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Title: The long-term effects of conventional and organic cropping systems, tillage managements and weather conditions on yield and grain quality of durum wheat (Triticum durum Desf.) in the Mediterranean environment of central Italy

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Keywords: Durum wheat; Organic farming; Subsoiling; Plowing; Weather conditions; Grain protein

content

Corresponding Author: Dr. Roberto Mancinelli, Ph.D.

Corresponding Author's Institution: University of Tuscia

First Author: Enio Campiglia

Order of Authors: Enio Campiglia; Roberto Mancinelli, Ph.D.; Ester De Stefanis; Stefano Pucciarmati; Emanuele Radicetti, Ph.D.

Abstract: Cropping system, tillage management and weather conditions can greatly affect durum wheat determining its grain yield and quality. For this reason the aims of this study were to evaluate the effects of cropping system (conventional vs organic), tillage management (plowed vs subsoiled soil), and the interaction between cropping system and weather conditions on yield and grain quality of durum wheat (Triticum durum L.). The study was part of a long-term experiment carried out in a rainfed Mediterranean environment of central Italy where a 3-year crop rotation (durum wheat tomato - chickpea) was compared in organic and conventional cropping systems. The field experiments refer to the period from 2005 to 2011. The combined effect of cropping system and weather conditions determined a strong variability on durum grain yield and quality parameters, this variability was greater in conventional than in organic cropping system. The durum grain yield was on average 15 % lower in organic compared to conventional, although the yield gap between the systems varied from 5 to 32 %. Organic grain yield was positively correlated with air temperatures in spring. High rainfall during the grain filling stage resulted in higher grain yield in conventional than in organic due to a severe weed infestation observed in organic although the protein content was similar between the systems. A severe water stress period starting from stem elongation determined a poor grain yield and scarce protein concentration, while high temperatures and water stress throughout the grain filling period determined a poor yield, yet high protein content in both cropping systems. Conventional wheat generally showed a higher test weight, vitreousness and gluten quality, while protein and gluten content were higher in conventional compared to organic when a regular rainfall distribution occurred throughout the wheat reproductive period. These results are probably due to a lower nitrogen supply in organic compared to conventional wheat. Yield and grain quality of durum wheat under different tillage managements such as plowed and subsoiled soil were comparable. Considering subsoiling as an important mode of conservation tillage, the results highlight that it is advisable to use a subsoiler for durum wheat production. Weed control and nitrogen supply appear to be the main factors which hinder yield production and grain quality especially when an excess of rainfall and low temperatures occur throughout the crop reproductive period in organic durum wheat production.

**Cover Letter** 

Dear Victor Sadras,

Editor in chief of Field Crops Research Journal,

we have recently submitted an article entitled "Yield and grain quality of durum wheat (Triticum durum Desf.) in conventional and organic cropping systems in the Mediterranean environment" n. FIELD-D-14-00492" to the Field Crops Research Journal which has been rejected. We would like to re-submit a new article regarding the same research which has been modified according to the reviewer's report and implemented with other measurements. The new article is entitled "The long-term effects of conventional and organic cropping systems, tillage managements, and weather conditions on yield and grain quality of durum wheat (Triticum durum Desf.) in the Mediterranean environment of central Italy".

We were encouraged by the reviewer n.1 who wrote: "The ms provide a valuable comparison between systems. It is of particular value since it contains good data from six years, which makes it possible to study long term effects of the systems, the relation between weather and main effects and the efficiency of the systems. By the thorough analyses of yield components it is possible to investigate the causes of the treatment\*year interactions, thus providing valuable information on how organic systems can be improved to reduce the yield gap compared to conventional agriculture." "There might be room for one efficiency paper and one weather interaction paper. The authors need to decide which story to build".

The reviewer's comments proved to be very useful for making new hypotheses and objectives of the research. In particular, according to the reviewer n.1, the new manuscript focuses on weather and cropping system interaction, moreover we have added new data regarding SPAD readings and TDR measurements in order to evaluate the differences between the cropping systems and soil tillage managements from several points of view (weeds, nitrogen and soil water availability). New hypotheses have been addressed in the discussion section.

Therefore, I kindly ask you to reconsider the paper for publication in the Field Crops Research Journal as research article.

The listed authors are: E. Campiglia, R. Mancinelli, E. De Stefanis, S. Pucciarmati, E. Radicetti

Your sincerely

Roberto Mancinelli

**Highlights (for review)** 

Organic and conventional durum wheat were cultivated in plowed and subsoiled soil

In the long run conventional wheat had higher grain yield and quality than organic

Organic grain yield was positively correlated with high air temperatures in spring

Conventional had better grain protein and gluten due to higher nitrogen availability

Plowing had a similar effect of subsoiling on wheat grain yield and quality

- 1 THE LONG-TERM EFFECTS OF CONVENTIONAL AND ORGANIC CROPPING
- 2 SYSTEMS, TILLAGE MANAGEMENTS AND WEATHER CONDITIONS ON YIELD
- 3 AND GRAIN QUALITY OF DURUM WHEAT (Triticum durum Desf.) IN THE
- 4 MEDITERRANEAN ENVIRONMENT OF CENTRAL ITALY
- 5 <sup>1</sup>E. Campiglia, <sup>1</sup>R. Mancinelli\*, <sup>2</sup>E. De Stefanis, <sup>2</sup>S. Pucciarmati, <sup>1</sup>E. Radicetti
- 6 <sup>1</sup>Dipartimento di Scienze e Tecnologie per l'Agricoltura, le Foreste, la Natura e l'Energia,
- 7 Università della Tuscia, via S. Camillo de Lellis, 01100 Viterbo, Italy
- 8 <sup>2</sup> Consiglio per la Ricerca e la sperimentazione in Agricoltura, Unità di Ricerca per la
- 9 Valorizzazione Qualitativa dei Cereali (CRA-QCE), via Cassia 176, 00191 Roma, Italy
- \*Corresponding author Tel.: + 39 0761 357556; fax + 39 0761 357558 e-mail address:
- 12 mancinel@unitus.it

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- 14 **KEY WORDS:** durum wheat, organic farming, subsoiling, plowing, weather conditions, grain
- 15 protein content

## 17 ABSTRACT

Cropping system, tillage management and weather conditions can greatly affect durum wheat determining its grain yield and quality. For this reason the aims of this study were to evaluate the effects of cropping system (conventional vs organic), tillage management (plowed vs subsoiled soil), and the interaction between cropping system and weather conditions on yield and grain quality of durum wheat (*Triticum durum* L.). The study was part of a long-term experiment carried out in a rainfed Mediterranean environment of central Italy where a 3-year crop rotation (durum wheat - tomato - chickpea) was compared in organic and conventional cropping systems. The field experiments refer to the period from 2005 to 2011. The combined effect of cropping system and weather conditions determined a strong variability on durum grain yield and quality parameters, this

variability was greater in conventional than in organic cropping system. The durum grain yield was on average 15 % lower in organic compared to conventional, although the yield gap between the systems varied from 5 to 32 %. Organic grain yield was positively correlated with air temperatures in spring. High rainfall during the grain filling stage resulted in higher grain yield in conventional than in organic due to a severe weed infestation observed in organic although the protein content was similar between the systems. A severe water stress period starting from stem elongation determined a poor grain yield and scarce protein concentration, while high temperatures and water stress throughout the grain filling period determined a poor yield, yet high protein content in both cropping systems. Conventional wheat generally showed a higher test weight, vitreousness and gluten quality, while protein and gluten content were higher in conventional compared to organic when a regular rainfall distribution occurred throughout the wheat reproductive period. These results are probably due to a lower nitrogen supply in organic compared to conventional wheat. Yield and grain quality of durum wheat under different tillage managements such as plowed and subsoiled soil were comparable. Considering subsoiling as an important mode of conservation tillage, the results highlight that it is advisable to use a subsoiler for durum wheat production. Weed control and nitrogen supply appear to be the main factors which hinder yield production and grain quality especially when an excess of rainfall and low temperatures occur throughout the crop reproductive period in organic durum wheat production.

