Combining multi-environmental trials and crop simulation to understand

soybean response to early sowings under contrasting water conditions

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Abstract - Anticipating sowing date in spring-sown crops is an agronomic strategy often suggested in Europe to escape water and heat stresses during the most susceptible growth periods occurring in early summer. This strategy was evaluated experimentally in soybean (*Glycine max* (L.) Merrill) by comparing early and conventional sowing dates for a range of cultivars grown in 18 "site x year" environments from southern France between 2010 and 2018. Conventional sowing often resulted in higher grain yields than early sowing, the latter being more favorable to grain size and protein concentration. STICS crop growth model was used to calculate dynamic abiotic (radiation, water, high and low temperatures, nitrogen) stress factors influencing the main crop physiological processes. Ecoclimatic and STICS-based agrophysiological indicators were used in a PLS (Partial Least Squares) regression as explanatory variables of grain yield (r^2 = 0.33). The key indicators were radiation interception from emergence to maturity, severe stomatal water stress during all the reproductive phase and high temperature since the beginning of grain filling. STICS crop model was used to characterize the 157 "cultivar x site x year x sowing date x irrigation" environments, according to water, heat and cold stresses importance in different phenophases. Then 5 profiles of abiotic stresses emerged from a clustering analysis in two steps by hierarchical ascending classification and K-means.

STICS-based environmental characterization was used to explain the relative success or failure of early sowing dates and early-maturing cultivars.

1. Introduction

Climate change is a recognized fact. Successive reports of the Intergovernmental Panel on Climate Change (IPCC) tend towards an historical global warming of around 1°C compared to the pre-industrial period (1850 - 1900) with increasingly unpredictable drought or precipitation events (IPCC, 2018). In Europe, intense heat waves in the South coupled with prolonged droughts, a global increase in air temperatures over the whole continent and more intense precipitation in the North are generally expected (Allen *et al.*, 2018). Crop cultivation in these future scenarios requires an understanding of plant response to environment to be able to anticipate needs in terms of breeding, crop management or cultivar choice. The study of genotype-environment-management interactions (GEMI) has often been conducted using statistical methods that allow the analysis of a large amount of data from multi-local and multi-year experiments (Allard and Bradshaw, 1964; Simmonds, 1981; Brancourt-Hulmel, 1999 ; Sudarić *et al.*, 2006; Zhe *et al.*, 2010 ; Assefa *et al.*, 2019). These methods, which highlight significant differences between environments, genotypes, and their interactions, require, however, a large amount of observed data and adapted experimental designs to successfully expand the results of statistical analyses. Because setting up field experiments is expensive, this statistical approach has been widely supplemented by the use of crop models that simulate at a daily time step the response of plant growth and development to the environmental (soil, climate) and management conditions (Chapman, 2008; Salmerón *et al.*, 2017).

Agro-environmental indicators as simulated by these models could be used to characterize crop growth conditions in summary patterns (Chenu *et al.*, 2011 ; Caubel *et al.*, 2015; Chenu, 2015). Caubel *et al.* (2015) defined ecoclimatic indicators as agroclimatic indicators (*e.g.* growing degree-days, number of rainy days, number of days with air temperature > 30°C) calculated over phenological periods (e.g. sowing-emergence,

flowering-maturity). These indicators are linked with the ecophysiological processes they characterize (e.g. crop establishment or grain filling). When calculated as outputs of dynamic ecophysiological models such as STICS (Brisson *et al.*, 2003; 2009), these indicators (*e.g* water or nutrient stress factors) could better represent the abiotic stresses perceived by the plants than ecoclimatic indicators. The term "agro-physiological" could qualify this family of indicators. Both ecoclimatic and agrophysiological indicators have been associated to analyze and predict crop yield data (Ly *et al.*, 2017 ; Boulch *et al.*, 2021).

The analysis of GEMI supported by crop growth models has widely developed to become one of the most frequently used methods in recent years (Van Eeuwijk *et al.*, 2016; Stöckle and Kemanian, 2020). In addition to the characterization of past events, modelling allows the exploration of future or unexperimented situations (Battisti *et al.*, 2017), but also of various crop management systems (Aminah *et al.*, 2017; Battisti *et al.*, 2018) and of real or virtual cultivars (Boote, 2011), which is very useful when applied for optimization of adaptation strategies (*e.g*. for designing ideotypes).

Soybean (*Glycine max* (L.) Merrill) is a thermophilic short-day plant whose yield potential is strongly impacted by water deficit, the reproductive phase (more particularly from pod enlargement to grain filling stage) being the main critical period (Doss *et al.*, 1974; Wani and Heng, 2012; Pardo *et al.*, 2015; Montoya *et al.,* 2017 ; Grassini *et al.*, 2021). In Europe, this crop is mainly cultivated in southern and continental parts, where more droughts and heat waves are expected in the near future (Rojas *et al.*, 2019). Several adaptive strategies could be foreseen to sustain the development of soybean areas (Maury *et al.*, 2015): (i) early sowings to escape water and heat stresses during the most sensitive phases of the cycle, (ii) choice of tolerant cultivars adapted to limiting water conditions, (iii) irrigation management to avoid severe water constraints. Early sowings would make it possible to avoid the water stress often encountered during the grain-filling phase, by shifting it later in the cycle. However, the effects of cool temperatures at the beginning of the cycle could adversely affect soybean development and growth by depressing seed germination and plant establishment (Lamichhane *et al.*, 2020a; Lamichhane *et al.*, 2020b), symbiotic nitrogen fixation (Zhang *et al.*, 1995) or photosynthesis (Allen and Ort, 2001). However, the effects of photoperiod on plant

development could increase the duration of the reproductive growth phase that determines pod set and grain filling (Schoving *et al*., 2020). Studies on early (Pedersen and Lauer, 2004; Salmeron *et al*., 2014; Kumagai, 2018) or late (Egli and Bruening, 2000; Bastidas *et al*., 2008; Hu and Wiatrak, 2012) soybean sowing times have been carried out mainly in the US, and mostly for maturity groups above III. In Europe, adopted maturity groups (MG) range from 000 (very early) to II (late) (Kurasch *et al*., 2017). Sowing earlier cultivars in an area where late cultivars are usually cultivated could also be of interest to avoid water or heat stress by shortening the crop cycle and anticipating the phenological phase of greater sensitivity to water deficit, *i.e.* R5 to R6 (Pardo *et al*., 2015). Kumagai (2018) concluded that early sowing is an effective option for reducing the risk of excess water stress in vegetative stage and increasing yield of soybean from GM IV in northern Japan.

In this study, we chose to investigate more precisely the effect of sowing date (especially early ones) on the performance of soybean cultivars of maturity groups usually grown in Europe under both irrigated and rainfed conditions. The objective was to identify and characterize environmental conditions that may impact soybean yield, in particular by testing sowing one month earlier than usual conditions and introducing early cultivars more often grown in northern situations. For this purpose, a network of field experiments was set up between 2010 and 2018 in southwestern France under rainfed and irrigated conditions for cultivars of maturity groups between 000 and II (Maury *et al*., 2015; Lamichhane *et al*., 2020a). Dynamic monitoring of crop growth and development until harvest were performed on all cultivars to enable the characterization of G x E x M interactions. Ecoclimatic and STICS-based agrophysiological indicators were used to decipher underlying mechanisms explaining soybean yield variation due to early sowing dates and early-maturing genotypes.

2. Materials and Methods

2.1. Experimental data

2.1.1. Experimental sites and design

A network of multi-local experiments was set up during 7 years (2010, 2011, 2012, 2013, 2014, 2017 and 2018) on 6 field phenotyping platforms from public and private

research in southern France to test cultivars, sowing dates and water management combinations, resulting in 18 site x year environments. The 13 soybean cultivars (from MG 000 to II) used in this network may have changed over the years, but three of them remained common to a large part of the experiments: Isidor, Santana and Ecudor. These cultivars were also representative of the maturity groups predominantly grown in southwestern France (MG I to II). The site, year, cultivar, and sowing date characteristics are presented in Table 1. From 3 to 9 cultivars were compared depending on the seasons. Two to three sowing date modalities were tested in each site, with at least one "early" date (from mid-February to beginning-April) and one "conventional" date (from mid-April to end of May) corresponding to the date when farmers usually plant soybeans in South-West of France. Late sowings (after end-May) sometimes occurred due to very wet springs. From 2013 onwards, a "water management" treatment (irrigated or not) was systematically added, the irrigated trials being watered with the help of tensiometric probes placed at different depths in the soil according to the Irrisoja method (Terres Inovia, 2019). The total irrigation applied varied between 65 and 290 mm depending on the sites, sowing dates and years. Different experimental layouts were used but they mainly resulted in split-plots and split-split-plots designs. Water management was the main treatment, sowing date was the sub-treatment and cultivars were arranged as sub-sub-plots. Three or four replications (B, statistical blocks) were present except in 4 experiments. The characteristics of each site-year and the modalities of the treatments carried out are described in Table 2.

2.1.2. Measurements

Weather data (minimum and maximum temperatures, precipitation, solar radiation, wind speed, relative humidity) were recorded daily at 2 m height in the vicinity of the experimental sites by an automatic climatic station from CIMEL (Cimel Electronique, Paris, France). The climatic water deficit (PET-P, mm) was calculated from the difference between potential evapotranspiration (PET) and precipitation (P) over the period from March 1st to September 30th. Each soil was characterized by physico-chemical analysis (e.g. texture, total N content) in the 0-90 cm before sowing. Soil water tension was monitored in a microplot of the Santana cultivar at 30, 60 and 90 cm depth by Watermark probes (Irrometer, Riverside, CA) for irrigation control purposes.

