradually decreased to 20/18 °C, maintained from March until the end of the experiment. Ventilation temperature was 25 °C from March onward. Global radiation outside and dry and wet bulb air temperatures in the greenhouses were measured every min. The average achieved environmental conditions are shown in **Fig. 4.7**.

Eggplant (cv. Cosmos) was sown on 20 October and planted on 10 December 1990 on rockwool at a density of 2.5 plants per m^2, with two shoots per plant. The first harvest was 11 February and the last on 18 July (end of experiment).

Each compartment contained 24 experimental plots, in which several subtreatments were multifactorially combined with the CO_2 treatments. All experimental plots were located in the rows under the ridge, whereas the rows under the gutters and near the compartment walls were guard rows. The ridge rows consisted of three experimental plots of 8 plants each, plus two guard plants in the beginning and three at the end. The subtreatments are not considered in this paper, as there were no interactions with C. Presented are the main CO_2 effects, averaged over all subtreatments. Further details on crop and environment were given in section 3.5.

Observations

The incidence of LTC was measured on 15 April, 27 May and 16 July. All leaves from one double guard row (58 plants) per compartment that were clearly affected by LTC (more than 10% yellow) were removed, weighed and measured. The severity of LTC was also visually assessed in all 24 plots per compartment on 12 April, 16 May and 28 June. Three observers independently scored on a relative scale from 0 to 5, for the degree of LTC. The three scores were averaged before statistical analysis.

Plant growth parameters were determined on 16 April on 3 plants per compartment. Leaf area and fresh and dry weight of leaves were measured; dry weight after 48 hours at 80 °C in a forced ventilated oven.

Leaf contents of K, Ca, Mg, P, N-total, NO_3, Fe, Zn and B were determined as described by De Bes (1986) on samples of 16 leaves. Leaves of two standard plots per compartment were previously labelled, and sampled on four dates between 18 March and 10 June, in three age classes (young, medium, old). The samples contained leaves with and without LTC. On 10 June also a sample of exclusively leaves with LTC was taken. Sampling for B analysis requires great care, because of the great non-uniformity. However, this is only the case at extremely high B contents, and negligible at low levels (Oertli, 1994).

Fruits were harvested every week from 11 February until 18 July. Fruits, separated into first and second class (smaller than ca. 180 gram), were counted and weighed for each experimental plot. Fruit DMC was determined four times between 24 June and 9 July on separate samples from four fruit growth stages, from low and high C. Fresh and dry weights of fruits and calyces were determined, as well as volumes of fruits.

Keeping quality was investigated for fruits of four harvesting dates between 28 March and 21 June. Ten harvested fruits of some selected plots were stored under controlled conditions (20 °C and 90% relative humidity). The firmness of fruits and quality of calyces were assessed directly after harvest and one and two weeks later.

4.6.3 Results

Leaves and chlorosis

The destructive plant measurements of 16 April showed no significant differences in plant fresh and dry weight and LAI between low and high C, possibly due to the small number of sampled plants. LAI was about 2.7. SLA was significantly smaller at high C than at low C (185 and 214 cm^2 g^{-1} DM, respectively), which is in accordance with literature (Acock & Pasternak, 1986).

The incidence of LTC revealed significant differences between low and high C in number, weight and area of yellow leaves (**Table 4.12**). High C induced 2 to 3 times as many yellow leaves than low C. The accumulated area of removed, chlorotic leaves was about 0.06 and 0.15 m^2 per plant for low and high C respectively. This is 0.16 and 0.38 m^2 per m^2 respectively, and related to the estimated final leaf area, it is 3.2 and 7.5%, respectively. In commercial situations sometimes far more severe damage by LTC prevails. The visual assessment of degree of LTC also revealed highly significant differences for CO_2 effects (**Table 4.13a**). SLA was clearly lower and DMC was higher at high C in the (partly) yellowed leaves (**Table 4.12e,f**).

Fruit production and quality

The production rate was always higher at high C than at low C and the difference increased during the season (**Fig. 4.7d**). Cumulative production (**Table 4.13b**) was increased by 10% at 25 March (6 weeks after first harvest) and by

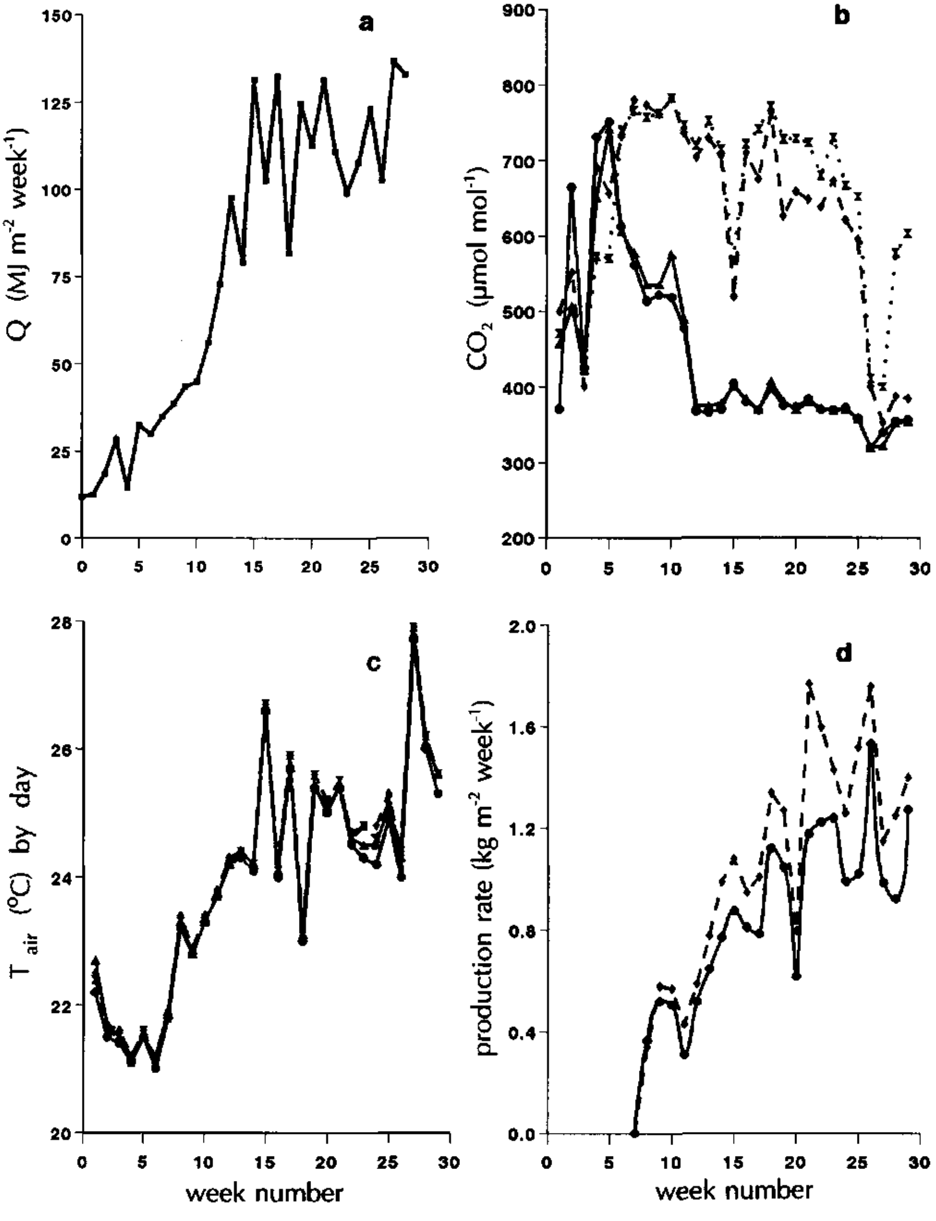

Fig. 4.7 *Results of the eggplant experiment with low CO_2 (———) and high CO_2 (- - - - - and . . . . . .), as observed in four compartments.*

a. *Q = global radiation outside the glasshouse (MJ m^{-2} $week^{-1}$)*

b. *CO_2 = average CO_2 concentration (μmol mol^{-1}, 9:00 - 18:00 h)*

c. *T_{air} = average air temperature (°C, 9:00 - 18:00 h)*

d. *production rate (kg m^{-2} $week^{-1}$), averaged over two replicates.*

Table 4.12 *Leaf tip chlorosis (LTC) of eggplants at low (413) and high CO_2 concentration (663 µmol mol^{-1} on average).*
a. *number of yellow leaves per plant*
b. *fresh weight of yellow leaves (g / plant)*
c. *area of yellow leaves (m^2 / plant)*
d. *yellow leaf area relative to estimated total leaf area (%)*
e. *specific leaf area (SLA) of yellow leaves (cm g^{-1} dry weight)*
f. *dry matter content (DMC in %) of the (partly) yellow leaves.*
All differences between low and high CO_2 are significant, except those with '='.
LSD is given for the accumulated values only (P=0.1, n=2).

date:	15 April		27 May		16 July		accumulated		
CO_2:	low	high	low	high	low	high	low	high	$LSD_{0.1}$
a. # y lvs	0.15	1.73	1.60	4.38	0.99	2.83	2.7	8.9	5.1
b. wt y lvs	1.57	9.08	9.4	20.7	7.8	19.0	18.8	48.7	14.7
c. area y lvs	0.01	0.03	0.03	0.07	0.03	0.05	0.06	0.15	.018
d. rel. area y lvs	0.8	3.0	2.4	4.8	1.2	2.7	3.2	7.5	0.90
e. SLA y lvs	219	186	241	195	191	167	217	182	18.9
f. DMC y lvs	14.9	17.7	14.7	16.6	16.6 =	16.8	15.5 =	16.9	n.s.

Table 4.13 *Effects of low and high C (413 and 663 µmol mol^{-1} on average, resp.) on some characteristics of eggplant:*
a. *degree of leaf tip chlorosis on a relative scale (0= no, 5 = severe yellow leaves);*
b. *cumulative production of fruits (kg m^{-2}) (class 1 and 2) on four dates;*
c. *cumulative number of harvested fruits per m^2 (class 1 and 2) on four dates.*

	date	low C	high C	$LSD_{0.05}$
a. degree of LTC (relative)	12 Apr	0.2	1.2	0.42
	16 May	0.8	1.9	1.08
	28 Jun	1.4	2.5	0.72
b. cum. prod. (kg m^{-2})	25 Feb	0.67	0.66	n.s.
	25 Mar	2.58	2.85	0.1
	27 May	10.83	13.27	0.7
	18 Jul	19.50	24.10	2.4
c. cum. number of fruits (m^{-2})	25 Feb	4.2	4.2	n.s.
	25 Mar	13.1	13.9	0.3
	27 May	43.1	49.7	1.3
	18 Jul	76.0	88.5	5.6

Table 4.14 **a.** *boron and* **b.** *magnesium concentration (μmol g^{-1} dry matter) of eggplant leaves. Variables: plant fruit load (FL, n = normal, l = low); leaf age (LA, y = young, m = medium, o = old) and CO_2 concentration (L = 413, H = 663 μmol mol^{-1} on average). Statistical significance discussed in section 4.6.4.*

		18 Mar		15 Apr		13 May		10 Jun		average	
FL	LA	L	H	L	H	L	H	L	H	L	H
a. B											
n	y	1.37	1.12	1.24	0.97	-	-	-	-	1.31	1.05
n	m	1.88	2.21	0.91	0.78	0.96	0.82	-	-	1.25	1.27
n	o	-	-	2.65	2.20	0.84	0.79	1.08	0.90	1.52	1.30
l	y	1.43	1.07	1.43	1.14	-	-	-	-	1.43	1.11
l	m	1.94	1.81	1.00	0.80	1.15	0.86	-	-	1.36	1.16
l	o	-	-	2.50	2.71	0.93	0.77	1.26	1.06	1.56	1.51
avrg		1.66	1.56	1.62	1.44	0.97	0.82	1.17	0.98	1.36	1.20
n+l	y[a]	1.40	1.10	1.34	1.06	-	-	-	-	1.37	1.08
b. Mg											
avrg		209	210	206	204	202	177	228	229	211	205

[a] *sample exclusively containing young leaves affected by leaf tip chlorosis*

24% at 18 July. Number of fruits harvested per m^2 was initially 6% higher and later 16% higher at high C (**Table 4.13c**). Average fruit weight was slightly higher at high C than at low C (**Table 4.13b,c**). The relative number of second class fruits was 7 - 11% of the total number of fruits and was not significantly different for low and high C.

The maturation period of the fruits (duration between anthesis and harvest) was determined frequently and showed no effect of C, as it was on average 32.5 and 32.3 days for low and high C, respectively. Also fruit DMC did not show an effect of C: on 16 April the average fruit DMC was 7.6 and 7.3%, on 9 July it was 8.2 and 7.9% and of the calyces it was 12.4 and 12.1%, for low and high C, respectively. The specific gravity of fruits was 0.57 and 0.56 g ml^{-1}, respectively. Fruit quality (firmness), assessed directly after harvest and one and two weeks later, was not significantly different but tended to be slightly better from high C. The quality of the calyx (colour green or brown, and the percentage rot) was on average not different in the two CO_2 treatments (data not presented).

Nutrient contents

The boron concentration (**Table 4.14a**) of the leaves was generally low. The differences in B data could not be tested with ANOVA, because of non-orthogonality of the data. Therefore regression analysis was applied. Boron content was significantly lower ($P<0.05$) in young leaves than in older leaves.

In all cases except one, leaf B content was lower at higher C. The negative effect of C on B content was found both in young and older leaves, but young leaves were most relevant, as LTC usually arises there. B concentration in young leaves was on average 0.29 µmol g^{-1} (21%) lower at high C than at low C ($P<0.01$). The sample with exclusively yellow leaves had an even lower B content than the other samples, consisting of leaves with and without LTC.

The concentration of magnesium (**Table 4.14b**) and other nutrients exhibited only non-systematic or small differences, insignificant with this low number of samples. So other nutrients than boron were considered irrelevant for the incidence of LTC.

