Non-linear mixed-effects modeling for photosynthetic response of *Rosa hybrida* L. under elevated CO_2 in greenhouses – Short communication

I. Ozturk[1], C.O. Ottosen[1], C. Ritz[2], J.C. Streibig[3]

[1]*Department of Horticulture, Faculty of Agricultural Sciences, Aarhus University, Aarslev, Denmark*

[2]*Department of Basic Sciences and Environment/Statistics, Faculty of Life Sciences, University of Copenhagen, Frederiksberg, Denmark*

[3]*Department of Agriculture and Ecology, Faculty of Life Sciences, University of Copenhagen, Taastrup, Denmark*

Abstract

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Photosynthetic response to light was measured on the leaves of two cultivars of *Rosa hybrida* L. (Escimo and Mercedes) in the greenhouse to obtain light-response curves and their parameters. The aim was to use a model to simulate leaf photosynthetic carbon gain with respect to environmental conditions. Leaf gas exchanges were measured at 11 light intensities from 0 to 1,400 $\mu mol/m^2s$, at 800 ppm CO_2, 25°C, and 65 ± 5% relative humidity. In order to describe the data corresponding to different measurement dates, the non-linear mixed-effects regression analysis was used. The model successfully described the photosynthetic responses. The analysis indicated significant differences in light saturated photosynthetic rates and in light compensation points. The cultivar with the lower light compensation points (Escimo) maintained a higher carbon gain despite its lower (but not-significant) quantum efficiency. The results suggested acclimation response, as carbon assimilation rates and stomatal conductance at each measurement date were higher for Escimo than Mercedes. Differences in photosynthesis rates were attributed to the adaptive capacity of the cultivars to light conditions at a specific day when the experiments were undertaken.

Keywords: miniature rose; irradiance; response curve; carbon assimilation

Exploring the differences in efficiency in energy conversion, and photosynthetic capacity in different cultivars and/or species under various abiotic conditions is an important strategy to investigate the underlying mechanisms in carbon assimilation. Even though pot-grown plants in greenhouses are supplied with standardized nutrients, water, and light levels, these plants still experience strong variation in light and temperature over the day and across seasons (Poorter et al. 2010). Studies shown that there is evidence for inter- and intra-specific variability in net photosynthesis rate of plants (Ottosen, Mentz 2000; Barman et al. 2008). This suggests that individual responses exhibit random deviations.

Studies of gas exchange provide a tool for evaluating the impact of abiotic conditions on crop produc-

tivity (Moon et al. 1987). In gas exchange studies of photosynthesis, it is common to use repeated measurements over time to estimate the response curve parameters. It is therefore essential to use a suitable statistical approach. Mixed models enable an appropriate representation of reality and provide us with a useful analysis tool. One of the main advantages of mixed models is that the ability to analyze repeated measurements on the same experimental units or data set (Fang, Bailey 2001; Pinheiro, Bates 2002).

The leaves of greenhouse-grown roses show high plasticity, and dynamic acclimation (Calatayud et al. 2007). The changing rates of photosynthesis in *Rosa hybrida* are also attributed to the increased leaf area due to training system (Matloobi et al. 2009) or the effect of CO_2 enrichment in the greenhouses (Baille et al. 1996), rather than the light intercepting capacity of leaves. The objectives of this study were to investigate the variation in photosynthetic parameters of the two *R. hybrida* cultivars at the leaf level and to determine whether any variation was associated with current light conditions on the day when the measurements took place.

MATERIAL AND METHODS

Plant material and growth conditions

The measurements conducted on eight week old *R. hybrida* cultivars, Escimo and Mercedes in a greenhouse at Research Centre Aarslev, Denmark from mid winter to early spring 2009. Plants were received from commercial growers and kept in the greenhouse at 25°C day and 20 ± 2°C night temperatures, at 65 ± 5% relative humidity. During gas-exchange measurements, the CO_2 concentration was elevated. Plants were provided with nutrient mixture and kept well watered. All plants were supplied with supplemental light (Powertone, Son-t Agro, 400W – Philips) for 18 h a day. The experimental period was four months. The measurement dates for Escimo were January 22, February 19, March 6, and April 28; for Mercedes they were January 20, January 22, March 9, and April 21.

