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High throughput field phenotyping (HTFP) of wheat and weed cover in field experiments using RGB images: assessment of crop-weed competition with a simple ecophysiological model

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Abstract

The early stages of growth for two winter wheat cultivars, Apache and Rubisko, were studied in field experiments based on destructive measurements and visible images. They cover the period from the three-leaf stage to tillering at four sampling dates. Maps of fractional vegetation cover (FVC) were established for both the crops and weeds. FVC was automatically determined from the images with an SVM-RBF classifier, using Bag of Visual Words vectors as inputs. The heterogeneity in populations and crop-weed competition were studied using descriptive and inferential statistics. The impact of weeds on crops was evaluated by comparing the results with simulations under unstressed conditions.

Keywords: competitiveness, weed, biomass, wheat, model.

Introduction

In the next few decades, the issues of chemical input reduction and climate change will require producers to pay particular attention to the regular and precise monitoring of crop growth. Biomass (BM) is one of the key variables in precision agriculture for crop management. It also enables addressing the negative impact of harmful organisms on crop growth by stress detection. Many remote sensors have been used so far to monitor plants over time for crop protection management (Weiss *et al.*, 2020). The emergence of artificial intelligence techniques such as machine learning (Suh *et al.*, 2018) and deep learning (Sa *et al.*, 2018), have also proved to be very efficient for locating weeds in cereals for site-specific weed management, allowing high-throughput field phenotyping (HTFP). Thus, digital image approaches have become an alternative solution for deducing plant biomass, especially at early growth stages (Casadesús and Villegas, 2014). However, to develop decision support tools, image data is no longer sufficient to make predictions. Additional information -for example, information provided by ecophysiological models- is needed. The main objective of this paper was the assessment of crop-weed competition using digital tools, combining images with a simplified ecophysiological model (Jeuffroy and Recous, 1999). It focuses on the estimation of crop biomass from a high-resolution visible imaging system to assess crop-weed competition (Caussanel, 1989). The first part describes the acquisition of data on two winter wheat cultivars, Apache and Rubisko, on four different dates. Then, an image-processing algorithm for crop-weeds discrimination is presented; this leads to the creation of vegetation maps (wheat vs. weeds) that help us to better understand crop-weed

competition through the weed pressure (WP) indicator. An ecophysiological model predicting wheat growth and identifying potential stress is also presented. Heterogeneity in populations and crop-weed competition was evaluated using descriptive statistics. In the second part, the results are analyzed and discussed to establish the portion of the wheat stress related to weeds. The potential of visible images to detect the presence of such a stress is discussed. The maintenance of a host population (i.e., weed) in a crop below a nuisance threshold through non-destructive measurements is a major challenge for spatio-temporal crop monitoring.

Materials and methods

Field experimental site: The study site was located in Dijon, Burgundy, France (47°18'32"N, 5°04'0.165" E). The experiment took place on micro-plots with a chalky-clay deep soil. Two cultivars (Apache and Rubisko) of winter wheat (*Triticum aestivum* L.) were sown on November 12, 2017, at a density of 345 seeds/m² on two plots of 15m × 1.20m (total area = 18 m²), each with 7 rows of plants spaced 15 cm apart. The experiments were conducted in 2018 during the early stages of wheat growth, from the three-leaf stage to tillering on four sampling dates. No nitrogen fertilizer was applied and weeds were not controlled. The most frequent species found were annual dicots and perennial dicots, which are not representative of wheat crops. The weed stand was considered as a single class in the supervised classification.

Data collection: Wheat and weed plants were characterized from RGB images acquired with a spatial resolution of 0.2 mm/pixel and from destructive measurements performed at four different dates spanning from mid-March to mid-April at the end of the vegetative stage (Figure 1). The plots were divided into four subplots representing 25% (area 1), 25% (area 2), 15% (area 3), and 35% (area 4) of the total area for the Apache cultivar. Each date corresponded to destructive measurements performed on a 0.342 m² quadrat with three replicates (R1 to R3).

