Climate Control Based on Stomatal Behavior in a Semi-Closed Greenhouse System ‘Aircokas’

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Abstract
Climate control in a greenhouse is usually based on maintaining a specific air temperature, sometimes adjusted to the available light level or the cost of energy. According to theoretical physiological knowledge optimal photosynthesis depends on the behavior of the stomata. No existing climate control strategy is based on measurement or modeling of the stomatal opening. A soft-sensor based on a crop energy-balance equation was developed. The validation of this sensor was started, but the method using a porometer and a silicone rubber impression proved to be inappropriate. A climate control strategy for stomatal opening based on the use of a high pressure misting system, an energy-balance equation and adjusted ventilation window control was developed. This system with the name ‘Aircokas’ was introduced in commercial greenhouse operations and used for various crops. A new approach for the representation of stomatal conductance is suggested.

INTRODUCTION
The worldwide rise in energy costs and the need for reducing CO₂ emission force greenhouse producers to develop and use more intelligent climate control strategies. The Dutch product board for Horticulture (PT) in cooperation with the Ministry of Agriculture, Nature and Food quality (LNV), formulated a plan for reduction of energy demand and CO₂ emission in greenhouse horticulture. One of the ambitions is that in 2020, every new built greenhouse will be equipped with so called (semi)closed energy systems. Compared to 1990, CO₂ emission will be reduced by 30–45% and the input of fossil energy will be minimized. To achieve this goal, 700 ha have to be equipped with semi-closed greenhouses before 2012. In general, ‘semi-closed’ means less heat and CO₂ losses through ventilation. This can be achieved with mechanical cooling systems, sometimes also used to collect solar heat in summer which is stored in subterranean natural water storage. However, the investment costs of such systems are very high and can only be covered by 20% extra production. Also, the power consumption of the fans, the pumps and the heat pumps to operate such a system reduce the total energy saving. Another limitation is the fact that the major crops, tomatoes and rose, are produced under artificial light. These users produce their own electricity with a combined heat and power machine (CHP), in many cases also supplying electricity to the national grid. This reduces the heat consumption dramatically and the co-generator also produces an immense amount of CO₂ which is available for plant growth.

‘Aircokas’ (Verkerke, 2008) (Fig. 1) is a semi-closed greenhouse concept based on the use of high pressure misting to raise the CO₂ level in the greenhouse by reducing ventilation losses at minimal costs, based on the principle of raising the enthalpy differences between inside and outside air. Mechanical cooling (50–100 W/m²) and an aquifer is used to collect and store solar energy. Plant production is maximized by plant reaction measurements and intelligent climate control based on an energy balance. This control system was developed in this project. Measurement and control of stomatal opening will directly influence plant performance under increased light conditions. Plant reaction to specific combinations of light, water, CO₂, temperature, VPD and air...
movement can be measured in terms of transpiration and CO₂ consumption. In this work, two methods to observe the stomatal opening were developed and tested: 1) direct measurement based on an impression of a 2 cm² area of an intact leaf made with dental gum and nail polish; 2) simulation of stomatal opening of an entire greenhouse crop based on the energy balance. Both methods were compared with a porometer measurement of stomatal conductance.

MATERIALS AND METHODS

Impression Method

For the production of impressions of the leaf surface, a method was used described by Wyers and Johansen (1985). Thin liquid Xanthopren Plus (Beyer Dental, Almere) was mixed with elastomeric hardener and directly applied on the downside of a leaf. After drying (2–3 minutes) the rubber impression was carefully peeled from the leaf (Fig. 2). A positive impression was produced by covering the rubber with colourless nail polish (Hema, long lasting). After 12 hours this film was removed and inspected with a microscope, magnification 10 (ocular) x 10 (objective). A photograph was taken of areas with stomata with a Leica digital camera. At the same magnification rate, photographs were taken of calibration glass plates with a grid of 50 x 50 µm (=0.0025 mm²) and 200 x 200 µm (0.04 mm²) to make an overlay of a fixed reference area. The photograph of one square of the grid was printed on A4 to allow hand measurements. In this image the characteristics (Fig. 3) pore width (b), guard cell length (l) and peristomatal groove distance (PGD) were measured. For a tomato crop ‘Mecano’, on a day with a maximum PAR light level of 670 μmol m⁻² s⁻¹ and an average value of 280 μmol m⁻² s⁻¹, every 30 minutes an impression of the lower and the upper leaf were taken of 2 plants from 15 minutes before sunrise till 15 minutes after sunset. Stomatal conductance values were collected with a porometer AP4 UM 3 (Delta–T Devices, UK) from the same spots on the leaves, 1 minute before the impressions were made.