Phenology was recorded following the Fehr and Caviness (1977) scale on all experiments with varying degrees of precision. At least three stages were scored (i) VE - emergence, (ii) R1 - appearance of the first flower, (iii) R8 - full maturity. In some trials (2017 and 2018), weekly monitoring allowed to identify the main growth stages (including R5 – beginning of grain filling and R7 – beginning maturity). At the end of the crop cycle, grain yield (at 0 % moisture) and harvest date were recorded on all experiments. Grain quality as determined by oil and protein (6.25 x N concentration) percentages was analyzed in one third of the experiments. Yield components on the main stem (number of pods m^{-2}, number of grains m^{-2}, thousand grain weight) were not systematically measured. A summary of site-year characteristics and ratings is presented in Table 2.

2.2. Data simulated by the STICS crop model

The STICS crop model (Brisson *et al.*, 2003; 2009) calibrated for soybean (Schoving, 2020) was used in this study to calculate abiotic stress factors (agrophysiological variables), and simulate missing phenology data in observations. The model runs on a daily time-step simulating leaf area index, aboveground biomass, soil water and nitrogen balances driven by daily weather data and soil characteristics.

The total dataset contained 227 simulation units (USM) created from the combination of experimental sites, years and cropping practices (cultivar, water management, sowing date). The training dataset was composed of 105 representative USMs and the remaining 122 USMs were used for validation (Schoving, 2020; Corrales *et al.*, 2022). Data training was built with USMs having a complete set of measurements as regards phenology (dates of R1, R5 and R7 stages), aboveground biomass, Leaf Area Index (LAI), and grain yield. USMs with less variables (no LAI) and less measurement dates were kept for validation dataset. The prediction of grain yield was successfully achieved by a linear regression model based on variables simulated by STICS (Corrales *et al.*, 2022).

Several stress factors calculated by the model and varying between 0 (intense stress) and 1 (no stress) may impact the main ecophysiological processes. The stomatal stress factor (*swfac)* and the turgor stress factor (*turfac)* are the two main water stress indicators affecting transpiration and leaf expansion respectively. They correspond to the ratios between actual and maximum values of transpiration (*swfac*) and leaf expansion (*turfac*). These ratios depend on the available water content in the root zone according to bilinear relationships (Brisson *et al.*, 2009). As expected from Hsiao (1973), relative leaf growth will

be first affected by soil desiccation then relative stomatal conductance will drop for lower water contents.

Theses stresses can have little impact on soybean performance when they act alone but can be reinforced in water-stressed conditions where N uptake is also deficient due to a multiplicative effect. Stresses related to high or low temperatures occurring during grain filling phase (*ftempremp*) may result in yield reduction if the temperatures are higher or lower than the *tmaxremp* and *tminremp* thresholds, 30°C and 5°C respectively. The characteristics of the STICS inputs and outputs (simulated variables) used in this study are presented in Table 3.

Table 1: Soybean cultivars tested in different site-years. Cultivars are ranked from earliest (MG 000) to latest (MG II) ones

Cultivar	Breeder	Registration in France	Maturity group (MG)	Auzeville	Béziers	En Crambade	Mauguio	Mondonville	Rivières
RGT SHOUNA	RAGT 2N	2014	OOO	2017, 2018	-	-	-	-	-
SULTANA	RAGT 2N	2009	OOO	2017, 2018	-	-	-	-	-
ES MENTOR	Euralis Semences	2009	OO	2018	-	-	-	-	-
SIGALIA	RAGT 2N	2008	OO	2018	2010	-	2010	2010	2010
SPLENDOR	Euralis Semences	2007	OO	-	2010	-	2010	2010	2010
SAREMA	RAGT 2N	2003	O	-	2010, 2011, 2012	-	2010	2010, 2011, 2012	2010, 2011, 2012
ES PALLADOR	Euralis Semences	2015	I	2018	-	-	-	-	-
ISIDOR	Euralis Semences	2004	I	2017, 2018	2010, 2011, 2012	2013, 2014	2010	2010, 2011, 2012, 2013, 2014	2010, 2011, 2012, 2013, 2014
SUMATRA	RAGT 2N	2004	I	-	2010	-	2010	2010	2010
SANTANA	RAGT 2N	2007	I/II	2017, 2018	-	2013, 2014	-	2011, 2013, 2014	2011, 2013, 2014
BLANCAS	Caussade Semences	2007	I/II	2017, 2018	-	-	-	-	-
ECUDOR	Euralis Semences	2006	II	2017, 2018	2010, 2011, 2012	2013, 2014	2010	2010, 2011, 2012, 2013, 2014	2010, 2011, 2012, 2013, 2014
FUKUI	Actisem	2002	II	-	2010	-	2010	2010	2010

Table 2: Summary of experiments carried out by site and year. The type of design (SP: Split-Plot, SSP: Split-Split-Plot, B: Block), sowing dates (in Day of Year, DOY), water management (IRR: Irrigated, DRY: Rainfed), soil type (according to Jamagne textural triangle), agronomic observed variables (GY: Grain yield; GNC: Grain nitrogen concentration; Oil: Grain oil concentration), and yield components (Plant, pod and grain numbers m^{-2}, thousand grain weight (TGW)) were reported. In all the experiments, at least the scorings of the phenological stages "emergence" (VE), R1, R5 and R8 were carried out.

Site	Latitude / Longitude	Year	Design	Cultivars tested	Sowing date (DOY)	Water Management	Soil type	Observed variables
Auzeville	43.53/1.48	2017	SSP + B	6	80, 130	IRR, DRY	Clay Loam	GY, GNC, Oil, TGW, pods m^{-2}, grains m^{-2}, plants m^{-2}
		2018	SSSP + B	9	114, 155	IRR, DRY	Silty Clay Loam	GY, GNC, Oil, TGW, pods m^{-2}, grains m^{-2}, plants m^{-2}
Béziers	43.34/3.21	2010	SP + B	7	55, 76, 112	IRR	Loam	GY, pods m^{-2}, plants m^{-2}
		2011	SP + B	3	67, 96, 132	IRR	Loam	GY, pods m^{-2}, plants m^{-2}
		2012	SP + B	3	76, 103, 131	IRR	Silt Loam	GY, GNC, TGW, pods m^{-2}, plants m^{-2}
En Crambade	43.43/1.65	2013	SSP + B	3	74, 115	IRR, DRY	Clay	GY, GNC, Oil, TGW, pods m^{-2}, grains m^{-2}, plants m^{-2}
		2014	SSP + B	3	73, 120	IRR, DRY	Clay	GY, GNC, Oil, TGW, pods m^{-2}, grains m^{-2}, plants m^{-2}
Mauguio	43.61/4.01	2010	SP	7	74, 98, 145	IRR	Clay Loam	GY, pods m^{-2}, plants m^{-2}
Mondonville	43.66/1.28	2010	SP	7	61, 92, 138	IRR	Silt Loam	GY
		2011	SP + B	3	80, 102, 124	IRR	Silt Loam	GY
		2012	SP	3	76, 97, 124	IRR	Silt Loam	GY, GNC, TGW, pods m^{-2}, plants m^{-2}
		2013	SSP + B	3	81, 147	IRR, DRY	Silt Loam	GY, GNC, Oil, TGW, pods m^{-2}, grains m^{-2}, plants m^{-2}
		2014	SP + B	3	126	IRR, DRY	Silt Loam	GY, GNC, Oil, TGW, pods m^{-2}, grains m^{-2}, plants m^{-2}
Rivières	43.91/1.96	2010	SP + B	7	62, 99	IRR	Clay Loam	GY
		2011	SP + B	3	70, 102, 131	IRR	Clay Loam	GY
		2012	SP	3	76, 108, 138	IRR	Clay Loam	GY, GNC, TGW
		2013	SSP + B	3	81, 126	IRR, DRY	Clay Loam	GY, GNC, Oil, TGW, pods m^{-2}, grains m^{-2}, plants m^{-2}
		2014	SSP + B	3	77, 126	IRR, DRY	Clay Loam	GY, GNC, Oil, TGW, pods m^{-2}, grains m^{-2}, plants m^{-2}

Table 3: Input and output variables of the STICS model considered in this study: name, description, range and unit.

Variable	I/O	Description, range	Unit
airg(n)	input	Daily amount of irrigation water	mm.d^{-1}
precip	input	Daily amount of water added to soil (precipitation + irrigation)	mm.d^{-1}
tmax(n)	input	Maximum daily air temperature	°C
tmin(n)	input	Minimum daily air temperature	°C
tmoy(n)	input	Mean daily air temperature	°C
trg(n)	input	Daily global radiation	MJ.m^{-2} d^{-1}
trr(n)	input	Daily rainfall	mm.d^{-1}
chargefruit	output	Number of filling grains	m^{-2}
CNgrain	output	N concentration in grains	% dry weight
huile	output	Oil concentration of grains	% dry weight
mafruit	output	Dry grain yield	t.ha^{-1}
p1000grain	output	Thousand grain weight	g
iflos	output	Date of flowering (R1)	julian day (DOY)
idrps	output	Starting date of grain filling (R5)	julian day (DOY)
imats	output	Starting date of physiological maturity (R7)	julian day (DOY)
irecs	output	Harvest date (H)	julian day (DOY)
inn	output	Nitrogen nutrition index (NNI), 0-2	unitless
innlai	output	Reduction factor of N deficiency on leaf growth based on NNI, from innmin to 1	unitless
inns	output	Reduction factor of N deficiency on biomass growth based on NNI, from innmin to 1	unitless
innsenes	output	Reduction factor of N deficiency on senescence based on NNI, from innmin to 1	unitless
fapar	output	Fraction of the photosynthetic active radiation (PAR) intercepted, 0-1	unitless
ftempremp	output	Temperature-related grain filling reduction factor, 0-1	unitless
swfac	output	Stomatal water stress factor, 0-1	unitless
turfac	output	Turgescence water stress factor, 0-1	unitless

2.3. Ecoclimatic and agrophysiological indicators: typology and calculation method

To classify and characterize environmental situations without taking into account the harvest performance of the cultivars under study, 41 indicators were calculated from daily variables simulated by the STICS crop model, weather data and field observations. They can be assigned to two groups of indicators:

- Ecoclimatic indicators: climatic indicators calculated on each phenological phase (*i.e.* rainfall sums over the emergence-flowering phase).