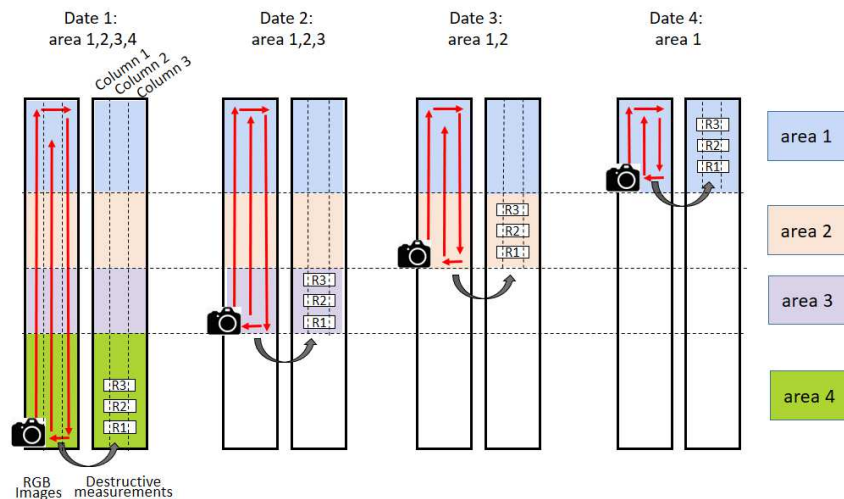


Figure 1. Experimental protocol applied to image and destructive measurements. The size of the plot (number of areas) decreased over time because of the destructive samplings. Area 1 is common to all dates.

Three variables were studied at each sampling date and for each stand: density; leaf area index (LAI), measured using a planimeter; and the dry matter biomass of aerial plant parts (BM, $\text{g}\cdot\text{m}^{-2}$). The wheat and weed plants were collected separately before to be weighed after oven drying at 80°C for 48h. Agronomists, experts in weed flora from the french national research institute for agriculture, food and the environment (INRAE), carried out the weed identification. For each date, the two plots were photographed using a Canon EOS 450D (Canon Inc., Tokyo, Japan) camera fixed vertically on a movable sensing platform made of PVC pipes 1 m above ground level so that each photo exactly covered one quadrat. As for a UAV platform, an orthomosaic photo of the plot with a $\sim 60\%$ overlap between successive images and a $\sim 40\%$ overlap between passes (3 passes = 3 columns) was constructed. Image Composite Editor (Version 2.0.3.0, 2015, Microsoft Corporation, Redmond, WA, USA), an image stitcher program, to create a panoramic image was used. Depending on the date, the image database ranged from ~ 250 images for date 1 to ~ 50 images for date 4. The daily photosynthetically active radiation (PAR, $\text{MJ}\cdot\text{m}^{-2}$) was measured by amorphous silicon sensors. These measurements are required as input data for the plant-growth model.

Image analysis: Before the use of a supervised crop/weed classification method, a pre-processing stage is required to discriminate between soil and vegetation based on a new vegetation index called MetaIndex, which is defined as a vote of six indices widely used in the literature (Gée *et al.*, 2020). The image processing for crop/weed discrimination used machine learning classification (support vector machine-radial basis function (SVM-RBF)) combined with the Bag of Visual Words technique. The training data set corresponded to 85% of the total dataset and comprised of 3841 thumbnail images for each class of plant. All the algorithms were implemented in Matlab (Version 2016b, The Mathworks, Natick, MA, USA). At the end of the procedure, two distinct vegetation maps were obtained and the fractional vegetation cover of each stand was calculated with: FVCc for wheat and FVCw for weeds. Subsequently, an indicator of weed pressure (WP) namely, the FVCw/FVCc ratio was defined to characterize the crop-weed competition. The results were compared to destructive measurements of above-ground biomass (BM), and calibration curves were deduced in order to feed a plant-growth model based on the Monteith equation with FVC.

Statistical analysis: An analysis of variance (ANOVA) was performed to test which factors (cultivar, area, column) showed a significant effect on both crop and weed vegetation cover in order to assess the crop/weed competition. The two variables, FVCc and FVCw, were specifically studied concerning three factors: the area (4 areas), the column (3 columns), and their interaction. Statistical analyses were performed in the R environment for statistical computing, using R version 4.0.3 (R Core Team, 2020) and RStudio (RStudio Team, 2020), an integrated development environment for R.

