

# Durum Wheat Quality, Yield, and Sanitary Status Under Conservation Agriculture

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**Abstract:** Conservation agriculture (CA) could be very effective for agricultural sustainability under Mediterranean environments, where farmers rely on short rotation based on durum wheat. In this work we investigated the effect of different combination of tillage treatments and crop sequence (conventional tillage and wheat monocropping, CT-WW; conventional tillage, and wheat following faba-bean, CT-WF; zero tillage and wheat monocropping, ZT-WW; zero tillage and wheat following faba-bean, ZT-WF) on yield, grain quality traits as well as on diseases incidence and severity in durum wheat (var. Saragolla). The results of a two-years of data of a long-term experiment (7-year experiment; split-plot design) are discussed. The CA approach (ZT+WF), which induced always the highest grain yields (6.1 t ha<sup>-1</sup> and 3.3 t ha<sup>-1</sup> in 2016 and 2017) thanks to an increased number of spikes m<sup>-2</sup> (296 *vs* 269 and 303 *vs* 287 spikes m<sup>-2</sup> in 2016 and 2017, respectively) as well as to a more pronounced ear length, demonstrated significant positive influences in terms of grain quality. It promoted grain protein accumulation (12.1% for ZT+WF *versus* 11.4% for ZT+WW and 12.4% for ZT+WF *versus* 10.6% for ZT+WW in 2016 and 2017), improved gluten quality (in terms of SDS sedimentation test) and colour of the grain. The abundance of crop residues determined a higher incidence and severity of *Zymoseptoria tritici* leaf symptoms under CA system; nevertheless the late appearance of infection was the main reason of not affecting yield and quality traits. The presence of faba-bean (WF) in the rotation significantly reduced leaf symptoms of *Z. tritici*.

**Keywords:** Conservation Agriculture; Durum Wheat; Faba-bean, Fungal diseases; Grain Quality.

## 1. Introduction

The representative farms of the inner hilly not irrigated Mediterranean environments, are characterized by low and erratic rainfall distribution and high temperatures, typically rely on the exclusively application of durum wheat monocropping or short rotations durum wheat - faba-bean, with significantly

instability of the yields and quality traits. Indeed, the exclusively application of conventional soil management, where deep ploughing represents the main tillage approach, has led to severe soil loss, organic matter depletion, CO<sub>2</sub> release, biodiversity constrains. It is, hence, such soil fertility reduction that, in combination with the climatic changes, has negatively influenced durum wheat performances over time [1].

A way to merge farms economic with social and environmental sustainability is represented by the adoption of Conservation agriculture (CA), an agronomic system developed to prevent soil erosion and compaction, saving costs for the implementation of cultivation practices [2]. It includes zero tillage, maintaining of crop residues in the field, adoption of the use of cover crops as well as of crop rotations [3, 4] : as results, soil layers are not reversed and crop residues and organic fertilizers are not incorporated in the processed layer [5]. Several data demonstrate that the balance between CO<sub>2</sub> emitted and captured is optimized [6, 7], soil organic matter, edaphic biodiversity and water retention are increased [8, 9], and the bioavailability of nutrients as well as the whole fertility of soil are improved [10] with consequent reduction of management costs [11].

Nevertheless, some concerns need to be investigated when CA is introduced in different environments; they principally relate to weed management, possible diffusion of wheat diseases, variable yields, seeds technological quality [12]. Among the main factors that could determine a greater severity of diseases under CA, the abundance of crop residues as well as continuous moist soil conditions, which allow survival and distribution of pathogen inoculum, are primary. At world level, and especially in Europe due to the low diffusion of CA, only few studies have been carried out on diseases in durum wheat managed according to these techniques [13] as well as on quality traits, and results are sometimes discordant. Data from Western Europe indicate low yield and quality decrease (8.5% less) when no tillage (NT) is compared to conventional tillage (CT). Studies on the effects of CA on disease incidence in Canada have highlighted the significant influence of wheat varieties and environmental conditions [14]. Similarly, Australian studies, have suggested some integrations of CA techniques to reduce the effects of pedoclimatic conditions [15, 16]. Previous work had stated that reduced tillage increased the presence of the soilborne pathogens thanks to favorable environmental conditions offered by cover crops and residues [17, 18]. On the other hand, a higher soil microbial activity, achieved under CA, should lead to decrease the virulence of the pathogens [19]. Cover crops and residues management play also, thanks to their effect on carbon and nutrient (especially N) dynamics in soil [20, 21], a key role in affecting technological, physiological and yield traits in durum wheat, especially in depleted soils; changes in N accumulation and release in soil would modify N uptake efficiency and synchronization [22, 23, 24]. Data regarding the effect of CA practices on durum wheat, kernel technological and their relationships with yields are missing.

