Comparison of continuous and spot measurements of radiation interception in barley

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Abstract

Modelling of crop yield requires accurate measurement of intercepted photosynthetically active radiation (PAR_i) and the calculated radiation use efficiency. Methods for measuring PAR_i are either spot or continuous in frequency. Spot measurements may not represent the true interception because of data paucity and variations in time and space. Seasonal PAR_i was compared using two spot measurements techniques: SunScan Canopy Analysis System (SS) and GreenSeeker® (GS) readings taken around noon and one continuous automated PAR (autPAR) technique (recorded every 5 minutes) in a replicated, rain-shelter experiment at Lincoln, Canterbury, New Zealand. The treatments were two barley cultivars (cv. Omaka and Dash) grown under three irrigation treatments ('low', 'medium' and 'high' water supply). The two cultivars differed in their PAR_i responses to irrigation treatments, most notably to the 'low' and 'medium' irrigation treatments, with Dash attaining lower values than Omaka. The autPAR technique gave higher PAR_i (>95%) and hence higher canopy cover values than the spot measurements [SS (90-94%), GS (86-90%)] at peak solar elevation (68.82° at 1200 hrs on 23 December 2014) and was the most appropriate technique for determining PAR_i before senescence commenced. However, autPAR does not discriminate between senesced and green leaves. Therefore, it resulted in an overestimation of PAR_i once leaf senescence commenced. The GS was the most appropriate method to use after the onset of leaf senescence, given the PAR_i represented the green leaves only. The diurnal variation in radiation interception under the autPAR technique showed the marked differences in leaf morphology between the cultivars. Interception for Dash was more variable, with a marked reduction around noon, while Omaka had a reasonably flat response throughout the day. The quantitative cultivar differences in canopy architecture may be used in models for predicting barley productivity under rain-fed conditions.

Additional keywords: *Hordeum vulgare*, automated ceptometers, canopy cover, photosynthetically active radiation, GreenSeeker®, SunScan Canopy Analysis System

Introduction

Techniques for measuring intercepted photosynthetically active radiation (PAR_i) are well documented (Norman and Campbell, 1989; Welles, 1990; Welles and Cohen, 1996; Chakwizira *et al.*, 2015). These include the use of instruments such as tube solarimeters (Szeicz *et al.*, 1964), ceptometers (O'Connell *et al.*, 2004), the SunScan Canopy Analysis System (Bréda,

2003) and the use of digital photographs as a surrogate for fractional ground cover (Steven *et al.*, 1986; Chakwizira *et al.*, 2015). These instruments are largely used for spot measurements in time and space (Wünsche *et al.*, 1995) and are therefore subject to large variation.

Comparisons of the efficiency of some of these techniques for determining total PAR_i have been reported (Steven *et al.*, 1986; Wünsche *et al.*, 1995; Chakwizira *et al.*, 2015). Instruments such as ceptometers and SunScan Canopy Analysis System do not discriminate between green and senesced leaves, and can therefore result in an overestimation of PAR_i. The use of GreenSeeker® (Crain *et al.*, 2012) to determine normalised difference vegetation index (NDVI) as a surrogate for fractional ground cover (Carlson and Ripley, 1997) has the practical advantage that reflectance values have high correlation with green tissue and less for dead matter.

The objective of this experiment was to compare three methods [SunScan Canopy Analysis System, GreenSeeker® and automated PAR sensors (daily integration)] for relativity using cultivars with differing canopy closure patterns and to compare whether spot and continuous measurements gave consistent results for barley (*Hordeum vulgare* L.) cultivars grown under different water treatments.

Materials and Methods

The experiment was conducted in a mobile rain shelter (Martin *et al.*, 1990) located at The New Zealand Institute for Plant & Food Research Limited, Lincoln (43° 37' S, 171° 28' E), Canterbury, New Zealand. Briefly, the site was situated on a deep (>1.6 m in depth), well drained Templeton silt loam, with a plant available water-holding capacity of about 190 mm/m of depth (Jamieson *et al.*, 1995a). These soils are classified as Immature Pallic soils in New Zealand soil taxonomy (Hewitt, 2010); Udic Ustochrept (Soil Survey Staff 1998). Physical characteristics of the soil have been reported by Martin *et al.* (1992). The site had been under perennial ryegrass (*Lolium perenne* L.) for the three years prior to the trial. Plot size was 3.6 m wide × 5.0 m long, with 0.4 m between plots. A new cladding (Ampelite Wonderglas GCGH18C, Ampelite NZ Ltd, Auckland) to replace the original fibreglass, was put on the rainshelter in June 2014.

The experiment was a randomised block design with six factorial treatments, replicated four times, giving a total of 24 plots. The treatments consisted of two cultivars of barley (cvs Omaka and Dash) and three irrigation treatments. Omaka and Dash are two commercially grown cultivars with contrasting morphologies (Barbour *et al.*, 2010). Omaka is a forage barley with a lax leaf type, and Dash is a high-yielding feed barley with upright leaf orientation. The trial was sown on 15 October 2014 to a target population of 250 plants/m^2 adjusted for germination test and mean kernel weight of 90% and 45.6 mg for Dash, and 82% and 51.4 mg for Omaka, respectively. Both cultivars were sown at row spacing of 0.15 m, giving a total of 21 rows per plot. The irrigation treatments consisted of (1) a ‘high’ treatment, where full potential evapotranspiration (ET) was replaced weekly; (2) a ‘medium’ treatment, where half ET was replaced twice (early and late December 2014), and (3) a ‘low’ treatment, where ET was replaced once only at growth stage 37 (Zadoks *et al.*, 1974), when water was applied to replace a week of measured crop water use by the ‘high’ treatment. Irrigation was applied using a drip line system and each plot had its own trickle irrigation supply, with emitters spaced 0.15 m × 0.15 m apart.