Stomatal Opening Prediction Model

Stomatal conductance was calculated for the entire crop based on infrared leaf temperature measurements and climate data for the moments of hand measurements. Photosynthesis and transpiration both depend on the solar energy absorbed by a leaf. The law of conservation of energy states that energy cannot be created or destroyed, it can only be changed from one form to another. Takakura et al. (2007) described a method to modify the Penman-Monteith equation into an energy balance equation based on the measurement of leaf and air temperature:

\[ R_n = E_t + Q + G \]  

where, \( R_n \) is net radiation over the canopy, \( E_t \) is latent heat transfer, \( Q \) is sensible heat transfer and \( G \) is heat transfer to the ground.

From the above equation evapotranspiration \( E \) can be calculated using the following formula:

\[ E = \frac{R_n - h(T - T_w) - G}{l} \]

here, \( h \) is the coefficient of the convective heat transfer, depending on air speed and Leaf Area Index (LAI), \( (T - T_w) \) is the temperature difference between the air and the leaf surface, \( l \) is the latent heat of vaporization of water.

By measuring \( T_{air}, T \) and net absorbed radiation in a crop, \( E \) can be calculated, if \( G \) is neglected. \( Q \) can be calculated based on \( (T - T_w) \). From the energy balance, the contribution of \( E_t \) can be calculated and compared with the actual \( E \). If the VPD between leaf and air is determined based on the measurements, an estimation can be made of the total stomatal pore opening in terms of stomatal conductance required to create the
calculated $E$. An example of the result of that calculation is in Figure 4.

**New Approach for the Representation of Stomatal Conductance**

In literature, several units are used to represent stomatal conductance. A fundamental representation is based on the transport of water vapor molecules from the mesophyll to the surrounding air because of a concentration gradient and with a diffusion constant. It is assumed that the vapor concentration in the mesophyll is equal to the saturation value for the actual leaf temperature. Transport of water vapor $E$ \([\text{mol m}^{-2}\text{s}^{-1}]\) then can be described as a diffusion process (1st Fick’s Law of Diffusion) in which water vapor molecules move because of the concentration gradient in the air.

$$E = Dw \times dWx / dx$$  \((3)\)

In which: $Dw$ = diffusion coefficient \([\text{m}^2\text{s}^{-1}]\), $dWx$ = concentration difference \([\text{mol m}^{-3}]\), $dx$ = transport distance \([\text{m}]\). Considering a concentration difference between the inside and the outside of the stomata, a simplified equation can be used:

$$E = G_{sw} \times (W_i - W_e)$$  \((4)\)

$G_{sw}$ is the effective conductance for water vapor in air \([\text{m s}^{-1}]\). $W_i$ = internal water vapor concentration \([\text{mol m}^{-3}]\), $W_e$ = external water vapor concentration \([\text{mol m}^{-3}]\).