Therefore, with this study undertaken in Mediterranean environment, based on a 7-year experiment combining two tillage practices (conventional tillage (CT) and zero tillage (ZT)) with two crop sequences (wheat monocropping (WW) and wheat – faba-bean (WF)), we tested the hypothesis that CA approach would not deplete technological profile of kernels as well as yields; besides, we wanted to investigate response in term of incidence and severity of fungal diseases and their possible influence on yield and technological traits.

## 2. Materials and methods

### 2.1 Site description

One long term field experiment has been carrying out in Teramo (Mosciano Sant'Angelo, Italy, 42°42' N, 13°52'E, 101 m a.s.l.). The area is characterized by a typical Mediterranean climate with a mean annual rainfall, recorded over a 58-year period, of 732 mm concentrated mainly between October and April. The mean of the maximum temperature ranges from 11 °C to 29 °C while the mean of the temperature minimum from 2 °C to 17 °C. The main soil characteristics are as follows: 23% sand, 45% silt, 32% clay, pH 8.1, 19.0% total CaCO<sub>3</sub> and 10.6% active CaCO<sub>3</sub>. Meteorological data were recorded with a meteorological station situated ~ 1 Km from the experimental field.

### 2.2 Experimental design and agronomic practices

The experiment consists in two soil tillage systems and two crop sequences arranged on a split-plot design with three replications, where tillage systems represent the main plots and crop sequences the sub-plots. CT and ZT were established in the same experimental field (15,000 m<sup>2</sup>) opportunely arranged to avoid any overlays during crop operations. They were imposed starting from the beginning of the experimentation (2010-2011), hence for ZT treatments a six/seven-year period of conversion (2010-2016/2017) was observed, respecting the requirements of the CT system standards to ensure reliable long-term production goals [25]. CT management included ploughing, complete soil inversion to 35-cm depth during the summer, with secondary tillage in autumn. ZT involved no soil disturbance with previous-crop residues retention and a Glyphosate (45%) application at the rate of 2 L ha<sup>-1</sup>, two weeks before sowing.

For each soil-management system, two different crop sequences (sub-plots) were randomly adopted: (i) durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) monocropping (WW); and (ii) durum wheat following faba-bean (*Vicia faba* L. var. *minor*) (WF). Each rotational area (sub-plots) was obtained by split each tillage system (main-plot) in area of equal size (1,200 m<sup>2</sup>) so hosting both crop sequences every year. Thesis ZT+WF allow the application of all the principles of CA.

The durum wheat and faba-bean cultivars were “Saragolla” and “Protabath”, respectively. In ZT approach, durum wheat was sown on 7<sup>th</sup> December and 23<sup>rd</sup> November in 2016 and 2017 respectively, with a direct seeder (Gaspardo Direttissima, Gruppo Maschio Gaspardo S.p.A., Campodarsego, PD, Italy) at a seeding rate of 350 seeds m<sup>-2</sup>. Nitrogen was applied as ammonium nitrate and urea in two applications at the whole rate of 150 kg N ha<sup>-1</sup>, splitted half on 22 March 2016 and 28 March 2017 at the beginning of stem elongation and half on 18 April 2016 and on 20 April 2017, at emergence of head complete

### ***2.3 Yield and merceological parameters***

At harvesting, (23 June and 19 June for 2016 and 2017, respectively) yield components and several commercial and technological quality parameters were determined. One square meter per each plot was harvested manually and grain yield measured at 13% moisture content. The number of spikes per unit area was estimated by counting spikes along one square meter plot surface and fifteen spikes per plot were collected for ear length and plant height determination; measurements were taken from the soil to the top of the spike. Thousand kernel weight (TKW) was calculated as the mean weight of three sets of 100 grains per plot and specific weight, expressed as kg hl<sup>-1</sup>, was measured with a Shopper chondrometer.

