Stomatal resistance modelling using the full factorial design: application to the New Guinea impatiens

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Abstract

The stomatal resistance Rs appears as a key parameter governing plant transpiration. For that reason, it deserved a particular attention of modelers. Up to now however, most models were multiplicative models established at a given plant growth stage and not valid at another growth stage. In this prospect, the objective of the present work is (1) to develop a dynamic model of Rs based on the full factorial design FFD, and (2) to validate the model at several plant growth stages inside a production greenhouse. The FFD is based on an optimization process which makes it possible to establish a polynomial relationship between Rs and the radiation, humidity and temperature. To implement the model, a set of experiments was conducted inside a 10 m^2 growth chamber equipped with 4 Impatiens New Guinea pots. Rs was directly measured with a porometer on 5 leaves. Nine scenarios were tested with extreme values for the 3 considered parameters: radiation $\in$[0-150] W m^{-2}, humidity $\in$[55-75]% and temperature $\in$[15-26]°C. These values were chosen from real conditions observed inside a greenhouse and taking account of the operational constraints of growth chambers. The polynomial model deduced from the experiments evidenced its ability to predict Rs from the climatic parameters (r^2= 0.99). It was then applied at several growing stages of a greenhouse Impatiens crop. Again, the model was able to correctly simulate the stomatal resistance (RMSE$\in$[186-309] s m^{-1} and $r^2 \in$[0.50-0.58] for measured Rs $\in$[40-2000]s m^{-1}. It also showed the prevailing role played on Rs by radiation during daytime and by humidity at night. The influence of the temperature however, seemed to be negligible. The FFD therefore appears to be a powerful tool to simulate satisfactorily the stomatal resistance at different growth stages. It could be useful to predict accurately the evolution of the plant transpiration.

INTRODUCTION

Cultivated potted plants inside greenhouses currently develop rapidly, requiring improved irrigation especially in locations where water resources are scarce. The control of irrigation necessitates a good understanding of water transfers inside the plants, and at the level of the stomata apertures in particular. Indeed, stomata control gas fluxes (water vapor and CO_2) between the leaf surface and the atmosphere (Buckley and Mott, 2013). The key parameter governing the stomata apertures is the stomatal resistance which strongly depends on the environmental factors, and which has been extensively studied in open field conditions over the past 50 years by modelers. In the last decade, the understanding of stomata dynamics improved with the advent of new technologies that make it possible the direct measurement of stomatal resistance (porometer diffusion or gas exchange analyzer) (Damour et al., 2010). However, very few models exist for plant cultivated in pots under greenhouse conditions (Casaroli et al., 2010). Most of these models are based on the multiplicative approach proposed by Jarvis (1976). The main disadvantage of the Jarvis approach is that many data covering a wide range of environmental conditions are required to estimate the model coefficients, and these coefficients often change over the time life of a leaf (Buckley and Mott, 2013).

In the present study, a new technique is used to model Rs by the design of experiments (DOE) which was originally developed by Box and Wilson (1951). Many designs are available, such as fractional or factorial, and two or three level designs (Mayers and Montgomery, 1995). All of them can be augmented by the center point. For the purpose of this work, the full factors design (FFD) was implemented (Lundstedt et al., 1998). To establish the model of Rs by FFD, different scenarios were conducted inside a growth chamber, the number of scenarios depending on the retained type of FFD. The model of Rs was then validated against data collected inside a greenhouse for several growth stages of Impatiens New Guinea plants. The FFD was finally applied to assess the main parameters impacting Rs and to determine the combined and individual effects of these parameters on the Rs response.

