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Research note:

Leaf cooling curves: measuring leaf temperature in sunlight

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Abstract. Despite the obvious benefits of using thermography under field conditions, most infrared studies at the leaf level are generally conducted in the laboratory. One reason for this bias is that accuracy can potentially be compromised in sunlight because reflected radiation from the leaf might affect the calculation of the temperature measurement. We have developed a method for measuring leaf temperature in sunlight by using thermal imagery to generate cooling curves from which the time constant for cooling, τ, can be calculated. The original temperature of the sunlit leaf may be determined by extrapolating backwards in time. In the absence of specular reflection, there is close agreement between the extrapolated sunlit temperature and the sunlit temperature recorded by the camera. However, when reflected radiation is high, the difference between the initial (incorrect) temperature determined from the sunlit image and the temperature extrapolated from the cooling curve can be > 2°C. Notably, our results demonstrate a close agreement between the extrapolated sunlit temperature and the temperature of the leaf approximately 1 s after being shaded, suggesting that this shaded image provides a good estimate of the original sunlit temperature. Thus, our technique provides two means for measuring leaf surface temperature in sunlight.

Keywords: infrared imaging, reflected radiation, specular reflection, thermography, time constant.

Introduction

The ability to accurately measure foliage temperature has been a goal of plant physiology and ecophysiology for decades. Major advances in this field occurred with the advent of infrared technology. Thermography has been employed widely in crop and plantation research as a tool for predicting plant water use and water stress (Fuchs *et al*. 1967; Jackson *et al*. 1979; Nielsen *et al*. 1984; Kimes *et al*. 1981; Paw U *et al*. 1989; Fuchs 1990; Jones *et al*. 2002; Luquet *et al*. 2003). As stomata close in response to water stress, transpirational cooling ceases, causing a rise in leaf temperature (Fuchs 1990; Luquet *et al*. 2003). Under warm, sunny conditions, this temperature may reach critical levels. Infrared imagery can map temperature fluctuations in foliage and therefore provide information about drought stress and water use in crops.

While measurements of gross canopy temperatures relative to air temperatures provide an indicator of average plant stress, individual leaf temperatures vary on a finer physiological scale. Recently, thermography has been used at the leaf level to track patterns of transpiration and stomatal closure (Jones 1999; Kummerlen *et al*. 1999; Prytz *et al*. 2003; Zwieniecki *et al*. 2004) and the progress of freezing in relation to water content (Ball *et al*. 2002). To date, research on single leaves has been restricted to laboratory studies (but see Zwieniecki *et al*. 2004). There are also many advantages to observing leaves in their natural environment, for example in direct sunlight, where heat stress is likely to be greatest. However, applying thermography in the natural environment has been hindered by the perceived problem of over-estimation of temperature due to reflected solar radiation in sunlit conditions. The amount of radiation reflected into a camera sensor can vary, with the greatest effect occurring when the camera, target and sun satisfy the condition for specular reflection, i.e. that the angle between the camera and the target is the same as that between the target and the sun (Vollmer *et al*. 2004). Most long-wave infrared cameras are bolometric and thus measure total energy falling

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on the detector without distinguishing between reflected radiation and radiation in thermal equilibrium with the leaf. Considering the potential applications of infrared technology in field-based studies, ascertaining the extent of specular reflection and how to overcome the dilemma it presents is a high priority for plant thermography.

For leaf-level studies, one approach to the problem of specular reflection is to simply eliminate it by shading the leaf immediately before taking an image. However, a concern here is that rapid leaf cooling might prevent an accurate measurement of the leaf's true sunlit temperature, particularly under field conditions where wind could accelerate cooling. Here, we outline a simple method for measuring the temperature of sunlit leaves with well-established portable infrared technology. We generate a cooling curve for an initially sunlit leaf that is suddenly shaded, calculate the time constant for cooling and extrapolate backwards in time to determine the initial temperature of the sunlit leaf. This procedure enables us to ascertain the extent to which reflected radiation affects the accuracy of readings. Further, by calculating the time constant for cooling, we can determine whether a rapidly shaded leaf can provide us with a good estimate of the leaf's original sunlit temperature.

Materials and methods

We obtained infrared images of leaves under field and laboratory conditions with a ThermaCAM SC2000 infrared camera with an uncooled microbolometer detector and built-in 24° lens (Flir Systems AB, Boston, MA). Field images were made of 24 Proteaceae species in the Royal Botanic Gardens at Mt Annan, New South Wales, Australia. These species varied widely in leaf size (1.5–360 cm^2), shape, thickness, hairiness and angle. Images were taken under hot, sunny conditions (~PAR 2000 $\mu mol\ m^{-2}\ s^{-1}$) with ambient temperatures reaching 41°C, relative humidity averaging 40% and wind speed not exceeding 5 $km\ h^{-1}$ All leaves were fully sunlit though the actual angle of the sun relative to the leaf surface and the camera lens varied, depending on the time of day and the angle of the leaf on the plant.