Description of the plant-growth model: The model was applied to both wheat cultivars. It calculates the dry matter biomass of the aerial parts of plants (Jeuffroy and Recous, 1999) at a daily time-step during the vegetative phase with no stress. Starting from the end of winter with an initial crop aerial biomass value ($\text{BM}_{\text{initial}}$, $\text{g}\cdot\text{m}^{-2}$) deduced from images the biomass was then calculated every day. The Monteith equation (Monteith, 1972, 1977) was calculated and the accumulated dry biomass was established depending on the photosynthetically active radiation (PAR) and other plant parameters such as the radiation intercepted by the plants (APAR) and the radiation use efficiency (ϵ). A detailed description can be found in Gée *et al.* (2019). The health status of the wheat was analyzed at four dates by comparing the wheat biomass simulated under unstressed conditions,

$BM_{simulated}$, to the actual biomass, $BM_{observed}$. The difference, $BM_{observed} - BM_{simulated}$, indicated the presence of crop stress due to weeds or other stressors, depending on the location and the date of observation, but did not explain its cause.

Results and discussion

Figure 2 presents the spatio-temporal evolution of the vegetation cover of the crop (FVCc) and weed (FVCw) stands for the Apache cultivar. Similar results were observed with the Rubisko cultivar. Over time, the wheat and weed growth can be finely described with a strong presence of weeds at the top left of the plot. A quantitative analysis can be carried out for both stands for each cultivars.

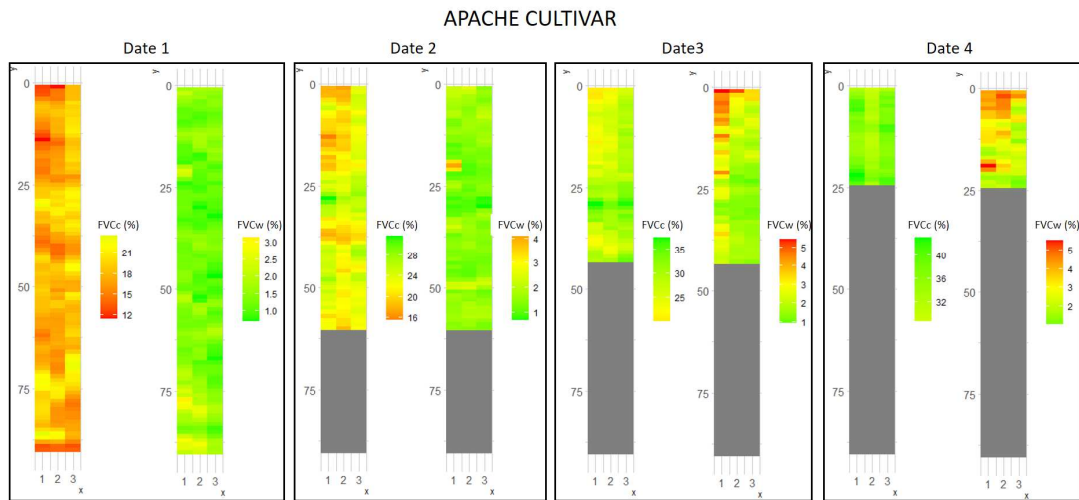


Figure 2. Example of FVCc and FVCw digital maps for Apache cultivar for the four dates. Red color is associated with low values of FVCc (wheat) and, conversely, with high values of FVCw (weeds).

Concerning the FVC values, Figure 3a shows that the stand of the winter wheat cultivar Apache is slightly more heterogeneous than the Rubisko ones.