MATERIALS AND METHODS

Modelling methodology

FFD is a collection of mathematical and statistical techniques which uses a sequence of designed experiments to obtain an optimal response. In the present case, FFD was applied to estimate the response of Rs to the variation of climatic parameters. Three parameters were chosen (global radiation, relative humidity and temperature) and each parameter had two levels. The 2^3 FFD was chosen by considering the time required to perform this experiment and a center point was added to take account of the non-linear relationships between the response and parameters. These non-linear relationships were evidenced in previous works conducted inside a greenhouse (Gang et al., 2012). For each parameter the maximum and minimum value were fixed by considering the growth chamber operating limits together with real conditions observed inside the greenhouse: the global radiation varied within the range $\in$[0-150] W m^{-2}, humidity $\in$[55-75] % and temperature $\in$ [15-26] °C. In order to simplify the calculation, it is appropriate to use coded variables with an interval [-1, 1], therefore 0 is in the middle of the design. The conversion of the original variables (rg, rh, tr) to the coded variables (RG, RH, Tr) and vice versa is given by the following formula (Eq. 1):

$$A = \frac{a_0 - a}{step} \text{ with } a_0 = \frac{a_{max} + a_{min}}{2} \text{ and } step = \frac{a_{max} - a_{min}}{2} \quad (1)$$

where *A* is the coded variable corresponding to *a* as the original variable.

A non-linear mathematical model was used to estimate the response of Rs to the variations of the climatic parameters. This model was deduced from the original model of 2^3 FFD given in Eq. 2.

$$Rs = a_0 + b_1.RG + b_2.RH + b_3.Tr + b_{12}.RG.RH + b_{13}.RG.Tr + b_{23}.HR.Tr + b_{123}.RG.RH.Tr \quad (2)$$

where RG is the coded global radiation, RH the coded relative humidity, and Tr the coded air temperature. This polynomial model contains a number of unknown coefficients (a_0, b_1, b_2, b_3, b_{11}, b_{12}, b_{13}, b_{23}, and b_{123}) to be determined by specific experiments. These experiments were based on different scenarios specified by the 2^3 FFD with one center point, meaning 9 different scenarios as described in Table 1. To implement the FFD, the Jmp^{TM} software was used with the input data obtained from experiments conducted inside a growth chamber.

Experimental setup

The experiments were launched both inside a 10 m^2 (5 m x 2 m) growth chamber located in Angers, France and inside a 100 m^2 (10 m x 10 m) greenhouse. A growth chamber was used because it offers the possibility to make the climatic factors (radiation, humidity, temperature) vary within a controlled range of values. Inside the growth chamber four Impatiens pots (*Novae-guinea*, cv. 'Sonic Scarlet') were used, after the 16^{th} week of plantation. The experiments inside the growth chamber were systematically performed in the morning in order to keep the same conditions for the different scenarios, and the plants were irrigated once before starting experiment. Experiments were also conducted during 16 weeks inside a greenhouse from March to July 2014 with Impatiens in order to collect the data to be used for validating the Rs model. Young plants were potted inside 0.74 l containers (8.7 cm height) filled in with fine peat (5-20 mm particle size) with peat bulk density =0.12 g cm^{-3}. Plants were equally distributed over four shelves, representing a total area of 18 m^2, and with an initial density of 28 plants per m^2. During the experiment the canopy covered 100% of shelves, and the plant density was decreased to favor plant growth (final density of 15 plants per m^2). Plants were irrigated twice a day at the beginning (6 am, 12 am) and three times (6 am, 2 pm, 12 am) at the end of the experiment because it was warmer. The irrigation was conducted by flooding the shelves with a complete nutrient solution. In addition, a shading screen was used and the roof vents were fully open to avoid high temperatures.

Climate and stomatal resistance measurements

Inside the growth chamber, the above canopy global radiation rg was measured by a radiometer (CNR1, Kipp&Zonen, Delft, The Netherlands, ±10 %). The temperature (tr) and relative humidity (rh) of the above canopy air were measured by shielded and ventilated sensors (Vaisala HMP45C, Campbell Scientific Ltd., Antony, France, accuracy ±0.1 °C for tr and ±2% for hr). They were directly connected to a computer in order to check simultaneously the value of different parameters. Rs was measured only for the upper leaves of the canopy by a porometer (AP4, Delta-T Device, United Kingdom). Five measurements were taken for different leaves in four pots at each scenario. A time interval of one hour was estimated to stabilize measurements of Rs from one climatic scenario to another.

