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LONG-TERM IMPACT OF TILLAGE SYSTEM ON *HETERODERA AVENAE* POPULATION DENSITY

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Foxi C., Carboni G., Mulé P., Ramassini W., Ruiu L., Troccoli A., Satta A. – Long-term impact of tillage system on *Heterodera avenae* population density.

An increase of conservative agriculture is being reported throughout the World but its impact on certain cereal pathogens and parasites is still a matter of debate. In this study, the long-term effects of direct drilling, conventional and reduced tillage, either under monoculture or crop rotation regimes, on the population density of *Heterodera avenae*, were evaluated. As expected, *H. avenae* was only exceptionally found in plots where durum wheat is cultivated in alternation with faba-bean. In the case of continuous wheat cropping, significantly higher infestation levels were instead recorded on conventional tillage systems (on average 112 females per g of root and 19 cysts in 200 g of soil); moderate, but under a growing trend, was the infestation level in reduced tillage plots (on average 24 females per g of root and 5 cysts in 200 g of soil); no nematodes were recovered in the case of no-tillage. Among the factors affecting nematode diffusion, specific consideration was given to the higher compaction level and organic matter content observed in the upper soil layers of no-tillage plots in comparison to conventional and, at a lower degree, to reduced tillage. These outcomes provide an important contribution to improve the application of conservation agriculture principles, which promotes the reduction of soil disturbance and crop rotation to limit damages by the cereal cyst nematode and to achieve a sustainable and profitable cropping.

KEY WORDS: Cereal cyst nematode, pest management, crop rotation, direct drilling, no-tillage.

INTRODUCTION

Conservation agriculture based on reduced tillage or direct drilling, crop rotation and residue management, plays an important role in reducing soil erosion, maintaining or increasing soil organic matter, and improving soil structure. For these reasons, an increase in conservation tillage methods is being reported throughout the world (CARBONI 2010; FAO, 2015; FRIEDRIC *et al.*, 2012; PRATLEY *et al.*, 1987).

The impact of tillage on certain cereal pathogens and parasites is controversial, as a result of studies highlighting either positive or negative effects depending on species, soil type, climate, cereal variety, tillage depth and intensity, in addition to other management practices (NEATE, 1988; ROGET *et al.*, 1996). For instance, the effects on take-all disease, caused by *Gaeumannomyces graminis* (Sacc.) Arx and Oliver, were reported to be unpredictable, while in Southern Australia, the severity of rhizoctonia root rot, caused by *Rhizoctonia solani* (Kühn), was shown to increase with the introduction of direct drilling (YARHAM, 1975, 1981; ROVIRA and VENN, 1985; DE BOER *et al.*, 1991). As reviewed by MINTON (1986), the results of studies on the impact of conservation tillage on nematode populations are significantly variable, in relation to the diversity of target species, management methods employed, and the response to previous crop residues. Among major pests of cereal crops (wheat, oats, barley, rye), infestations of the cereal cyst nematode, *Heterodera avenae* Wollenweber are often

associated with significant yield losses (DROPKIN, 1989). This species is widely distributed throughout the world, especially in the regions where wheat is intensively grown, like Italy (including main islands: Sicily and Sardinia), and where this pest has been reported in association with durum wheat decline for many years (INSERRA *et al.*, 1978; AMBROGIONI and MARINARI PALMISANO, 1985; GRECO *et al.*, 1993; D'ADDABBO and SASANELLI, 1994). Only few and inconclusive studies have been devoted to understanding the actual role of soil management practices on the density and distribution of *H. avenae* populations. For instance, ROVIRA & SIMON (1982) and ROGET & ROVIRA (1985) reported a significant reduction of the damages caused by this nematode species in soils subjected to direct drilling in South Australia, while DE BOER *et al.* (1991) found no response to tillage systems.

More in-depth studies on the effects of tillage system on nematode population density, have been conducted with the soybean nematode *Heterodera glycines* Ichinohe, highlighting variable results apparently depending on diverse experimental conditions (TYLER *et al.*, 1983, 1987; BAIRD and BERNARD, 1984; HERSHMAN and BACHI, 1995; KOENNING *et al.*, 1995; WORKNEH *et al.*, 1999; CHEN *et al.*, 2001; NOEL and WAX, 2003). Besides, a significant impact of agronomic practices (i.e. tillage, crop rotation) on the development of *H. glycines* populations were more recently documented (MELAKEBERHA *et al.*, 2015).

