A non-destructive method of individual leaf area estimation for potato

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Abstract

Non-destructive methods of measuring individual leaf area (LA) are important in physiological and agronomic studies where successive measurements of the same leaf are required. Ideally, such methods should be easy to complete, accurate and inexpensive. This work reports on a method to estimate individual LA from terminal leaflet length measurements in three indeterminate potato (*Solanum tuberosum*) cultivars. The models coefficient of determination (R^2) ranged from 0.89 to 0.98 for different cultivars, depending on the model used, and was as high as 0.92 for the combined data for different cultivars and crop growing seasons. The method should reduce workload of LA data collection in the field and minimize the risk of canopy damage during this operation. The equations created could be validated for different seasons and cultivars and applied in future potato modelling studies of leaf growth and, consequently, transpiration, photosynthesis and plant productivity.

Additional keywords: leaf growth, leaf model, plant productivity; *Solanum tuberosum* L., terminal leaflet length

Introduction

Individual leaf area measurements are important in canopy growth and development analyses where canopy expansion is associated with changes in individual leaf area. This is particularly useful in mechanistic modelling of canopy light interceptance (Squire, 1995) which drives dry matter accumulation. Individual leaf area can be measured using destructive or non-destructive methods. Destructive methods (*e.g.* tracing, blueprinting, photographing, or a conventional planimeter) require the removal of the leaf from the plant. This impedes repeated measurements of the same leaf. Moreover, damage to the crop canopy could compromise other measurements on the same experiment (Cristofori *et al.*, 2007; Olfati *et al.*, 2010).

Conversely, accurate, non-destructive measurements permit repeated sampling of the same plants over time, which excludes the chance of biological variation from sequentially sampling different plants (Swart *et al.*, 2004). Computerized analytical equipment and software are available to measure leaf area non-destructively (Brodny *et al.*, 1986). A portable scanning planimeter (Daughtry, 1990; Demirsoy, 2009), for example, is reportedly a fast, accurate non-destructive method of estimating leaf area. However, it is only appropriate for small plants with few leaves (Nyakwende *et al.*, 1997). Other methods such as software image analysis (Bignami & Rossini, 1996; Rodríguez *et al.*, 2000), are accurate and only require a digital camera. Nevertheless, the processing of images is time consuming which limits its use.

The use of equations to combine linear leaf dimensions to estimate leaf area is inexpensive, rapid and non-destructive (Pandey & Singh, 2011). Indeed, many studies have used leaf length (L, cm) and width (W, cm), or some combination of these variables to estimate leaf area in different plant species (Blanco & Folegatti, 2003; Olfati *et al.*, 2010; Pandey & Singh, 2011).

In potato crops, individual leaf area estimations using non-destructive methods are usually derived from the measurements of W and L of the compound leaf (refer to Figure 1). Later, a factor (f) is used to calibrate these parameters. Individual leaf area (LA) is then expressed as:

Equation 1 $LA = f \times W \times L$

In the literature, f ranges from 0.45 (Vos & van der Putten, 1998), to 0.74 (Fleisher & Timlin, 2006).

Estimates of individual potato leaf area using only leaf length or leaf width have also been reported (Firman & Allen, 1989; Silva *et al.*, 2008; Busato *et al.*, 2010). However, measuring the leaf length and, or, width in young, fragile potato leaves, or in a mature potato canopy with a high density of foliage, involves the risk of damaging the leaves. This is a particular problem in field experiments, where intensive handling of potato vines for sequential leaf measurements increases the chance of canopy damage, can be ergonomically difficult and tiring for the sampler. Moreover, the lack of consensus about leaf area estimation from linear leaf measurements can compromise potato data integrity (*e.g.* when the unification of different international potato data bases are required) and precludes direct leaf area comparisons generated from different estimation approaches.

Therefore, the aim of this work was to develop a simple and quick non-destructive method for estimating leaf area using the minimal amount of linear measurement for three potato cultivars to reduce both measurement discomfort in the field and the risk of canopy damage. Objectives were: 1) to simplify individual leaf area estimation from the current methods (where L and, or, W, are used) by using the individual terminal leaflet length (LL, Figure 1) as the explanatory variable; 2) to compare the proposed method with the current ones; 3) to produce a model that estimates individual leaf area across different potato cultivars based on LL.

