Analysis and modelling of the combined effect of plant nitrogen concentration after fertilization and development stage on rice radiation use efficiency

Analisi e modellizzazione dell'effetto combinato della concentrazione di azoto nella pianta dopo la fertilizzazione e dello stadio di sviluppo sull'efficienza d'uso della radiazione del riso

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Abstract

The equation describing aboveground biomass (AGB) accumulation as a function of intercepted photosynthetically active radiation and radiation use efficiency (RUE) is used in many crop growth models and is one of the conceptual bases of the methods for the estimation of dry matter productivity from satellite data. It has been observed that the increase in rice nitrogen (N) concentration following the N fertilization at the panicle initiation (PI) seems to temporarily increase the biomass accumulation rate. In terms of modelling, this could be translated in an increase in RUE. A growth chamber experiment has been carried out during 2006 in order to analyze these processes in a controlled environment. Measured variables were AGB and plant nitrogen concentration (PNC) (separately for blades and pseudoculms), leaf area index, chlorophylls concentration, stomatal conductance, and RUE. Two different N levels were applied: one able to maintain PNC at the critical threshold, the other able to assure PNC close to the luxury consumption (critical threshold and luxury consumption are concepts to be considered here according to the N dilution theory). The different behavior of the plants under the two N fertilization levels allowed deriving a model for simulating the RUE increase basing on PNC after the PI fertilization. The model developed has been compiled as a COM DLL (TOPFERT.DLL) and tested under the WARM simulation environment using measured data. The WARM accuracy improved with the use of the DLL (RRMSE = 15.6% compared to 25.8%; modelling efficiency = 0.97 compared to 0.92). The formalization of the acquired knowledge in a reusable and free of charge software component should favor the dissemination and validation activities of the model developed.

Keywords: Radiation use efficiency, rice, plant nitrogen concentration, growth, crop model, WARM.

Riassunto

L'equazione che descrive l'accumulo di biomassa aerea (aboveground biomass; AGB) in funzione della radiazione fotosinteticamente attiva intercettata e dell'efficienza d'uso della radiazione (RUE) è usata in molti modelli di crescita ed è uno dei concetti chiave nei metodi di stima della produttività basati su dati satellitari. E' stato osservato che l'aumento della concentrazione di azoto in piante di riso (plant nitrogen concentration; PNC) in seguito alla concimazione all'iniziazione della pannocchia (panicle initiation; PI) sembra determinare un temporaneo aumento del tasso di crescita. Questo può essere tradotto, in termini modellistici, con un aumento della RUE. Per analizzare i processi coinvolti nel fenomeno è stato allestito nel 2006 un esperimento in ambiente controllato. Le variabili misurate sono state AGB e PNC (in entrambi i casi separatamente per lamine e pseudoculmi), l'indice di area fogliare, la concentrazione di clorofilla, la conduttanza stomatica e la RUE. A piante di riso allevate in camera di crescita all'inizio della differenziazione della pannocchia sono stati applicati due diversi livelli di concimazione azotata: uno in grado di mantenere il PNC in prossimità della soglia critica, l'altro in grado di portare la concentrazione di azoto vicino a livelli di consumo di lusso (soglia critica e consumo di lusso sono qui da intendersi alla luce della teoria della diluizione dell'azoto). Il diverso comportamento delle piante in risposta alle diverse concimazioni ha permesso di sviluppare un modello per la simulazione dell'incremento di RUE in funzione di PNC in seguito alla concimazione alla PI. Il modello è stato compilato come DLL COM (TOPFERT.DLL) e testato nell'ambiente di simulazione di WARM usando dati misurati. La DLL ha permesso di aumentare l'accuratezza di WARM (RRMSE = 15.6% rispetto a 25.8% ottenuto senza la DLL, efficienza di modellizzazione = 0.97 rispetto a 0.92). La formalizzazione della conoscenza acquisita in un componente software riutilizzabile e gratuito dovrebbe favorire la diffusione e la validazione del modello sviluppato.

Parole chiave: Efficienza d'uso della radiazione, riso, concentrazione di azoto nella pianta, crescita, modello colturale, WARM.

Introduction

The concept of radiation use efficiency (RUE) as factor allowing to derive aboveground biomass (AGB; always expressed as dry matter hereafter) from the fraction of solar radiation intercepted by the canopy was initially proposed by Warren Wilson (1967). This relationship was successively provided with robust theoretical bases by Monteith (1977), whose name is now linked to the derived equation. Among the different definitions of RUE flourished in the last decades (e.g. amount of carbon assimilated per photon flux unit of photosynthetically active radiation intercepted by the crop; amount of carbon produced or moles of CO_2 assimilated) the one which probably derives directly from the observation of data is the following: RUE is the slope of the linear regression between AGB and intercepted radiation, under non-saturating radiation.