Gas exchange measurements

Measurements were carried out on the first or second youngest fully developed leaf on the top of the stem. Values of net photosynthetic rate, (P_N) μmol (CO_2)/m²s, leaf transpiration rate, (*E*) mmol (H_2O)/m²s, stomatal conductance to CO_2, (g_s) mmol (CO_2)/m²s, sub-stomatal CO_2 concentration (C_i) (ppm), and vapour pressure deficit (VPD, kPa) between air and internal leaf were provided by the CIRAS-II (PP Systems, Amesbury, MA, USA) portable infrared gas analyzer, on which a double sided leaf cuvette (PL5-U, PP Systems, Amesbury, MA, USA) was mounted. During gas exchange measurements, artificial light was supplied by the controllable LED light resource (PL5-U, PP Systems, USA). Leaf temperature, humidity and external air CO_2 concentration (C_a) in the cuvette were controlled by the CIRAS-II and continuously monitored. The corresponding values were 25°C, 65 ± 5%, and 800 ppm, respectively. Light-response curves were made by decreasing photosynthetic photon flux density (PPFD) from 0 to 1,400 μmol/m²s in 11 steps until steady state values were obtained. The estimated parameters were R_D (dark respiration), P_{Nsat} (photosynthesis rate at saturating light and elevated CO_2), α (the apparent efficiency of light energy conversion on an incident light basis, quantum efficiency) (Saroussi, Beer 2007; Le Roux et al. 1999). A light compensation point (LCP) parameter was deduced from the Mitscherlich function (Tosserams et al. 2001; Heschel et al. 2004). Net photosynthesis rate presented in the analysis was derived from at least four single leaf measurements at each measurement date.

Statistical methods

Data consisted of two layers. First, there were the large-scale experimental units corresponding to the measurement dates; these units were similar to the notion of blocks in a standard randomized block design. Second, there are the individual within-date measurements, constituting the light response curve at a given date and thus under specific experimental conditions, as determined by the cultivar used. The statistical analysis was carried out by using the nonlinear mixed-effects regression (NLME) procedure in R open source statistical software (Ritz, Streibig 2008; R Development Core Team 2008).

RESULTS

At elevated CO_2 concentration level, P_N of Mercedes reached a plateau at a PPFD value of 1,200 μmol/m²s

Table 1. The non-linear mixed-effects regression (NLME) analysis. Estimated photosynthesis-related parameters obtained from light response curves for each cultivar

Estimated parameters	Escimo	Mercedes
R_D (μmol (CO_2)/m^{2}s)	-0.98 ± 0.38[a]	-1.58 ± 0.38[a]
$P_{N\text{sat}}$ (μmol (CO_2)/m^{2}s)	22 ± 0.5[a]	16 ± 0.5[b]
α (μmol/mol)	$0.0035 \pm 2.2 \times 10^{-4}$[a]	$0.0040 \pm 3.3 \times 10^{-4}$[a]
LCP (μmol/m^{2}s)	15.28 ± 2.95[a]	23.63 ± 3.59[b]

R_D – dark respiration recorded at 0 μmol/m^{2}s photosynthetic photon flux density, $P_{N\text{sat}}$ – light and CO_2 saturated rate of photosynthesis, α – quantum yield, LCP – light compensation point at P_N – 0. Parameters are presented with standard error of means. Different letters within a row designate statistically significant difference at the 0.001 level

