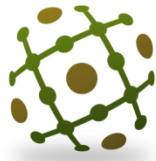


SCIENTIFIC REPORTING



MultiHemp

Multipurpose hemp for industrial bioproducts and biomass
(Ref n. 311849)

WP3 – Optimisation of hemp cultivation and crop modelling
Deliverable 3.3 “Report on the use of the crop model to simulate
the effect of G x E x M on fibre yield and quality”

Authors:

STEVEN M. DRIEVER¹, KAILEI TANG^{1, 2}, TJEERDJAN STOMPH¹, XINYOU YIN¹, PAUL C. STRUIK¹, STEFANO AMADUCCI²

Affiliations:

¹ Centre for Crop Systems Analysis, Plant Sciences, Wageningen University & Research, PO Box 430, 6700 AK, Wageningen, The Netherlands

² Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, via Emilia Parmense, 84, Piacenza, Italy

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2. Introduction

There is relatively little information for hemp on effects on biomass or seed yield of either agronomic factors such as nitrogen (Struik et al., 2000) or environmental factors (Cromack et al., 1997; Amaducci, 2003), let alone the interaction between genotype, environment and management (G×E×M). In this task, this is investigated and a model is made to aid hemp breeding, leading to better fibre yield and quality.

To study the complex G×E×M interaction for hemp, a crop model is an important research tool. Most ecophysiological crop models can reveal how G×E comes about (e.g. Dingkuhn (1996)), but rarely can elucidate the genetic basis of the underlying model parameters, let alone explain the genotypic differences. However, when ecophysiological modelling is combined with genetic mapping approaches, the possibility is created to include genotypic differences. In this combined approach it is possible to dissect complex traits (such as biomass yield) into different underlying component traits. Also, it can integrate effects of genetic factors of the component traits over both time and space at the whole crop level. Although much information exists about leaf- or plant level processes, the step towards the crop level is rarely made. Furthermore, the combined modelling approach allows prediction of the response of complex traits of various genetic make-ups to different environmental conditions. The potential of this method is very big: it can help to pre-select genotypes for specific environments or management practices, e.g. for local field trials, but can also produce a so-called “ideo-type” (ideal genotype). The latter is a direct benefit of the genetic extension of the model, to include molecular markers for specific identified beneficial traits to direct breeding efforts. This G×E×M modelling approach has been successfully applied to different crops and to various complex traits (Yin et al., 2000; Prudent et al., 2010; Tardieu and Tuberosa, 2010).

The Genotype-by-Environment interaction on CROp growth Simulator (GECROS, Yin and van Laar (2005)) is such a model framework, which has been developed for the specific purpose of studying G×E×M interactions. It can be used to examine biomass production in many arable crops in response to both environmental and genotypic characteristics. In the model framework, current physiological knowledge and the interaction with the environment is mechanistically summarized. It is designed specifically for input parameters that are closely related to traits that breeders score for selection, e.g. stem length, flowering time, etc. Apart from processes such as growth and development, it also includes mechanistic sub-models of nitrogen uptake and use, respiration and photosynthesis. However, the model was initially tested for crops like cereals and legumes whereas growth and development characteristics of hemp are very different from those of the main agro-crops, especially under low-input environments where G×E×M was very different, as manifested experimentally within this WP. Therefore, more time was invested than originally planned at the proposal stage, on refining the GECROS model and also developing new protocols of parameterizing various subroutines of the model; in particular the subroutines of photosynthesis (as described below in this report). This effort was time consuming, because the sub-model for photosynthesis is a unique feature of GECROS which uses the detailed biochemical model of Farquhar et al. (1980), or FvCB model, and it's responses to environmental factors. As photosynthesis is the basis of primary plant production, this particular feature of the model framework allows detailed analysis of environmental effects on the growth and production of the bioenergy or bio-fibre crops like hemp. With the inclusion of specific mechanistic and physiologically relevant sub-models, selection of genetic markers for the specific, most important physiological parameters is possible, which would normally not be identified in conventional genetic analysis alone (e.g. GWAS).

In this task, we parameterize and validate the GECROS model for the crop hemp. This task was performed in three stages. In the first stage, G×E interaction of the phenology of hemp was modelled, according to the phenology model as described by Amaducci et al. (2012). The phenology model forms intrinsic part of GECROS and was parameterized with data on sowing and flowering time of different cultivars from field trials at different locations (experiments in 2013, 2014 and 2015) and sowing dates (2016). In the second stage, leaf- and canopy photosynthesis was parameterized by leaf- and canopy level measurements, to validate the canopy photosynthesis simulated with the photosynthesis model in GECROS. In the third stage, the parametrized GECROS model was used to simulate biomass of hemp under different environmental

conditions and the effect of N supply. The model was validated by comparison of the observed- and measured growth and biomass in the field trials at the different locations (experiments in 2013 to 2016).

3. G×E interaction: modelling of flowering time

As reviewed by Amaducci et al. (2012) and Tang et al. (2016), hemp flowering time, which is mainly determined by G×E interaction, is a predominant factor for both stem and seed production. Subsequently, the choice of planting date and available cultivars with differences in photoperiod sensitivity are important determinants of the final hemp yield, both for stem and seed. To inform decisions about this, Amaducci et al. (2012) proposed a phenological model, similar to that used in GECROS (Yin and van Laar, 2005), to estimate time between emergence and flowering (50% of flowers are open, hereafter flowering time) in field-grown hemp cultivars. The set of parameters needed for this model is relatively small and the model comprises of a set of non-linear equations that describe the individual effect of air temperature and photoperiod, as detailed below.