The effectiveness of the Monteith equation led to its implementation in many crop models (e.g. EPIC, Williams *et al.*, 1984; Cabelguenne *et al.*, 1999; the CERES family of models, Jones *et al.*, 1984; Jones and Kiniry, 1986; Uehara and Tsuji, 1993; STICS, Brisson *et al.*, 2003; WARM, Confalonieri *et al.*, 2006a). In spite of its simplicity, the RUE-based approach for AGB accumulation has not proved to be less accurate than more complex approaches (Spitters, 1990; Jamieson *et al.*, 1998; Confalonieri *et al.*, 2006a), such as those implementing a mechanistic modellization of gross photosynthesis, respiration, etc. Moreover, this approach has been used for tree models (e.g. Medlyn, 1998) and is currently the basis of the method for estimating dry matter productivity from satellite data (Laguette *et al.*, 1998).

Different studies show that actual RUE values change during crop cycle depending on various factors. Campbell *et al.* (2001a; 2001b) show that, before anthesis, RUE decreases significantly in correspondence to an increase of leaf area index (LAI). After anthesis, the decrease in RUE is related to the proportional reduction of leaf nitrogen (N) concentration because of translocation and dilution processes (Wall and Kanesmasu, 1990; Gent and Kiyomoto, 1992; Balocchi, 1994; Hasegawa and Horie, 1996). Choudhury (2001) underlines the effect of high irradiative levels on saturating the enzymatic chains involved with photosynthesis and therefore on decreasing RUE. Sinclair and Horie (1989) studied the RUE variability for different varieties. Therefore, it is evident that a deep knowledge of the factors which act on RUE and of their variable effect during crop development is fundamental for a better understanding of the physiological processes involved and for the implementation of effective crop simulation models.

In particular, all the studies carried out to analyze the factors influencing RUE were largely focused on the effects of either suboptimal N availability, water stress or senescence phenomena on RUE. In the case of rice crop, it has been observed (Hasegawa and Horie, 1996) that N uptake rate immediately increases after top dressing fertilization at the panicle initiation (PI), pushing plant nitrogen concentration (PNC) to high values, especially because of the blades N concentration, with a concurrent increase in AGB accumulation rate. A better understanding of the effects of the related ecophysiological processes is the key for an accurate prediction of crop growth and, therefore, of the final yield. The Authors' hypothesis is that in rice part of the new available N is directed towards the synthesis of Ribulose 1,5 bisphosphate carboxylase/oxygenase (RuBisCO) the most important CO_2 assimilative enzymes which largely contributes to the total blades nitrogen concentration.

Dynamics of leaves N-concentration can probably be described by a bell curve, with an ascending phase due to the accumulation of synthesized Rubisco and a decreasing phase caused by dilution and translocation processes. If we invert the Monteith model for AGB accumulation to estimate RUE, we can notice an increase in RUE itself for some days immediately after PI fertilization. Therefore, it is possible to hypothesize that, for some days after PI fertilization, RUE increases as a consequence of PNC increase, also if PNC is above the N critical threshold, and therefore that N dilution theory is not completely suitable in these conditions. Critical N concept is to be intended here according to the N dilution theory (Greenwood *et al.*, 1986; Justes *et al.*, 1994; Sheehy *et al.*, 1998), therefore indicating, for a given value of AGB, the PNC below which crop growth is N-limited. Preliminary simulation studies show that even a small increase in RUE (less than 10%) for a limited period of time (e.g. two weeks) leads to a substantial yield increase.

No information is available in the literature about the effects of high levels of PNC due to top-dressing fertilization on RUE. Moreover, the almost ubiquitous presence of the Monteith equation in all the levels of analysis of agricultural systems (from plot to large scales) makes the improvement of the concept of RUE a crucial step forward for agroenvironmental research.

In order to better understand these dynamics, a growth chamber experiment was carried out as part of the exploratory research project TOPFERT "Effect of TOP-dressing nitrogen FERTilization on radiation use efficiency: physiology, crop modeling and remote sensing" (http://agrifish.jrc.it/marsstat/topfert/), funded by the IPSC of the European Commission. This paper presents the data collected to analyze the effects of top-dressing N fertilization on RUE and their formalization in a module - compiled as a Dynamic Link Library (DLL) - for simulating the increase in the AGB accumulation rate following the fertilization at PI. A preliminary test of the developed DLL is also shown.

