

Project title: Estimating of CO₂ offtake in a long season UK tomato crop.

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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headlines

- A photosynthesis model has been developed to take account of seasonal variation. Using this, season-long estimates for crop CO₂ offtake were 26.4% (Piccolo) and 23.1% (Avalantino) in 2016.
 - Offtake was greatest in the early season, when CO₂ dosage was best matched with low productivity as a result of low leaf area and radiation receipts.

Background

Optimising CO₂ Management for the UK Tomato Industry

CO₂ enrichment is an industry standard, with growers applying in excess of 300 kg ha⁻¹ hr⁻¹ to target CO₂ concentrations above 900ppm, which has been supported by investment in supply systems (e.g. high volume CHP units). In addition, difficulties in quantifying how much CO₂ is used by the crop relative to that lost to the environment has limited grower attempts to maximise the proportion of enriched CO₂ absorbed into new biomass (CO₂ offtake) and optimise the use of CO₂ in their production systems. CO₂ dosing regimes can be based on the economics of provision (e.g. spark spread dictating when maximum economic returns can be achieved from CHP use and electricity sales to the National Grid) or on technical capacity (e.g. heat storage volume). By formalising a biologically-informed approach to CO₂ dosing, growers may be provided with a scientific route to optimising the application and use of CO₂ resource available to them. An ability to make better informed decisions regarding CO₂ application may offer direct cost savings to growers through reduced application, or realise higher marketable crop yield from current CO₂ enrichment regimes.

Project PE 021 began to address this challenge by developing tools for non-destructively estimating crop CO₂ offtake. Grower measurements of radiation were input into a model for photosynthesis which could estimate hourly rates of CO₂ uptake. When compared against leaf area estimates of the crop, the gross mass of CO₂ absorbed could be estimated and compared against grower records of CO₂ dosing to determine overall crop CO₂ offtake. Measurements made over the summer period of 2014 and 2015 indicated that UK tomato crops were capable of utilising up to 23% of the CO₂ applied as enrichment based on current estimates. Once preliminary estimates for CO₂ offtake could be reliably produced, two key areas for subsequent examination were identified. Firstly, estimates of CO₂ assimilation of an early season UK tomato crop which could be used to calculate CO₂ offtake. This would allow

estimating of crop offtake with greater accuracy, supporting optimisation of CO₂ enrichment. Secondly, to further explore how enrichment strategies could be updated to increase over CO₂ offtake. Evidence was to be collated as to changes in how the crop utilised CO₂ from which changes in demand could be inferred and used to inform preliminary development of variable CO₂ dosing guidelines.

Enhancing Estimates of CO₂ Offtake

Project PE 021 developed a photosynthesis-based model that used measurements of light levels to estimate hourly rates of photosynthesis. When combined with estimates of crop foliar area, an estimate for the mass of CO₂ taken up by the crop across the season could be made. The PE 021 model was based on measurements taken over the summer period, and therefore included a potential margin of error due to over estimates of crop responses to light in the early season, particularly during the onset of fruiting. Further data were required however from the entire season to test the accuracy of the current model during the main and shoulder periods of the growing season. A month-by-month estimate of crop CO₂ offtake would allow growers the opportunity to assess their CO₂ use efficiency and highlight potential areas where changes could be made to further optimise CO₂ use efficiency beyond current stand practice.

Variable CO₂ Dosing

Changes in light availability combine with biological cycles in the crop to define how well the crop can use light to drive CO₂ assimilation. Peaks in assimilation or crop demand for CO₂, were identified from the limited dataset accumulated for PE 021: accumulated evidence indicated that typical tomato crops demonstrated variation in their ability to utilise CO₂ which peaked between 12 – 3pm in response to a combination of environmental factors and time of day. By exploring how CO₂ demand changes within the crop over a daily time course, evidence as to the significance of daily cycles in crop CO₂ demand were to be sought. If significant daily changes in crop CO₂ use were reliably identified, these could be used to provide preliminary guidelines for variable CO₂ dosing that would allow growers to change their enrichment strategy to better match variable crop demand rather than the flat-line approach to dosing that is typical of current practice. To achieve this, measurements of photosynthesis were related to assimilatory area and solar radiation. Through a combination of increased accuracy of CO₂ offtake estimation and the development of preliminary tools for variable CO₂ dosing, progress towards an optimised CO₂ management strategy for UK growers could be made.

Project Aim

To further develop a gas exchange/modelling technique to predict CO₂ use in the early growth

phase of a long season tomato crop.

Project Objectives

- To generate integrated estimates of canopy carbon fixation by non-destructive gas exchange measurements in a long-season tomato crop.
- Identify any impact of environment and crop photosynthetic potential on carbon fixation.
- To collect sufficient data to supplement model outputs of PE 021 and PE 021a to improve estimates of CO₂ offtake by a long season crop.
- To interact with the industry representatives on a monthly basis or sooner as appropriate and communicate the outputs of the project in both verbal and written forms.

Summary

Approach

An area of 0.60ha of the category 3 Avalantino, and an area of 0.77ha of the category 1 cherry tomato cultivar Piccolo, were used for the trial between February and November 2016. Both cultivars were grown according to commercial standards, with average CO₂ enrichment levels of 937ppm (Piccolo) and 877ppm (Avalantino). Measurements were taken on a monthly basis to quantify CO₂ uptake and the changes in the physiological condition of the crop to identify changes in CO₂ demand. Analysis of the results was undertaken to estimate crop CO₂ offtake, and to test the significance of daily variation on CO₂ uptake.

Estimating Crop CO₂ Offtake

Crop offtake was estimated for February – November 2016 inclusive. Measurements of hourly radiation measurements were input into the PE 021 model for estimating hourly rates of photosynthesis. When combined with monthly estimates of canopy leaf area, it was possible to give hourly estimates for the mass of CO₂ assimilated which could be compared against grower records of CO₂ application. Instantaneous measurements of photosynthesis under ambient conditions were made on a monthly basis. Photosynthesis estimates from the original PE 021 model showed significant deviation from observed estimates. The PE 021 model was developed from a crop grown under CO₂ levels of 500ppm, considerably below those seen in the 2016 crop and were seen to routinely underestimate the rates of photosynthesis when compared with observed measurements. To resolve this, a new model was developed that included three separate sub-models which could be applied separate to the early, middle and late season to enhance the accuracy of the CO₂ offtake estimates. Offtake estimates varied significantly across the season, as summarised in **Table i**. Offtake estimates using the 2016 model were greatest in February (39.3%, Avalantino) and May (35.1%, Piccolo) and least in

April (15.9% Avalantino, 21.8% Piccolo). On average, the CO₂ offtake was greater for Piccolo (26.4%) than Avalantino (23.1%). The offtake estimate for Piccolo using the 2014 model (20.5%) was comparable for the offtake estimate from 2015 (19.4%). The low offtake estimate for Avalantino was due to a combination of a high level of CO₂ application combined with a low leaf area as a result of low planting density (3.3 heads/m). The sharp decrease in offtake in February for both cultivars was attributable to lower light levels and total leaf area combined with a sharp increase in CO₂ applications.

Table i. Summary of 2016 seasonal offtake estimates

	Year	Model	Cultivar		
			Avalantino	Piccolo	Roterno
Offtake (%)	2016	2014	17.98	20.52	
		2016	23.13	26.41	
	2015	2014		19.42	23.66
CO ₂ Applied (kg/ha/day)	2016		1504	1789	
	2015			1265	1240
CO ₂ Conc. (ppm)	2016		937	877	
	2015			979	847

Developing Variable CO₂ Dosing Guidelines

The second primary goal of this project was to test for the incidence of predictable daily changes in the crop's ability to utilise CO₂. Changes in the ability of the crop to absorb CO₂ over the course of a day (a diurnal cycle) would be characterised by a decline in assimilation in the afternoon period that was not attributable to light levels. Such an effect would be the result of stomatal closure in response to a water deficit, an accumulation of sugars in the leaf due to the plant being unable to utilise them (e.g. for new growth) at the rate at which they were produced, or biochemical changes caused by exposure to high levels of light damaging photosynthetic machinery. A peak in assimilation between 12 – 15:00h was demonstrated in the 2014 work for PE 021, and this project set out to test whether such a cycle was routinely present in a commercial crop, and how this interacted with environmental inputs. The rationale to this was that if such cycles were routine and predictable, CO₂ dosing could be targeted to periods of high demand, lowering wastage when the crop was less responsive. Two key indicators of CO₂ demand were used: CO₂ response curves, and light responsive curves which were measured over the course of the season. These would enable the CO₂ demand of the plant to be quantified in a way that could be linked back to corresponding environmental measurement. Such measurements were made on a monthly basis over the course of the season, and then subject to analysis to identify the occurrence of statistically significant interactions. This analysis demonstrated two key effects: firstly, the position in the season was the most significant factor when explaining variation in CO₂ uptake potential. This

can be seen in the significant changes in light and CO₂ response that were observable over the course of the growing season (**Figure i**). Secondly, light response was highly linked to incident radiation levels (as these provide the raw energy input for photosynthesis alongside directing how photosynthetically active the plant is) and CO₂ response was linked with temperature (due to biochemical changes linked with higher rates of reaction). Time of day was indicated to have only minor significance once environmental and seasonal factors had been taken into account. Monthly collections of data meant that it was not possible to fully explore variation within each season block (spring, summer or autumn), but the analysis indicates that seasonal variation and instantaneous environmental conditions are better predictors of crop CO₂ demand than time of day alone. In addition, prior research indicates that the diurnal effect in tomato is likely to be great in the December to March period as the crop adapts to changes in light levels in early spring. This may mean that significant effects are less likely to be detectable in the summer period.

From these results, a variety of conclusions can be drawn. The behaviour of the crop in the early season, and its rapid response to the onset of fruiting and high light levels has a significant effect on CO₂ use potential (and also offtake potential; as discussed above). On the available data it is not possible to fully appraise this, but a key feature of subsequent work may be to more fully explore the effect of seasonal variation in the early spring period. In addition, it is likely that environmental conditions are more likely to have a significant effect on CO₂ uptake than a straight forward cycle related to time alone. To respond to this, variable dosing could be implemented that tracked environmental inputs directly (especially PAR), but current facilities available to growers are likely to be insufficient when compared with the rapidity of response that will be required if this were to be implemented in a commercial setting. Alternatively, measures could be implemented to ensure a more consistent environment to ensure that environmental inputs are manipulated to align with flat-line CO₂ enrichment. For example, the use of supplementary lighting outside of set PAR thresholds is seeing limited uptake in the UK industry, and this has the potential to ensure a constant light source is available to promote a constant uptake of CO₂ by the crop.

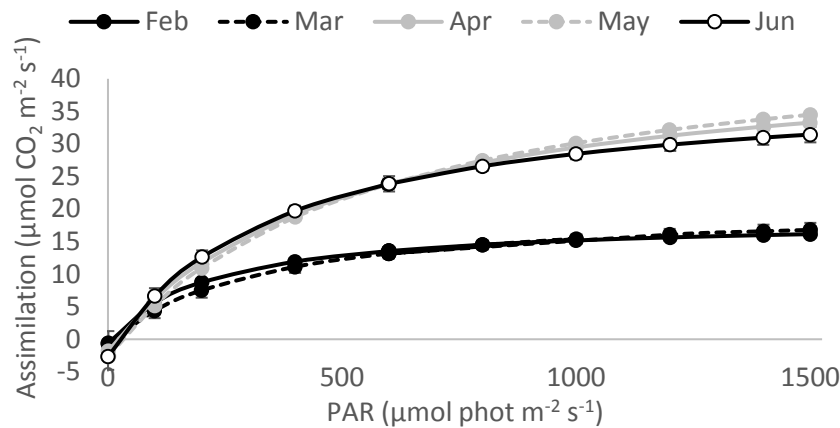


Figure i. Changes in light responses in the early season for Piccolo.

Summary

Conclusions

- Large changes in crop responses to light and CO₂ have been observed across the season, with increases over spring peaking over the summer period, before declining after September.
- Seasonal variation supported the development of a new photosynthesis model, allowing crop offtake to be estimated with greater accuracy than prior work. Season-long estimates for crop offtake were 26.4% (Piccolo) and 23.1% (Avalantino). The lower level of offtake in Avalantino was largely due to a lower leaf area, a consequence of lower plant density compared with Piccolo.
- Offtake was greatest in the early season, when CO₂ dosage was best matched with low productivity as a result of low leaf area and radiation receipts.
- Strong seasonal variation and a limited monthly dataset meant that it was not possible to observe diurnal changes in photosynthesis. This may be greatest in the early season, with only limited diurnal changes seen in the summer months as the crop adapts to periods of high productivity with heavy fruit load, which helps to buffer internal restrictions on CO₂ uptake.
- Large reductions in photosynthetic potential within the canopy have been observed, meaning that the lower canopy is up to 40% less productive than the whole-plant average under conditions of constant light. This is a result of leaf aging and the crop adapting to increased proportions of shade in the lower canopy. Leaf biochemistry is wound down and nitrogen is recycled for use in the upper canopy, lowering photosynthetic capacity and the ability of the leaf to utilise high light levels.

Conclusions of PE 021 and 021a

- The UK tomato industry currently uses around 0.27m tonnes of CO₂ a year (assuming applications rates of 370 kg ha⁻¹ h⁻¹, over 225ha of UK glasshouse production area for a 12 hour day for a 270 day growing season). At a typical cost of £80/tonne, CO₂ enrichment costs the UK industry almost £21.6 m. While this cost is necessary to achieve required yields, the efficiency of CO₂ enrichment has not been quantified. Current enrichment practice can be based on technological limitations or economic arguments (e.g. spark spread) rather than a biological approach to crop need, limiting the efficacy of CO₂ usage that can be achieved.
- A robust method of calculating CO₂ offtake has been devised and tested, using leaf photosynthesis and solar radiation measurements. CO₂ offtake ranges from 6.2% to 26.4% for two sites measured between 2014 and 2016. The cost of CO₂ wastage from current practice could be between £15.9 - 20.3 m per year, offering considerable scope for improvement.
- There is some evidence of a stronger crop response to CO₂ enrichment in the morning compared with the afternoon, which may be stronger in the early season. To demonstrate a clear economic benefit for growers, further evidence to support this is required.
- Significant variation is seen in crop photosynthetic responses over the course of a season as the crop rapidly responds to changing weather conditions in spring and autumn. This is also strongly influenced by crop growth stage, fruit load and the removal of the growing point at the end of the season.
- Shading by leaves in the lower canopy means that photosynthesis in the lower canopy can be almost 40% lower than the whole-plant average rate of photosynthesis. This presents a key area of under exploited potential to drive increased CO₂ offtake and stimulate marketable yield above those currently achieved by UK growers under glass.

Financial Benefits

CO₂ enrichment in the UK tomato industry will typically see dosage rates of around 370 kg ha⁻¹ h⁻¹ to achieve sufficient CO₂ concentrations for economically viable yields. This has an updated estimates of CO₂ offtake, indicating that a crop can achieve upwards of 23.6% offtake of enriched CO₂. Assuming CO₂ is provided to the 225ha of UK tomato production, 12 hours a day for a growing season of 270 days, 0.27 million tonnes of CO₂ are emitted. By continuing

to enhance the accuracy of this estimate, the potential for industry lobbying for a proportionate reduction in the Climate Change Levy is supported. This has direct potential to realise a reduction in costs for growers using typical gas-fired CHP and boiler systems.

This project also highlighted an additional range of factors that can impact crop offtake. Higher levels of enrichment, to a crop planted at a lower density, can decrease offtake by over 3%. Taking an average CO₂ cost of £80/tonne, adjustments to crop density and enrichment level have the potential to maximise the proportion absorbed by the crop. Enhancing offtake by 3% would reduce the cost of wasted CO₂ by £648k annually to the UK industry, which could be achieved by adjustments to existing management practice.

The scope for reducing CO₂ wastage by enhancing offtake further has also been identified. While this may not be realistically achievable using variable dosing in current production settings, the potential for increasing overall offtake by maintaining environmental inputs such as the use of supplementary lighting below given PAR thresholds holds significant potential to boost overall CO₂ offtake by ensuring that input routinely meets both crop potential and CO₂ enrichment rates. In addition, further enhancement to CO₂ offtake could be achieved through additional exploitation of the lower canopy. Quantification of this layer has demonstrated that the lower canopy has considerable yet underutilised potential to assimilate CO₂, and adjustment to grower practice to further drive productivity in this section of the crop. By using methods such as lighting management, there is potential to boost productivity in the lower 30%, potentially leading to an increase in offtake by 50% overall. Should this be realised, overall crop uptake could be increased to over 35%, reducing CO₂ wastage by £2.7m. This could be achieved by lowering overall CO₂ applications, or by utilising CO₂ in a fashion that maximises economic returns by maximising the proportion that is converted into marketable yield.

Action Points

- Consider how CO₂ increases are made in the early season. Incremental increases in CO₂ between April and May would help to sustain high early-season offtake by ensuring that dosing matches ambient light levels and crop leaf area, maximising the proportion that can be used by the crop.
- Planting at a higher density to sustain a large leaf area in the crop may act to enhance CO₂ offtake, but care must be taken that this does not negatively impact light penetration into the lower canopy or restrict air movement so that humidity and disease pressures are controlled.

- Methods of enhancing light availability in the lower canopy such as de-leafing strategy, angled wire arrangements or supplementary lighting provision to enhance lower canopy photosynthesis could be a key route to exploiting unrealised potential of the lower canopy for enhanced CO₂ offtake.

Future Work

- While CO₂ offtake calculations have been calculated with additional levels of accuracy across an entire season, there is only evidence from a single full year at a single grower site to supplement measurements made over the summer months of 2014 and 2015. Additional measurements over other seasons, grower sites and cultivars will be required to further test the findings of this project.
- Broaden data set for diurnal changes in a commercial tomato crop, with increased monthly sampling to allow better separation of the effects of season, environment and time of crop CO₂ demand. A broader evidence base will allow for more accurate identification of diurnal cycles in tomato that can be used to inform variable CO₂ dosing.
- Further evaluate changes in crop CO₂ demand in the early and late season to identify the effects of fruit load and environmental change on crop productivity. Test how these can be manipulated (e.g. by using early season supplementary lighting) and how this can impact seasonal productivity.
- Explore how productivity in the lower canopy can be driven to achieve overall increases in crop CO₂ uptake. Light availability could be enhanced by changes to crop management strategy (e.g. de-leafing or supplementary lighting) to boost CO₂ uptake, increasing offtake and marketable yield outputs.