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#### 1. INTRODUCTION

Wheat is one of the most important cereal crops in the world in terms of cultivated area (Wilcox and Makowski, 2014). It is generally grown as a rainfed crop even in semi-arid areas where large fluctuations occur in the amount and frequency of rainfall from year to year. Durum wheat (*Triticum durum* Desf.) is the main cereal grown in the Mediterranean regions, although it is characterized by an insufficient yield stability due to inadequate and adverse weather patterns characterized by irregular rainfall distribution and high temperatures during the grain filling stage

1 (Lopez-Bellido et al., 1996). In this kind of environment annual and especially seasonal rainfall can 2 strongly influence durum wheat response to agronomical inputs such as soil tillage, fertilizers, 3 chemical herbicides and fungicides accounting for the major variation in yield and grain protein

content (Rahimizadeh et al., 2010).

Currently, low prices for cereal grains coupled with changes in environmental policies and government support programs, have provided strong incentives for producers to seek alternative production opportunities, giving importance to soil and environmental quality and stimulating changes in cropping systems (Gan et al., 2003). In this respect, there is a growing interest in the adoption of conservation tillage practices which are based on minimizing soil disturbance and maximizing plant residue coverage with the aim of maintaining high production levels and healthy environment by reducing energy and labor costs and improving soil fertility (Hobbs, 2007; Hobbes et al., 2008). Subsoiling is an important technique of conservation tillage which reduces soil compaction, increases soil impermeability and water retention ability (Zhang et al., 2014). Considering that the majority of Italian farmers currently plow the soil for durum wheat cultivation, we hypothesized that subsoiling rather than plowing may determine a high water supply to the wheat especially throughout the reproductive period when droughts frequently occur.

Organic farming may represent another practical alternative for reducing the environmental impact of durum wheat production in Italy (Bevilacqua et al., 2007). However, considering that organic and conventional may differ greatly in terms of amount and sources of fertilizers and crop protection strategies, a different response in terms of grain yield and quality could be expected in durum wheat. In this respect, a recent review regarding the crop yield gap between organic and conventional agriculture (De Ponti et al., 2012) showed that organic wheat yield was on average 27 % less than conventional, although there was a large variability in the yield gap (from -60 to +30%). The Authors concluded that the wheat yield gap between organic and conventional increases as the conventional yield increases and they mainly attributed this effect to a low nutrient stress and a better pest, disease and weed control than that obtained in conventional compared to organic

systems. In fact, conventional wheat relies on chemical inputs such as synthetic fertilizers and herbicides since they are known to be effective means for increasing crop yield. However, we hypothesize that organic systems respond differently to meteorological inputs than conventional systems, therefore the large variability in the yield gap between organic and conventional wheat is probably due to the different agricultural practices as well as the different weather conditions occurring throughout the cropping period. Up to now few studies have investigated long-term interactions between weather conditions and agricultural practices adopted in conventional and organic durum wheat. The main objectives of this study were therefore (a) to evaluate the long-term effects of the cropping systems (conventional versus organic), tillage management (plowed versus subsoiled soil) and (b) to investigate the interactions between cropping systems and weather conditions on grain yield characteristics and quality parameters of durum wheat in the rainfed Mediterranean conditions of Central Italy.

## 2. MATERIALS AND METHODS

# 2.1. Description of the study area and climate

The experiment was carried out at the experimental farm "Nello Lupori" of the University of Tuscia in Viterbo, Italy (85 km NW of Rome, lat. 42°25', long. 12°04', alt. 310 m a.s.l.) during six consecutive growing seasons (from 2005-2006 to 2010-2011 season). The study was part of a long-term experiment (hereafter called ORG-CONV) carried out on volcanic soil classified as *Typic Xerofluvent* with the following characteristics: sand 45%, silt 17%, clay 38%, and pH 6.79 (water, 1:2.5). The climatic conditions at the experimental site are typical of the Mediterranean environment with minimum temperatures just below 0 °C in the winter and maximum temperatures above 35 °C in the summer. Annual rainfall (mean 800 mm, considering a long-term 30-year period) is mostly concentrated in autumn and spring, the potential evapo-transpiration rarely exceeds 7 mm per day in summer. Complete weather data (including temperatures, rainfall, and

potential evapo-transpiration) were obtained from an automatic meteorological station located 100 m from the experimental site.

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## 2.2. Field set up and crop management

The ORG-CONV long-term experiment was established in 2001 in order to compare organic vs. conventional cropping systems and plowed vs. subsoiled soil. In the conventional system traditional agricultural practices have been adopted, while the organic system has been managed according to the Council Regulation n. 834/2007 concerning organic production and the labeling of organic products and repealing Regulation n. 2092/91 (EC, 2007). A 3-year crop rotation was established in both cropping systems [pea (*Pisum sativum* L.), durum wheat (*Triticum durum* Desf.) and tomato (Lycopersicon esculentum Mill.)]. In the organically-managed cropping system, the crop rotation was implemented with common vetch (Vicia sativa L.) and sorghum (Sorghum vulgare L.) cover crops, which were green manured before tomato transplanting and pea sowing, respectively. Since 2008-2009 pea has been substituted with chickpea (Cicer arietinum L.) and sorghum with oilseed rape (Brassica napus L.). Furthermore, two tillage managements were compared: (i) inversion layer soil tillage consisting in moldboard plowing at a depth of 30 cm as main soil tillage, followed by secondary tillage with a disk harrow for seed bed preparation (hereafter called plowed soil), and (ii) non-inversion layer soil tillage consisting in subsoiling at a depth of 20 cm as main soil tillage, followed by secondary shallow tillage with disk harrow for seed bed preparation (hereafter called subsoiled soil). The treatments were replicated three times according to a randomized complete block design. Considering that the three main crops in rotation were simultaneously cropped every year, the experimental field included 36 plots (3 crops x 2 cropping systems x 2 tillage managements x 3 replications).