The site was prepared by ploughing (0.15 m depth), followed by power harrowing. Ten soil samples to a depth of 0.3 m were randomly taken from the experimental area on 10 April 2014, evenly mixed, and a representative sample taken for chemical analyses. Average soil test results were as follows: pH 6.0, phosphorus (Olsen P) 17 mg/kg, potassium (K) 260 mg/kg, calcium (Ca) 1,375 mg/kg, magnesium (Mg) 80 mg/kg, sodium (Na) 40 mg/kg, sulphate sulphur (S) 4 mg/kg soil and available nitrogen (N) 160 kg/ha. The amounts of soil nutrients were determined as 'MAF quick-test units' (Mountier *et al.*, 1966) and converted into mg/kg dry soil using the following conversion factors: P, ×1.1; Ca, ×125; K, ×20; Mg, ×5; Na, ×5; S, × 1.0 (Chapman and Bannister, 1994).

Basal fertiliser at 250 kg/ha superphosphate (0-9-0-11) and 200 kg/ha Cropmaster® 15 (15-10-10-8) was applied through the drill at sowing. Additional N fertiliser was applied at 50 kg N/ha as urea (46% N) on 2 December 2014 with 2.8 mm of irrigation. This gave a total of 80 kg N, 42 kg P, 20 kg K and 43.5 kg S applied per ha throughout the growing season.

Measurements

Soil moisture

A single neutron probe (NP; Model 503DR Hydroprobe, Instro Tek Inc., Raleigh, North Carolina, USA) access tube and time domain reflectometer (TDR; Model CS650 Water Content Reflectometers, Campbell Scientific Inc., Utah, USA) wave guide was installed in each plot following seedling emergence. Measurements of volumetric soil water content (SWC) were made for each plot at weekly intervals beginning on 10 November 2014 until 3 March 2015. Measurements were made in 0.3 m increments to a depth of 1.5 m. The 0-0.3 m depth was measured using TDR, and thereafter measurements were made using the NP in 0.3 m increments to 1.5 m depth. It was assumed that drainage losses were negligible for the soil type of this experiment (Jamieson *et al.*, 1995a). Additionally, automated TDR sensors were installed in each plot measuring SWC at the following depths: 0-0.15 m in and between the crop rows, 0.15-0.3 m, and then in 0.3 m increments from 0.3 m to 1.8 m depth (total of eight sensors per plot). These were connected to a data logger (Model CR1000, Campbell Scientific Inc., Utah, USA) and SWC recorded at 15 minute intervals. The automated TDR and NP system are fully described in Michel *et al.* (2015).

Radiation interception

The three methods of determining intercepted PAR_i were: SunScan Canopy Analysis System (SS) (Delta-T Devices, Cambridge, United Kingdom), Trimble GreenSeeker® (GS) (Trimble Agriculture Division, Colorado, USA) and continuous automated photosynthetically active radiation (autPAR) sensors (Model PAR/LE, Solems S.A. Palaiseau, France). Light interception was measured twice weekly, between 1100 h and 1400 h using the SS and GS crop sensing systems and once every 5 minutes with an autPAR. These methods (and instruments) have previously been described: the SS (Bréda, 2003), GS (Singh *et al.*, 2006; Crain *et al.*, 2012) and autPAR (Munz *et al.*, 2014) and therefore only the measurement details will be described here.

Average light interception for each measurement date for the SS method was calculated from five measurements taken in each plot, with the probe initially being inserted at ground level, perpendicular to the plant rows. Measurements from the probe and ‘above canopy’ point sensor were recorded on a hand-held computer (Trimble Recon). The difference between the above- and below-canopy sensors defined the amount of radiation intercepted by the canopy. As the season progressed, the lower leaves began to senesce and the probe was inserted into the canopy above the senesced leaves to reduce the effect of senesced leaves blocking light from the wand sensors. Leaves were considered dead when half their area had yellowed through senescence.

For the GS method, the average reflectance values on each measurement date were calculated from approximately 30 measurements obtained from a 4 m × 0.6 m area of each plot. These measurements were taken from a height of about 0.6 m over the crop canopy. The GS sensors collect 10 readings per second and this information was stored in a hand-held computer (Trimble Recon). These reflectance values were converted into a normalised difference vegetation index (NDVI), which has been used previously to approximate light interception (Carlson and Ripley, 1997). As the reflectance values are influenced by the amount and colour of soil, bare soil readings were taken after all the plots were measured and a corrected NDVI value was calculated by using a scaled NDVI formula (Carlson and Ripley, 1997):

$$\frac{NDVI - NDVI_o}{NDVI_s - NDVI_o}$$

Where $NDVI_o$ and $NDVI_s$ correspond to the values of NDVI for bare soils (leaf area index=0) and a surface with a fractional ground cover of ≥95% (full canopy cover), respectively.