- Agrophysiological indicators: based on water, nitrogen or thermal stress variables calculated by STICS integrating the soil type and crop management (*i.e.* number of days when water stress exceeds a given threshold).

The two families of indicators were calculated for three phenological phases: 1 - Emergence to Flowering (VE-R1), 2 - Flowering to Beginning of Grain Filling (R1-R5), 3 - Beginning of Grain Filling to Harvest (R5-H). The observed dates of the phenological stages were used to force this calculation when possible. In the case of 'no record', the date simulated by STICS was used. Four ecoclimatic indicators were calculated for each phase: the sum of precipitation (*cum_precip*), the number of days with temperatures above 28,

30, 32°C (*ndays_tmax_n*) or below 2, 4, 6°C (*ndays_tmin_n*), and the sum of global radiation (*cum_trg.n*). A phenological indicator characterizing the duration of each phase (in number of days) was selected (*ndays_phase*). Two indicators derived from the variables calculated by the model were used: the number of days where *swfac* is less than 0.2, 0.4 and 0.6 (*ndays_swfac_n*), a *swfac* value equal to 1 representing an absence of water stress, and the cumulated values of daily fractions of the photosynthetic active radiation intercepted (*cum_fapar)*. The list of indicators and their characteristics are given in Table A (Supplementary Information).

The objective was to represent as completely as possible and with no redundancy the abiotic stress conditions (water, radiation, temperature, nitrogen) that could impact soybean performance.

2.4. Data analysis

2.4.1. Choice of indicators for grain yield explanatory model by PLS regression

A classification of the most important indicators explaining the grain yield at 0% humidity was carried out using PLSR (partial least square regression) with the *pls* package of the R software (version 3.2.2; [R Core Team (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna]). Multicollinearity occurs when independent variables (here the indicators) in a regression model are correlated. PLSR is a very common way to deal with highly correlated explanatory variables in the multiple linear regression.

This regression was carried out on all the phasic indicators calculated for all the individual situations after being centered and reduced. In order to simplify *a posteriori* interpretation, it was decided to finally retain only 4 indicators per phenological phase. The performance of PLSR was evaluated by calculating RMSE (Root Mean Square Error), bias (MBE), efficiency (EFF) and the coefficient of determination (R^2). These model performance indicators and their calculation formula are reported in Table 4.

Table 4: Performance indicators for the evaluation of the PLS analysis and their equations.

Performance Indicator	Equation	Unit
Root Mean Square Error (RMSE)	$RMSE = \sqrt{\dfrac{1}{n}\sum_{i=1}^{n}(\hat{y}_i - y_i)^2}$	Unit of y e.g days, t.ha^{-1}
Mean Bias Error (MBE)	$MBE = \dfrac{\sum_{i=1}^{n}(\hat{y}_i - y_i)}{n}$	Unit of y e.g days, t.ha^{-1}
Coefficient of Determination (R²)	$R^2 = 1 - \dfrac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2}$	Unitless
Efficiency (EF)	$EF = (1 - \dfrac{\sum(y_i - \hat{y}_i)^2}{\sum(y_i - \bar{y})^2}) \times 100$	%

Where y_i is the observed value for the *i* observation, $\hat{y}_i$ is the predicted value and $\bar{y}$ is the average of observations.

2.4.2. Classification of environments

A two-step classification of the individual conditions was performed using the indicators (12 in all) of the three phenological phases selected after the PLSR analysis. It was decided to carry out the classification at the cultivar response level to take into account the phenology of the soybeans from the different maturity groups studied, as this phenology might lead to potentially different durations and intensities of stress for the same location and sowing date (157 "cultivar x site x year x sowing date x irrigation" combinations in all). Initially a hierarchical ascending classification according to Ward's method (Ward and Hook, 1963) after calculating Euclidean distances squared (HCPC function of the *FactoMineR* package) was carried out with the R software to determine the number of classes to be retained. In a second step, this classification was refined with the k-means algorithm (*kmean* package) according to the method of Hartigan and Wong (1979) by setting the number of classes retained in the previous step as the value of k and using the Euclidean squared calculation method for the distances between classes. The

performance of the classification was evaluated by calculating the ratio of the sums of squares between classes to the sum of the total squares.

Each class was characterized by the end-of-cycle observations of each situation. An analysis of variance and a Student Newman Keuls test at *p < 0.01* were performed on these data to highlight differences between classes. These analyses were performed with the R software using the *stat* and *agricolae* packages.

2.5. Analysis of variance of experimental data

The experiments were analyzed individually according to their own experimental design (see Table 2) for 6 agronomic variables: grain yield, thousand grain weight, grain protein concentration, grain oil concentration, pod number m^{-2}, and grain number m^{-2}. The effects of cultivars, sowing dates, water management and blocks were analyzed using R software and the functions *sp.plot* and *ssp.plot* of the *agricolae* package applied to split plot and split split plot devices respectively. As the design implemented in 2018 on the Auzeville site was slightly more complex, we chose to consider the two water management systems (dry and irrigated) separately as split-split plots.

2.6. Simulation of a water stress indicator with STICS in contrasting sites x years: effect of early sowing date on escape from stress

The STICS model was used to simulate the *swfac* indicator at a daily step in the most contrasting "soil x climate" combinations of southern France (3 locations : Hagetmau (Landes), Lat 43.66°N/Long -0.59°E, oceanic climate ; Auzeville (Haute-Garonne), Lat 43.53° N/Long 1.48°E, semi-oceanic climate ; Béziers (Hérault) – Lat 43.35°N/Long 3.25°E, Mediterranean climate) over 28 historical years (1990-2017) and for two levels of available soil water content per site (90 and 180 mm). The objective was to explore the proportion of years where early sowing date (D1 = March 20th) of a GM I soybean cultivar resulted in less water stress in unirrigated conditions ("escape effect") in comparison with two more conventional sowing dates (D2 = April 15th; D3 = May 5th).

3. Results

3.1. Climatic conditions

The climatic conditions experienced during the various "site x year x management" combinations made it possible to cover contrasting situations in terms of temperature, precipitation, and water management. The seasons 2010 and 2011 were particularly dry and warm (climatic water deficit of 702 and 683 mm from March 1st to September 30th), while in 2013 and 2014 the deficit was low (337 and 283 mm). The ombrothermic diagrams for each 'site-year-sowing date' are shown in Figure A (Supplementary Information).

3.2. Effects of crop management on soybean production: highlighting cultivar x crop management interactions

Among the 18 site x year experiments from Table 2, 4 did not have statistical blocks (replications) and could not be analyzed with the ANOVA models. The data produced in Mauguio-2010, Mondonville-2010, Mondonville-2012 and Rivières-2012 were nevertheless used in the following stages of this study to characterize the environments.

The analyses of variance for the split-plots (7 site-years) and split-split-plots (7 site-years) were summarized in Table 5. These analyses showed a most frequent very significant effect (*0.001 < p < 0.01*) of water management (non-irrigated vs. irrigated) on grain yield, thousand grain weight (TGW), grain protein and oil concentrations and number of grains per m² for all cultivars combined. The number of pods per m² was also significantly affected by water regime (*0.01 < p < 0.05*). For all these variables, apart from oil concentration, supplemental sensor-based irrigation increased agronomic performances of soybean crop. The sowing date had a most frequent very significant effect (*0.001 < p < 0.01*) on yield and number of pods per m² and a significant effect (*0.01 < p < 0.05*) on TGW, grain protein concentration and number of grains per m². Conventional sowing resulted more frequently in higher grain yields than early sowing, the latter being more favorable to TGW and grain protein. No systematic effect was observed regarding the number of pods and grains per m². Cultivar choice had a very significant effect (*0.001 < p < 0.01*) on all previous variables except oil concentration and number of pods per m² (*0.05 < p < 0.1*). Later cultivars (MG I to II) increased grain yield, number of grains and pods per m². Early cultivars (MG 000 to 0) had higher TGW and protein levels. Interactions between water management and sowing date were significant for grain yield, protein and oil concentrations, and number of grains per m² (*0.01 < p < 0.05*). Irrigation

coupled with conventional sowing improved grain yield while irrigation combined with early sowing resulted in higher protein levels. The choice of a later cultivar coupled with a conventional sowing date showed a very significant increase in grain yield (*0.001 < p < 0.01*) compared to an early variety. Interactions between water management and cultivar or between the three treatments investigated did not show very significant differences nor any systematic ranking for the agronomic variables.

Table 5: Summary of statistical analyses by site-year for agronomic variables. The effects of different factors and their interactions are reported: WM: Water management, SD: Sowing date, CV: Cultivar. Boxes are colored according to the level of significance most frequently represented in the statistical analyses (14 ANOVA in all) for the source of variation considered. The annotations give the conditions for maximizing the observation concerned, for example "irrigation" allows a very significant increase in "Dry yield" (0.001 < p < 0.01). (-) indicates no clear effect of a factor or factors in interaction.

