



Australian Government

Cotton Research and Development Corporation

SUMMER SCHOLARSHIP REPORT: 2015-2016 SEASON

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| 1. | Project Title | : | Monitoring leaf hydration of cotton non-destructively with ZIM-probes during irrigation cycles
(Maximum 15 words) |
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| 2. | Proposed Start Date | : | December 2015 |
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SUMMER SCHOLARSHIP REPORT

1. Executive Summary:

Cotton production in Australia is limited by water availability, with seasonal variability affecting both irrigated and dryland cotton. Optimal water use in commercial cotton production today requires effective measurement techniques that can be used by farmers to inform their irrigation schedules. Existing methods of measuring water status include those which measure soil, atmospheric and/or plant conditions. The aim of this experiment was to test the potential application of ZIM-probes in irrigated cotton. ZIM-probes are a novel, non-destructive technology used for the continual, remote measurement of leaf hydration. ZIM-probes are a contact, plant based method of measurement. The preliminary findings of this study demonstrated that ZIM-probes detect changes in cotton water status between irrigation events and are potentially more sensitive in detecting signs of water stress than traditional techniques. Features of the probe output patterns have been identified that indicate calibration parameters could be developed to optimise the timing of irrigation. Further studies are also needed to remedy clamping problems encountered due to unusual properties of cotton leaves.

2. Background:

There is a continual demand to increase water use efficiency in Australia, particularly in cotton and other irrigated crops. Irrigated cotton is a heavy consumer of water, using an average of 7.8 ML ha⁻¹ (Cotton Australia 2016). Furthermore, water is one of most limiting inputs in cotton production, but adequate supply is critical for growth, fibre quality and yield. There is an inherent need to increase water use efficiency of Australian cotton production systems and decrease water consumption for the long-term sustainability of the industry. Improving irrigation scheduling and developing technology that helps growers determine the optimum time to irrigate will contribute to the efficiencies required.

The most common technology used to aid irrigation scheduling in cotton include devices that directly measure atmospheric and soil moisture content and indirectly determine plant water status (Dugdale et al. 2004; White and Raine 2008; Conaty et al. 2012). Crop water status is quantified by monitoring one or several of these factors at the same time. All existing methods have both advantages and limitations. Plant based measurements are arguably the most accurate method of measuring water status for irrigation, as they are a direct measurement of the plant condition, and the cumulative response to external and internal factors. However, all currently used methods of measurement on cotton are indirect or destructive and labour intensive. New tools are required.

This project evaluated ZIM-probes, which are plant-based sensors, originally invented to monitor relative changes in leaf turgor (a component of water status) in orchards and vineyards (Zimmermann *et al.* 2008; 2013). The advantages of the ZIM-technology include direct continuous measurements that can be remotely accessed via the internet and once set up, have relatively low labour requirement. However, the technology may not be applicable to all irrigation formats (e.g. furrow/flood irrigation) and measurements can be confounded by leaf injury during initial clamping of the probes to leaves or after long-term attachment. Leaf structure can also influence the output from the probes because the sensor needs to make good contact with the leaf surface, avoiding raised veins and delicate leaves may need restraining to support the 5 g device (Bramley et al 2013). This project tested a variety of methods of clamping the probes to cotton leaves and compared the data collected from an irrigated cotton crop in the field against traditional measurements of water status to determine potential applicability of ZIM-technology to monitoring water status of cotton.

3. Aims and Objectives:

The aims of the project were to:

- (1) Determine whether ZIM-probes accurately monitor water status of cotton and,
- (2) Identify patterns of ZIM-probe output and how these relate to changes in leaf water status, and
- (3) Determine whether output from the probes could be used for irrigational scheduling.

4. Materials and Methods:

The ZIM system and probe principles have been described elsewhere (Zimmermann et al 2008). In summary, miniature pressure sensors clamped to leaves by magnets detect the change in leaf turgor as the plant dehydrates and rehydrates in response to water availability and transpiration. The pressure output (termed patch pressure, P_p) can, thus be used as an indication of the changing water status of the plant.

A large part of the project was involved in method development. Two separate experiments were conducted consisting of a glasshouse trial to establish the best method for clamping the probes on cotton and a field trial to assess the output P_p under typical irrigated conditions.

The glasshouse trial was conducted at the Narrabri campus of the University of Sydney using cotton plants (Coker 310 due to restrictions on the use of GM crops at IA Watson) grown from seed. When plants reached tenth-node stage of development, different methods for clamping probes to cotton leaves were tested, including probe orientation and position on the leaf, distance between magnets and use of a support mechanism (Bramley et al. 2013). Successful clamps were determined through visual inspection for leaf injury and observation of P_p output graphs over 2-3 days.