Several interrelated factors should be considered for these kinds of studies, including tillage system, crop rotation,

over time persistence of soil management methods, and the deriving soil features like porosity and organic matter content, which may significantly influence the nematode population density and distribution (BOUWMAN *et al.*, 2001; AVENDAÑO *et al.*, 2004).

The aim of this work was to investigate how long-term applied tillage systems (conventional, reduced or no-tillage), crop regimes (monoculture or rotation) and the deriving main soil features (organic matter content and soil compaction), affect the *H. avenae* population density and distribution.

MATERIALS AND METHODS

STUDY SITE AND EXPERIMENTAL DESIGN

The study was conducted in the period 2013-2014 in southern Sardinia (39°25'10" N, 09°05'09" E, 89 m a.s.l.), on experimental fields of the Cropping Systems Research Unit of the Agricultural Research Agency of Sardinia (AGRIS) (Italy), where long term tillage systems were repeatedly applied from 2003. According to the U.S.D.A. Soil Taxonomy classification, soil was classified as Inceptisols with vertic characteristics (VerticEpiaquept) (SOIL SURVEY STAFF, 2010). A summary of the main physical and chemical features of the top 30 cm soil are reported in Table 1. The experiment was based on a split-plot design with three blocks. Three types of tillage systems were applied in the main plots (2400 m² each):

- conventional tillage (CT): mold board plowing (25-30 cm depth), disk harrowing (15 cm depth) and tine harrow (5-7 cm depth) to prepare a proper seedbed;
- reduced tillage (RT): disk harrowing (15 cm depth) after a treatment with glyphosate (3.5-5 l ha⁻¹ of commercial product) to eliminate weeds;
- no tillage or sod seeding (NT): weeding in pre-sowing as above and direct drilling with a no-till drill.

Durum wheat (*Triticum durum* Desf.) cv Karalis and faba bean (*Vicia faba* L. var. *minor*) cv Prothabat 69, representing widely distributed varieties in the study area, were used in this research. In sub-plots (1200 m² each) two different crop successions were applied: continuous wheat (CW) and legumes (faba bean) – wheat rotation (LW). Ordinary seeders were used for CT and RT plots seeding

operations. Durum wheat and legumes were sown in 17.5 cm wide rows from December to January depending on meteorological and field conditions, at a seeding rate of 190-200 kg ha⁻¹ for durum wheat and 200-220 kg ha⁻¹ for faba bean. Fertilizers were applied to wheat plots (200 kg ha⁻¹ diammonium phosphate) at sowing, incorporated by drill in CT and RT plots, or through surface-broadcast in NT plots before seeding. At the beginning of wheat tillering stage, an additional fertilization was given in order to reach 80 units N per hectare. During the growing season, weed control in wheat crops was based on the application of specific herbicides in a mixture including Clodinafop-propargyl (250 ml ha⁻¹) and Tribenuron-methyl (20 g ha⁻¹). Weed management in faba bean crops consisted of pre-emergence treatments with Pendimethalin (5 kg ha⁻¹).

PLANT AND SOIL SAMPLING

Monitoring of *H. avenae* population density was carried out during each year by collecting plants and soil samples from the experimental field and their subsequent inspection in the laboratory under a stereo-microscope.

Three plants were randomly collected in each sub-plot (n=54) in May, when *H. avenae* females could be directly seen on the root system, after gently washing to remove any soil debris preventing females detachment. The number of *H. avenae* females per root portion (c.a. 0.2 g) was counted and their number per g of root was calculated.

Three soil samples surrounded by plant rhizosphere (ca 1 Kg) were randomly collected from each sub-plot (n=54) at the end of July with a small spade operating within a range of 5-20 cm deep down, after removing the upper soil layer. Soil sub-samples (200 g) were processed in the laboratory with a Fenwick can in order to extract nematode cysts.

IDENTIFICATION OF *HETERODERA AVENAE*

The identification of *H. avenae* was based on morphological and morphometrics analysis of the main diagnostic characters of cists (n=30) and of second stage juveniles (n=30) (HANDOO, 2002; SUBBOTIN *et al.*, 2010). Measurements were made using a compound microscope (Zeiss Axioscope) equipped with a digital camera (Leica DMC 2900) and a specific software (Leica Application Suite, Leica microsystem®). To confirm the results of morphological identification, species-specific genetic analysis based on the mitochondrial cytochrome oxidase subunit 1 (COI) were conducted on a random sample (n=10) (TOUMI *et al.*, 2013).