1 SCIENCE SECTION

1.1 Introduction

Increasing investment in CO₂ production technologies by the UK tomato industry has meant CO₂ concentrations above 900ppm can be routinely targeted which require dosage of 190 – 580 kg/hr/ha depending on glasshouse construction and the extent of venting (Nederhoff, 2004). Concentrations at this level mean that growers can achieve high marketable yields and photosynthesis is generally limited only by light levels. Current energy prices make this application rate economical, but shifts towards renewable fuel sources (e.g. biomass) from which siphoning off pure CO₂ can be prohibitively expensive or future changes in wholesale energy prices may promote the requirement for effective CO₂ management to ensure that maximum benefit can be derived from the CO₂ applied.

Historically, CO₂ dosing has been based on limited supplies available to growers, or the prohibitive cost of pure food-grade CO₂. Dosing was targeted at the perceived peak of the cost/benefit curve derived from the economics of supply (Chalabi *et al.*, 2002; Short *et al.*, 2002; Adams *et al.*, 2009). The consequences of excessive dosage have also been evaluated, such as increased crop CO₂ acclimation (e.g. Besford, 1990; Qian *et al.* 2012), CO₂ toxicity (Hao *et al.*, 2006) or pollution damage from contaminants in improperly treated flue gases (e.g. NO_x, CO and CH₄) (Short *et al.*, 2002). Current best practice is based on economic or technological considerations, and does not necessarily link to CO₂ provision with the demand of the crop and its ability to convert this resource into marketable yield. An ability to make better informed decisions regarding CO₂ application may offer direct cost savings to growers through reduced application, or promote maximum yield to be derived from the applied CO₂.

Previous work has attempted to link environmental management with modelled CO₂ assimilation in tomato crops (e.g. Nederhoff, 2004) and which in turn has been used to simulated yield prediction. Such an approach can pay scant attention to the impact of variability in photosynthesis caused by innate biological limitations and spontaneous seasonal morphological and physiological changes that affect crop function. Environmental conditions such as sub-optimal light/CO₂ from intercanopy shading (Ehret *et al.*, 2011) or reductions in photosynthesis caused by diurnal cycles in light availability (Hao *et al.*, 2006) could exacerbate this variability. Furthermore, factors such as restricted rooting volume, canopy air movement or crop behaviour may impact photosynthetic efficiency and measureable assimilation in a glasshouse crop (Qian *et al.*, 2012). As a result, growers have little upon which to base judgements regarding CO₂ dosage for UK vented glasshouse crop production systems.

1.1.1 Estimating CO₂ Offtake

Project PE021 was undertaken to provide an evidence base to support optimised CO₂ dosing, and develop methodology that could be used by commercial growers to calculate CO₂ offtake and measure how much of the CO₂ supplied was actually captured by the crop against the proportion vented to the atmosphere. Currently, growers can only assess the efficacy of their CO₂ usage through evaluation of gross yield outputs, but this does not demonstrate the efficiency at which CO₂ is being used. Of the bulk CO₂ applied to the crop, a significant proportion will be lost to the atmosphere, particularly in the summer period when vents are fully open. Mechanistic analysis of CO₂ losses to the environment are technically complex and highly site-specific, meaning that growers are unable to accurately estimate CO₂ use efficiency.

Preliminary work conducted in the summer of 2014 at Cornerways Nursery, Norfolk, was undertaken to develop a photosynthesis-based model of CO₂ assimilation upon which estimates of whole crop CO₂ use could be based using grower records of radiation receipts and estimations of crop foliar area. Estimates indicated that between 6.2% (Piccolo) and 8.2% (Roterno) of the applied CO₂ was being absorbed by the crop. In a similar crop monitored in 2015 at Stubbins Nursery, Fen Drayton, offtake estimates of 19.4% (Piccolo) and 23.7% (Roterno) were generated. The total mass of CO₂ per plant was comparable between the two sites, but differences in CO₂ dosing rate (311 kg/ha/hr for Cornerways vs. 190 kg/ha/hr for Stubbins) resulted in the significant differences in estimated offtake.

Three key elements are present in these findings. Firstly, direct economic benefit to growers from monitoring CO₂ use could be derived from enhancing the “green” credentials of the industry with the potential to exert influence at the policy-making level to reduce carbon tax levies. Secondly, the lack of a direct link between dose and concentration was highlighted: cultivation and engineering practices means that CO₂ application rates may achieve different CO₂ concentrations at different sites, highlighting the importance of grower-specific advice for individual grower businesses. Thirdly, these data indicate that between 76 – 94% of the applied CO₂ is currently being lost representing a significant opportunity for optimisation of use efficiency by increasing the proportion of CO₂ absorbed by the crop.

While the sampling for PE021 was robust, the conclusions and derived models require further validation. The data was collected from two sites from only the second half of a long season in both years, and data was collected over a limited number of occasions from a relatively small sample size for each cultivar. The photosynthesis model also assumed that crop responses to light and CO₂ would be constant, although this is unlikely to be supported given potential changes in assimilation potential that may occur within the crop, or in response to temporal or environmental changes. Furthermore, the existing photosynthesis model was

developed for a crop grown at 500ppm, which is considerably less than industry-standard enrichment levels of between 800 to 1000ppm.

Given these areas of uncertainty, it was necessary to sample a replicate and extended data set to test the assumptions of non-variable rates of photosynthesis which are uncoupled from time and environmental input. To augment the data set for PE021, work was undertaken at Stubbins Nursery, Cambridgeshire, between February and November 2016. CO₂ provision at Stubbins is sourced from a high-volume CHP unit, allowing for increased parity of results with industry standards in addition to the Cornerways data generated in the first phase of PE021. This would provide a useful comparison between an industrial waste CO₂ source versus a standard CHP enrichment system with which to compare CO₂ uptake and use. Measurements from the full season would also allow greater quantification of early and late season responses, allowing variation in CO₂ uptake to be accounted for in the photosynthesis model for CO₂ offtake.

Individual measurements of crop photosynthesis under ambient conditions were made, and these were used to test the existing photosynthesis model, and to support the development of an alternative model if deemed appropriate. To support this, A/C_i curves were planned to compare assimilation (*A*) against leaf internal CO₂ concentrations (*C_i*) to quantify the effects of varied CO₂ concentration upon assimilation. This approach would allow crop assimilation to be tested at CO₂ concentrations between 0 – 1500ppm and represent the full range of enrichment levels used by growers and experienced by UK tomato crops. Light response curves would also be measured and used to quantify the effects of changing light intensity upon assimilation to allow comparisons to be drawn between measurements made at separate points in the season. Lastly, measurements of changes to photosynthesis within the canopy were examined to quantify whole-canopy responses to light.

By fully validating the model developed in PE021, and broadening understanding of how a typical tomato crop responds to CO₂ and light, we envisaged that a significant step would have been taken towards the development of a responsive strategy for efficient CO₂ management.

1.1.2 Enhancing CO₂ Offtake

A primary target for optimising CO₂ usage is a close alignment of dosage with the biological demand of the crop. Standard practice is to target enrichment to a flat-line level, achieving the maximum possible concentration across daylight hours. The biological ability of the crop to utilise CO₂ is unlikely to remain constant, instead varying over a range of daily and seasonal timescales. Application of high levels of CO₂ at times when the crop is under reduced capacity to utilise it will reduce the efficiency at which it is used, lowering offtake.

A range of potential causes could result in a decreased capacity of the crop to utilise CO₂. A crop which is stressed will be unable to assimilate CO₂ at a level comparable with a non-stressed crop (Ehret *et al.*, 2011). Stomatal closure triggered by transient water stress such as that experienced at the top of the crop on hot, bright days, large vapour pressure deficits (drying atmosphere) or a cold root zone will promote stomatal closure (Norisada *et al.*, 2005). Diurnal cycles in stomata opening may also result from innate biological cycles (McKee *et al.*, 2000) whereby stomata partially close in the midday/early afternoon period. Under these conditions, penetration of CO₂ into the leaf will be reduced, lowering assimilation even under conditions of high light. Extended periods of high light can also lead to damage to light absorption machinery, reducing the efficiency of photosynthesis and lowering CO₂ assimilation rates. This is particularly notable in stressed plants, or those grown in low night temperatures (Liu *et al.*, 2006).

Stomatal closure and reduction in photosynthesis can also result from the phenomenon of end-product inhibition as a result of unbalanced sink:source interactions. In this instance, the rate of CO₂ assimilation and new sugar formation in the leaf (the source) outpace the rate at which the sugars can be exported and utilised in active tissues such as the root or developing fruit (sinks). The accumulation of sugars will promote the down regulation of photosynthesis, stomatal closure and the ultimate reduction in the rates of CO₂ independently of light levels. The timing of such an event will be linked to the environment and plant condition, but cultivars with a relatively large leaf area and a high source:sink ratio, such as Piccolo, are likely to suffer yield penalties under less than optimal atmosphere CO₂ concentrations because of a concurrent decline in photosynthesis (Ho *et al.*, 1996).

The occurrence of diurnal changes in the potential of the tomato crop to assimilate CO₂ have been demonstrated in PE021. Results from 2014 demonstrated that a peak in assimilation was typically encountered between 12-15:00h (**Figure 1**) which was independent of light conditions. CO₂ response curves measured in 2015 showed a decline in the potential of the crop to utilise CO₂ under saturated light conditions (**Figure 2**). Diurnal variation in tomato has been reported in a number of instances. Ayari *et al.* (2000a) reported peaks in assimilation in tomato grown at 1000ppm that corresponded with an 8-hour period after sunrise that corresponded to a level of stomatal limitation restricting CO₂ influx into the leaf (Ayari *et al.*, 2000b). Edwards (2011) recorded daily cycles in leaf starch accumulation that could be indicative of increased incidence of end-product inhibition as a result of sink saturation whereby leaf export of carbohydrate is beyond the level at which it can be utilised in growing areas.

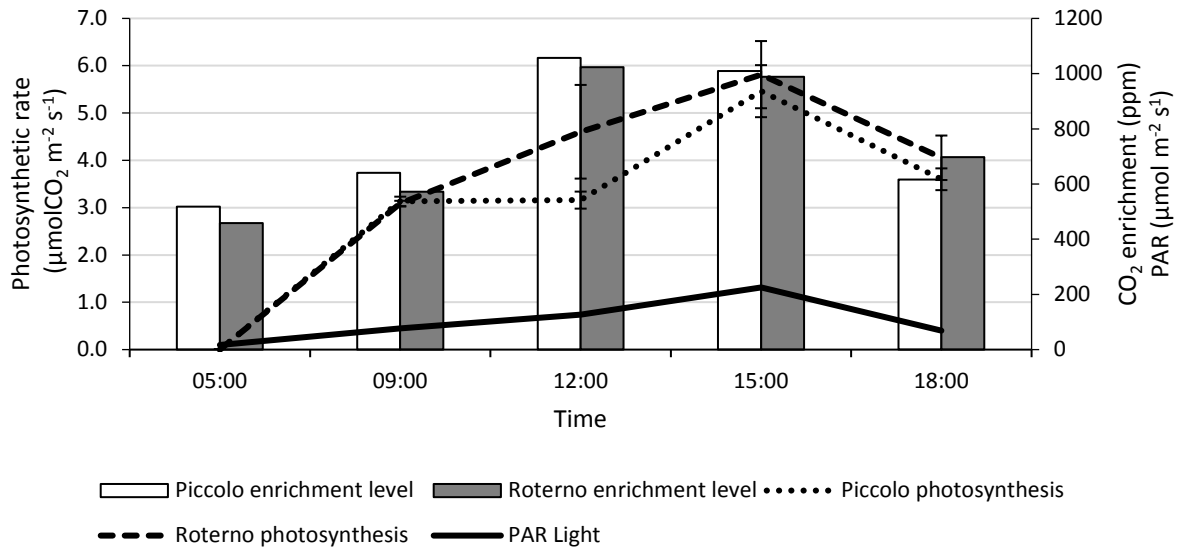


Figure 1. Diurnal changes in assimilation as measured in 2014. Measurements made in July demonstrate increased levels of assimilation in the morning which peaked at 3pm before declining.

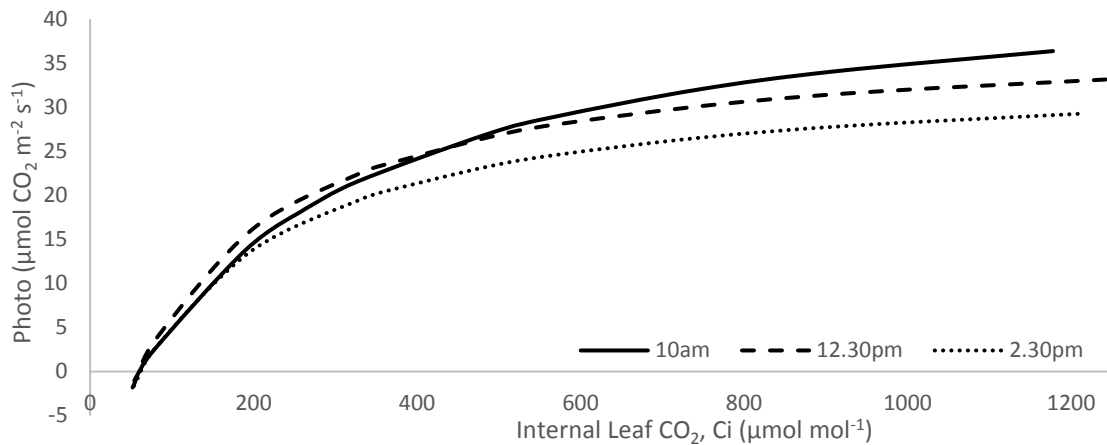


Figure 2. Changes in CO₂ response over a day. Measurements were taken from a single row of Roterno plants on the 25th August. Changes in both the maximum rate of photosynthesis and Carboxylation Efficiency (initial slope below 200 µmol mol⁻¹ CO₂ are evident between 10am and 2.30pm.

Therefore, it was considered likely that an observable diurnal variation was occurring in UK grown tomato crops. By quantifying the extent and time periods of diurnal variation, it would be possible to define periods of peak and slump demand in CO₂ by the crop. These could be used to match enrichment targets against, offering the potential to reduce overall CO₂ application, lowering wasting and enhancing CO₂ offtake. These effects contribute to the ability of the crop to act as a variable absorber of CO₂, placing the biological nature of the system at odds with the current commercial practice to flat-line levels of CO₂ enrichment. Sustaining high levels of enrichment in the early morning or late afternoon periods when the crop has increased biological constraints on its ability to utilise CO₂ will act to decrease crop CO₂ offtake, lowering the efficiency at which this resource is utilised.

In addition to the time period of diurnal variation, it will be necessary to overlay environmental responses on any observed differences in photosynthetic behaviour during the diurnal cycle. For example, reduced CO₂ demand may occur after periods of high light/high productivity, but demand may remain more constant under periods of more continuous lower light levels. This project was established to test for the presence of diurnal variation and to measure environmental changes that may underpin this as a preliminary step to generating the evidence base required by growers to guide decisions regarding variable dosing.

Two key parameters were taken as indicators of crop CO₂ demand: light response and CO₂ response (A/C_i curves). These measures indicate how controlled changes to CO₂ and PAR impact assimilation allowing changes in underlying leaf responses to be assessed. These parameters were to be assessed across the season on a monthly basis to allow year-long quantification of the ability of the crop to utilise CO₂. The work was conducted in close communication with industry representatives which allowed sampling and experimental processes to be adapted closely to industry norms while feeding forward into the development of industry-ready CO₂ management guidelines.

1.1.3 Summary

This project was undertaken with two key objectives. Firstly, to use data accumulated from a full long season to test and refine the estimate of CO₂ offtake using the approach successfully demonstrated in PE021. Secondly, accumulated evidence would be analysed to test for the presence of diurnal variation in a commercial tomato crop, and these use any relationship demonstrated as the foundation for variable CO₂ dosing guidelines.

Project Aim:

To further develop a gas exchange / modelling technique to predict CO₂ use in the early growth phase of a long season tomato crop.

Project Objectives:

- To generate integrated estimates of canopy carbon fixation by non-destructive gas exchange measurements in a long-season tomato crop.
- Identify any impact of environment and crop photosynthetic potential on carbon fixation.
- To collect sufficient data to supplement model outputs of PE021 and PE021a to improve estimates of CO₂ offtake by a long season crop.
- To interact with the industry representatives on a monthly basis or sooner as appropriate and communicate the outputs of the project in both verbal and written forms.

1.2 Materials and Methods

1.2.1 Site and Crop Details

Measurements were taken from a commercially grown crop at Stubbins Nursery in Cambridgeshire between February and December 2016. Crops of Avalantino (large vine) and Piccolo (cherry) grown in separate compartments were used for data collection which corresponded to the Roterno and Piccolo crops examined in the 2015 season. These were planted out on 6th January (Piccolo) and 13th January 2016 (Avalantino) as 62 day old plants. Leaf area measurements were taken on a monthly basis, and gas exchange measurements were taken according to the schedule given in below in **Table 1**. Hourly radiation values, CO₂ dosing and weekly de-leaving numbers and fruit yield were collected from Stubbins across the 2016 season.

Table 1. Measurement schedule for the 2017 season.

Month	Measurement			
	Spot Assimilation	Light Curves	Whole Canopy	A/Ci Curves
February	05/02 & 25/02		11/02	18/02
March	17/03	03/03		10/03 & 31/03
April	14/04	14/04		21/05
May	05/05	12/05		19/05
June	21/06		09/06	21/06
July		28/07		14/07 & 21/07
August	12/08		18/08	25/08
September	30/09	22/09		15/09
October	20/10		31/10	18/10
November	10/11	23/11		14/11

Plants were grown in rockwool blocks placed on 100x8x15cm Botanicair Breeze® slabs with two taproots per slab fed by drip irrigation. Plant densities were 4.2 heads m⁻² (Piccolo) and 3.4 heads m⁻² (Avalantino). The same rows of plants were sampled over the course of the project to maximise sample variation, and sampled plants were always taken from the centre of the row. The crop received no supplementary lighting, and sampled plants were subject to the same cultural practices as the commercial crop. Average day-time CO₂ enrichment levels across the season were 877ppm (Piccolo) and 936ppm (Avalantino), with average weekly dosage rates of 12170kg ha⁻¹ and 10229kg ha⁻¹ respectively with CO₂ being sourced from a high-volume CHP system.