The field experiment was conducted in two irrigated fields, planted with Sicot 74 BRF, on 'Willawood', Merah North. The grower used furrow irrigation from a pump water source and personal determination of the timing of irrigation. ZIM-probes were clamped on three different plants in four replicate locations throughout the two fields. Microclimate probes were also placed in one of the locations to collect temperature and humidity measurements, using the ZIM system. Additional physiological measurements were taken at each field location, which included: stomatal conductance using a porometer, canopy temperature using a pyrometer and leaf water potential using a pressure chamber. These measurements were collected between 11 am and 2 pm on every second day, between two complete irrigation cycles.

At the time of this report, analysis of data is only in the preliminary stage and will form the basis of the student's thesis, which will be submitted in October.

5. Results:

The most effective clamping was when the probes were placed on the most recently fully expanded main stem leaf in both the glasshouse and in the field and when clamped on flat, veinless surfaces of the leaves, with the sensor placed on the underside of the leaf (Fig. 2a). Optimal clamp pressure on leaves was achieved by altering the distance between the magnets and observing the output using the ZIM clamping device. Glasshouse leaves needed supporting, but field leaves were able to hold the probe weight without a support (Fig. 2a). Patch pressure in several probes could not be maintained for more than 1-2 days after initial clamping. In these cases, the gossypols exuded by cotton leaves may have interacted with the silicon material embedding the pressure sensor in the lower magnet, as the probe output was influenced by temperature after the unsuccessful clamping, which is an indicator of silicon deterioration. These probes need to be repaired by the manufacturer. Tests were conducted using tape and varnish to protect the probe's silicon sensor, but were unsuccessful. However, it was found that probes with a newer silicon compound in the sensor were not influenced by this and remained active. Thus, only data from new probes placed in the field were used for the analysis.

Some probes could be clamped without adjustment for significant periods in the field. Only slight discolouration due to loss of chlorophyll was observed after removing the probes after around one month (Fig. 2b).



Figure 2. Examples of a ZIM-probe clamped on a cotton plant in the field (a) and leaf patch after removal of the probe (indicated by arrow) at the end of the experiment (b). Note the slight discoloration due to loss of chlorophyll in (b).

Fig. 3 shows the typical diurnal fluctuations in patch pressure (P_p) output for a probe clamped in the field between 7 - 20 February 2016. The field was irrigated twice during the period. Transmission was lost on one night due to GSM provider issues. The diurnal P_p pattern of cotton is similar to the pattern observed for other plant species, where P_p increases during the day when the leaf dehydrates and decreases at night when transpiration ceases and the plant recovers. As the crop became more stressed between irrigation events, a depression in daytime peak P_p began to occur (indicated by arrows), but after irrigation the peaks recovered (Fig. 3). In addition, the night-time trough in P_p was reached just before dawn before irrigation events, but occurred earlier in the evening after irrigation. While peak P_p amplitudes varied depending on individual probes, all effective probes followed these same trends. In particular, night-time recovery of P_p was found to be achieved much earlier in the evening directly following an irrigation, and P_p recovery was observed to occur much later in the evening as plants became more water stressed.