4.6.4 Discussion

Leaf boron content

The leaf boron concentrations measured (**Table 4.14a**) were below those reported for B deficiency: in tomato, the normal B content is between 3 and 10 µmol g^{-1} (dry weight) and deficiency occurs around 1 µmol g^{-1} (Shorrocks, undated). In tomato a B content of 1.2 µmol g^{-1} caused deficiency symptoms (Gupta, 1983); B levels of 0.5-0.9, 2.1 and 3.0 µmol g^{-1} corresponded with severe, considerable and slight deficiency symptoms, respectively (Adams & Winsor, 1974). Baevre (1990) observed in tomato plants growing in peat, a leaf B content above 3 µmol g^{-1}, and positive effects of increased B supply.

It is generally accepted that boron (like calcium) is almost exclusively translocated by the mass flow in the xylem and hardly redistributed (Michael et al., 1969; Mengel & Kirkby, 1978; Oertli, 1994), so that the leaf B concentration depends on the transpiration. This explains also the lower B concentration in young leaves compared to older leaves.

The calculated transpiration (section 3.5.2) was only slightly reduced over the whole cultivation period, but if considered over the period from the start of CO_2 enrichment until the date when leaves were first harvested for analysis (12 February until 18 March) the calculated transpiration was about 15% lower at high than at low C. In this period the sampled young leaves had expanded and it is presumable that this period had been critical for the development of B deficiency in the growing young leaves.

Boron content in young leaves was 21% lower at high C than at low C, both on 18 March and 15 April. This difference is larger than the calculated difference in transpiration. It may be explained by a additional dilution effect, as the DM production increased considerably by CO_2 enrichment. It is beyond the scope of this work to determine the magnitudes of the transpiration effect and the dilution effect on the leaf B concentration, because this requires measurements on individual leaves.

The lower B concentrations at high C were not caused by a lower B supply, as all four compartments received the same nutrient solution. Samples of the solution taken from the rockwool slabs, had B concentrations of about 100 µmol l^{-1} (at pH 6.2) in February and around 60 µmol l^{-1} (at pH 6.3) later in the season, which is supposed to be sufficient (Sonneveld & Straver, 1989). B uptake is favoured by a lower pH of the nutrient solution (Adams & Winsor, 1974; Mengel and Kirkby, 1978) and by a higher B supply (Sonneveld & De Bes, 1984, Baevre, 1990). However, recent observations demonstrate that a higher supply can not always prevent deficiency symptoms (Sonneveld, 1992, pers. comm.).

Recently, the experiment was repeated (Rijsdijk et al., 1993b), but extended with some levels of B supply. A reduced B supply hardly decreased the leaf B content. Only an extremely low supply gave a significant lower content and severe LTC. High C clearly aggravated LTC, but in spite of this, also remarkably increased the fruit production. These results confirm those presented above, but also this second experiment could not elucidate the background of boron in this respect.

It can be conclude that the observations confirm the hypothesis: high C decreased leaf conductance and transpiration (section 3.5.2); high C was correlated with reduced leaf B concentration; high C aggravated leaf tip chlorosis; leaves with LTC had the lowest B concentration. It is the most likely explanation that B deficiency, because of reduced transpiration at high C, caused the observed LTC. However, more specific research on boron is desired to further confirm the causal relationship between B and LTC, and if required, to find ways to increase the leaf B content.

Fruit production

Accumulated fruit production was 24% higher at elevated C than at low C. This CO_2 effect on fruit production is in the order of magnitude as generally reported in the literature (Kimball, 1986; Mortensen, 1987). So CO_2 enrichment increased fruit production, despite an increase of LTC. The light interception capacity of the canopy was relatively little affected by LTC, because of the large leaf area (LAI of about 5. For comparison: tomato often has an LAI of about 2, mainly due to the cultivation measure of removing older leaves). Obviously the small negative effect of reduced active leaf area was more than compensated for by the increased photosynthetic rate per unit leaf area.

These compensation aspects were verified with a simulation model for light interception and photosynthesis (Gijzen, 1992). The model calculations yield a rather crude approximation, as it was made for fixed conditions (air temperature 25 °C, global radiation outside 600 W m^{-2} and reference C of 500 µmol mol^{-1}). It does not account for the light interception by yellow leaves and for the fact that a partly yellow leaf is still partly active. These two latter simplifications will partly compensate for each other.

The model demonstrates that a 10% decrease in LAI would cause 6.5% reduction in calculated canopy photosynthesis if LAI 1.5 and 1.35 were compared; a 3% reduction if LAI of 3 and 2.7 were compared and only 1% if LAI of 5 and 4.5 were compared. Increasing C from 413 to 663 µmol mol^{-1} would increase the simulated canopy photosynthesis by 23% at LAI of 1.5 and by 21% at LAI of 5. So it is obvious that the positive effect of CO_2 enrichment is far larger than the effect of a (small) loss of leaf area by LTC. A marked reduction of LAI would be disadvantageous at low LAI, i.e. severe LTC is undesirable in early spring, whereas a (limited) reduction of LAI is acceptable in a full grown canopy. The increasing difference in fruit production between low and high C during the season is explained partly by the declining impact of leaf loss by LTC on canopy photosynthesis.

The total production was clearly favoured by CO_2 enrichment, despite increased leaf tip chlorosis. The recommended C in greenhouse cultivation is usually 700 to 900 µmol mol^{-1} (Mortensen, 1987). The C actually achieved in summer is far lower, as it is determined by technical, climatic and economic factors (Nederhoff, 1994a,b). The results of this experiment show that in spring (when high C can be achieved), it is advisable not to supply CO_2 excessively in a young eggplant crop (with a low LAI), for instance not to exceed 700 µmol mol^{-1}, in order to prevent or limit the incidence of LTC.

4.7 SYNTHESIS AND DISCUSSION

4.7.1 Structure

Section 4.7 first gives an overview for each crop species of the observed growth and production in relation to the CO_2 concentration. A hypothesis is proposed to explain the species-specific features. Subsequently, the various aspects introduced in section 4.1 (growth rate, light use efficiency, fruit set, DM allocation, leaf morphology, fruit production and fruit properties) are discussed. Section 4.7.5 presents an hypothesis, which is used to explain several observations (section 4.7.6 - 4.7.9). The overall effect of C on fruit production is discussed (section 4.7.10) by comparing the observed fruit production to the 'CO_2-rule'.

4.7.2 Growth and production of cucumber

Vegetative growth

In cucumber (section 4.3.1), a low and a high CO_2 treatment were applied, with average C of 364 and 620 µmol mol^{-1}, respectively, over the period 28 July until 19 October. The two C treatments were applied in triplicate and were multifactorially combined with two pruning treatments: the plants were either unpruned or fruit-pruned (2/3 of the inflorescences removed).

There were many interactions between the C treatments and the pruning treatments. Vegetative growth was increased by high C in the first two weeks of CO_2 enrichment, but after four weeks, most vegetative parameters were not significantly different between low and high C, at least in the unpruned cucumber crop (**Table 4.1a**). Also in other studies it was found that vegetative growth was stimulated by high C in the first weeks, while the differences in plant size soon levelled off or disappeared completely (Aoki & Yabuki, 1977; Klapwijk & Wubben, 1984; Kriedemann & Wong, 1984; Peet, 1986; Rijsdijk et al., 1992). In the fruit-pruned crops, however, CO_2 enrichment resulted in a higher weight of leaves and stems, and these differences still existed after some months (**Table 4.1**).

The vegetative dry weight gain between 28 July and 19 October was about equal for low and high C in the unpruned crop, while it was significantly increased by high C in the fruit-pruned crop (**Table 4.2**). On several observation dates, SLA and LAR were often not significantly affected by C or were lower at high C, particularly in the fruit-pruned crops (**Table 4.1**).

Fruit pruning significantly enhanced vegetative growth, for instance visible by a larger LAI (**Table 4.1**). This is explained by the fact that growing shoots became the major sinks for assimilates instead of fruits. These results agree with work of Marcelis (1993a) with cucumber, where decrease of the number of fruits per plant favoured vegetative growth. Fruit pruning did not significantly affect SLA in the current work. Also Marcelis (1993c) observed that SLA of cucumber plants did not respond to fruit pruning, except that it decreased when all fruits had been removed.

Fruit production

The onset of cucumber fruit production was sometimes found to occur a few days earlier in a CO_2 enriched crop than at low C (Enoch et al., 1970; Nederhoff & Schapendonk, 1985). Rijsdijk et al. (1992) found that the period between anthesis and harvest was one day shorter at high C, whereas Enoch et al. (1976) did not find such an effect. In the present work, the harvest started on the same day, but the first harvest was greater at high C than at low C. The different observations are explainable, because the time for harvesting a cucumber does not depend on ripeness, but on weight or size, and a harvestable size is reached earlier under high C. The period of fruit growth is influenced also by other factors, for instance temperature, light and number of competitive fruits (Marcelis, 1993a,b,c, respectively). This aspect was not quantified in the present work.

Fruit production (fresh as well as dry weight) was always bigger at higher C. In both experiments with cucumber, both the number of fruits and the average fruit weight were increased by high C, whereas fruit DMC was not significantly affected. In expt 1 in the unpruned treatment, fresh weight fruit harvest increased by 34%, 2/3 of which was caused by an increased number of harvested fruits and 1/3 by increased average fruit weight. Dry weight fruit production increased by 32%, 4/5 of which can be ascribed to the increased assimilation rate (apparent from an increased rate of dry weight gain) and 1/5 to a shift in DM allocation in favour of the fruits. In the fruit pruning treatment, the effect of C was a 20% increase in fresh weight fruit production, completely owing to increased number of fruits. Also dry weight fruit production increased by 20%, almost completely caused by increased assimilation under high C.

Fruit pruning reduced the number of harvested fruits only slightly. The relatively small reduction is explained by the increased growth of new lateral shoots with inflorescences in the fruit-pruned crops (section 4.7.5).

4.7.3 Growth and production of sweet pepper

Vegetative growth

Of sweet pepper (section 4.4) the two extreme CO_2 treatments are compared here: '250' and '450' with average C of 301 and 454 µmol mol^{-1}, respectively, over the period 8 August - 22 October.

High C tended to reduce the vegetative growth with respect to weights of leaves and stems and leaf area (**Table 4.15**). LAI was smaller, due to reduced absolute leaf weight per plant and reduced SLA. However, LAI was still reasonably large. Reduced vegetative growth is ascribed to competition for assimilates by growing fruits. This is particularly important in sweet pepper (Daunicht & Lenz, 1973; Hall, 1977; Rylski, 1986; Hubert, 1988; Klapwijk, 1988; Van Uffelen, 1988; Bhatt & Srinivasa Rao, 1989), but occurs also in eggplant (Claussen, 1976), tomato (Van Gurp, 1991), cucumber (Marcelis, 1993a) and other crops (Gifford & Evans, 1981). Because a CO_2 enriched sweet pepper crop bears more fruits (owing to better fruit set and less abortion of young fruits, section 4.4), the competition with vegetative growth is stronger in a CO_2 enriched crop than in an unenriched crop. Vegetative growth in sweet pepper plants is also restricted artificially because newly formed lateral shoots are topped, to prevent further growth. However, plants in low and high C were topped exactly equally.

Fruit production

Production was increased by increased fruit number, while average fruit weight was unaffected. The observed improvement in fruit production and reduced vegetative growth at high C imply that the DM allocation was appreciably shifted from vegetative to generative (reproductive) organs by CO_2 enrichment (see below, section 4.7.8). This shift in allocation caused a considerable part (1/3) of the increase in sweet pepper dry weight fruit production, while the other 2/3 was caused by increased CO_2 assimilation rate.

The relative difference in fruit production was 46 %, at C increasing from about 300 to 450 µmol mol^{-1}. This appears to be a great response, compared to for example Fig. 1.1. The main explanation is probably earliness: the CO_2 enriched plants had a higher early production than the non-enriched plants. It can be seen that the relative difference levelled off in the course of time (**Table 4.7**). In the short harvest period, this earliness still had an impact on the total harvest data. Secondly, C considered is low, and hence the C effect strong. Thirdly, the shift in DM allocation is appreciable, and additional to the increased photosynthesis.

The results generally correspond to previous reports on sweet pepper: Enoch et al. (1970) also found better fruit set; Milhet & Costes (1975) found far better fruit set and no difference in average fruit weight after CO_2 enrichment; Daunicht & Lenz (1973) found a considerable increase in partitioning of DM to the fruits at elevated C.

Table 4.15 **a.** *Growth and dry matter allocation of sweet pepper over the period 8 August - 22 October. Dry weight gain of various plant segments and of vegetative, generative and total above-ground plant tissue, and weight ratios. Absolute growth rate (GR) is dry weight gain per day. Light use efficiency (LUE) is dry weight gain per amount intercepted PAR.*
b. *Some plant parameters at the end of the experiment (see also Table 4.4 - 4.7).*

CO_2 'treatment' and (average):	'250' (306)	'450' (448)	LSD $_{0.05}$
a. dr wt gain 8 Aug - 22 Oct			
leaves (g m^{-2})	68.1	58.6	n.s.
stems (g m^{-2})	98.7	83.9	n.s.
fruits harvested (g m^{-2})	75.8	165.2	26
fruits on plant (g m^{-2})	89.1	60.2	n.s.
fruits pruned (g m^{-2})	10.4	19.1	n.s.
vegetative (g m^{-2})	167	143	n.s.
generative (g m^{-2})	175	244	n.s.
total (g m^{-2})	342	386	n.s.
ratio leaf / total (%)	19.9	15.2	3.1
ratio generative / total (%)	51.2	62.6	6.4
GR (g m^{-2} d^{-1})	4.56	5.15	n.s.
LUE (g MJ^{-1})	1.7	2.1	n.s.
b. stationary 22 October			
LAI	3.3	2.5	0.54
SLA (cm^2 g^{-1})	326	278	16
LAR (cm^2 g^{-1})	144	122	12
DMC leaves (%)	13.9	15.2	0.7
DMC stems (%)	12.8	13.9	1.2

4.7.4 Growth and production of tomato

Vegetative growth

Tomato (section 4.5) was grown at two CO_2 concentrations after 13 June: 370 and 510 µmol mol^{-1}. At high C compared to low C, weights of leaves and stems were higher, SLA and LAR were lower (**Table 4.16**) and DMC and starch content of leaves and stems were higher (**Table 4.9d,e**). These phenomena are characteristic for the 'short leaves syndrome' (SLS, sections 4.1.6 and 4.5). SLS was stimulated by a number of factors, e.g. high C, low plant density (i.e. high irradiance per leaf), fruit pruning. Two crops that were extreme with respect to SLS are compared here: crop-1 with plant density 3.1, without pruning, hardly affected by SLS; crop-2, with plant density 2.1 and with fruit pruning, suffering severely from SLS.