The soil was tilled in September of each year according to the experimental procedures, while seedbed preparation was carried out following the first autumn precipitations. Durum wheat was sown in all plots on the same day with a conventional planter machine with row spacing of 12.5 cm

and at a depth of 3-4 cm in order to avoid any difference due to sowing date. Colosseo, a winter durum wheat cultivar widely used in the region, was sown at the seeding rate of 450 seeds m<sup>-2</sup> in November when the soil moisture become suitable for seed germination. In the conventional cropping system, fertilization was carried out with 80 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> as triple superphosphate applied at seedbed preparation and 120 kg ha<sup>-1</sup> of nitrogen which was applied at the beginning of the tillering stage in January (30 kg ha<sup>-1</sup> of nitrogen as calcium nitrate), at the end of the tillering stage in February (40 kg ha<sup>-1</sup> of nitrogen as ammonium nitrate), and at the beginning of stem elongation stage in March (50 kg ha<sup>-1</sup> of nitrogen as ammonium nitrate). In the organic cropping system, fertilization was carried out with the same amount of nitrogen as for the conventional cropping system and commercial organic fertilizers were applied at seeding. Weeds were controlled by means of a herbicide in the conventional (Mesosulfuron-Metile 3% + Iodosulfuron.Metil-Sodium 3% + Mefenpir-Dietile 9%) and by mechanical weed harrowing in the organic (tine harrows). All plots were harvested mechanically at crop maturity on the same day in July.

## 2.3. Measurements and wheat grain quality analysis

Soil moisture content was measured in the 0-20 cm soil layer by using the Time Domain Reflectrometry technique (TDR 300 Soil Moisture Meter, Spectrum Technologies, Inc., Plainfield, IL - USA) with 20 cm probe which was vertically placed along the central axis of each plot. TDR measurements were taken every ten days from the 1<sup>st</sup> of March to 30<sup>th</sup> of June of each experimental year. Ten TDR readings were taken in each plot and averaged.

At wheat anthesis, the chlorophyll content of the wheat leaves (hereafter called SPAD readings) was estimated using the SPAD-502 (Minolta, Osaka, Japan). SPAD readings were measured on the same day on fully developed laminae of the wheat flag leaf. Ten readings, one reading per plant, were taken in each replication and averaged (Minotti et al., 1994).

At wheat physiological maturity, the aboveground biomass of the wheat plants from eight 1 m long adjacent rows, corresponding to a 1 m<sup>2</sup> area per plot, was manually cut at ground level and was

analyzed for determining the yield components. At the same time and in the same area the weed aboveground biomass was cut at ground level and collected. The wheat plant height (with awns excluded), the number of fertile spikes and the kernels per spike were measured. Thousand grain weight (hereafter called TGW) was calculated as the mean weight of five sets of 100 grains per plot. In order to determine the dry weight, samples from all plant (wheat and weeds) were oven dried at 65 °C until constant weight. Harvest index (hereafter called HI) of each plot was calculated as the ratio between grain weight and the total wheat aboveground biomass. Grain yields were determined by harvesting with a Walter Wintersteiger cereal plot combine-harvester. Each plot sample was weighed and three sub-samples were dried in order to determine moisture content. The harvested grains were accurately mixed and about 5 kg of grain samples were randomly taken from each plot, cleaned and used for wheat grain quality determination. Test weight or the weight per hectoliter (weight per unit volume, hereafter called TW) reflects the density and the volume occupied by the grains. TW was measured on three samples of 250 g per plot and expressed as kg hL<sup>-1</sup> obtained with a Shopper chondrometer equipped with a 1 L container. The percentage of vitreous kernels was determined according to the method given by ISO (1987). Wheat kernels were cut transversely. The percentage of vitreous kernels was determined by examining the cross-section of 100 cut kernels. Vitreous grains have a dark translucent appearance, while opaque grains are yellow and starchy. The grains were ground with a laboratory mill (Cyclotec, mod. 1093-Tecator/Hoganas, Sweden) equipped with a 1.0 mm sieve prior to the other analyses. Moisture content was analyzed with a thermo balance at 120° (Sartorius MA40, Gottingen, Germany) and ash content according to 08-12 standard method (AACC, 1995) with a few modifications. An ash gravimetric analysis was carried out on 5 g samples at a temperature of 580°C for 16-24 hours. After cooling, a little water was added to the sample (lixiviation step) which was once again placed in the muffle furnace at 580°C for two hours. Protein content was determined by means of a Kjeldhal nitrogen analysis (N x 5.7) and was expressed as percentage on a dry weight basis (AACC, 1976). Gluten content was estimated according to ISO (2006) method. Gluten quality was evaluated by SDS (sodium dodecyl

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sulfate) sedimentation test using a solution of 3% sodium dodecyl sulfate. The standard 56-70 method (AACC, 1995) was adopted for assessing the SDS sedimentation test. The results were expressed in milliliters (mL) of the interface line between solid (ground sample) and liquid (solution) in a measuring cylinder. All parameters were measured each year except for 2009 where gluten content, gluten quality (SDS test) and ash content were not measured due to logistic problems.

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### 2.4. Statistical analysis

For all parameters, the analysis of variance (ANOVA) was performed using JMP statistical software package, version 4.0 (SAS Institute, Cary, NC) in order to test the main effects of year, cropping system, tillage management and their interactions. All data were tested for normality to determine if transformation was necessary. The analysis of variance was carried out for the 6-year period, considering the year as a repeated measure across time. Percentage data were transformed before analysis as angular transformation in order to homogenize the variance (Gomez and Gomez, 1984). The data reported in the tables were back transformed. A two-way factorial experimental design was adopted for the wheat yield, grain quality characteristics and weed aboveground biomass where the cropping system was a treatment, the tillage management the other treatment and the year was considered as repeated measure. The main effect and interaction means were compared using Fisher's protected LSD test at 5 % of probability level (P < 0.05). A canonical discriminant analysis (CDA) was used in order to investigate the two dimensional associations between the wheat yield characteristics or wheat grain quality with the conventional and organic cropping system. The results of the CDA were summarized in a two-dimensional canonical discriminant structure plot, where a vector diagram based on the total canonical coefficient of each wheat characteristic from the canonical functions was combined into the same plot for each year. Group means were determined and reported on axes defined by the first and second canonical functions to describe the degree of similarity on wheat characteristics between the cropping system groups. The

wheat characteristics were represented as vectors whose length indicates the degree of association with direction in ordination space (Kenkel et al., 2002). Differences in wheat yield and quality among cropping systems and tillage groups were evaluated with the MRPP analysis (multi-response permutation procedure) in accordance with the recommendations of McCune and Grace (2002). The multivariate MRPP analysis tests the null hypothesis that two or more a priori defined groups do not differ in respect to composition (Zimmerman et al., 1985) and it provides a test statistic (Tstatistic) with an associated probability value (McCune and Grace, 2002). T-statistic describes the separation among groups (the more negative T, the stronger the separation). The MRPP analysis was performed with the squared Euclidean distance as a measure of dissimilarity using BLOSSOM software (Cade and Richards, 2001) and the years were analyzed separately for both cropping systems and tillage groups. Correlations between meteorological variables (temperatures and rainfall) were calculated on a monthly (starting from March to June) and multi-monthly basis and all possible combinations were investigated in order to identify the periods in which each meteorological variable was most likely to affect the yield and protein content of wheat grain in both conventional and organic cropping systems. Linear regressions were performed on data regarding SPAD readings and grain protein, as well as SPAD readings and gluten content.

