Measuring 'wilting' and 'pod density' of canola genotypes with an optical sensor unit (GreenSeeker)

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ABSTRACT

A GreenSeeker optical remote sensing unit was used to measure the degree of wilting during the course of a single day in plots of diverse canola genotypes. There were clear and significant differences between genotypes for wilting. There was significant interaction between genotypes and days, depending on the weather conditions experienced by the plants on each day. This wilting character merits further investigation as it may be related to a genotype's ability to respond successfully to drought stress.

The GreenSeeker was also used in a novel, reversed mode to point skywards to measure pod density at crop maturity. Pod density was also assessed visually on a 0-5 scale by two assessors. Significant genotype differences for pod density via optical readings were observed, and these correlated well with pod density assessments made visually. Both measurements of pod density were correlated with seed yield.

INTRODUCTION

Using an indirect selection approach to improve a crop's tolerance to stress requires rapid, non-destructive techniques to quantify traits of interest. Hand held remote optical sensor technology offers the opportunity to make rapid, accurate and non-destructive measurements of important traits in the field.

Recent advances in data processing and storage, optical engineering and miniaturization has resulted an wide range of optical instruments to measure plant characteristics related to stress. One of these sensors is the GreenSeeker: a hand held unit that generates light at two specific wavelengths bands in the visible (red, 660 nm) and infra-red (NIR, 770 nm) regions and measures the light reflected off the target. This internal illumination allows the unit to be used in any lighting condition, day or night. A microprocessor within the sensor analyses the reflected light and calculates NDVI (a normalised differential vegetation index).

The GreenSeeker unit has been used to measure fractional ground cover over time and hence crop vigour (Moroni et al. 2009, elsewhere in this volume). During measurements on a field trial under extreme drought stress it was observed that certain canola genotypes wilted more than others during the course of the day. It was decided to estimate this degree of wilting and to see whether it might be a suitable trait to indicate drought tolerance – in this case the ability of the plant to remain fully hydrated all day. Of course, if water is limited it is also possible that wilting and therefore reduced water-use in the heat of the day may be advantageous to plant survival. It was unclear how the extent of wilting might be related to crop yield in dry conditions.

When measuring NDVI, the GreenSeeker is basically contrasting the extent of green vegetation relative to brown soil when pointed towards the ground. It was considered possible that this contrast could be reversed at the end of the season to measure pod density. This was possible because pods are tan or brown in colour and could be contrasted by an artificial green background held above the mature crop at a fixed height.

MATERIALS AND METHODS

The plots measured were in a field trial at Wagga Wagga in 2008 (drought block). The details of this experiment and the genotypes included are described by Moroni et al. (2009, elsewhere in this volume). NDVI readings were taken on the 1 October 2008 and 2 October, in the early morning and the late afternoon on each day. These times were chosen to represent the times of the day when the plants were expected to be least water stressed, and then maximally stressed. The two sampling days had quite different weather conditions. October 1 had a maximum temperature of 18.3 ^{o}C, with 44% relative humidity, and resulted in a pan evaporation figure of 3.7 mm for the day. In contrast, October 2 was 24.2 ^{o}C, humidity 31%, and pan evaporation of 6.8 mm. There was a warm northerly wind on October 2. Overall, October 2 was a much more stressful day for the plants, given that they were already experiencing severe soil water deficit due to the drought conditions.

A wilting index, on a 0-100% scale, was used to describe the increase in wilting during the day relative to the morning value. Wilting index (WI) was calculated as:

$$\mathbf{WI = (NDVI_{am} - NDVI_{pm}/NDVI_{am}) * 100}$$

Analysis of wilting index data was carried out in Genstat v.11 software using REML to estimate spatial, genotype and day effects, plus genotype x day interaction.

The day before harvest the same experiment was measured for pod density. Two observers rated each plot on a 0-5 scale from poor pod set to high (good) pod set. The plots were then measured with an inverted GreenSeeker unit with an artificial green-coloured flat sheet attached to the frame of the unit as the background.

In this case, raw data for both visible red light (660 nm, VIS) and infra-red light (770 nm, NIR) was collected, as well as the indexes NDVI and RVI, which were calculated as: normalized differential vegetation index (NDVI) = (ρNIR-ρVIS)/(ρNIR+ρVIS), and ratio vegetation index (RVI) = (ρRED/ρNIR).