The approximated LAI of tomato seemed to be about equal at low and high C (**Table 4.16b**), in spite of a tendency to higher leaf weight at high C (**Table 4.16a**). The overall effect of C was a reduction of leaf area (**Table 4.9**). In crop-2 (with SLS) the approximated LAI was small, both at low and high C (**Table 4.16b**). Low values of LAI have been observed often in tomato, for instance De Koning (1993) reported an LAI as low as 1.3 for a producing crop in summer. A small leaf area is partly caused by picking the older leaves, which is a normal procedure in practical tomato cultivations. Notice that crop-2 had a 30% lower plant density than crop-1, so the difference in LAI between crop-2 and crop-1 must be ascribed for the greater part to the lower plant density and only for a small part to reduced leaf area per plant (**Table 4.11c**).

Fruit production

The relation between C and tomato fruit production was complicated and variable (**Table 4.11e**). There was a relation (probably causal) between C and SLS (section 4.5.4) and also a relation between SLS and response of production to C. In the crop with severe SLS (crop-2) there was only a statistically insignificant tendency to increased fruit production at high C. This disappointing effect of C in crop-2 is explainable, as SLS is a symptom of relative assimilate abundance, and CO_2 enrichment aggravated this problem.

In the sound crop (crop-1), the fresh weight of harvested fruits increased by 14%, and the fresh weight of fruits on the plant by 31% by high C, resulting in 18% increase of the total fruit dry weight production in crop-1. This increase must be ascribed to increased assimilation only, as the allocation was not significantly changed (**Table 4.16a**). High C favoured the fruit weight still on the plants (to be harvested in August) far more than the fruits production harvested by the end of July. This is explicable, because the earlier harvested fruits were grown partly before the start of the CO_2 treatment (section 4.5.3). In tomato, the results of a treatment are apparent only after a while, due to the long fruit growth period.

Table 4.16 **a.** *Growth and DM allocation of tomato over period 10 May - 31 July. Estimated dry weight gain of various plant segments and of vegetative (veg.), generative (gen.) and total above-ground plant tissue, and weight ratios. Absolute growth rate (GR) is dry weight gain per day; light use efficiency (LUE) is dry weight gain per amount intercepted PAR. low C = 370, high C = 510 μmol mol^{-1} average.*
b. *Some plant parameters at the end of the experiment (see also Table 4.9 - 4.11).*

	crop-1 [a]		crop-2 [b]		$LSD_{0.05}$
	low C	high C	low C	high C	
a. growth 10 May - 31 Jul					
leaves on plant (g plant^{-1})	60.4	73.3	65.0	76.7	14.9
leaves pruned (g plant^{-1}) [c]	18.8	18.8	18.8	18.8	-
stem (g plant^{-1})	68.4	78.9	85.8	99.4	9.8
fruits harvested (g plant^{-1})	161.9	180.8	159.8	169.3	12
fruits on plant (g plant^{-1})	79.7	103.8	64.4	68.8	22
vegetative (g plant^{-1})	148	171	170	195	23
generative (g plant^{-1})	242	285	224	238	24
total, veg.+gen. (g plant^{-1})	389	456	394	433	36
total, veg.+gen. (g m^{-2})	1223	1432	788	866	96
ratio leaf / total (%)	20.5	20.2	21.2	22.0	2.7
ratio gen. / total (%)	61.9	62.4	57.0	55.1	3.8
GR (g m^{2} d^{-1})	14.9	17.5	9.6	10.6	1.2
LUE (g MJ^{-1})	2.8	3.4	2.2	2.4	0.25
b. stationary 31 Jul					
LAI (approximated) [d]	3.3	3.2	2.0	1.8	0.03
SLA of top leaves (cm g^{-1})	174	141	145	116	22
LAR (cm g^{-1})	81.7	67.7	61.4	50.2	11

[a] *crop-1, sound crop, density 3.1 plants per m^2, without fruit pruning*
[b] *crop-2, with SLS, density 2.1 plants per m^2, with fruit pruning*
[c] *average weight over all treatments*
[d] *LAI could not be measured, but was approximated from leaf dry wt, SLA and plant density*

4.7.5 Hypothesis explaining species-specific features

Tomato, sweet pepper and cucumber showed similarities and differences in response to C (**Table 4.17**). One difference is that cucumber (unpruned treatment), in contrast to tomato and sweet pepper, did not show a clear reduction of SLA and leaf area and no increase in DMC of vegetative organs at high C. Another difference is that in tomato the DM allocation was not affected by C, whereas it was in cucumber and particularly in sweet pepper. The specific growth and production behaviour are explained below by sink and source relationships.

The source activity, i.e. canopy photosynthesis, determines the availability of assimilates. It depends mainly on light and C and on LAI. The sink strength is the demand for assimilates, determined by number, size and physiological age of fruits and by number of growing points and immature leaves per unit ground area.

Important facts are that sink rather than source determines the DM partitioning (section 4.1.4), and secondly that a relatively high source compared to sink means an overproduction of assimilates. Such a sink limitation, i.e. low sink/source ratio, usually causes storage of assimilates in leaves and reduction of SLA. There are some cases reported, where sink limitation caused suboptimal fruit production (e.g. Peet, 1984; see also section 4.5.4).

Table 4.17 *Summary of the CO_2 effects on growth and production as observed in cucumber (section 4.3.1), sweet pepper (section 4.4) and tomato (section 4.5). Data from Tables 4.2, 4.3, 4.15 and 4.16. Symbols: ≈ no tendency, ↑ increase, ↓ decrease, with increasing C; between brackets: statistically insignificant tendency.*

	cucumber	sw.pepper	tomato
leaf area index	≈	↓	↓
SLA	≈	↓	↓
(dry) weight leaves	≈	(↓)	↓
(dry) weight stems	≈	(↓)	↑
DMC leaves	≈	↑	↑
DMC stems	≈	(↑)	↑
fruit production	↑	↑	↑
average fruit weight	↑	≈	(↑)
DMC fruits	≈	≈	≈
allocation to fruits	↑	↑	≈

Cucumber

Cucumber plants are basically never sink-limited, in contrast to the other crops investigated. Cucumber plants continuously produce new lateral shoots, which are usually not removed. They bear at least one inflorescence per leaf axil, and sometimes more, particularly after many fruits have been harvested (Drews, 1979). In a normal productive crop, a considerable number of flowers abort (Marcelis, 1992), indicating that there are more than sufficient potential sinks formed. Because new shoots with inflorescences appear rapidly and because fruits are harvested after a short growth period (10 to 20 days, Rijsdijk et al., 1993a), sink strength can adapt (increase or decrease) rapidly to fit to the average assimilate production. So a cucumber plant can easily maintain a proper balance between vegetative and generative growth. Because the assimilates available can always be used in sinks, and need not to be stored in leaves and stems, SLA in cucumber was not severely affected by C (section 4.7.9).

Tomato

A tomato plant has only one main shoot with one vegetative growing point, as all lateral shoots are removed at appearance. After each third leaf, one truss is formed. The number of flowers per truss is different between cultivars and may vary slightly throughout the season, but it is generally limited (order of magnitude 7-15 flowers per truss). So the number of flowers present on a plant at a certain moment is bound by a maximum, in contrast to the abundant flowering of sweet pepper, eggplant and cucumber.

In tomato, as in other crops, the number of flowers that set fruit after pollination, depends basically on the amount of assimilates available. In the present experiment, however, the effect of increased assimilation by high C on fruit set was almost nil, because under the favourable light conditions (summer), virtually all flowers grew into fruits. So high C can hardly increase the number of generative sinks on the plants, i.e. CO_2 supply did not affect the sink strength. As the DM allocation is regulated by sink strength, rather than source activity, it is explicable that DM allocation was not changed by CO_2 supply in tomato.

The growing period of a tomato fruit is 45 to 60 days (Rijsdijk et al., 1993a), which is far longer than that of cucumber. The limited variation in number of fruits and the long fruit maturation period cause that there is far less possibility for adjusting the sink strength in tomato than in cucumber. In periods with high light and particularly when CO_2 is supplied, the CO_2 assimilation rate can exceed the demand for assimilates. A surplus of assimilates is stored in leaves and stems, which increases the leaf thickness, i.e. reduces SLA. An oversensitive response of a tomato crop may even cause the 'short leaves syndrome' (sections 4.5 and 5.3.3).

Sweet pepper

In sweet pepper, the features and also the proposed underlying mechanism, are least clear. During a short period with favourable conditions, many flowers may set fruit. Subsequently, over a longer period, the fruits will grow and mature (fruit growth duration 48 to 64 days, Rijsdijk et al., 1993a). Because in this period the major part of the assimilates is attracted by the growing fruits, the vegetative growth and the formation of new flowers and young fruits is temporarily inhibited (Daunicht & Lenz, 1973; Hall, 1977; Rylski, 1986; Van Uffelen, 1988; Hubert, 1988; Klapwijk, 1988; Bhatt & Srinivasa Rao, 1989; Van Gurp et al., 1991). This results in a clearly cyclic fruit production (Van Uffelen & Goebertus, 1985).

Due to increased fruit set under high C, the generative sink strength is higher at high than at low C, which explains the shift in DM allocation towards the fruits (section 4.7.8). Because fruit growth inhibits vegetative growth and formation of new fruits, the sink strength is fixed for a longer period and cannot easily adapt to increased assimilate supply, as described for cucumber. As only a more or less fixed amount of assimilates is demanded by the sinks, the assimilation may sometimes be abundant for a period of time. These assimilates can be stored in leaves and stems, which explains that also in sweet pepper, SLA is clearly lower at higher C (section 4.7.9).

4.7.6 Growth rate (GR) and light use efficiency (LUE)

Growth rate (GR) and light use efficiency (LUE) were given for cucumber in section 4.3.1 and are calculated here for sweet pepper and tomato. GR and LUE in the present study are based on above-ground dry weight gain. Notice that LUE depends on the LAI, because light interception is a function of LAI (section 4.1.2).

In cucumber (section 4.3.1), high C increased GR and LUE by about 25%, both in the unpruned and the fruit-pruned crop. LUE of cucumber was 3.4 and 4.3 g MJ^{-1} (g dry weight per MJ intercepted PAR), for low and high C, respectively (**Table 4.2**). This is an increase of 10 % per 100 μmol mol^{-1} CO_2.

With sweet pepper (section 4.4), GR and LUE seemed considerably increased by CO_2 enrichment, as the observed LUE values were 1.7 and 2.1 g MJ^{-1} (**Table 4.15**). However, these data do not allow a firm conclusion about effects of C on LUE, because of too few samples and too large variability between samples.

In tomato (section 4.5), GR was increased by CO_2 supply by about 17% in crop-1 and by about 10% in crop-2. LUE data of crop-1 were 2.8 and 3.4 and of crop-2 2.2 and 2.4 g MJ^{-1}, at low and high C, respectively, which was an increase of 21% in crop-1 and of 9% in crop-2 (**Table 4.16**). It must be emphasized that these calculations are made over the whole period between planting and ending of the experiment, while the CO_2 treatments were started only at 13 June. No samples were taken at that date, because no plants could be missed from the plots. Hence the growth over the actual treatment period were approximated by using estimates for the plant weight on 13 June, based on similar tomato plantings (De Koning, 1994, pers. comm.). The approximated relative effects of C on GR and LUE over the treatment period are at maximum 2% higher than the figures mentioned.

De Koning (1993) observed that GR (rate of above-ground dry weight gain) of tomato, ranged from about 5 to 25 g m^{-2} d^{-1}, with intercepted PAR ranging from about 1 to 8 MJ d^{-1}, and LUE ranging from 3 to 5 g MJ^{-1}. Heuvelink (1995b) observed twelve tomato cultivations and established a LUE of 2.5 g dry weight per MJ PAR received. Challa et al. (1994) derived a LUE of 3.1 g MJ^{-1}.

Summarizing, observed was an increase in LUE by CO_2 enrichment, varying from 6 (tomato crop-2) to 11 (cucumber) and 15 (tomato crop-1) % per 100 µmol mol^{-1} CO_2. The LUE values observed in cucumber and tomato compared reasonably with those reported in the literature for tomato. Sweet pepper had a lower GR and LUE than the other crops. Bruggink & Heuvelink (1987) also found a far lower growth rate of sweet pepper than of tomato and cucumber in young plants. The observed effects of C on GR are discussed in section 4.7.10, where they are compared to the 'CO_2-rule'.

4.7.7 Fruit set

Carbon dioxide obviously promoted fruit set and production of sweet pepper (section 4.4). The number of harvested fruits increased by 46% by CO_2 supply. This is ascribed to the direct positive effect of C on the rate of CO_2 assimilation and on carbon translocation (Ho, 1977). Positive effects of C on fruit set were also found by other authors, e.g. Enoch et al. (1970), Milhet & Costes (1975) in sweet pepper and Calvert & Slack (1975) in a winter planting of tomato. It must be emphasized that fruit set is usually limited by source in tomato in early spring (due to low light), while in summer fruit set of tomato is usually not critical. The growth of young fruits in cucumber (either winter or summer planting) is usually not considered a problem.

4.7.8 Allocation of dry matter

In the literature, a wide range of percentages of DM allocation to fruits can be found (section 4.1.4). In the present studies, the DM allocation was determined at the end of the cultivation and includes all biomass grown during the cultivation, including harvested and removed biomass, except roots. So **Tables 4.2, 4.15** and **4.16** present the long-term cumulative DM distribution. The DM allocation found for cucumber was similar or higher than the reported values. Those for sweet pepper and tomato were lower than generally reported. This is explicable, because the present experiments with sweet pepper and tomato covered relatively short periods, while the fruit growth period is long. It is normally observed in those two crops that the share of the fruits in the cumulative biomass production gradually increases during the season (Rijsdijk et al., 1993a).