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#### 3. RESULTS

### 3.1. Weather conditions, soil moisture, and wheat phenological stages

Rainfall for the whole wheat cropping season (November-June) varied considerably throughout the 6-year experimental period ranging from 375 mm in 2006/2007 to 899 mm in 2009/2010, as well as during the grain filling period (April-June) (Fig.1). The spring period was particularly dry in 2006 and 2009 (82 and 111 mm from April to June, respectively), while it was exceptionally rainy in 2008 and 2010 (268 and 337 mm from April to June, respectively). Consequently the drought period (rainfall – evapotranspiration) began in the middle of April in 2006 and at the beginning of June in 2008 and 2010 (Fig. 1). These observations were confirmed by

the soil water content measurements (Fig. 2). Maximum air temperatures varied considerably especially during the grain filling period, a fast increase in the maximum temperatures in spring was recorded in 2009 when values above 27 °C were already observed in the second half of May. The minimum air temperatures dropped below 0°C several times throughout the winter period in most years reaching a minimum peak in February 2008 (-2.6°C). However no cold damage was detected on wheat during the experimental period. The wheat crop emerged regularly around the 2<sup>nd</sup> week after sowing in all years. Wheat tillering generally began in December, stem elongation at the end of March, the heading-anthesis stage at the beginning of May and wheat physiological maturity was extremely variable ranging from the first decade of June in 2006 and 2009 to the third decade of June in 2008 and 2010.

## 3.2. Leaf SPAD values, yield, yield components and weed aboveground biomass of wheat

The SPAD readings at wheat anthesis, which characterized the chlorophyll content of the flag leaf of the wheat, tended to be higher in conventional than in organic, although significant differences were only observed in 2007, 2009 and 2011 (Fig. 3). The SPAD reading values were particularly low in 2006, 2007 and 2011 in organic, and in 2006 and 2010 in conventional.

Regarding wheat yield and yield components, the tillage management treatment was never significant, while there was a significant year x cropping system interaction ( $P \le 0.05$ ) except for plant height which ranged from 59.1 to 70.7 cm in 2009 and 2011, respectively (Table 1). Grain yield and straw of durum wheat were generally higher in conventional than organic cropping system (on average 3.40 and 6.10 vs. 2.86 and 5.30 t ha<sup>-1</sup> of DM, respectively), even if they varied among the growing seasons with the highest values observed in 2007 (on average 3.84 and 7.56 t ha<sup>-1</sup> of DM, respectively). In the conventional cropping system, the grain yield was higher in 2007 and 2010, intermediate in 2008 and 2011, and lower in 2009 and 2006 (on average 3.95, 3.43, and 2.83 t ha<sup>-1</sup> of DM, respectively). In the organic cropping system the highest values of grain yield were observed in 2007 and 2011 and the lowest in the other years (on average 3.36 vs. 2.61 t ha<sup>-1</sup> of DM,

respectively). HI was generally similar in both conventional and organic cropping systems (on average 35.6 %), except in 2008 and 2011 where it was higher in conventional than organic (on average 37.5 vs. 34.4 %, respectively).

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There was also a significant cropping system x year interaction on the yield components of durum wheat (P  $\leq$  0.05, Table 2). The number of fertile spikes ranged from 179.5 in 2009 to 361.5 per m<sup>2</sup> in 2007 and it was similar among the cropping systems (on average 272 n. m<sup>-2</sup>), except in 2008 and 2010 when the number of fertile spikes was higher in conventional than organic. The number of kernels per spike varied from 19.5 in 2008 to 35.0 in 2009, and it was generally higher in conventional than organic even if it only significantly differed between the cropping systems in 2007 and 2010. Thousand grain weight (TGW) ranged from 42.6 g in 2007 to 52.1 g in 2011, and it was significantly higher in organic than conventional in 2007, 2010 and 2011 (Table 2). The results of the canonical discriminant analysis (CDA) on the yield and yield components of durum wheat for each year of the study are reported in Fig. 4. The first two canonical variables generally accounted for approximately 70% of the total variance with low variation among the years. The yield and yield components of durum wheat tended to differ according to the cropping system. Grain yield, kernel, and fertile spike vectors were generally in the same ordination space of conventional cropping system, while TGW and plant height vectors were in the same ordination space of organic cropping system. This trend was mainly observed in the rainy springs in 2008 and 2010, while in the dry springs of 2006 and 2009 it was less pronounced. The MRPP analysis was in accordance with the CDA analysis, which indicated that yield and yield components were mainly influenced by the cropping system, although the T values differed considerably among the years (Table 4). The T statistics for distinct previously-defined groups were generally significantly negative (P < 0.05) in 2007, 2008, 2010 and 2011. The strongest separation between the cropping systems was observed in the wet years of 2008 and 2010 (Table 4).

Test weight (TW), vitreousness, and gluten quality measured by means of the SDS test were only affected by the main effects of cropping system and year (Table 3). Regarding the cropping

systems, TW, vitreousness, and gluten quality (SDS test) were higher in conventional (on average 80.90 kg hl<sup>-1</sup>, 90.83 %, and 37 mL, respectively) than in organic (79.56 kg hl<sup>-1</sup>, 87.46 %, 36 mL, respectively). Concerning the year, TW was higher in 2006, 2007, 2009 and 2011 compared to 2008 and 2010 (on average 81.45 vs. 77.78 kg hL<sup>-1</sup>, respectively). The vitreousness was higher in 2009 (92.27 %), intermediate in 2006, 2007 and 2011 (on average 89.97 %), and lower in 2008 and 2010 (86.35 %), while gluten quality (SDS test) was similar among the years except in 2007 when it was higher (on average 44 vs. 34 mL, respectively). There was a significant cropping system x year interaction on protein and gluten content. They were generally higher in conventional than in organic, even if significant differences were only found for gluten in 2006, 2007 and 2011, and for protein in 2007 and 2011. The lowest protein and gluten content values were observed in 2006 both in organic and conventional (Table 3). However, protein and gluten content values were positively correlated with the SPAD readings, measured at wheat anthesis (Fig. 5). A significant cropping system x year interaction was also observed for ash content values. They were significantly lower in conventional than in organic in 2007, 2008 and 2010. In both cropping systems the best ash contents were observed in 2006 and 2011 when they were approximately 1.80 % of the dry matter. The canonical discriminant analyses (CDA) of the grain quality parameters performed for each year are reported in Fig. 6. The first two canonical variables explained 68.6, 75.9, 68.1, 95.2, 72.6, and 75.8% of the total variance in 2006, 2007, 2008, 2009, 2010, and 2011, respectively. Vitreousness, protein and gluten content vectors were generally in the same ordination space of the conventional cropping system, while TW and ash vectors seemed to be associated with the organic cropping system (Fig. 6). Results of the MRPP analysis suggested that different cropping systems are characterized by somewhat different grain quality parameters of durum wheat. In the cropping system previously-defined T value groups were negatively high (from -2.0 to -6.0) and always significant (P  $\leq$  0.05), while the values of T statistics were generally low and significant only in some years between the tillage management groups (Table 4).

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Weed aboveground biomass at wheat harvesting was significant for year x cropping system (P  $\leq 0.05$ ) interaction (Fig. 7). It varied from 134.73 g m<sup>-2</sup> in 2010 to 32.91 g m<sup>-2</sup> in 2006, and it was generally higher in the organic compared to the conventional, even if it only significantly differed between the cropping systems in 2007, 2008 and 2010.