Light intercepted by the canopy was also measured by autPAR sensors. These had a detection surface of 286 mm × 3 mm (858 mm^2). The sensors were installed in three replicates of each of the ‘low’ and ‘high’ irrigation treatments for both cultivars (12 plots), across two rows of the barley crop, and levelled. Three extra sensors were installed in the open to measure total incoming PAR. These were levelled and their height was adjusted throughout the growing season to be similar to that of the crop canopy. All the sensors were connected to a data logger (Model CR1000, Campbell Scientific Inc., Utah, USA) and recorded PAR every five minutes. The amount of PAR intercepted by the crop canopy was calculated as the difference between the total incoming PAR and the PAR measured by the sensors installed under the crop canopy.

Both the SS and GS values were plotted against the autPAR and the established relationship was used to calculate a conversion coefficient (CC), as a reciprocal of the coefficient of the x variable in the linear equation established. These relationships were also compared with the 1:1 line; the closer the plotted points were to this line the closer the relationship between the methods, and vice-versa.

The green leaf area index (GLAI; m^2/m^2) was determined from the four samples harvested for dry matter measurement throughout the season. A representative sub-sample of 20 stems per plot from approximately 300 g retained for dry matter yield measurements was partitioned into green and senesced leaves, stems and ears. The area of the green leaf per quadrat was determined using a leaf area meter (LI-COR Model LI-3100; Lincoln, Nebraska, USA) and used to calculate GLAI. The accumulated GLAI and a critical LAI (LAI_{crit}: LAI at which the crop was intercepting ≥95% of PAR) of 4-5 m^2/m^2 (McKenzie *et al.*, 1999; Kemanian and Stockle, 2003) was used to describe canopy development over the season.

Data analyses

All statistical analyses were carried out in GenStat (14th Edition, VSN International Ltd, UK). Data were assessed using exploratory data analysis (EDA) (Good, 1983), analysed separately initially to obtain an understanding of results within each method, and then for those treatments and dates in which all data were available. Fits of interception data over time followed a non-linear increase, a plateau, and then a decline. Regression for the non-linear phase until peak was fitted using a logistic model (Cox, 1958). A sequence of increasingly complex models was fitted, allowing separation of parameter estimation of the logistic curve for different treatments and methods. This aided the description of how crop PAR_i behaviour differed between treatments, and whether the methods for measuring PAR_i changed as crops showed increased interception.

For PAR_i analyses, the growth and peak phases for the three methods were important and therefore are described fully. The canopy decline toward the end of the season was included in the analysis, but it was assumed that late-season processes had little influence on crop productivity. Parameter estimates for interception patterns defined by logistic fits to data for differing cultivars, irrigation treatments and method of measurement were analysed by analysis of variance (ANOVA).

Regression lines for the diurnal radiation interception were fitted with a quadratic equation for Omaka barley and a pairwise relationship for Dash barley. Variability associated with predicted means was evaluated by the least significant difference (LSD) tests (α=0.05) and standard error of the mean (SEM) where applicable. The canopy was deemed closed when the crops were intercepting ≥95% of the incoming radiation (Brougham, 1958).

Results and Discussion

The green leaf area index (GLAI) for both Dash and Omaka did not differ (P=0.78) throughout the growing period but increased (P<0.001) with water supply (Figure 1), from an average of 1.77 (1.67-1.87) m^2/m^2 for the 'low' and 'medium' irrigation treatments to 4 m^2/m^2 for the 'high' irrigation treatments. Peak GLAI was attained about 55 days after emergence (DAE) for the different water treatments. Furthermore, the barley crops under water stress ('low' and 'medium') did not attain the LAI_{crit} of 4-5 m^2/m^2 (McKenzie *et al.*, 1999; Kemanian and Stockle, 2003), implying they did not fully close their canopies (Figures 2 and 3).

The duration of green leaves of 80-100 DAE (Figure 1), also extrapolated from using PAR_i as a surrogate for GLAI (Figure 2a and b) was comparable with values reported previously for 'Triumph' barley crops (Jamieson *et al.*, 1995b) grown under differing rates and timing of irrigation at the same site. These authors also showed that senescence was earlier for water-stressed crops than for fully irrigated crops, similar to trends in Figure 2.

There was an interaction (P<0.001) between cultivar and irrigation treatments through time (Figure 2) for PAR_i. Specifically, irrigation had no effect on PAR_i during the first 30 DAE, while the cultivars differed (P<0.001), with Dash intercepting lower PAR_i values than Omaka under the three methods. However, beyond 30 DAE, both irrigation and cultivars treatments affected ($P<0.001$) the PAR_i under the three methods, with more pronounced cultivar differences under the 'low' and 'medium' irrigation treatments. The time to reach peak PAR_i differed with cultivar and irrigation for each of the three methods of determining PAR_i (Figures 2 and 3). Dash grown under 'low' and 'medium' irrigation had consistently lower PAR_i values, and failed to reach full canopy cover (≥95% of incoming PAR) (Figure 2). This was more pronounced when the SunScan Canopy Analysis System (SS) was used. Even though there were no statistical differences among the methods in PAR_i measured under the 'high' irrigation treatments (also supported by similar GLAI in Figure 1), only the automated PAR sensor (autPAR) measurements attained full cover (≥95% of incoming PAR) (Figures 2-4).