In most cases, however, stomatal conductance is expressed as \([\text{mol m}^{-2}\text{s}^{-1}]\) that is in the same unit as transpiration. It may be clear that by this representation the distinction between the conductance of a stoma and the circumstances in the mesophyll and outside the stoma, both together responsible for the transport of water vapor, will be lost. The “stomatal conductance” for water should indicate the relation between the concentration difference for water vapor over the stomata and the transpiration. It should not represent the transpiration itself. The difference in vapor pressure \([\text{kPa}]\) can be used, since the definition $VPD$ is well-known by horticulturists and fits better to the intuitive sense of mass flow caused by pressure differences. A new unit for stomatal conductance that indicates the following relation between transpiration and $VPD$ is suggested:

$$E = G_{sw} \times VPD$$  \((5)\)

A representation of stomatal conductance at crop level can be expressed by this equation:

$$E_{crop} = G_{swcrop} \times VPD_{crop}$$  \((6)\)

In which: $E_{crop}$ \([\text{mol m}^{-2}\text{s}^{-1}]\) is the crop transpiration, $G_{swcrop}$ \([\text{kg m}^{-2}\text{s}^{-1}\text{kPa}^{-1}]\) the crop (canopy) stomatal conductance and $VPD_{crop}$ \([\text{kPa}]\) the crop vapor pressure difference.

**RESULTS AND DISCUSSION**

There was a good correlation between pore width and peristomal groove distance (PGD) (Fig. 5). This is the same result as described in Lawson et al. (1998). However, this method is very labor intensive and is not useful for direct climate control. No correlation was found between porometer conductivity and pore width or guard cell length. Correlation between porometer conductivity and PGD was small (Fig. 6). The calculated $G_s$ showed no correlation with porometer $G_s$ pore width, guard cell length or PGD on November 9. A second measurement on December 14 showed a better result for the porometer value (Fig. 7). Therefore, no conclusion can be made.

Kaiser and Kappen (2001) indicated that a small pore area is sufficient for 90% diffusion. For CO$_2$, 8% pore area and for water vapor, 24% opening is enough. Since the guard cell length is more or less constant and long (Table 1), a small pore width will have
great impact. The resolution of the measurement technique was too small for reliable results. Validation of the calculation model will now be attempted using a balance to measure development of plant weight and water content in the substrate in relation to water supply, plant transpiration and energy balance. The lack of correlation between the porometer values and the measured stomatal dimensions may be explained by the nature of the measuring technique. The assumption that the effect of the measuring clip on the opening of the stomata may be neglected if the measurement period is shorter than 10 seconds, may not be valid for small differences in pore width. The energy balance is changed when the clip is mounted on the leaf. Also the number of stomata under the clip is not known and may not be comparable to the microscope image.

ACKNOWLEDGEMENTS
The Dutch Ministry of Agriculture, Nature and Food Quality and The Dutch Product Board for Horticulture, financially supported this research.

Literature Cited

Tables

Table 1. Dimensions of tomato stomata during a day.

<table>
<thead>
<tr>
<th>Time</th>
<th>Guard cell length (μm)</th>
<th>PGD (μm)</th>
<th>Pore width (μm)</th>
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<tr>
<td>8.18</td>
<td>27.2</td>
<td>16.2</td>
<td>3.5</td>
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<td>26.9</td>
<td>16.2</td>
<td>3.3</td>
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<td>27</td>
<td>16.6</td>
<td>4</td>
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<tr>
<td>4:48</td>
<td>28.3</td>
<td>16.4</td>
<td>1.7</td>
</tr>
</tbody>
</table>
Figures

Fig. 1. Working principle of an “Aircokas”.

Fig. 2. Dental gum impression of a leaf.

Fig. 3. Measured dimensions of stomata.
Fig. 4. Calculated stomatal conductance $G_{\text{avg}}$ of a tomato crop 11-9-2007.

$y = 1.2433x - 16.864$

$R^2 = 0.8379$

Fig. 5. Relation between measured pore width and peristomal groove distance PGD for a tomato crop 11-9-2007: 8 am–5 pm.

$y = 7E-07x^{7.4902}$

$R^2 = 0.5643$

Fig. 6. Relation between porometer $G_s$ and PGD for a tomato crop 11-9-2007: 8 am–5 pm.

$y = 1.3079x - 18.477$

$R^2 = 0.5392$

Fig. 7. Relation between Calculated $G_{s\text{c}}$ (canopy) and porometer $G_s$ (leaf) for a tomato crop 12-14-2007: 8 am–12 am.