Observation	WM	SD	CV	SD x WM	SD x CV	WM x CV	SD x WM x CV
Grain dry yield (t ha^{-1})	Irrigation	Conv. sowing	Late MGs	Conv. sowing x Irrigation	Conv. sowing x Late MGs	(-)	
TGW (g)	Irrigation	Early sowing	Early MGs	(-)	(-)	(-)	
Grain protein concentration (%)	Irrigation	Early sowing	Early MGs	Irrigation x Early sowing	(-)	(-)	
Grain oil concentration (%)	Rainfed	(-)	(-)	(-)	(-)	(-)	(-)
Pod number m^{-2}	Irrigation	(-)	Late MGs		(-)	(-)	

Grain number m^{-2}	Irrigation	(-)	Late MGs	(-)	(-)	(-)	

Statistical significance

	(0.001 < p < 0.01)
	(0.01 < p < 0.05)
	(0.05 < p < 0.1)
	NS

These statistical analyses have highlighted various situations that are conducive to early sowing. Table 6 shows the ranking of "site-year-water management" conditions based on grain yield response to early sowing ($p < 0.01$). The 2018 experiment in Auzeville that tested conventional *vs.* late sowing dates was not included in this ranking. However, it should be noted that the irrigated management of this experiment showed better performance in conventional sowing, whereas there was no difference in performance between the two sowing dates in the absence of irrigation.

Table 6: Site-Year-Water Management ranking by grain yield response to early sowing date. In the left and right columns are considered only those situations where there is a significant difference between early and conventional sowing dates ($p < 0.01$), while in the center column there was no significant difference in grain yield between sowing dates. Site-years conducted only under irrigation (IRR) are shown in italics. DRY refers to unirrigated management.

Early sowing > Conv. Sowing	Early sowing = Conv. Sowing	Early sowing < Conv. Sowing
2011_BEZIERS_IRR	2013_EN CRAMBADE_IRR	*2010_BEZIERS_IRR*
2013_EN CRAMBADE_DRY	2013_MONDONVILLE_DRY	*2010_RIVIERES_IRR*
2017_AUZEVILLE_DRY	2013_RIVIERES_IRR	*2011_MONDONVILLE_IRR*
	2014_EN CRAMBADE_IRR	*2011_RIVIERES_IRR*
	2014_RIVIERES_DRY	*2012_BEZIERS_IRR*
	2014_RIVIERES_IRR	2013_MONDONVILLE_IRR

Out of the 18 "site x year x water management" conditions, 8 resulted significantly in lower yields in early sowing (sowing before the beginning of April), 7 showed no difference between the two treatments and 3 showed higher yields in early sowing. These three situations were Béziers-2011 (irrigated), En Crambade-2013 (non-irrigated) and Auzeville-2017 (non-irrigated). No significant difference in yield between early and conventional sowings was observed when these last two sites-years were irrigated. Early sowing increased yield in these two unirrigated "site x year" conditions, all cultivars combined. Conversely, the situations Mondonville-2013 (irrigated), Rivières-2013 (non-irrigated) and En Crambade-2014 (non-irrigated) resulted in lower performance in early sowing while the opposite water management systems did not result in significant differences between sowing dates. The experiment carried out in 2014 at Rivières produced no difference in yield according to sowing dates or water management.

Figure 1 shows the grain yield of 5 cultivars (Sultana, RGT Shouna, Isidor, Santana, Ecudor) as a function of the cropping conditions encountered (site-year-sowing date-water management) ranked by increasing average yield as an indicator of the environment potential. The average yields observed ranged from 1.55 t ha^{-1} for the site with the lowest potential to 4.74 t ha^{-1} for the best one. Situations that did not show significant differences between early and conventional sowing were mostly found in the upper third of the environments (10 situations out of 14). On the other hand, no pattern was found for situations with a significant difference in yield between the two sowing dates.

The cultivar Ecudor showed a higher yield than the other genotypes in Béziers, whatever the pattern. This was not the case for cv Isidor, which yielded less in this site, particularly in 2010. Conversely, this cultivar was superior to the others in Mondonville in 2010. Santana seemed more adapted to high-potential environments since it performed similarly or better in the ten best situations represented. The early varieties Sultana and RGT Shouna were only tested in 2017 and 2018 in Auzeville. These cultivars were able to perform similarly to late varieties in three situations: in 2017 for the conventional sowing date (irrigated and non-irrigated) and in 2018 for the conventional non-irrigated sowing

date. In all other situations, the performance of the early cultivars was lower than the late ones.
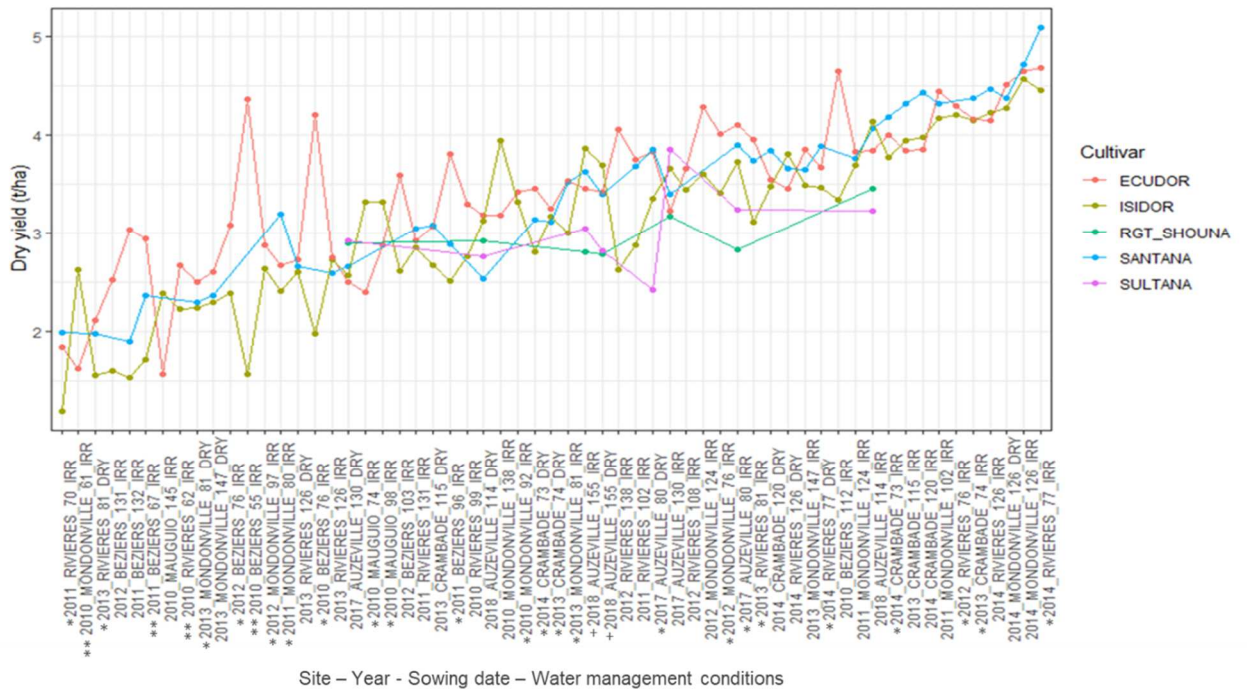

Figure 1: Diagram of grain yields at 0% moisture (t ha^{-1}) for 5 cultivars according to the "site - year - sowing date - water management" situations. The environments were ranked by average yield increasing from left to right. The sowing dates were reported in DOY. (**) Very early sowings (DOY ≤ 69: 10 March); (*) early (69 < DOY ≤ 98: 8 April); ( ) conventional (98 < DOY ≤ 149: 29 May); (+) late (DOY ≥ 150).

3.3. Calibration and evaluation of STICS model for some ecophysiological variables
STICS resulted in good performances for simulating phenology (R1, R5, R7 stages), LAI and aboveground biomass (Table 7). As illustrated for 4 conditions on Figure 2, the level and time-course of aboveground biomass was correctly simulated with STICS and was consistent with the intensity and timing of water stress as indicated by *swfac*.

Table 7 – Performance indicators of the STICS-soybean model for phenology, leaf area index and aboveground biomass (DOY: Day of Year ; n.a = not available). RMSE: Root Mean Square Error (unit of the considered variable), MBE: bias (unit of the considered variable), EF: efficiency (%), R²: coefficient of determination (0-1)

	Calibration				Evaluation			
	RMSE	MBE	EF	R²	RMSE	MBE	EF	R²
R1 date (DOY)	5.3	-0.10	89	0.89	7.0	-3.50	86	0.92
R5 date (DOY)	4.8	-0.26	90	0.90	8.4	-2.39	77	0.80
R7 date (DOY)	8.7	4.74	72	0.82	6.6	4.82	71	0.92
Leaf area index (unitless)	1.39	-0.25	43	0.60	n.a	n.a	n.a	n.a
Aboveground biomass (t ha^{-1})	1.67	0.46	67	0.70	2.12	-0.74	36	0.64
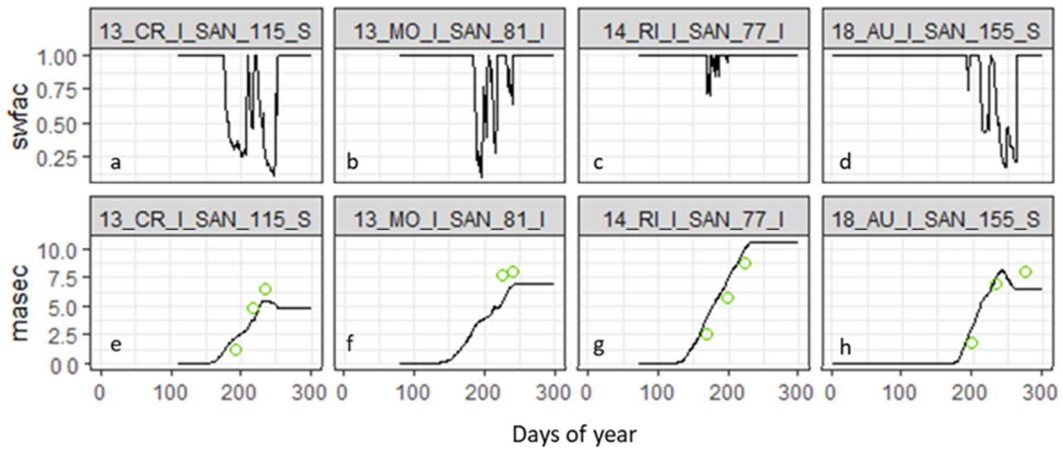
Figure 2: Example of contrasting simulated water stress (*swfac*), aboveground biomass (*masec*, t.ha^{-1}), for cv Santana (GM I/II). Black lines represent model simulations and green circles the field observations. Conditions represented are: En Crambade-unirrigated-2013 (a, e), Mondonville-irrigated-2013 (b, f), Rivières-irrigated-2014 (c, g), and Auzeville-unirrigated-2018 (d, h).