Model description and parameterization for phenology

The parameterization of the phenological model for hemp is described by Tang et al. (2016), which was published as part of this task, and is summarized below.

Phenology of hemp is characterized by three different phases; the basic vegetative phase (1), photoperiod induction phase (2) and flower development phase (3). The developmental rate (R_{dev}) can therefore be calculated using accumulated fractional development, until unity is reached:

$$R_{dev}(x) = \begin{cases} \frac{fT(1)}{D_1} & x = 1 \\ \frac{fT(2) \cdot fP}{D_2} & x = 2 \\ \frac{fT(3)}{D_3} & x = 3 \end{cases} \quad \text{Equation 1}$$

where D_x are the physiological developmental days, that indicate the number of required days to complete a particular phenological phase ($x = 1, 2, 3$) when conditions are optimal. fT and fP are the thermal and photoperiodic factors. The duration of the flower development phase ($x = 2$) was assumed to be 1 day for optimal air temperature and day length conditions.

The rate of change in development in response to air temperature ($fT(x)$) was described nonlinearly, with initial quasi-exponential to linear response and an optimum, followed by a steep decline (Yin et al., 2003):

$$fT(x)[x = 1,2,3] = \begin{cases} 0 & T \leq T_b, T \geq T_c \\ \frac{T_c - T}{T_c - T_o} \cdot \left(\frac{T - T_b}{T_o - T_b} \right)^{(T_o - T_b)/(T_c - T_o)} & T_b < T < T_c \end{cases} \quad \text{Equation 2}$$

where T (°C) represents the mean hourly air temperature, T_b (°C) the base air temperature for development, T_c (°C) the air temperature at which development halts and T_o (°C) represents the air temperature optimum where rate of development is maximal. A sigmoid, so-called switch-off function was used to model the response to day length as a photoperiodic factor (fP):

$$fP = \frac{K^n}{K^n + P^n} \quad \text{Equation 3}$$

where P is the day length (in hours), K is the parameter determining the switch-off point (in hours, for day length giving $fP = 0.5$) and n is the cultivar specific photoperiod sensitivity.

Among the 13 parameters of the model, the days required to complete the basic vegetative phase under the optimal condition (D_1) and the slope of the photoperiod response curve in the photoperiod sensitive phase (n) were considered as intra-crop genotype-specific parameters, while the other parameters were

crop-specific (Amaducci et al., 2012; Tang et al., 2016). D_1 and n were estimated by minimization of the difference between observed and predicted flowering time. Other parameters were used as previously determined by Amaducci et al. (2012). Evaluation of fitting was performed using relative root mean square error (RRMSE,%) and modelling efficiency (EF), as described by Tang et al. (2016).

Model validation for phenology

The predictability of this phenology model was validated using data sets from field trials in 2013 with 14 commercial hemp cultivars (Table 1), grown in four different locations (Latvia, Czech Republic, France, Italy). Average temperature in the growing season (May-October) ranged from 16.0 in the Czech Republic (CZ) to 21.6 °C in the southernmost location, Italy (IT). The latter location was characterized by a hot and dry summer, where other locations were cooler and more humid. Precipitation ranged from 227 mm in the hot, dry location (IT) to 419 mm in the wettest coolest location (Latvia; LV). Maximum day length differed by 2 hr between the most northern (LV) and southern location (IT). Cultivars were sown in randomized complete block design (four replicates), with 40 m² plot size, between end April – Mid May (depending on local conditions). Further details are described by Tang et al. (2016).

Table 1. List of origin and sexual type of tested cultivars. From Tang et al. 2016

Cultivar	Abbreviation	Origin	Sexual type
Beniko	BEN	Poland	Monoecious
Bialobrzeskie	BIA	Poland	Monoecious
Epsilon 68	EPS	France	Monoecious
Fedora 17	FED	France	Monoecious
Felina 32	FEL	France	Monoecious
Férimon	FER	France	Monoecious
Futura 75	FUT	France	Monoecious
Markant	MAR	Netherlands	Monoecious
Monoica	MON	Hungary	Monoecious
Tygra	TYG	Poland	Monoecious
CS	CS	Italy	Dioecious
KC Dora	KC	Hungary	Dioecious
Tiborszallasi	TIB	Hungary	Dioecious
Tisza	TIS	Hungary	Dioecious

For each cultivar, development was measured by daily monitoring to determine emergence date (50% of seedlings appeared) and twice weekly to determine the flowering state (onset of flowering, full flowering, end of flowering).

With these data, the parameters for flowering time (the time needed to complete the basic vegetative phase, D_1) and steepness of the photo-inductive phase (n) were estimated (Table 2). The parameterized model predicted the observations well, with values of EF ranging from 0.85-1 for the cultivars in the current study. Still, the presented parameter as estimates by Tang et al. (2016) were done using a single year and thus a somewhat limited dataset. The values estimated of Tang et al. (2016) were higher for D_1 , but lower for n , in comparison with previous studies (Amaducci et al., 2008; Amaducci et al., 2012). Given that previous estimates were based on relatively larger data sets (Amaducci et al., 2008; Amaducci et al., 2012), a larger dataset would be needed to assure a that a wide range of environments can be simulated. For further implementation of this important phenology model into GECROS, field trials were conducted at different locations (experiments in 2013, 2014 and 2015) and sowing dates (2016) for consolidating the simulation of G×E interaction of hemp. This dataset comprised of 31 cultivars, of which seven selected cultivars are presented here as an example. Parameterization of other cultivars are described by Tang (PhD Thesis, in preparation). The selected cultivars include previously analysed cultivars (Beniko, Bialobrzeskie, CS and Futura 75) and cultivars not previously analysed (Antal, Carmagnolo and Uso 31). These cultivars

were grown over the period between the years 2013-2016 in locations in Italy (IT), Czech Republic (CZ), France (FR), Latvia (LV), the Netherlands (NL) and the United States (US), as described in Table 3.

