# STUDY OF TRANSIENT RESPONSE OF PLANT LEAF USING INFRARED IMAGING AND EFFECT OF LIGHT ON STOMATAL OPENING

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By

WASEEM AHMAD BHAT IIT GUWAHATI

Dept. of Bioengineering and Biosciences



Department of Alechanical Engineering INDIAN INSTITUTE OF SCIENCE BANGALORE -560 012

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## **ABSTRACT**:

Infrared thermography can be used as a tool for studying the surface temperature of a plant leaf non-invasively. The main aim of this project is to study the transient temperatue response of a leaf towards constant thermal radiation incident upon it. Knowing the temperature as function of time we can then compare it with evaporation rates which can be measured with a precision weighing balance. The main problem in the correct evaluation of stomatal conductance at leaf level is due to the need of performing a measurement in a completely non-invasive method. Thermography helps us to know the stomatal conductance indirectly at different temperatures without actually disturbing the leaf. The main advantage of thermographic method is the possibility to acquire information about instantaneous conditions of transpiration over a large number of plants, with no need of sampling avoiding with and any contact plants.

In my work I also tried to understand how the heating and cooling curves of a leaf are different than some other materials having approximately same heat capacity and mass. The behavior is different in the two cases as expected. It turns out that transpiration is highly effective way of regulating the temperature of leaf. Lastly, I tried to analyse the effect of visible light on stomatal opening and also the individual components of light. The results showed that response towards blue light is as much as ten times more than red light. Stomata barely respond to green light.

Keywords: Infrared Thermography Stomata

Transpiration

## 1. Introduction:

Now-a-days, the sustainable management of water resources represents a priority for agriculture, especially in waterless regions, in fact approximately 70% of the worldwide water use is committed to agriculture [1]. For plants, water is a central molecule in all physiological processes, and its availability determines the distribution of plant species and their productivity. Accordingly, drought represents one of the major constrains to both crop productivity and quality, reducing average yields of 50% and over [2]. The strategies of plant adaptation to drought stress include different mechanisms. Thermal imaging by infrared thermography represents a suitable system for studying the energy balance at both leaf and canopy level and can be used for the estimation of evapotranspiration rates [3] which in turn can be directly compared with digital weighing balance. The basic principle of this technique is the relationship between leaf temperature and leaf transpiration. Authors demonstrated that, for porous materials, at room temperature, there is a linear relationship between the cooling due to the evaporation and recorded by IR detector and the flux rate [4]. Therefore, temperature variations of leaf surface depend strongly on the plant water status, which, in turn, is a function of the stomatal conductance [5]. Furthermore, infrared thermography can be a useful approach for both proximal and remote sensing of plant biotic stresses, for irrigation scheduling in arid environments [6], as well as for screening the stomatal functionality in different lines of a crop of interest [7–9]. Combined procedures and technologies can improve the plant water-use efficiency [10]. In this context, under drought conditions, the employ of antitranspirants may improve the water-use efficiency, often assumed to express the irrigation system performance and also defined as the ratio between the crop biomass and the amount of water consumed by the crop itself, including rainfall, irrigation water and plant transpiration [11]. There have been attempts to know water content of leaf and relate it to stomatal conductance. One of such attempts is the paper regarding use of leaf mounted thermal sensor for the measurement of water content of leaf. This paper describes proof of principle experiments demonstrating a microfabricated thermal

sensor capable of detecting changes in the water content of leaves. The device consists of a resistive heater and 2 thin film thermocouples (TFTCs) formed on a polyimide substrate. The heater induces a thermal gradient within a sample brought into contact with the device, which is monitored by the TFTCs. Changes in the thermal gradient can be related to the properties of the sample. In particular, monitoring the water content of a leaf has been demonstrated in this paper[12]. The problem with the above method is that the thermal sensors have to come in contact with the leaf itself which may change the rate of change of water content of the leaf.