3.4. Selection of relevant indicators for grain yield

The PLSR analysis of the contribution of the 41 indicators to grain yield resulted in an efficiency of 32.4% on the four selected axes (Table 8). This analysis enabled the indicators to be ranked according to their contribution to predicting final performance. The indicators, their description and ranking are shown in Table A in Supplementary Information.

Table 8: Performance indicators of the PLS model. Axes: number of axes retained, RMSE: Root Mean Square Error (q.ha^{-1}), MBE: bias (q.ha^{-1}), EF: efficiency (%), R^2: coefficient of determination (0-1).

Axes	RMSE	MBE	EF	R^2
4	6.49	0.04	32.4	0.33

Following this step of ranking, 4 indicators per phenological phase were kept according to the following decision rule: 1- value of the VIP (Variable Importance in Projection), 2 - removal of "duplicate" indicators: thus, for example, if the number of days where Tmax > 28°C is retained, then the equivalent variable for Tmax > 30°C was not taken into account if it was ranked lower in the classification, 3- the duration of the three phases in days were kept by default. The selected indicators and their characteristics are presented in Table 9. The values of *fapar* and the duration (in days) of each phase were retained for the 3 phases considered namely Emergence-Flowering (VE-R1), Flowering-Beginning of grain filling (R1-R5), Beginning of grain filling-Harvest (R5-H). The intensity of water stress *swfac* was retained for phases R1-R5 and R5-H through the number of days where *swfac* < 0.2. The temperature-related indicators selected for each phase depended on their respective impacts. The number of days where Tmin is less than 2°C was used only for the first phase VE-R1, the number of days where Tmax exceeds 32°C impacted the first two phases VE-R1 and R1-R5 and the last phase R5-H was characterized by the number of days where Tmax is greater than 28°C.

Table 9: Selected indicators after classification by PLSR. The indicators are classified by phenological phase and by decreasing VIP (Variable Importance in Projection). Emergence-Flowering (VE-R1), Flowering-Beginning of grain filling (R1-R5), Beginning of grain filling-Harvest (R5-H).

Phase	Indicator	Type	Unit	VIP
VE_R1	cum_fapar_VE-R1	agrophysiological	Unitless	1.71
	ndays_phase_VE-R1	agrophysiological	Days	0.91
	ndays_tmax_32_VE-R1	ecoclimatic	Days	0.97

	ndays_tmin_2_VE-R1	ecoclimatic	Days	0.90
R1_R5	cum_fapar_R1-R5	agrophysiological	Unitless	0.85
	ndays_phase_R1-R5	agrophysiological	Days	0.29
	ndays_swfac_0.2_R1-R5	agrophysiological	Days	1.04
	ndays_tmax_32_R1-R5	ecoclimatic	Days	0.88
R5_H	cum_fapar_R5-H	agrophysiological	Unitless	1.19
	ndays_phase_R5-H	agrophysiological	Days	1.40
	ndays_tmax_28_R5-H	ecoclimatic	Days	1.78
	ndays_swfac_0.2_R5-H	agrophysiological	Days	1.39

3.5. Classification of environments according to indicators

The Hierarchical Clustering on Principal Components (Figure 3) resulted in a final number of classes of 5 (from 157 genotypes x environments). The second classification by k-means resulted in a percentage explanation of the variation between classes of 43.1%. The first 3 axes of the Principal Component Analysis of "genotype x site x year x management" explained 57.6% of the variation in environments. The summary of individual agronomic situations contained in each class is shown in Table B in Supplementary Material.
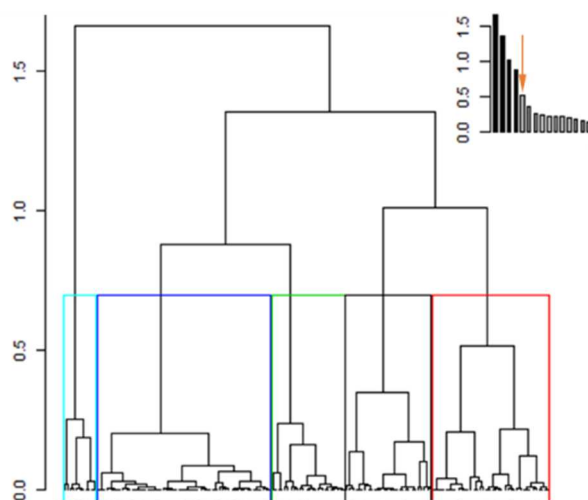
Figure 3: Graphical representation of the hierarchical ascending classification of environments prior to analysis by the k-means algorithm.

Each class was characterized by the center-reduced values of the 12 indicators used for classification and by the mean values of the field observations in each agronomic situation concerned. This information was displayed in Figure 4.

- Class 1: Water stress and high temperatures in the reproductive phase. This class is characterized by intense water stress during phases R1-R5 and R5-H but also by high temperatures (Tmax > 28°C) at the end of the cycle. The R5-H phase was also longer in this class, which increased the cumulative *fapar*. The yield, number of pods and grains per m², and oil concentration of the seeds were negatively impacted by these conditions, with class 1 showing significantly lower performance for *p < 0.01* with the SNK test. On the contrary, the TGW and grain protein were increased. This class also had the lowest number of plants per m². Class 1 includes 13 situations from Mauguio and Mondonville from the seasons 2010 and 2013 (10 situations out of 13), all sowing dates combined and for mostly irrigated situations.

- Class 2: Water stress during flowering and high temperatures at the end of the cycle. Water stress during the R1-R5 phase was more frequent and more intense in this class than in the others, resulting in a decrease in cumulative *fapar* on R1-R5. The number of days with Tmax > 28°C at the end of the cycle was slightly above average. The conditions in this class also affected grain yield, protein content and TGW, as this class ranked last in the SNK test for these variables. Class 2 includes 12 situations and is largely dominated by the Béziers eastern site (10 situations) from 2010 to 2012. All situations included in this class were irrigated and a range of sowing dates was practiced.

- Class 3: "Average" situations, low water stress. In this class, the values of the centered and reduced indicators were all around 0. This is the class with the highest number of situations with a total of 61. In this class, the highest values were observed for yield, number of grains per m², TGW and oil concentration. The sites represented in this

class are mainly Rivières, Mondonville and En Crambade. Most of the situations were irrigated, except in 2014, which was particularly rainy, especially at the end of the season.

- Class 4: Cold at the beginning of the cycle, low water stress. This class is characterized by cool temperatures during the VE-R1 phase, which increased the duration of this phase and thus the cumulative *fapar*. Abiotic stresses were low, with values around 0, but the R1-R5 phase was relatively warm. Yield, number of grains and pods per m², oil concentration and TGW were enhanced by these conditions. This class, comprising 37 situations, is particularly indicative of early sowing dates, regardless of water management. However, some conventional sowing dates (En Crambade-2013 and Rivières-2014) were assigned to this class as rather cold temperatures were observed in springs 2013 and 2014.