Table 2. Parameter values for cultivars of the days needed to complete the basic vegetative phase (BVP) under optimal conditions (*D1*), steepness parameter of the photo-inductive phase (*n*). *RRMSE*: relative root mean square error, and *EF*: model efficiency. From Tang et al. (2016)

Cultivar	<i>D1</i>	<i>n</i>	<i>RRMSE</i>	<i>EF</i>	
BEN	50.2	21.7	5.8	0.89	
BIA	43.7	23.9	3.9	0.95	
EPS	43.9	25.8	1.3	0.99	
FED	37.2	21.4	5.8	0.91	
FEL	39.1	25.2	2.2	0.98	
	2012 ^a	11.1	33.4	9.3	0.74
	2008 ^a	13.2	47.0	13.0	-0.29
FER	44.8	24.3	5.6	0.91	
FUT	25.1	33.6	1.2	1.00	
	2012 ^a	14.7	39.6	5.6	0.92
	2008 ^a	19.7	52.1	2.2	0.99
MAR	41.9	12.6	3.9	0.91	
MON	26.8	34.0	1.4	1.00	
TYG	42.0	25.5	5.0	0.93	
CS	51.6	77.4	3.9	0.85	
KC	32.5	50.9	2.6	0.97	
TIB	60.9	14.1	2.9	0.95	
	2012 ^a	20.6	38.6	10.6	0.19
	2008 ^a	18.5	66.7	13.3	0.50
TIS	61.5	15.6	3.1	0.95	

^a: 2008 indicates parameters from (Amaducci et al., 2008) and 2012 indicates parameters from (Amaducci et al., 2012)

Table 3. Cultivars, years and locations used for validation of GECROS phenological model.

Cultivar	Abbreviation	Year	Location(s)
Antal	ANT	2015	IT
		2016	IT
Beniko	BEN	2013	IT, CZ, FR, LV,NL
		2015	IT
		2016	IT
Bialobrzieskie	BIA	2013	CZ, FR, LV, IT, NL
		2014	FR, LV, IT
		2015	US, IT
		2016	FR, LV, IT, NL
Carmagnolo	CAR	2015	IT, US
		2016	IT
CS	CS	2013	CZ, FR, LV, IT, NL
		2015	IT
		2016	FR, LV, IT, NL
Futura 75	FUT	2013	CZ, FR, LV, IT, NL
		2014	FR, LV, IT
		2015	IT, CZ, US, FR, LV
		2016	FR, LV, IT, NL
Uso 31	USO	2015	US, IT
		2016	FR, LV, IT, NL

To capture the variation of hemp phenology in the simulation of hemp growth and yield, the above described phenology model was integrated into the framework of GECROS, specifically, into sub-model called PHENO (Yin and van Laar, 2005). In this sub-model, the developmental stage (ϑ , unitless) is considered at 0 for seedling emergence, 1 for full flowering and 2 for physiological seed maturity. This developmental stage is accumulated as developmental rate (ω_i , in day⁻¹) and expressed for each stage as:

$$\omega_i = \begin{cases} f(T)/m_V & \vartheta \leq \vartheta_1 \text{ or } \vartheta \geq \vartheta_2 \\ f(T)f(P)/m_V & \vartheta_1 < \vartheta < \vartheta_2 \end{cases} \quad \text{Equation 4}$$

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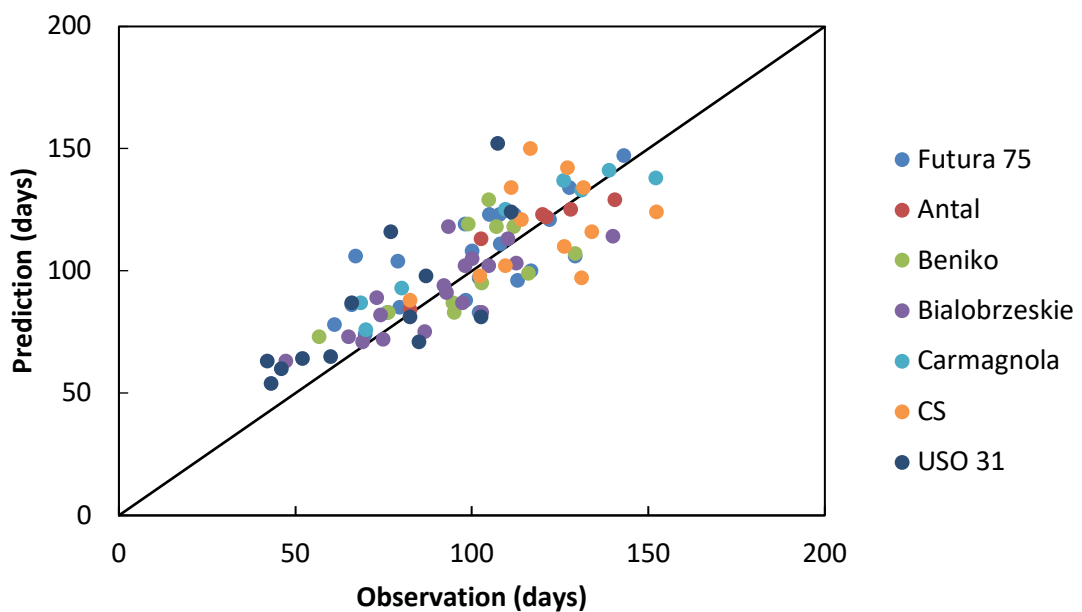
$$f(P) = \frac{K^{P_{sen}}}{K^{P_{sen}} + P^{P_{sen}}} \quad \text{Equation 5}$$

m_V is the minimum number of thermal days for the vegetative stage, $f(T)$ was expressed as The parameters m_V and photoperiod sensitivity (P_{sen}) are intra-crop genotype-specific and were estimated with the dataset collected from field trials conducted at different locations (experiments in 2013, 2014 and 2015) and sowing dates (2016, see Table 3). The other parameters were after Amaducci et al. (2012). Results of this parameterization for each cultivar are shown in Table 4.