It is of interest to consider if the CO_2 concentration affected the DM allocation. The hypothesis was that sink rather than source determines the allocation (section 4.1.4). CO_2 supply influences primarily the source. In some experiments, sink was manipulated additionally by fruit pruning.

In the unpruned cucumber crop (section 4.3.1), the share of the fruits in the dry weight gain increased by CO_2 enrichment at the expense of leaf growth (**Table 4.2**). This is in accordance with the hypothesis, because the generative sink (number of fruits) was remarkably increased by CO_2 enrichment, owing to formation of new shoots with inflorescences. The higher demand for assimilates by the fruits increased the assimilate flow towards the fruits. It appeared that in this unpruned crop, sink strength of fruits in competition for assimilates was predominant above that of the vegetative sinks, as fruit growth was favoured relatively more than vegetative growth by CO_2 enrichment. This is probably partly a numerical matter, as there were more generative than vegetative sinks in the unpruned treatment.

Reducing the number of generative sinks by means of fruit pruning stimulated the growth of lateral shoots with inflorescences. So the loss of generative sinks was compensated for by formation of new vegetative and generative sinks. Also on the new shoots, frequently 2/3 of the new inflorescences were removed. The ultimate result of CO_2 enrichment in the fruit-pruned crop was a strong increase both in vegetative and generative dry weight gain, so that obviously no clear change in DM allocation occurred.

In sweet pepper the contribution of fruits in the total dry weight gain was far higher at higher C, while the dry weight gain of leaves and of stems was lower, not only relatively but even absolutely. This caused that the percentage generative of total dry weight gain was 63% at high C, compared to 51% at low C (**Table 4.15**). This is a consequence of competition: a CO_2 enriched crop has a better fruit set (**Table 4.6** and section 4.7.7) and hence a larger number of fruits on the plant. These fruits form a stronger sink strength, attracting more assimilates at the expense of vegetative organs. According to the hypothesis, increased fruit sink strength is responsible for increased DM allocation to fruits. This corresponds to findings of other authors (section 4.7.3) that fruit growth suppresses the vegetative growth in sweet pepper.

In tomato, the estimated DM allocation was compared between low and high C in crop-1 (with high density, without pruning, little SLS) and in crop-2 (with low density, fruit pruning, severe SLS). The contribution of fruits in biomass production was significantly lower in crop-2 than in crop-1, which is a direct consequence of fruit pruning. This was also found in tomato by Hurd et al. (1979). In contrast to cucumber, tomato could not compensate for the removed fruits by forming extra shoots or fruits. So reduction of generative sink strongly reduced DM allocation to the fruits. This in accordance with the hypothesis on DM allocation (section 4.1.4), that sink rather than source determines the DM allocation.

There was no significant effect of C on DM allocation (**Table 4.16**), which is also in accordance with this hypothesis. CO_2 enrichment did not change the sink strength in tomato, shown by almost equal numbers of fruits harvested in the two CO_2 treatments. This experiment was done in summer, when tomato usually sets fruit easily (notice that this tomato crop was sink-limited, section 4.5). In contrast to the present work, Frydrych (1984) observed that CO_2 enrichment in tomato plants increased the DM allocation to the fruits. In that case, however, fruit set was enhanced by CO_2 supply, leading to a higher generative sink strength. Thus these observations also fit the hypothesis.

4.7.9 Morphological characteristics

Specific leaf area (SLA)

SLA was reduced by elevated C in sweet pepper, tomato and eggplant. The reduction was 15 to 20%, at C increasing by 150 to 250 $\mu mol\ mol^{-1}$. Because SLA is affected by so many other factors, it was not attempted to derive a relation between SLA and C from few data.

In general, SLA is reduced by high C, as well as by bright light. This is usually ascribed to a surplus of carbohydrates relative to the assimilate demand i.e. sink limitation (Hurd, 1968; Jolliffe & Ehret, 1984; Acock & Pasternak, 1986) or more particularly to accumulation of starch in leaves (Madsen, 1976;

Goudriaan & De Ruiter, 1983; see Farrar & Williams, 1991). Other authors observed more densely packed palisade mesophyll cells and also more layers of these cells (see Acock & Pasternak, 1986). In the present tomato experiment, the leaf starch content was significantly higher at elevated C, but the starch content observed on 31 July was too low to explain the difference in SLA. Therefore, obviously the amount of structural DM per leaf area was increased.

Reduction of sink strength basically has a similar effect on SLA as increasing the source. SLA tended to be lower in a fruit-pruned crop of tomato (**Table 4.10**), but not in cucumber (**Table 4.1**). The fact that in cucumber SLA was not influenced by C and fruit pruning, is explicable because this crop can maintain a properly balanced sink/source ratio (section 4.7.5). According to the literature, fruit pruning reduced the SLA in various experiments, e.g. in sweet pepper and tomato (Frydrych, 1984), in sweet pepper (Bhatt & Srinivasa Rao, 1989), but in cucumber only after complete fruit removal (Marcelis, 1993d).

Hence, reduced SLA can be regarded as characteristic of morphological adaptation of a canopy to assimilate abundance, which can be a consequence of high C. The 'short leaves syndrome' in tomato can be regarded an extreme form of this adaptation, enforced by other effects (sections 4.5 and 5.3).

Reduced SLA is particularly undesirable in canopies with a low LAI (as usually in tomato): such a crop would favour from increased leaf area (increased light interception capacity) and not from increased leaf thickness. In a canopy with a large LAI (for example full-grown eggplant, section 4.6.4, or sweet pepper), the effect is less harmful. A low SLA seems in any case a dissipation of energy, as the biomass is not used for increase of production, nor for increase of the production capacity.

Dry matter content of vegetative organs

The DMC of vegetative parts was slightly increased in sweet pepper leaves (**Table 4.5**), tomato leaves and stems (**Table 4.9d**) and tended to be increased in eggplant leaves (**Table 4.12f**). In cucumber, DMC of leaves and stems was not consistently influenced by C (**Table 4.1**). An increased leaf DMC was often reported. According to Idso & Kimball (1988), the DMC of plants is not influenced by C, except that leaves can have a higher value, owing to storage of starch. Indeed in tomato a higher DMC was accompanied by a higher leaf starch content, but this could not completely explain the difference in DMC. The absence of such a tendency in cucumber is ascribed, again, to the easily adjustable sink/source ratio (section 4.7.5).

4.7.10 Fruit production

Quantity of fruit production

The total DM and fruit production of the four crops investigated were shown in previous tables, and are summarized in **Table 4.18.** The relative effects of C per unit CO_2 (% per µmol mol^{-1}, abbreviated here to % μ^{-1}) are calculated. The figures for relative fresh and dry weight fruit production response are equal, because fruit DMC was not affected by C. After Slack et al. (1988), the absolute increase in harvested fruit weight per week per µmol mol^{-1} CO_2 was calculated.

The response of DM production to C was highest in tomato and lowest in sweet pepper. The response of fruit production to C (absolute increase in dry weight and relative increase in dry or fresh weight) appeared by far the strongest in sweet pepper. This is explainable by earliness, low C (far from saturation) and shifted DM allocation in favour of the fruits (section 4.7.3).

Table 4.18 *Approximated effects of CO_2 concentration on fruit production in cucumber (Table 4.2), sweet pepper (Table 4.7 and 4.15), tomato (Table 4.16) and eggplant (Table 4.13). Fruit production observed at low (L) and high (H) C are compared in absolute (abs.) or relative (rel.) sense.*

	CO_2	cucumber		pepper	tomato		eggpl.
		without pruning	fruit-pruned		crop-1 sound	crop-2 SLS	
harvest duration (weeks)		12	12	7	5	5	22
average CO_2 level	L	364	364	306	370	370	413
(µmol mol^{-1})	H	620	620	448	510	510	663
total DM production	L	819	874	342[a]	389	394	-
(g m^{-2})	H	1021	1092	386[a]	456	433	-
rel. effect on total DM	L						
production (% μ^{-1})	H	0.096	0.097	0.086[a]	0.12	0.07	-
fresh weight harvested	L	16.79	17.15	2.69	8.40	5.12	19.5
(kg m^{-2})	H	22.11	20.66	3.96	9.33	5.37	24.1
abs. effect on fresh weight harvest (g m^{-2} week^{-1} μ^{-1})		1.73	1.14	1.28	1.33	0.36	0.84
abs. effect on dry weight harvest (g m^{-2} week^{-1} μ^{-1})		0.062	0.041	0.100	0.074	0.020	0.064
rel. effect on fresh & dry weight harvest (% μ^{-1})		0.12	0.08	0.33	0.08	0.03	0.09

[a] *data from Table 4.15, covering a shorter period than in Table 4.7*

With respect to the absolute difference in fresh weight of fruits, cucumber shows the strongest response to C and eggplant the weakest. Also sweet pepper responded less than cucumber, despite the strong response in production of fruit dry weight. These results are explainable by the differences in fruit DMC (cucumber 3.6, tomato 5.6, eggplant 7.6 and sweet pepper 8%, independently of C). The second cucumber crop and tomato crop-2 lagged behind in fruit production, explainable by fruit pruning. Also other authors found that cucumber responded stronger with respect to fresh weight fruit production, than tomato (Slack & Hand, 1985; Slack et al., 1988).

Production compared to the 'CO_2-rule'

The observed relative effects of C on fruit production are compared here to the expected relative effect at a particular C, based on the CO_2-rule (eqn. 2.7b). This rule describes only the effects of C on DM production, so if there is an additional influence of C on DM allocation, this will cause a discrepancy between observed and expected relative effect of C. Also possible negative effects interfering with the fruit production process might be revealed by this comparison. The relative effects are expressed in % (µmol mol^{-1})$^{-1}$, which is abbreviated to % μ^{-1}.

In cucumber, grown at C of 364 and 620 µmol mol^{-1}, respectively, the expected relative C effect was about 21%, or 0.08 % μ^{-1}. The observed relative effect on growth rate (GR, i.e. rate of DM production) was 0.095 % μ^{-1}, which exceeds the expected value. The observed relative effect on fruit production was 0.12 % μ^{-1} in the normal cultivation, and 0.08 % μ^{-1} in the fruit-pruned crop. This difference between the two crops can be explained only partly by the shift in DM allocation under high C in the normal cultivation.

In sweet pepper, the C range considered was 306 to 448 µmol mol^{-1}, and the expected relative effect was 21% (or 0.15 % μ^{-1}). The relative effect of C on DM production (although calculated over only a part of the production period) was 0.09 % μ^{-1}. The relative effect on fruit production was extremely high (46% or 0.33 % μ^{-1}), which was ascribed to several effects (section 4.7.3).

Eggplant was investigated in the C range between 413 and 663 µmol mol^{-1}. The expected relative effect was 0.07, and the observed effect was 0.09 % μ^{-1}.

Tomato was investigated in the range 370 to 510 µmol mol^{-1}, where the expected relative effect was about 0.10 % μ^{-1}. The observed relative effect of C on DM production was 0.12 % μ^{-1}. The total fruit production, with the fruits on the plants included (section 4.5.3 and **Table 4.11e**), increased by 0.12 % μ^{-1}, which exceeds the expected relative effect. Crop-2 responded far less, due to SLS and partial deflowering.

Generally, the CO_2-rule yielded too small values (except in tomato crop-2), when applied to fruit production. In sweet pepper, an additional effect of increased DM distribution to fruits and somewhat earlier production in CO_2-enriched crops (**Table 4.7**) can explain a part of the discrepancy between expected C and observed C effect. It will be discussed in section 5.2 whether conditions as light flux and leaf area index can explain the underestimation by the CO_2-rule. A preliminary conclusion is that generally the CO_2-rule provides a conservative approximation of the effect of C on fruit production.

4.7.11 Fruit properties

Weight and dry matter content of fruits

The average fruit weight was generally increased by CO_2 enrichment (cucumber, **Table 4.1**; tomato, **Table 4.9i**; eggplant, **Table 4.13b,c**), as reported by many authors (e.g. Acock & Pasternak, 1986). Only in sweet pepper, C had no significant effect on the average fruit weight (**Table 4.7b**), in accordance with Milhet & Costes (1975). This might be explained by the fact that the fruits that were set additionally (owing to CO_2 enrichment) were mainly fruits on lateral shoots, which usually lag behind in growth.

Fruit DMC tended to decrease slightly at high C in tomato (**Table 4.9j**) and eggplant (section 4.6.3), and was not significantly affected by C in cucumber (**Table 4.1**). No effect of C on fruit DMC was expected (section 4.1.5). The reduction in DMC that was observed sometimes might be explained by indirect effects, for instance by the fact that a reduced stomatal conductance and transpiration at high C may increase the relative water content. DMC of tomato fruits was higher in the fruit-pruned crop than in the unpruned crop (**Table 4.10j**), which corresponds to the fact that fruit DMC increases with decreasing number of fruits on the plant Marcelis (1993b).

Fruit quality

Various aspects of fruit quality have been considered in several experiments. In earlier work (Nederhoff & Schapendonk, 1985), cucumber fruits were judged for the colour, directly after harvest and after storage under controlled conditions. The fruits of the non-enriched crop were initially slightly greener, which was explained by higher irradiance in the less dense canopy, but the difference levelled off during two weeks of storage. In sweet pepper (section 4.4.3) and tomato (section 4.5.3), no significant differences in external fruit quality were observed. There was a tendency for increased blossom end rot in tomato at high C, but it was not significant. Blossom end rot was discussed in relation to calcium (section 4.5.4). Eggplant fruits (section 4.6.3) from low and high C did not differ with respect to specific mass and occurrence of second class fruits. Also no CO_2 effect was found on firmness, colour of fruit and calix and percentage calix rot of eggplant fruits, either directly after harvest or after storage under controlled conditions. The fact that hardly any aspect of fruit quality was significantly affected by C, agrees with the literature (section 4.1.5).

5 Final discussion

5.1 RESULTS CONSIDERED

The work presented in the previous three chapters has enhanced the insight in effects of the CO_2 concentration on some important physiological processes of greenhouse fruit vegetable production. The following results can be considered as new contributions to the existing knowledge: data files with photosynthesis measurements, the 'CO_2-rule', quantification of stomatal response to C, improved insight in the relation between stomata and transpiration, demonstration of the insignificance of the effect of C on leaf temperature and of the absence of an endogenous 'midday depression' in the transpiration and photosynthesis measurements, analysis of differences between crops regarding sink/source ratio and DM partitioning, hypothesis for the incidence of 'short leaves' in tomato and advice for effectively preventing it, explanation for the incidence of leaf tip chlorosis in eggplant, test of intermittent CO_2 supply.