- Class 5: Warm at the beginning of the cycle, low water stress. The number of days with temperatures above 32°C on phases VE-R1 and R1-R5 was the highest in this class. Water stress remained in the average and the high temperature episodes at the end of the cycle were lower than in the other classes. The yield was slightly lower than in classes 3 and 4, despite the same number of grains per m². The TGW and the oil concentration of the grains were impacted by the conditions experienced by the plants in this class. Composed of 34 situations, class 5 mainly includes late sowing dates, all water management combined. However, 4 situations in 2013 correspond to early sowing dates (Mondonville and Rivières sites).
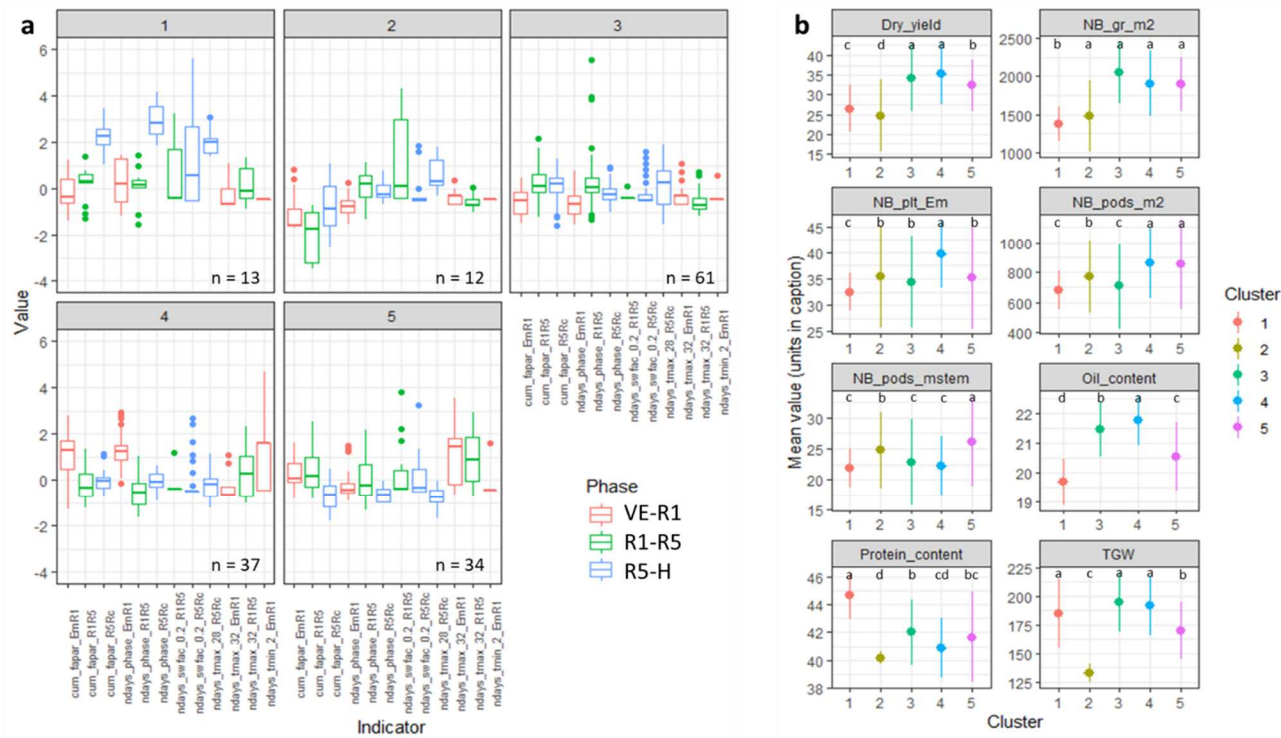
Figure 4: Graphical representation (a) of the centered-reduced values of the indicators characterizing each class for the different phases of the cycle VE-R1, R1-R5 and R5-H. The number of situations making up each class has been plotted on the corresponding graph, (b) the mean field observations and their standard deviation for each class. Dry_yield: Grain yield (q ha^{-1}, 0 % moisture), NB_gr_m^2: Number of grains per m^2, NB_plt_Em: Number of plants per m^2, NB_pods_m2: Number of pods per m^2, NB_pods_mstem: Number of pods on the main stem, Oil_content: Seed oil concentration (%), Protein_content: Seed protein concentration (%), TGW: Thousand Grain Weight (g). The overall effect of the clusters on the observed variables is very significant at the threshold p < 0.001. Means with a common letter are not significantly different for p < 0.01 according to the Student Newman Keuls test.

3.6. Simulation of swfac, fapar and Qfix for different environmental classes

Figure 5 shows the dynamics of the water stress indicator *swfac*, the fraction of absorbed radiation *fapar* and the amount of nitrogen symbiotically fixed Qfix for the cultivar Ecudor in 5 representative situations from the 5 previously determined classes.

The first class, represented by the first column, shows significant water stress beginning at flowering (red dotted line) and persisting until harvest despite several irrigation applications. This stress impacted *fapar* with a drop between the onset of flowering and the beginning of grain filling (red and green dotted lines). This sharp decrease was due to the reduction of green leaf area index after R1. When water stress is severe, senescence of older leaves at the bottom can occur dramatically followed by leaf growth at the top of the plant when the constraint is raised.

Symbiotic N2 fixation was markedly impacted by these conditions from flowering onwards with a maximum of 150 kg ha^{-1} of nitrogen fixed. However, the grain protein concentration was still high in this class. Coupled with a low TGW, this suggests that symbiotic fixation was sufficient to accumulate N in grains, which also benefited from a concentration effect due to low yield.

For class 2, *fapar* was decreased on the R1-R5 phase just after an intense water stress episode around the flowering stage. These conditions limited symbiotic N2 fixation to a maximum of 200 kg ha^{-1}. Protein content and TGW were the lowest ones in this class. Therefore, it can be assumed that poor radiation interception during both R1-R5 and R5-H phases was strongly detrimental to grain filling and nitrogen concentration, as it was the main difference with class 1.

Classes 3 and 4, which had the best performance in terms of grain yield and TGW, were the least impacted by water stress during the R5-H phase, during which nitrogen remobilization towards the grains takes place. Nitrogen fixation and *fapar* were not limiting in these situations with N fixed of 300 kg ha^{-1} and *fapar* close to 0.9 between R1 and harvest. This resulted in high yield and oil concentration values while protein concentration was reduced by a dilution effect.

In the class 5 environment, cv. Ecudor experienced water stress during the grain filling phase (R5-H). This water limitation did not influence *fapar* but the accumulation of Qfix was temporarily stopped between R5 stage and harvest. This late water stress had a direct

impact on grain filling and consequently on TGW and oil concentration which were reduced as compared to classes 3 and 4.
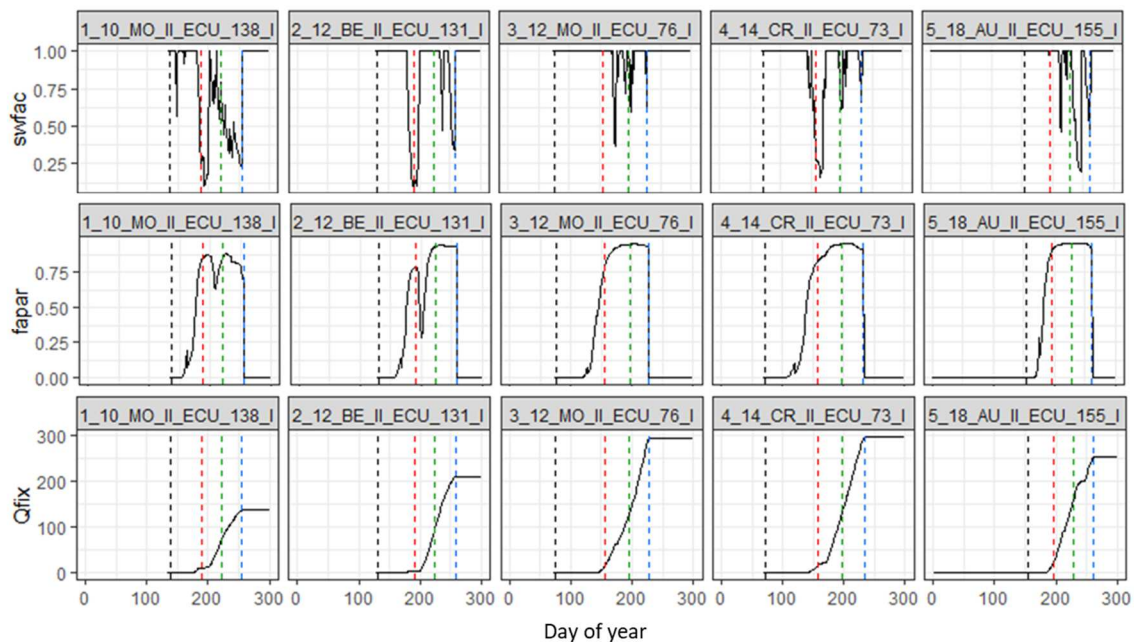

Figure 5: Dynamics of simulated *swfac*, *fapar* (unitless) and symbiotic fixation Qfix (kg N ha^{-1}) for the cultivar Ecudor (MGII) in 5 representative situations of the previously defined environment classes, arranged in ascending order from left to right, the first number in the code being the class number. The vertical dotted lines represent the different phenological stages. Black: sowing, red: flowering, green: beginning of grain filling, blue: harvest. The individual situations represented are from left to right (DOY, day of year): Mondonville-2010-sowing DOY138-irrigated, Béziers-2012-sowing DOY131-irrigated, Mondonville-2012-sowing DOY76-irrigated, En Crambade-2010-sowing DOY73-irrigated, Auzeville-2018-sowing DOY155-irrigated.

3.7. What explains the good performance of early sowing in some situations ?

Figure 6 displays the dynamics of the *swfac* water stress indicator for 4 cultivars belonging to contrasting maturity groups (Sultana-MG 000, Isidor-MG I, Santana-MG I/II, Ecudor-MG II) and grown in Auzeville-2017 without irrigation. Significantly better yield was observed for early sowing (21 March) compared to conventional sowing (10 May). Regardless of the genotype investigated, water stress was more pronounced on the two phases R1-R5 and R5-H for the early sowing date, whereas it was concentrated on the R5-H

phase for conventional sowing. In the case of early sowing, the duration and intensity of water stress was greater on the R1-R5 phase and as intense but shorter during the R5-H phase. For conventional sowing, water stress was relatively short and low on phase R1-R5 but was very pronounced during the R5-H filling phase, both in intensity and duration. The duration of water stress and its intensity showed an increase with the maturity group (from left to right), especially for the conventional sowing date. Shifting the cycle in early sowing made it possible to avoid the water deficit starting on day 200, with harvesting around day 220 versus 250 in conventional sowing.
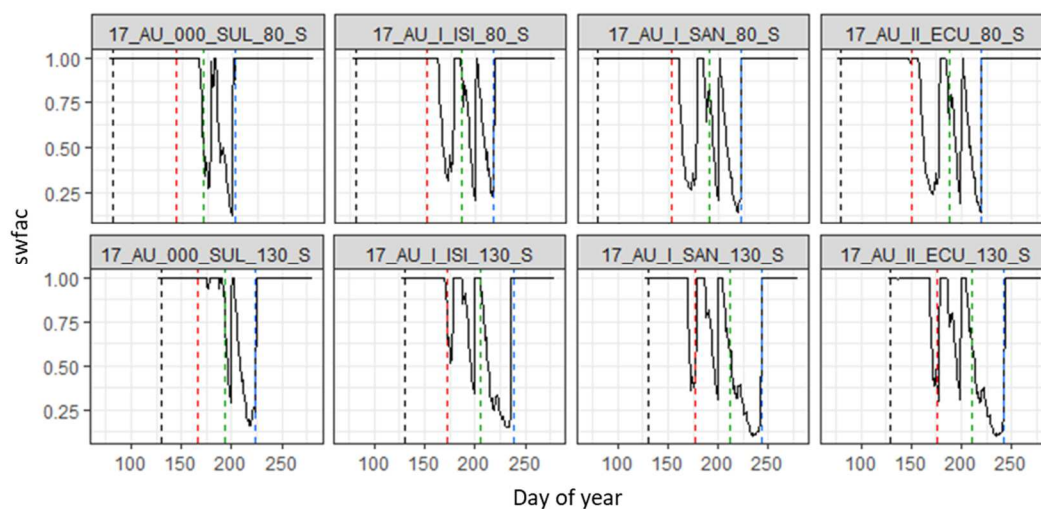
Figure 6: Dynamics of the *swfac* water stress indicator for 4 cultivars (Sultana-MG 000, Isidor-MG I, Santana-MG I/II, Ecudor-MG II) in the Auzeville experiment in 2017 conducted without irrigation. The first row corresponds to early sowing (March 21, DOY 80) and the second to conventional sowing (May 10, DOY 130). The vertical dotted lines represent the different phenological stages. Black: sowing, red: beginning of flowering, green: beginning of grain filling, blue: harvest.