Materials and methods
Experimental data
The data used to develop the model were collected in 2006 during an experiment conducted in a growth chamber at the University of Milan. Three subsequent batches were cultivated and the environmental stability inside the chamber justifies treating their data as experimental replicates. The choice of working in growing chamber, in fact, served to guarantee, constant, verifiable and suitable environmental conditions during the entire course of the experiment, avoiding the effects of meteorological fluc-

tuations. Growth chamber size is 2.5 m × 2.0 m × 3.0 m and, during the experiment, it has been provided with sensors connected to a HOBO datalogger (Micro-DAQ.com, Ltd., Contoocook, USA) for monitoring the chamber micro-environment. This allowed to control light intensity (provided by 400 W lamps with an uniform radiation spectrum comparable to natural sunlight), photoperiod and temperature (16 h light at 25°C; 8 h dark at 21 °C), humidity (between 76% and 78%), and ventilation.

Rice (*Oryza sativa* L. cv Gladio; Indica type) was sown in tubs (surface: 56.5 cm × 36.5 cm; depth: 30 cm) under a regular grid in order to reach a final plant density of 300 plants m^{-2}. The soil (Entisol; CEC: 8.9 meq $(100g$ dry soil$)^{-1}$; C = 1.05%; N = 0.09%), was taken from a rice paddy (Tab. 1).

Based on average values of crop nutrient uptake, each tub received the same amount of basal fertilizer (101.5 kg P_2O_5 ha^{-1}; 224.3 kg K_2O ha^{-1}) which was incorporated by hand into the soil before sowing. The crop was flooded after the emission of the third leaf; a constant 5 cm floodwater level was successively maintained until the end of the experiment.

The experimental factor was the amount of N (via urea) top-dressed at the PI (code 30 of the BBCH scale for rice; Lancashire *et al.*, 1991); two levels (0 and 160 kg N ha^{-1}; respectively T0 and T1 hereafter) were applied. Plants were sampled the day before the fertilization at PI and every two days after the same fertilization for ten days, for a total of six sampling dates for each replicate. Each time, and for both treatments, the following variables were estimated: stomatal conductance, AGB, pseudoculms and blades biomasses, PNC, pseudoculms and blades N concentrations, leaf area index (LAI), chlorophylls A and B concentration, and RUE. Stomatal conductance was determined in vivo using a CIRAS-2 (PP-SYSTEM, UK) on the last completely extended leaf. The plants were randomly harvested from tubs, assuming a sample size of 5 plants per tub.

Since only the leaf blade area gives a significant contribution to rice LAI (Yoshida, 1981), leaf blades were separated from the other plant tissues and analyzed using the following destructive method. Before being dried, the blades were placed on a sheet of paper along with two red squares of standard size in order to derive the dimensions of the green area of the blades. Two digital photos were acquired (Canon, IXUS 50) for each sample and results were averaged. The Adobe Photoshop Magic Wand tool, set up with a tolerance between 15-30%, was used to count the number of green pixels in each image and compare them with the number of pixels in the red squares. LAI was probably slightly underestimated because the blades were moderately curled during images acquisition. The criterion for separating the blades from the pseudoculms was to consider as blades only those completely expanded. After LAI determination, blades and pseudoculms were oven dried at 105 ˚C until constant weight to determine AGB. Nitrogen and carbon concentration were measured separately for blades and pseudoculms using an Elemental Analyzer (model NA 1500, series 2, Carlo Erba, Italy) and then PNC was obtained from them using the blades and

Tab. 1 – Characteristics of the soil (topsoil: 0.00 – 0.40 m layer) used in the growth chamber experiments.
Tab. 1 *– Caratteristiche del suolo (strato superficiale di 0.40 m di spessore) usato nella sperimentazione in camera di crescita.*

	Units	value
Sand	%	84.40
Clay	%	4.70
Silt	%	10.90
pH (H_2O)	-	6.00
pH (KCl)	-	4.80
CEC*	meq $(100$ g$)^{-1}$	8.90
C org.	%	1.05
SOM**	%	1.90
N tot	%	0.09
C/N	-	13.00
Ca	meq $(100$ g$)^{-1}$	2.40
Mg	meq $(100$ g$)^{-1}$	0.30
Na	meq $(100$ g$)^{-1}$	0.10
K	meq $(100$ g$)^{-1}$	0.20

*: cation exchange capacity
**: soil organic matter

pseudoculms biomasses. Part of the blades were separated and stored in test tubes at -80°C to determine chlorophyll content by spectrophotometric analysis (model Cary 50 which registers wave length absorption at 470 nm, 646.8 nm and 663.2 nm).

Statistical analysis

All variables were analyzed at each sampling time using ANOVA, and considering the three batches as blocks. Global differences between T0 and T1 trends were analyzed using a mixed model, where tubs are considered subject, and the sampling in time are evaluated as repeated measures within the same subject. According to Verbeke and Molenberghs (2000), the not homogeneous Toeplitz covariance structure within subject in time was used.