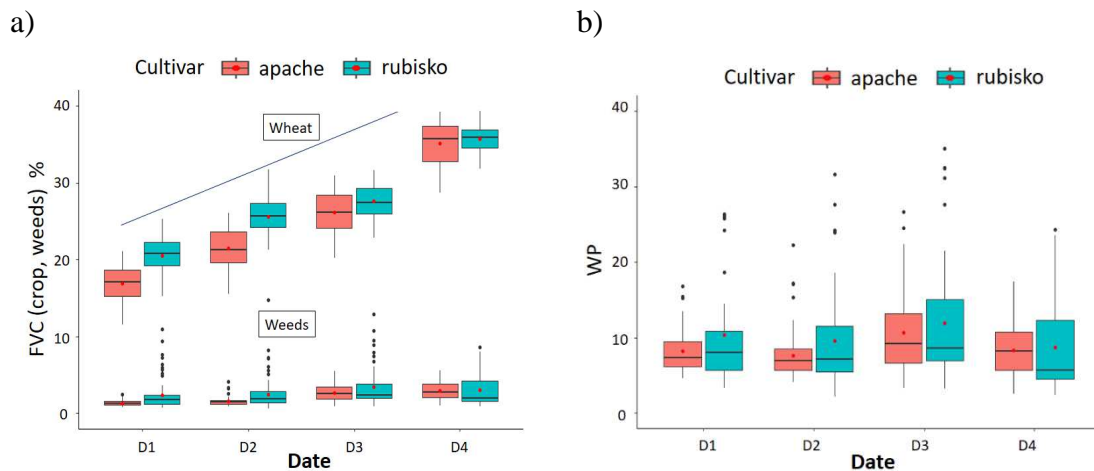


Figure 3. Temporal evolution of a) FVCc and FVCw and b) weed pressure indicator (WP) for both the Apache and Rubisko cultivars.

In addition, at the early dates, the FVC values of the Apache cultivar appear to be slightly lower than those of Rubisko, which is explained by it being a variety with a less-dense tillering. As for the weed stand, the FVCw values were similar for both cultivars, with slightly more heterogeneity observed for Rubisko. One possible explanation for this is higher weed diversity in the Rubisko plot. Over time and for both cultivars, weed populations increased slightly and became more heterogeneous, probably due to the emergence of new weed flora. In order to assess the impact of weeds on wheat development, a weed pressure indicator (WP) was developed. Figure 3b indicates the temporal evolution of the WP. For both cultivars, this slightly increased until date 3, with a higher dispersion of the values, and then it decreased. This dispersion is associated with an increase in the spatial heterogeneity of weed flora and is particularly clear in the Rubisko plot.