### ***2.4 Grain quality analysis***

Grain sub-samples collected from each windows plot were milled with Knifetec TM 1095 (Foss, Hillerød, Denmark) to obtain a fine powder and whole-meal flour was used to evaluate some quality-related parameter. Grain N content was determined by the standard Kjeldahl method. Grains protein concentration (GPC, %) was calculated multiplied by 5.7 [26] and expressed on a dry weight basis. An SDS sedimentation volume test using 0.6 g of flour sample was performed as an indicator of gluten strength.

Grain yellow index was determined using a Minolta Chroma Meter colorimeter for colour space measurements while carotenoid pigments were evaluated by extraction in solution of n-butanol saturated with water in samples of 50 mg of flour and measured in a spectrophotometer at 435 nm.

Ash content was determined by incineration in a muffle oven at 550 °C over night.

### ***2.5 Survey of symptoms of fungal diseases***

For each thesis (CT+WW, CT+WF, ZT+WW, ZT+WF) visual assessments of fungal disease symptoms were carried out. In each thesis three plots were selected (each of which represented a repetition). Each plot measured 16 m<sup>2</sup> and was appropriately spaced from the others. Two surveys were carried out on 22 May 2016 and 26 May 2017, when durum wheat was at phenological stages of hard dough. For each plot, leaf symptom incidence was calculated by dividing the number of symptomatic plants by the number of standing plants.

The severity of leaf symptoms were calculated by assigning at each plant a percentage value obtained from the ratio of the attacked surface versus the whole plant surface. Percent disease severity was calculated from the McKinney index as follow:  $\Sigma N \times 100 / (Y \times Z)$  where  $\Sigma N$  = sum of severity rating in each plant;  $Y$  = number of standing plants,  $Z = 100$ , which is the maximum rating in the disease assessment scale [27].

## 2.6 Isolation of pathogens from symptomatic leaves

On 26 May 2017, 15 plants showing symptoms of a fungal disease were collected from each plot of the different 4 thesis and transferred to the laboratory where 2 leaves per plant were harvested. Thus, each of the 4 thesis was represented by a group of 90 leaves. Each leaf was subsequently separated into 2-3 sections of about 7-9 cm and collocated in 14 cm Petri plates where was previously put a disc of tissue paper wetted with distilled water, to create a moist incubation chamber conditions. The leaf section of each thesis have been kept separated, in order of point out possible differences in the detected microflora. The plates were then kept at  $20 \pm 2^\circ\text{C}$  and photoperiod of 12 hours of light. After 24, 48, 72 and 96 hours, the leaf samples were monitored with a Leica DMRM 301-371.010, assembled with a digital camera Leica DFC295 (2048x1536 pixel), magnification of 40x, to assess the presence of fungal propagules and proceed to pathogen identification. Images were processed with the software Leica Application Suite (LAS), V4.1.

At 24-48 hours time, some of the pycnidia observed on the lesions of the symptomatic leaves had formed conidial cirrhi. All these cirrhi were collected, covered with a drop of sterile water, fixed on slides and observed under the optical microscope to verify the presence of conidia. After 72-96 hours the formation of additional cirrhi was assessed; besides, some of the pycnidia (3-4 for leaf portion), which had not generated cirrhi, were collected, placed on slides containing a few drop of sterile water and crushed with another slide. Each crushed pycnidium was observed under the optical microscope.

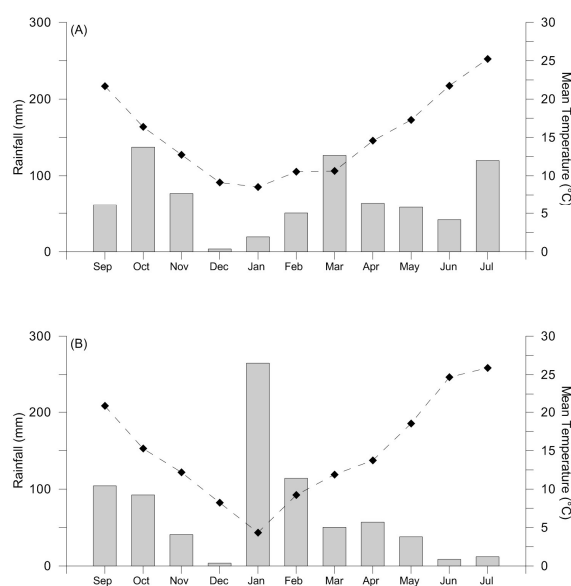
## 2.7 Statistical analysis

A two-way analysis of variance (ANOVA) was applied to test (F-test) the effects of soil management (main factor) and crop sequences (secondary factor) on all tested variables. If the ANOVA detected significant differences, the means were compared based on the standard error of the difference (SED) between means (data in Tables). All the statistical analyses were performed using R software [28].