Two GreenSeeker measurements were made per plot on two replicates of the 3-replicated trial. Each measurement consisted of 20 readings made in sequence from about 1.5 m inside the end of the plot out towards the edge of the plot. The readings were taken between rows 3 & 4, and between 5 & 6 of each 8-row plot. The depth of the pod layer in each plot was then recorded as the difference (in cm) between the top of the canopy and the position of the lowest pod in the canopy. At harvest, the seed yield (g/plot converted to t/ha) was calculated for each plot. For the purposes of presentation in this paper, RVI readings, pod density scores, pod layer depth, and seed yield were simply averaged across both plots of the same genotype.

RESULTS

There were detectable changes in the ‘wilting index’ (WI) in most genotypes between morning and afternoon and, not surprisingly, this change was significantly greater ($P<0.001$) on the hotter day (2 October). The range in wilting index was ~0 to10% on 1 October, 5 to 20% on 2 October. There were significant main effect of genotype for WI ($P<0.001$), and a significant interaction for genotype x day ($P<0.001$). Of interest is not only the overall wilting index for a particular genotype but the change in wilting from the cooler to the hotter day. Six genotypes (Fig. 1) showed significantly greater wilting on 2 October compared to 1 October (as assessed by the LSD at 5%). The other 24 genotypes showed smaller positive or negative changes which were not significant.

Genotype means for pod density, RVI readings, and seed yield were over a wide range. There was a good correlation between RVI and both the pod density scores (Fig 2a) and seed yield (Fig 2b). There was no correlation between RVI and pod layer depth (data not shown).

DISCUSSION

Measuring water stress in plants is a slow and time-consuming task. Most techniques such as conductance, pressure balance, relative water content etc. are not suitable for screening of large breeding populations. The use of remote sensing technology, such as the GreenSeeker sensor, offers a rapid way to estimate the degree of wilting in broad leaf crops, such as canola. We have assumed that upon leaf dehydration a significant drooping of the leaf blade will occur (i.e. wilting). This positional (or orientation) change in the leaves will expose more ground (soil) relative to when the plant is not wilting. It is this difference that we aim to assess with the Greenseeker. In this context, we assumed that the optical reflective characteristics of plant leaves (as detected by the Greenseeker sensor) are the same under wilting and non-wilting conditions. This assumption remains to be tested.

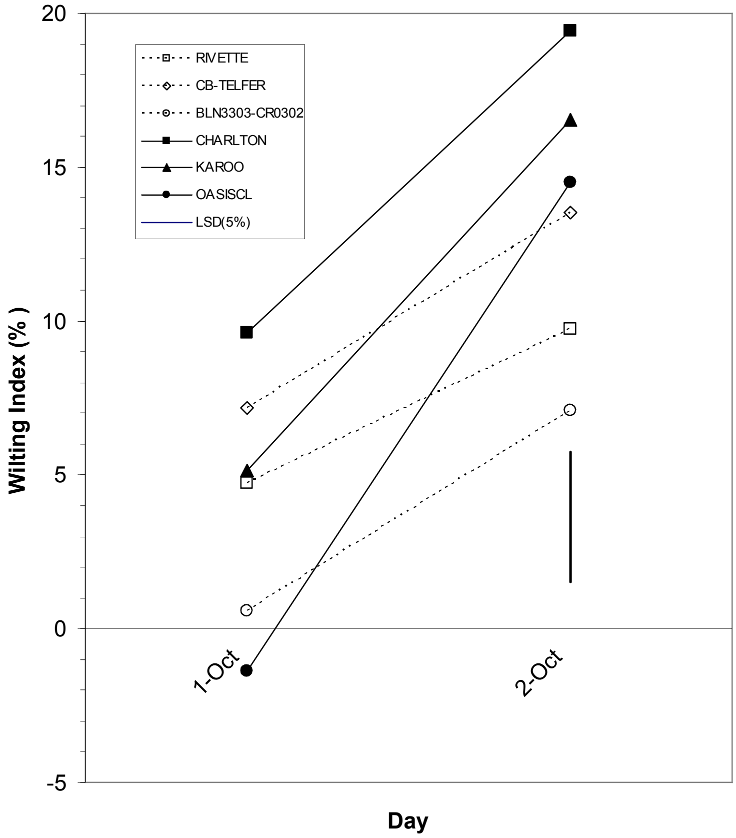

Fig. 1. Predicted genotype means of wilting index (%) for six Brassica genotypes (all *B. napus* except OASIS-CL = *B. juncea*) grown in a field plot trial at Wagga Wagga, NSW in 2008. These six genotypes showed significantly increased wilting index on 2 October compared to 1 October (other 24 genotypes not shown). The LSD (5%) is shown as a vertical bar.