Table 4. Parameter estimation of each cultivar for the phenology sub-model PHENO (of GECROS) for seven different cultivars grown in different environments. m_V : minimum thermal days for vegetative phase (days). p_{sen} : photoperiod sensitivity (hour^{-1}).

Cultivar	m_V	p_{sen}
Antal	27	8.7
Beniko	29.2	1.8
Bialobrzeskie	23.9	2.5
Carmagnola	17.6	21
CS	35.8	1.4
Futura	27.1	3.4
Usa	14.4	11.3

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Figure

4. G×E interaction: model aided analysis of hemp leaf and canopy photosynthesis

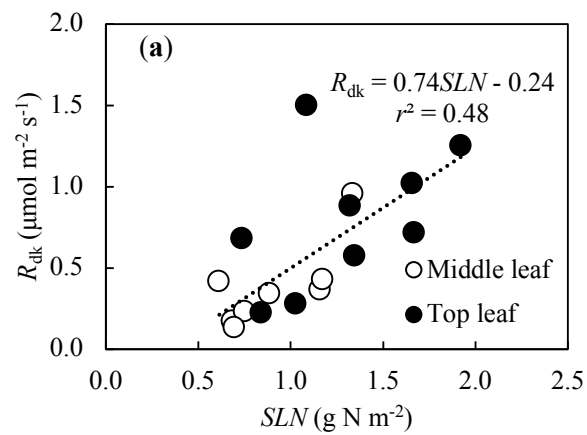
Photosynthesis
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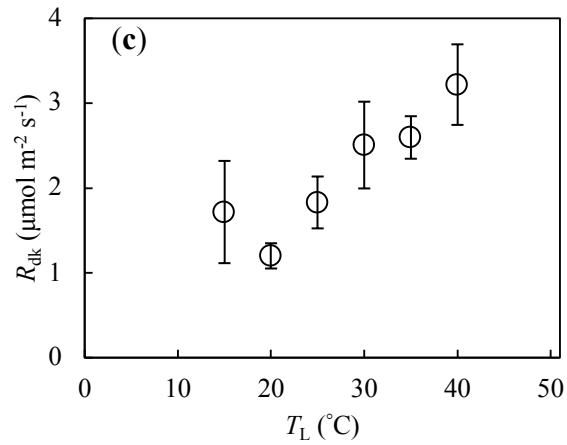
Table

Parameter	Unit	Hemp
<i>Respiration</i>		

R_{d-}	Slope	μmol	0.85
	Intercept	μmol	0.03
E_{Rd}		J	21634
	e^-		
$J_{\text{max-}}$	Slope	μmol	132.9
	Intercept	μmol	54.4
$E_{J\text{max}}$		J	67292
$D_{J\text{max}}$		J	114701
$S_{J\text{max}}$		J	375
K_{2LL}		mol	0.21
			0.37
ϑ		-	0.70 ^a
	Rubisco		
$V_{\text{cmax-}}$	Slope	μmol	76.2
	Intercept	μmol	12.6
E_{vcmax}		J	63024
	TPU		
$T_{\text{p-}}$	Slope	μmol	4.2
	Intercept	μmol	4.3
E_{Tp}		J	34417
	g_m		
δ		-	2.12
g_{m0}		mol	0 ^a

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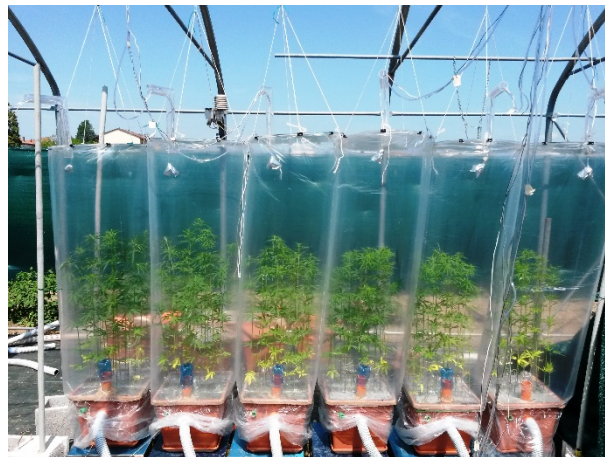
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To calculate canopy photosynthesis and transpiration, raw data were first filtered and corrected for anomalies, such as short time fluctuations of air CO_2 concentration and water vapour. Subsequently, $\text{CO}_{2,\text{dif}}$ and VP_{dif} were corrected for system error with recorded data from the period without plants in the

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$$A_{c,\text{net}} = \frac{u_e \text{CO}_{2,\text{dif}}}{s} - TE_c \text{CO}_{2,\text{in}}$$

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$$T_c = \frac{u_e VP_{\text{dif}}}{s(P - (VP_{\text{in}} + VP_{\text{dif}}))} \quad \text{Equation 7}$$

where u_e is air flux into the chamber; s is the ground area of plant chamber; P is the air pressure inside the chamber. The standard air pressure (101.3 kPa) was used as a proxy of P in the present study although a slight overpressure was maintained inside the plant chamber (about 10 Pa) to avoid any flux of ambient air through possible leaks. The effect of overpressure on $A_{c,\text{net}}$ and T_c was considered negligible ($\ll 1\%$).