The results obtained fulfil the main objectives and related research questions (section 1.4). Considering canopy photosynthesis (P, chapter 2), the observed effects of C on P were presented by a set of regression equations. These generalized the results and demonstrated the effect of C on certain photosynthesis characteristics, in reply to research question Ia. In view of the aim to establish a rule of thumb for C effects, the 'CO_2-rule' was proposed (see next section). Another objective was to test a simulation model for canopy photosynthesis to be applied in climate control and decision support systems. After two well-known models were tested, it was concluded that certain applications would require a model that accounts better for effects of C on P. It is anticipated that a more elaborated model, assembled from described physiological knowledge, probably gives a better performance (Gijzen et al., 1995).

The effect of C on DM production and fruit production was quantified in several experiments (chapter 4), in view of research question Ib. The observed effects of C on production were compared to the CO_2-rule, because this is an easy and generic description of the CO_2 effect. In one particular case (tomato crop-2), the observed C effect was lower than that predicted by the CO_2-rule, due to negative side effects of CO_2 enrichment. Two side effects of high C were investigated: reduced transpiration (chapter 3) and morphological adaptation (chapter 4). The discussion on side effects (section 5.3) gives an answer to research questions IIa & IIb. Also intermittent CO_2 supply, intended to avoid side effects of high C, is discussed further (section 5.4).

5.2 THE 'CO_2-RULE'

The CO_2-rule proposed was: $\mathbf{X = (1000/C)^2 * 1.5}$ (eqn. 2.7b), with X the relative effect of an increase in C (in % per 100 µmol mol^{-1}). The merits of a rule of thumb need no discussion, but important is its validity, i.e. its capability for predicting the effect of C. It must be considered what the consequence is of not accounting for the interaction of CO_2 with light and with temperature; and whether the rule is applicable at low and high LAI, etc.

A point of discussion is, why the observed effects of C on fruit production were higher than the effect of C on P calculated by the CO_2-rule, as seen in section 4.7.10. It was expected that fruit production, an overall result, would show a lower response than the photosynthesis-based CO_2-rule. It is possible that the crops in the photosynthesis experiments were adapted to high C (although it was attempted to avoid this), whereas in the later experiments these adaptations were prevented better. Another possible explanation is an effect of earliness under high C, for instance in the sweet pepper experiment (section 4.7.3). A third possible explanation is that the average light flux played a role.

In order to establish the validity and restrictions of this CO_2-rule, and to test the significance of interactions of C and light, the CO_2-rule is compared to calculations with a canopy photosynthesis model (Gijzen, 1994, pers. comm.). This model is based on the leaf photosynthesis model of Farquhar et al. (1980), the light interception model of Gijzen (1992) and Spitters et al. (1989). This model is preferred for testing the CO_2-rule, because it is probably at present the most appropriate for this purpose. It comprises a detailed calculation of the response of P to the main environmental factors, based on described physiological knowledge. The model will be discussed in great detail in other studies (Gijzen et al., 1995; Heuvelink, 1995a). Some comparisons between model and rule are presented in **Fig. 5.1**.

Light is by far the most important factor determining the response of P to C, far more important than temperature, and also more important than LAI, if LAI exceeds 2 (**Fig. 5.1**). At low light, the CO_2-rule overestimates the response of P to C. At medium high light, the relative effect simulated by the model agrees very well with the relative effect approximated by the CO_2-rule, irrespective of LAI and temperature. The interaction with light explains why in **Fig. 2.3** the CO_2-rule compared very well with the regression lines of expts 1, 2 and 3 (with relatively high light conditions, see **Table 2.1**), while the rule overestimated the observations of expts 4, 5 and 6 (lower light conditions, **Table 2.1**). Notice that the CO_2-rule was established arbitrarily after comparison with the regression lines (section 2.6).

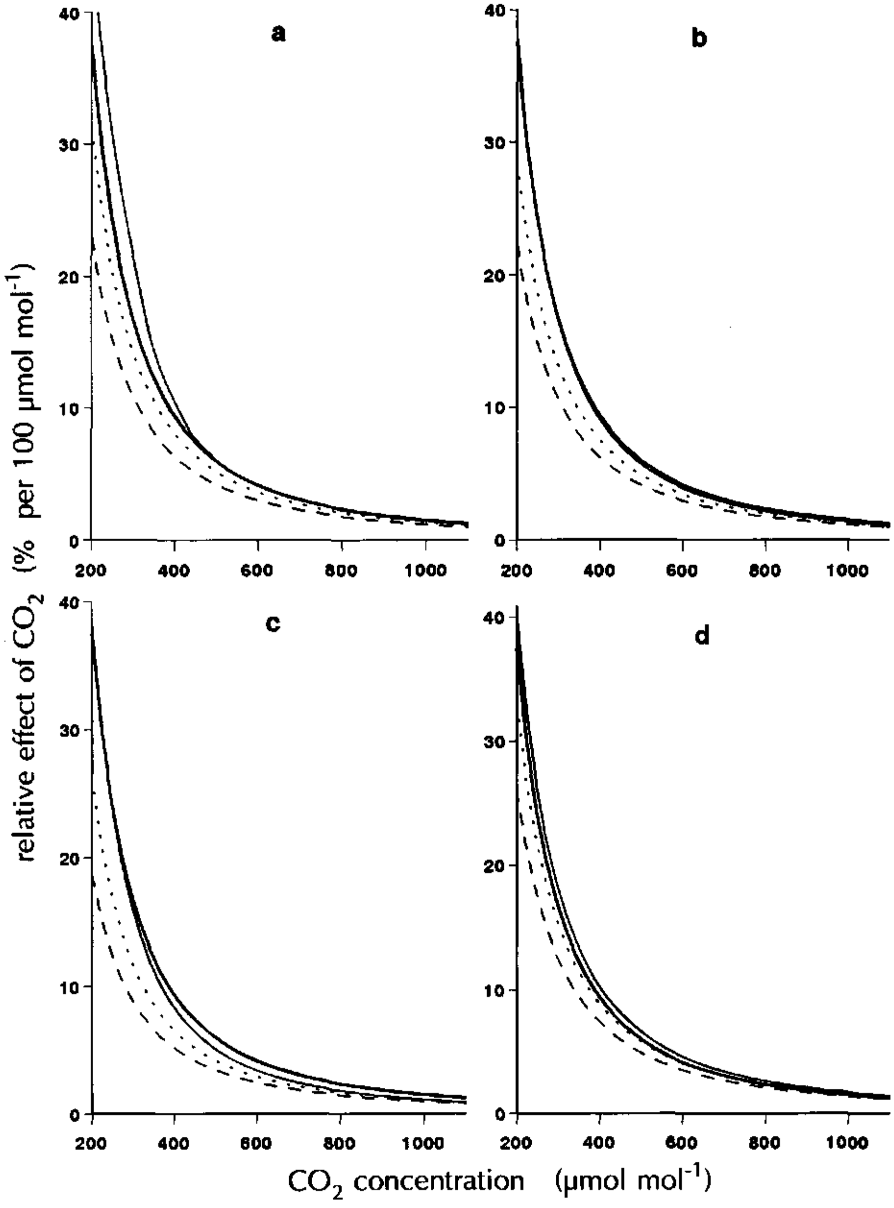

Fig. 5.1 Comparison of the relative effect of the CO_2 concentration (in % per 100 µmol mol^{-1}) calculated by the 'CO_2-rule' and by the model of Gijzen (1994).

——— 'CO_2-rule' [$X = (1000/C)^2 * 1.5$]

- - - - - model of Gijzen at low light (100 µmol m^{-2} s^{-1} PAR intercepted)

. . . . model of Gijzen at medium light (500 µmol m^{-2} s^{-1} PAR intercepted)

——— model of Gijzen at high light (1000 µmol m^{-2} s^{-1} PAR intercepted).

a. *LAI=1, T=25 °C;* **b**. *LAI=5, T=25 °C;* **c**. *LAI=3, T=20 °C;* **d**. *LAI=3, T=30 °C.*

The higher relative response to C at high light also explains that the observed responses of fruit production were higher than estimated by the CO_2-rule, as discussed in section 4.7.10. Since the CO_2-rule was derived from photosynthesis measurements under moderate light conditions, the CO_2-rule revealed a moderate response to C. The effects of C on production, in contrast, were studied mainly under summer light conditions.

Finally, the CO_2-rule is compared to the CO_2-production curve, which was derived previously from data from the literature (**Fig. 1.1**). The two curves compare favourably (**Fig. 5.2**), which confirms the conclusion that the CO_2-rule may be used to approximate the effect of C on production. The reason why the curve based on the literature is somewhat lower than that of the CO_2-rule, is again the average light flux. Many experiments referred to in Fig. 1.1, were done under poor light conditions, due the practical problem of obtaining high C under high radiation influx.

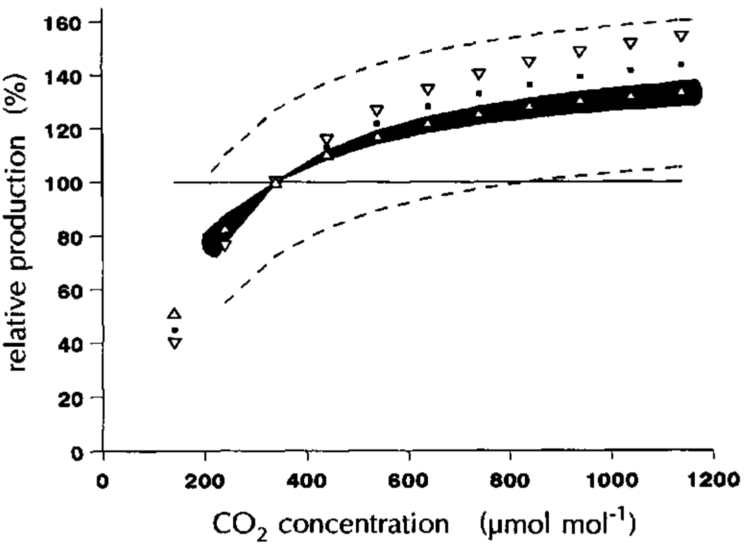

Fig. 5.2 Relative production (%, with 100% at 340 μmol mol^{-1}) of greenhouse fruit vegetable crops, according to the literature (Fig. 1.1) and according to the CO_2-rule.
▬ 95% confidence interval of mean of the observations,
- - - - - *95% confidence interval of the observations*
symbols: ■ *X = 1.5 *\ (1000/C)²* ▲ *X = 1.2 * (1000/C)²* ▼ *X = 1.8 * (1000/C)²*

The conclusion is that the rule is roughly applicable to instantaneous (and also daily) canopy photosynthesis, as well as to production. If production must be predicted for unfavourable light conditions, or if clear negative side effects (e.g. leaf deformation) are expected, the CO_2-rule may yield an overestimation. In this case the multiplication factor (1.5 in eqn. 2.7b) may be adjusted downwards. Similarly, when clear positive effects are known to occur in a certain case (e.g. increased DM allocation to the harvestable parts, or high light influx), one can account for these effects by increasing the multiplication factor. The lower and higher boundary of the multiplication factor will be in the order of 1.2 and 1.8, respectively, as was estimated on the basis of the photosynthesis regression lines (section 2.6.3) and production data from the literature. Fig. 5.2 shows these lower and higher boundaries of the CO_2-rule.

As the CO_2-rule agrees reasonably well with the curve obtained from data of various flower and vegetable crops (**Fig. 5.2**), it can be concluded that the CO_2-rule can be used as a generic rule for greenhouse crops. It must be emphasized again that it is not more than a rule of thumb, not appropriate to account for refined differences.

5.3 SIDE EFFECTS OF CO_2 ENRICHMENT

Possible side effects of high C are partial stomata closure and reduced transpiration, secondly morphological adaptation, and thirdly reduced photosynthetic efficiency. Two side effects have been dealt with in different chapters, and here below these are considered in a more integral discussion.

Partial stomatal closure does reduce the transpiration, but only to a small extent (section 3.7.2). This appeared not to cause direct problems, neither regarding increased leaf temperature (section 3.7.4), nor regarding fruit quality or smaller fruit quantity (except in tomato crop-2). Possible indirect effects of reduced transpiration, however, might arise from a reduced uptake of nutrients. This is similar to reduced nutrient uptake at low transpiration, due to high air humidity. In this case the reduced nutrient content appeared to be most pronounced in the apex and youngest leaves (Armstrong & Kirkby, 1979; Ehret & Ho, 1986). Several authors reported a reduced leaf mineral concentration in response to high C (Porter & Grodzinski, 1984; Eng et al., 1985; Peet et al., 1986; Kuehny et al., 1991).

In the eggplant experiment, high C aggravated leaf tip chlorosis (LTC), probably due to boron deficiency in young, rapidly growing leaves. In the eggplant cultivation under investigation, the production was not negatively affected by LTC, because the effect of the loss of some active leaf area was not disadvantageous in this crop with a large leaf area. However, reduced uptake of a nutrient might also be at the basis of SLS, which certainly is a harmful phenomenon. There may be a relation or interaction between Ca and B (Armstrong & Kirkby, 1979; Jyothi & Shanmugavelu, 1985; Jones et al., 1991b). The effects of Ca and B deficiency are being studied further in new projects at the Glasshouse Crops Experimental Station in Naaldwijk (De Kreij, 1994, pers. comm.).

Reduction of specific leaf area (section 4.7.9) is considered a characteristic of morphological adaptation of the canopy to high C, or more in general, adaptation to assimilate abundance (i.e relative sink limitation). An extreme form of this adaptation is the 'short leaves syndrome' in tomato. However, this is not solely caused by sink limitation, but also by reduced calcium translocation at high C. The conclusion that reduced stomatal conductance and morphological adaptation are both involved in SLS, causing the weak response of fruit production to C, may be an answer to research question II for this particular case (section 1.4.2). It is of interest to investigate if calcium is involved in general in the adaptation of SLA to the conditions.