MEASURE OF SOIL COMPACTION AND WATER CONTENT

In November 2013 (10 years after the trial start) soil compaction measurements were conducted. Measurements were made when the whole profile was at field capacity (approximately 24 hours after a soaking rain), using an electronic penetrometer (Penetrologger - Eijkelkamp) with an internal GPS, which allowed to measure, with ultrasonic waves, soil resistance to the penetration every centimeter up to 80 cm depth (max pressure: 10 MPa). This instrument allowed us to obtain a series of compaction curves (twenty for each sub-plot, 360 in total) (KI-YUOL *et al.*, 2010; SERVADIO *et al.*, 2005). The soil humidity in the top 5 cm soil profile was also measured (% in volume) with a humidity sensor (ThetaProbe –Eijkelkamp) directly connected with the penetrometer.

DETERMINATION OF SOIL ORGANIC MATTER (SOM)

In November 2013 (10 years after the trial start), soil samples were collected before sowing in each sub-plot at

Table 1 – Chemical and physical characteristics of the experimental field soil.

Soil characteristics	
Texture	Light-clay
Sand (%)	44.0±5.2
Silt (%)	30.1±3.1
Clay (%)	25.9±4.1
pH (in water)	8.4±0.06
Organic matter (%)	2.1±3.5
Total N (%)	1.0±0.2
Assimilable P (ppm)	16.2±6.0
Assimilable K (ppm)	235.7±37.1
C.S.C. (me/100g)	18.5±2.1
E.S.P. (%)	0.5±0.1

two different depth (0-5 cm and 5-20 cm) in order to study the principal variations of chemical and physical parameters: among them the amount of soil organic matter (SOM), which has major importance in our study. The soil samples were analyzed according to official methods (MINISTERO RISORSE AGRICOLE, 1994).

STATISTICAL ANALYSIS

All data were analysed by general linear model (GLM) of ANOVA, and means were separated by Fisher's least significant differences (LSD, $\alpha=0.05$) test. The factors considered were year, block, tillage system, cropping system, and their respective interactions. The number of females per gram of root and the number of cysts in 200 gram of soil were log-transformed before analysis ($\log(x+1)$) because they did not meet the assumption of variance homogeneity. For these variables, untransformed means are reported on tables.

RESULTS

HETERODERA AVENAE MONITORING

Table 2 shows mean values and variability range of the two variables "females in roots" and "cysts in soil" as a function of year, tillage system (conventional, minimum and no-tillage) and rotation (continuous wheat vs legumes – wheat rotation). In 2013, the number of females per g of root in plants from continuous wheat cropping, varied between 0 and 264, with an average value significantly higher in comparison to all other thesis, with the sole

exception of data recorded the next year in the same plots (tillage system $\times$ rotation: $df=2$, $F=28.94$, $P<0.0001$). In the case of continuous cropping and reduced tillage in 2013, only one plant (among 9 plant samples) was infested by *H. avenae* with a density of 102 females per g of root. However, the following year, in the same sub-plots the number of infested plants increased to 6 (corresponding to 67% of sampled plants) with a number of females per g of root (38) significantly higher in comparison to sub-plots featured by rotation, regardless of tillage system, and by continuous cropping and no-tillage.

A similar trend was observed considering the number of cysts in 200 g of soil (tillage system $\times$ rotation: $df=2$, $F=30.25$, $P<0.0001$). In 2013, the average number of cysts in plots with continuous wheat cropping and conventional tillage, was significantly higher compared to all other thesis in the same year. On the other hand, this value increased significantly for the same plots the following year (26 vs 12). In the case of continuous wheat cropping and reduced tillage systems, no cysts were found in 2013, while 5 sample (out of 9) were infested with an average density of 11 cysts/sample, significantly higher in comparison to all other thesis, with the sole exception of the mean value determined in 2013 for continuous cropping and conventional tillage systems.