To assess canopy gross photosynthesis ($A_{c,\text{gross}}$), canopy respiration (R_c) was estimated. As $CO_{2,\text{dif}}$ at night is mainly due to R_c , this allowed direct estimation, assuming day- and night-time R_c follow the same relation with T_{air} , both described with the same Arrhenius function:

$$R_c = R_{c,25} \exp \left[\frac{E_{Rc}(T_{\text{air}} - 25)}{298R(T_{\text{air}} + 273)} \right] \quad \text{Equation 8}$$

where $R_{c,25}$ is the value of R_c at 25 °C, E_{Rc} is the energy of activation and R is the universal gas constant (= 8.314 J K⁻¹ mol⁻¹). Thus $A_{c,\text{gross}}$ was calculated as:

$$A_{c,\text{gross}} = A_{c,\text{net}} + R_c \quad \text{Equation 9}$$

To estimate light and nitrogen extinction coefficients, PAR and SLN were assumed to be attenuated exponentially, based on LAI:

$$\frac{I_i}{I_0} = e^{-k_L LAI_i} \quad \text{Equation 10}$$

$$\frac{SLN_i}{SLN_0} = e^{-k_{tn} LAI_i} \quad \text{Equation 11}$$

where LAI_i is the LAI at depth i ; k_L is light extinction coefficient; I_i/I_0 is the ratio of light intensity at depth i and the top of canopy; k_{tn} is SLN extinction coefficient, SLN_i/SLN_0 is the ratio of SLN at depth i and the top of canopy. The k_L and k_{tn} were estimated by fitting canopy profiles of light intensity and SLN , respectively.

Calculation of potential canopy gross photosynthesis and transpiration

The Sun-Shade model described in GECROS (Yin and van Laar, 2005; Yin and Struik, 2017) was used to calculate potential canopy gross photosynthesis ($A_{cp,\text{gross}}$) and potential canopy transpiration (T_{cp}). This model was adopted from previous studies (Pury and Farquhar, 1997; Wang and Leuning, 1998), in which the canopy is divided into sunlit and shaded fractions and each fraction is modelled separately with a single-layer leaf model. The prediction of canopy photosynthesis by Sun-Shade model is similar to that of a multi-layer model (Pury and Farquhar, 1997) while the Sun-Shade model is computationally more efficient. In this canopy model, leaf photosynthesis rate was simulated using a modified FvCB model coupled with a stomatal conductance model (Yin and Struik, 2009). Parameterization of the leaf photosynthesis model was presented in a companion study. These parameters are presented in Table 5. The canopy related parameters LAI , k_L (for diffuse light) and k_{tn} were derived in this study from data collected in previous experiments. Leaf angle that was used to calculate the light extinction coefficient of direct light was fixed at 15 °, based on an average value measured with a goniometer. Thus, the $A_{cp,\text{gross}}$ and T_{cp} were calculated using instantaneous recordings of environmental parameters in each plant chamber: C_a , VP , T_{air} and I_{inc} .

Simulation of actual canopy gross photosynthesis

The calculation of $A_{cp,\text{gross}}$ and T_{cp} indicated that canopies had limited water availability under certain conditions. Therefore, the actual canopy gross photosynthesis ($A_{ca,\text{gross}}$) was simulated assuming that the effect of water stress on canopy photosynthesis is largely mediated by the stomata (Chaves, 1991). The response of stomata was estimated considering the difference of the measured T_c and simulated T_{cp} as:

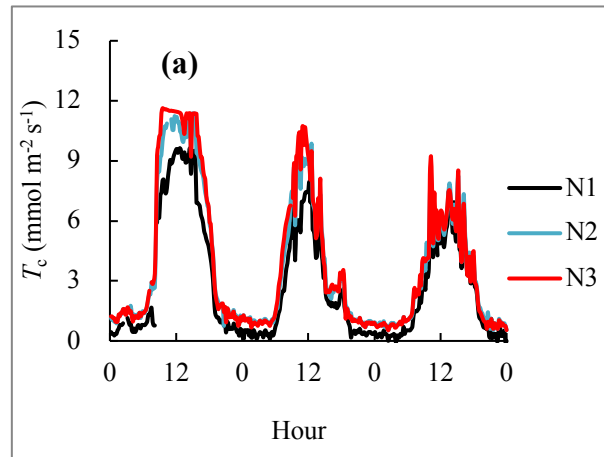
$$r_{sw,a} = (T_{cp} - T_c)[s(r_{bh} + r_t) + \gamma(r_{bw} + r_t)]/(\gamma T_c) + r_{sw,p}T_{cp}/T_c \quad \text{Equation 12}$$

where s is the slope of saturated vapour pressure curve; r_{bh} , r_{bw} , r_t , and $r_{sw,p}$ are boundary layer resistance to heat, boundary resistance to water, turbulence resistance and stomatal resistance to water transfer in

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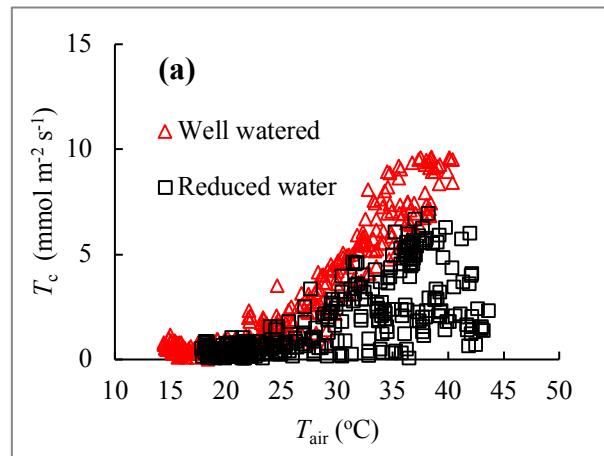
Validation