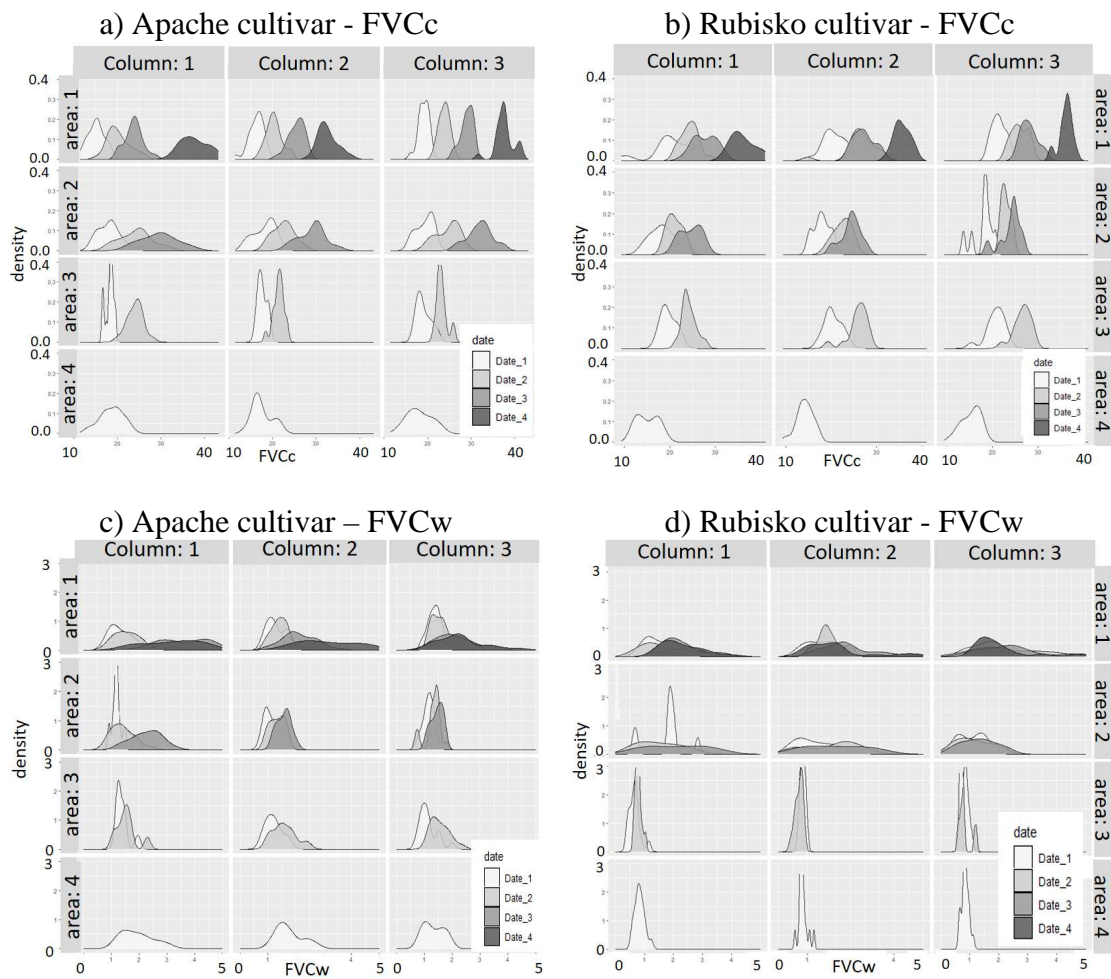


Figure 4. Temporal evolution of the distribution of values depending on the area (4 horizontal rows) and column (3 vertical columns) for a) FVCc (%) for the Apache cultivar b) FVCc (%) for the Rubisko cultivar c) FVCw (%) for the Apache cultivar and d) FVCw (%) for the Rubisko cultivar.

A detailed description of the two variables FVCc and FVCw was carried out using ANOVA analysis. Whatever the date, no significant correlation between these two

variables was observed (Kendall's test). Moreover, the distributions of FVCc and FVCw were not normal regardless of the area and the column (Figure 4). Subsequently, different non-parametric tests were used to test the influence of the two factors: area and column. In addition, the temporal evolution of FVCc showed an increasingly wide distribution on the other dates due to an increase in leaf area and a greater dispersion of values was observed indicating spatial variation in the wheat growth (Figures 4a-b). The variance comparison demonstrated that the factor area as well as the column factor had a significant influence on FVCc for the first three dates for the Apache cultivar. The interaction (area vs. column) is also significant at the first two dates (Test of Scheirer Ray Hare). For the Rubisko cultivar, the column factor is not significant, nor is the interaction. Concerning the weeds (Figures 4c-d), for date 1, the distribution is clearly different according to the area, the column, and the wheat cultivar. It exhibits different shapes. Several non-parametric tests were used for the FVCw variable to evaluate the influence of the two factors area and column. In the Apache plot, with the area factor as well as the column factor, a significant effect was observed. For areas 2 and 3, the test of Scheirer Ray Hare did not indicate a significant effect. Whereas, concerning the Rubisko plot, only the area factor presented a significant influence on the FVCw variable.

Plant-growth model to characterize wheat growth status: the simulated aboveground biomass of the crop, $BM_{\text{simulated}}$, was compared to the actual biomass, BM_{observed} , at the four dates and for each cultivar. The model starts with the initial value of the crop aboveground biomass (BM_{initial} , $g.m^{-2}$). It is normally fed by destructive biomass measurement, which has been substituted, by proximal sensing data (FVCc) from calibration (Figure 5). Merienne *et al.*, (2019) demonstrated that FVCc and BM were highly linearly correlated ($r^2 = 0.93$) for the Apache cultivar. Concerning the Rubisko cultivar, a new correlation was obtained with a stronger correlation ($r^2 = 0.98$). The results are presented in Figure 5. The results confirm that this calibration method for estimating the aerial biomass from the parameters obtained from the image can be generalized to the early growth stage of wheat crops.