Gas Exchange Analysis

In order to support the use of the model produced in PE021 to estimate assimilation, the responses of the crop to varying CO₂ concentration and light levels were assessed. Plants

subject to analysis did not have fruit picked for a minimum of 48 hours before measurement in order to prevent disruption due to distorted sink/source relationships. The youngest fully expanded leaf was deemed to be physiologically typical of each plant (Mulholland *et al.*, 2000). Study PE021 indicated similar photosynthetic rates between top, middle and bottom cross sections of the canopy and that leaf six was the youngest fully expanded leaf in both cultivars.

Gas exchange measurements were taken using a Li-Cor LI-6400XT Portable Photosynthesis System (Li-Cor, USA), and data relating to net CO₂ gas exchange ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), intercellular CO₂ concentration ($\mu\text{mol CO}_2 \text{ mol}^{-1}$) and transpiration rate for evaporative water loss ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) were collected at each sample point.

1.2.2 Spot Measurement of Photosynthesis

1.2.3 Crop Responses to Varying CO₂

Crop responses to variation in CO₂ concentration were measured on the three sampling dates noted above. On each sampling date at least three replicate measurement sets were made of each cultivar between 09:00 and 17:00, alternating readings between each cultivar. In order to assess the crop responses to CO₂, assimilation (A) was measured over a range of internal CO₂ concentrations (C_i). These data would be sufficient to allow the plotting of A/C_i curves from which the carboxylation efficiency and carbon compensation point of each cultivar can be determined following the methodology of Farquhar *et al.* (1980).

The Li-Cor's external air supply was scrubbed of CO₂ before supplementing from an internal CO₂ supply to deliver CO₂ concentrations in the cuvette of 50, 100, 300, 500, 700, 800, 1000, 1200 and 1500ppm. The Li-Cor cuvette was set to maintain a leaf temperature of 23°C and a PAR level of 1500 $\mu\text{mol phot m}^{-2}\text{s}^{-1}$, a level at which light was judged to be in saturation. This range was selected as it was representative of concentrations found during normal cultivation, cover the range of a rectangular hyperbola A/C_i curve for tomato (Mulholland *et al.*, 2000) whilst allowing the calculation of derived values (see below). Measurements began after readings had stabilised (at least 5 minutes) and a minimum period of 120s was allowed between CO₂ intervals to allow readings to be stabilised.

Analysis of the gas exchange data was undertaken following the principles of Farquhar *et al.* (1980). The net exchange of CO₂ ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) was plotted against intercellular CO₂ concentration ($\mu\text{mol CO}_2 \text{ mol}^{-1}$, which is equal to ppm) to generate an A/C_i curve, onto which was fitted a curve fitting a rectangular hyperbola curve fitting the equation $y = A + B / (1 + Dx)$

using Genstat5 (Payne *et al.*, 2011) where y is the net exchange of CO₂ and x is intercellular CO₂ concentrations.

From this equation could be derived the maximum carbon assimilation rate under experimental conditions (A_{Max} , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), the carbon compensation point (the CO₂ concentration at which photosynthesis/respiration breaks even: Γ , ppm CO₂) and the carboxylation efficiency (CE, the efficacy of the plant at fixing carbon, $\mu\text{mol m}^{-2} \text{ s}^{-1}$). Using these values it was possible to compare gas exchange parameters of Piccolo and Avalantino, and to predict carbon assimilation at given internal CO₂ concentrations.

1.2.4 Crop Responses to Varying Light Levels

In order to test any difference in response to changing light levels, a set of four light response curves were constructed for each cultivar using measurements taken on the schedule given in **Table 1**. Measurements were taken in a similar fashion as above, except that a constant CO₂ level of 800ppm (Avalantino) and 950ppm (Piccolo) was set to match ambient conditions. Assimilation was recorded at PAR values of 0, 100, 200, 400, 600, 800, 1000, 1200, 1400 and 1500 $\mu\text{mol phot m}^{-2}\text{s}^{-1}$, the results of which were used to construct light response curves.

1.2.5 Canopy Level Analysis

To quantify variation in photosynthesis within the canopy, spot measurements of assimilation were made under ambient light and CO₂ conditions as measured from the top of the plant. Four consecutive leaves were measured in the top, middle and bottom canopy sections of three plants of each cultivar, over three time points on each measurement date. Measurements were taken at four dates over the 2016 season.

Estimates of crop carbon assimilation were made using the methods developed in PE021. In addition to hourly radiation values, estimates of foliar area present in the canopy were necessary.

Measurements of the length (leaf petiole base at point of attachment to the stem to the terminal leaflet tip, l) and breadth (width of the widest point, b) were taken of every leaf on the vine. These measurements were taken on a monthly basis between February and November. Once a quarter, destructive leaf measurements were made using a Delta-T leaf area meter of 10 leaves for the top, middle and bottom canopy sections of each cultivar and used to define allometric coefficients for use in predicting leaf area following the methodology of Mulholland *et al.* (2000). Estimated leaf area (E) was given by the equation $E = l \times b \times c$. The coefficient c was cultivar-specific and defined from the destructive area measurements, and different coefficients were applied to the top, middle and bottom layers of the canopy with each layer containing one third of the total leaf number on each vine. Co-efficient values are

given below in **Table 2**, although the data for these relationships are presented in **Figure 3** and **Figure 4** in the results section.

Once average whole plant leaf areas were calculated, these were compared against planting density to give foliage density for each cultivar (cm^2 leaf/ m^2 of ground). The foliage density could then be scaled up to give a total leaf area. Monthly foliar measurements allowed crop leaf area across the season to be estimated.

Table 2. Allometric coefficients (*c*) used to estimate leaf area from length and breadth measurements.

Section	Piccolo	Avalantino
Top	0.207	0.258
Middle	0.321	0.335
Bottom	0.392	0.357

1.2.6 Estimating CO₂ Offtake

Hourly assimilation rates were estimated through two routes. The original photosynthesis model developed in 2014 was utilised, alongside the development of a second model based on gas exchange measurements from the 2016 season.

2014 Model of Photosynthesis

Hourly radiation values (Wm^{-2}) obtained from grower records were combined with the estimates for foliage density ($\text{cm}^2 \text{ m}^{-2}$) to provide the inputs for estimations of crop CO₂ offtake. The estimated hourly assimilation rate (A_H , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) was calculated using light intensity (L , Wm^{-2}) and two constants (m and d) in the equation $A_H = mL + d$. The m and d values were taken from the analysis of PE021 following the negative intercept model with a maximum assimilation rate occurring at 750 Wm^{-2} . PE021 used the same values for m and d for each cultivar, but these varied with the light intensity values (see **Table 3**).

Table 3. Constants used for determining assimilation (m and d) across a range of light intensity values.

Light Intensity (Wm^{-2})	m	d
<40	0.0953	-1.008
40 – 750	0.0325	1.506
>750	0.000	25.874

The negative y intercept represents net CO₂ release in darkness due to base respiration, while the 750 Wm^{-2} threshold represents the point at which light is no longer limiting photosynthesis. Gas exchange measurements in 2015 supported the use of 750 Wm^{-2} as an upper limit to assimilation rates.

2016 Model of Photosynthesis

A new photosynthesis model (the 2016 model) was developed based on the spot measurements of crop photosynthesis described in **Section 2.2.2**, using a statistical methods outlined in **Section 2.3.3**. In this instance, PAR was used as an input ($\mu\text{mol phot m}^{-2} \text{s}^{-1}$) to allow alignment with gas exchange measurements. Grower records of radiation in Wm^{-2} were converted to PAR using the West Midlands conversion factor developed by McCartney (1978) of $2.3 \mu\text{mol J}^{-1}$ (e.g. 100 Wm^{-2} equates to $230 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR). In a similar fashion to the 2014 model, light intensities were used to estimate A_H as $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$.

Estimating Crop CO₂ Offtake

The estimated assimilation rate from both models (A_H) was then used to calculate the amount of CO₂ absorbed and summed for each month. The scaling factor κ was used to convert A_H to a mass of CO₂ absorbed per cm² per hour. This was then multiplied by the foliage density to give a total mass of CO₂ absorbed per month. This could then be compared directly against the mass of CO₂ applied to the crop.

$$\kappa = 3600 \left(\frac{44}{1000000} \right) \left(\frac{1}{1000} \right)$$

1.3 Results

1.3.1 Development of the Allometric Leaf Area Model

Destructive measurements of leaf area were made in March, June, July and October. Total leaf area was measured, alongside leaf length and breadth. Measured area vs. LxB measurement were plotted and subject to linear regression analysis to define an allometric leaf area model for the top, middle and bottom canopy section of each cultivar (**Figure 3** and **Figure 4**).

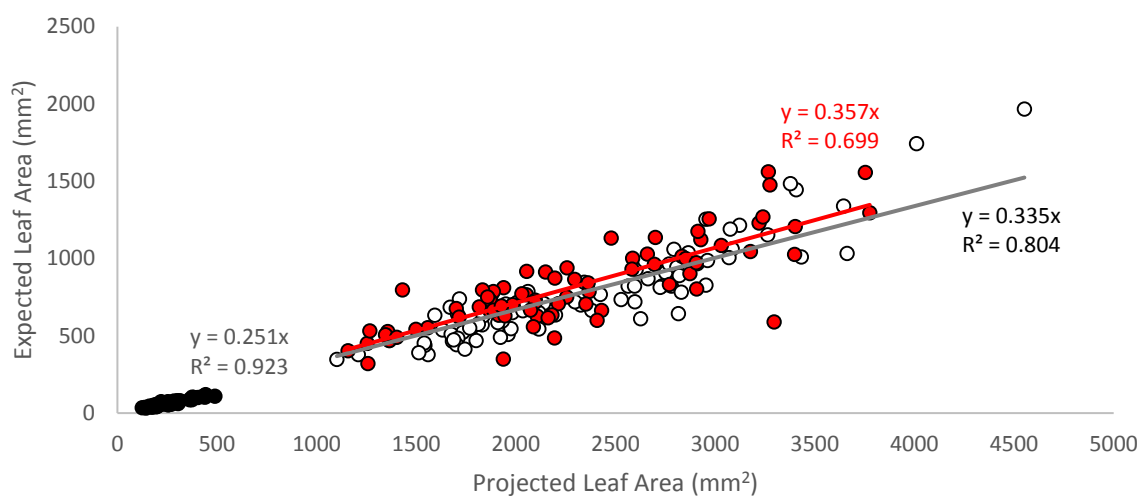


Figure 3. Estimated and projected leaf area for Avalantino across the season for top (●), middle (○) and bottom (●) canopy sections from four measurement dates across the 2017 season. Linear regression analysis has been plotted for each canopy section.

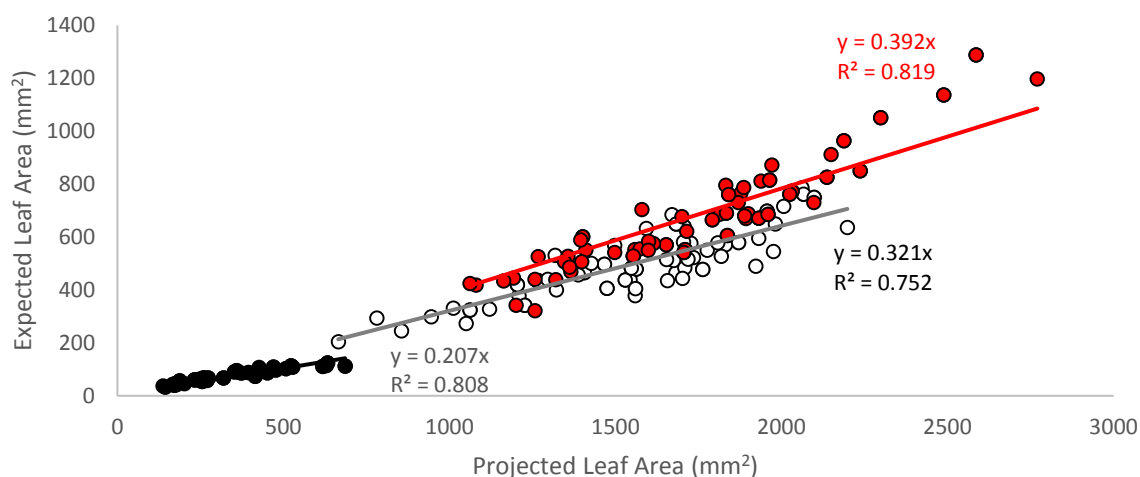


Figure 4. Estimated and projected leaf area for Piccolo across the season for top (●), middle (○) and bottom (●) canopy sections from four measurement dates across the 2017 season. Linear regression analysis has been plotted for each canopy section.

From these data, linear regression analysis was used to define allometric relationships between the projected leaf area and the expected leaf area, as given in **Table 4**. These were then utilised to make estimates for whole-canopy leaf area using the length/breadth measurements that had been recorded on a monthly basis. The coefficients from the 2017 season are largely comparable to those that had been defined in previous years.

Table 4. Estimated leaf allometric coefficients derived from destructive leaf area measurements.

Section	Piccolo 2015	Piccolo 2016	Roterno 2015	Avalantino 2016
Top	0.222	0.207	0.222	0.258
Middle	0.341	0.321	0.326	0.335
Bottom	0.366	0.392	0.358	0.357

1.3.2 Monthly Leaf Area Estimates

Monthly collection of leaf length and breadth measurements for each leaf on 10 plants of each cultivar were used to estimate the leaf area of each canopy section and whole plant head using the allometric coefficients outlined in **Section 2.3.1**, monthly averages of which are given in **Table 5**. Average leaf number across the season was greater in Piccolo (17.2 leaves/plant) than Avalantino (16.1 leaves/plant), although Piccolo achieved a lower average whole-head area (8041.8 cm²/head) than Avalantino (8863.5 cm²/head). A similar relationship was seen in 2015 whereby a smaller leaf number but greater leaf area was seen in Roterno when compared with Piccolo. The lower planting density of Avalantino (3.4 heads/m² vs. 4.2 heads/m² in Piccolo) resulted in the lower LAI for Avalantino (3.0 in Avalantino compared with 3.4 in Piccolo averaged across the season).

Table 5. Average estimates of total leaf areas for each cultivar by canopy section, total leaf number and LAI (leaf area index, m² of leaf per m² of ground) on a monthly basis across 2016 (n = 10 per cultivar per month, except March and June where n = 20).

	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct
Avalantino									
Top	219.7	332.9	1675.3	574.6	660.8	521.5	656.3	663.6	2249.9
Middle	3152.3	3072.6	2846.7	4504.6	4692.8	4547.8	3964.3	4212.6	3150.2
Bottom	3226.3	4154.9	2744.6	5172.5	4763.9	5451.4	5028.6	4862.4	2668.6
Total	6598.4	7560.4	7266.5	10251.7	10117.6	10520.8	9649.2	9738.6	8068.8
Leaf No.	14.3	15.8	15.1	19.7	17.6	18.4	17.4	18.1	8.4
LAI	2.2	2.6	2.5	3.5	3.4	3.6	3.3	3.3	2.7
Piccolo									
Top	281.0	412.9	997.4	497.6	329.0	327.6	238.8	547.7	1427.9
Middle	4094.5	3881.7	3549.8	3817.6	2881.3	3140.8	2090.1	4044.0	3134.1
Bottom	2743.2	4346.8	4295.2	5629.3	3917.3	4589.5	3159.7	5340.7	2660.6
Total	7118.7	8641.3	8842.3	9944.5	7127.6	8057.9	5488.6	9932.4	7222.6
Leaf No.	16.6	17.1	17.5	21.0	16.3	18.7	16.4	18.1	13.4
LAI	3.0	3.6	3.7	4.2	3.0	3.4	2.3	4.2	3.0

1.3.3 Modelling Photosynthesis for Non-Destructive Estimates of CO₂ Offtake

The estimation of plant leaf area in **Section 2.3.2**, combined with grower records of radiation values, were used as primary inputs for the estimation of crop CO₂ offtake over the 2016 season. A dual approach was taken: implementation of the model previously defined in 2014, and the development of a new model based on crop measurements in 2016 to reflect the change in grower site and cultivar.

1.3.4 Comparability of the 2014 Model to 2016 Data

The 2014 photosynthesis model developed for PE021 as summarised in **Section 2.2.6** combined with grower measurements of radiation to give hourly estimations for the rate of net CO₂ assimilation by the crop. The 2014 model was also applied to the observed spot measurements of photosynthesis described in **Section 2.2.2** to test the applicability of the original model to measurements made in the 2016 season. Median PAR measurements were input into the 2014 model, and compared against observed rates of assimilation (**Figure 5**). The 2014 model routinely underestimated the observed net assimilation rates, particularly in the May – August period. On this basis it was deemed necessary to reformulate the photosynthesis model to enhance the comparability between observed and estimates photosynthesis rates.

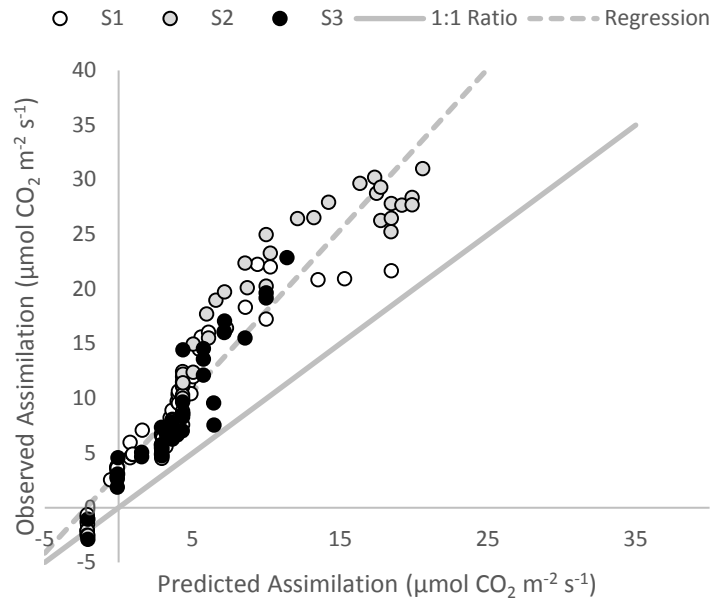


Figure 5. Predicted rates of net assimilation based on the 2014 model plotted against observed net assimilation rates seen in spot measurements for combined Piccolo/Avalantino data. S1: Feb – Apr, S2: May – Aug, S3: Sep – Nov 2016. A regression analysis of the complete data set is plotted ($r = 0.90$).