SLS and LTC are considered no serious drawback of CO_2 enrichment per se, but the result of a number of factors. As both occur under particular conditions only, there are measure to prevent them, without ceasing the CO_2 supply. Deficiency of B, probably causing LTC, occurred mainly in young, relatively fast growing leaves (transpiring organs) after a period of low transpiration. The assumed Ca deficiency in the tomato apex (a non-transpiring organ), which is probably at the basis of SLS, was associated with a low sink/source ratio. As discussed previously, good production results can be achieved if the CO_2 supply is continued (at an acceptable rate), while SLS in tomato is avoided by increasing sink/source ratio or in eggplant by tolerating some LTC, as this is not necessarily disadvantageous. Only in extreme cases, a modest CO_2 supply is recommended.

As long as a canopy is not closed (i.e. does not completely cover the soil area), high C promotes the leaf growth, thus increases the light interception and canopy photosynthesis (Gifford & Evans, 1981). After canopy closure, leaf area growth has less effect on light interception. In addition, the increase of leaf area may be restricted by reduction of SLA of newly formed leaves grown under high C. These processes explain the strong positive effect of high CO_2 on plant growth early in the cultivation, and the declining effect later (section 4.7.2). Besides these morphological adaptations, also physiological adaptation occurs, as declining photosynthetic efficiency per unit leaf area (CO_2 exchange rate, CER) after long-term CO_2 enrichment (Hurd, 1968; Tanuka & Fujita, 1974; Clough & Peet, 1981; Dugal et al., 1990). It has been suggested that this photosynthetic adaptation to elevated C might be related to feedback inhibition by nutrient deficiency (Dugal et al., 1990; Bunce, 1992). This might affect the photosynthetic efficiency in several ways. There are, for example, indications that the Ca concentration in the xylem sap influences the stomatal conductance reaction pattern (Atkinson et al., 1990). New research on photosynthetic adaptation on the scale of whole crops in greenhouses is intended by Heuvelink (1994, pers. comm.).

5.4 INTERMITTENT CO_2 SUPPLY (ICS)

Two experiments were done with ICS, one with cucumber (section 4.3.2) and one with sweet pepper (section 4.4). It must be emphasized that ventilation was not done intermittently, but in the standard way, i.e. on the basis of temperature. This was done in order to avoid effects of temperature and humidity (section 4.1.8) and hence deterioration of the crop and fruit quality (Slack et al., 1988).

ICS clearly reduced the CO_2 expenditure and also reduced the average CO_2 concentration achieved. Hence it is logical that the increment in production was lower than with constant high C. Mostly, the resulting fruit production was reduced about proportionally to the reduction in C. These results agree with those of Slack & Hand (1985) and Willits & Peet (1989), who found no particular favourable effects of 'partial' or 'fractional' enrichment. The adaptations of the crop to C were not clearly less pronounced under ICS than under a constant C. The treatments with low-frequent intermittent supply appeared to give slightly better results, but the differences were insignificant.

Various reports about ICS are available in the literature. Clough & Peet (1981) introduced ICS, intermittent on the basis of days. Their results were intermediate to those of continuous low and high C, i.e. growth was about proportional to the average C. Mortensen (1984) compared ICS on hourly basis with continuously high C and with a treatment without enrichment in chrysanthemum and saintpaulia cultivars. The results were different between species and between cultivars. The same author concluded later that continuous high C generally had effects superior to those of ICS (Mortensen, 1986). In an experiment under sub-tropical conditions (Zipori et al., 1986), ICS combined with intermittent ventilation, gave better results than selective enrichment only when the ventilation windows were closed. Dugal et al. (1990) reported that ICS yielded the same daily sum of photosynthesis as constant high C did. However, the photosynthesis measurements were made on excised leaves of tomato seedlings, and the measuring conditions (particularly humidity near the leaves) were not representative for a full-grown producing crop in a greenhouse. This has probably affected the stomatal conductance in an unrealistic way.

Based on the presented experiments and the literature, the following considerations are made:

- intermittent supply greatly reduces the expenditure of CO_2 compared to supply to a constant level, particularly under ventilation;
- a limited CO_2 supply (e.g. ICS) under a normal ventilation regime can accomplish only a limited increase of the average C;
- fruit production was often found to be in accordance with the average C;
- thus ICS can accomplish only a limited increase in fruit production;

- in cucumber, the efficiency of CO_2 utilization was about equal in a constant high C and in an ICS treatment; only low-frequent ICS seemed more efficient;
- in sweet pepper, ICS gave similar morphological adaptations as constant C did (at comparable average C), only low-frequent ICS seemed to induce relatively less morphological adaptation;
- no clear physiological advantages or disadvantages of intermittent CO_2 supply could be observed compared to constant level supply.

The choice of a CO_2 regime is always a matter of economic optimization. When the product price is low, it is not feasible to increase the production at the expense of much CO_2. Under unfavourable economic conditions, it might therefore be useful to save on carbon dioxide expenditure by using ICS. So ICS can be a feasible option, if the expenditure of CO_2 must be greatly reduced for economic reasons, or if the CO_2 supply must be stopped for technical reasons, for example if a CO_2 generator (burner) must be stopped frequently because of excessive heat production.

5.5 EVALUATION OF THE APPROACH

At the end of this study, the pros and cons of the approach and the consequences for the validity of the conclusions are considered. The aim of this study was to investigate the effect of C on greenhouse crops under practical conditions. All experiments were done in full-grown canopies in medium-size greenhouses.

The reason for using relatively large-scale facilities was to achieve realistic conditions, for instance with respect to interactions between stomatal conductance, greenhouse air humidity, leaf temperature and transpiration (feedback effects). Small-scale experiments may easily lead to incorrect conclusions in this respect (section 3.1.1). The present work demonstrated a decoupling between stomata and transpiration in canopies in greenhouses, so that the response of the transpiration rate to variations in C was restricted.

For canopy photosynthesis and transpiration research, it was necessary to use whole canopies rather than a few plants in a cuvette, as the light interception and the climatic conditions should be comparable to those in real greenhouse cultivations (discussed in section 2.8). Fruit production can only be studied in canopies in their reproductive phase, i.e. in crops of a considerable size and age. Adaptation of a crop to the conditions requires a certain period of time. There points are all arguments to conduct experiments on the scale of a real greenhouse.

For investigating climatic conditions, it is inevitable to use a separate greenhouse compartments for each treatment level. So an experiment with a number of CO_2 concentrations, set up with sufficient replicates for statistical analysis, requires a large number of identical greenhouse compartments. These should be equipped with sophisticated technical facilities, in order to obtain practical conditions. There are probably no greenhouse research facilities in the world that fulfil these requirements. The facilities of the Glasshouse Crops Research Station in Naaldwijk are well equipped, but the number of identical compartments is still limited. Most experiments were done with not more than two or three replicates. Hence, the results showed sometimes a clear tendency, but the natural variability in responses and the limited replications impeded the statistical significance of some results, e.g. in case of C effects on growth of sweet pepper (section 4.4).

Another drawback of the approach was that the practical problems that occur in normal greenhouse cultivations, also bothered the experiments. For instance, high radiation forced the ventilation windows to be opened, which impeded to maintain high C at high light influx. Although this condition resembles practical horticulture, it is an omission in the data, which hindered firm conclusions about for instance maximum photosynthesis rate and midday depression.

Another disadvantage of working in large greenhouses is that the conditions could not be manipulated as in small-scale or in laboratory experiments. For instance in the transpiration measurements, the air humidity could not be controlled without affecting the air temperature (by heating or ventilating) and/or the canopy transpiration. Therefore, air humidity was not actively controlled, but was recorded as it was, and used as input factor in the analysis.

The set up of the study was to investigate several topics one by one. Measurements on canopy photosynthesis were made in a separate series of experiments and the measurements on leaf conductance were done in another series. In both series, C was deliberately maintained equal in different compartments (except on measuring days), to avoid differences between crops. Hence, the long-term effect of C on growth and production had to be studied in a new series of experiments. It was considered a disadvantage that the observations on various aspects could not be combined, or at least be done in the same cultivation. Hence, for example the rule of thumb for CO_2 effects, derived from photosynthesis measurements, was compared to fruit production data observed in other experiments. Yet certain observations cannot fully be explained, because several factors were different in the various series of experiments.

Recommendations for practical horticulture

Preventing morphological adaptation to high C

CO_2 enrichment reduces the specific leaf area (SLA, leaf area per unit leaf dry weight), as a consequence of increased assimilate production. CO_2 enrichment might disturb the balance between assimilate production by the source and assimilate utilization in the sinks. As an example in tomato, high C increases the source strength, but cannot affect the sink strength (the apex and a limited number of trusses). A too low sink/source ratio in tomato severely reduces the SLA, and even causes leaf deformation, in the form of small and curled leaves, called 'short leaves syndrome' (SLS). This may occur also in other crops (e.g. ornamentals) with comparable conditions.

It is recommended as a method to minimize the harmful effects of increased source, to increase the shoot density. This decreases the source by mutual shading of leaves and increases the sink by increasing the number of vegetative and generative sinks per unit ground area. The shoot density can be enhanced either by choosing a more narrow plant distance, or in a winter planted year-round crop, by maintaining an extra shoot on the plants in spring. In other crops, particularly in cucumber, the sink strength (number of growing shoots and fruits) easily adapts to the increased source strength. When measures to prevent adaptation are not effective, the CO_2 supply may be restricted.

CO_2 supply in relation to stomata closure

Increase of the CO_2 concentration decreases the stomatal conductance considerably. The net overall effect of CO_2 supply on transpiration of a crop in a greenhouse, however, is a relatively small decrease (the extent of the effects depends on the conditions). The reduction in transpiration is restricted by adapted air humidity and leaf temperature (feedback effects).

Reduced transpiration may reduce the translocation of the nutrients calcium and boron, which is disadvantageous in the long term. Particularly during a longer period of low light conditions, it is required to give extra attention to maintaining sufficient transpiration (by ventilating, heating, preconditioning) and nutrient uptake (supply and uptake conditions). Only if a serious threat for insufficient nutrient uptake exists, it is advisable to restrict the

CO_2 supply. This is not very disadvantageous under poor light, because the potential photosynthesis is low, and thus the deprivation small.

Under high light conditions, the transpiration is sufficiently high, so that a reduction caused by high C is usually not critical for nutrient translocation. However, it is possible that local nutrient deficiencies occur as a consequence of increased phloem import at the expense of calcium containing xylem import. Hence, it is recommended to be aware of the Ca supply to non-transpiring organs (fruits, apex). Reduced transpiration will theoretically increase the leaf temperature, but this effect appeared to be small.

Concern for a 'midday depression'

No evidence was found for an <u>endogenous</u> midday depression or declining photosynthetic and transpirational activity during the course of a day. Hence there is no reason from this view point, to cease the CO_2 supply during midday. The potential CO_2 uptake is highest at high light, i.e. at midday. However, a critical requirement is that the water supply and water uptake capability of the crops are optimal.

Maximum CO_2 concentration

Aiming for CO_2 concentration at about threefold the outside value (i.e. 1000 μmol mol^{-1} at present) has been recommended for some years already. Arguments were that canopy photosynthesis tends to saturate at about that level, whereas the risk for possible disadvantages increases (see above). In case of supply of flue gases from a combustion installation, the risk of accumulation of harmful gases is an extra reason for restricted supply. The results of the present work, conducted in semi-practical greenhouses, substantiate the general recommendation, from a physiological point of view.

Under some conditions, 1000 μmol mol^{-1} might be supra-optimal, for example in tomato when the sink/source ratio is obviously disturbed. Eggplant has an extraordinary strong stomatal sensitivity to C. Reduced transpiration may induce leaf tip chlorosis, but this is not necessarily harmful. Whenever the effect of CO_2 enrichment is suspected, also in other crops, it is advised to restrict the supply. At twofold the outside concentration, a large positive effect on P is obtained, due to the saturation (3/4 of the effect of threefold the outside level, according to the CO_2-rule) and the negative effect on stomata is acceptable, as the stomatal response is linear (3-4 % per 100 μmol mol^{-1}).

Minimum CO_2 concentration

At a high light influx, the absolute quantity of CO_2 uptake by the crop is of course greater than at low light, and the relative effect of high C appears more pronounced at high light (**Fig. 5.2**). CO_2 enrichment is conflicting with the high rate of ventilation, which normally accompanies high radiation. It is recommended for bright weather conditions to consider the supply of CO_2 at least to maintain the outside concentration, because of the relatively strong effect of increased C at high light and at low C (**Fig. 5.1** and **5.2**). According to the CO_2-rule, an increase in C from 250 to 350 µmol mol^{-1} generally increases the production by 25%, and at high light even more. The feasibility and the technical solution (pure CO_2, heat storage) must be considered for individual cases.

Intermittent CO_2 supply (ICS)

Intermittent CO_2 supply can be recommended as a method to reduce the expenditure of CO_2. However, the effect of ICS on production is reduced in parallel with the reduction in average C. No clear advantages nor disadvantages were observed regarding plant physiological responses. When the CO_2 supply must be strictly limited for economic reasons, or should be done intermittently for technical reasons, there is no reason to advise against ICS.

CO_2-rule

The 'CO_2-rule' derived in this study was: $\mathbf{X = (1000/C)^2 * 1.5}$, with X the relative effect of an C increase, in % per 100 µmol mol^{-1}. It can be used as a rule of thumb for CO_2 effects, i.e. as a tool for macro studies and global explorations concerning CO_2 enrichment. It provides a global approximation of the effect of C on canopy photosynthesis and also on fruit production. The dependence of the C effect on the light conditions is ignored in the CO_2-rule. Whenever a certain positive condition is known (e.g. high light intensity or favourable assimilate distribution to fruits) or a certain negative condition (SLS in tomato), these effects can be taken into account additionally by adjusting the multiplication factor (now 1.5) to about 1.2 at unfavourable conditions or to 1.8 at favourable conditions.