Modelling criteria

The general guideline for deriving the algorithms related to the studied processes was that the model should have been able to work also at large scales, and therefore with a reasonable requirement in terms of data needs for being parameterized and fed.

T0 was approximately maintained around the critical N dilution curve (Greenwood *et al.*, 1986). All the variables measured on this treatment were used for defining a sort of basal condition for rice. T1 was used for evaluating the effect of the PI fertilization on an unstressed crop.

The modelling activities, aimed at finding a possible relation between actual PNC and RUE, resulted in a model which was coded in Visual Basic 6 and compiled as COM Dynamic Link Library (TOPFERT.DLL). The developed model is meant to be complementary to the critical N concept between the PI and the beginning of heading stages for high N levels, since the classical theory is considered by the Authors valid in the other phases of the rice cycle and in case of limiting N conditions.

Model testing

The model developed was tested using data coming from a field experiment carried out in Opera (MI, Italy) during 2004 and accurately described by Confalonieri *et al.* (2006b, 2006c). The test was performed under the WARM modelling environment (version 1.9.4, 5 September 2006; the WARM crop model and the related modelling environment are the subject of a special issue of the Italian Journal of Agrometeorology (issue 11, 2006)). Measured PNC_{act} data were used as input of the TOPFERT module instead of simulated ones to reduce the uncertainty in the evaluation of the new module. The use of PNC_{act} data simulated by WARM would have mixed the uncertainty due to the module for the simulation of PNC_{act} with that related to the processes formalized in the TOPFERT module.

After giving to WARM all the input necessary for the simulation, the WARM's integrated tool for automatic calibration was used to adjust crop model parameters both with and without TOPFERT.DLL. The method used for automatic calibration was the Evolutionary Shuffled Simplex (Duan *et al.*, 1992; Acutis and Confalonieri, 2006) assuming as objective function the minimization of relative root mean square error (RRMSE, minimum and optimum=0%), calculated on AGB data.

To evaluate the performance of the model in the two configurations, the following indices of agreement were used: RRMSE, the modelling efficiency (EF, $-\infty \div 1$, optimum=1, if positive, indicates that the model is a better predictor than the average of measured values), the coefficient of residual mass (CRM, $-\infty \div +\infty$, optimum=0, if positive indicates model underestimation) and the parameters of the linear regression equation between observed and predicted values.

Results and discussion

Experimental results

Blades and pseudoculms biomasses and total AGB are shown in Fig. 2. For the three variables considered, the trend is similar for both the N levels: although it is possible to notice some differences between T0 and T1 for blades biomass, they are not statistically significant.

LAI changes between the two N treatments: while T0 is stable at about 1.0 m^2 m^{-2} for the whole examined period, T1 increases after the fertilization (especially between the second and fourth day (+0.25 m^2 m^{-2}) after the fertilization). The greatest difference between the average values obtained for the two levels (0.27 m^2 m^{-2}) was measured at day 4 and 10. In any case, all differences were not statistically significant (data not shown).
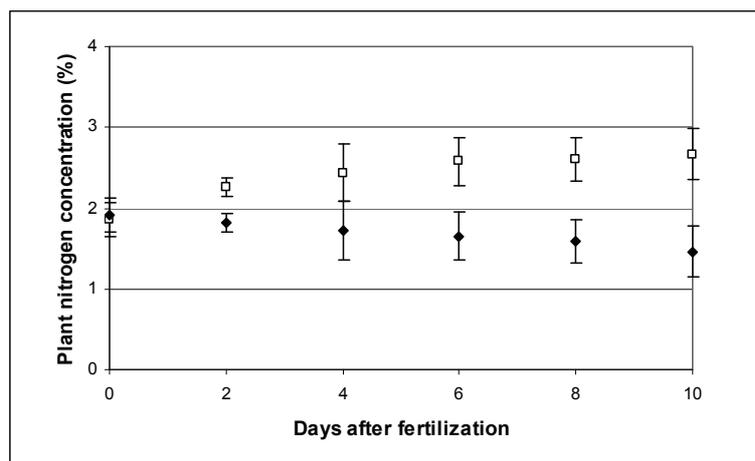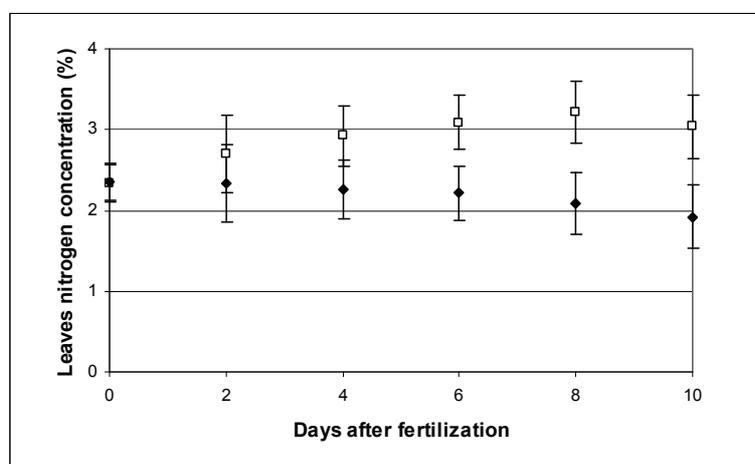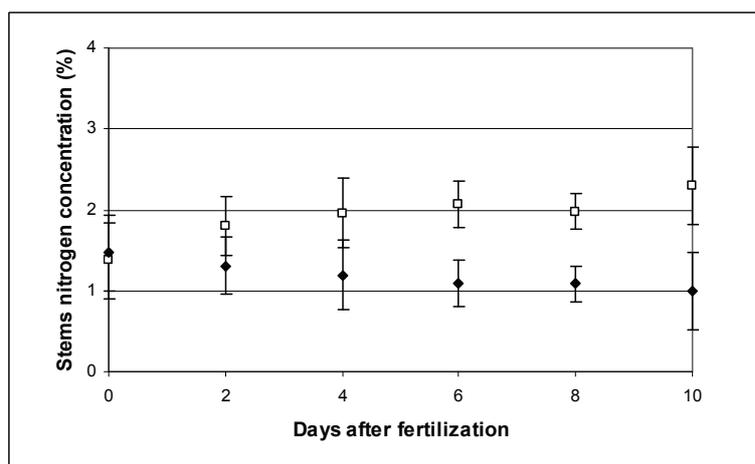