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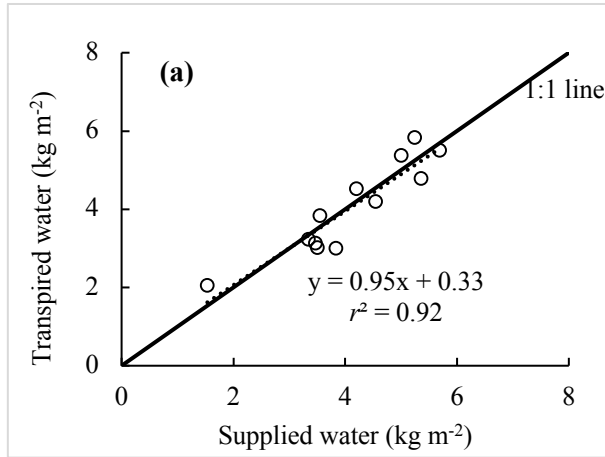


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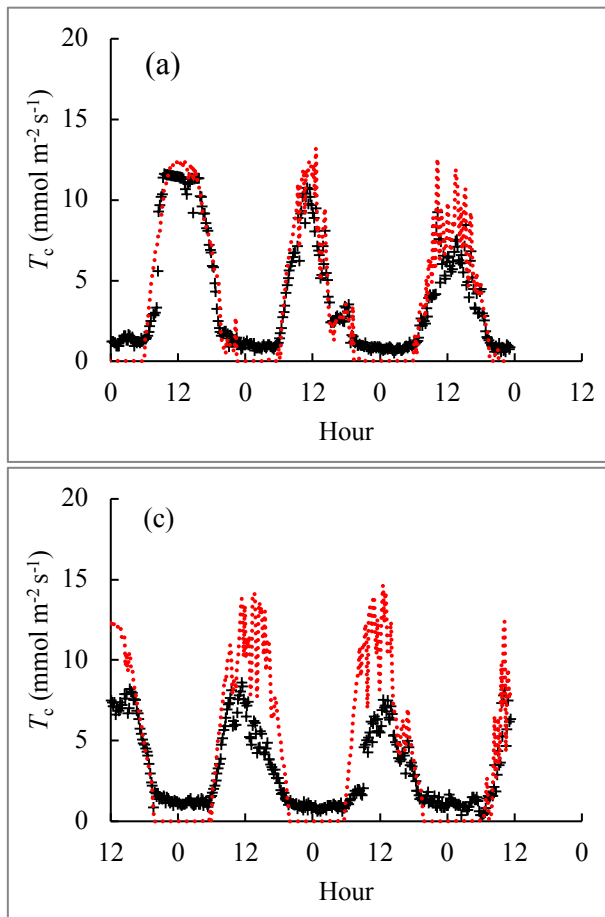


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Taking the above into account, the effect of stomatal closure on the simulation of $A_{cp, \text{gross}}$, the stomatal

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$A_{c, gross}$ matched well with measurements (Figure 8). The daily integrated values of simulated $A_{c, gross}$ with the model had an r^2 of 0.86 and an $rRMSE$ of 13.6%. Therefore, it is concluded that stomatal closure as caused by water stress is important to take into account in the model when simulating canopy photosynthesis, for which this model is applicable. This is especially relevant to situations where simulations are done on field-grown crops that are non- or irregularly irrigated.

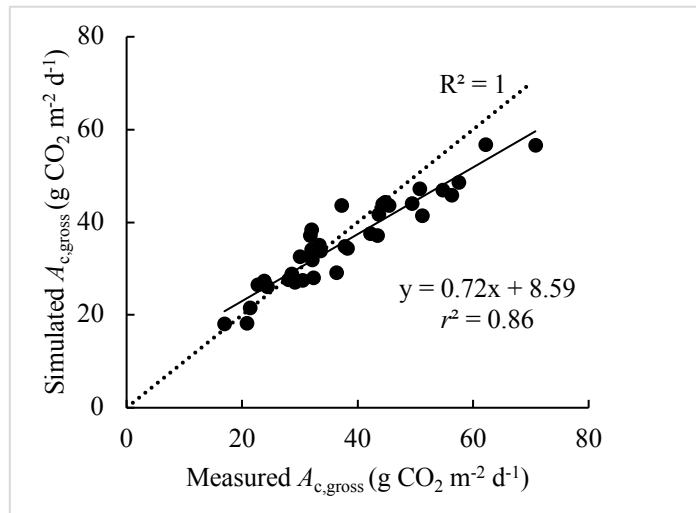


Figure 8. Simulated gross canopy photosynthesis ($A_{c, gross}$) against measured. Values were simulated by accounting the effect of water deficit on canopy photosynthesis by accounting the response of $r_{sw,a}$

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in conclusion, both leaf- and canopy gross photosynthesis can be accurately simulated under different conditions, including different nitrogen- and water levels. The model presented for simulating canopy gross photosynthesis includes the effect of stomatal closure and can simulate diurnal fluctuations, which allows sufficient accuracy for the hourly time steps used by GECROS. The results presented above validate the model physiologically, with correct scaling from the leaf- to the canopy level. They validate the correct interaction of photosynthesis with the environment with daily water, light and temperature variations. Furthermore, it validated the interaction with management (e.g. nitrogen supply). These interactions form the basis of simulation of growth, development and biomass.