This is where thermography has advantage over other techniques. It is a non-invasive method to know the transient temperature response of a leaf. The results obtained by infrared thermography are more close to actual values than by any other technique. There have been attempts to use thermography in very innovative and efficient ways over the last few years and this technique has great promise for further findings.

## 2. Theory:

## 2.1 Thermography

Infrared radiations are emitted by all objects with a temperature above absolute zero according to Black Body Radiation Law.Thermography makes it possible to see one's environment without visible light.The amount of radiation emitted by an object depends on it temperature.Greater the temperature of the object ,greater will be the amount of radiations emitted.Thermographic cameras allow us to differentiate between temperature variations. When viewed through a thermal imaging camera, warm objects stand out well against cooler backgrounds; humans and other warm-blooded animals become easily visible against the environment, day or night. As a result, thermography is particularly useful to the military and other users of surveillance cameras. In general thermographic cameras detect radiations in the infrared range of the electromagnetic spectrum (7500-14000 nm).The images taken by thermographic cameras are known as thermograms.

Infrared radiation is energy radiated by the motion of atoms and molecules on the surface of object, where the temperature of the object is more than absolute zero. The intensity of the emittance is a function of the temperature of the material. In other words, the higher the temperature, the greater the intensity of infrared energy that is emitted. As well as emitting infrared energy, materials also reflect infrared, absorb infrared and, in some cases, transmit infrared. When the temperature of the material equals that of its surroundings, the amount of thermal radiation absorbed by the object equals the amount emitted by the object

Some physiological changes in human beings and other warm-blooded animals can also be monitored with thermal imaging during clinical diagnostics. Thermography is often used for lava detection, breast screening, allergy detection, and in veterinary use. It can also be used to detect swine flu by Airport personnel.Firefighters use thermography to see through smoke, to find persons, and to localize the base of a fire. Maintenance technicians use thermography to locate overheating joints and sections of power lines & rail wheels (e.g. Rajdhani Express), which are a sign of impending failure.Building construction technicians can see thermal signatures that indicate heat leaks in faulty thermal insulation and can use the results to improve the efficiency of heating and air-conditioning units.

The appearance and operation of a modern thermographic camera is often similar to a camcorder.A camcorder is a video device combining a video recorder and a video camera.



Fig 1. Infrared image of hands showing variation of temperature in different parts of hand.



Fig 2. Infrared image of a cooling plant leaf

## 2.2 Stomatal Regulation

How do plants "decide" on the most appropriate stomatal aperture for any given situation? The physiology of stomatal regulation has been an active area of research for several decades, but there are still many questions that remain unanswered. It is clear that there is more than one control system involved. In fact, stomata seem to sense a variety of environmental parameters and respond accordingly. The precise response depends on the crop species (and perhaps cultivar) as well as the previous history of the plant.

#### Factors affecting stomatal opening: -

Light affects stomatal regulation both directly (blue light perceived by guard cells) and indirectly (PAR increasing photosynthesis and decreasing leaf internal CO<sub>2</sub>). Although the signal transduction pathway is not fully understood, it is known that these effects cause K+ ions to be actively pumped into guard cells. The resulting decrease in guard cell solute potential causes water to enter the guard cells, thus increasing turgor and causing the stomatal pore to increase in size. A decrease in leaf (or even root) water potential causes the opposite to occur: K+ and water exit guard cells and the stomata close. Abscisic acid (ABA) is known to play a role in inducing stomatal closure under water stress.

#### 2.2.1 Light:

Light has both direct and indirect effects on stomata. Blue light (especially) can bring about stomatal opening directly. The mechanisms by which the light is perceived, and the subsequent signal transduction pathway, are currently under investigation. Light can also indirectly cause stomatal opening via photosynthesis, by decreasing the leaf internal  $CO_2$  concentration. Plants have evolved multiple photoreceptor systems to monitor light quality, quantity and direction. It is well known that stomatal morphogenesis is controlled by genetic as well as environmental factors and in general, an increase in light intensity results in an increase in stomatal. Light is perceived by various photo receptors and stomatal movements are regulated by both blue and red light. The blue light response of stomata appears to be strongly affected by red light. It is shown that blue light-

induced stomatal opening is mediated by the blue light receptor phototropins and cryptochromes. Recent findings suggest that the light control of stomatal development is mediated through a crosstalk between the cryptochrome-phytochrome-COP1 signaling system and the mitogen-activated protein kinase signaling pathway. Blue light is required for the activation of phototropins, plant-specific Ser/Thr autophosphorylating kinases, and the activated phototropins transmit the signal to the plasma membrane H<sup>+</sup>-ATPase for its activation.