# 3. Results

## 3.1 Meteorological data and soil characteristics

The amount and distribution of rainfall and the range of monthly averages of temperatures, registered during the two year-experiments, are reported in Figure 1. During the period anthesis-end of grain filling (march-may), rainfalls were 248 mm in 2016 and lowered to 145 mm in 2017 (about 50% less). Differences emerged also during the vegetative phase: in 2016, between sowing and anthesis, crops received 262.4 mm of rain while in 2017 505.2 mm, mainly concentrated in January. Although the monthly average temperature of the whole cropping cycles was almost the same (15.3 and 15 °C for 2016 and 2017, respectively), during the reproductive period the values of the second year were generally higher (16.1 and 17.2°C in 2016 and 2017 respectively).



**Figure 1:** Means of monthly rainfall (bars) and temperatures (symbols) registered during the durum wheat crop cycle in two different growing seasons, 2015-2016 (A) and 2016-2017 (B) at Mosciano Sant'Angelo, Teramo.

### 3.2 Yield and yield components

Data regarding grain yield, ears m<sup>-2</sup>, ears length and plant height for the different combination of tillage treatments and crop sequence are presented in Table 1.

**Table 1:** Influence of different combination of tillage treatments and crop sequence on wheat Yield ( $\text{t ha}^{-1}$ ), number of spikes per square meter (ears,  $\text{num m}^{-2}$ ), ear length (cm) and plant height (cm), recorded at harvesting in 2016 and 2017.

	2016			2017		
	ZT	CT	Overall mean	ZT	CT	Overall mean
<b>Yield (<math>\text{t ha}^{-1}</math>)</b>						
WW	5.4	5.5	5.4	3.2	3.3	3.2
WF	6.1	5.5	5.8	3.3	3.2	3.2
Overall mean	5.8	5.5	5.6	3.2	3.2	3.2
<i>Soil management</i>		<i>n.s.</i>			<i>n.s.</i>	
<i>Crop Sequence</i>		<i>*(7.76)</i>			<i>n.s.</i>	
<i>Soil management x Crop Sequence</i>		<i>*(10.98)</i>			<i>n.s.</i>	
<b>Ears (<math>\text{num m}^{-2}</math>)</b>						
WW	269	255	262	287	262	275
WF	296	271	284	303	286	294
Overall mean	283	263	273	295	274	284
<i>Soil management</i>		<i>n.s.</i>			<i>** (1.37)</i>	
<i>Crop Sequence</i>		<i>n.s.</i>			<i>n.s.</i>	
<i>Soil management x Crop Sequence</i>		<i>*(4.1)</i>			<i>n.s.</i>	
<b>Ear length (cm)</b>						
WW	7.18	7.40	7.29	6.60	6.22	6.41
WF	7.35	7.72	7.53	6.53	6.17	6.35
Overall mean	7.27	7.56	7.41	6.57	6.19	6.38
<i>Soil management</i>		<i>n.s.</i>			<i>*(0.09)</i>	
<i>Crop Sequence</i>		<i>** (0.03)</i>			<i>n.s.</i>	
<i>Soil management x Crop Sequence</i>		<i>n.s.</i>			<i>n.s.</i>	
<b>Plant Height (cm)</b>						
WW	71	72.7	71.8	66.1	63.3	64.7
WF	69.7	70.7	70.2	62	63.8	62.9
Overall mean	70.3	71.7	71	64	63.5	63.8
<i>Soil management</i>		<i>n.s.</i>			<i>n.s.</i>	
<i>Crop Sequence</i>		<i>n.s.</i>			<i>n.s.</i>	
<i>Soil management x Crop Sequence</i>		<i>n.s.</i>			<i>n.s.</i>	

CT+WW: conventional tillage and wheat monocropping; CT+WF: conventional tillage and wheat following faba-bean; ZT+WW: zero tillage and wheat monocropping; ZT+WF: zero tillage and wheat following faba-bean. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; *n.s.* = not-significant. In brackets: standard error of differences between means (s.e.d.). Degrees of freedom: Soil management, 1; Crop Sequence, 1; Soil management x Crop Sequence, 1; Residual, 4.