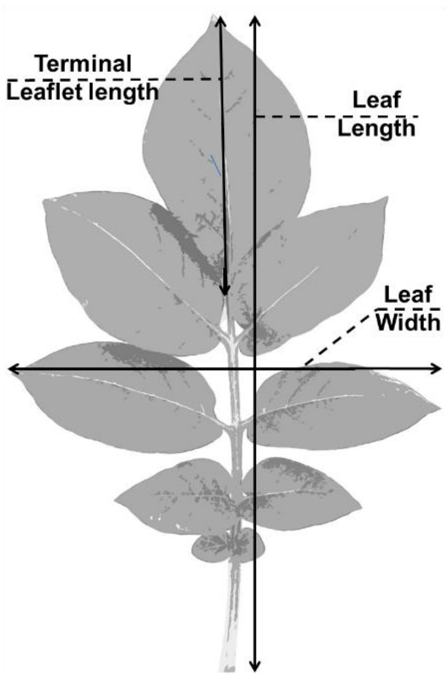

Figure 1: Morphological illustration of the compound potato leaf. Arrow indicates the longitudinal distance between the base and the top of the terminal leaflet (terminal leaflet length, LL), leaf length (L) and leaf width (W).

Materials and Methods

Briefly, the data used in the current work were collected from a field experiment conducted in 2011-12 at Lincoln, New Zealand (43°39'S and 172°28'E) and from a commercial potato grower in 2016 in Timaru, New Zealand (44°39'S and 171°25'E).

In 2011-12, two New Zealand grown cultivars of contrasting yields ('Bondi' and 'Fraser') and an internationally known standard cultivar ('Russet Burbank') were used. These are all main-crop potato cultivars with an indeterminate growth habit. A detailed description of the experiment and potato morphological characteristics is given in Oliveira *et al.* (2016). The seed potatoes were hand planted on 14 October 2011. Best management practices (refer to Oliveira *et. al.*, 2016) were applied to keep the crop free of weeds, pests and diseases and supplied with adequate nutrients and water. Plots were 21 m long and 1.6 m wide with two rows. Plant spacing was 0.35 m within the row and 0.8 m between rows, with each cultivar planted in three replicates following a completely randomized design. Each plot was separated by a 1.6 m empty row and two buffer plants were used at the end of each plot row.

Six plants were sampled from each plot (three plants on each row). The plants were hand lifted using a fork on eight occasions (40, 47, 53, 61, 67, 75, 82 and 97 days after planting; DAP) from 23 Nov. 2011 to 19 Jan. 2012. The 1^{st} to the 5^{th} samplings were scheduled to occur between the phenological stages 40 and 41 (BBCH-scale (Hack, *et al.*, 1993)) and the 6th to the 8th samplings between 41 and 46 (BBCH-scale). The samples were taken sequentially from the western end of each plot. To minimize any confounding effect of canopy competition among plants, the first plant of the row was skipped at each sampling event. A sub-sample of one main stem of each plant was randomly selected from each one of the six plants sampled.

In this paper a main stem refers to the "true stem" developed directly from the seed potato. Above-ground level, the main stem produces a leaf and, potentially, a branch at each node (first level of foliage) which terminates in an inflorescence (first inflorescence). Lateral branches are also produced on these main stems. They can arise on the second (n-1) and third (n-2) axillary nodes below the first inflorescence (apical lateral branches) and at lower node positions (e.g. n-13 and n-14; basal lateral branches) of the main stems. These above-ground lateral branches represent the second level of growth that can also terminate in an inflorescence (second inflorescence). Later, a third and higher levels may appear. In this study the topmost above-ground lateral branch (n-1 or n-2) to appear on each new level of growth was considered as a continuation of the main stem.

According to McCauley & Evert (1988), the morphology of the potato leaves can range from simple to pinnately compound on the most basal leaves and are generally odd pinnate (with three major pairs of lateral leaflets and a number of folioles) on the upper leaves of the potato stem. Therefore, in this work the terminal leaflet length (L_L, Figure 1) of all leaves on the main stem was measured using a ruler. However, in the absence of a terminal leaflet (e.g. on those simple leaf present on the base of the potato stem) the length of the leaf blade (without the petiole) was measured as a proxy for L_L. On the first four samplings the measurements were taken on the main stem leaves below the first inflorescence (first level of growth). The fifth and sixth leaflet measurements were taken between the first and second main stem inflorescences. The last two measurements were taken on every leaf formed above the first inflorescence (*e.g.* second, third and fourth levels of growth) on the main stem. To measure leaf area a Li-Cor 3100 Area Meter was used and leaves from each individual node of the main stem were grouped from the six sub-samples before being passed through the leaf area meter. A total of 867 potato leaves (321 from 'Bondi', 264 from 'Fraser' and 282 from 'Russet Burbank') were measured for L_L (cm) and leaf area (LA; cm^2) (Figure 1).