Summary

Growing under glass offers the opportunity to benefit from the stimulating effect of elevated CO_2 concentration (C) of the air on plant growth. In greenhouse horticulture, CO_2 enrichment is commonly applied to enhance the production. In chapter 1 of this dissertation, the history of CO_2 enrichment in greenhouses is reviewed and the current methods for CO_2 supply are described. The generalized effect of C on the production of greenhouse crops is demonstrated by a CO_2-curve, based on literature data.

The main effect of elevated C is an increase of the net CO_2 assimilation rate; other possible effects of C are influences on flowering, fruit set, dry matter allocation to fruits and dry matter content of fruits. Also negative side effects may occur. The objective of this thesis is analysis and quantification of the main and side effects of C in the range 200 to 1100 μmol mol^{-1} on growth and production of tomato (*Lycopersicon esculentum* Mill.), cucumber (*Cucumis sativus* L.), sweet pepper (*Capsicum annuum* L.) and eggplant (*Solanum melongena* L.), grown in greenhouses. All experiments were performed with full-grown crops in semi-practical greenhouses, under normal operating conditions.

Photosynthesis

The effect of C on canopy net photosynthetic CO_2 assimilation rate (or photosynthesis, P) was investigated in chapter 2. Particular aims were to derive a rule of thumb for overall calculations concerning the effect of C on P, and to test a model to be used in climate control and decision support systems. Measurements were done in six cultivations, each using some greenhouse compartments. The method involved an accurate estimation of the greenhouse CO_2 balance, using nitrous oxide (N_2O) as tracer gas to determine on-line the exchange rate between greenhouse and outside air. The obtained photosynthesis data comprised an estimated inaccuracy of about 10%, except larger errors under particular circumstances in 5% of the data.

A set of regression equations, relating P to PAR, C and LAI, was fitted to the measurements, and explained 83 to 91% of the variance. The main canopy photosynthesis characteristics calculated with these fitted regression equations were reasonably in accordance with data from the literature or could be explained by the conditions.

A rule of thumb ('CO_2-rule') was derived from the fitted regression equations. This CO_2-rule stated **$X = (1000/C)^2 * 1.5$**, with X in % per 100 μmol mol^{-1}, and C in μmol mol^{-1}. It approximates the relative increase of canopy photosynthesis, caused by a 100 μmol mol^{-1} increase at a certain C.

Two models for canopy photosynthesis (Acock et al., 1978 and Thornley, 1976) were examined by comparing them with the experimental photosynthesis data. Measured environmental conditions and measured LAI were input to the model. The (modified) models performed reasonably, but the Thornley model in particular did not adequately represent the effect of C on P. The photosynthesis measurements were analyzed for the occurrence of time-dependent variations in P, irrespective of the conditions (e.g. 'midday depression'). No indications were found of an endogenous depression or decline in the diurnal course of P.

Transpiration

The effects of C on conductance (*g*) of leaves (i.e. of stomata) and on rate of crop transpiration (*E*) were investigated in chapter 3. In sweet pepper, tomato and cucumber, measurements were done in greenhouse compartments that were usually exposed to equal C, but on measuring days, simultaneously exposed to different C.

Leaf conductance was measured on the upper leaves, using a steady state diffusion porometer. Measurements were done during a number of days in several greenhouse compartments. Multiple regression equations, describing the effects of PAR, VPD-leaf-air, C and optionally leaf temperature on *g*, were fitted to the measured data. The fitted equations demonstrated that 100 μmol mol^{-1} increase in C in the range 300 to 1200 μmol mol^{-1} reduced *g* by about 3 to 4% in sweet pepper, tomato and cucumber and by about 11% in eggplant. These relative effects are valid at any level of C, VPD and PAR, if VPD and PAR would remain constant. In eggplant, *g* was measured in two glasshouse compartments where the crops were exposed continuously to low or high C. The stomatal behaviour of eggplant did not adapt to long lasting CO_2 conditions.

Generally, partial stomata closure reduces the leaf transpiration, which reduces the air humidity (in the boundary layer and in the surrounding greenhouse air) and may slightly increase the leaf temperature. So an increase in C usually leads to a marked increase in VPD-leaf-air, which enforces the effect of elevated C on *g* and counteracts the effect of C on *E*. These phenomena, known as hydrologic and thermal feedback effects, were observed.

Rates of crop transpiration, measured with three weighing lysimeters per compartment, were highly correlated to radiation. The effect of C on *E* was analyzed by combining the regression equation for *g* (see above) with the Penman-Monteith equation for *E*. Calculations by this combination, using measured environmental conditions as input data, agreed reasonably with measured *E*.

The relation between *g* and *E* was expressed by a coupling factor, *K* (relative change in calculated *E* divided by relative change in calculated *g*). This was estimated to be mostly less than 0.2, except at low radiation. This implies that the reduction of the transpiration rate of greenhouse crops caused by (moderate) CO_2 enrichment is usually small or even negligible, except under low light conditions. In eggplant, however, the reduction can be larger, due to the stronger stomatal response to C. It was, for instance, 15% in a certain period in spring.

No time-dependent variation or 'midday depression' was observed in the transpiration measurements (as in the photosynthesis measurements). Also no significant effect of C on average leaf temperature was established.

Growth and production

The effects of C on growth and production are studied by investigating the underlying processes in five experiments (chapter 4). Some features were investigated only in one species, that was known to respond clearly in this respect.

In an autumn cultivation of cucumber, the effects of C on vegetative and generative parameters were quantified. Two CO_2 concentrations (364 and 620 μmol mol^{-1} on average) were maintained in triplicate, multifactorially combined with two treatments of fruit pruning (unpruned and 2/3 of inflorescences removed). High C stimulated vegetative growth only in the first weeks. Later, mainly fruit production was favoured by high C, thus increasing the relative allocation of biomass to the fruits. DMC of vegetative parts and SLA were not markedly affected by fruit pruning nor by C in this cucumber experiment.

In sweet pepper, the number of harvested fruits was increased by 46% by high C (450 μmol mol^{-1}) compared to low C (about 300 μmol mol^{-1}), whereas the average fruit weight was not affected. The vegetative growth was lower at high C. This implies that the allocation of biomass to the fruits was strongly increased by high C, owing to increased sink strength by fruits (at high C more fruits were set). Leaf DMC was higher and SLA was smaller at high C.

Tomato in summer commonly suffers from a type of morphological adaptation, called here 'short leaves syndrome' (SLS), characterized by severely reduced SLA. As a working hypothesis, it was postulated that SLS is a consequence of an oversupply of assimilates relative to the sink capacity (i.e.

sink-limitation or low sink/source ratio). In twelve glasshouse compartments, the sink/source ratio was varied by maintaining two levels of CO_2 (350 and 550 µmol mol^{-1}) multifactorially combined with two plant densities and three pruning treatments. CO_2 enrichment and wider planting enhanced SLS, i.e. decreased the leaf area and SLA. In crops with little SLS symptoms, CO_2 enrichment considerably increased the fruit production, whereas in crops with severe SLS, CO_2 enrichment aggravated SLS and had no significant effect on fruit production.

The mechanism proposed to explain SLS in tomato, is that local calcium deficiency arises through two processes. The first is that high C increases the assimilate availability (source strength), implying that the phloem flux into the sinks increases, at the expense of the calcium-containing xylem influx. The second process is that high C reduces the transpiration and hence the translocation of calcium. This leads to a lower Ca content, possibly (according to the literature) most pronounced in the apex. It is often reported that a low Ca content in the apex reduces the area of newly formed leaves. The combination of small leaves (due to Ca deficiency in the apex) and thick leaves (due to storage of assimilates) is manifest as SLS.

As the stomata of eggplant appeared to be more sensitive to C than those of the other crops investigated, this species is particularly of interest to study a nutrient-based disorder. Eggplant often shows chlorosis on the distal end of the leaves (leaf tip chlorosis, LTC) in spring, particularly under high C. An experiment was done to investigate the effects of C on LTC, on leaf boron content and on fruit production of eggplant. Two levels of CO_2 (413 and 663 µmol mol^{-1}) were maintained in duplicate. The results support the hypothesis that LTC is related to reduced boron content in young, fast growing leaves. LTC and boron deficiency were significantly more severe at high than at low C. However, fruit production was 24% higher at high C than at low C, despite more severe LTC. This is explicable, because this crop had a large LAI (of about 5), so the loss of some active leaf area hardly affected canopy photosynthesis.

Analysis of the growth and production data obtained in the five experiments, revealed the following:

- Light use efficiency (LUE) was increased by CO_2 by about 10 to 15% per 100 µmol mol^{-1} increase in C.
- Fruit set of sweet pepper was greatly increased by high C.
- Allocation of biomass (i.e. DM distribution) to fruits was increased by high C in sweet pepper and cucumber, explained by increased sink strength.

- (Except in cucumber) increase of C by 150 to 250 μmol mol^{-1} reduced the specific leaf area (SLA) by 15 to 20% and slightly increased the dry matter content (DMC) of vegetative organs, owing to assimilate abundance.
- Fruit production (dry weight) was affected by C the most in sweet pepper, owing to increased CO_2 assimilation and increased DM allocation to fruits at high C.
- Cucumber showed the largest increase in fresh weight per unit CO_2, explainable by a low fruit DMC.
- Fruit quality was not influenced by C, which was in accordance with the literature.

The observed effects of C on fruit production were compared with the CO_2-rule (see above), which was derived from photosynthesis measurements. In all crops, the observed effect was larger than the expected effect, based on this CO_2-rule. This was ascribed mainly to favourable light conditions in the production experiments.

Intermittent CO_2 supply (ICS) was tested, which was proposed in the literature as a method to maintain a high CO_2 use efficiency. In cucumber (expt 2) two continuous CO_2 concentrations and two ICS treatments were compared in duplicate. ICS reduced the CO_2 use considerably, but reduced the increment in production about proportionally. In sweet pepper, three continuous levels, two ICS regimes and a control were compared in duplicate. The results with respect to setting and yield were not better at ICS than at continuous CO_2 levels, if related to average, measured C.

Conclusions

- Partial stomata closure hardly has an effect on leaf temperature nor any direct effect on fruit production. A possible indirect effect of reduced conductance in the long term is reduced nutrient contents in the crop. This may play a role in certain features, for example leaf tip chlorosis in eggplant (associated with boron deficiency) and short leaves in tomato (explained by local calcium deficiency).
- No endogenous 'midday depression' (dip or decline in photosynthesis and transpiration), was observed in the experiments, under the condition that the crop was well supplied with water and capable of adequate water uptake.
- High C generally increased the light utilization (LUE), fruit set (except in tomato), allocation of dry matter to fruits, dry matter content of vegetative organs and considerably increased fruit production. High C generally reduced SLA.
- Many features were explicable by considering the effect of C on the sink/source ratio. High C basically increases the source, but it may indirectly

increase the sink strength as well (by better fruit set in sweet pepper and formation of more new shoots in cucumber).

- In the CO_2-rule [$X = (1000/C)^2 * 1.5$, with X in % per 100 µmol mol^{-1} and C in µmol mol^{-1}] the multiplication factor (1.5) may be adjusted to the conditions (in particular the average light level): upwards to about 1.8 in case of favourable (light) conditions, or downwards to about 1.2 under poor (light) conditions. The CO_2-rule agreed reasonably well with the CO_2-curve, which was a generalized description of the effect of C on production.
- Intermittent CO_2 supply (ICS) under standard ventilation could accomplish only a limited increase in average C, and hence a limited increase in production. No physiological advantages (nor disadvantages) of ICS were observed.

Samenvatting

Telen onder glas biedt de mogelijkheid maximaal te profiteren van het stimulerende effekt van verhoogde CO_2-concentratie van de lucht (C) op plantengroei. In de glastuinbouw wordt op grote schaal CO_2-dosering toegepast om de produktie te verhogen. In hoofdstuk 1 van deze dissertatie wordt kort de geschiedenis van CO_2-dosering in kassen belicht en gangbare methoden voor dosering beschreven. Het effekt van CO_2 op produktie van kasgewassen wordt weergegeven door middel van een CO_2-curve, gebaseerd op literatuurgegevens.

Het belangrijkste effekt van CO_2-dosering is verhoging van de netto fotosynthese snelheid. Andere mogelijke effekten zijn beïnvloeding van bloei, vruchtzetting, droge stof verdeling naar de vruchten en droge stof gehalte van de vruchten. Ook kan CO_2-dosering nadelige neveneffekten oproepen. Doelstelling van dit proefschrift is het analyseren en kwantificeren van de hoofd- en neveneffekten van C in het bereik van 200 tot 1100 μmol mol^{-1} op groei en produktie van de gewassen tomaat (*Lycopersicon esculentum* Mill.), komkommer (*Cucumis sativus* L.), paprika (*Capsicum annuum* L.) en aubergine (*Solanum melongena* L.) in kassen. Alle experimenten werden uitgevoerd met volgroeide gewassen in semi-praktijk kassen, onder normale teeltcondities.

Fotosynthese

Hoofdstuk 2 is gericht op onderzoek naar het effekt van C op de netto CO_2 assimilatie (of fotosynthese, P) van het gewas. Speciale doelstellingen hierbij zijn het afleiden van een vuistregel om globaal het effekt van C op P te kunnen schatten, en het testen van een model, geschikt voor klimaatregeling en beslissingsondersteunende systemen. In zes experimenten werden in meerdere afdelingen metingen verricht. De gebruikte methode berust op een nauwkeurige schatting van de CO_2-balans van de kas. Daarbij werd gebruik gemaakt van lachgas (N_2O) als tracergas om on-line de ventilatiesnelheid te bepalen. De verkregen fotosynthesedata bevatten een onnauwkeurigheid van ongeveer 10%, afgezien van grote relatieve fouten die onder bepaalde omstandigheden optreden (in ca. 5% van de data).

Uit de metingen is een set van regressievergelijkingen afgeleid, die P relateren aan PAR, C en LAI. Hiermee kon 83 tot 91% van de variantie verklaard worden. De belangrijkste gewasfotosynthesekarakteristieken die met deze gefitte regressievergelijken werden berekend, waren redelijk in overeenstemming met data uit de literatuur of konden goed verklaard worden door de omstandigheden.

Een vuistregel voor CO_2 effekten is afgeleid met behulp van de regressievergelijkingen. Deze CO_2-regel, die luidt $X = (1000/C)^2 * 1.5$, berekent bij benadering het relatieve effekt op de gewasfotosynthese van een verhoging van 100 μmol mol^{-1} bij een zekere CO_2-concentratie C (X in % per 100 μmol mol^{-1}, and C in μmol mol^{-1}).