(Fig. 1b) and of Escimo at a value of 1,400 μmol/m^{2}s (Fig. 1a), with a $P_{N\text{sat}}$ of 16 (± 0.5) μmol(CO_2)/m^{2}s and 22 (± 0.5) μmol (CO_2)/m^{2}s, respectively. The estimated parameters between cultivars with respect to α, were not significantly different (Table 1). $P_{N\text{sat}}$ were, howerver, significantly different with higher values shown by the cv. Escimo. An analysis showed that the LCP of the cultivars was also significantly different. Despite its lower quantum efficiency (nonsignificant), Escimo was characterized by a significantly higher $P_{N\text{sat}}$ and lower LCP (Table 1). With leaf and ambient temperature ≈ 25°C, relative humidity 65 ± 5%, and a subsequent VPD in the range of 0.8–1.2 kPa, the cultivar Mercedes illustrated a proportional increase in transpiration (*E*) and stomatal

conductance (g_s) with increasing light. Escimo was characterized by a particular drop in the same parameters at 800 μmol/m^{2}s PPFD (data not shown).

The variation in P_N at the higher light intensities and over time was evident. The fitted response curves based on the NLME analysis illustrated the inter-date variability (Fig. 1a, b). The intra-date and inter-date specific estimated variance components indicated that the inter-date variation was approximately four times as large as the intra-date variability (2.581 vs. 0.622, respectively). The NLME analysis showed that the photosynthesis rates of both cultivars across the experimental period from January 20 to April 28, 2009 were significantly different at the asymptotic values. Under actual envi-
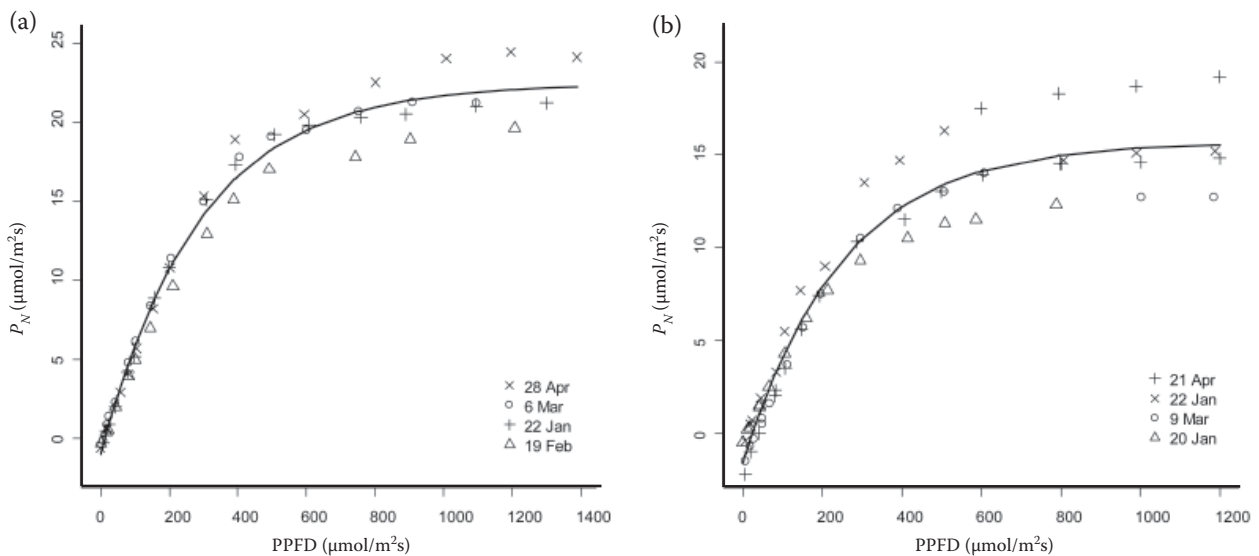

Fig. 1. Light response curves for Escimo (a) and light response curves for Mercedes (b) for each measurement day at constant values of leaf temperature (25°C), relative humidity (65 ± 5%), and at elevated CO_2 at 800 ppm; where P_N represents net photosynthetic rate, R_D – dark respiration, $P_{N\text{sat}}$ – photosynthesis rate at saturating light and elevated CO_2, α – the apparent efficiency of light, and the non-linear mixed-effects regression photosynthetic photon flux density (PPFD). The fitted curve based on non-linear mixed-effects regression is shown (solid line). Measurement dates are arranged from the highest $P_{N\text{sat}}$ observed to the lowest

ronmental conditions, P_N and g_s at each measurement date were higher for Escimo than Mercedes.