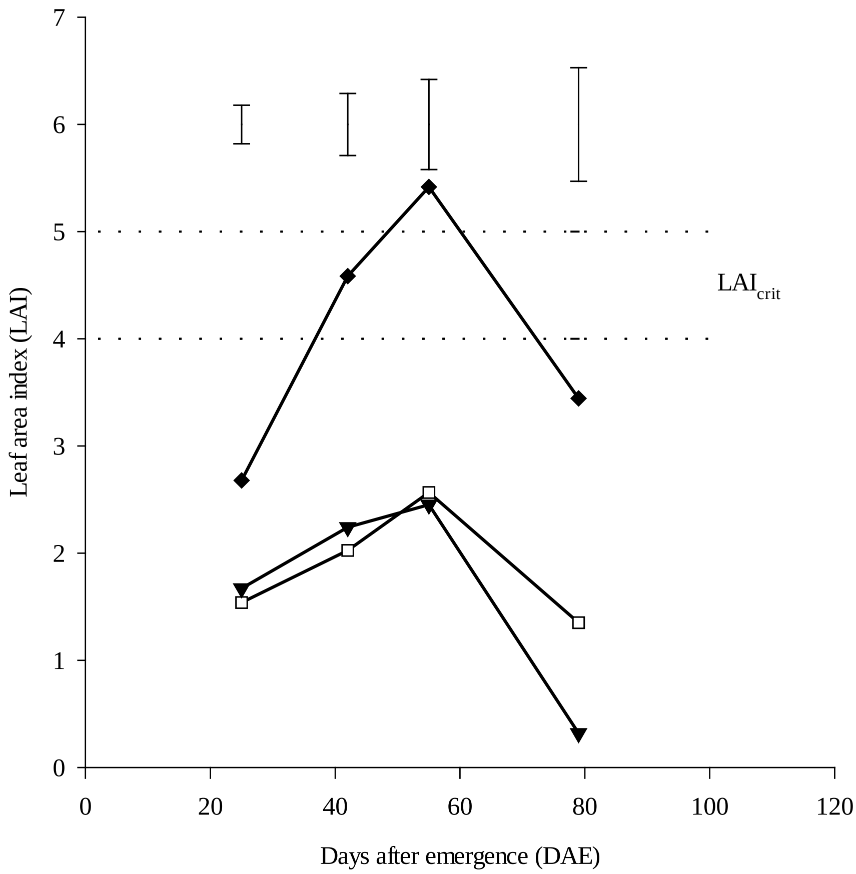


Figure 1: The accumulation of green leaf area index through the growing period for a barley crop grown under high (●) medium (□) and low (▼) irrigation treatments at Lincoln in the 2014-15 season. There was no significant difference between the two cultivars, and therefore data was combined across cultivars. The dotted lines represent the critical leaf area index (LAI_{crit}) for barley (McKenzie *et al.*, 1999; Kemanian and Stockle, 2003). Bar represents 5% LSD with 30 df.