Fig. 1 - Plant (1.a), blades (1.b), and stems (1.c) nitrogen concentration data. Black rhombus and white square represent, respectively, T0 (0 kg N ha^{-1} distributed at the panicle initiation) and T1 (160 kg N ha^{-1}).

Fig. 1 - *Concentrazione di azoto nella pianta (1.a), nelle foglie (1.b) e negli steli (1.c). I rombi neri e i quadrati bianchi rappresentano, rispettivamente, T0 (0 kg N ha^{-1} distribuito alla iniziazione della pannocchia) e T1 (160 kg N ha^{-1}).*
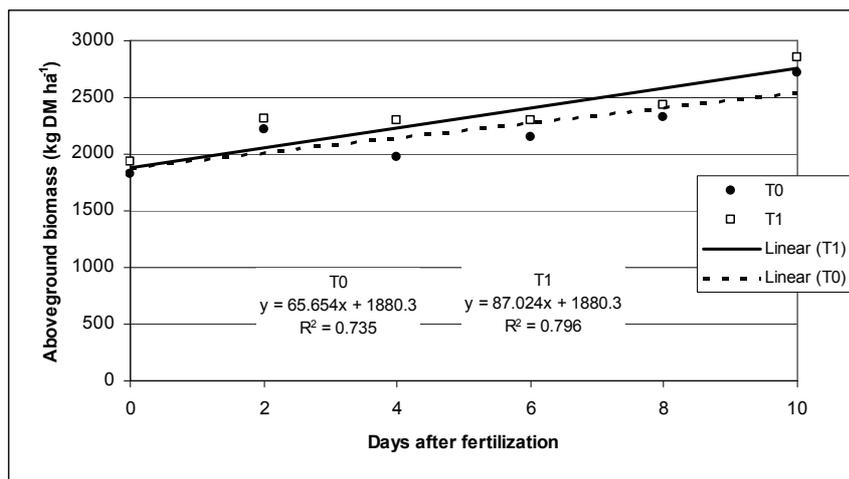
Fig. 2 - Interpolation of aboveground biomass values using linear functions.
***Fig. 2** - Interpolazione dei valori di biomassa aerea usando funzioni di primo grado.*

Also PNC was measured separately for blades and pseudoculms in order to understand N dynamics in the different plant organs during the examined period (Fig. 1.a, 1.b, and 1.c). For blades, the trends of T0 and T1 are significantly different (Fig. 1.b). On day 0 (zero days after the fertilization), both groups of plants show similar values of N concentration; after only 2 days (day 2), N concentration starts to increase for the fertilized plots and, for the unfertilized ones, it remains constant. On day 4, N concentration for T1 continues to increase while the value measured for T0 begins to decline as a consequence of the dilution processes. The same trends are visible for pseudoculms N concentration, although the absolute values are considerably lower than in the blades (Fig. 1.c). The main part of the absorbed nitrogen is immediately allocated - and stored - in the blades, the most important organs involved in the photosynthetic activities. The analysis of total PNC (Fig. 1.a) confirms the trend already discussed for blades. The differences shown in the charts were confirmed as significant by ANOVA, and in all the cases the best fit function was a quadratic curve. Although the T0-plants were unfertilized, it is possible to notice that their PNC values were only slightly below the critical N threshold during the examined period. This could be explained considering (i) the initial soil N content in relation to the low plant N demand, explained by the suboptimal irradiative level measured in the growth chamber, (ii) the intense mineralization process typical of soil with high sand content and (iii) the absence of leaching. According to the critical N curve (Greenwood *et al.*, 1986) and its parameterization proposed by Confalonieri and Bocchi (2005), T1-plants can be considered under a luxury consumption N regime, since, with the exception of the first sampling date, they present PNC values higher than those determined by the critical curve.