The degree of wilting seen in a genotype has various possible interpretations. A genotype with high wilting may have a poor root system in terms of depth or root density and is therefore unable to extract enough water to maintain turgor, especially in the hotter parts of the day. Alternatively, the plant may have an adequate root system and water supply but may not have the required conductance capacity to deliver water to the leaves. Conversely, wilting may be a “deliberate” strategy to conserve moisture and reduce heat stress by reducing the incident radiation and making the leaves droop – closing the stomata to achieve this has the added effect of saving transpired water but at the expense of photosynthesis. Wilting *per se* is not necessarily an undesirable occurrence (unless permanent wilting point is reached) and may well be a survival strategy employed by the plant to conserve the limited water soil left while waiting for further rainfall. In other words, stay alive until conditions improve.

In soybean several accessions have been identified with a “slow wilting” pattern (Hufstetler et al. 2007) that has some relation to yield in stressful environments. At present the physiology and genetics basis for this trait are poorly understood; it is not clear whether wilting is the result of a single mechanism, or an integration of several (Hufstetler et al., 2007).

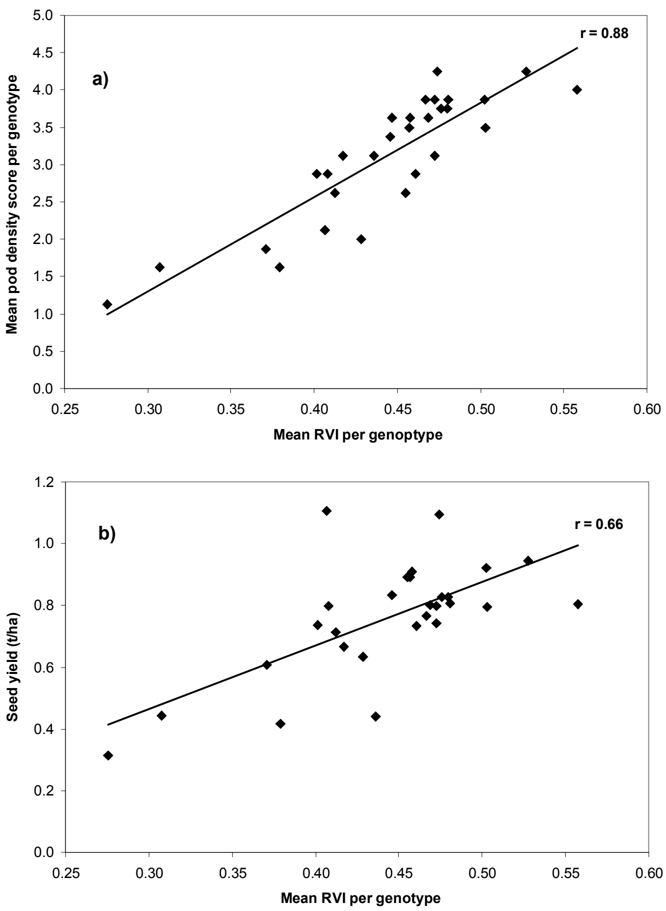

Fig. 2. Simple genotype means of ratio vegetation index (RVI) readings for 28 Brassica genotypes (all *B. napus* except OASIS-CL = *B. juncea*) grown in a field plot trial at Wagga Wagga, NSW in 2008. a) RVI versus visual pod density score (0-5 scale); b) RVI versus seed yield (t/ha).

Wilting response is being further investigated in canola experiments in 2009, and is being related to rooting characteristics, crop vigour, canopy temperature, water use, carbon isotope discrimination, and yield components.

Pod density assessment by RVI measurements shows promise as a tool for the prediction of yield since it was correlated well with actual seed yield, and visual pod density scores. Interestingly, the depth of the podding layer was not correlated with RVI or yield, indicating that since these layers vary in depth between genotypes, there must be variation in the "concentration" of pods, caused by some differences in plant architecture.

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