Inside the greenhouse, the global radiation, relative humidity and temperature were measured. The same sensors were used as the ones used inside the growth chamber. All the above-mentioned parameters were measured every 3 s and averaged online over 10 min periods with a data logger system (CR5000, Campbell Scientific Ltd., Antony, France). Rs was measured for leaves of the upper part of canopy (or sunlit leaves) and also for leaves of the bottom part (or shaded leaves). Five measurements were done for each sunlit and shaded leaves of different plants. The measurements of Rs were replicated every half an hour.

RESULTS AND DISCUSSION

Stomatal resistance modelling under growth chamber conditions

Table 1 shows the experimental design matrix with the 9 scenarios considered to provide the input information for DOE models (by Eq. 2). This table also provides the value of Rs measured for each scenario and the corresponding standard deviation. The experiments were carried out in order to determine the effect of the climatic parameters on the Rs response. Table 1 clearly indicates that the effect of rg on the Rs response is the most significant. A simple comparison between scenarios (150 W m^{-2}, 55%, 15°C) and (0 W m^{-2}, 55%, 15°C) shows that Rs increases from 267 to 1410 s m^{-1} just in response to a variation of rg. The regression equation based on the first-order model with three parameters and their interaction terms (Eq. 3) confirms that RG is the most important parameter with the highest coefficient.

$$Rs= 590-454.RG -104.RH -58.Tr + 124.RG.RH -35.RG.Tr +20.HR.Tr -8 .RG.RH.Tr \quad (3)$$

with RG=(rg-75)/75; HR= (hr-65)/10; Tr=(tr-20.5)/5.5

The accuracy of the model (Eq. 3) was assessed from the coefficient of determination (r^2). A value of r^2=0.83 was obtained. In a second step, some improvements of the model were undertaken in order to increase its accuracy. The first modification was to consider that the highest order multiplicative interaction term (RG.RH.TR) was negligible (Myers, 1995). This term was replaced by RG^2 because RG represents the most important parameter impacting the Rs response. The second modification was suggested after several sensitivity tests showing that the intercept=590 s m^{-1} would be better replaced by a RG^3 term. Taking account of these modifications, a new model (Eq. 4) was obtained with r^2=0.99 far better than the model given by Eq. 3.

$$Rs=-115.RG -139.HR-39.Tr +139.RG.HR +43.RG.Tr +11.HR.Tr +661.RG^2-368.RG^3 \quad (4)$$

This model therefore evidences that RG is the prevailing parameter influencing the Rs response with higher coefficient. HR appears to have a less degree of influence.

Analysis of the interaction effects

To understand how the three parameters impact the Rs response, a further analysis was conducted. The interaction plot shown in Figure 1 depicts the effect of a given parameter on the Rs response for the highest and lowest level of another parameter. Interaction occurs when one parameter does not produce the same effect on the Rs response at different levels of another parameter. Therefore, if the curves of the same graph are parallel, there is no interaction. On the contrary, when the curves are far from being parallel, the two parameters interact. Thus Figure 1 confirms the significance of rg/hr (Fig. 1 A and C) and rg/tr (Fig. 1 B and E) interactions. It can be observed that in both cases (rg/hr and rg/tr interactions), Rs decreased when the curve moved from lower to higher level. From Figure 1 it can also be seen that hr effect is small when rg is at high level and large when the rg is at low level (Fig. 1 A and C). By contrast, tr effect is small when rg is weak and large when rg is high (Fig. 1 B and E).

Surface response (Rs)

Figure 2 shows a 3D plot of the response of Rs according to rg and hr which are the most significant parameters; tr was fixed at the center value. It helps visualize the shape of the Rs response and its non-linearity. It can be seen also that both rg and hr have a negative effect on Rs response, i.e when rg and hr increase, Rs decreases.