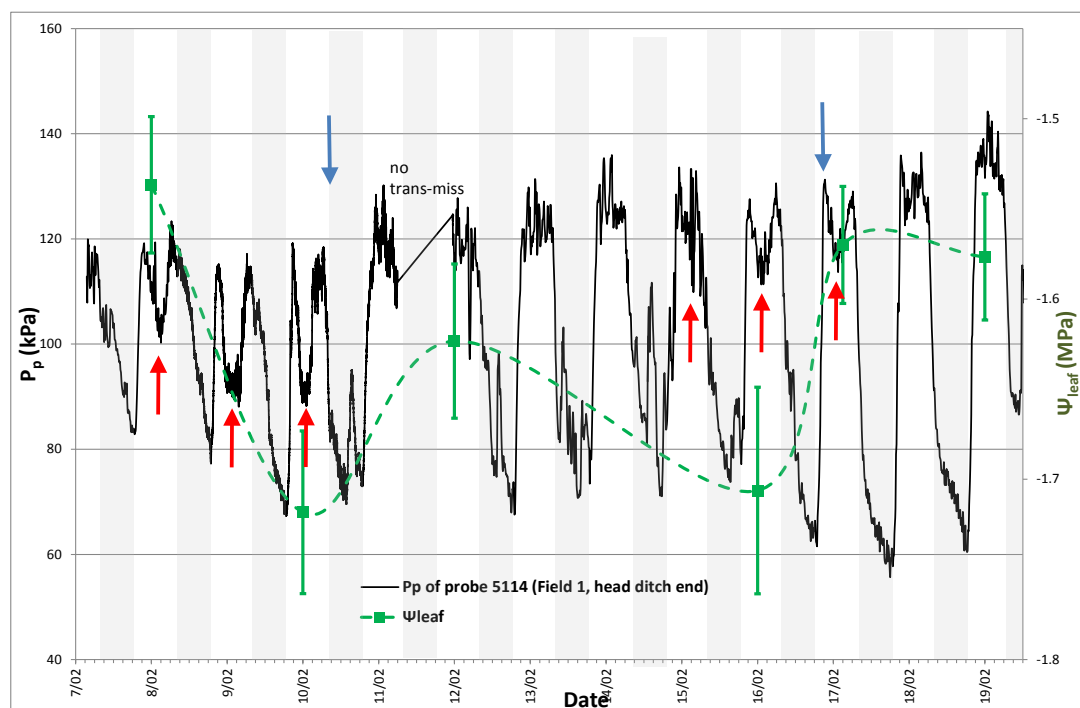


Figure 3: ZIM-probe patch pressure (P_p) output for probe 5114 in field 1. White and grey blocks represent day and night, respectively. Blue arrows indicate irrigation events and red arrows indicate day-time inversion of P_p suggesting midday closure of the stomata and hence, partial recovery of turgor. Leaf water potential (Ψ_{leaf}) measured with a pressure chamber are also shown on the y-axis for comparison.

The physiological measurements explain some of the observed patterns in P_p (Fig. 3 and 4). While there was only a small variation in midday leaf water potential (Ψ_{leaf}) throughout the irrigation cycle (Fig. 3) due to closure of stomata (Fig. 4), the data in both testing locations show Ψ_{leaf} was most negative prior to irrigation and partially recovered following irrigation (Fig. 3). Similarly, when taking into account the corresponding ambient temperature, canopy temperature decreased slightly following an irrigation event, before increasing

again (Fig. 4). Canopy temperature also tended to be higher when stomatal conductance was lower (Fig. 4). More physiological measurements over a diurnal period, will further quantify the P_p patterns.