It has also been suggested that the guard cell response to red light is in part an indirect response to red-light-driven intercellular  $CO_2$  uptake in the mesophyll. For example, it has been shown that chloroplast-containing guard cells in albino sections of variegated leaves do not respond to photosynthetically active radiation, but are sensitive to blue light and  $CO_2$ , bringing into question a direct role of guard cell photosynthesis on red-lightmediated stomatal opening in intact leaves.

#### 2.2.2 Carbon dioxide concentration

Stomata open as leaf internal  $CO_2$  declines, in order to reduce the resistance to  $CO_2$  diffusion into the leaf. Thus, as photosynthesis increases, stomata open. If photosynthesis decreases, leaf internal  $CO_2$  increases, and stomata close. Grasses such as corn are very sensitive to leaf internal  $CO_2$ , and stomata open and close rapidly in response to natural fluctuations in photosynthesis. Drastically increasing  $[CO_2]$  in the air around a leaf will usually cause at least transient stomatal closure.



Fig 4. Carbon dioxide concentration and stomatal opening.

#### 2.2.3 Temperature

Leaf temperatures may indirectly affect stomatal opening in several ways. For instance, changes in temperature affect the photosynthetic rate, and therefore alter leaf internal CO<sub>2</sub> stomata respond on the minutes time scale to such changes in CO<sub>2</sub>, as we have seen above. Also, high temperatures cause leaf internal vapor pressure and therefore transpiration to increase. This may lead to a reduction in leaf water potential, causing stomata to close.Temperature may also have a more direct effect on stomata. In most species, deleteriously high leaf temperatures may induce stomatal opening, even when leaf internal CO<sub>2</sub> is not limiting to photosynthesis. (The effect occurs even in darkness.) This appears to be a strategy designed to decrease leaf temperatures through evaporative cooling.



Fig 3. Rate of photosynthesis vs Temperature.

#### 2.2.4 Leaf Water Status

As leaf water potential drops, stomata tend to close. Again, this effect may be direct or indirect.If water potential is very low, the guard cells themselves, and the surrounding subsidiary cells,may lose turgor, causing stomatal closure directly.Much more often, the response to leaf water potential is indirect. As leaf water potential drops, the content of ABA in the leaves increases. This ABA appears to sensitize the stomata to othersignals that would normally cause closing, and so the average stomatal aperture is decreased.

#### 2.2.5 Soil Water Potential

Obviously, as soil water content drops, leaf water potential also declines (and xylem tension increases). Thus, low soil water potentials indirectly causes stomatal closure by causing a decrease in leaf water potential. However, it is now well established that low soil water content can lead to stomatal closing even in the absence of a change in leaf water potential. It appears that as the soil dries, the roots "sense" the lower soil water potential, and synthesize ABA. This ABA is transported to the leaves in the transpiration stream (i.e., via the xylem), and produces the same effect on stomata as leaf source ABA.

This mechanism may allow plants to anticipate drought conditions and respond accordingly, even before the leaf water potential has been affected.Some species also open stomata in response to extremely high soil water content. This can occur even at night, and presumably has the advantage of depleting soil water and thus preventing root anaerobiosis.