Validation of model with data collected inside a greenhouse for different growth stages

Once the coefficient of the polynomial model were adjusted and discussed, the second step consisted in the model (Eq. 4) validation with data collected at different growth stages from plants grown inside a greenhouse. The results of Rs measured with a porometer and predicted by the model (Eq. 4) are shown for weeks 5, 11, 12 and 16 after plantation in Figures 3, 4, 5 and 6, respectively. They globally demonstrate the capacity of the model to correctly predict Rs with value of r^2 greater than 0.50 for weeks 11, 12, 16 although a lower value of r^2=0.20 was obtained for the 5th week. This poor performance of the model for week 5 may be explained by the fluctuations of the measured rg (Fig. 7) caused by clouds. These fluctuations of rg are taken into account by the model of Rs, but in reality for week 5, the stomata did not have time enough to respond to these fluctuations of rg. From previous experiments, it was shown that the time required by Rs to answer to a variation of the climatic parameters was estimated before in the growth chamber and equal to 1 hour minimum. This aspect was not taken into consideration in the model given by Eq. 4, which could be considered as one of the model limits.

CONCLUSIONS

In this study, the FFD methodology was used to estimate Rs as a function of several climatic parameters, and also to understand how these parameters impact Rs. To reach this goal, a set of nine scenarios was established inside a growth chamber under controlled climatic conditions. Results revealed that the global radiation is the most important parameter, while the relative humidity only becomes a significant parameter for low values of the radiation. The model was then validated against data collected for plants cultivated inside a greenhouse. It provided good agreement with measured values of Rs for different growth stages except for cases of overcast days when fluctuations in the radiation were recorded. In the present work, a simple FFD with just two levels was tested and although it showed pretty good prediction of Rs, other designs could also be tested with more levels and therefore more scenarios in order to improve the accuracy of the model to predict Rs.

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Tables

Table 1. Scenarios and input data

Scenario (rg, hr, tr)	Mean Rs value (s m^{-1})	Standard deviation (s m^{-1})
150 W m^{-2}, 55%, 15°C	267	36
150 W m^{-2}, 55%, 26°C	91	10
150 W m^{-2}, 75%, 15°C	254	31
150 W m^{-2}, 75%, 26°C	102	38
45 W m^{-2}, 60%, 22°C	265	18
0 W m^{-2}, 55%, 15°C	1434	315
0 W m^{-2}, 55%, 26°C	1410	381
0 W m^{-2}, 75%, 15°C	848	33
0 W m^{-2}, 75%, 26°C	885	189