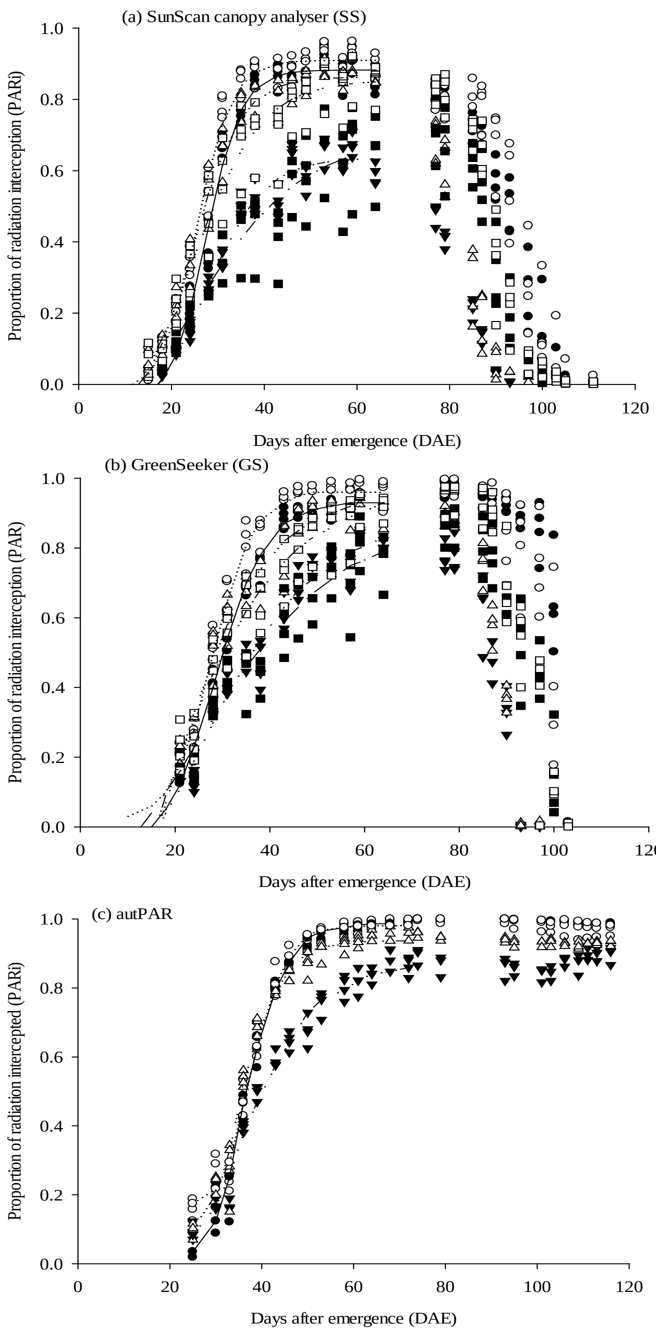


Figure 2: The proportion of intercepted photosynthetically active radiation (PAR_i) over the growing season determined by (a) SunScan Canopy Analysis System, (b) GreenSeeker® and (c) automated PAR sensors (daily integration) for two barley cultivars [Omaka, open symbols and Dash, closed symbols] grown under high (●○), medium (■□) and low (▼∆) irrigation treatments at Lincoln in the 2014-15 season.

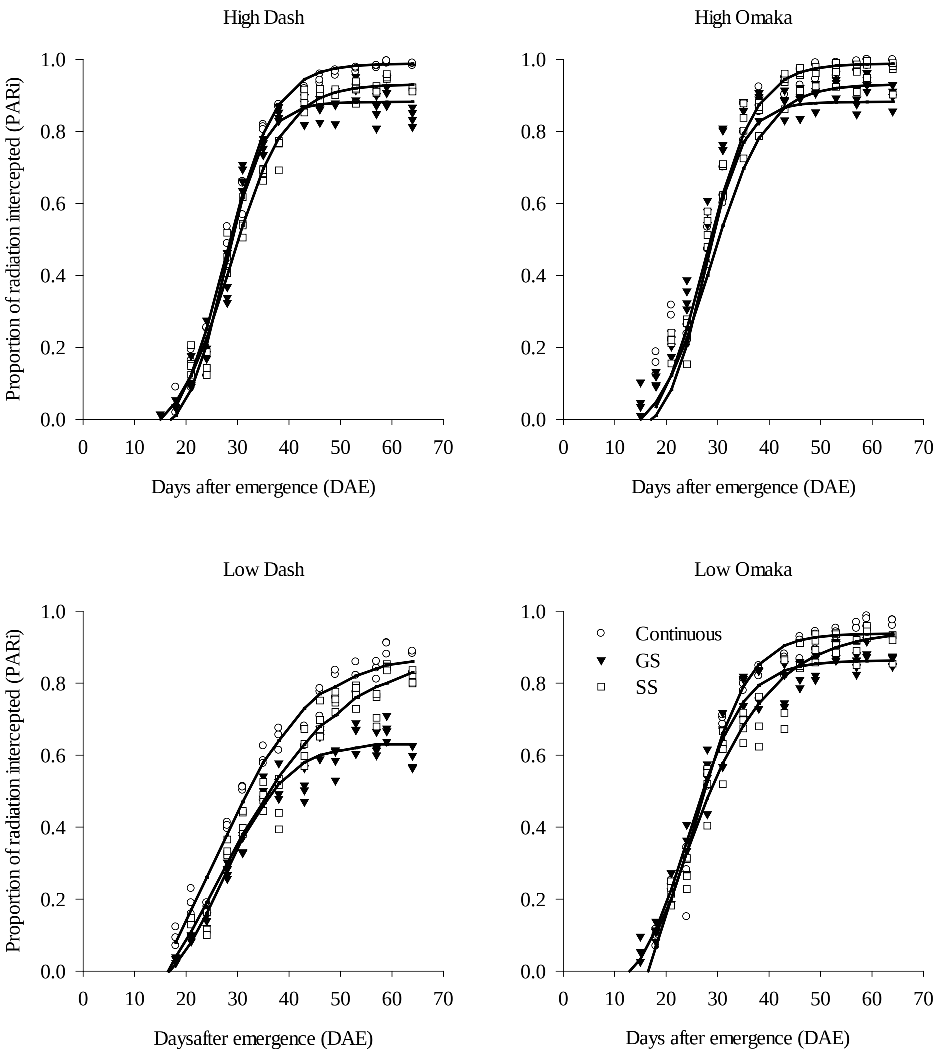


Figure 3: The proportion of intercepted photosynthetically active radiation (PAR_i) over the growing season determined by SunScan Canopy Analysis System (SS; ☐), GreenSeeker® (GS; ▼) and automated PAR sensors (autPAR; O) for two barley cultivars (Omaka and Dash) grown under 'low' and 'high' irrigation treatments at Lincoln, New Zealand, in the 2014-15 season.