The concentration of chlorophylls A and B in the blades does not show any significant difference between the two treatments. A possible explanation is that the main part of the N absorbed is used for the synthesis of Rubisco; this hypothesis is coherent with the increase in blades N concentration, with the increase in photosynthetic activity and with the lack of increase in chlorophylls concentration.

RUE was calculated by inverting the Monteith's equation (Monteith, 1977) and using the measured values of AGB, incident PAR, LAI, a value of 0.52 for the extinction coefficient for solar radiation (k) (Boschetti *et al.*, 2006), and a factor accounting for thermal limitation to growth (T_{lim}) estimated according to Confalonieri *et al.* (2006a). For each sampling date, the Monteith's equation was applied to determine RUE for T1 and T0 (data not shown) Average values of 5.22 g MJ^{-1} and 5.96 g MJ^{-1} respectively for T0 and T1 were obtained. An examination of some of these values shows that not all of them are completely consistent with typical RUE values for rice crops under optimal growing conditions (normally equal to 3.2 g MJ^{-1} for Indica-type and 2.7 g MJ^{-1} for Japonica-type Italian varieties; Boschetti *et al.*, 2006). Factors which probably influenced the obtained data were (i) reduced radiation in growth chambers, (ii) inadequate sampling size for AGB determination and (iii) high experimental error typical of LAI measurement. Although the data we obtained could be considered high, they are coherent with some of the values reported by Campbell *et al.* (2001) and with the evidences discussed by Sinclair and Muchow (1999), who observed for rice a large fraction of carbohydrate production, as clue to higher potential RUE than other C3 species.

In order to assess the effects of nitrogen fertilization on RUE and to bypass the uncertainty in AGB determination discussed above, it was decided to interpolate measured AGB data using a linear function and to use the equations derived to estimate RUE (Fig. 2). The use of a linear function instead of other curves is justified by the shape of the logistic curve describing crop growth, which assumes a nearly linear shape around PI. Both the regressions were significant (P<0.05; R^2 = 0.735 and 0.796 respectively for T0 and T1). To reduce the impact of the uncertainty in LAI estimation, for each treatment, the average of the LAI values measured during the examined period was used. The ratio between RUE values determined for T0 and T1, calculated with AGBs from the regressions, was equal to 1.141762, which means that, in the explored conditions, nitrogen fertilization at the PI determines about a 15% increase in RUE.

Model development
Basing on the nitrogen dilution theory (Greenwood *et al.* (1986)) as formulated by Justes *et al.* (1994) and its parameterization proposed by Confalonieri and Bocchi (2005), the following equation was developed to simulate the critical N concentration as a function of development stage between PI and heading (Eq. 1):

$$N_{crit} = -1.1736 \cdot DVS + 2.1688 \qquad \text{(eq. 1)}$$

where:

o N_{crit} (%) is the plant nitrogen concentration below which RUE starts to decrease due to nitrogen limitation;

o DVS (-; 0.00 – 2.00) is a development stage numerical code (0.00: emergence; 0.60: PI; 0.90: heading; 1.00: flowering; 2.00: physiological maturity).

Although Greenwood *et al.* (1986) and other authors (e.g. Justes *et al.*, 1994; Sheehy *et al.*, 1998) proposed the nitrogen dilution theory relating N_{crit} to AGB, we chose to relate the same variable to DVS because experimental evidences appeared to show the unsuitability of the dilution theory for rice between PI and beginning of heading development stages. The hypothesis we developed for explaining the changes in biomass accumulation rate between these two stages (changes in gene expression with increased synthesis of RuBisCO immediately after the PI and dilution before heading) was strictly related to DVS. Therefore, we considered DVS as the driving variable for the considered phenological phase. Eq. 1 is not meant to be an alternative to the classical nitrogen dilution theory but only a premise to build a model, valid between PI and heading, specific for groups of cultivars having similar features, and only for non N-limited conditions, independent from the value of AGB. Eq. 1 was empirically obtained by relating (i) PNC values simulated according to the classical dilution curve (Greenwood *et al.*, 1986) parameterized by Confalonieri and Bocchi (2005) and (ii) corresponding simulated DVS values. Data for these simulations are those reported in Confalonieri *et al.* (2006b). The regression which led to Eq. 1 was considered a suitable model for explaining data ($R^2 = 0.97$; $p < 0.001$).