Figures

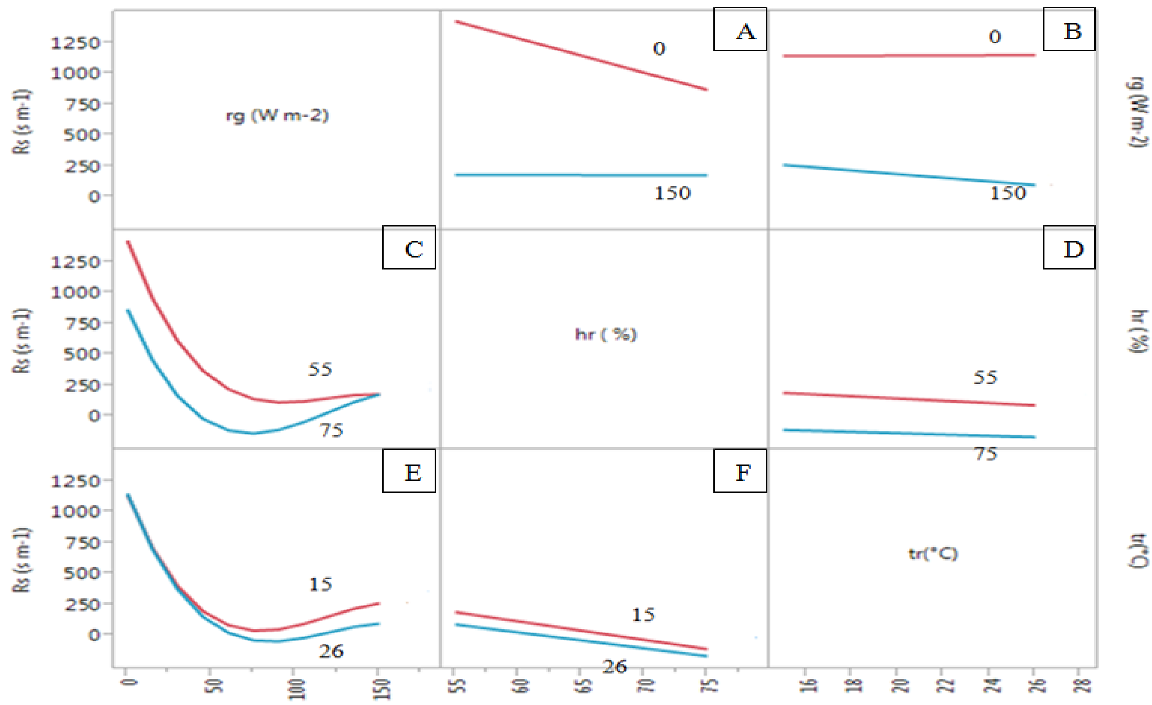


Fig. 1. Interaction plot for the three considered parameters: rg, hr and tr.

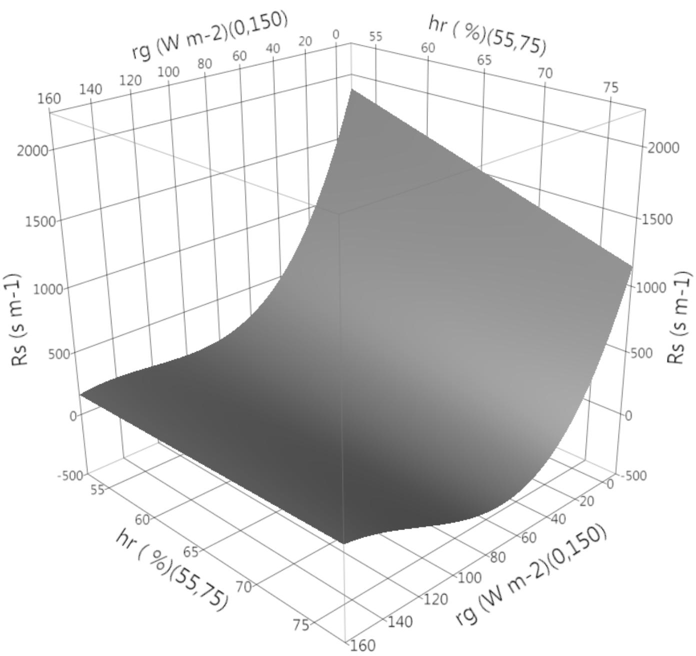
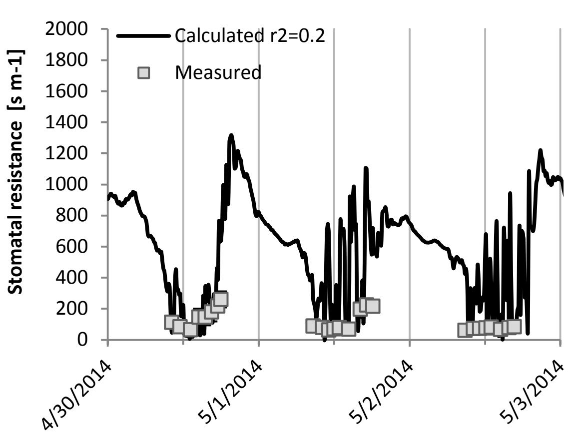


Fig. 2. Response surface of Rs to rg and hr.