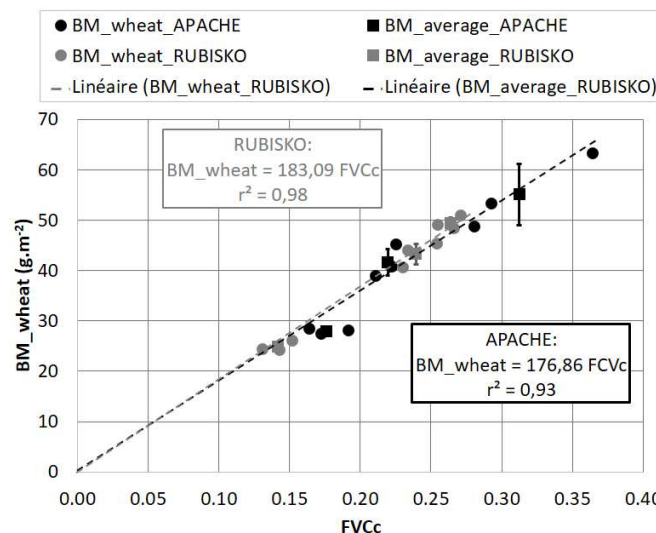


Figure 5. Linear regression (dashed line) between BM and FVC displayed for wheat Apache cultivar (black) and Rubisko cultivar (grey). For each date, there are three replicates (the filled circle) and the solid squares represent the mean and the standard deviation (vertical errors bars) values for each date.

Figure 6 presents the deviation (%) between BM_{observed} and $BM_{\text{simulated}}$ for each image acquired for each cultivar and for the last three dates. The $BM_{\text{simulated}}$ of date 2 is compared with the images of date 2 located at the same place of the image of date 1 and so on. One can notice that in most cases, the predicted biomass is greater than the experimental one, indicating a negative value of deviation and thus reflecting a stress with no explanation of its origin.

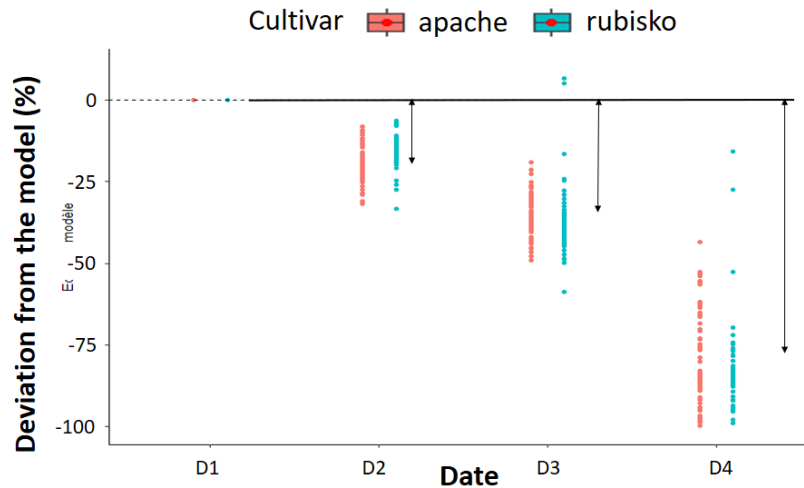


Figure 6. Temporal evolution of crop growth status through the deviation from the model for both cultivars (red color for Apache and blue color for Rubisko).

Over time, the deviation increased and almost similar behavior was observed whatever the cultivar. In order to understand the crop-weed competition, it is necessary to correlate these results with those of the weed pressure indicator (Fig. 3b). For the last three dates, the WP values hardly increase, whereas the deviation from the model increases. Consequently, on date 4 the majority of stress is probably not related to weeds. The main hypothesis is that this stress is related to the lack of nitrogen supply. However, this hypothesis remains unchecked, as no precise measurement has been performed to quantify the nitrogen in plants and soil.

Conclusion

Digital farming solutions (visible images, statistical analysis of extracted parameters and plant-growth model) have been used to characterize the health status of two winter wheat cultivars (Apache and Rubisko). With the spatial-temporal mapping of crop and weeds, areas with a high weed density or low wheat growth have been highlighted with the future aim of concentrating on agricultural interventions. This led us to assess the crop-weed competition with a simplified ecophysiological model. The results of this study demonstrate the potential of visible images to identify stress and, based on the observation of weed pressure, it is shown that the major stress is not always due to weeds. This simple and fast method based on proximal detection allows high-throughput field phenotyping and can be transposed to UAV images. It offers promising results in agroecological cropping systems, where high responsiveness is a major challenge for site-specific weed management.

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