1.3.5 Reformulating the Photosynthesis Model

Estimates of crop CO₂ uptake were based on methods developed for PE021. Direct measurement of the rate of photosynthesis of the 6th leaf under ambient conditions made across the season (the spot measurements outlined in **Section 2.2.2**) were subject to regression analysis to determine a model that related incident PAR reception with an observed rate of photosynthesis. The spot measurement data was separated into three seasonal periods: February – April (S1), May – August (S2) and September – November (S3) on the basis of the trends shown in responses to CO₂ and to light response as discussed in **Section 2.3.9** and **Section 2.3.8**.

Spot measurements of photosynthesis were grouped by date, time, block and radiation level (which was constant for each of the 10 plants used per replication), and the median assimilation value used for each measurement group. No significant effect of cultivar was identifiable, so a single analysis was run on a combined Piccolo/Avalantino dataset (as was employed in the 2014 model used in PE021).

The light response curve analysis was used to identify the rate of dark respiration for each period. Measurements of photosynthesis made at PAR intensities of 0, 100 and 200 μmol phot m⁻² s⁻¹ were analysed by linear regression to identify the extent of negative photosynthesis at 0 PAR (i.e. the rate of CO₂ emitted through respiration in the absence of

photosynthetic oxygen evolution in darkness). Dark respiration estimates were included in the spot measurement data set as no other ambient darkness measurements were available, the values for each period are given in below in **Table 6** .

Table 6. Dark respiration rates used for each season block based on linear regression analysis of the light responses curves taken on a monthly basis.

Season	Dark respiration rate ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$)
1 (February – April)	0.798
2 (May – August)	2.107
3 (September – November)	1.641

Exponential Curve Fitting

The median net assimilation values from both the curve fitting data and the dark respiration data (averaged across cultivars), adjusted by the respiration rate for the relevant season, were used to investigate the photosynthetic response to increasing PAR. An exponential curve with the form $\text{Assimilation Rate} = A + B \cdot R^{\text{PAR}}$ was fitted to the adjusted assimilation values. The analysis was carried out in Genstat 16th Edition using the Standard Curves tool. Initially, a single curve was fitted for all the data, across all three seasons. Next parallel lines were fitted, with a single value for R but varying values for A and B for each season. Finally separate non-parallel lines were fitted for each season, with varying values for A, B and R for each season. The analysis showed that fitting separate lines for each season gave a significant improvement in the fit to the data, as summarised in **Table 7**.

Table 7. Accumulated analysis of variance for model fitting separate exponential curves for each season to Assimilation Rate vs. PAR data.

Source	d.f.	Sum sq.	Mean Sq.	v.r.	Prob > F
PAR	2	9997.78	4998.89	2266.69	< 0.001
Season (separate A)	2	135.46	67.73	30.71	< 0.001
PAR*Season (separate B)	2	133.78	66.89	30.33	< 0.001
Separate nonlinear (separate R)	2	17.88	8.94	4.05	0.020
Residual	128	282.29	2.21		
Total	136	10567.19	77.70		

Fitting final exponential curves with constrained origin

To ensure that the estimated net assimilation rate at 0 PAR matched the estimated dark respiration rate for each season, an exponential curve was fitted for each season with the origin constrained so that at 0 PAR, the predicted net assimilation would be equal to the estimated dark respiration rate for that season. The curves were fitted to the adjusted net

assimilation median values for each season, using the Standard Curves tool in Genstat 16th Edition with the option 'Constrain origin'. This fitted exponential curves of the form $\text{Assimilation Rate} = A + B \cdot R^{\text{PAR}}$ with the condition that $A = -B$. The value of A was then adjusted for each season by subtracting the dark respiration rate, to give a model for each season that predicts net assimilation including the effect of respiration. The asymptote region of the S3 model is constrained by the lack of observations from light intensities above 650 PAR, but environmental measurements did not exceed this level often during this period, minimising the effect of the inaccuracy beyond this PAR level. The fitted models are summarised in **Table 8** and illustrated in **Figure 6**, and the goodness of fit between observed and estimated rates of photosynthesis are given in **Figure 7**.

Table 8. Fitted models for each season. Net Assimilation = $A + B \cdot R^{\text{PAR}}$.

Season	A	B	R	% Variance
February – April	22.508	-23.307	0.997	96.6
May – August	29.587	-31.694	0.997	98.5
September – November	23.391	-25.032	0.997	86.6

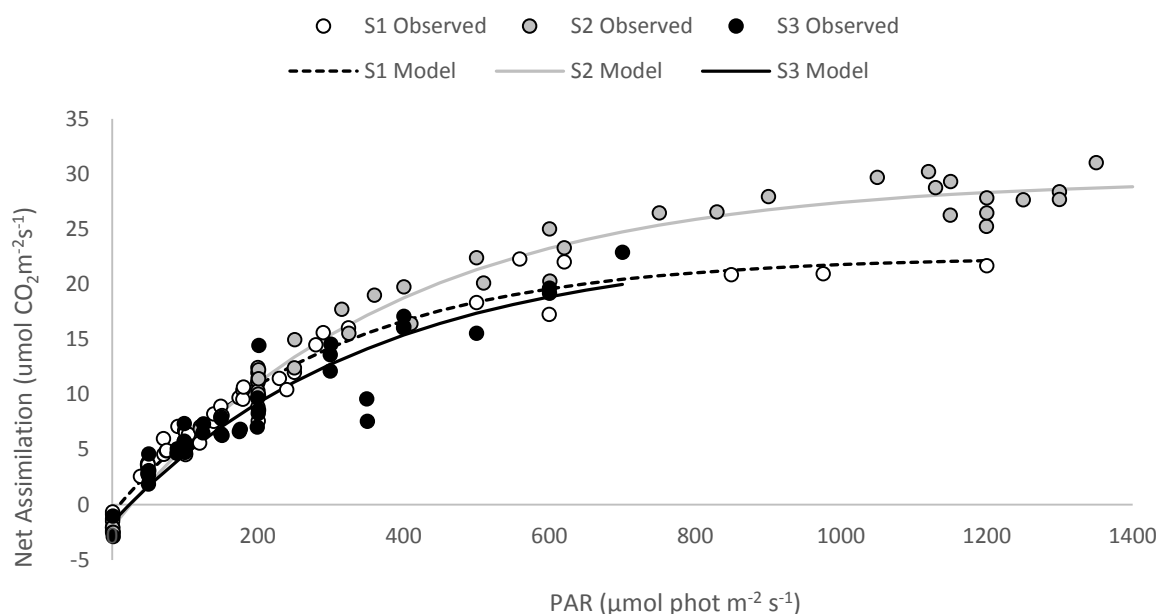


Figure 6: Median observed rates of net assimilation against PAR taken from spot measurements made across the 2016 season, from combined Piccolo/Avalantino data in season period 1 – 3. The model fitted against each season period is given. S1: Feb – Apr, S2: May – Aug, S3: Sep – Nov 2016.

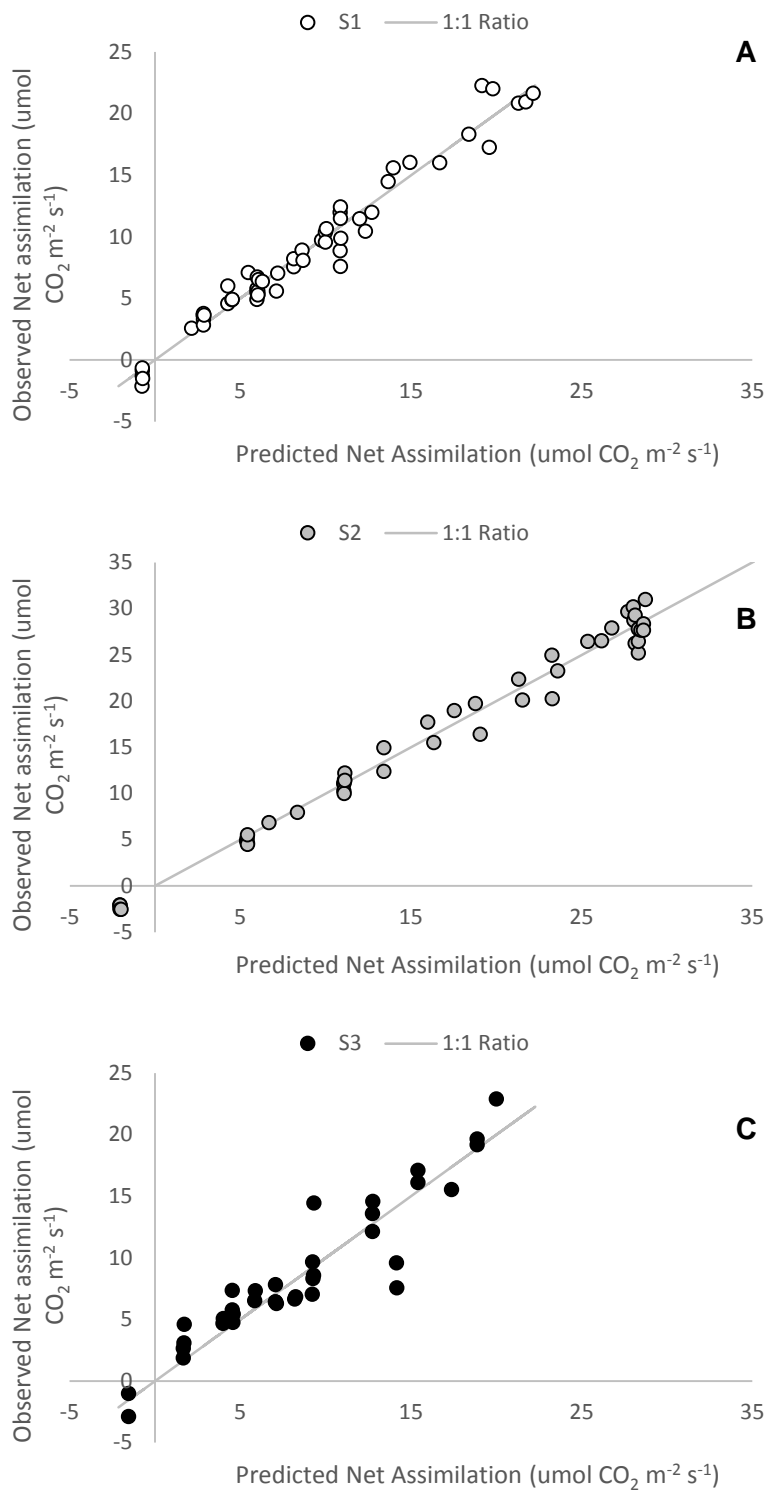


Figure 7. Observed against predicted net assimilation estimated using the model parameters defined in Figure 7/Table 8. Median PAR values for a combined Piccolo/Avalantino dataset were used to generate predicted rates of photosynthesis, and are plotted against observed rates. Separate models were applied to separate parts of the 2016 season: **A:** February – April, **B:** May – August, **C:** September – November.

The fitting of an exponential curve is in contrast to the approach used in the 2014 model: this utilised separate linear models to estimate rates of photosynthesis over three discrete ranges of radiation 50 Wm^{-2} , $50 - 750 \text{ Wm}^{-2}$ and above 750 Wm^{-2} as discussed in **Section 2.2.6**. The shape of the exponential curve fitted the data well, and in fitting the curves it was not necessary to first make assumptions about appropriate values of PAR at which changes of rate might occur; the exponential curve allows a more gradual change of rate. A summary of the models is given in below in **Figure 8**.

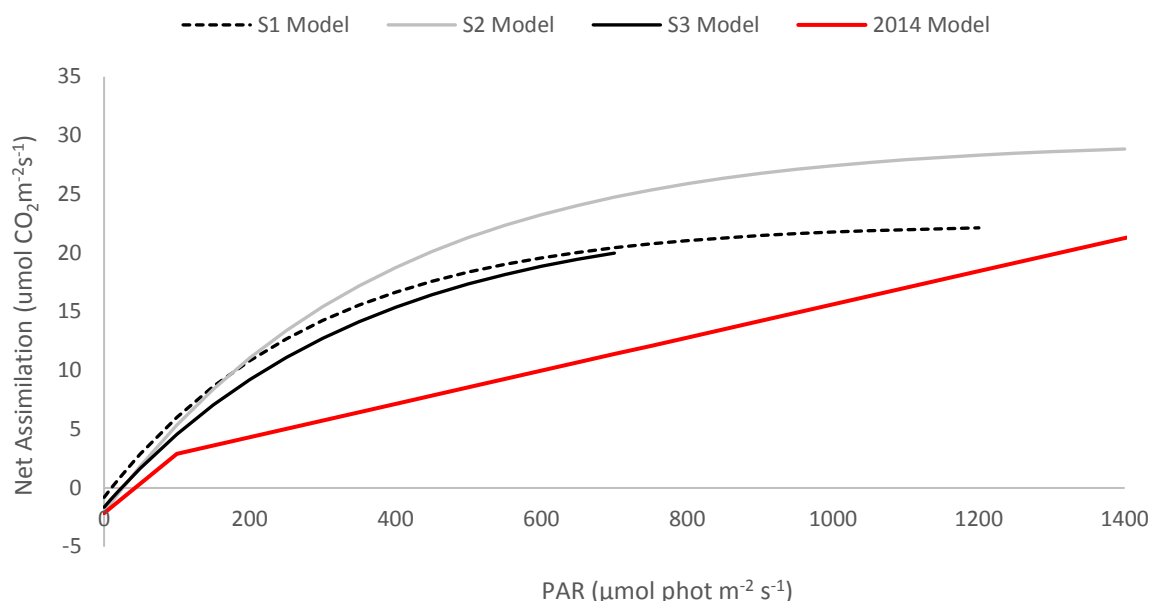


Figure 8. Comparison of the models developed for the 2016 season data relative to the linear model developed in 2014. Separate models were applied to separate parts of the 2016 season based on measurements taken from three seasonal blocks: **S1**: February – April, **S2**: May – August, **S3**: September – November.

1.3.6 Estimating CO₂ Offtake

Hourly radiation measurements taken at the glasshouse roof level were used as primary input for estimating hourly rates of photosynthesis. Technical issues at the grower site prevented the accumulation of data across the season, but a period between 1st February and 15th September was covered by records. Radiation measurements obtained as Wm^{-2} , which were the units required for input into the 2014 model. Conversion of radiation measures in Wm^{-2} into PAR ($\mu\text{mole phot m}^{-2} \text{ s}^{-1}$), and a conversion factor of $2.3 \mu\text{mol/J}$ was used to determine incident PAR (McCartney & Unsworth, 1978). Direct measurements of light transmission were not available, but a standard figure of 0.85 (Kozai & Kimura, 1977) was taken for the rate of light transmission through the glass. Summary radiation statistics are given in **Figure 9**. The hourly radiation/PAR values were input into the respective model and used to give a mass of CO₂ absorbed per m² of leaf per hour as summarised in **Section 2.2.6**. Total mass of CO₂ per m² of leaf absorbed between 1st February and 15th September 2016 was estimated at 3.42kg using the 2014 model, and 4.65 using the 2016 model. The discrepancy in estimates

between the two models increased across the season, with significant differences in estimates CO₂ uptake being shown in the September period (**Figure 10**).

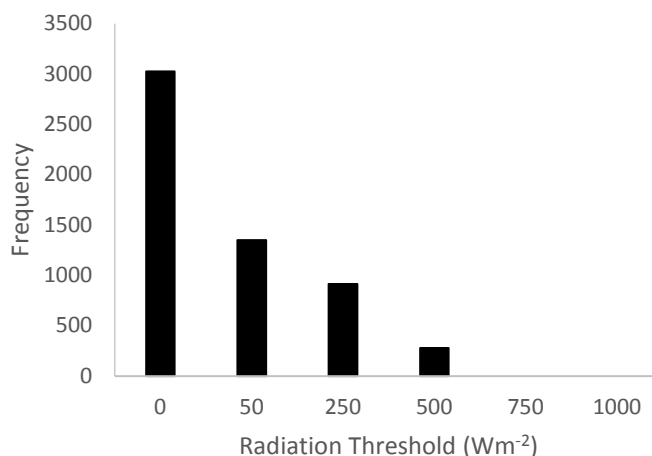


Figure 9. Radiation receipts at the trial site. The number of hours that exceeded the radiation thresholds used in the assimilation model are given for the period 1st February – 15th September 2016.

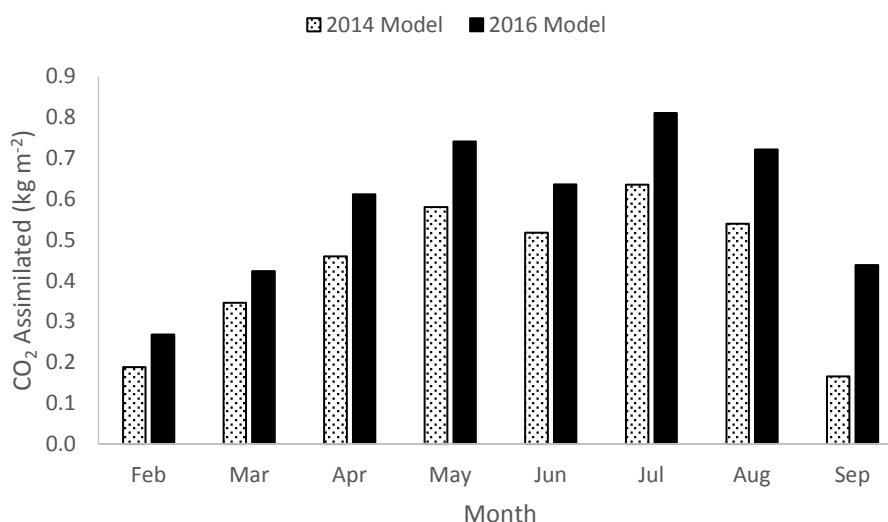


Figure 10. Estimates of CO₂ uptake per m² of leaf across between 1st February – 15th September 2016 according to the 2014 and 2016 photosynthesis models.