### 3.4. Relationships between wheat grain characteristics and meteorological variables

In order to evaluate the relationships between cropping systems and weather patterns on yield and protein content of durum wheat grain, all possible monthly and multi-monthly combinations of mean air temperatures and cumulative precipitations during the shooting, grain filling and grain ripening periods were considered (Table 5). The monthly or multi-monthly air temperatures were never significantly correlated in the conventional cropping system both for grain yield and protein content, while in the organic cropping system the grain yield was positively correlated from March ( $R^2 = 0.627$ ) to May ( $R^2 = 0.564$ ), with the highest correlation value in the March-April period ( $R^2 = 0.896$ ). As already observed with air temperatures, the monthly and multi-monthly precipitations were not correlated with the grain yield and protein content in the conventional cropping system (Table 5), except for the cumulated rainfall in May which was positively correlated with the grain yield. In the organic cropping system, rainfall negatively affected the grain yield in the March – April period ( $R^2 = -0.723$ ) and April ( $R^2 = -0.792$ ), while protein content was positively correlated in the April – June and May – June periods ( $R^2 = 0.552$  and 0.594, respectively, Table 5).

# 4. DISCUSSION

The climatic conditions were extremely variable among the experimental years which is typical of the Mediterranean climate. The amount of rainfall observed during the wheat growing seasons from 2005 to 2011 was slightly higher than the 30-year long-term average (on average 866 vs. 801 mm). However, differences were above all observed in the spring, particularly throughout the grain filling period, where low annual rainfall values were observed especially in 2006 and

2009. In general higher precipitations corresponded to lower temperatures during the spring. Three types of weather pattern were distinguished during the wheat reproductive period according to air temperatures and water stress (rainfall - evapotranspiration): very dry when the water stress and high temperatures generally began at the end of March and in the middle of April (2006 and 2009 year, respectively), moderately dry when the water stress and high temperatures began in the middle of May (2007 and 2011 year); and wet when the water stress and high temperatures began in June (2008 and 2010 year). Grain yield and grain quality characteristics were generally sensitive to yearby-year variations in temperatures and in the amount of rainfall during the wheat cropping season. The best wheat yield performance was observed in the years when a moderate water stress generally occurred from the end of May onwards (in 2007 and 2011), especially in the organic system. In our experiment an excess of rainfall and soil water availability in spring, which occurred in 2008 and 2010 (Fig. 1), only favored the yield in the conventional where the weeds were well controlled by the herbicide, while it caused a strong increase of weed proliferation in the organic (Fig. 7) thus reducing grain yield performance (Table 1). Considering that in the six experimental years the largest grain yield gaps between organic and conventional were observed in 2008 and 2010, an efficient weed control strategy appear to be essential for obtaining a satisfactory grain yield when the wheat reproductive period is particularly wet. A shortage of rainfall and soil water availability combined with high temperatures in spring significantly reduced grain yield, accelerated cell death and caused earlier wheat maturity as occurred in 2006 and 2009. The yield reduction was similar in both organic and conventional wheat which showed the lowest productive performance of the whole experimental period.

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The results of the grain quality assessment highlighted that durum wheat cultivated under the conventional cropping system generally reached the highest values particularly for test weight, vitreousness and gluten quality, while protein, gluten content and ash varied considerably from year to year due to differences in temperatures and rainfall. This great variability in some of the grain quality parameters between conventional and organic may have been caused by a greater sensitivity

of different weather conditions on mineralization-driven N supply deriving from organic fertilizers (Smith et al., 2007). This hypothesis is supported by the results of the correlation analysis between meteorological variables and grain yield which indicate that there was a positive and significant correlation between high spring temperatures and yield only in the organic cropping system (Table 5). Considering that mineralization of organic fertilizers mainly depends on temperature and moisture, low spring temperatures could have determined a low N mineralization rate in organic soil thus supplying less nitrogen in organic compared to conventional wheat. This conclusion is supported by the leaf chlorophyll concentrations (SPAD reading values of wheat flags leaves) which were significantly lower in organic than in conventional (Fig. 3). Although the same amount of nitrogen fertilization was applied to both cropping systems, it is conceivable that the quantity of nitrogen available to the wheat from organic fertilizers was significantly lower than from mineral fertilizers. Therefore, a good strategy could be to increase the amount of N applied with organic fertilizers compared to that applied with mineral fertilizers in order to compensate for the lack of available nitrogen in organic wheat. In particular it may be advisable to apply organic fertilizers with high available nitrogen such as slurries or blood meal in the reproductive period of the wheat. However the lowest values in protein and gluten content, both in conventional and organic, were observed in 2006 when a long period of drought occurred starting from stem elongation and lasted throughout the whole wheat reproductive period (from the end of March to the beginning of June). Severe water stress conditions may have reduced not only the number of fertile spikes but also impeded the filling and translocation of nitrogen to the grain (Garrido-Lestache et al., 2005). High spring temperatures in combination with water stress during the grain filling period, as occurred at the end of May 2009, caused an increase of protein content and vitreousness. Therefore, in our experiment an early and prolonged water stress determined a decrease both of protein and grain yield, while an improvement in grain quality and a reduction in grain yield were observed when water stress was combined with high temperatures during the grain filling period. An excess of rainfall and soil water availability during the grain filling stage, which occurred in 2008 and 2010,

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determined a similar protein and gluten content in both conventional and organic wheat grain. A surplus of water may have drastically reduced the mineral nitrogen availability due to nitrogen loss by leaching (Doltra et al., 2011) especially in conventional where the nitrogen was mainly replenished by mineral fertilization and therefore more easily leachable. This hypothesis is confirmed by the SPAD reading values of wheat flag leaves which were similar between organic and conventional wheat in 2008 and 2010 (Fig. 3). In agreement with Rharrabti et al. (2003) the results show that high rainfall during the grain filling stage significantly reduced TW and vitreousness thus affecting the density and shape characteristics of the grain. Therefore, both an excess and a severe lack of water during the anthesis and grain filling period negatively affected wheat grain quality especially in conventional where the advantage in protein content compared to the organic seems to disappear.

Tillage management did not affect durum wheat, in fact the grain yield was comparable throughout the 6-year period in plowed and subsoiled soil (Tab. 4), and only an increase of the test weight was observed in plowed soil compared to subsoiled soil. A higher soil water content was expected in the subsoiled compared to plowed soil especially in the year when a long period of drought occurred during the grain filling stage period. Although the soil water content tended to be higher in subsoiled soil, no significant differences were observed between the soil tillage managements (Fig. 2). In conservation tillage systems, a higher water content is usually associated with the placement of crop residues on the soil surface which increases water infiltration and reduces soil water evaporation (Smith and Elliot, 1990). In our case the disk operation, which was performed for seed bed preparation, buried most of the residues of the preceding crop, therefore these effects may be reduced or even disappear. Subsoiling would have probably been more effective and efficient if it had been combined with an appropriate planter machine without further soil tillage, which would have left more residues on the soil surface.