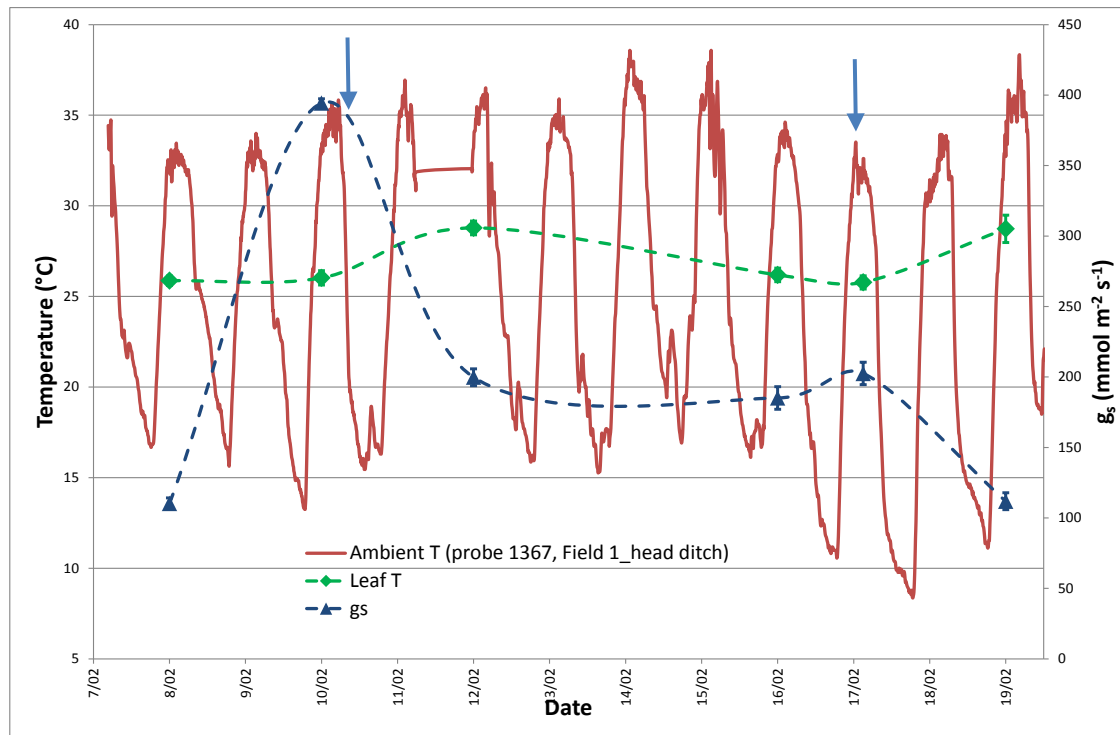


Figure 5: Ambient temperature (solid red line) monitored continuously with ZIM-system, leaf temperature (green dotted line) and stomatal conductance (g_s , blue dotted line) corresponding to plants in the vicinity of probe 5114 in Figure 2. Timing of irrigation events are indicated by blue arrows.

6. Discussion and Conclusions:

The results indicate that P_p data collected by ZIM-probes in the field could be a useful tool complementing other technologies for irrigation scheduling. The patterns in P_p for cotton showed two distinct signs of crop stress, including the formation of inverse peaks during the middle of the day time, and slower recovery of P_p at night time. The formation of these inverses during the middle of the day indicate the crop is becoming water stressed, and may be closing its stomata to conserve water. This is supported by the measurements of stomatal conductance during midday, which tended to be low. Additional measurements of stomatal conductance over a diurnal period will support these observations. Slower recovery of P_p at night-time also indicated crop stress, as P_p is inversely related to turgor (Zimmermann et al. 2008), it indicates that the plant is not able to regain full turgor at night due to water stress. Slower night-time recovery with increasing water stress has previously been observed for other crop species (Bramley et al. 2013, Ehrenberger et al. 2012). This could be confirmed in the future through conducting pre-dawn measurements of leaf water potential. Overall, signs of crop stress were able to be observed earlier using ZIM probe data than through traditional water status measurements, despite measurements taking place every second day. Probes can also remain on leaves for long periods without requiring re-clamping. If this were possible for a whole season, the system would have very low labour requirements once initially installed. Additional testing is needed to determine whether re-clamping is necessary as the plant grows.

Application of probes on cotton leaves was not as simple as anticipated due to the high density of leaf glands exuding gossypols that reacted with the probe's silicon material covering the pressure sensor. Only a small number of new probes, with a new silicon polymer, were able to be used in this study. High venation and small field leaves further hampered the clamping technique. Hence, the probe-testing phase of the trial took longer than planned and consequently, probes were only applied in the field for a short period. A greater amount of data should be collected, ideally over at least a full season, to ascertain in more detail the accuracy and applicability of the technology to cotton irrigation scheduling. Measurements of soil moisture content would also complement the analysis and provide a whole system perspective.

Despite the initial problems, data collected during this experiment indicate that ZIM-probes can monitor the water status of cotton. Accessing the data remotely is also a useful feature of the technology. If effective clamping can be routinely achieved, the ability to monitor water status continuously provides a resolution of information not previously achievable. Midday closure of the stomata and rehydration processes can be observed in real time. Both the midday depression in P_p and the slower recovery in night-time P_p with increasing water stress may be useful and easily identified parameters for determining the level of water stress. Calibration profiles for the use of irrigation scheduling could be developed for different growers and growing regions. Thus, the preliminary data of this project indicates that ZIM-probes could potentially have a role in scheduling irrigation events for cotton, but further field testing and development is recommended.

7. Highlights:

ZIM-probes are a plant-based sensor that monitor relative changes in plant water status non-destructively. This project demonstrated that ZIM-probes can be clamped to cotton leaves in the field and the data collected identified patterns of water stress not obtainable by other indirect methods. These preliminary findings indicate that ZIM-probes could potentially be an effective mechanism for monitoring plant water status in cotton, and with further testing and development could be used to inform irrigation scheduling in the future

8. Future Research:

Future research is needed to resolve the clamping problems and obtain consistent clamps every time the probes are connected to the crop. Data should be collected over at least one or two seasons alongside complementary technology such as soil moisture probes and including physiological parameters over diurnal periods or other times of the day/night. The data could then be used to develop calibration profiles for timing of irrigation events and test their implementation on field trials where the irrigation cycle can be manipulated. Long-term trial will also determine whether probes will need re-clamping over the duration of a season. Further testing will also give a better indication of whether ZIM probes could be used as the sole device for monitoring water status, or whether other complementary methods would also be needed in the farm setting.

9. Presentations and Public Relations:

Data will be presented at the 2016 University of Sydney PBI annual field day on the 7th of September.

10. Reference List:

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