#### 2.3 Energy Balance of Leaf

The law of thermodynamics states that energy can neither be created nor destroyed, but it can be converted from one form to another. In simple terms leaves convert solar enegy received in the form of radiation into usable form of chemical energy. Leaves in a crop canopy that are illuminated by sunlight absorb energy primarily as shortwave and longwave radiation. They dissipate this energy in different forms, through a number of physical processes, including:

- 1. Emittance of longwave radiation
- 2. Convection (movement of warm air away from leaves)
- 3. Evaporation of water (transpiration)
- 4. Conduction (warming of their surroundings by direct contact)
- 5. Storage of energy, either as heat energy in tissues, or chemical potential energy in the products of photosynthesis.

When plants are exposed to high temperatures, drought stress or intense thermal radiations, they tend to cool themselves via convection, conduction through soil and transpiration through stomata. Cooling through tiny epidermal pores called stomata accounts for most of cooling mechanism of leaves. Apart from radiating longwave radiation, plants mainly lose energy via conduction, convection and radiation.

Conductance: Heat may be dissipated via conductance. That is. direct contact between the plant tissue and some other object, such as the soil, may allow transferred from object heat energy to be the warmer to the cooler one. Estimating conductance is difficult from a computational standpoint. However, for individual sunlit leaves, conductance represents a very minor portion of the total energy balance, and can therefore be safely ignored.

Transpiration: It is the process of water movement through a plant and its evaporation from aerial parts, such as from leaves but also fromstems and flowers. Water is necessary for plants but only a small amount of water taken up by the roots is used for growth and metabolism. The remaining 99-99.5% is lost by transpiration. Leaf surfaces are dotted with pores called stomata, and in most plants they are more numerous on the undersides of the foliage. The stomata are bordered by guard cells and their stomatal accessory cells (together known as stomatal complex) that open and close the pore. Transpiration occurs through the stomatal apertures, and can be thought of as a necessary "cost" associated with the opening of the stomata to allow the diffusion of carbon dioxide gas from the air forphotosynthesis. Transpiration also cools plants, changes osmotic pressure of cells, and enables mass flow of mineral nutrients and water from roots to shoots. Evaporation of water in the interior of the leaf results in cooling due to the latent heat of vaporization required to allow the water to enter the gas phase. It is a simple matter to multiply the rate of transpiration by the molar latent heat of vaporization to determine the energy dissipated.

Convection: The other major process by which leaves can dissipate heat energy is the process of convection. Because the leaf is warmer than the surrounding air, heat is transferred from the leaf to the air. This warmer air is moved away from the leaf due to normal turbulence, and the leaf experiences a net loss of heat.

The rate at which convection removes heat from the leaf depends on (a) the temperature difference between the leaf and the air and (b) the conductance to movement of warm air away from the leaf (gA).

This is directly analogous to the process of diffusion: convective heat loss = (leaf temp. air temp.)  $\times$  gA  $\times$  29.2 J mol 1 °C 1 ,where 29.2 J mol 1 °C 1 is the (approximate) molar heat capacity of air. gA is a function of both windspeed and physical properties of the leaf itself that affect air turbulence near the leaf surface (a thick "boundary layer" of unstirred air next to the leaf tends to decrease gA)

3. Experimental apparatus:

APPARATUS	USAGE
1. Fluke Ti 400 infrared camera	Videos and images of transient
	responses.
2. Fluke Telephoto Lens	To Focus on Single Leaf.
3. GPA5202 weighing balance	To measure mass.
4. Tripod stand	To hold thermal imaging
	camera.
5. Plant	To carry out leaf temperature
	analysis.
6. Polythene(Polyethylene)	To cover soil pot so as to
	prevent evaporation of soil
	water.
7. Stainless steel strip	To make artificial leaf.
8. Black spray	To coat steel strip in order to
	increase its emissivity.
9. IR lamp (150 watt)	To incident IR radiation on
	plant leaf.
10.Simple stand	To hold the lamp.
11.Thermocole (polystyrene)	To make artificial leaf.

In my experiment I used an infrared camera Fluke TI400, a 150w IR lamp, a plant having large leaves, stand (to hold the lamp), steel plate, thermocole, black spray,

a tripod (for holding the Infrared camera) and a weighing balance. Further, I needed software to analyse data. I also had to work with matlab. Thermal camera helped me to take videos of transient temperature response of plant leaf and also still images. Then I used software to gather thermal data from the still images. I also measured transpiration rates by precision weighing balance and tried to relate it with amount and type of the radiation falling on the leaf.