When early sowing resulted in significantly higher grain yield (Table 6), water stress as indicated by *swfac* was more intense (low values) in conventional sowing either during R1-R5 or R5-H or both. When conventional sowing was more performant, it was systematically associated to lower water stress levels during one or two growth periods. When similar yields were observed, no difference in stress intensity was simulated between the two sowing dates. This generally corresponded to situations where water was not yield-limiting

with the exception of 2013_Mondonville_DRY where water stress was high for both sowing dates.

3.8. When is it worth sowing an early maturing cultivar ?

In some situations, no significant difference in yield was observed between early and late cultivars. This was the case of Auzeville-2017 for the conventional sowing date (May 10) and for Auzeville-2018 for the non-irrigated crop at a conventional sowing date (April 24). Figure 7 shows the dynamics of the *swfac* water stress indicator for the cultivars Sultana (MG 000) and Ecudor (MG II) in these situations. In all three situations, Sultana experienced less water stress than the cultivar Ecudor, both in intensity and duration. The early cultivar was only impacted by severe water stress during the late grain-filling period (R5 to harvest), so pod and grain numbers, which are set up during the R1-R5 phase, were not affected. On the contrary, cv. Ecudor was subject to water stress throughout the reproductive phase, with particularly long and intense water stress from the beginning of the grain-filling phase. This timing of the stress had a direct influence on TGW which was depressed.
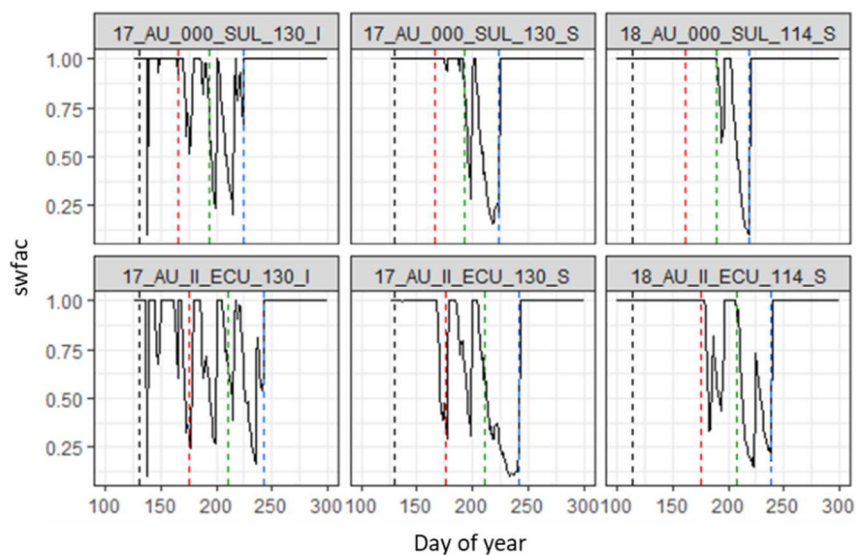
Figure 7: Graphical representation of the water stress experienced by the cultivars Sultana (MG 000) and Ecudor (MG II) for three situations where yields were not significantly different between early and late cultivars. The situations represented are from left to right: Auzeville-2017-sowing DOY130-irrigated then non-irrigated, Auzeville-2018-sowing DOY114-non-irrigated. The vertical dotted lines represent the different phenological stages. Black: sowing, red: flowering, green: beginning of grain filling, blue: harvest.

3.9. Virtual evaluation of the water stress escape strategy with early sowing

The *swfac* water stress indicator was simulated over 28 years with STICS for three sowing dates in 6 virtual environments (location x soil) from southern France. Table 10 reports for each of these 6 environments the percentage of years where the number of days with *swfac* < 0.4 was minimum for early sowing, considering either R1-R5, R5-Harvest or R1-Harvest phases. The higher this percentage, the most efficient would be early sowing for escaping water stress as was suggested in § 3.7 for selected agronomic conditions. As expected, water stress (as indicated by *swfac*) increased from Oceanic to Mediterranean climates and was higher for shallow soils (ASWC = 90 mm).

The frequency of years escaping water stress through early sowing was globally higher in soils with low water availability (90 mm). In such conditions, early sowing was the most efficient for reducing the number of stress days especially during the R1-R5 period. In deep soils (180 mm), the escape strategy was less efficient except in Béziers and water stress was reduced during R5-H period in a greater proportion than in shallow soils, as water deficit was delayed by higher initial soil water content.

In order to give an overview of these results, the percentage of years where early sowing was the most efficient strategy for water stress escape was plotted against the mean water stress indicator (*swfac*) over R1-H period. We can conclude clearly that the success of early sowing for escaping water stress increases with the intensity of the stress (Figure 8).

Table 10. Frequency of water stress escape by early sowing (%) over 28 historical years (1990-2017) for three locations (Hagetmau, Auzeville, Béziers), two available soil water content (90, 180 mm) and three phenological phases (R1-R5, R5-H, R1-H). The locations have been chosen to represent the diversity of soil x climate conditions in southern France. Here, the water stress indicator for each location x soil combination and each phase was the number of days with *swfac* below 0.4. Three sowing dates: early sowing date (March 20th) and two more conventional sowing dates (April 15th; May 5th).

Location	Climate	Available soil water (mm)	Mean number of days (R1-H) with swfac < 0.4 (3 sowing dates, 28 years)	% of years where water stress was minimum for early sowing		
				R1-R5	R5-H	R1-H
Hagetmau	Oceanic	90	22.6	54	18	36
Auzeville	Semi-oceanic	90	29.6	61	29	45
Béziers	Mediterranean	90	34.9	36	36	36
Hagetmau	Oceanic	180	10.6	4	11	7
Auzeville	Semi-oceanic	180	22.0	18	29	23
Béziers	Mediterranean	180	34.0	71	21	46
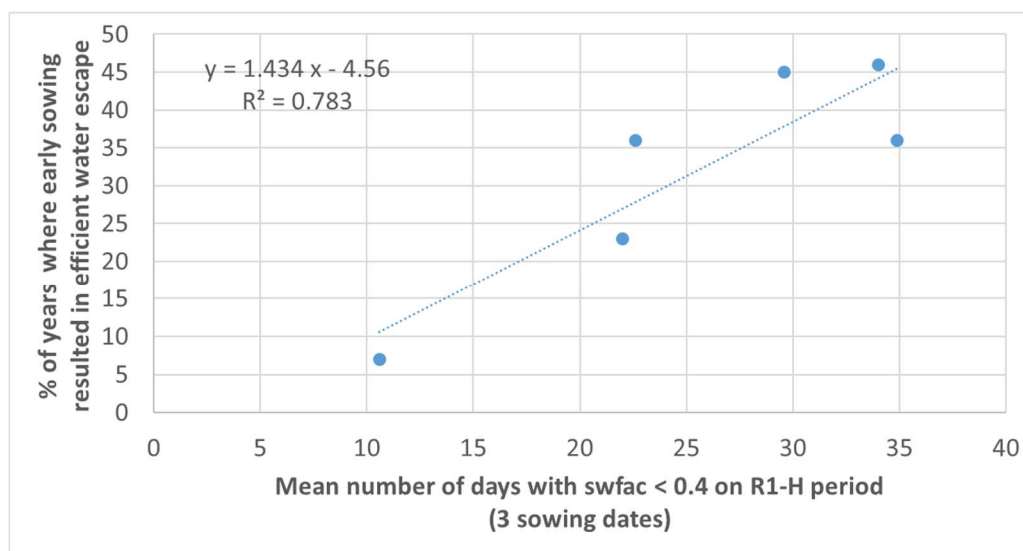
Figure 8 - Relationship between the percentage of years where early sowing resulted in efficient escape from stress and the mean intensity of water stress from R1 to harvest in 6 site x soil conditions : simulation with STICS on Hagetmau, Auzeville and Béziers (1990-2017) for 2 available soil water content (90, 180 mm).

4. Discussion

In this study, we investigated the influence of pedoclimatic environments and crop management on the performance of soybean cultivars belonging to maturity groups commonly grown in Europe. The analysis was not performed with an AMMI model specific to the study of G x E x M interactions (Sudarić *et al*., 2006) but by site-year analyses depending on the lay-out employed. Indeed, the experimental designs and cultivars investigated were not common to all site-years, which complicated the use of this type of

statistical tool. The aim of the experiments carried out in this study was mainly to understand the effects of sowing date on soybean performance, by testing contrasting varieties and water management coupled with a diagnosis of G x E x M interactions via a crop growth model. Thus, a "complete" phenotyping (numerous traits, with dynamic measurements) on a limited number of phenotyping platforms was preferred to the implementation of a large multi-local network. Dynamic crop models are now complementary to the "statistical" methods used to understand and predict the performance of genotypes in various environments (Van Eeuwijk *et al*., 2016 ; Boulch *et al*., 2021). Contrasting water management (with or without irrigation) from 2013 onwards has however confirmed the importance of water input for soybean performance, with this treatment having the most significant effect ($p < 0.001$) on the different yield components investigated. This effect of irrigation has already been widely demonstrated in the literature (Korte *et al*., 1983a ; Korte *et al*., 1983b; Karam *et al*., 2005; Montoya *et al*., 2017).