The leaf area estimates given in **Section 2.3.2** were combined with planting densities and planting area data to give a total monthly foliage estimate (**Table 9**). Piccolo was planted in an area of 7704m² at a density of 4.2 heads/m², and Avalantino planted over an area of 6000m² at a density of 3.4 heads/m². By combining daily estimates of CO₂ offtake with monthly estimates of foliage area, a total value for CO₂ uptake by the crop could be determined.

Table 9. Estimated foliar area expressed as Foliage Density ($\text{m}^2 \text{ha}^{-1}$) and Total Foliar Area for the trial area (m^2).

	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct
Foliage Density									
Avalantino	22434	25705	24706	34856	34400	35771	32807	33111	27434
Piccolo	29898	36294	37138	41767	29936	33843	23052	41716	30335
Tot. Foliar Area									
Avalantino	13461	15423	14824	20913	20640	21462	19684	19867	16460
Piccolo	23034	27961	28611	32177	23063	26073	17759	32138	23370

Complete monthly records for radiation and CO_2 application were only available for the February to September period, so offtake estimation was restricted to this period. Radiation measurements and foliar density were input into both the 2014 and 2016 photosynthesis models, and were compared with grower records of CO_2 application to estimate crop offtake on a monthly basis (**Table 10**).

For Avalantino, the greatest crop offtake from the total mass of CO_2 supplied by the grower was estimated to have occurred in February (39.3%, 2016 model), with the smallest offtake record in April (15.9%, 2016 model). Total crop CO_2 offtake over the February – September period was 17.98% (2014 model) / 23.13% (2015 model). For Piccolo, peak offtake was estimated in March (34.2%, 2016 model) with lowest offtake recorded in August (20.6%, 2016 model). Total crop CO_2 offtake for Piccolo was 20.52% (2014 model) / 26.41% (2016 model). The discrepancy in estimates between the 2014 and 2016 model averaged 22%, but was greatest in the early period of the season.

Monthly changes in offtake are attributable to a number of early factors. The high offtake recorded in February and March was due to low rates of CO_2 application, while the decrease in offtake in April was because of increased CO_2 application without concurrent increases in foliar area and/or radiation: CO_2 application in February was 16% (Avalantino) and 26% (Piccolo) of that applied in April. The decline in offtake in June is attributable to a drop in radiation receipts for both cultivars, and a decline in foliar area for Piccolo. A summary of the relationship between the causal factors and estimated offtake for both cultivars is summarised in **Figure 11** below.

Table 10. Summary figures for monthly CO₂ assimilation estimates and offtake estimates for the 2016 season.

		Month							Total
		Feb	Mar	Apr	May	Jun	Jul	Aug	
Avalantino	Foliar Area (m ²)	13461	15423	14824	20913	20640	21462	19684	
	2014 CO ₂ Uptake (kg)	0.19	0.35	0.46	0.58	0.52	0.64	0.54	3.27
	Model Total CO ₂ Uptake (kg)	2533	5336	6811	12146	10683	13632	10618	61759
	2016 CO ₂ Uptake (kg)	0.27	0.42	0.61	0.74	0.64	0.81	0.72	4.21
	Model Total CO ₂ Uptake (kg)	3617	6540	9071	15496	13123	17403	14206	79456
	CO ₂ Applied (kg)	9202	28441	57004	59684	60760	69414	59077	343583
	2014 Model Offtake (%)	27.53	18.76	11.95	20.35	17.58	19.64	17.97	17.98
	2016 Model Offtake (%)	39.31	22.99	15.91	25.96	21.60	25.07	24.05	23.13
	Discrepancy (%)	29.97	18.41	24.91	21.62	18.59	21.67	25.26	22.27
	Piccolo	Foliar Area (m ²)	23034	27961	28611	32177	23063	26073	17759
2014 CO ₂ Uptake (kg/m ²)		0.19	0.35	0.46	0.58	0.52	0.64	0.54	
Model Total CO ₂ Uptake (kg)		4335	9673	13146	18688	11937	16561	9579	83919
2016 CO ₂ Uptake (kg/m ²)		0.27	0.42	0.61	0.74	0.64	0.81	0.72	4.21
Model Total CO ₂ Uptake (kg)		6189	11856	17507	23842	14663	21142	12817	108016
CO ₂ Applied (kg)		21334	34661	80369	67908	66953	75523	62171	408919
2014 Model Offtake (%)		20.32	27.91	16.36	27.52	17.83	21.93	15.41	20.52
2016 Model Offtake (%)		29.01	34.20	21.78	35.11	21.90	27.99	20.62	26.41
Discrepancy (%)		29.97	18.41	24.91	21.62	18.59	21.67	25.26	22.31

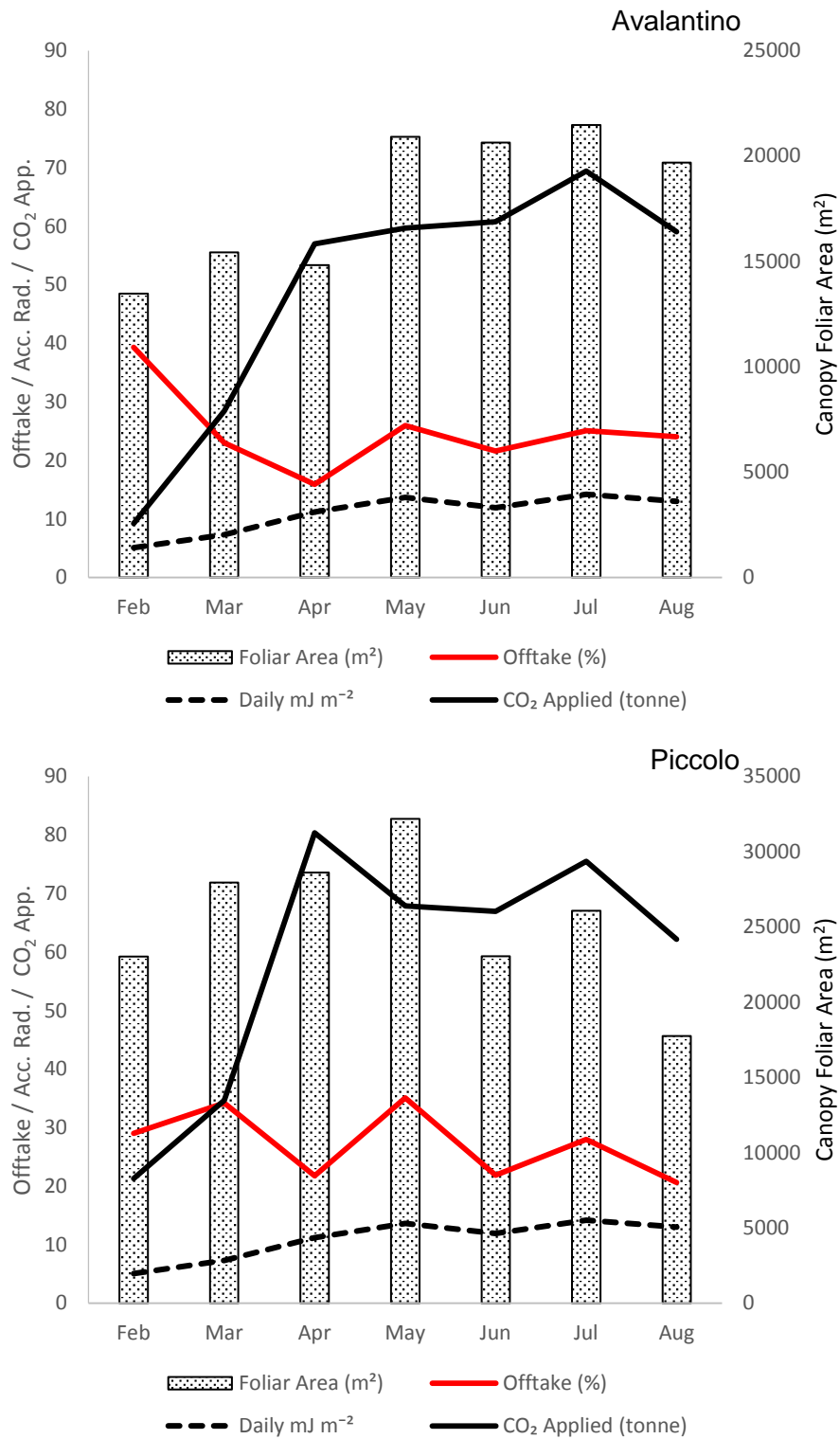


Figure 11. Relationship between causal factors of offtake estimates using the 2016 model. Monthly accumulated radiation (Acc. Rad.) is given at mJ m⁻², total CO₂ application in Tonnes, and the foliar area of the crop (m²) is compared with estimated CO₂ offtake.

1.3.7 Canopy Effects

At five points across the season canopy-level measurements of assimilation were made under ambient conditions using PAR levels set at the top of the plant. Four consecutive leaves from the top, middle and bottom canopy section were measured, giving a total of 12 leaves per plant. In instances where more than 12 leaves were on a plant the leaves were taken from the centre of each canopy section, although this resulted in non-continuous canopy section in these instances (see **Table 5**). Three plants were measured on each date, over three time points during day light hours, with measurements being made in February, March, June, August and October. This spread of measurements was deemed sufficient to encapsulate changes in the plant during establishment, onset of fruit and peak productivity.

A typical photosynthetic assimilation profile showed peak assimilation in the middle canopy layer (representing the youngest fully expanded leaves) before declining into the lower canopy, with the lower leaf layer showing the smallest level of assimilation at a constant light level (**Figure 12**). The lower canopy also showed the greatest level of variation as a result of differences in leaf position (e.g. projecting out into the pathway vs. into the canopy centre).

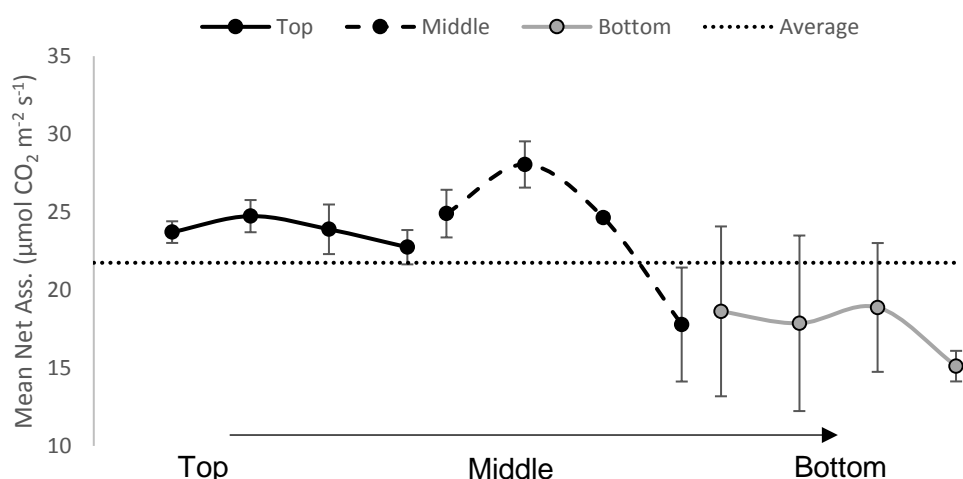


Figure 12. Average assimilation in an Avalantino canopy, measured 9th June at 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR. Corresponding leaves within each canopy section were averaged at each position, and the average rate of assimilation across all leaves measured is plotted.

Because of variation in leaf number, measurement conditions and variation between leaf position significant relationships could not be identified by direct comparison between measurement sets. Alternatively, an average rate of assimilation was determined for each plant at each measurement point and the observed rates of assimilation determined as a proportion of the average rate of assimilation, with average values given for each monthly measurement for each cultivar. Leaves in the upper canopy section of Avalantino were typically more productive, with an average relative assimilation rate of 1.08, compared with 1.00 and 0.82 in the middle and lower canopy respectively. Conversely, the middle canopy

section of Piccolo showed higher relative rate of assimilation across the season, averaging 1.05 compared with 1.01 and 0.85 in the upper and lower canopy sections respectively. In both cultivars the lower canopy typically showed lower rates of assimilation than the upper and lower canopy section.

Table 11. Assimilation rates of canopy sections as expressed as a ratio of each measurement against the whole plant average. Average relative rate for each canopy section is given at T_{Tot}, M_{Tot} and B_{Tot}. Avalantino plants had few than 12 leaves in February and October, resulting in * values for the B4 leaf. Values are colour coded within each month grouping.

		Avalantino					Piccolo				
Section		Feb	Mar	Jun	Aug	Oct	Feb	Mar	Jun	Aug	Oct
Top	1	1.08	0.90	1.19	1.32	1.33	0.88	0.92	0.99	0.93	1.03
	2	1.06	1.00	1.09	1.17	1.02	0.89	0.94	1.11	1.04	1.20
	3	1.00	0.99	1.10	1.26	0.89	1.00	0.98	1.19	1.03	1.20
	4	1.02	1.01	1.06	1.34	1.18	1.06	1.05	1.06	1.04	1.28
Middle	1	1.03	1.09	1.06	1.28	1.01	1.13	1.08	1.11	0.99	1.42
	2	1.04	1.07	1.19	1.17	0.87	1.08	1.13	1.22	1.08	1.11
	3	1.01	1.02	1.10	0.98	1.15	1.01	1.12	1.13	1.07	0.83
	4	1.04	1.00	0.90	0.98	0.71	1.04	1.08	1.02	1.10	0.92
Bottom	1	0.95	0.97	0.90	0.70	1.00	1.11	1.05	1.02	0.97	0.64
	2	0.89	0.97	0.88	0.58	0.91	0.95	1.03	0.75	0.87	1.17
	3	0.88	1.00	0.84	0.59	0.74	0.99	0.85	0.75	0.98	0.76
	4	*	0.98	0.67	0.63	*	0.84	0.75	0.65	0.91	0.45
T _{Tot}		1.01	0.95	1.08	1.24	1.10	0.95	0.93	1.06	0.98	1.15
M _{Tot}		1.00	1.02	1.03	1.07	0.88	1.07	1.04	1.09	1.03	1.04
B _{Tot}		0.87	0.95	0.80	0.61	0.88	0.90	0.95	0.77	0.91	0.73

Canopy-level relationships showed significant variation, particularly in line with radiation levels. In instances where lower PAR levels were used, assimilation rates were more constant between different canopy sections such as the March measurements for Piccolo and Avalantino which were taken at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR which showed variance from the whole-plant average between 0.90 – 1.09 (Avalantino) and 0.75 – 1.13 (Piccolo). Conversely, measurements taken in June at 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR saw variance between 0.69 – 1.31 (Avalantino) and 0.63 – 1.29 (Piccolo). This variation was due to an apparent difference in response to increased light levels between canopy sections. Avalantino crops measured at low (250 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and high (1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) PAR in July showed a disproportionate increase for rates of assimilation in the middle canopy that was not observed in the upper and lower canopy sections (**Figure 13**). Therefore, while these data shed some light on changes in the relative assimilatory potential for the canopy section there is too much variation in supporting factors (e.g. PAR levels, time) to properly account for variation demonstrated in the rates of assimilation between separate canopy sections.

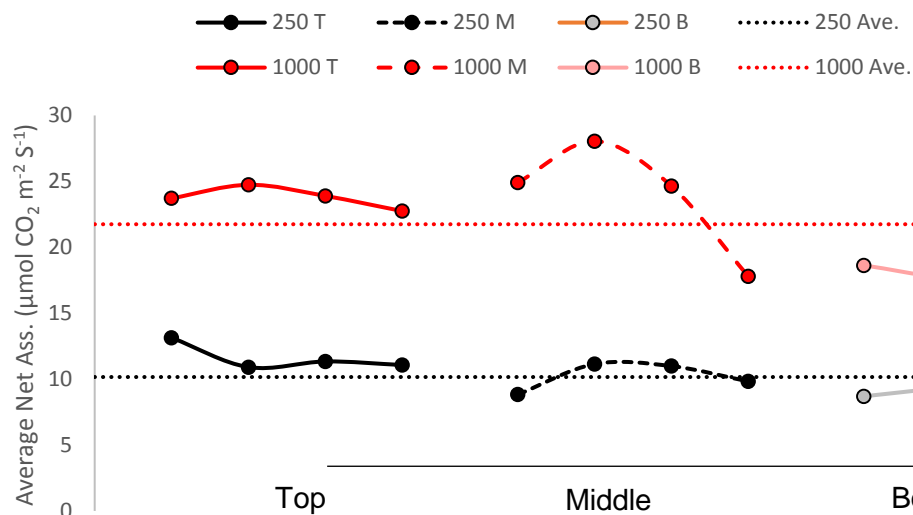


Figure 13. Average rates of assimilation in an Avalantino canopy measured at 250 and 1000 m² s⁻¹ PAR, June 2016. The average whole-plant assimilation rate is plotted for each PAR level.

1.3.8 Light Responses

Crop responses to varying light conditions were made on a monthly basis, with either two or three curves measured per cultivar (**Figure 14**), except technical issues resulted in only one curve being recorded for September in Piccolo. Both cultivars showed a depressed response to light in February and March, with a marked increase in light response in April, coincident with the onset of heavy fruit load. Light responses between April and July remained relatively constant. Piccolo recorded a decline in light response in August, but a significant decline in light response was not seen in Avalantino until September. After this point, light responses rapidly declined until November, where maximum rates of assimilation were less than a sixth of that seen in the summer period.

In the early and late season, the onset of light saturation occurred at relative low levels below 300 µmol phot m⁻² s⁻¹ PAR. This increased proportionately across the summer where Piccolo was reaching saturation above 1000 µmol phot m⁻² s⁻¹ PAR. Generally, the Avalantino response to light was somewhat depressed compared with Piccolo.