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5. G×E×M interaction: biomass and stem yield at different nitrogen fertilization

With the phenological and physiological parameterization of the crop model for hemp validated, as described above, it is now possible to simulate biomass- and stem yield of a crop for a given environmental condition. In principle, the model would allow for assessment of biomass and growth on a day-to-day basis, since this is the time step at which the GECROS model framework can simulate. However, there are limitations that need to be taken into account. In this part, the limits of the model are explored, by simulation of a hemp crop for a given environment (Italy, 2015) and compared to measured values of periodic harvest for biomass and stem yield.

Simulation of biomass and stem yield

For a crop grown in Italy (2015), biomass was simulated on the basis of weather data and model parameters determined for cultivar Futura 75. For five moments in the growing season, plants were harvested and total aboveground biomass and stem yield were determined. As shown in Figure 9, the simulated biomass (blue line) followed a similar trend as the measured biomass (red circles), for both biomass and stem yield. However, earlier emergence of the crop was simulated and subsequently, a higher biomass and stem yield were simulated than was observed at each harvest date. It was also noted that observed values showed increasing variation from around 60 days after sowing to the end of the growing season. Although at each harvest date, the model over-estimated the biomass and stem yield, compared to observed values, but the slope of both simulated and observed were similar. Nevertheless, the final biomass and stem yield were simulated correctly. This implies that the model, in essence, can accurately predict the growth rate and final yield. However, there may be several factors that may have caused later emergence than simulated, caused by factors which are not included in the model.

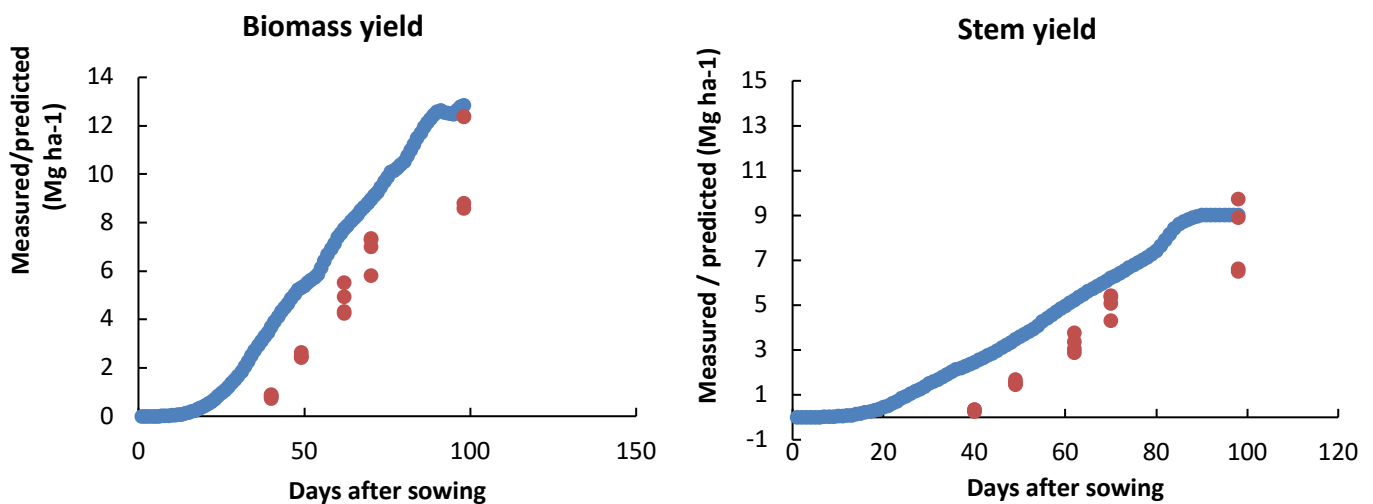


Figure 9. Simulated (blue line) and measured (red circles) of a hemp crop (cv. Futura 75) over a growing season in Italy (2015). Red circles represent averages of 5 harvested plants.

Using the same model, parameters, cultivar and environmental conditions as above, stem yield was simulated for different nitrogen fertilization rates. Nitrogen fertilization treatments were: 0 (no additional fertilization), 30, 60 and 120 kg ha⁻¹ of additional nitrogen. The model was used to simulate the final stem yield and compared to the final observed stem yield for each treatment. As shown in Figure 10, the model showed higher predicted (blue circles) than measured stem yield (red squares, Figure 10). The largest differences were found for the lowest nitrogen treatments (0 and 30 kg ha⁻¹ additional nitrogen), but differences were relatively small for the high nitrogen treatments between measured and predicted values.

Nonetheless, the general trend for the response of stem yield to the amount of nitrogen fertilization was similar between predicted and measured values. As shown in Figure 10, both simulated and predicted values suggest that the effect of nitrogen fertilization over 60 kg ha⁻¹ does not result in a higher stem yield, which is a useful insight for crop management decisions. The similarity in the trend between measured and predicted values also implies that the model does capture the interactions of the plant with nitrogen fertilization correctly, but that either parameterization may need fine-tuning to simulate this interaction accurately or that other external factors (not included in the model) may reduce stem yield in the field.