On 28 November 2016 a total of 15 'Russet Burbank' plants were sampled from a commercial potato crop planted in mid-October and grown under best management practices. A sample of one main stem was randomly selected from 15 different potato plants. This time 10 leaves on each sample were measured on the first and second level of the main stem for L_L, L, W and LA, using the same methods previously described.

Data were analysed using GenStat version 14 (VSN International). Leaf area and leaf linear values (L_L, L, W, LxW) were considered the dependent and independent variables, respectively. Exponential, Quadratic, Bi-linear, or Linear, and Linear log transformed (or Linear log-log; linear regression performed for the log of the linear values against the log of leaf area (Firman & Allen, 1989) regressions were fitted between dependent and explanatory variables. The regressions performances were assessed on their biological logic, level of simplicity, coefficient of determination (R^2), significance of regression coefficients; using the t and F test at 5% significance (Busato *et al.*, 2010). The 2011-12 data are presented as averages of the three replicates.

In the results and discussion section the field data collected in 2016 are presented first for easier interpretation of the results. Initially, this paper compares the proposed method that estimates leaf area from L_L, with the conventional methods that use L, W, or a combination of these two measurements. The data are then combined for the three cultivars and the two field seasons to build a more robust equation for leaf area estimation.

Results and Discussion

The results showed that the terminal leaflet measurement method, which uses only L_L to estimate leaf area, represents a much simpler (e.g. less labour and time consuming) non-destructive way of measuring individual leaf area compared with the commonly applied method of leaf area estimation from two linear measurements (L and W). This could largely improve individual leaf area data collection in the field.

Although the Exponential, Quadratic and the Bi-linear or linear regressions fitted to L_L did not improve the individual LA estimation compared with the current methods used, there was a close fit (R^2=0.95) for the Linear log-log regression performed between LL (cm) and LA (cm^2) in 'Russet Burbank' (Table 1). This coefficient of determination was greater than the Linear log-log method tested using LxW (R^2 of 0.75) (Table 1) and comparable with the R^2 from the models fitted between LA and the commonly used, single linear leaf measurements (L, W; R^2 of 0.97 and 95, respectively) in this work, and in previous potato studies (R^2=0.96; Firman & Allen, 1989). In addition, the R^2 of 0.95 was greater than in other published results (e.g. R^2<0.87, Silva *et al.*, 2008; R^2<0.92, Busato *et al.*, 2010) where L, W and LxW was used as the predictors of LA. Thus, the L_L method can be successfully used to predict individual potato LA.

Table 1: Coefficient of determination (R^2), and equations for regressions fitted between leaf length (L) and leaf area (LA), leaf width (W) and LA, terminal leaflet length (L_L) and LA and, Log10 of L, W and L_L and Log10 LA of 'Russet Burbank' measured on 28 Nov. 2016 at Timaru, New Zealand.

Equations	Method	R^2	Equation(s)
Exponential	L_L	**0.84**	$LA=25.7\times1.22^{L_L}$
	L	0.93	$LA=88.1\times1.04^{L}$
	W	0.90	$LA=87.7\times1.07^{W}$
	L*W	0.96	$LA= -543.9\times1.00^{L*W}$
Quadratic	L_L	**0.88**	$LA= -19.5-8.55L_L+2.74L_L^2$
	L	0.94	$LA= -42.1+5.21L+0.15L^2$
	W	0.91	$LA= -17.1+ 4.66W+0.41W^2$
	L*W	0.96	$LA= -6.77+0.47L*W-7.94x10^{-5}L*W^2$
Bi-linear or Linear	L_L	**0.90**	†$LA_I=11.3L_L-38.2$; $LA_{II}=54.9L_L-377.2$
	L	0.95	$LA_I=5.59L-28.3$; $LA_{II}=13.9L-163.2$
	W	0.91	$LA_I=6.71W-11.7$; $LA_{II}=20.4W-157.8$
	L*W	0.96	$LA=0.41L*W+2.83$
Linear log-log	L_L	**0.95**	$\log_{10} LA=3.35(\log_{10}L_L)-1.19$
	L	0.97	$\log_{10} LA=2.13(\log_{10}L)-0.76$
	W	0.95	$\log_{10} LA=1.80(\log_{10}W)+ 0.02$
	L*W	0.75	$\log_{10} LA=9.04x10^{-4}(\log_{10}L*W)-1.44$

Note: † LA_I = first phase of the Bi-linear model; LA_{II} = second phase of the Bi-linear model.