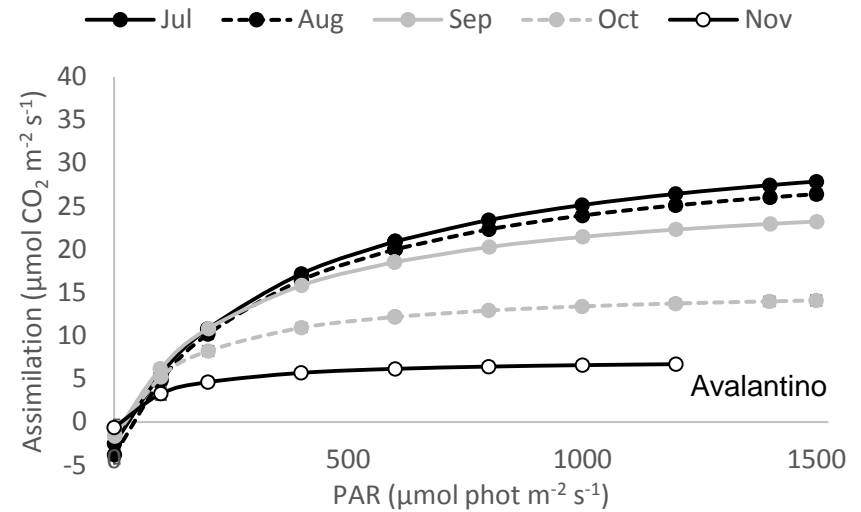
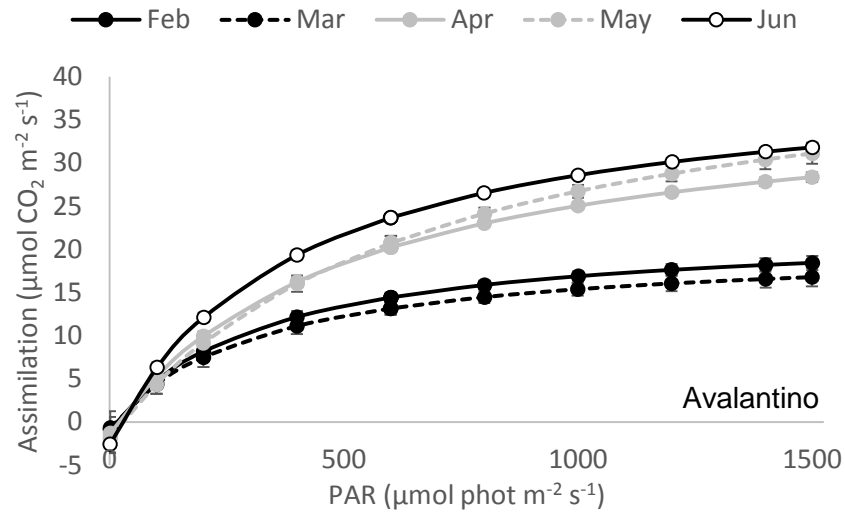
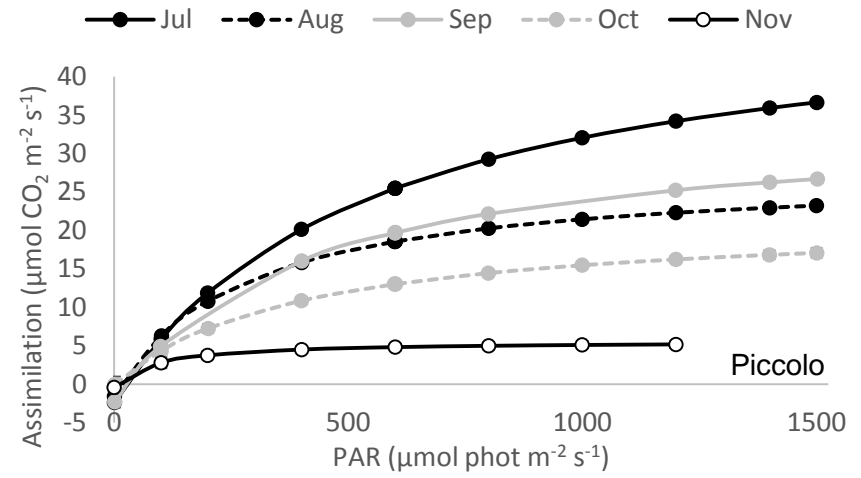
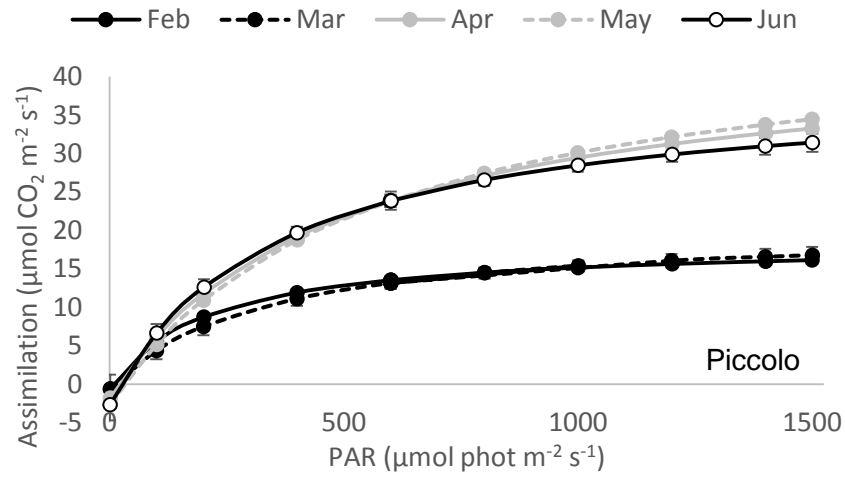


Figure 14. Average light response curves across the 2016 season. Curves for February, March, April, May and July were based on three separate curves, with the remainder based on two curves made on the same date. As an exception, the Piccolo curve for September is based on a single set of measurements.

1.3.9 CO₂ Responses

Monthly measurement of crop responses to changing CO₂ concentrations were made between February and November. Measurements of assimilation over a controlled range of CO₂ concentrations at saturated light level were made. A minimum of three sets of measurements were made on a monthly basis, and used to construct average A/C_i curves for each month (**Figure 15**). From the A/C_i curves could be derived explanatory factors of the maximum rate of photosynthesis (A_{Max}), carboxylation efficiency (CE) and the CO₂ compensation point (**Table 12**). The CO₂ responses of both cultivars showed marked increases across the early season until a peak was reached in June and July. During this time the A_{Max} increased for both cultivars, along with the carboxylation efficiency. An increase for 0.1 to 0.30 (Avalantino) and 0.25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was detected between February and July. This represents greater investments in leaf biochemistry (e.g. enzymes) allowing the plant to increased efficiency of CO₂ uptake, increasing uptake at CO₂ concentrations below saturation. On average, carboxylation efficiency was comparable between Avalantino and Piccolo, although the latter showed a 0.05 $\mu\text{mol m}^{-2} \text{s}^{-1}$ reduction in peak CE over June and July. Peak CO₂ response was sustained until August, but both cultivars demonstrated a decline in CO₂ response from September, with November responses below that seen in the spring (**Figure 11**). Mathematically determined A_{Max} values remain comparable over this period (**Table 12**), although these will only be reached at CO₂ far beyond those found in a typical glasshouse environment.

Table 12. Derived photosynthetic parameters for both cultivars, averaged by month. A_{Max} : Predicted maximum rate of assimilation, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, CC: CO₂ compensation point, $\mu\text{mol mol}^{-1}$, CE: Carboxylation efficiency, $\mu\text{mol m}^{-2} \text{ s}^{-1}$. The August – September average for 2015 is given for the category 3 Roterno as measurements are not available for Avalantino.

	Avalantino			Piccolo			
	A_{Max}	CC	CE	A_{Max}	CC	CE	
Feb	24.43	64.48	0.10	21.07	61.86	0.11	
March	24.43	64.48	0.10	21.07	61.86	0.11	
April	33.89	67.45	0.16	39.81	70.44	0.16	
May	39.73	58.26	0.24	39.90	57.87	0.25	
Jun	38.37	57.00	0.30	42.68	58.45	0.25	
Jul	43.82	59.18	0.30	36.96	60.45	0.25	
Aug	45.31	63.32	0.24	41.48	60.08	0.24	
Sep	38.54	74.53	0.20	38.90	58.55	0.20	
Oct	45.31	63.32	0.24	41.48	60.08	0.24	
Nov	38.54	74.53	0.20	38.90	58.55	0.20	
Average	37.24	64.65	0.21	36.22	60.82	0.20	
Aug/Sep	2016	41.93	68.92	0.22	40.19	59.31	0.22
Average	2015	35.35	61.83	0.23	38.15	61.68	0.2

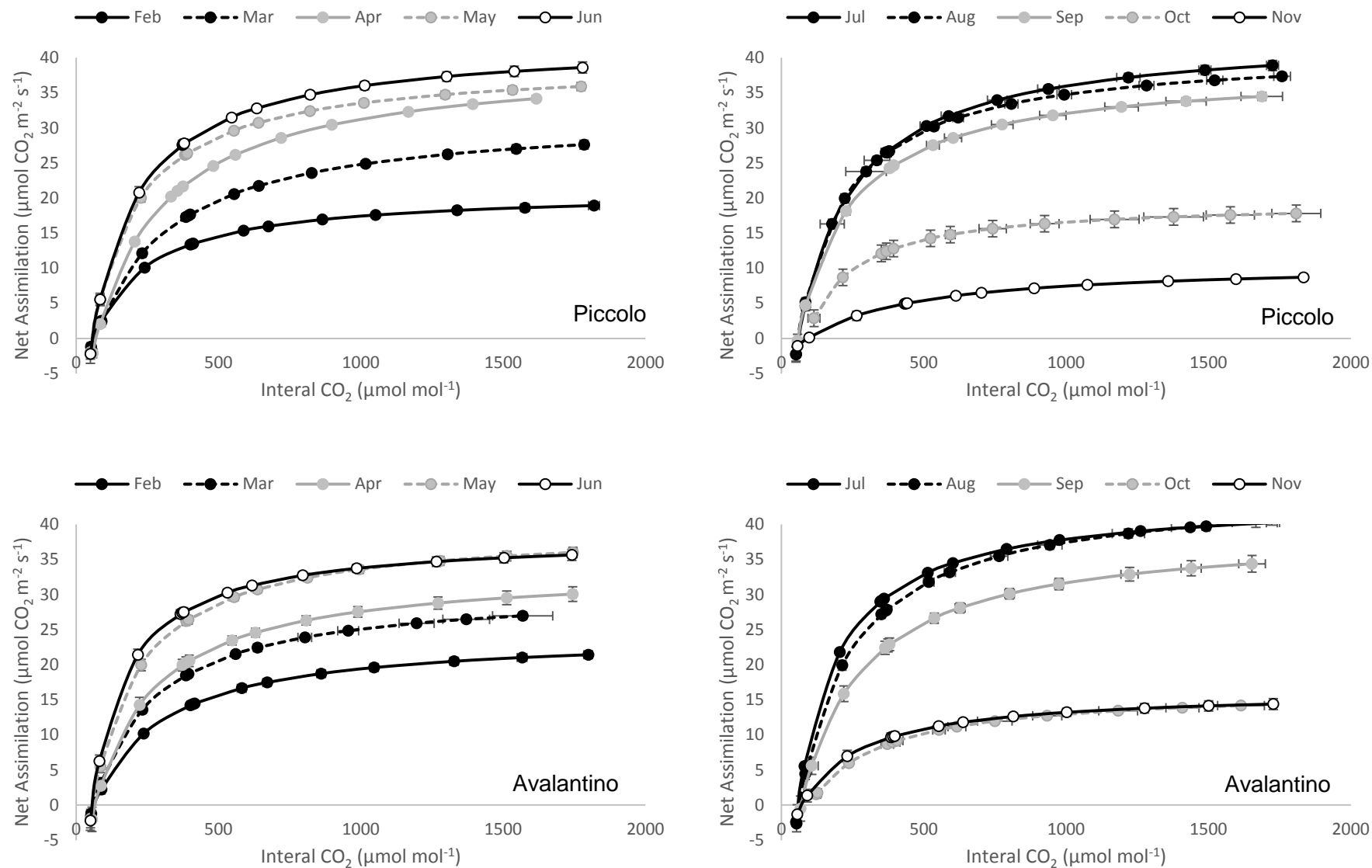


Figure 15. A/C_i curves across the 2016 season for Piccolo and Avalantino. Each curve represents the average of a minimum of three measurement sets per month.

1.3.10 Yield Outputs

Average weekly marketable yields were 0.56 kg m⁻² for Piccolo and 1.01 kg m⁻² for Avalantino, compared with 0.55 kg m⁻² for Piccolo and 1.06 kg m⁻² for Roterno grown in the same area in 2015. Piccolo yields were much more linear than Avalantino, showing reduced rates of increases with high levels of accumulated radiation (**Figure 16**). The proportion of unmarketable produce did not increase proportionality, with much higher proportions and unmarketable produce at higher radiation levels, particularly in Avalantino.

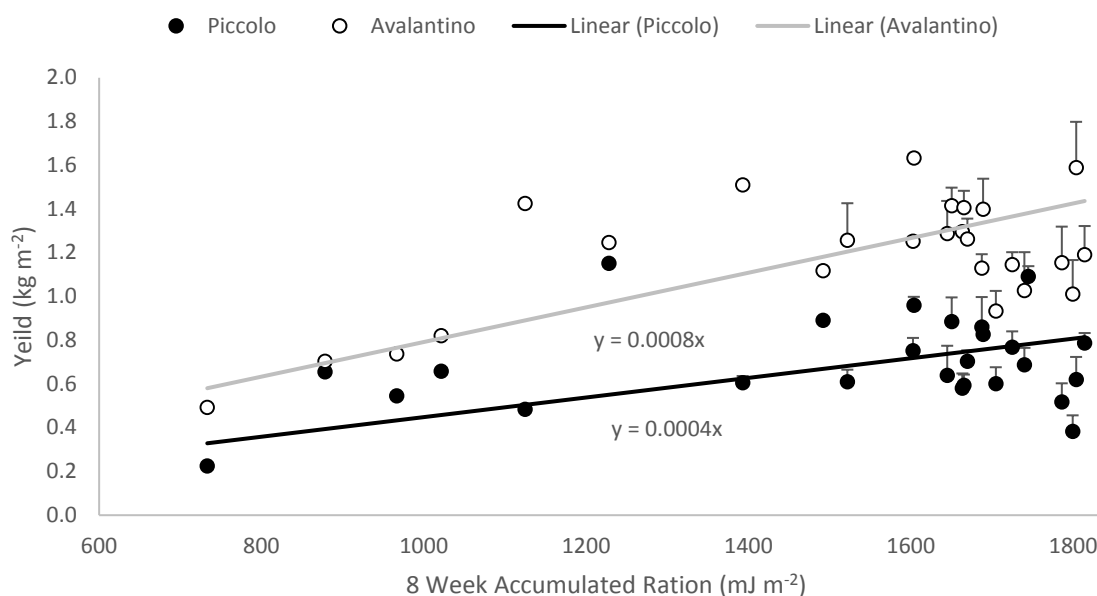


Figure 16. Yield responses to 8-week accumulated radiation measures. Marketable yield is plotted, with the positive error indicating additional yield recorded as waste.

1.3.11 Diurnal Variation

To explore the effects of time and environment on the ability of the crop to utilise CO₂, two sets of data were combined into a single analysis: the individual response curves to PAR (**Section 2.2.4**) and to internal CO₂ concentration (**Section 2.2.3**). Using these data it was possible to explore how time, past radiation levels (measured as accumulated radiation, MJ m⁻²) and temperature (measured as thermal time, °C hr) could interact to explain crop CO₂ assimilation potential. 28 separate light response curves (representing 336 measurements) and 36 CO₂ response curves (from 468 measurements) were used in the analysis.

Response to PAR

Data from the light response curves (**Section 2.2.4**) by date and time of measurement. Groups of measurements taken at a median time (taken from the c.40 minute period required to construct each curve) from 10am up to 1pm were assigned to time group '2'. Groups of measurements taken at a median time from 1pm up to 3pm were assigned to time group '3'.

An exponential curve with the equation $Net\ Assimilation = A + B * R^{PAR}$ was fitted to each time group for each date. The fitted curves were examined visually to check fit.

The data was restricted to dates where measurements had been taken at both time groups '2' and '3', for both cultivars 'A' and 'P', during seasons '1' or '2' (there was insufficient data from season '3' to allow comparison of the effect of season '3', particularly due to the limited number of curves taken for Piccolo), and where there was data relating to temperature and radiation levels from grower records. .

From each curve, the value of the asymptote (the parameter 'A' from the exponential equation) was extracted and the area under the fitted curve between 0 $\mu\text{mol phot m}^{-2} \text{s}^{-1}$ and 1500 $\mu\text{mol phot m}^{-2} \text{s}^{-1}$ PAR was calculated ('AUC1500'). The asymptote and 'AUC1500' were used as the response variables describing the photosynthetic response to radiation, and biologically represented the maximum rate of assimilation under saturated light and CO₂.

A forward stepwise regression was carried out to compare the effects of the following factors on the asymptote and 'AUC1500':

- Season: '1' (February-April) or '2' (May-August).
- Cultivar: 'A' (Avalantino) or 'P' (Piccolo).
- Time group: '2' (10am-1pm) or '3' (1pm-3pm).
- Background cumulative radiation level over time group duration 'RadTimeGrp': 'M' (< 5 mJ m^{-2}) or 'H' ($\geq 5 \text{ mJ m}^{-2}$).
- Background cumulative radiation level on date before 10am 'RadT1Grp': 'L' (< 2 mJ m^{-2}) or 'M' ($\geq 2 \text{ mJ m}^{-2}$).
- Cumulative thermal energy over time group duration 'ThermTimeGrp': 'M' (< 72.5 , °C hr) or 'H' (≥ 72.5 , °C hr).
- Cumulative thermal energy on date before 10am 'ThermT1Grp': 'M' (< 190 °C hr) or 'H' (≥ 190 °C hr).