Infrared Imaging camera



#### Infrared Imaging camera

## 4. Experimental setup:

There are two major targets of my experimental setup. One is to know the temperature transient response of a plant leaf surface when an incident IR radiation falls on it till its temperature saturates to a constant value. We further try to compare the time responses of heating and cooling curves. The other part is to know how light quality and intensity affect the transpiration rate. It turns out that only some particular wavelengths affect stomatal opening and closure. The detailed explanation of my experiments is as follows.

#### 4.1. Transient Temperature Response of leaf surface:

In this part of the experiment in which IR radiation is directed towards a plant. Thermal images were acquired every thirty seconds. The radiation falling on the plant leaf increases the temperature of the leaf till it saturates. At saturation there is a balance between energy loss and energy gain of the leaf. It gains energy through radiation and loses via convection, radiation, conduction and transpiration. Of the above heat loss processes, evaporation is the most effective one. For taking the temperature readings, I used an infrared camera Fluke Ti 400 and took images and videos which I later processed to plot variation of temperature at a point, along and perpendicular to the main vein of leaf and parallel to main vein of the leaf. The following graphs depict the transient temperature variation.



Fig 5. Temperature variation of three points on three different leaves of a plant on which IR radiation from a 150w lamp is incident.





Fig 6. One still image for above experiment showing temperature of three points p1,p2, p3 and average temperature.



Fig 7. Temperature variation along central vein of leaf.



Fig 8. It shows the cooling of a leaf when incident IR lamp is removed.



Fig 9. Temperature variation perpendicular to central vein of leaf.

Fig



Fig 10. Temperature variation along a line parallel to central vein.

From the above graphs various conclusions can be drawn. IR images make it very clear that veins have lesser no. of stomata than the rest of the area of the leaf surface. Also the saturation temperature at the edges of the leaf is lesser than that in the interior region. The time responses of heating and cooling are approximately the same.

4.2. Comparison between transient responses of a leaf and artificial leaf made from black coated stainless steel:

This comparison didn't serve our purpose as metals get heated and cooled in much less time than a typical plant leaf so we couldn't compare the results in the two cases. One might argue that what sort of comparison is the one where you completely neglect material properties but in reality we only need to have the mass and thermal heat capacity of the two materials to be same in order to compare their heating and cooling properties. The time responses are definitely lesser in metals. I also tried to do similar comparison with a polystyrene leaf but unfortunately the black spray digested it. Then we tried to paint it black but it absorbed the black color. So we were forced to do it without a coating. The results were absurd as white colored objects have very less emissivity.



Fig 11.Transient temperature responses of a metal and a leaf.

4.3. Temperature variation of a steel leaf coated with black:

The following graph shows the transient temperature response of a metallic leaf coated black. We kept shifting the distance between the lamp and the metallic strip till we finally got a saturation of about fifty degrees. But as expected the response time is very less as compared to that of a plant leaf.



Fig 12. Temperature variation of three points on the black coated metallic leaf.

4.4. Cooling curves of a leaf attached to a plant and an isolated leaf:

In order to get clearer picture of effect of transpiration on cooling of leaf, I tried to carry this experiment on a just detached leaf so that mass remains approximately same along with the properties. What I saw is that if I keep the distance same as in the case of attached leaf, the saturation temperature is much higher than the one achieved in attached leaf case which proves that transpiration has the cooling effect on a plant. In order to compare the curves, we increased the distance between the isolated leaf and the attached leaf so that saturation temperatures are approximately same and we could compare the cooling curve



Fig. 13. Isolated and attached leaf response.