In the 2011-12 experiment, leaf area sizes ranged from 0.2 to 196 cm^2 in 'Bondi', 0.03 to 109 cm^2 in 'Fraser' and 0.15 to 146 cm^2 in 'Russet Burbank'. The individual leaf area against L_L fits (Table 2) indicated that all four regressions tested could be used to describe the variation between these two variables. However, the Quadratic, Bi-linear and Linear log-log regressions had a higher R^2 ($0.92<R^2<0.98$) compared with the exponential regressions tested ($0.89<R^2<0.96$). When all data were combined for the three cultivars and the two 'Russet Burbank' growing seasons, R^2 was improved by the Linear log-log fit ($R^2=0.92$) compared with the other relationships ($0.88<R^2<0.90$) (Table 2). A similar improvement of the regression fit with the Linear log-log has been previously reported (Firman & Allen, 1989). The relationship established (log L_L against log LA) therefore allows LA to be accurately estimated from L_L measurements.

Table 2: Coefficient of determination (R^2) and equations for regressions fitted between terminal leaflet length (LL; cm) and leaf area (LA; cm2) of 'Bondi', 'Fraser' and 'Russet Burbank' measured from 23 Nov. 2011 to 19 Jan. 2012 and on 28 Nov. 2016 at Lincoln and Timaru, New Zealand, respectively.

Equations	Cultivar	R^2	Equation(s)
Exponential	#'Russet Burbank'	0.89	$LA=22.2\times1.24^{L_L}$
	'Fraser'	0.96	$LA=3.64\times1.55^{L_L}$
	'Bondi'	0.92	$LA=5.71\times1.56^{L_L}$
	Combined	0.88	$LA=27.3\times1.22^{L_L}$
Quadratic	#'Russet Burbank'	0.92	$LA=15.2-14.6L_L+3.00L_L^2$
	'Fraser'	0.98	$LA=7.71-9.02L_L+2.73L_L^2$
	'Bondi'	0.93	$LA=13.8-16.9L_L+4.77L_L^2$
	Combined	0.90	$LA=3.40-5.96L_L+2.38L_L^2$
Bi-linear	#'Russet Burbank'	0.92	†$LA_I=9.9L_L-23.84$; $LA_{II}=51.6L_L-337.2$
	'Fraser'	0.98	$LA_I=5.29L_L-8.20$; $LA_{II}=25.1L_L-96.1$
	'Bondi'	0.93	$LA_I=8.00L_L-9.81$; $LA_{II}=45.6L_L-187.8$
	Combined	0.90	$LA_I=16.7L_L-40.0$; $LA_{II}=52.9L_L-352.6$
Linear log-log	#'Russet Burbank'	0.96	$\log_{10} LA=3.04(\log_{10}L_L)-0.86$
	'Fraser'	0.95	$\log_{10} LA=3.48(\log_{10}L_L)-0.99$
	'Bondi'	0.93	$\log_{10} LA=3.05(\log_{10}L_L)-0.49$
	Combined	**0.92**	$\log_{10} LA=2.96(\log_{10}L_L)-0.69$

Note: † LA_I = first phase of the Bi-linear model; LA_{II} = second phase of the Bi-linear model. #'Russet Burbank': data combined for 2011-12 and 2016 samplings.

This novel method will reduce the amount of work and time required to estimate LA from linear leaf area measurements in potato studies. Moreover, the risk of leaf damage during sequential leaf measurements in field experiments will be reduced, as the methodology is much less invasive to the canopy compared with previous methods where L (Firman & Allen, 1989) and, or W is used (Busato *et al.*, 2010). It seems that these complications involved in the process of individual potato leaf area measurement in field experiments has reflected on the small number of scientific reports on sequential individual potato leaf area for the whole canopy in field studies. To our knowledge, such difficulties have been overlooked in potato studies where leaf area models are created from leaf length and, or, width. This might partially be explained by the fact that these models are generally calibrated using pot-grown potato crops and later used in potato pot-based studies. Indeed most reports of individual potato leaf area expansion were performed in pot experiments in glasshouses (e.g. Vos & Biemond, 1992; Vos & van der Putten, 1998) and growth chambers (e.g. Fleisher & Timlin, 2006), where individual plants can be moved around to avoid the difficulties of data collection mentioned earlier. Moreover, these reports do not account for all leaves in the crop main stem which represents a caveat for canopy expansion modelling work. Here, the relationships were fitted using data collected from all main stem profiles and throughout the crop canopy development phase which allowed the estimation of leaf area regardless of leaf age and position on the plant (Figure 2). This is an advantage compared with other potato models of leaf area estimation built from data collected at a single point in time during the crop development (Firman & Allen, 1989) and with no regard to small leaf area (e.g.<100 cm^2, Silva *et al.*, 2008; Busato *et al.*, 2010).