Comparison of the three methods at 'low' and 'high' irrigation (Figure 3) showed distinct cultivar differences, particularly for the water-stressed crops. Dash grown under 'low' irrigation took longer to attain peak PAR_i values across the methods and also had lower values (60-70% cover) than Omaka, which attained cover of >80% for similar treatments. The autPAR attained the highest peak PAR_i values and the GS had the least for both cultivar and irrigation treatments. This suggests that the autPAR was the more accurate method for determining PAR_i until the crop reached full canopy cover and senescence commenced.

Only the crops under 'high' irrigation treatments attained the LAI_{crit} (Figure 1) and therefore closed their canopies. Furthermore, only the autPAR method showed the crops attaining full cover (≥95% PAR) for the 'high' irrigation treatments

(Figures 2, 3 and 4). The 'high' irrigation treatments were at full cover between 30-75 DAE (Figure 1), with the canopy opening up thereafter. This meant that these crops were not capturing maximum PAR from about 75 DAE to the final harvest and hence not accumulating optimum dry matter. However, the autPAR showed that the same crops continued to have closed canopies (Figure 2 and 3), implying the crops were capturing maximum PAR. The implication for this, was that autPAR was a valuable tool to show canopy development up to the point when GLAI falls below the LAI_{crit}, after which, the technique overestimated PAR_i. Because autPAR does not discriminate between senesced and green leaves (Figure 2c), the data cannot be used for modelling crop growth through the whole season. This is because only radiation interception during the early part of the season would be representative of the PAR_i driving dry matter production, while in the later part of the season, the 'intercepted' radiation useful for crop production would be lower than the figures reported here (Figure 2c). Using autPAR as a way of estimating canopy cover would thus overestimate both PAR_i and crop productivity (Chakwizira *et al.*, 2015) but underestimate the calculated radiation use efficiency (RUE; g DM/MJ) (Monteith, 1977).

On the contrary, for the SS and GS, PAR_i decreased with time after the start of senescence (Figure 2a, b) from about 75 DAE (Figure 1). Both the SS and GS showed that crops did not attain full canopy (≥95% PAR; Figure 2 and 3), even under the 'high' irrigation treatments for both cultivars (Figure 4). This was despite the fact that there were no statistical differences in GLAI for the crops grown under 'high' irrigation treatments (Figure 1). Both the 'low' and 'medium' irrigation treatments did not attain full cover (Figure 1), which was also corroborated by the lower PAR_i for the same treatments across the different methods (Figure 2) and cultivars (Figure 3).

The differences among the cultivar and methods of determining PAR_i were most clear when interception had reached its peak values (Figures 3 and 4), particularly for the crops grown under 'high' irrigation treatment (Figure 4). The GS consistently had the lowest values: 86% for Dash and 90% for Omaka compared with 92% and 94%, respectively, under the SS method. Dash had lower cover, for all the methods of determining PAR_i (Figure 4). The diurnal pattern determined for autPAR (Figure 4) at peak interception, showed that radiation interception for Omaka varied little throughout the day while Dash had reduced interception between 1130 and 1400 hrs. This could be attributed to the upright nature of leaves (thus lower extinction coefficient; k, an attenuation coefficient; Steven *et al.*, 1986) for Dash and the changes in the elevation of the sun throughout the day; for example, the elevation was 53.44° at 0900 hr, 68.82° at 1200 hr and 42.57° at 1500 hr. One of the key features of solar radiation to crop ecology is the angle of incidence of the sun's rays, usually specified by the solar elevation (Monteith, 1969; Szeicz, 1974). Brougham (1958) reported that diurnal changes of radiation interception was more pronounced in crops with distinct k values such as ryegrass compared with clovers; again this highlights the effect of k (0.4-0.5 for ryegrass and 0.9-1 for clovers; McKenzie *et al.*, 1999) in radiation interception. Furthermore, Szeicz (1974) reported that crops with high values of k, such as kale (approximately 0.9) (Chakwizira *et al.*, 2011), showed negligible response to solar elevation. The difference in radiation interception between the two cultivars in the current experiment (Figure 4) showed that Dash had a lower k value than Omaka, and subsequently intercepted less radiation, particularly around noon. The contrasting morphologies between the cultivars alluded to in Barbour *et al.* (2010) was clearly shown in Figure 4.