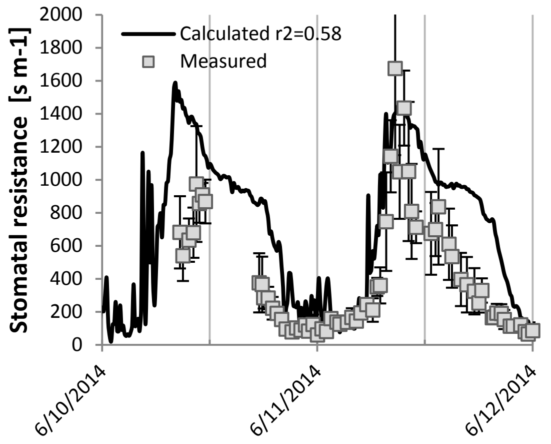
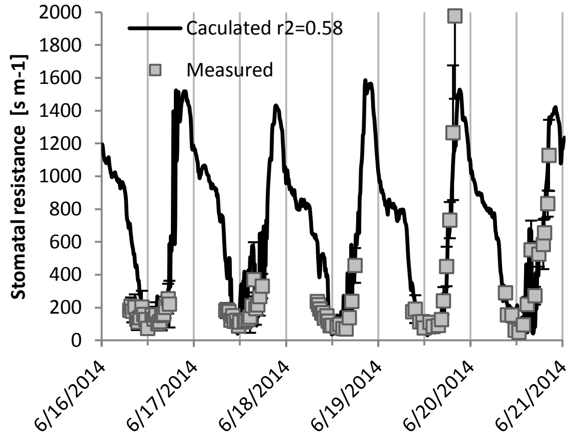


Fig. 3.Validation of the model of Rs against data collected inside a greenhouse 5 weeks after plantation.

Fig. 4. Validation of the model of Rs against data collected inside a greenhouse 11 weeks after plantation.



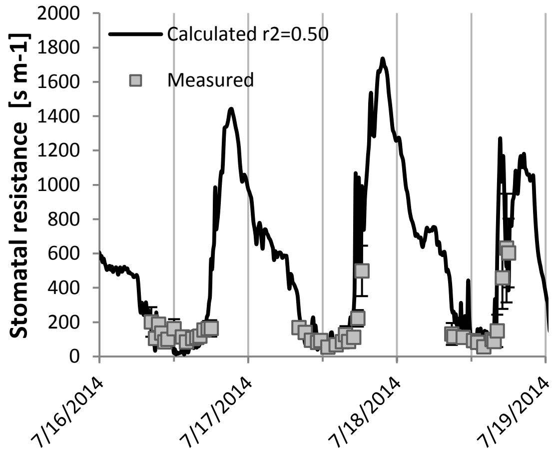


Fig. 5. Validation of the model of Rs against data collected inside a greenhouse 12 weeks after plantation.

Fig. 6. Validation of the model of Rs against data collected inside a greenhouse 16 weeks after plantation.

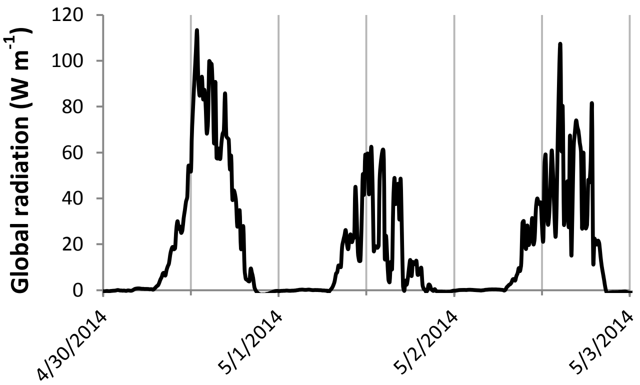


Fig. 7. Global radiation 5 weeks after plantation.