Future potato work could focus on the validation of the current Linear log-log model (using L_L as the explanatory variable) for a wider range of potato crops (e.g. early crops) and management practices (e.g. non-irrigated crops). This could review the opportunity of using L_L as a proxy for detecting potato canopy limitations early in the growing season.

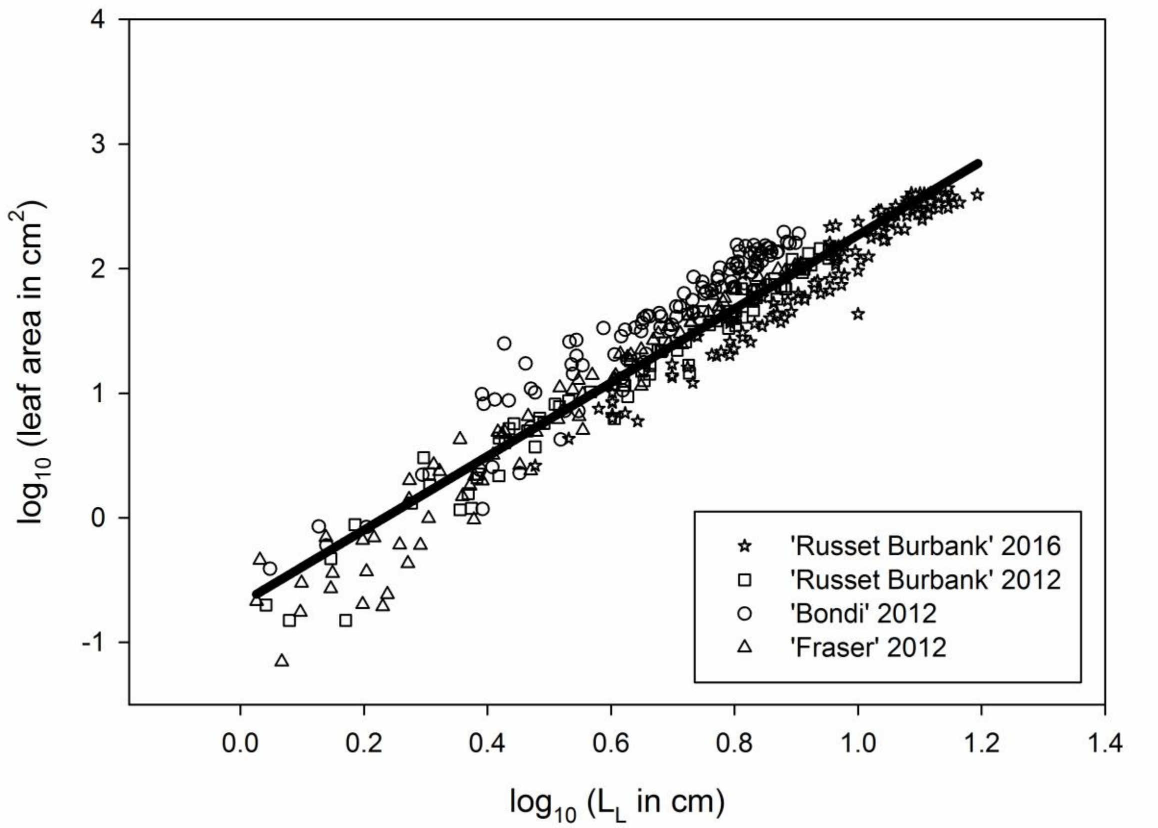

Figure 2: Relationship between log10 (terminal leaflet length; L_L) and log10 (leaf area; LA) for combined data from data collected on 2011-12 for 'Russet Burbank,' 'Bondi' and 'Fraser' and in 2016 for 'Russet Burbank'. Solid regression line: log10LA=2.96(log10LL)-0.69; R^2=0.92.

Conclusion

An inexpensive, rapid, reliable, and non-destructive method for measuring potato leaf area using minimal linear measurements was found. The Linear log-log relationship established between terminal leaflet length and leaf area was able to accommodate the effect of changes in leaf area during canopy development and for different node positions in the vertical main stem profile. This information will be useful for potato agronomists and physiologists interested in modelling leaf expansion and the estimation of leaf area index particularly in field-based studies.

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