## 5. CONCLUSION

Although these results are related to a single cultivar of durum wheat and specific crop management techniques, they provide useful information on the behavior of conventional and organic durum wheat under various weather conditions over time. In particular this study highlights that the combined effect of cropping system and weather conditions determined a strong variability on durum grain yield and quality parameters, which were higher in organic than in conventional system. Conventional durum wheat generally had a higher grain yield compared to organic, although the size of the yield gap between the two cropping systems was highly variable, especially when the grain filling stage was characterized by high precipitation which determined an strong increase of weed proliferation in the organic system. An extended water stress starting from the stem elongation stage determined the worst wheat yield performance which was similar in both conventional and organic, while high air temperatures in spring were positively correlated with grain yield only in organic. Conventional wheat also showed a better grain quality in terms of test weight, vitreousness and gluten quality, while protein and gluten content were higher in conventional compared to organic only when a regular rainfall distribution occurred throughout the wheat reproductive period. These results may be due to a lower nitrogen availability in the organic compared to conventional which was evident in the heading-anthesis stage. In contrast, yield and grain quality of durum wheat under different tillage managements such as plowed and subsoiled soil were comparable. Considering subsoiling as an important conservation tillage technique, the results highlight that the use of the subsoiler for durum wheat production is advisable in the Mediterranean environment of Central Italy, although subsoiling alone did not increase soil moisture compared to plowing. However, weed control and nitrogen supply appear to be the main limiting factors which strongly depend on weather conditions and can determine the magnitude of the yield gap between organic and conventional cropping systems, especially when an excess of rainfall or low temperatures occur during the wheat reproductive period. Further research should take into account different durum wheat genotypes which are more suitable for organic farming as well as flexible

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- agricultural strategies that are able to improve or maintain grain durum wheat yield and quality
- 2 under different weather conditions.

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2 3 **Figure 1.** Rainfall ( $\square$ ), minimum ( $\longrightarrow$ ) and maximum air temperatures (--), and  $\Delta$  (cumulated 4 rainfall - cumulated evapotranspiration) at 10-day intervals at the experimental site of the 5 September – July period from 2005/2006 to 2010/2011. 6 7 8 Figure 2. Soil moisture content of durum wheat as influenced by cropping system and soil tillage 9 throughout the period from March to June. Error bars represent standard error from mean (n= 30). 10 CONV = Conventional cropping system; ORG = Organic cropping system; PS = Plowed soil; SS = 11 Subsoiled soil. 12 13 14 Figure 3. The effect of interaction of the year x cropping system on the SPAD readings at wheat 15 flag leaf stage. Values belonging to the same characteristic without common letters are statistically different according to LSD (P < 0.05). 16 17 18 Figure 4. Biplot from a canonical discriminant analysis (CDA) of durum wheat grain yield and yield components from 2006 to 2011 experimental years. TGW = Thousand grain weight; HI = 19 20 Harvest index. 21 22 Figure 5. Relationships between the SPAD readings and the grain protein content, and the SPAD 23 readings and the grain gluten content. Data correspond from 2006 to 2011 growing seasons and the significance level is \*\*\* significant at  $P \le 0.001$ . 24 25 26 Figure 6. Biplot from a canonical discriminant analysis (CDA) of durum wheat characteristics from

**Figure 7.** The effect of interaction of the year x cropping system on the weed aboveground biomass

at wheat harvesting. Values belonging to the same characteristic without common letters are

2006 to 2011 experimental years. TW = Test weight. Vitr.= Vitreousness.

statistically different according to LSD (P < 0.05).

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Figure captions

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**Table 1.** The effect of interaction of the year x cropping system on the plant height, wheat grain yield, wheat straw, and harvest index (HI) in durum wheat. Values belonging to the same characteristic without common letters are statistically different according to LSD ( $P \le 0.05$ ) in rows for each cropping system (upper case letters), and columns for each year (lower case letters). CONV = Conventional cropping system; ORG = Organic cropping system.

	Plant height	Grain yield			raw	ŀ	ΗI	
	(cm)	(t ha <sup>-1</sup> of dry matter)		(t ha <sup>-1</sup> of o	dry matter)	(%)		
Year		CONV ORG		CONV	ORG	CONV	ORG	
• • • •								
2006	61.2 b	2.75 cA 2.62	bA	4.61 cA	4.30 bA	37.4 aA	37.9 aA	
2007	70.5 a	4.10 aA 3.58	aB	8.27 aA	6.85 aB	33.1 bA	34.3 bA	
2008	70.2 a	3.44 bA 2.63	bB	5.88 cA	5.18 abA	36.9 abA	33.7 bB	
2009	59.1 b	2.91 cA 2.62	bA	5.17 cA	4.70 bA	36.0 abA	35.8 abA	
2010	70.5 a	3.79 aA 2.58	bB	7.08 bA	4.94 abB	34.9 bA	34.3 bA	
2011	70.7 a	3.41 bA 3.13	aA	5.57 cA	5.82 aA	38.0 aA	35.0 abB	
Cropping System (CS)	ns	***			*	***		
Soil Tillage (ST)	ns	ns		1	ns	ns		
CS x ST	ns	ns		1	ns	ns		
Year (Y)	***	***		*	**	***		
CS x Y	ns	***			*	***		
ST x Y	**	ns		1	ns	ns		
CS x ST x Y	ns	ns		1	ns	ns		

<sup>\*, \*\*, \*\*\*,</sup> or ns: significance at  $P \le 0.05$ ,  $P \le 0.01$ ,  $P \le 0.001$  or  $P \ge 0.05$ , respectively.

**Table 2.** The effect of interaction of the year x cropping system on the fertile spikes, the kernels and the thousand grain weight (TGW). Values belonging to the same characteristic without common letters are statistically different according to LSD ( $P \le 0.05$ ) in rows for each cropping system (upper case letters), and columns for each year (lower case letters). CONV = Conventional cropping system; ORG = Organic cropping system.

		spikes		nels	TGW			
	(n. 1	m <sup>-2</sup> )	(n. sp	oike <sup>-1</sup> )	(g)			
Year	CONV	ORG	CONV	ORG	CONV	ORG		
2006	217.7 dA	222.7 dA	26.3 cA	25.9 bA	49.8 aA	50.3 abA		
2007	357.0 aA	361.5 aA	30.8 bA	25.0 bB	42.6 dB	44.6 cA		
2008	322.2 bA	259.4 cB	21.4 dA	19.5 cA	47.1 bA	48.3 bA		
2009	202.5 dA	179.5 eA	35.0 aA	34.5 aA	44.5 cA	44.4 cA		
2010	318.3 bA	293.8 bB	30.9 bA	20.4 cB	44.1 cdB	46.1 bcA		
2011	273.0 cA	256.7 cA	26.7 cA	24.3 bA	50.4 aB	52.1 aA		
Cropping System (CS)	n	ıs	*:	**	ns			
Soil Tillage (ST)	n	ıs	r	ıs	*			
CS x ST	;	k	:	*	ns			
Year (Y)	**	**	*:	**	***			
CS x Y	;	k	:	*	*			
ST x Y	n	ıs	r	ns	ns			
CS x ST x Y	n	ıs	r	ns	ns			

<sup>\*, \*\*, \*\*\*,</sup> or ns: significance at  $P \le 0.05$ ,  $P \le 0.01$ ,  $P \le 0.001$  or  $P \ge 0.05$ , respectively.

**Table 3.** The main effect of year on the test weight (TW), vitreousness, and gluten quality (SDS test), and the effect of interaction of the year x cropping system on the protein content, gluten content, and ash content in durum wheat grain. Values belonging to the same characteristic without common letters are statistically different according to LSD ( $P \le 0.05$ ) in rows for each cropping system (upper case letters), and columns for each year (lower case letters). CONV = Conventional cropping system; ORG = Organic cropping system.