Leaves of three *Eucalyptus pauciflora* Sieber ex Spreng. plants growing in pots outdoors were used for laboratory experiments at The Australian National University, Canberra, Australia. Our objective with the laboratory experiment was to determine the degree to which reflected radiation can pose a problem during thermography. We maximised this effect by heating the leaves with a heat lamp (Ceramic 150 W Pandorel bulb and 30 cm diameter aluminium reflector; Vaucluse and ASP, Adelaide, SA) directed at an angle similar to that of the camera lens to raise the leaf temperature to ~10°C above the ambient temperature of 23.5°C. Under laboratory conditions, relative humidity was ~45% and light levels were negligible (PAR < 50 $\mu mol\ m^{-2}\ s^{-1}$).

In the camera controls, leaf emissivity was set at 0.95 (Jones 1999; Jones *et al*. 2002), and ambient temperature, relative humidity, and the distance between the leaf and the lens were entered according to ambient conditions before each series of measurements. The camera lens was set perpendicular to the main plane of the leaf's surface at a distance of 0.5–1 m from the leaf when using the standard 24° built-in lens and 0.1–0.2 m when using the close-up lens. An image series consisted of 60–90 frames taken approximately one second apart. A single leaf was imaged in each time series, with the first three frames taken of the leaf in full sunlight. Immediately after the third frame was taken, the leaf was shaded. In the field, this was done manually with a sheet of cardboard and all subsequent images were made as the leaf cooled. In the laboratory, instead of shading the leaf, the heat lamp was removed immediately after the third image was taken. Because the points subsequent to the initially lamp-lit ones are of images made when the lamp is turned off (the 'shaded' leaf), the shape of the cooling curve is unaffected by the quality of the light to which the leaf was subject during the heating phase. Using the ThermaCAM Researcher 2000 software on a PC computer, an area of standard size was drawn in the same position on the leaf of every image and the average temperature of the pixels within this area was recorded. Hereafter, leaf temperature recorded by the camera will refer to the average temperature of this defined area.

Assuming the cooling of a shaded leaf is accurately described by a single time constant, τ, the change in temperature of the leaf, dT, over a time interval, dt, is:

$$dT = -(T - T_a)\,dt/\tau. \quad (1)$$

Here, T is the leaf temperature at time t, T_a is the temperature at which leaf cooling asymptotes. Integrating the equation above and applying the boundary condition, $T(t=0) = T_0$, where T_0 is the initial sunlit temperature of the leaf, gives:

$$T(t) = (T_0 - T_a)\,e^{-t/\tau} + T_a. \quad (2)$$

Taking logarithms of both sides of Eqn 1 and rearranging the terms gives:

$$\ln[(T - T_a)/(T_0 - T_a)] = y = -t/\tau\ . \quad (3)$$

The negative inverse of the slope of the line in a plot of the left hand side of Eqn 1 (y) *v.* time provides τ. Note that the error in y can be determined by taking differentials of the left-hand side of Eqn 3:

$$dy = dT/(T - T_a). \quad (4)$$

For a fixed error in the temperature measurement, the error in y diverges as T approaches T_a. In our analysis, we fit Eqn 4 to data recorded within the first two cooling time constants ($\tau \times 2$) or approximately the first 30 s for the leaves we have studied. Here, the error in y is low and an accurate value of the cooling time constant can be determined.

The original sunlit temperature can now be determined by extrapolating backwards in time. If the temperature of the first shaded image is T_1 measured at time t_1 (the time interval between shading the leaf and the first image after shading) Eqn 2 can be extrapolated to T_0, the temperature at $t = 0$:

$$T_0 = T_a + (T_1 - T_a)\,e^{t_1/\tau}. \quad (5)$$

In the experiments presented here, the precise timing of leaf shading was prone to variation because of human error. The time interval t_d was 1 ± 0.5 s. Error associated with τ was the standard error of the regression, which averaged 0.5 s. Note that the estimates of uncertainty in the measured temperature have been made from the recorded data and do not include error associated with the estimation of emmissivity or any systematic effects due to the factory calibration of the camera. The absolute accuracy of a typical infrared camera can be as low as 2°C (Flir Systems AB), although if adjusted before every image is more likely to be approximately 0.5°C.