The classification of a date in early sowing brings together situations of very early and early sowing (~1.5 months to 1 month compared to the conventional sowing period) since, depending on the sites, it was possible to plant more or less early. In Béziers, for example, where soil warming was earlier, humidity lower and soil texture sandier, the first sowing was possible at the end of February, whereas in En Crambade, where the soil is clayey, sowing was not possible until mid-March on average. Generalizing yield responses to sowing dates, cultivars, and water management from individual statistical analysis of each experiment would result in a loss of information. Indeed, the method we used was able to aggregate together situations presenting different levels of significance of a management on a crop performance variable, even if grouping by classes of significance (*i.e*. 0.01< p <0.1) made it possible to limit this problem. There is no universal answer on the effect of early sowing on soybean yield, particularly due to the water stress dynamics experienced by the cultivar during reproductive period. Nevertheless, it was possible to identify situations conducive to early sowing, which occurred before the beginning of April. These situations, encountered in 2017 at Auzeville and in 2013 at the En Crambade site, were both unirrigated. In these two situations, early sowing, compared to conventional sowing, avoided severe stomatal water stress from the beginning of the seed filling phase, this phenological phase (R5 - R6) being the most sensitive to water shortage in soybean (Pardo *et al*., 2015). Therefore, this suggests the interest of early sowing of soybean for escaping water stress conditions as was also

reported by Di Mauro *et al*. (2019). The use of STICS on historical climatic data confirmed the interest of this strategy in the most water-limited environments.

The indicators calculated from climatic data or simulated by the STICS model were selected on the assumption that water stress, temperature and intercepted radiation were the factors that had the greatest impact on soybean performance. The PLSR results on these indicators showed 33 % explanation of yield variation. Critical temperatures based on phenological phases were identified. For example, values of air temperature above 28°C during the grain-filling phase had a negative impact on grain yield. We expected critical temperature close to this threshold during grain filling for soybeans (Egli and Wardlaw, 1980; Kumagai and Sameshima, 2014; Zhang *et al*., 2016). Lower critical temperatures (< 28°C) for grain filling phase have also been reported for some soybean cultivars (Choi *et al*., 2016). The temperatures above these thresholds are very harmful for grain yield, as the slope of the decline above the optimum is significantly steeper than the incline below it (Schlenker and Roberts, 2009). Among the 41 indicators initially calculated, we finally selected 12 variables to classify and characterize cropping situations (cultivar x sowing date x water management x location x year). The performance with these 12 indicators was satisfactory since the inter-class variation was 43.1%. The classification was deliberately based on individual agronomic situations that integrate the varietal dimension in order to take into account this effect and not only the environmental effect as has been done (Löffler *et al*., 2005 ; Chenu *et al*., 2011). Thus, the classification takes into account the phenology of soybean cultivars belonging to different maturity groups and therefore the positioning of sensitive phases. The 5 selected classes were characterized by the calculated indicators and the values of field observations. Classes 1 and 2, despite supplemental irrigation, showed the highest levels of stomatal water stress (*swfac*) and the lowest yields. The low irrigation volumes (around 20 mm) and their staggering in time did not allow the crop to escape the water deficit already well established in these situations. Irrigation at the beginning of the cycle also contributed to producing a higher biomass (from 6 to 10 t ha^{-1}), which further increased water requirements. Water stress impacted the functioning of symbiotic activity by limiting the amount of nitrogen fixed at less than 150 kg N ha^{-1}, mainly for the earliest cultivars. This constraint directly impacted grain filling and thus the final grain yield. The decrease in yield was accompanied by an increase in grain protein concentration and a decrease in oil

concentration in class 1. In a meta-analysis, Rotundo and Westgate (2009) reported that protein accumulation would be less affected than was oil accumulation by water deficit.

This confirms the sensitivity of soybeans to water deficit, particularly during the grain filling phase (Pardo *et al*., 2015 ; Farooq *et al*., 2017; Anda *et al*., 2020 ; Boulch *et al*., 2021). These environments could be of particular interest for a drought-tolerant cultivar selection. It would be interesting to simulate these situations under non-limiting irrigation to estimate the achievable yield of cultivars in these site-years.

Contrary to expectations, low temperatures at the beginning of the cycle (below 2 °C during the VE-R1 phase) did not have a negative impact on grain yield (class 4 close to class 3, without major stress). The hypothesis that early sowing would decrease the number of grains per unit area due to lower photosynthetic performance as related to low temperatures in the early part of the cycle (Maury *et al*., 2015) was not verified. This reduction in photosynthetic efficiency could be compensated by an increase in the amount of radiation intercepted by the canopy via an increase in the duration of the R1-R5 phenological phase. Indeed, the number of grains and the TGW of class 4 were at the same level as those of class 3. Since these classes were made from the 3 cultivars present in all experiments, it was not possible to determine an environment more suitable for the performance of one specific cultivar. However, in the site-year-management trials, the cultivar Santana showed better performance in high-potential situations (Figure 1), while the cultivar Ecudor was particularly well adapted to the Mediterranean conditions in Béziers, where water management and thermal constraints were higher.

The use of the STICS crop model to characterize *a posteriori* the abiotic stresses experienced by the cultivars in different cropping situations allowed to validate the impact of water stress (here through the stomatal stress indicator *swfac*) on the establishment of yield components or other processes such as symbiotic fixation. It is the timing, intensity and duration of this stress that determine the final performance of a cultivar for a given sowing date, as severe water stress from pod filling onwards is very detrimental to soybean yield establishment. Thus, situations allowing early sowing to achieve higher yields than conventional sowing (Auzeville 2017 and non-irrigated En Crambade 2013) resulted in stress profiles that were out of step with the conventional date. The hypothesis of escaping water stress in early sowing was therefore validated, especially for late cultivars that have a longer reproductive phase than the early ones. However, the effect of water stress on leaf

senescence as simulated by STICS was probably too strong resulting in an overestimation of both leaf area index and *fapar* decrease.

The same response to water stress was demonstrated in situations where early cultivars reached the same yields as late cultivars (Auzeville 2017 non-irrigated). Indeed, it was the intensity and duration of water stress, which was lower than that experienced by a late cultivar, that enabled these early cultivars to achieve similar yields. The early cultivars only performed satisfactorily in conventional sowing, which can be explained by the more determinate nature of the crop, which means that cold-related flower abortion early in the cycle (Ohnishi *et al.*, 2010) cannot be caught up on the upper nodes later on, as is the case for the more indeterminate late-maturing cultivars. However, this is only a first step in understanding the response of soybean to environmental situations. Indeed, the choice of the "determinate" growth formalism in the calibration of soybean with STICS instead of the "indeterminate" formalism does not allow any visualization of the establishment of organs (pods), nor the source-sink relationships that result from the competition for carbon resources in the case of indeterminate flowering. In this study, grain yield depends on biomass accumulation only. However, it would be interesting to predict the implementation of yield components according to the photo-thermal conditions induced by a shift in sowing date to envisage adapted "cultivar-sowing date" combinations. An extension of the R1-R5 phase would, for example, makes it possible to increase yield components in favorable situations (Kantolic and Slafer, 2001). This type of study would require a new calibration of the STICS model by activating the formalisms "indeterminate" and "impact of stress on development" (acceleration of development rate in case of water stress). Before such a calibration, simulating the response of a cultivar from a later maturity group (MG III) would allow to test the interest to lengthen the crop duration with early sowing.

It will also be possible to calibrate the STICS model for each cultivar studied and not by maturity group to improve the sensitivity of the analyses. The possible response of cultivars to water deficit could be adjusted from the water stress response parameters (e.g. *psiturg* and *psisto* for critical water potentials of cell expansion and stomatal closure, respectively) to better represent the varietal response in different environments.

5. Conclusions

The objective of this study was to evaluate and understand the effect of various sowing dates (with a focus on early sowing) on the performance of soybean cultivars grown in contrasted water conditions. This understanding was based on a statistical analysis of cultivar performance by field experiments coupled with environmental characterization through agronomic modeling. This combined analysis made it possible to finely characterize the pattern of stress events experienced by the plants during the different phenological phases. In most cases, conventional sowing performed better than early sowing due to the photo-thermal conditions encountered by the plants. In fact, in the case of conventional sowing, even if the development is slowed down by lengthening photoperiods, the temperatures are sufficient for growth to proceed at a satisfactory rate. In the case of early sowing, on the contrary, we observe plants at R1 having accumulated less biomass since the temperatures encountered are sub-optimal (colder) and the short photoperiods accelerate the development of the plant. This effect may be surprising for new soybean growers. However, early sowing proved to be interesting in situations presenting severe water stress during the grain filling phase. Indeed, by anticipating crop development, grain filling occurred in less limiting situations and drying out was accelerated after the R8 stage (maturity). This strategy of escaping water stress through early sowing seems to be more valuable for late vs. early cultivars. Heat stress during grain filling should not be neglected for soybeans, despite the thermophilic nature of this species. A temperature above 28°C strongly impacts soybean performance with depressive impact on final TGW. In-depth studies of the implementation of yield components (number of nodes, pods, and seeds) will be necessary to identify possible soybean cultivation strategies in water-constrained situations. These strategies may involve adapting sowing date, maturity group and water management in a consistent way.

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