The analysis treated observations taken on the same date but on different cultivars or at different times of day as independent. Interaction terms were not included in the stepwise regression, as the data did not cover all of the possible combinations of factors (as background thermal energy and radiation were not controlled/most combinations would be limited to a single data point).

An analysis of variance was carried out for the terms that the forward stepwise regression showed to provide the best fit model (**Table 13 & Table 14**). For both asymptote and 'AUC1500', 'RadTimeGrp' and 'Season' were found to explain the most variation in photosynthetic response. The values of the asymptote and 'AUC1500' were higher when 'RadTimeGrp' was high, and were higher in season 2 compared with season 1.

Table 13. Accumulated analysis of variance for best fit model from forward stepwise regression for asymptote of Assimilation Rate vs. PAR curve. 76.4% variance accounted for.

Source	d.f.	Sum sq.	Mean Sq.	v.r.	Prob > F
RadTimeGrp	1	1187.42	1187.42	80.61	< 0.001
Season	1	131.99	131.99	8.96	0.006
Residual	25	368.27	14.73		
Total	27	1687.68	62.51		

Table 14. Accumulated analysis of variance for best fit model from forward stepwise regression for 'AUC1500' (area under Assimilation Rate vs. PAR curve between PAR=0 units and PAR=1500 units). 72.8% variance accounted for.

Source	d.f.	Sum sq.	Mean Sq.	v.r.	Prob > F
RadTimeGrp	1	866786168	866786168	63.46	< 0.001
Season	1	146823015	146823015	10.75	0.003
Residual	25	341476592	13659064		
Total	27	1355085776	50188362		

The results of this analysis indicate crop response is primarily dictated by the radiation level (RadTimeGrp) and Season. The combination of these two features accounts for over 75% of the observed variation in observed assimilation rates. The inclusion of radiation is explicable through the energetics of photosynthesis and biological condition. Under periods of high light, coincident rates of assimilation will be proportionately higher. In a similar fashion, the seasonal variation in light response outlined in **Section 2.2.4** indicates strong correlation between season and observed assimilation rates. The second feature of this analysis is that time of day is not detectable in these as a significant factor on observed rates of assimilation in response to light.

A paired t-test analysis was carried out to compare responses at time 2 (10am-1pm) and 3 (1pm-3pm) on each date for each cultivar. In contrast to the stepwise forward regression and analysis of variance, this analysis did not make the assumption that measurements taken from the same cultivar on the same date but at different times of day were independent. It was however assumed that measurements taken from different cultivars on the same date were independent. The analysis showed that the area under the light response curve up to 1500 $\mu\text{mol phot m}^{-2} \text{s}^{-1}$ (including both the light-limited and light-saturated phase) was greater on average at time 2 compared with time 3 (paired $t(13) = 3.19$, $p = 0.0071$). The asymptote (AMax) (paired $t(13) = 3.60$, $p = 0.0033$), or saturated rate of assimilation, at time 2 was 3.88 $\mu\text{mol m}^{-2} \text{s}^{-1}$ greater on average than at time 3. These diurnal effects are small relative to the between-date variation in photosynthetic response.

Response to CO₂ Concentration

Data on response to increasing levels of CO₂ at saturated levels of PAR (approx. 1500 ppm) were grouped by date and time of measurement as in the analysis of factors affecting photosynthetic response to increasing levels of PAR. An exponential curve with the equation $Net\ Assimilation = A + B * R^{CO_2}$ was fitted to each time group for each date. The fitted curves were examined visually to check fit.

The data was restricted to dates where measurements had been taken at both time groups '2' and '3', for both cultivars 'A' (Avalantino) and 'P' (Piccolo), during seasons '1' or '2' (as above, and in similar fashion there was insufficient data from season '3' to allow comparison of the effect of season '3'), and where there was information on both background radiation and thermal energy.

From each curve, the value of the asymptote (the parameter 'A' from the exponential equation and which corresponds to A_{Max}) was identified and the area under the fitted curve between CO₂ = 0 ppm and CO₂ = 1500 ppm was calculated ('AUC1500'). The asymptote and 'AUC1500' were used as the response variables describing the photosynthetic response to radiation.

A forward stepwise regression was carried out to compare the effects on the values of the asymptote and 'AUC1500' of the factors described in the section 'Analysis of factors affecting photosynthetic response to radiation: 'Season', 'Cultivar', 'Time group', 'RadTimeGrp', 'RadT1Grp', 'ThermTimeGrp' and 'ThermT1Grp'.

An analysis of variance was carried out for the terms that the forward stepwise regression showed to provide the best fit model (**Table 15** and **Table 16**). For both asymptote and 'AUC1500', 'Season', 'TimeThermGrp' and 'Cult' were found to explain the most variation in photosynthetic response to CO₂. The values of the asymptote and 'AUC1500' were higher in season 2 compared to season 1, were higher when 'TimeThermGrp' was high, and were lower for Piccolo.

Table 15. Accumulated analysis of variance for best fit model from forward stepwise regression for asymptote of Assimilation Rate vs. PAR curve. 73.2% variance accounted for.

Source	d.f.	Sum sq.	Mean Sq.	v.r.	Prob > F
TimeThermGrp	1	832.00	832.00	79.13	< 0.001
Season	1	149.41	149.41	14.21	< 0.001
Cult	1	57.70	57.70	5.49	0.026
Residual	32	336.46	10.51		
Total	35	1375.58	39.30		

Table 16. Accumulated analysis of variance for best fit model from forward stepwise regression for 'AUC1500' (area under Assimilation Rate vs. CO₂ curve between CO₂ = 0 ppm and CO₂ = 1500 ppm. 80.3% variance accounted for.

Source	d.f.	Sum sq.	Mean Sq.	v.r.	Prob > F
Season	1	1529858652	1529858652	118.88	< 0.001
TimeThermGrp	1	246566053	246566053	19.16	< 0.001
Cult	1	102420170	102420170	7.96	0.008
Residual	32	12869258	12869258		
Total	35	65447461	65447461		

Analysis of the CO₂ responses indicated that over 80% of the variation present within the results is accountable by a combination of season, thermal time (temperature) and cultivar. In a similar fashion to light, significant seasonal variation to CO₂ response was seen in the crop as outlined in **Section 2.2.3**. The significant effect of cultivar could be due to minor differences in carboxylation efficiency that were identified between the cultivars (**Table 12**), or the fact that the cultivars were grown under slightly different environmental conditions. Lastly, thermal time (as a measure of temperature) has a significant effect on CO₂ uptake. Temperature effects of CO₂ uptake are primarily observable at the biochemical level and key enzymes follow typical kinetic relationships. Secondly, time as a specific feature does not appear to have a significant effect on CO₂ uptake potential within the data used for this analysis.

A paired t-test analysis was carried out to compare responses at time 2 (10am-1pm) and 3 (1pm-3pm) on each date for each cultivar. In contrast to the stepwise forward regression and analysis of variance, this analysis did not make the assumption that measurements taken from the same cultivar on the same date but at different times of day were independent. It was however assumed that measurements taken from different cultivars on the same date were independent. The analysis showed that the area under the A/Ci curve up to 1500 ppm (including both the CO₂-limited and CO₂-saturated phase) was greater on average at time 2 compared with time 3 (paired $t(17) = 2.57$, $p = 0.0198$). The asymptote (A_{Max}) (paired $t(17) = 2.32$, $p = 0.03308$), or saturated assimilation rate, at time 2 was $1.97 \mu\text{mol m}^{-2} \text{s}^{-1}$ greater on average than at time 3. These diurnal effects are small relative to the between-date variation in photosynthetic response

1.4 Discussion

1.4.1 General Crop Responses

This project has generated a unique evaluation of crop performance over the course of a full season. Significant changes in crop behaviour have been recorded as the crop responded to

increased fruit load leading to a large increase in light response in Spring (**Figure 14**) and a progressing increase in CO₂ response over the year as the crop adapts to higher light levels in the summer (**Section 2.3.9**). These features have combined to illustrate a dynamic crop response to the immediate glasshouse environment, and has generated a breadth of evidence that will be directly relevant to current practice. In terms of light response, Piccolo reaches a maximum assimilation rate greater than that of Avalantino (**Figure 14**) although this is achieved at PAR levels unlikely to be routinely encountered in the crop. In addition, Avalantino retains its photosynthetic potential for longer into the autumn period, although this may be a result of increased light penetration into the canopy as a result of the lower foliar area and planting density (**Table 9**). The seasonal effects in spring are of particular interest as these may dictate the potential of the crop to assimilate CO₂ both within this period and across the season, particularly as this period may impact and acclimation in the crop to high CO₂ concentrations (Besford, 1993). It may be of direct relevance to growers if the adaptive period between February and May can be brought forward, allowing the crop to reach peak productivity at an earlier point in the season. If this response is primarily linked with light, then the provision of supplementary lighting may offer a route to enhance adaptation rates in the crop. A similar effect may be demonstrable during the autumn period: productivity may be sustainable for longer if supplementary lighting can be used to buffer declines in ambient PAR levels, although this would be set against a context of an increasingly aging crop.

Compared with previous studies, both cultivars responded to CO₂ in a typical fashion (HDC, 2002), reaching maximum rates of photosynthesis above 800 – 1000 ppm, although both cultivars achieved this maxima at lower CO₂ concentrations earlier in the season (**Figure 15**). Piccolo demonstrated a lower A_{Max} and carboxylation efficiency than Avalantino on average across the season (**Table 12**), which confirms findings from 2015 that Piccolo is more likely to be susceptible to lower CO₂ concentrations than either Avalantino or Roterno. This difference is also identified as significant in the analysis of photosynthetic potential (**Section 2.4.4**), and is the only crop-derived parameter that impacts photosynthetic potential identified in the analysis.

1.4.2 Canopy-level Effects

Typical cultivation practice will create a highly heterogeneous vertical environment, many aspects of which will directly impact the crop's ability to utilise CO₂. PAR availability will be greatest at the top of the plant, with increasing proportions of shade increasing with canopy depth, resulting the large proportions of the middle and lower canopy being continually shaded. CO₂ enrichment may be supplied at ground level, and wet-pipe/grow pipe heating acting to create different temperature and humidity gradients within the canopy. Onto these

physical variations are overlaid biological variability, most significantly increasing leaf age with canopy depth and increased proximity to sugar sinks in the maturing fruit and roots.

While it is beyond the scope of this project to fully evaluate the effects of canopy conditions on photosynthesis, some analysis was undertaken in an attempt to quantify the effects of canopy variability on overall CO₂ offtakes. Offtake estimates to date have been based on the 6th eldest fully expanded leaf, which was identified as the most physiologically robust leaf in the 2014 phase of PE021. Leaves younger than this are still developing and have yet to reach physiological peak, while leaves below this point are starting to undergo senescence whilst adapting to the increasing proportion of shade in the lower canopy. Acock *et al.* (1978) note that the top 26% of the canopy is responsible for assimilating 66% of the CO₂ fixed by the plant, an effect of a combination of reduced PAR availability and the reduced ability of the lower canopy to utilise light. Therefore, measurements were undertaken in an attempt to provide a whole-plant estimate of photosynthesis rate relative to the 6th leaf which could be utilised as a basis for the introduction of a corrective coefficient that would adjust offtake estimates to include the reduced assimilatory potential of the middle and lower canopy.

The evidence presented in **Section 2.3.7** supports previous findings that assimilation potential peaks in the middle canopy layer. Typically, above-average rates of assimilation were identifiable within the middle canopy section (Piccolo) or in the upper or upper/middle margin (Avalantino). Assimilation potential differed by upwards of 70% in some instances between canopy layers (Avalantino, August, **Table 11**) this was typically associated with periods of high light. A_{Max} rates have been shown to decline within the canopy with lower canopy layers less able to utilise higher levels of PAR (Dueck *et al.*, 2011) as the leaves adapt to higher levels of shade in the canopy. This includes declines in leaf nitrogen (and therefore leaf protein, including reductions in enzymes such as Rubisco and those involved with the Calvin cycle) as nutrients are withdrawn from the leaves and relocated to active growing sites. This allows nutrients to be recycled to where they will have the greatest benefit, while also lowering the respiration load on the individual level as less productive cells will have lower maintenance costs to sustain them. This is mirrored in the lower dark respiration rate in the spring period compared with the summer (**Table 6**) which corresponds with the reduced response to PAR in the spring period (**Figure 14**). The increased variation in assimilation at high light levels is most likely as a result of changes in the leaves potential to utilise high levels of PAR caused as a result of differences in investment in leaf architecture and photosynthetic machinery. The limited data collected this season resulting from the use of ambient conditions when making measurements, especially the lack of comparable PAR intensities, has limited the extent to which this data can be utilised as the breadth of variables that cannot be accounted for is significant.

As a result, only limited conclusions can be drawn relating to changes in the lower canopy impacting variable crop CO₂ demand. This work supports the use of the middle canopy layer as the defining portion of the crop which will show the greatest potential for CO₂ uptake, and the use of the 6th leaf as a the typical model for development of a variable dosing regimen. Therefore, while it has not been possible to define a sufficiently reliable relationship between canopy variation and whole-crop CO₂ offtake and demand, a sufficiently robust estimate can be derived using the leaf measurement methodology detailed in this report.

1.4.3 Estimating CO₂ Offtake

Comparisons of Photosynthesis Models

A primary objective of this project was to provide an estimate for CO₂ offtake which incorporated measurements made from the early season period and which could be used to augment data from PE021. Project PE021 was able to produce estimates for CO₂ offtake for two contrasting grower sites in 2014 and 2015, although these estimates were passed primarily on observations made over the summer period. The original photosynthesis model upon which offtake estimates were based was formulated in 2014, combining measurements made between July and October of a Piccolo and Roterno crop grown with an enrichment level of 500ppm. This model comprised of three linear relationships that were applied at different PAR intensities that were applied across the season as outlined in **Section 2.2.6**. When estimates of the 2014 model were compared against observed measurements (**Figure 17**), assimilation was significantly underestimated when compared against observed measurements. The 2014 model was based on measurements of a crop grown at 500ppm, compared with average CO₂ concentrations of 930ppm (Avalantino) or 880ppm (Piccolo). Based on the A/C_i curves given in **Figure 15**, increasing the CO₂ concentration to these levels will have a sizeable effect on net assimilation rates, leading to the discrepancy identified in the observed and estimated rates of assimilation.

The development of the 2016 photosynthesis model addresses to key areas of error in the 2014 model. Firstly, by replicating measurements under the increased ambient CO₂ concentrations, adjustments for higher net rates of assimilation can be accounted for. Secondly, the 2014 model was based on light responses measured over the summer period. As demonstrated in **Section 2.2.4**, significant changes in light responses over the course of the season renders a single-use model unsuitable as its accuracy will be impacted by including an overly variable dataset. By developing separate models for the spring, summer and autumn periods, the accuracy of photosynthesis estimates will be enhanced (**Figure 17**).

The 2016 model will still include variability caused by time of day, temperature and any diurnal effects, but as these would not be separable in a reliable fashion using a data source that could be accumulated in a cost-efficient fashion. This variability is reflected in spread of observed vs. predicted measurements, particularly in the later stages of the season (**Figure 17**). Despite the spread of observed vs. estimates photosynthesis rates, the fit for the 2016 model is considered sufficiently tight to justify its application for the estimation of offtake.

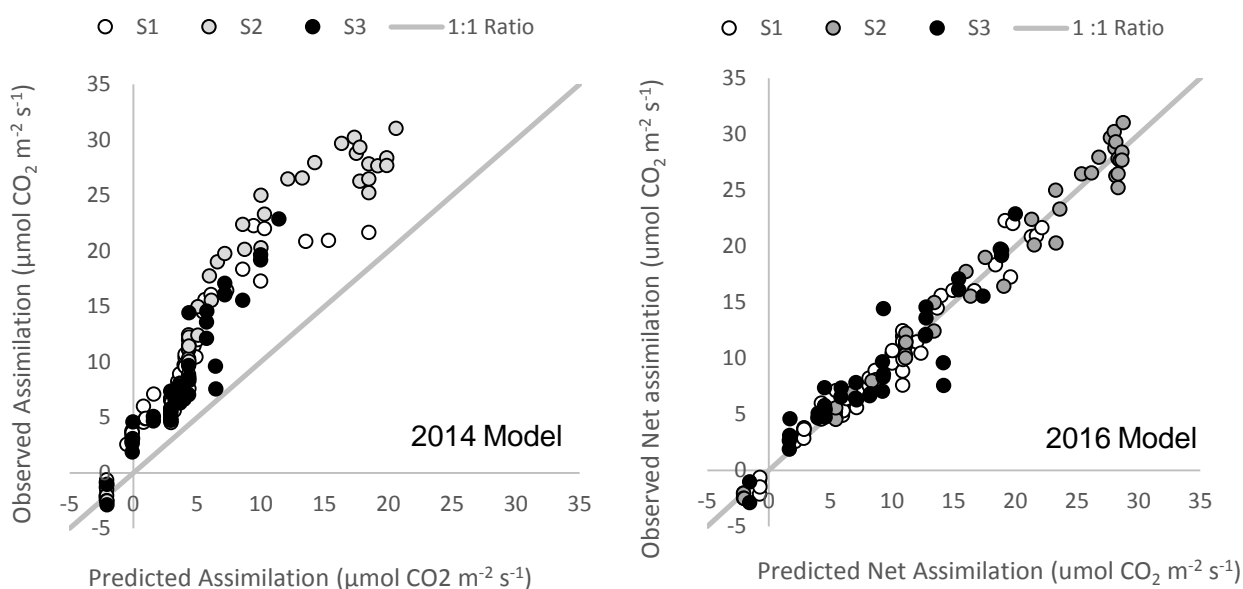


Figure 17. Comparison of 2014 and 2016 photosynthesis models. Observed measurements of assimilation are plotted against estimated rates of assimilation using the respective model. A 1:1 ratio is plotted. S1: Feb – May, S2: June – Aug, S3: Sep – Nov.

Estimations of Crop CO₂ Offtake

Use of the photosynthesis models and foliar area estimates allowed estimation of CO₂ offtake across the season. Complete radiation and CO₂ records were only available between weeks 1 – 35 (ending 4th September), constraining the offtake estimation to this period. Summary values of offtake in the 2015 and 2016 season are given in **Table 17**.