DISCUSSION

The light saturation and corresponding $P_{N\text{sat}}$ values seemed high compared to Baille et al. (1996) and Jiao et al. (1991). This might be explained by the fact that when all the leaves along the flower stems have a fully developed photosynthetic mechanism, the highest photosynthetic capacity is found in the uppermost leaves below the flower bud (Gonzalez-Real, Baille 2000), and it is these leaves that were measured in this study. The saturation irradiance and $P_{N\text{sat}}$ values were on the other hand, slightly lower than the values observed by Kim and Lieth (2003), and Urban et al. (2007).

A higher $P_{N\text{sat}}$ was evident on a clear sunny day for Escimo. This may reflect an increased plasticity of Escimo under conditions of high irradiance when other factors were non-limiting. The plasticity was related to the higher values of g_s recorded at sunny days. The lower level of R_D and g_s suggested that Mercedes might be more sensitive to VPD, since the high air flow rate inside the cuvette might have decreased the humidity on the leaf surface of Mercedes more than on Escimo.

Due to the experimental design, different measurement dates created a random effect and NLME suggested that the measurement dates (varying natural light conditions) significantly influenced the capacity for CO_2 assimilation. It was reported that the age and the position of the leaves have an effect on assimilation rates in rose plants, which was associated with a varying gradient of nitrogen in young and older leaves (Pasian, Lieth 1989). Position, age and elongation of the leaves of both cultivars, in our study, were approximately the same. However, differences in water and nutrient status and source-sink relationship may have contributed to the differences in photosynthetic rates. It was also shown that photosynthetic leaf nitrogen and Rubisco capacity are associated with seasonal acclimation to the light conditions in *R. hybrida* leaves (Gonzalez-Real, Baille 2000). We found an interaction between natural light and photosynthetic activity on April 28 for Escimo and on April 21 for Mercedes. There is evidence indicating that the light is the most important determinant of variation in leaf related characteristics and characteristics related to the water balance of plant (Poorter 1999).

CO_2 enrichment reduced R_D in the two rose cultivars. The parameter estimates regarding α were similar to those reported by Peek et al. (2002). It was shown that the quantum efficiency is an intrinsic characteristic, and its upper ceiling in C_3 plants is set firmly by physiochemical limitations (Skillman 2008). Under steady-state and non-stressed conditions, quantum yield is generally regarded as constant (Ehleringer, Bjorkman 1977; Oberhuber et al. 1993). The significantly lower LCP value of Escimo suggested that this cultivar might be more tolerant to lower light levels than Mercedes, as LCP was reported to be a good indicator for shade tolerance (Timm et al. 2002). The variability in LCP might be attributed to photosynthetic induction caused by frequently changing natural light conditions (Ogren, Sundin 1996; Pearcy 1990).

Mixed model approach allow regression analysis of data collected outside of a fixed design matrix and for unbalanced or incomplete data sets (Nothdurft et al. 2006). The variability in response, due to the random effect, was successfully represented. The Mitscherlich function explained the data and provided us with parameters of biological relevance. The NLME was found to be appropriate to predict the leaf photosynthesis, mainly because of its ability to incorporate random predictors into the model.

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Corresponding author:

Isik Ozturk, Aarhus University, Faculty of Agricultural Sciences, Research Center Aarslev,
Kirstinebjergvej 10, DK-5792 Aarslev, Denmark
phone: + 45 899 919 00, fax: +45 899 934 90, e-mail: Isik.Ozturk@agrsci.dk