A nitrogen-surplus factor ($\alpha_{[DVS]}$) was calculated, from the data measured for T1, as a function of development stage (Eq. 2):

$$\alpha_{[DVS]} = \frac{PNC_{TF[DVS]}}{N_{crit[DVS]}} \qquad \text{(eq. 2)}$$

where:

o $PNC_{TF[DVS]}$ (%) is the plant nitrogen concentration measured at the development stage DVS on T1 plants;

o $N_{crit[DVS]}$ (%) is the critical nitrogen concentration derived using Eq. 1.

Although Eq. 2 is empirically derived from data collected in the specific conditions of the growth chamber experiments, the weather and management conditions were defined to avoid the influence of factors compromising a priori its general validity. We are carrying out specific field experiments to test the validity of Eq. 2 in other conditions.

Data calculated using Eq. 2 were fitted using DVS as independent variable and the following quadratic function for α (Eq. 3) was obtained ($R^2 = 0.88$, $p = 0.04$):

$$\alpha_{[DVS]} = -13.19 \cdot DVS^2 + 22.247 \cdot DVS - 6.9382$$

$$\text{(eq. 3)}$$

A second term, $\beta_{[DVS]}$, was computed using Eq. 4 and the RUE data estimated during the growth chamber experiments:

$$\beta_{[DVS]} = \frac{RUE_{TF[DVS]}}{RUE_{ref}} \qquad \text{(eq. 4)}$$

where:

o $RUE_{TF[DVS]}$ (kg MJ^{-1}) corresponds to the radiation use efficiency estimated after the PI fertilization on the data collected on T1 (fertilized plants);

o RUE_{ref} (kg MJ^{-1}) is the value of radiation use efficiency measured under conditions characterized by irradiance lower than 25 MJ m^{-2} day^{-1}, average daily temperature between 25 °C and 40 °C, when the plants have not reached yet the beginning of flowering stage, and under unstressed conditions for nutrients and water availability and absence of weeds, pests and diseases. For this study, the value of RUE_{ref} is considered equal to that measured on the unfertilized plants growing into the growth chamber.

The values of β derived from the first results of the growth chambers experiments were find to be not depending from the development stage. A constant value of 1.141762 was used for the first version of the model. However, other data will be available from other ongoing researches. For this reason, the structure of the model implemented in the DLL includes the possibility of using a function instead of a single value for β.

A third term, $\gamma_{[DVS]}$, is calculated as the ratio between β and α (Eq. 5).

$$\gamma_{[DVS]} = \frac{\beta_{[DVS]}}{\alpha_{[DVS]}} \qquad \text{(eq. 5)}$$

Under the assumptions of (i) non limiting conditions for water, temperature, weeds, pests and diseases, (ii) development stage prior to flowering, and (iii) irradiance levels preventing the saturation of enzymatic chains, γ is only a function of development stage. The following equation (Eq. 6) can be written:

$$\frac{RUE_{act[DVS]} \cdot \left(RUE_{ref}\right)^{-1}}{PNC_{act[DVS]} \cdot \left(N_{crit[DVS]}\right)^{-1}} = \gamma_{[DVS]} \qquad \text{(eq. 6)}$$

where:

o $RUE_{act[DVS]}$ (kg MJ^{-1}) is the actual RUE after the PI fertilization;

o $PNC_{act[DVS]}$ (%) is the actual PNC after the panicle initiation.

Supposing the availability of $PNC_{act[DVS]}$ (%) (e.g. output from a module for plant nitrogen uptake), Eq. 6 can be arranged as:

$$RUE_{act[DVS]} = \gamma_{[DVS]} \cdot RUE_{ref} \cdot \frac{PNC_{act[DVS]}}{N_{crit[DVS]}} \quad \text{(eq. 7)}$$

In this way, under the assumptions enunciated above, $RUE_{act[DVS]}$ (kg MJ^{-1}) can be determined and used for the computation of daily biomass accumulation in models using a radiation use efficiency approach.