	=	Vitreousness	SDS test		tein y matter)	Glu (% of dry		Ash (% of dry matter)		
Year		(%)	(mL)	CONV	ORG	CONV	ORG	CONV	ORG	
2006	81.08 a	91.08 ab	34.83 b	10.4 cA	9.8 cA	6.3 dA	5.6 dB	1.84 cA	1.80 dA	
2007	80.83 a	89.05 ab	44.08 a	12.0 aA	10.2 cB	9.2 aA	7.6 bB	2.01 aB	2.13 aA	
2008	77.82 b	87.78 b	34.25 b	11.5 bA	11.2 bA	7.9 cA	7.6 bA	1.89 bB	1.98 cA	
2009	82.20 a	92.27 a		12.6 aA	11.9 aA					
2010	77.74 b	84.92 b	34.67 b	11.4 bA	11.9 aA	8.3 bcA	8.8 aA	2.03 aB	2.08 bA	
2011	81.68 a	89.78 ab	34.42 b	12.4 aA	10.7 bB	8.6 bA	6.6 cB	1.79 dA	1.73 eB	
Cropping system (CS)	*	***	*	***		<b>*</b> *	***			
Soil tillage (ST)	*	ns	ns	n	ıs	n	S	**		
CS x ST	ns	ns	ns	n	ıs	ns		ns		
Year (Y)	***	***	***	***		***		***		
CS x Y	ns	ns	ns	**	***		***		***	
ST x Y	ns	ns	ns	ns		ns		*		
CS x ST x Y	ns	ns	ns	ns		ns		ns		

<sup>\*, \*\*, \*\*\*,</sup> or ns: significance at  $P \le 0.05$ ,  $P \le 0.01$ ,  $P \le 0.001$  or  $P \ge 0.05$ , respectively.

**Table 4.** Test statistic (T) from the multi-response permutation procedure (MRPP) for the multiple paired comparisons to evaluate the main effects of cropping systems and tillage on wheat yield characteristics and wheat grain quality from 2006 to 2011 experimental years. P is the probability of significant differences among groups.

	2006		2007		2008		2009		2010		2011	
	T	P	T	P	T	$\boldsymbol{P}$	T	$\boldsymbol{P}$	T	P	T	P
Wheat yield characteristics												
Cropping system	0.349	0.574	-3.625	0.006	-4.213	< 0.001	-1.037	0.143	-6.245	< 0.001	-1.946	0.043
Soil tillage	-1.663	0.067	1.187	0.940	-0.276	0.347	-0.808	0.193	1.025	0.971	-0.637	0.225
Wheat grain quality												
Cropping system	-2.408	0.026	-5.959	< 0.001	-2.049	0.037	-2.836	0.023	-2.743	0.019	-5.624	< 0.001
Soil tillage	-0.944	0.160	-0.299	0.288	-3.433	0.004	-0.726	0.245	-3.114	0.012	-2.735	0.020

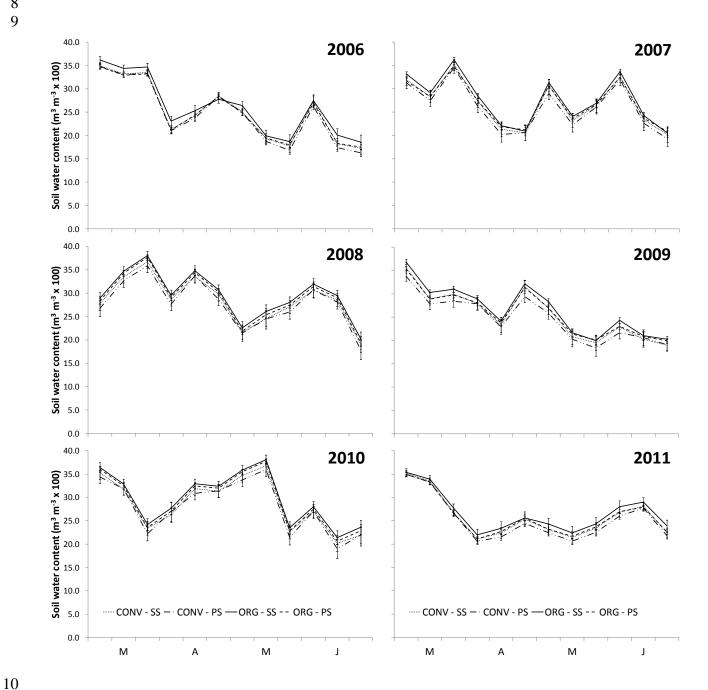
**Table 5.** Correlation coefficients between grain yield or protein content of durum wheat and monthly, multi-monthly air temperatures or monthly, multi-monthly cumulated rainfall in the periods March – June from 2006 to 2011 in conventional and organic cropping systems. \*, \*\*, \*\*\* and ns significance at  $P \le 0.05$ ,  $P \le 0.01$ ,  $P \le 0.001$  or  $P \ge 0.05$ , respectively.

				Monthly or	r multi-mon	thly air tem <sub>l</sub>	peratures			
	Mar	Mar-Apr	Mar-May	Mar-Jun	Apr	Apr-May	Apr-Jun	May	May-Jun	Jun
Conventional	cropping s	system								
Grain yield	$+0.471^{ns}$	$+0.338^{\text{ns}}$	$+0.181^{ns}$	$+0.140^{ns}$	$+0.189^{ns}$	$+0.071^{ns}$	$+0.061^{ns}$	$-0.043^{\text{ns}}$	$+0.004^{ns}$	$+0.031^{ns}$
Protein	$+0.202^{ns}$	$+0.072^{ns}$	+0.121 <sup>ns</sup>	$+0.196^{ns}$	$+0.012^{ns}$	$+0.076^{ns}$	$+0.167^{ns}$	$+0.426^{ns}$	$+0.311^{ns}$	$+0.192^{ns}$
Organic cropp	oing systen	n								
Grain yield	$+0.627^{*}$	+0.896***	+0.867***	+0.842***	+0.857***	+0.819***	+0.795***	+0.564*	$+0.494^{ns}$	$+0.398^{ns}$
Protein	$-0.056^{\text{ns}}$	-0.359 <sup>ns</sup>	-0.382 <sup>ns</sup>	-0.310 <sup>ns</sup>	-0.582*	-0.553*	-0.384 <sup>ns</sup>	-0.237 <sup>ns</sup>	-0.159 <sup>ns</sup>	-0.093 <sup>ns</sup>
				Monthly or	multi-montl	nly cumulate	ed rainfall			
	Mar	Mar-Apr	Mar-May	Mar-Jun	Apr	Apr-May	Apr-Jun	May	May-Jun	Jun
Conventional			o acans	0.4.4 <b>0</b> ns	0 0 <b>=</b> 4 ns	o acons	o 4 ==ns	0.744*	0.22078	0.00 <b>~</b> ns
Grain yield	$-0.057^{\text{ns}}$	$-0.153^{\text{ns}}$	$+0.203^{\text{ns}}$	$+0.142^{\text{ns}}$	$-0.051^{\text{ns}}$	$+0.208^{ns}$	$+0.157^{\text{ns}}$	+0.544*	$+0.329^{\text{ns}}$	$-0.005^{\text{ns}}$
Protein	$-0.001^{\text{ns}}$	$-0.237^{\text{ns}}$	$-0.079^{\text{ns}}$	$+0.001^{\text{ns}}$	$-0.189^{\text{ns}}$	$-0.064^{\text{ns}}$	$+0.001^{\text{ns}}$	$-0.021^{\text{ns}}$	$+0.030^{\text{ns}}$	$+0.501^{\text{ns}}$
Organic cropp	ping systen	n								
Grain yield	$+0.029^{\text{ns}}$	-0.723***	$-0.164^{\text{ns}}$	$-0.175^{\text{ns}}$	-0.792***	$-0.162^{\text{ns}}$	$-0.179^{ns}$	$-0.023^{\text{ns}}$	$-0.040^{\text{ns}}$	$-0.022^{ns}$
Protein	$-0.211^{ns}$	$+0.127^{ns}$	$+0.255^{ns}$	$+0.496^{ns}$	$+0.206^{ns}$	$+0.299^{ns}$	$+0.552^*$	$+0.357^{ns}$	$+0.594^*$	$+0.479^{ns}$