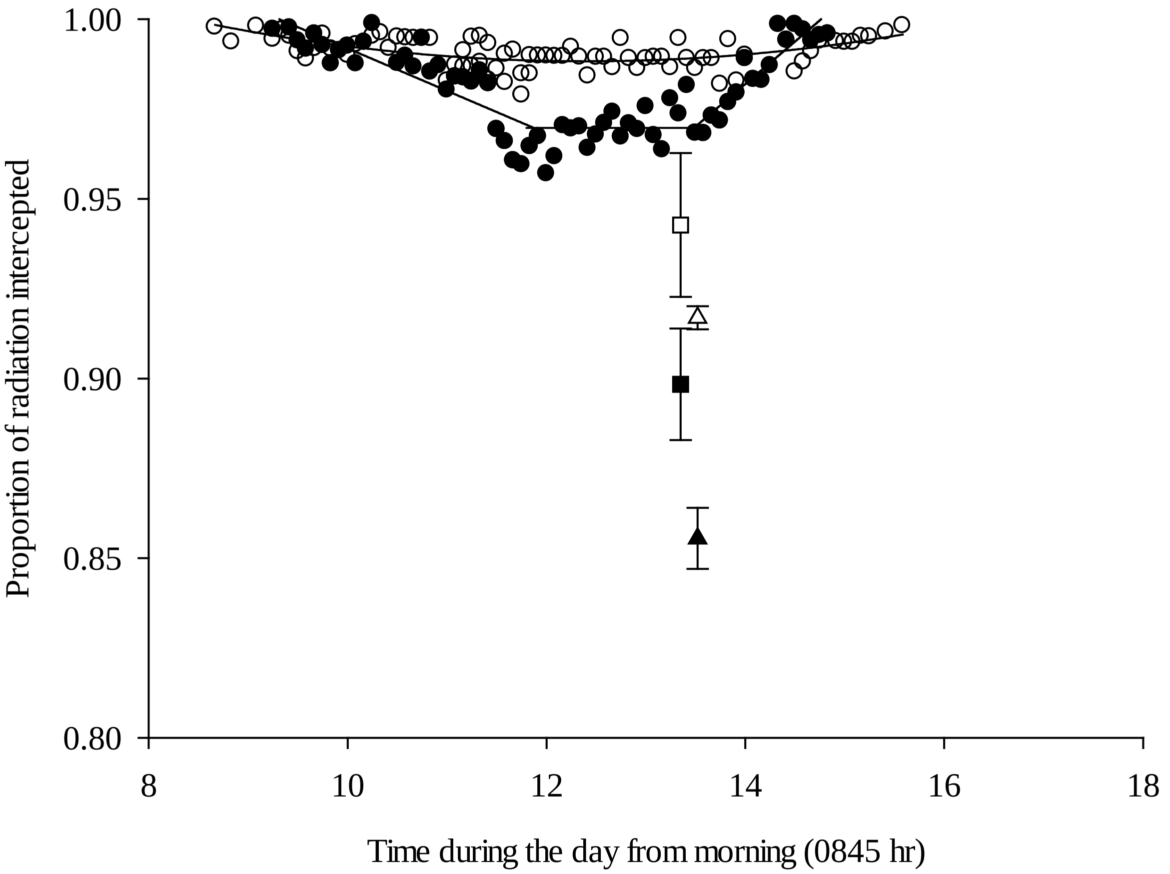
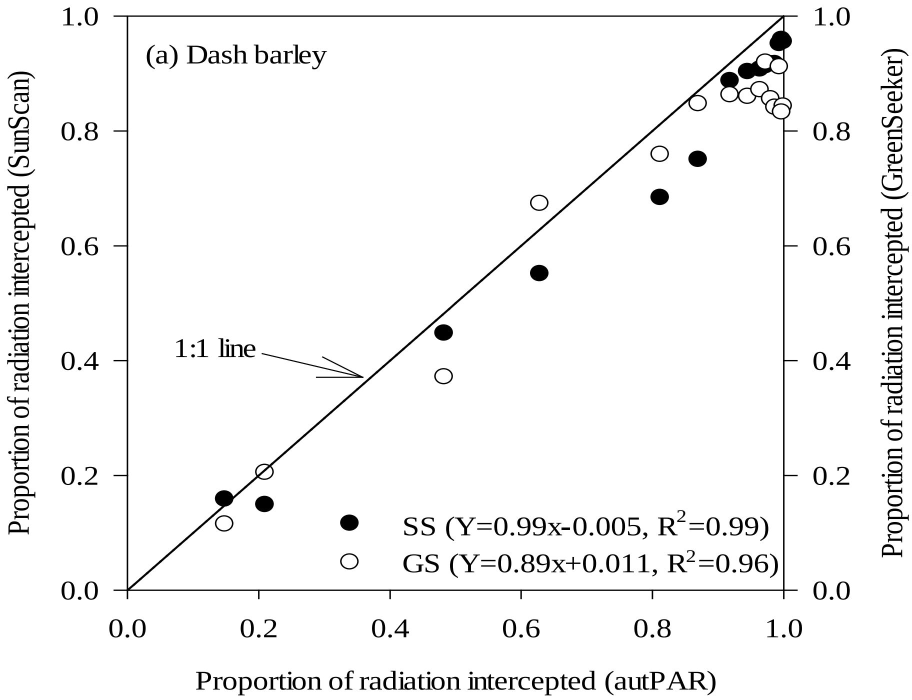


Figure 4: Diurnal variation of radiation interception for the autPAR (○●) at peak GLAI (Figure 1; 55 DAE) and spot measurement for SS (□ ■) and GS (△ ▲) for Omaka (open symbols) and Dash (closed symbols) barley on a cloudless day (23 December 2014, solar elevation of 68.82° at 1200 hrs) at Lincoln, New Zealand. Spot measurements have been plotted on averaged time for the different replications for each treatment. Vertical bars represents standard error of the mean (SEM).