The use of ratios between RUE measured after the fertilization and reference RUE avoids including in the al-

Tab. 2 - Indices of agreement between measured data and those simulated by WARM in its standard version and in that including TOPFERT.DLL.
Tab. 2 - *Indici per la valutazione dell'accordo tra i dati misurati e quelli simulati da WARM in configurazione con e senza TOPFERT.DLL.*

	RRMSE (%)	EF	CRM	Slope	Intercept (t DM ha-1)	R2
WARM	25.83	0.92	0.09	0.90	0.82	0.94
WARM-TOPFERT	15.59	0.97	0.00	0.98	0.11	0.97
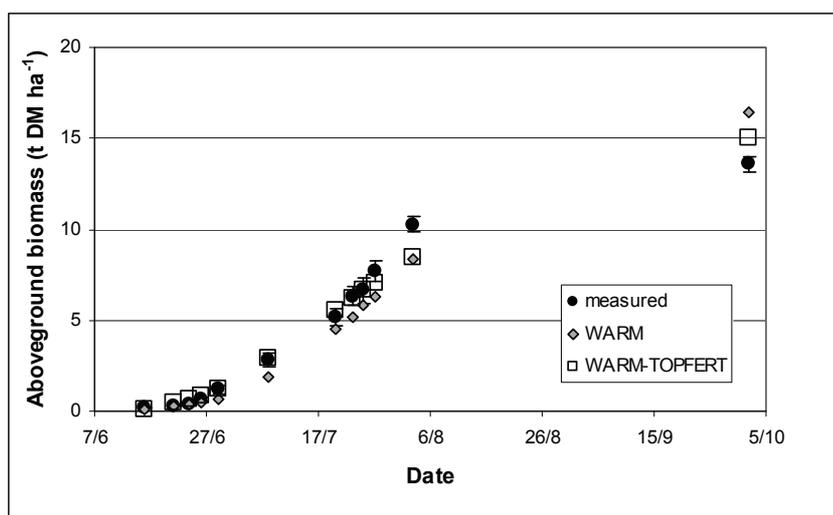
Fig. 3 - Comparison between data measured in Opera (MI, Italy) during 2004 and those simulated by the standard WARM model and the version of the WARM model including TOPFERT.DLL.
Fig. 3 - *Confronto tra i valori misurati ad Opera (MI, Italy) nel 2004 e quelli simulati da WARM in configurazione con e senza TOPFERT.DLL.*

gorithms effects due to the particular conditions where measurements are carried out.

Model testing

Fig. 3 and Tab. 2 show an example of the comparison between measured data and those simulated by WARM in the standard configuration and in that including the new TOPFERT.DLL. It is possible to notice (Fig. 3) that WARM is not able, without the DLL, to correctly reproduce the crop behavior in the central part of the crop cycle, underestimating AGB values. This is mainly due to the model inadequacy in simulating the high growth rate in that part of the season and to reproduce the decrease in the same rate during the final part of the crop cycle. The DLL allows the model to maintain a certain efficiency in converting radiation during the first and final phases and to increase this efficiency in the central part of the growth curve, matching the measured data with more accuracy. This is mainly explained by a lower basal value for RUE obtained with the use of the TOPFERT module. The increase in RUE after the PI fertilization allows WARM to simulate high AGB accumulation rates in the linear phase of the growth curve and to come back to the basal RUE values after heading. This results in a better prediction of AGB at harvest. The indices of agreement (Tab. 2) confirm these considerations. In particular,

RRMSE reaches 16 % (it is 26 % without using the DLL), EF is greatly improved and the parameters of the linear regression assume always better values.

The use of the Evolutionary Shuffled Simplex assured a reproducible and objective calibration of the WARM parameters both with and without the new model developed. Therefore, it is possible to affirm that the different performances shown in the two cases by WARM are only due to the introduction of the new algorithms.

The software component TOPFERT.DLL

TOPFERT.DLL is a MS Windows library implementing routines for estimating the effect of panicle initiation fertilization on rice radiation use efficiency. It can be downloaded at the web sites http://agrifish.jrc.it/marsstat/topfert/archive.htm together with a help file including the documentation of the implemented algorithms and a guide (including examples of code) for its use within a visual basic project.

The DLL can be used with all the simulation models based on the radiation use efficiency approach. TOPFERT.DLL can be used in MS Excel spreadsheets and in code developed using a Windows programming language (Visual Basic 6, C++, Delphi, etc.). A .NET component implementing the same algorithms is under development, to assure the full compatibility with the .NET environment.

The DLL can be freely distributed by modellers and developers in their own applications.

Conclusions

Data achieved during the growth chamber experiment appears to be adequate for giving a first evaluation of the effects of top-dressing nitrogen fertilization on RUE. Even if the biochemical and physiological foundations are still not completely known, a relationship between the RUE increase and the nitrogen fertilization at PI has been confirmed and described by an algorithm obtained by the analysis of experimental data. The implementation of the algorithms developed in a COM DLL (TOPFERT.DLL) makes the acquired knowledge available for the use in all the models based on the RUE approach, coded in the most used programming languages. This way of implementing the developed module can be considered particularly suitable for a wide diffusion and therefore for a reliable validation of the knowledge acquired.

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