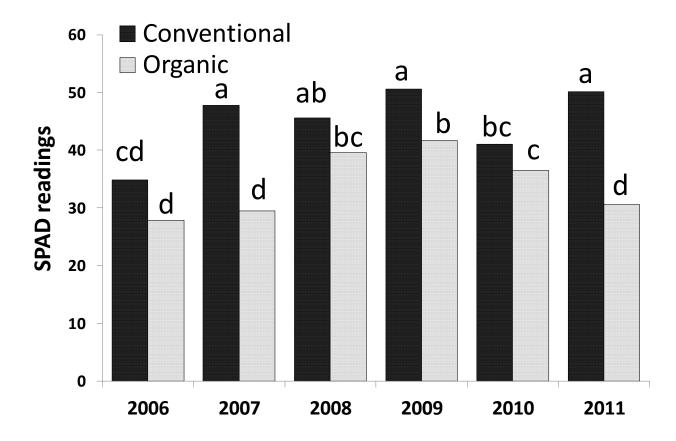
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**Figure 1.** Rainfall ( $\blacksquare$ ), minimum ( $\longrightarrow$ ) and maximum air temperatures (--), and  $\Delta$  (cumulated rainfall - cumulated evapotranspiration) at 10-day intervals at the experimental site of the September - July period from 2005/2006 to 2010/2011.

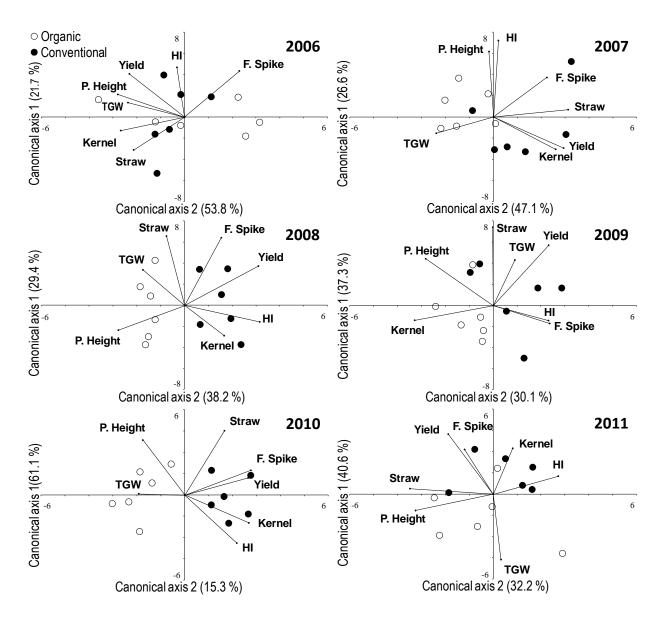
**Figure 2.** Soil moisture content of durum wheat as influenced by cropping system and soil tillage throughout the period from March to June. Error bars represent standard error from mean (n= 30). CONV = Conventional cropping system; ORG = Organic cropping system; PS = Plowed soil; SS = Subsoiled soil.



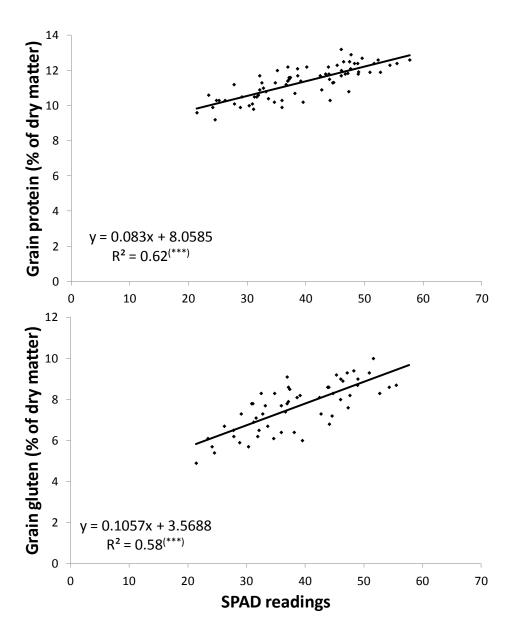
**Figure 3.** The effect of interaction of the year x cropping system on the SPAD readings at wheat flag leaf stage. Values belonging to the same characteristic without common letters are statistically different according to LSD (P < 0.05).



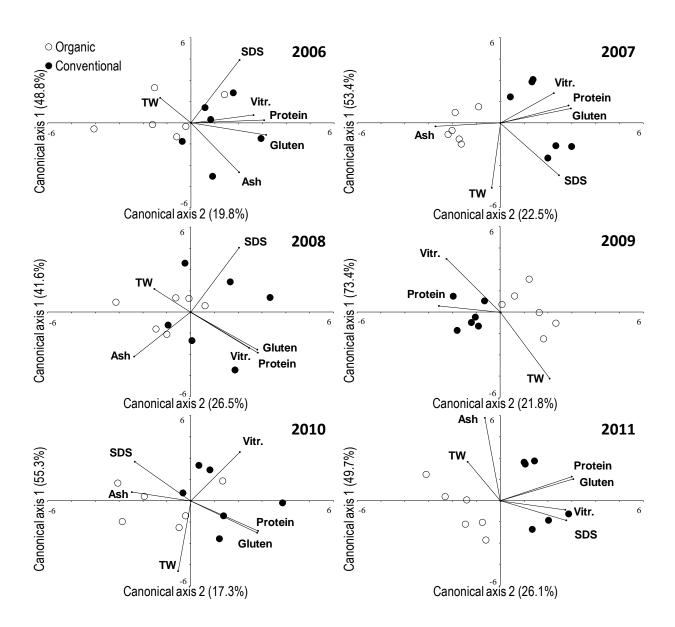
**Figure 4.** Biplot from a canonical discriminant analysis (CDA) of durum wheat grain yield and yield components from 2006 to 2011 experimental years. TGW = Thousand grain weight; HI = Harvest index.



**Figure 5**. Relationships between the SPAD readings and the grain protein content, and the SPAD readings and the grain gluten content. Data correspond from 2006 to 2011 growing seasons and the significance level is \*\*\* significant at  $P \le 0.001$ .



**Figure 6.** Biplot from a canonical discriminant analysis (CDA) of durum wheat characteristics from 2006 to 2011 experimental years. TW = Test weight.\_Vitr.= Vitreousness.



**Figure 7.** The effect of interaction of the year x cropping system on the weed aboveground biomass at wheat harvesting. Values belonging to the same characteristic without common letters are statistically different according to LSD (P < 0.05).