Higher yield values were registered in 2016,  $5.6 \text{ t ha}^{-1}$  vs  $3.2 \text{ t ha}^{-1}$  in 2016 and 2017 respectively, when were also observed significant differences between treatments. The combination ZT and WF gave the highest value ( $6.1 \text{ t ha}^{-1}$ ), while the other treatments did not differ between each other. The higher yields were related to a significant increasing in the number of ears  $\text{m}^{-2}$ : in both years ZT+WF combination gave the highest values

(296 and 303 spikes  $m^{-2}$ ) while CT WW the lowest (255 and 262 spikes  $m^{-2}$ ). Moreover, regarding ear length, crop sequence resulted significant in 2016, with WF exhibiting the highest value (7.53 vs 7.29 cm for WF and WW, respectively), while soil management in 2017 with ZT showing 6,57 cm and CT 6,19 cm. In 2016 in general wheat plants reached higher heights than 2017 and no significant differences were induced by the different treatments.

### 3.3 Grains quality traits

TKW and specific weight values are shown in table 2; for this variable no significant influences were registered among thesis. In general, higher values were obtained in 2016 both for TKW (73.7 and 54.7 g in 2016 and 2017, respectively) and specific weight (84.8 vs 81.6  $Kg\ hl^{-1}$  in 2016 and 2017, respectively).

**Table 2:** TKW (g) and Specific weight ( $kg\ hl^{-1}$ ) of durum wheat affected by different combination of tillage treatments and crop sequence, in 2016 and 2017

GPC was significantly increased by WF sequence with induced significantly higher values in both								
			2016		2017			
TKW (g)								
WW			75.5	71	73.2	56.1	55	55.6
WF			77.8	70.3	74.1	56.0	51.7	53.9
Overall mean			76.6	70.7	73.7	56.1	53.4	54.7
Soil management			n.s.		n.s.			
Crop Sequence			n.s		n.s			
Soil management x Crop Sequence			n.s.		n.s.			
Specific weight (kg hl <sup>-1</sup> )								
WW			84.5	84.6	84.5	81.4	80.8	81.1
WF			85.7	84.4	85.0	81.8	82.2	82.0
Overall mean			85.1	84.5	84.8	81.6	81.5	81.6
Soil management			n.s.		n.s.			
Crop Sequence			n.s		n.s			
Soil management x Crop Sequence			n.s.		n.s.			

CT+WW: conventional tillage and wheat monocropping; CT+WF: conventional tillage and wheat following faba-bean; ZT+WW: zero tillage and wheat monocropping; ZT+WF: zero tillage and wheat following faba-bean.

\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; n.s. = not-significant. In brackets: standard error of differences between means (s.e.d.). Degrees of freedom: Soil management, 1; Crop Sequence, 1; Soil management x Crop Sequence, 1; Residual, 4.

years (12.1 vs 11.0 % and 12.2 vs 10.3 % in 2016 and 2017, respectively) (Table 3); soil management did not induce any significant differences. The SDS collected values are shown in table 3. Significant differences were observed only in 2016 for both treatments, soil management and crop sequences. ZT exhibited the higher values than CT (3.6 vs 3.4 ml) and WF than WW (3.8 vs 3.4 ml). Yellow index (YI) resulted always increased by WF with respect to WW (Table 3), while the effect of soil management registered a significant interaction



with crop sequence. In particular, when ZT was combined with WF it gave always higher YI values than CT; besides, the ZT-WW combination did not induce any higher YI value than CT. Carotenoid and Ash concentrations do not seem influenced by soil management nor crop sequence treatments (Table 3).

**Table 3:** Influence of different combination of tillage treatments and crop sequence on wheat quality traits: Grains protein concentration (GPC, % s.s.), SDS sedimentation test (SDS, ml), Yellow Index (%) Carotenoid ( $\mu\text{g g}^{-1}$ ), and Ash (%) recorded at harvesting in 2016 and 2017.