#### 4.5. Transpiration in presence of IR radiation

There have been many attempts in the past to know which wavelengths photosynthesis of light alter the rate of transpiration and which doesn't. It turns out that some wavelengths affect it and some do not. Also different wavelengths which affect photosynthesis to a different extent. It is known that blue light affects photosynthesis and hence stomatal conductance ten times more than red light. Experiments also show that green component of visible light does not affect photosynthesis. In this part of the experiment, I tried to see the effect of IR radiation on transpiration rate. It turns out that transpiration rate increases and achieves a never steady state.





Fig 13. Water evaporated Vs time graph. At t=75 min IR light is switched off. Rate of transpiration decreases by 3.6 times when we turn off IR lamp.



Fig 14. Evaporated mass Vs Time when IR lamp is turned on.



Fig 15. Evaporated mass when IR lamp is turned off at time equal to 75(0C)

We can also show the transpiration rate as a function of time as in the next graph. It is just the manipulation of the above data into a more comprehensive form. It is quite clear from the below graph that when IR radiation is incident transpiration rate initially increases and then saturates finally until the lamp is again turned off.



Fig 16. Transpiration rate of a plant as a function of time. At t=0s to t=4500s IR lamp is turned on and kept undisturbed. After this time period it is turned off.



Fig 17. Evaporation rate and Temperature variation in the same graph.

4.6. Comparison between evaporation rates in dark and in visible light:

It is the curvature of the guard cells controlled by turgor pressure that controls the size of the stomata. When there is visible light available and the plant is able to perform photosynthesis, carbon dioxide is utilized. So there is need to bring in more and more carbon dioxide. This thing can happen only if there is an increase in the stomatal aperture or the number of stomata increase. The following experiment was performed with a plant whose soil pot was well covered in order to prevent any evaporation from the soil. Plant was kept on a precision weighing balance and kept inside a dark room. Water loss and time was noted to calculate transpiration rate. Similarly, experiment was carried out in a room in which fair amount of visible light was present. What I saw is there was initially no difference in evaporation rate but eventually the evaporation rate for the plant inside visible room overshoots. The graph is given below.



Fig. 18. Comparison of evaporation rates a plant first kept in visible light and then in dark.

5. Results and Discussion:

Our experiment started with analysis of transient temperature of a point on a plant leaf. We get the entire description by plotting temperature against time. Then I tried to analyse temperature along the central vein of leaf and perpendicular to it. The conclusion was that at edges of the leaf saturation temperature of the leaf is lesser than at the centre. Also we could see that veins cool slower than the rest of leaf surface, thereby suggesting that veins contain lesser number of stomata. When the analysis was done on the black coated metallic leaf, it turned out that metals took very little time to get heated or cooled. Also when the analysis was done on isolated leaf and attached leaf, effect of transpiration on saturation temperature clearly suggested that transpiration is a cooling phenomena.

One more interesting thing was that when we persisted with at saturation for some time, there was a dip of about 2.5 (0C) in the saturation temperature suggesting some kind of late response of stomata. In the last part of the experiment we tried to study the effect of light of different wavelengths on stomatal conductance. The results suggested that IR increased the transpiration rate upto 5 mm/day when it was only around 0.72 mm/day. Further, I tried to compare transpiration rates of the plant kept in the dark and that in the visible light. The conclusions were positive. Initially, there was not much of a difference between the evaporation rates but with time the graph for the one present in visible light overshoots the other one.

6. Scope for Improvement:

We may like to develop an equation for cooling of the leaf by combining the radiative, conductive and convective heat losses in a single mathematical equation. We can also study cooling of isolated and attached leaf and at the same time keep track of the changes in mass. The results can be improved to a very great extent by using a plant having flat leaves which are approximately at same height. Analysis will be more logical if we can do the above analysis with LEDs so as to know the effect of heat on the stomatal conductance. Also we can perform the transpiration experiment with higher precision balances in order to get better readings. We know that only certain wavelengths of light affect the stomatal conductance. In future we can try combinations of wavelengths and see if one component acts as a stimulant for the other. There is a great potential to develop drought and stress resistant plant species by combining and utilizing the techniques of thermography and genetics.

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