Results and discussion

Examples of cooling curves of leaves from different Proteaceae species under field conditions are presented in Fig. 1*a*, *c*, *e*. Although a slight breeze was present during the field thermography, creating minor ripples in the curves, the cooling rate was easily obtained from the curves and τ could be determined using a good fit of the regressed

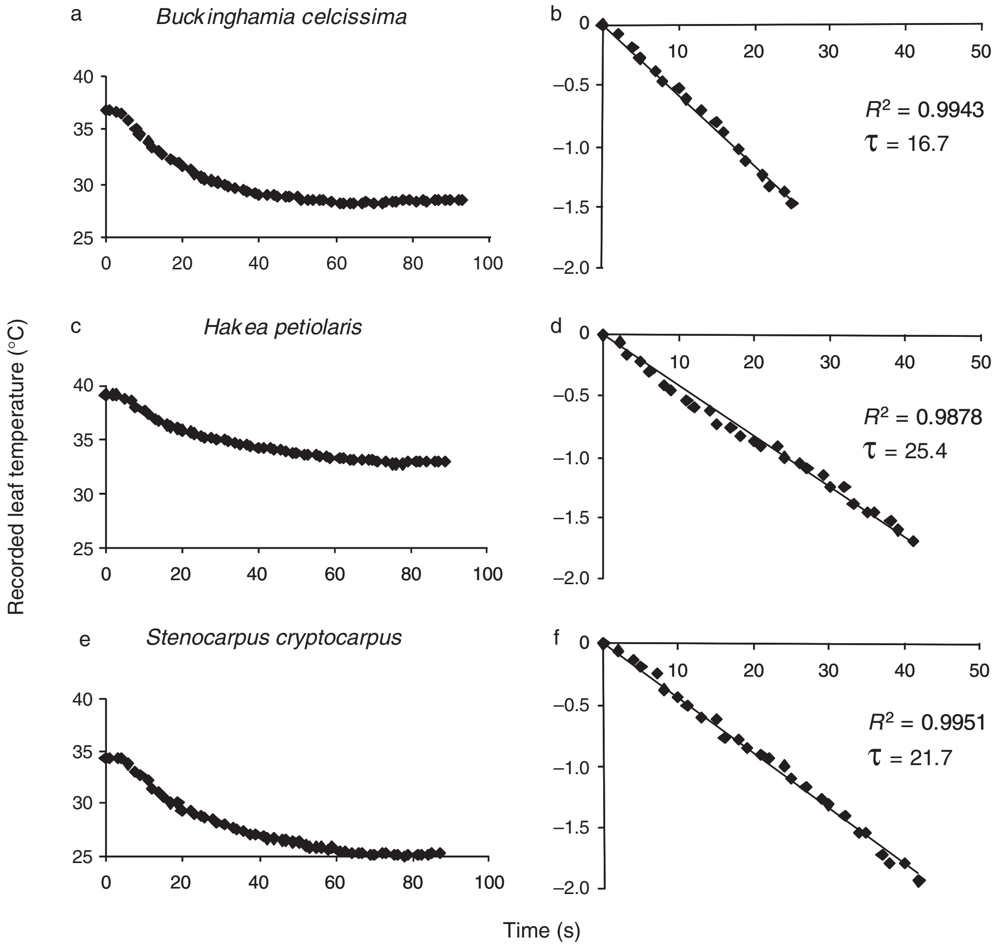

Fig. 1. An example of cooling curves of Proteaceae leaves showing the recorded temperature as they cooled in response to shading under field conditions (*a*, *c*, *e*). The first three points on these graphs are from images of the leaves in full sun, subsequent points represent the leaves after being shaded. The corresponding log regressions derived from Eqn 3 are displayed to the right of each curve (*b*, *d*, *f*).

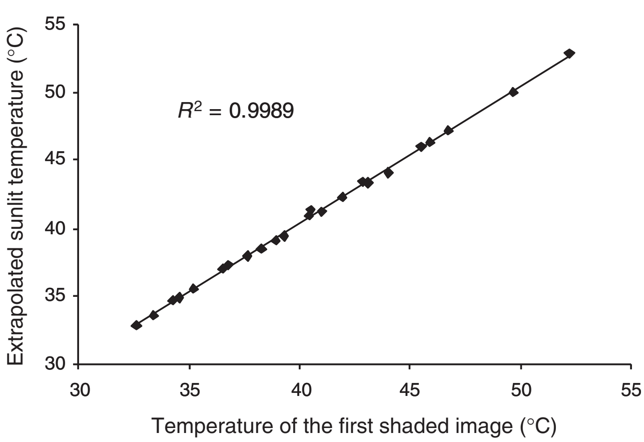

Fig. 2. Correlation between the recorded temperature of leaves approximately 1 s after being shaded and their extrapolated sunlit leaf temperature based on cooling curves of leaves of 24 Proteaceae species under field conditions.