2016				2017		
GPC (% s.s.)	ZT	CT	Overall mean	ZT	CT	Overall mean
WW	11.4	10.6	11.0	10.6	10.1	10.3
WF	12.1	12.2	12.1	12.4	11.9	12.2
Overall mean	11.7	11.4	11.6	11.7	11.4	11.6
Soil management		n.s.			n.s.	
Crop Sequence		** (0.16)			** (0.15)	
Soil management x Crop Sequence		n.s.			n.s.	
SDS (ml)						
WW	3.4	3.3	3.4	3.8	3.4	3.6
WF	3.8	3.5	3.6	3.9	3.9	3.9
Overall mean	3.6	3.4	3.5	3.8	3.6	3.8
Soil management		** (0.01)			n.s.	
Crop Sequence		* (0.07)			n.s.	
Soil management x Crop Sequence		n.s.			n.s.	
Yellow Hindex (%)						
WW	21.2	21.3	21.2	21.4	21.4	21.4
WF	22.5	21.8	22.1	22.4	21.9	22.1
Overall mean	21.8	21.5	21.7	21.9	21.7	21.7
Soil management		n.s.			n.s.	
Crop Sequence		** (0.17)			** (0.14)	
Soil management x Crop Sequence		* (0.20)			* (0.19)	
Carotenoid ( $\mu\text{g g}^{-1}$ )						
WW	8.0	8.2	8.1	8.0	8.1	8.0
WF	8.1	8.3	8.2	8.3	8.4	8.4
Overall mean	8.1	8.2	8.1	8.2	8.3	8.3
Soil management		n.s.			n.s.	
Crop Sequence		n.s.			n.s.	
Soil management x Crop Sequence		n.s.			n.s.	
Ash (%)						
WW	1.78	1.74	1.76	1.73	1.75	1.74
WF	1.77	1.79	1.78	1.76	1.78	1.77
Overall mean	1.77	1.76	1.77	1.74	1.77	1.75
Soil management		n.s.			n.s.	
Crop Sequence		n.s.			n.s.	
Soil management x Crop Sequence		n.s.			n.s.	

CT+WW: conventional tillage and wheat monocropping; CT+WF: conventional tillage and wheat following faba-bean; ZT+WW: zero tillage and wheat monocropping; ZT+WF: zero tillage and wheat following faba-bean.

\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; *n.s.* = not-significant. In brackets: standard error of differences between means (s.e.d.). Degrees of freedom: Soil management, 1; Crop Sequence, 1; Soil management x Crop Sequence, 1; Residual, 4.

### 3.4 Survey of symptoms of fungal diseases

The monitored wheat plants showed the same symptoms on the leaves, and only this type of symptoms. These symptoms were chlorosis and necrosis, apparently attributable to *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous. Any alteration on the grain was observed. In 2016, all treatments showed higher values of incidence and severity of leaf symptoms than 2017 (Table 4).

**Table 4:** Incidence and severity of *Septoria tritici* blotch (*Zymoseptoria tritici*) leaf symptoms in wheat subjected at different combination of tillage treatments and crop sequence in 2016 and 2017

Cropsequence		Incidence (%)			Severity (%)		
Soil	Management	ZT	CT	Overall Mean	ZT	CT	Overall Mean
<b>2016</b>							
	WW	54.47	17.33	35.90	15.48	2.03	8.75
	WF	28.43	14.52	21.47	3.48	0.70	2.09
	Overallmean	41.45	15.92	28.69	9.48	1.37	5.42
	Soil management		** (1.67)			** (0.23)	
	Crop Sequence		** (1.59)			** (0.71)	
	Soil management x Crop Sequence		** (2.26)			** (1.01)	
<b>2017</b>							
	WW	38.54	9.09	23.81	7.11	0.48	3.79
	WF	19.89	8.59	14.24	2.65	0.50	1.58
	Overallmean	29.21	8.84	19.02	4.88	0.49	2.68
	Soil management		** (0.97)			* (0.57)	
	Crop Sequence		** (1.19)			** (0.32)	
	Soil management x Crop Sequence		** (1.66)			** (0.44)	

CT+WW: conventional tillage and wheat monocropping; CT+WF: conventional tillage and wheat following faba-bean; ZT+WW: zero tillage and wheat monocropping; ZT+WF: zero tillage and wheat following faba-bean.\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; *n.s.* = not-significant. In brackets: standard error of differences between means (s.e.d.). Degrees of freedom: Soil management, 1; Crop Sequence, 1; Soil management x Crop Sequence, 1; Residual, 4.