Twee modellen voor gewasfotosynthese (Acock et al., 1978 en van Thornley, 1976) zijn onderzocht door ze te vergelijken met de experimenteel bepaalde fotosynthese gegevens. De (enigszins aangepaste) modellen voldeden redelijk, maar vooral het Thornley model berekende het effekt van C op P niet adekwaat. De fotosynthesemetingen werden ook geanalyseerd voor het optreden van een tijdsafhankelijk variatie in P, onafhankelijk van de omstandigheden (b.v. een zogenaamde 'midday-depression'). Er werden geen aanwijzingen gevonden voor het bestaan van een endogene depressie of daling in P gedurende de dag.

Transpiratie

Het effekt van C op geleidbaarheid (*g*) van blad (of van stomata) en op de snelheid van verdamping door het gewas (*E*) zijn onderzocht in hoofdstuk 3. In paprika, tomaat en komkommer werden metingen gedaan in verschillende kascompartimenten, waar gelijke CO_2-concentraties werden aangehouden. Alleen op meetdagen werd het nivo verschillend ingesteld. Metingen werden verricht gedurende verscheidene dagen in meerdere kasafdelingen.

Geleidbaarheid werd gemeten met een steady-state diffusie porometer. De metingen werden geanalyseerd met multiple regressie. De gefitte vergelijkingen beschrijven het effekt van PAR, VPD-blad-lucht, C en optioneel bladtemperatuur op *g*. De analyse toonde aan dat per 100 μmol mol^{-1} verhoging van C (in het bereik 300 tot 1100 μmol mol^{-1}) *g* gereduceerd werd met ongeveer 3 à 4% in paprika, tomaat en komkommer en met circa 11% in aubergine. Deze percentages gelden bij ieder nivo van C, VPD en PAR, mits VPD en PAR constant blijven. Bij aubergine werd *g* gemeten in twee kasafdelingen, een met constant hoog en een met altijd laag C. Het gedrag van de stomata vertoonde geen aanpassing aan C waarbij het gewas opgroeide.

Partiële sluiting van stomata veroorzaakt een daling van de verdamping, die vervolgens de luchtvochtigheid verlaagt (in de grenslaag en in de kaslucht) en mogelijk de bladtemperatuur enigszins verhoogt. Dit heeft tot gevolg dat de VPD-blad-lucht merkbaar toeneemt, wat het effekt van verhoogd C op *g* versterkt en het effekt van C op *E* tegenwerkt. Deze zogenaamde hydrologische en thermische feedback effekten werden in de waarnemingen opgenomen.

Gewasverdamping, gemeten met drie weegschaal-lysimeters per kasafdeling, was sterk gecorreleerd met straling. Het effekt van C op *E* werd bestudeerd door gebruik te maken van de genoemde regressievergelijkingen voor *g*, in combinatie met het Penman-Monteith model voor *E*. Berekeningen met deze combinatie, waarbij gemeten klimaatomstandigheden als invoergegevens dienden, bleken redelijk overeen te komen met de metingen.

De relatie tussen *g* en *E* werd tot uitdrukking gebracht met een zogenaamde koppelingsfaktor, *K* (relatieve verandering in berekende *E* gedeeld door relatieve verandering in berekende *g*). Volgens berekeningen was deze *K* meestal lager dan 0.2, behalve bij lage straling. Dit betekent dat afname in verdamping als gevolg van (gematigde) CO_2-dosering gering is en meestal verwaarloosbaar, behalve bij weinig licht. Bij aubergine kan het effekt echter sterker zijn, vanwege de sterkere stomataire gevoeligheid voor C, zo werd bijvoorbeeld 15% reduktie vastgesteld over een periode in het voorjaar.

Er werd geen tijdsafhankelijke variatie ('midday depression') gevonden in de metingen (evenmin als in de fotosynthesemetingen). Ook werd geen effekt van C op de gemiddelde bladtemperatuur vastgesteld.

Groei en produktie

Het effekt van C op groei en produktie werd geanalyseerd door de onderliggende processen te bestuderen in vijf experimenten (hoofdstuk 4). Sommige aspekten werden slechts in een gewas bekeken, waarvan bekend was dat het duidelijk reageert in dit opzicht.

In een herfstteelt van komkommer werd het effekt van C op vegetatieve en generatieve parameters gekwantificeerd. Twee CO_2-concentraties (364 en 620 μmol mol^{-1} gemiddeld) werden aangelegd in drievoud, multifactorieel gecombineerd met twee vruchtdunningsbehandelingen (ongedund en met verwijdering van 2/3 van de vruchtbeginselen). Hoog C stimuleerde de vegetatieve groei alleen in de eerste weken. Daarna werd vooral de vruchtproduktie gestimuleerd. De droge stof verdeling naar de vruchten was hoger bij hoog C dan bij laag C. DMC (droge stof gehalte) van de vegetatieve delen en SLA (specific leaf area) werden niet door C beïnvloed in dit experiment met komkommer.

Bij paprika was het aantal geoogste vruchten 46% hoger onder hoog C (450 μmol mol^{-1}) vergeleken met laag C (ongeveer 300 μmol mol^{-1}), waarbij het gemiddeld vruchtgewicht ongeveer gelijk was. De vegetatieve groei was minder bij hoog C dan bij laag C. Dit houdt in dat de relatieve verdeling van biomassa naar de vruchten sterk was toegenomen door hoog C, tengevolge van een sterkere sink-werking door de vruchten (bij hoog C waren meer vruchten gezet). DMC van de bladeren was hoger en SLA was lager bij hoog C in paprika.

Tomaat heeft in de zomer vaak te lijden van bepaalde morfologische aanpassingsverschijnselen ('kort blad' of 'short leaves syndrome', afgekort SLS), vooral gekenmerkt door sterk gereduceerde SLA. Als werkhypothese werd gesteld dat SLS veroorzaakt wordt door een overmaat van assimilaten, relatief ten opzichte van de sink capaciteit (dus lage sink/source verhouding ofwel sink-limitering). In twaalf kascompartimenten werd de sink/source verhouding gevarieerd door twee CO_2-concentraties aan te houden (350 en 550 µmol mol^{-1}) en dit te combineren met twee plantdichtheden en drie snoeibehandelingen. CO_2-dosering en ruimere plantafstand verhevigden het optreden van kort blad. In gewassen met geringe mate van SLS, kon CO_2-dosering de vruchtproduktie behoorlijk verhogen, terwijl in gewassen met ernstig kort blad, CO_2 geen statistisch betrouwbaar effekt had op de produktie.

Ter verklaring van kort blad in tomaat wordt een mechanisme voorgesteld, gebaseerd op calcium gebrek, dat kan ontstaan door twee processen. Ten eerste verhoogt CO_2-dosering de beschikbaarheid van assimilaten (source sterkte), wat inhoudt dat er een grotere floeemstroom de sinks bereikt. Dit gaat ten koste van de calcium-bevattende xyleemstroom. Ten tweede wordt door hoog C de verdamping wat verminderd, zodat het transport van calcium afneemt. Dit kan lokaal het calcium gehalte verminderen, wellicht (volgens de literatuur) met name in de apex. Het is bekend dat een laag calciumgehalte in de apex tot gevolg heeft dat nieuw gevormde bladeren kleiner blijven. De combinatie van klein blad (door lokaal calcium gebrek) en dik blad (door opslag van de overmatige assimilaten) manifesteert zich als SLS.

Omdat de stomataire reaktie op C gevoeliger bleek te zijn bij aubergine dan bij de andere onderzochte gewassen, was aubergine bij uitstek geschikt voor onderzoek aan afwijkingen in verband met voeding. Aubergine vertoont in het voorjaar vaak bladpuntvergeling (leaf tip chlorosis, LTC), vooral onder hoog CO_2. In een experiment werd het effekt onderzocht van C op LTC, boriumgehalte van het blad en vruchtproduktie bij aubergine. Twee nivo's van CO_2 (413 en 663 µmol mol^{-1}) werden in duplo aangelegd. De resultaten ondersteunden de hypothese dat LTC veroorzaakt wordt door een laag boriumgehalte in jonge, snelgroeiende bladeren. LTC en boriumtekort waren ernstiger bij hoog dan bij laag C. De vruchtproduktie was echter belangrijk hoger (24%) bij hoog dan bij laag C, ondanks LTC. Dit is verklaarbaar, omdat dit gewas een hoge LAI had (ongeveer 5), zodat enig verlies van aktief blad nauwelijks de gewasfotosynthese kon beïnvloeden.

Analyse van de vele groei- en produktiegegevens brachten onder andere de volgende zaken aan het licht:

- De lichtbenuttingsefficientie (LUE) was 10 tot 15% hoger per 100 μmol mol^{-1} stijging in C.
- Vruchtzetting van paprika werd sterk gestimuleerd door verhoogd C.
- Verdeling van biomassa (droge stof) naar de vruchten was hoger onder hoog C in paprika en komkommer, wat verklaard werd door verhoogde sink-sterkte.
- Behalve in komkommer, zorgde een verhoging in C met 150 tot 250 μmol mol^{-1} voor een verlaging van de SLA met 15 tot 20% en een kleine verhoging van het DMC van de vegetatieve organen, tengevolge van assimilatenoverschot.
- Vrucht produktie (droge stof) werd het sterkst beïnvloed door C in paprika, ten gevolge van een verhoogd CO_2 assimilatie en verhoogde droge stof verdeling naar de vruchten bij hoog C.
- Komkommer vertoonde de sterkste toename in vers gewicht produktie per eenheid CO_2, te verklaren door een laag DMC van de vruchten.
- Vruchtkwaliteit werd niet beïnvloed door C, wat overeenkomt met de literatuur.
- In alle gewassen was het waargenomen effekt van C op produktie groter dan het effekt berekend met de CO_2-regel (zie boven). Dit kon worden toegeschreven aan betere lichtomstandigheden in de produktie-experimenten.
- Intermitterend CO_2 doseren (ICS) werd getest onder normale ventilatie. In komkommer verlaagde ICS het verbruik van CO_2, maar verkleinde het gunstige effekt op de produktie in ongeveer gelijke mate. Bij paprika bleken zetting en produktie niet beter bij ICS dan bij een constant CO_2-nivo, gerelateerd aan gemiddelde C.

Konklusies

- Partiële sluiting van stomata heeft nauwelijks effekt op bladtemperatuur en geen direkt effekt op produktie. Op de lange termijn heeft hoog C mogelijk een indirekte invloed in de vorm van verlaagde concentratie van nutrienten in het gewas. Dit kan leiden tot bijvoorbeeld bladpuntvergeling in aubergine (door boriumgebrek) en kort blad in tomaat (samenhangend met calcium gebrek).
- In de metingen werden geen aanwijzingen gevonden voor een endogene 'midday depression' (dip of daling in fotosynthese en verdamping), althans onder de voorwaarden van goede watervoorziening en wateropnamecapaciteit.
- CO_2-dosering verhoogde meestal de lichtbenutting (LUE), vruchtzetting (behalve in tomaat), droge stof verdeling naar de vruchten, droge stof gehalte van de vegetatieve delen en de produktie, en verlaagde de SLA.

- Veel verschijnselen konden verklaard worden door een effekt van C op de sink/source verhouding: hoog C verhoogt de source, maar kan indirect ook de sink versterken, bijvoorbeeld door betere zetting in paprika en meer scheutgroei in komkommer.
- De CO_2-regel [**$X = (1000/C)^2 * 1.5$**, met X het relatieve effekt van CO_2 in % per 100 µmol mol^{-1}, and C in µmol mol^{-1}], kan aangepast worden aan de omstandigheden, met name het lichtnivo. De vermenigvuldigingsfaktor (1.5) zal bij gunstige (licht) omstandigheden ongeveer 1.8 bedragen en bij minder goede (licht) omstandigheden ongeveer 1.2. De CO_2-regel was redelijk in overeenstemming met de CO_2-curve op basis van literatuurgegevens.
- Intermitterend CO_2 doseren (ICS) kon onder normale ventilatie omstandigheden het gemiddelde CO_2-nivo slechts beperkt verhogen, en daardoor de produktie beperkt stimuleren. Er werden geen bepaalde fysiologische voor- of nadelen gekonstateerd.

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Curriculum vitae

Elly (Elisabeth Maria) Nederhoff werd geboren in Gouda op 20 oktober 1956. Na de lagere school doorliep zij de β-richting van het Coornhert Gymnasium in Gouda. Zij raakte bekend met de glastuinbouw via vakantie- en zaterdagwerk. In 1975 begon zij aan de toenmalige Landbouw Hogeschool in Wageningen en koos de plantkundige orientatie. Zij onderbrak de studie voor bestuurswerk bij een studentenvereniging en behaalde vervolgens in 1979 het kandidaatsdiploma. Voor stage werkte ze vier maanden bij 'Besor Experimental Station' in Israël aan fotosynthesemetingen in kassen en drie maanden bij 'Soltuna' op Curaçao aan diverse teeltexperimenten. Haar afstudeervakken waren tuinbouwplantenteelt, plantenfysiologie en meet- & regeltechniek. Voor dit laatste vak deed zij vier maanden experimentele metingen in kassen van het Proefstation in Naaldwijk. In 1982 behaalde zij het Wageningse ingenieursdiploma in de studierichting Tuinbouwplantenteelt.

Na het afstuderen werkte ze drie jaar in tijdelijk dienstverband in het kader van onderzoek naar energiebesparing bij de sektie Kasklimaat van de afdeling Teelt en Kasklimaat van het Proefstation voor Tuinbouw onder Glas in Naaldwijk. Naderhand werd de aanstelling omgezet van tijdelijk naar permanent en in 1988 van full-time naar half-time. Zij werkte onder meer aan ventilatie, licht, assimilatie belichting, fotosynthese, CO_2 en luchtverontreinigingseffekten op gewassen en maakte deel uit van de werkgroep 'kasklimaatbesturing van de jaren negentig'. In de periode 1990-1994 heeft zij zich toelegd op het onderwerp CO_2 en hierover een proefschrift geschreven.