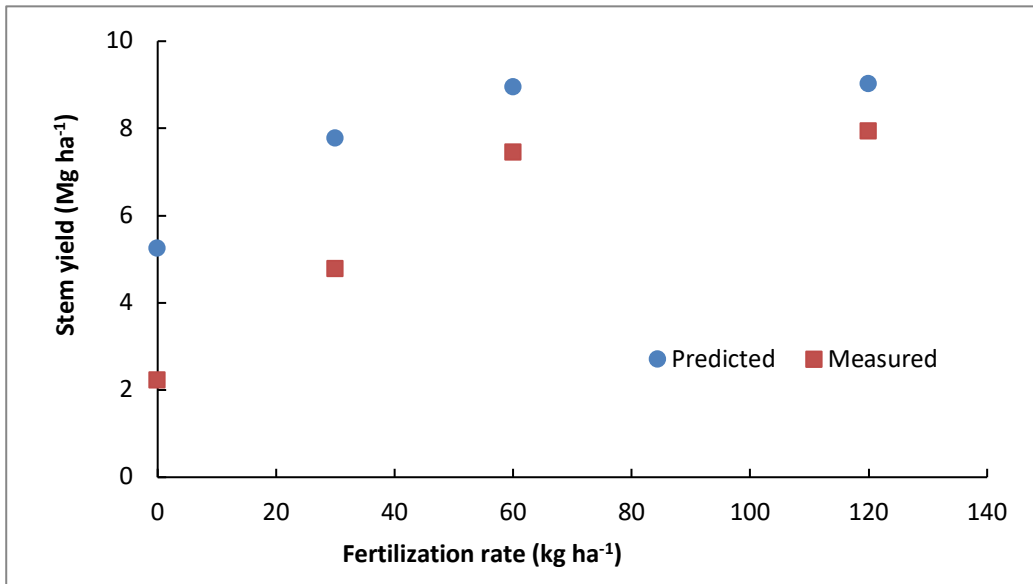


Figure 10. Simulation (blue circles) and measured (red squares) final stem yield of a hemp crop grown at four different additional nitrogen fertilization rates for a hemp crop (cv. Futura 75) grown in Italy (2015).

The two simulation studies above show that the model can, in essence, predict crop growth, development and yield. However, the studies also show where the model is limited. Indeed, the model is constructed around the phenological and physiological processes in the plant and does not include external factors other than the environment (e.g. temperature, light, etc.) and management (e.g. nitrogen supply). The mechanistic understanding and inclusion of phenology and physiology into crop models already form a great improvement over other crop model frameworks (Yin and Struik, 2008; Hammer et al., 2010; Yin and Struik, 2010). However, there is also room for improvement. In the simulation studies above, it is not fully understood what was causing the discrepancies found. In the case of emergence, factors such as growth of quick growing weeds or the level of compaction of the soil may have been of influence. Or, in the case of nitrogen fertilization, a drought spell, soil compaction or other short term stresses may have been of influence. Although the model is capable of capturing the long term growth and yield dynamics, short term stresses and influences of factors such as weeds are much more complex to model.

6. Perspectives on the use of the newly parameterized hemp model

In general, the parameterized and validated model for hemp was able to simulate genotype by environment by management interactions (G×E×M) adequately. The flowering time, an important parameter for both seed and fibre production, could be predicted well for a range of genotypes (cultivars) and environments (locations and weather). Through a validated interaction between environment, management and physiology, e.g. photosynthesis, the essence of effects of e.g. available nitrogen could be simulated. Although the actual biomass and stem yield during the growth period under certain situation was not simulated exactly, both the rate of development and the final biomass and stem yield were predicted accurately. This model has now been parameterized for a number of genotypes (cultivars), including those most used in commercial production (e.g. Futura 75). The model can be extended with new genotypes in future, following the same parameterization protocol as described in this report. Given the mentioned possibilities of the model, this model has the potential to become a valuable tool for both growers and breeders in the ongoing development of a sustainable hemp production.

For growers, the model has the potential to be used to search for a suitable variety for their current location, or a location of interest. If the grower can provide information on their agronomic practice (e.g. N available for the plant or to be fertilized) and the weather at their location (e.g. weather over the past 10 years), the model would be able to predict an expected flowering time, as well as a final biomass and stem yield of the cultivars of interest. This will indicate which varieties are likely to perform well a specific location, with a specific management strategy. It can help to narrow down the number of candidate cultivars, to be tried out in test plots on their land. The benefit for the grower in using the model is therefore mainly in speeding up and narrowing down the pre-selection of (newly available) cultivars before testing, saving time, resources and money.

For breeders, the model can provide a tool to design and breed for cultivars adapted to specific environments or management practices, or for a particular purpose (e.g. fibres, seeds, or both). In the model, each phenological, photosynthetic or other physiological parameter can be linked to a quantitative trait locus (QTL), a genetic marker that allows for selection in the breeding process. The use of such a crop model can greatly facilitate the selection process, as traits can be evaluated *in silico* under different environments and management practices. This has been made possible as the interactions of the trait with the environment and management have been parameterized and validated. There are several examples where the GECROS model framework was successfully used in combination with QTL- or association mapping for this specific purpose in other species such as rice (Gu et al., 2014) and barley (Yin et al., 2000; Yin et al., 2005). The development of this type of combined physiological modelling and genetic analysis has great potential for improving (functional) genetic analysis (Yin and Struik, 2008), gene by environment interaction (Yin, 2013) and crop system-biology (Yin and Struik, 2010; Yin et al., 2016). Moreover, there is great potential to exploit these type of models in combination with ever-expanding genotyping- and phenotyping strategies (Cobb et al., 2013). These strategies are now also possible for hemp. It is expected that with further study and availability of sufficient genetic markers for hemp, the currently developed model for hemp can be adopted and operational for association mapping and genetic analysis within 2-3 years. Therefore, the currently developed crop model for hemp is an important step towards facilitating and improving breeding strategies for hemp to enable multi-purpose production, as well as their responses under current and future climates.

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