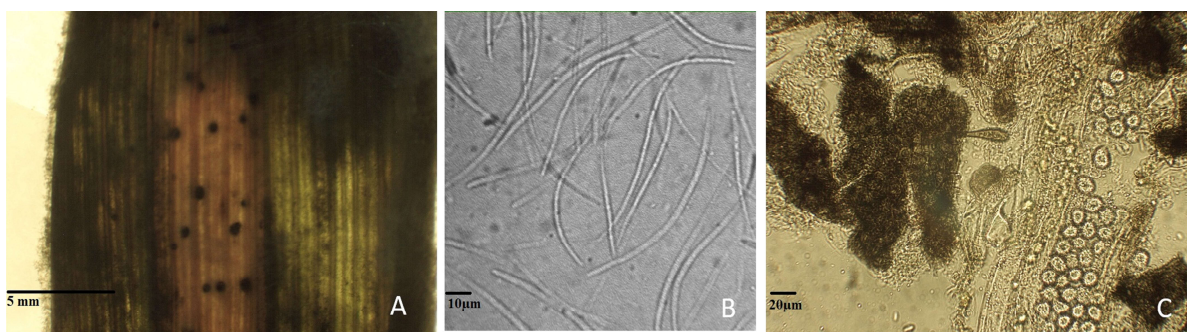
ZT showed significantly higher values of incidence as well as severity of symptoms with respect to CT treatments in both 2016 and 2017 years (Table 4). ZT+WW plots registered always higher incidence values, 54.47 % and 38.54 % respect to ZT+WF, 28.43% and 19.89%, in 2016 and 2017, respectively, (Table 4).

In the ZT plots, the introduction of rotation with faba-bean (WF) was much more effective in lowering the severity of symptoms which was 15.48% and 3.48% in 2016, and 7.11% and 2.65% in 2017, in ZT+WW and ZT+WF, respectively (Table 4).

In the CT plots, the introduction of WF was less effective in reducing leaf symptoms respect to ZT plots. In 2016, differences of symptom incidence between CT+WF and CT+WW were not significant, while symptom severity was significantly different, resulted 0.70% in CT+WF and 2.03% in CT+WW (Table 4). In 2017, no differences was observed between CT+WF and CT+WW, in terms of both incidence and severity.

### 3.5 Isolation of pathogens from symptomatic leaves

In the laboratory, the microscopic observations of the leaf samples, coming from the investigated 4 theses, made it possible to highlight the presence of pycnidia on the lesionated surface of the leaf, in a variable percentage from 26 to 35 per cm<sup>2</sup> of damaged leaf surface (Figure 2A).



**Figure 2:** A) Pycnidia of *Zymoseptoria tritici* on natural infected wheat leaf. B) Asexual spores (conidia) of *Zymoseptoria tritici*. C) Crushed pycnidia collected from natural infected wheat leaf.

No difference was recorded between the number of pycnidia, both fertile and sterile. At 24-48 hours, for every thesis and fragment of injured leaf, 13-15% of pycnidia showed cirrus formation. In subsequent times, no further formation of cirrhi from the pycnidia present on the damaged leaves was noticed. The subsequent observations of the cirrhi under the microscope confirm the presence of conidia, morphologically attributable to *Zymoseptoria tritici* [29, 30] (Figure 2B). The crushing on the slide of the pycnidia, taken at 72-96 hours, in the damaged leaf portions of each thesis, and the subsequent observation under the microscope, did not allow to observe any propagation element inside the corpuscle (Figure 2C).

#### 4. Discussion

Under Mediterranean areas, seven/eight years application of ZT have materialized with the building up of a permanent soil cover which become fundamental in such areas where rainfall are scarce and erratic, with drought frequently occurring during grain filling of durum wheat. In comparison with CT, characterized by bare soils due to continuous inversion of upper layers, ZT allowed a mulching accumulation 0.93 cm thick corresponding to about 2.00 t ha<sup>-1</sup> on average. Furthermore, it results greater under WW (1.40 *vs* 0.46 cm, about 3.06 *vs* 0.95 t ha<sup>-1</sup>), although it has to be considered that the residues from combination ZT+WF are composed by a mixture of straw and faba-bean which, once in the soil, behave differently than pure straw mulch accumulated under WW. The quicker decomposition of the ZT+WF favour a fast and more abundant release of several nutrients [31] as in the case of K<sup>+</sup> [32] and produces higher N amounts thanks to N-fixing faba-bean process [33].