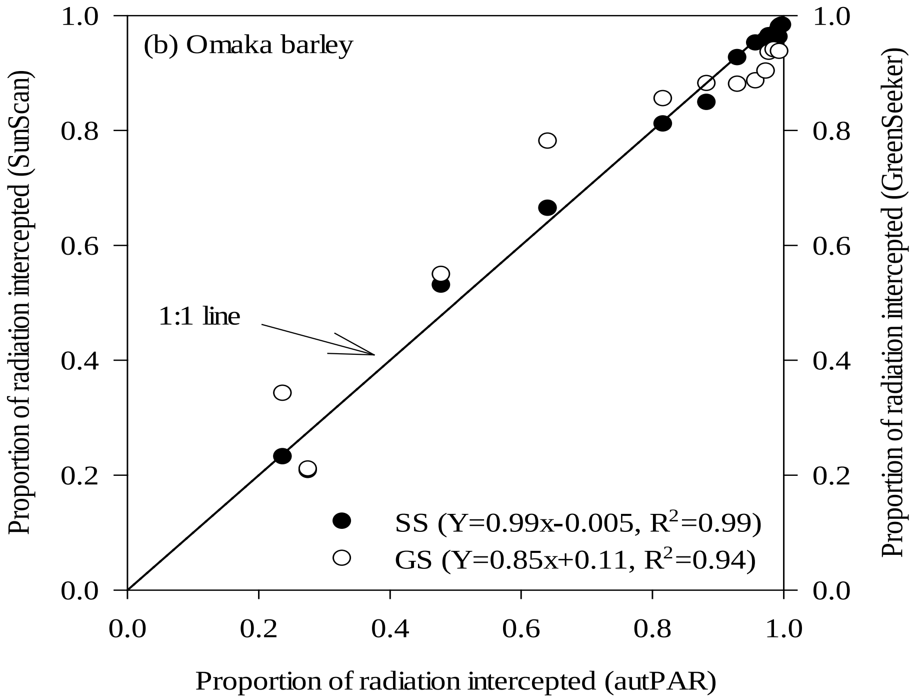


Figure 5: The relationship between the proportion of radiation intercepted determined by the SS (●) and GS (○) to the autPAR for (a) Dash and (b) Omaka barley grown at Lincoln in the 2014-15 season. The 1:1 line indicates perfect agreement.

The autPAR method is the most appropriate for determining PAR_i before senescence commences. As the autPAR is an integral of the PAR_i throughout the day, it gave a more accurate representation of daily interception before canopy closure compared with both SS and GS. This was confirmed by the autPAR being the only method showing the fully irrigated crops attained full canopy cover (Figure 4). The GS, because it discriminates between senesced and green leaves, would be the most appropriate for the period after leaf senescence has started. The SS, in the broader sense has the same disadvantages as the autPAR if the probe is not positioned above the dead matter. Furthermore, the placing of the probe 'above' the senesced leaves brings in subjectivity: in the individual definition of senesced leaves, maintaining a level probe across multiple rows and the assumption of uniform senescence across the rows. These results suggest that accuracy of modelling barley crops depends on understanding the methods of measuring PAR_i, morphological characteristics of specific cultivars and effects of key resources, for example water supply, on canopy development.

The separation between methods was more distinct for Dash than for Omaka, and this was more pronounced at the 'low' irrigation treatments (Figure 3). This is important, particularly for modelling purposes, as the method used to estimate canopy cover helps to determine the total PAR_i by the crops and subsequently the calculated RUE, both important parameters for estimating crop productivity (Monteith, 1977) and efficiency of resource use (Teixeira *et al.*, 2014). The difference between the cultivars, particularly for the 'low' and 'medium' irrigation treatments, suggests that barley crop models should incorporate cultivar differences in canopy development and architecture to accurately estimate barley productivity under water-stressed (rain-fed) conditions. Although GS showed the least cover, the fact that it discriminated between green and senesced leaves means it might be the most appropriate method for estimating PAR_i, particularly after crops have reached peak PAR_i towards the end of the season. For the SS method, even though every effort was made to exclude senesced leaves, the technique is still prone to error as the probe was inserted horizontally across five rows and assumed uniform senescence. There would still be chances of either over-estimating PAR_i by including some senesced leaves or under-estimating by excluding some green leaves.

The direct comparison between the spot and automated PAR measurement methods up to canopy closure (Figure 5) showed that for both cultivars, the SS and autPAR, had a closer relationship ($R^2 \geq 0.98$), with a conversion coefficient (CC) of ≤1.04. There was relatively weak relationship ($R^2 \leq 0.96$) between the GS and autPAR, with a CC of between 1.12 and 1.18. The overall CC values (1.04-1.18) reported here are comparable with the 1.08 and 1.10 established (45° and 90°, respectively) by Chakwizira *et al.* (2015) for barley, when radiation interception was determined by the digital photographs (comparable to GS) regressed against Sunfleck ceptometer (comparable to autPAR) results.

Conclusions

Both barley cultivars had similar GLAI development patterns throughout the growing season. However, GLAI decreased with water stress from a mean of 4 m^2/m^2 for the 'high' irrigation treatment to 1.77 (1.67-1.87) for the water stressed crops.

Continuous PAR (autPAR) gave higher PAR_i and hence higher canopy cover values than the SS and GS methods and was the most accurate method for determining PAR_i before senescence commenced. However, because autPAR does not discriminate between senesced and green leaves, the method overestimated PAR_i once leaf senescence commenced. The GS gave the best representation of canopy cover after the onset of leaf senescence due to its sensitivity to the presence of green leaves.

Acknowledgements

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