Piccolo

Use of the 2014 model indicates that offtake in the 2016 season (20.52%) is comparable with the 2014 estimate (19.42%). The 1.1% increase is despite an increased daily rate of CO₂ application, which was 522kg/ha/day greater than that seen in 2015 (a 30% increase). A CO₂ concentration of 877ppm was achieved with the lower rate of dosage which is still well within the saturated region of the Piccolo A/C_i curves (**Figure 15**), indicating that assimilation is still light limited within this range. Foliar density of Piccolo averaged 33203 m² ha⁻¹ in August/September 2015, compared with 32384 m² ha⁻¹ in the same period of 2016, with a comparable radiation receipts (**Figure 18**). The apparent increase in offtake is correlated with the high level of offtake seen in the early season (**Figure 11**): low levels of CO₂ dosing in February and March, combined with comparable foliar areas meant that high crop offtake in Piccolo was identifiable in the early season which buffered the reduced rates of offtake seen in April and June. The 2016 model estimated CO₂ offtake for Piccolo at 26.41%, 5.91% greater than the 2014 model. The discrepancy in estimates between the two models was greatest in February, but averaged at 22% over the season (**Table 17**).

Avalantino

The offtake estimate for Avalantino using the 2014 model was lower compared with Piccolo in 2015/16 and the category 3 Roterno that was grown in the same area in 2015. Avalantino was grown as a category 3 cultivar, although its productivity as somewhat intermediate between category 2 and 3 in terms of individual fruit weight, particularly in the summer period when CO₂ concentrations are depressed. As a result, the Avalantino crop was grown under a much higher CO₂ concentration than Roterno in 2015 (average daily dosage of 1504 kg/ha/day achieving 937ppm on average compared with 1240 kg/ha/day achieving 847ppm in Roterno). Planting density in Roterno in 2015 was 3.8 heads/ha, while Avalantino was grown at a lower density of 3.3 heads/ha to maximise light penetration into the canopy. The reduced planting density meant that the foliar area of Avalantino was almost half that achieved by Roterno: a density of 19775 m² ha⁻¹ was achieved in August/September for Avalantino compared with 42674 m² ha⁻¹ in Roterno. The decreased leaf area, combined with the reductions in foliar area, meant that overall CO₂ offtake of Avalantino was 17.98% compared with 23.66% for Roterno as estimated by the 2014 model despite similar radiation receipts. According to the 2016 model, the Avalantino crop offtake was 3.28% less than that estimated for Piccolo.

Table 17. Summary of offtake estimates and crop CO₂ enrichment. Average CO₂ applications across the season are expressed as kg/ha/day to negate variation in the daily timespan of enrichment across the day. CO₂ concentration is averaged across the season.

	Year	Model	Cultivar		
			Avalantino	Piccolo	Roterno
Offtake (%)	2016	2014	17.98	20.52	
		2016	23.13	26.41	
	2015	2014		19.42	23.66
CO ₂ Applied (kg/ha/day)	2016		1504	1789	
	2015			1265	1240
CO ₂ Conc. (ppm)	2016		937	877	
	2015			979	847

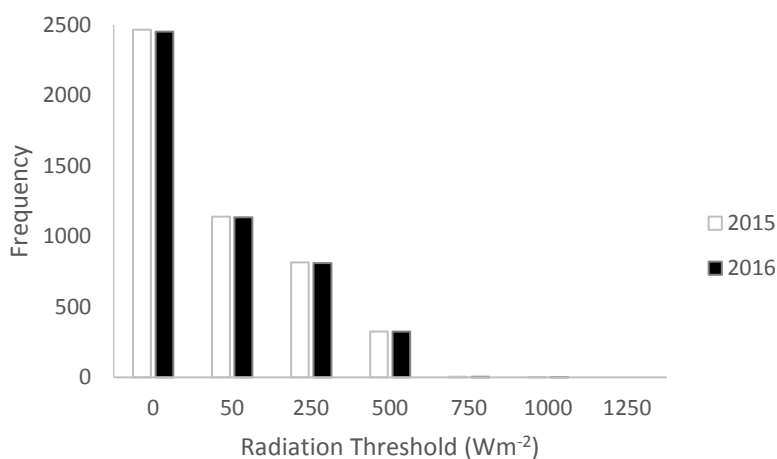


Figure 18. Frequency of hourly radiation receipts over comparable date range March to September 2015/16

1.4.4 Evidence for Diurnal Effects

A key feature of this project was an exploration of any effect of time upon the crop's ability to utilise CO₂. Current enrichment practice is to apply a flat-line approach to enrichment, it was proposed that an ability to apply CO₂ in a way that followed crop demand would allow an enhanced rate of CO₂ offtake by lowering wastage as a result of avoiding high dosing at periods when the crop was liable to be less responsive to high levels of CO₂. Preliminary evidence for the development of variable dosing would be an appraisal of daily variation in photosynthetic potential, and how this interacted with other environmental parameters, specifically temperature and light levels.

A precursor to this was the indication from data collected in 2014/15 that assimilation was peaking between 12:00 and 15:00h, indicating that underlying changes within the crop were impacting its ability to utilise CO₂. Analysis of the CO₂ and light response data given in **Section 2.3.10** indicates that season and resource input (primarily light level and

temperature) has a predominate effect on photosynthetic potential of the crop. Given the significant changes in the response to PAR and CO₂ demonstrated in **Figure 14** and **Figure 15** respectively, it is evident that a large adaptation in the crop is seen across the growing season, with a peak in the potential to utilise both PAR and CO₂ being seen in the summer months. This variation was responded to in the development of the photosynthesis model in **Section 2.3.3** by developing a separate model for each seasonal period. However, only limited data is available for light/CO₂ response, with typically three response curves per cultivar per parameter per month.

The presence of “Date” in the analysis creates a potential issue as it covers a wide variety of parameters, most significantly seasonal response. It also covers a range of features that are difficult to measure, including variables within the crop that were not included in the measurements taken during the trial. The analysis presented in **Section 2.3.11** indicates that once seasonal variation was removed, a significant effect of time on CO₂ demand could be demonstrated. This was achieved by directly comparing light and CO₂ responses within time group 2 (10:00 – 13:00h) and time group 3 (13:00 – 15:00h) in an analysis which did not assume that measurements taken from each cultivar were independent, assimilation potential at time 2 (as indicated by A_{Max} and integrated curve values) was shown to be significantly higher at time 3. This indicates that peak photosynthetic potential (and hence CO₂ demand) was encountered within time group 2, between 10 – 13:00h). As these measurements were taken under a range of PAR/CO₂ concentrations that were constant between each assessment, this support the midday peak in photosynthesis observed in the 2014/205 data.

However, the seasonal variation observed in the data means that diurnal effects are small compared with other sources of variation in crop CO₂ demand. The limited data collected within each seasonal group means that it has not possible to fully address any variation in the response to time with greater refinement than that used in the analysis as the variation in photosynthetic parameters caused by the seasonal adaption of the crop makes it difficult to draw comparisons between measurements at separate points in the season.

A second limiting feature of these data is that an environmental influence is seen on assimilation potential: light response curves relate to PAR levels, and CO₂ responses to temperature. Biochemical changes may have occurred, such as the activation of enzymes and typical enzyme kinetic relationships which will directly impact the overall rate of CO₂ assimilation. This is a second limitation of the dataset. As measurements were made only under ambient conditions, there was a high level of aliasing between parameters (e.g. high light levels coincided with the early afternoon period) which meant that it was more difficult to interpret the effect of each in isolation. Had more repetitions be possible within each month,

greater chance of contrasting ambient conditions (e.g. overcast vs. bright day) occurring within the same seasonal block may be seen.

Therefore, while the presence of a significant effect of time on CO₂ assimilation potential of the crop has been observed, there is insufficient data to fully define the occurrence of a routinely demonstrable diurnal cycle as “time” in isolation has been shown to be an ineffective predictor of CO₂ assimilation relative to season and light/temperature inputs. This position may be resolvable through three scenarios:

1. A relationship between crop photosynthetic potential and daily time is stronger than that detected, but insufficient data has been collected to define it fully. This would require additional data collection to take place within each seasonal group to mitigate both the effects of seasonal variation, and aliasing between related parameters such as time and light levels. With greater replication, separation of these parameters would be possible and the effect of time on crop CO₂ more definable.
2. The scenario of (1) is correct, but the pool of data necessary to fully test the link between time and crop photosynthetic potential is unobtainable in practical terms. By collecting measurements from a commercial crop, the lack of control over causal factors combined with the significant seasonal variation would effectively preclude from making meaningful conclusions for the dataset that is collectable.
3. Time does not have a significant independent effect on crop CO₂ demand, and instead it is predominately a product of environment factors (e.g. PAR) in combination with seasonal responses which show an instantaneous response and therefore one which cannot be responded to on a timescale that is relevant to grower practice.

For instances where significant diurnal variation of large magnitude has been described in crops, it is typically linked with environmental parameters that are unlikely to impact tomato production. These include high soil water deficits and limited nitrogen availability (e.g. Mohotti & Lawlor, 2002) or where conditions of high light and CO₂ are combined with a restricted ability of the crop of utilise assimilated carbon (Long *et al.*, 1994). As the tightly controlled environment typical tomato production is unlikely to see such environmental effects, support could be given to the third scenario. However, given that diurnal variation in glasshouse-grown tomato has previously been reported it is likely to be a combination of the first and second scenarios.

The diurnal variation in tomato reported by Ayari *et al.* (2000a) was mainly limited to the early spring period, where daily variation in CO₂ uptake potential was recorded until February in an over-wintered lit crop. After this point, progressive adaptation resulted in a level relationship with no variation with time that was not attributable to PAR levels. Given the wide range of

adaptation seen in these data, and the likelihood that photoinhibitive conditions were likely to be encountered for only brief periods of time (such as high, direct light on a given section of the crop) it may be seen that diurnal variation is limited to the early spring season. For the summer period high levels of productivity may buffer any negative environmental effects, prevent the onset of diurnal variation. Given the decline of crop responses in the autumn period the onset of diurnal variation in this period may also occur, but insufficient data is available to test this. Further analysis has implied that diurnal variation was seen in leaves that had developed in the winter period and were due to a stomatal limitation not seen in younger leaves that had developed later on in the season (Ayari *et al.*, 2000b). Qian *et al.* (2012) report that diurnal variation in tomato was only recordable in plants with low fruit load, which would also correlate the occurrence of this effect in the spring/autumn period where fruit load is low. High fruit load in the summer months will provide a strong sink strength, allowing rapid removal of sugars from the leaves and preventing the onset of photoinhibition. Therefore, the absence of a well-defined diurnal cycle may be a result of limitations of this effect to the early spring and late autumn periods.

1.4.5 Variable Dosing and CO₂ Offtake

While this project has identified the presence of a significant effect of time on the ability of the crop to use CO₂, insufficient data is available to fully test for the presence of a diurnal cycle of any size and predictability. This is a result of significant seasonal variation precluding analysis of individual variables that may impact crop CO₂ demand. In addition, diurnal effects may predominately occur during the early spring and late autumn periods. In the summer, photosynthesis is more directly connected with incidental environmental conditions rather than a predictable pattern. The effects of venting in the summer period will also mean that high levels of CO₂ dosing will be required to maintain productivity, further limiting the potential to implement a variable dosing strategy. The poor definition of a diurnal cycle in tomato means that variable dosing guidelines cannot be formulated on the basis of this data alone, and that replicate measurements over multiple years and from multiple grower sites are liable to be required to provide sufficient evidence as to generate defensible variable daily dosing guidelines for the UK tomato industry.

Early season offtake is significantly higher than that seen in the later periods (**Table 10**). Closed venting within this period will further limit CO₂ wastage. If diurnal effects are significant in UK tomato production, it is most likely to occur during this period as the crop adapts to increasing light levels as it approached the summer period. Given that offtake is already significant for this period, it may not be possible to enhance this further even if a variable dosing regimen were implemented. Should further work be pursued, focused, high volume data collection should be targeted at the early and late season to better understand how the

crop is responding to the significant changes that are occurring during this period, and how this influences productivity in high summer.

1.4.6 Enhancing Crop CO₂ Offtake

Variable CO₂ dosing linked with diurnal variation in crop CO₂ demand represented a potential route to enhancing crop CO₂ offtake. Variable dosing would allow CO₂ to be applied when it is most required, and therefore reduce the potential for wastage while conserving the proportion taken up by the crop. However, the lack of any significant diurnal variation detectable in the crop means that alternative methods should be considered that will enable the wider UK tomato industry to enhance CO₂ offtake by adapting current practice.

One area that this project has identified is the under exploitation of the lower canopy region. The lower canopy which represents over a third of the foliar area of the crop (**Table 4**) has been demonstrated to have significantly impaired ability to utilise CO₂ demonstrated by the decrease in assimilation potential evident in **Section 2.3.7**. As a result, a significant proportion of the leaf area is currently severely underutilised. This correlates with the observation of Acock *et al.*, 1978) that the lower 75% of the canopy is only responsible for 34% of the CO₂ uptake.

The lower canopy is routinely shaded, meaning that the lower leaves have adapted to lower PAR levels, lowering their ability to utilise light and uptake CO₂ compared with the level found higher up in the plant (**Figure 12**). **Figure 13** demonstrates the variability in lower canopy assimilation potential, highlighting that a decline in a leaf's ability to utilise CO₂ is more linked with adaptation to its environment (e.g. shaded in the canopy vs. lit in the pathway) rather than a direct response to aging.

An alternative route to enhancing CO₂ offtake could be the increased exploitation of the lower canopy layer. The use of a supplementary light source offers a significant opportunity to drive CO₂ uptake in the lower canopy to enhance overall crop productivity. A second light source offers an additional source of PAR which is independent of external conditions (cloud, sun angle) to provide an energy input for enhanced CO₂ assimilation. The provision of an additional light source will also have direct effects on plant growth and the leaf life cycle. Higher light levels promotes the plant to maintain productivity in the lower canopy layers for long as it seeks to utilise the additional resource. In cucumber crop, light emitting diode interlighting increased the A_{Max} of the lower middle by 36% and the lower canopy sections by 112%, while the upper canopy layers were unaffected (Trouwborst *et al.*, 2010). Comparable effects have been seen in tomato when diffuse lighting has been used to boost light penetration to the lower canopy, where high haze increased A_{Max} of tomato of the middle canopy by 19% without significant effects on the upper canopy (Li *et al.*, 2014). In both

instances, leaf nitrogen was more than double the non-lit control and was comparable if not exceeding levels in the upper canopy. Supplementary lighting provision slows leaf senesce, reducing adaptation to a shaded canopy and enabling leaves to sustain higher productivity and assimilate more CO₂ compared with unlit conditions. Therefore, the underutilisation of the lower canopy implied these results could be enhanced through management of light in the lower canopy to drive whole-plant CO₂ uptake.

As an alternative to seeking variable CO₂ dosing, measures could be taken instead to treat the crop as a variable CO₂ absorber. Modification of the lower canopy to enhance PAR availability would allow a large proportion of the canopy to utilise CO₂ to a greater extent, giving the possibility of achieving greater CO₂ offtakes. This would have the additional benefit of increasing the availability of sugars for yield output, increasing the return on investment made for CO₂ provision. This could be achieved through a variety of routes, including supplementary lighting and diffuse coatings to enhance light penetration to the lower canopy, or updated crop management practice to reduce upper canopy light interception.

1.5 Conclusions

- Significant early and late season changes in crop response to CO₂ and light have been demonstrated, indicating that the crop has considerable plasticity to adapt to wide range of conditions. There is potential to better link this adaptation with resource provision, to either reduce CO₂ application in the early season to match lower demand, or to increase light availability to drive early season adaptation at a faster rate.
- Higher enrichment levels and the seasonal variation set a requirement for the development of a new photosynthesis-based model for estimating crop offtake. The new model combined three curvilinear relationships applied to separate periods across the season to give an enhanced estimates of crop CO₂ uptake relative to the original linear model developed in PE021.
- Crop offtake estimates according to the new model suggest that Piccolo had an average offtake of 26.4% compared with Avalantino with an offtake of 23.1%. The discrepancy is due to a lower leaf area achieved through reduced planting densities and lower plant leaf areas in Avalantino, restricting CO₂ uptake.
- Offtake was greatest in the early season when low levels of CO₂ were applied. Lowest offtake was estimated in April as a result of increased CO₂ application despite low levels of leaf area and incident PAR, restricting uptake. Better linkage better enrichment rates of crop/environmental condition in the early and late season may act to enhance overall offtake.

- Significant variation was seen in assimilation potential within the canopy. Assimilation peaked in the middle canopy, before rapidly declining in the lower canopy. Middle canopy leaves showed an increased ability to respond to high PAR levels relative to the lower canopy which showed a relatively depressed response to PAR as a result of adaptation to lower light levels in the inner canopy.
- Assessment of light/CO₂ use potential across the season did not indicate the presence of diurnal variation. This may be due to the large variation in seasonal effects and response to light/temperature may be concealing any effects linked with time as the limited data set means that it is not possible to test for interactions in isolation. Alternatively, diurnal variation may be most significant in the early spring period before the crop adapts to high light/temperature conditions.
- This evidence accumulated by the project demonstrates that a number of significant effects are occurring in the early spring season which may impact the crops ability to utilise CO₂ both within that period and across the year. Combined with the potential for diurnal effects in this period, and the changes to offtake that may be achievable as a result of modification to growing practice, a key area for future work may be early season crop behaviour and how this can be manipulated using resource inputs of light and CO₂.
- Significant changes in photosynthesis potential within the canopy have also been demonstrated. The lower canopy adapts to near-continuous shade by lowering the ability of older, lower leaves to respond to light and assimilate CO₂. Manipulation of the lower canopy to encourage the retention of peak photosynthesis potential may offer a key to enhancing CO₂ offtake by driving CO₂ uptake in a section of the canopy that is currently under exploited.

1.6 Knowledge and Technology Transfer

Monthly meetings have been held with industry with industry representatives throughout the project, and results from the project have been fed back to the industry via Tomato Working Party meetings. A presentation summarising the project results was also given at the AHDB Growsave event, Kenilworth on the 28th September 2016.

1.7 References

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1.8 Appendices

This will include all supporting statistical analyses, raw data, and additional relevant photographs not incorporated elsewhere.