Under such soil ameliorated conditions, i.e. increased rainfall infiltration, reduced evaporation rates [24, 34] and better nutrients availability (especially N, under WF), we have found positive increasing of yield components, yields as well as some technological traits of durum wheat. Indeed, yields improvement took place especially in the dryer year, confirming the effectiveness of CA approach toward water and N soil availability. According to Carr et al. [35], single grain weight was not affected by tillage systems, although De Vita et al. [36] and Di Fonzo et al. [37] report higher grain weight under no-till with respect to conventional tillage, possibly because soil water and N are conserved [38]. The variability of climatic conditions among the two years of experimentation have, in general, affected the TKW. At this regard, Royo et al. [39] indicated that water deficit occurring during the reproduction stage reduces both duration and rate of grain filling, and hence lower mean grain weight.

An analogous trend was observed for specific weight and previous findings are pretty contradictory: some researchers indicate higher hectolitre weight under no-till [36, 37, 38], while others report no influence of tillage systems on TKW [35, 40, 41].

The quality of end-products of the durum wheat chain is strongly related to the quality of the grains, which, in turn, is mainly determined by the genotype and environment, it merges that crop management plays a very important role. In particular, good technological traits such as grain protein concentration and gluten quality, are of primary importance for production of pasta [42, 43], which is mainly consumed in European and North American countries. Our work indicates that residue management and tillage treatments significantly affect GPC and protein quality, although limited and controversial results are found in literature; while Carr et al. [35] reported no effect, López-Bellido et al. [38, 41] indicated that conventional tillage induced higher GPC than no-till. Probably such inconsistency was due to growing and crop management conditions

[44]. Our results confirm that conservation approach induce increasing both in GPC and its quality [36, 37] under environmental conditions which seems generally favourable to higher GPC [45, 46]. Nevertheless, the significant diversity in annual rainfall distribution, which characterizes Mediterranean environments, induce a strong variability of such trait which was effectively softened by CA system. Besides, positive correlations were found between GPC and SDS volume [43, 47] indicating that increasing GPC, through CA approach, it in turns could lead to an increasing in gluten strength and quality. Crop residues management has also demonstrated to be a useful strategy to improve the colour of the grain, which is notorious to depend not only to genetic factors but also to growing conditions [48, 49, 50], and that has important implications in marketing of durum wheat based products.

The lower expression of *Z. tritici* symptoms under CT is probably due to the absence of crop residues and consequently to the reduced inoculum of the fungus [10, 18]. However, under ZT the higher expression of the symptoms of *Z. tritici* did not affect quantitative and qualitative characteristics of the grain [15]. Crop rotation caused a consistent reduction of leaf symptoms under ZT treatments in both years [15, 51], although grain characteristics of ZT+WW were not dissimilar to ZT+WF. In plants affected by the disease no direct damage to grains were found because of the delayed appearance of the infection; indeed, infection appeared at the end of May, during grain filling, without affecting earlier stages when starch is accumulated in stem and roots and remobilized later on, contributing to the grain filling at the extent of 20% [52, 53]. In seasons with low rain during the end of the crop cycle (2017), cirrhi developed from pycnidia located on the leaves, could result scarce [29, 54] inducing a decrease of the cycles of infection, although the late disease appearance seem to be the main reason of not affecting yields, as demonstrated in 2016 when climate conditions were favorable to disease symptoms expression [54].

Since the evaluations on the quantitative and qualitative characteristics of the grain were carried out collecting both healthy and symptomatic plants, any damage to grains in infected plants may be found not relevant for final yield.

## 5. Conclusions

Our work proves that under Mediterranean climates the application of CA exerts full benefits already after a six/seven-year period of CA adoption. In addition to higher yield, it ensures also higher GPC accumulation in kernels thanks to the improved soil water and nutrient availabilities.

Faba-bean (WF) included within the rotation caused a consistent reduction of leaf symptoms of *Z. tritici* under ZT treatments normally subjected to the diffusion of wheat diseases. However, the late appearance of the infection appeared to be decisive in avoiding yield damages. Future studies are needed to evaluate

strategies to avoid or delay fungal infections, thus combining the positive effects of CA techniques with a sanitary status that preserves grain quality and quantitative traits thus reducing economic damage threshold.

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