slope (Fig. 1*b*, *d*, *f*). For the 24 field leaves, τ averaged 17 ± 1.08 (s.e.) seconds. For all curves made in the field, the extrapolated sunlit temperature did not differ from the first recorded sunlit temperature by more than 0.8°C (results not shown). Similarly, there was a close agreement between the first shaded image and the corresponding extrapolated sunlit image (Fig. 2), with the difference averaging $0.4°C \pm 0.18$ (s.e.) and never exceeding 0.8°C. Because τ was well under one minute, we can be confident that our results are not biased by the effect of stomatal closure in response to shading, as the half-time for stomatal response to a drop in light level is usually 2–5 min (Jones 1992).

For all field images, the error associated with τ averaged 0.01°C (range 0.002–0.028°C). The error based on variation in the timing of leaf shading averaged 0.2°C (range 0.1–0.4°C). Thus, the error introduced by uncertainty in the timing of shading dominated the overall error in the extrapolated temperature in our experiments.

The close agreement between the recorded sunlit and extrapolated sunlit temperature for our field data suggests that reflected radiation did not introduce major error to the measured temperature. There may be cases where the sun, leaf and camera satisfy the conditions for specular reflection and the error due to reflected radiation is large. In other cases, such as the field data presented here, it may be insignificant. It is obviously preferable to choose a viewing angle that avoids reflected radiation. However, imaging a sunlit leaf generally requires the camera to be positioned such that its angle, relative to the leaf, will be similar to that of the sun, thereby increasing the likelihood that specular reflection will occur. Our laboratory experiment was aimed at determining the extent of error possible when reflected radiation from a leaf is high.

A typical cooling curve made in the laboratory for a *Eucalyptus pauciflora* leaf is shown in Fig. 3. In the curve shown, the 'sunlit' temperature of the leaf determined immediately before removing the heat lamp was 32.5°C (Fig. 3). The leaf temperature of the first 'shaded' frame was 30.2°C (Fig. 3). The extrapolated 'sunlit' temperature was 30.4°C. In this case, there would be an error of 2.1°C if the recorded 'sunlit' data were used. Similar results were obtained for the other laboratory images. These data demonstrate that, first, extrapolation can remove any effects of reflection in calculating temperature and second, that the first shaded image provides a good estimate of the temperature of the sunlit leaf.

Conclusion

Uncertainty about the effects of reflected radiation from the sun has hindered field-based thermography, particularly at the leaf and sub-leaf level. By creating cooling curves and extrapolating the original sunlit temperature, we demonstrate that reflectance can produce a measurement error of > 2.0°C. In contrast, across all measurements (made in both the laboratory and field), the temperature of a leaf measured within 1 s of shading was never more than 0.8°C lower than the extrapolated sunlit temperature. The time constant for leaf cooling, τ, averaged 17 s. Therefore, imaging a leaf within 1 s of shading provides a comparatively good estimate of the sunlit leaf temperature. A higher level of accuracy of absolute temperature measurement could only be obtained with a more accurate camera. If such a camera becomes available, extrapolating to the original sunlit temperature would remove any uncertainty in the temperature measurement with respect to specular reflection. To take advantage of a more accurate camera, extrapolation with accurate timing (for example, by deploying an automatically triggered shutter) would be essential.

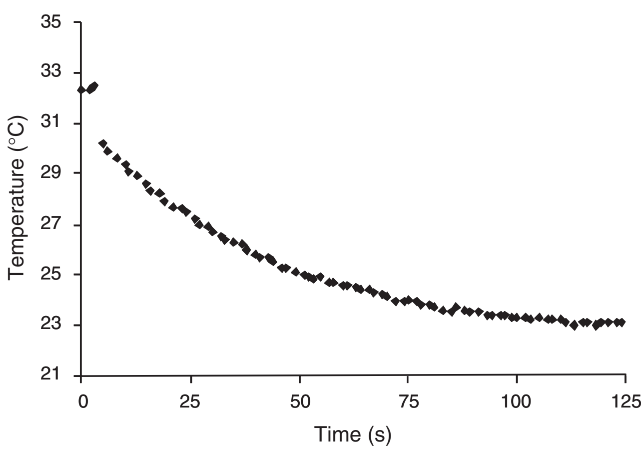

Fig. 3. A cooling curve of a *Eucalyptus pauciflora* leaf showing its recorded temperature as it cooled in response to shading under laboratory conditions. The first three points on the graph are based on images of the leaf exposed to direct radiation from a heat lamp, subsequent points represent the leaf after the heat lamp was removed.

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