SOIL COMPACTION AND WATER CONTENT

Statistical analyses of data compaction, grouped into classes of 5 cm depth in the first 20 cm, are reported in Table 3. Soil compaction was significantly influenced by tillage system ($df=2$, $F=93.79$, $P<0.001$ for 0-5 class;

Table 2 – Infestation level of *H. avenae* on wheat (average, minimum e maximum values) in relation to soil management operations and cropping systems in two consecutive years.

Year	Tillage system	Rotation	n	Females per g of root n	Cysts in 200 g soil n
2013	Conventional tillage (CT)	CW	9	107 a (0-264)	12 b (0-27)
		LW	9	0 c	1 c (0-3)
	Reduced tillage (RT)	CW	9	11 c (0-102)	0 c
		LW	9	0 c	1 c (0-1)
	NO-tillage (NT)	CW	9	1 c (0-7)	0.2 c (0-2)
		LW	9	0 c	0.1 c (0-1)
2014	Conventional tillage (CT)	CW	9	118 a (30-242)	26 a (11-50)
		LW	9	0 c	0 c
	Reduced tillage (RT)	CW	9	38 b (0-140)	11 b (0-47)
		LW	9	0 c	0 c
	NO-tillage (NT)	CW	9	0 c	0 c
		LW	9	0 c	0.1 c (0-1)

Means in the same column with different letters are significantly different (GLM of ANOVA, followed by LSD test, $P<0,05$); rotation treatments are identified by CW (continuous wheat cultivation) and LW (legume-wheat rotation).

Table 3 – Water content and soil penetration resistance (means $\pm$ SD) in relation to soil management operations and cropping systems.

Agronomic management		Water content 0-5 cm (% volume)	Soil penetration resistance (MPa)			
			0-5 cm	5-10 cm	10-15 cm	15-20 cm
Conventional tillage	CW	18.05 $\pm$ 2.13 b	0.35 $\pm$ 0.04 cd	0.71 $\pm$ 0.09 d	0.87 $\pm$ 0.18 c	0.86 $\pm$ 0.21 c
	LW	19.58 $\pm$ 2.19 b	0.31 $\pm$ 0.04 d	0.53 $\pm$ 0.06 e	0.69 $\pm$ 0.06 d	0.75 $\pm$ 0.11 c
Reduced tillage	CW	19.25 $\pm$ 0.70 b	0.46 $\pm$ 0.09 bc	0.95 $\pm$ 0.21 c	1.46 $\pm$ 0.13 b	1.53 $\pm$ 0.06 ab
	LW	18.23 $\pm$ 0.67 b	0.53 $\pm$ 0.07 b	1.22 $\pm$ 0.21 b	1.63 $\pm$ 0.18 a	1.59 $\pm$ 0.14 a
NO-tillage	CW	26.23 $\pm$ 0.86 a	0.90 $\pm$ 0.11 a	1.67 $\pm$ 0.06 a	1.61 $\pm$ 0.06 ab	1.40 $\pm$ 0.07 b
	LW	25.27 $\pm$ 1.57 a	0.98 $\pm$ 0.20 a	1.68 $\pm$ 0.11 a	1.64 $\pm$ 0.13 a	1.45 $\pm$ 0.14 ab

Means in columns followed by different letters are significantly different at $p = 0.05$ according to Fisher's least significant differences test (LSD); rotation treatments are identified by CW (continuous wheat cultivation) and LW (legume-wheat rotation).

$F=178.85$, $P<0.001$ for 5-10 class; $F=145.62$, $P<0.001$ for 10-15 class; $F=115.45$, $P<0.001$ for 15-20 class) but not by rotation. The interaction tillage system $\times$ rotation was significant only for 5-10 ($df=2$, $F=8.37$, $P=0.001$) and 10-15 class ($F=5.28$, $P=0.011$).

The differences of compaction levels among soils featured by different tillage systems diminished in the deeper layers, and became non significant below 45 centimeters ($df=2$, $F=2.49$, $P=0.101$).

Compaction influenced significantly also the water content (% in volume) in the top 5 cm layers ($df=2$, $F=91.16$, $P<0.001$): higher compaction and sod seeding caused a reduction in porosity (especially macro-porosity) if compared to soil under other tillage systems. As a consequence of macro porosity reduction, these layers are characterized by a lower level of oxygenation, as (micro) pore spaces are filled by water instead of air.

SOIL ORGANIC MATTER (SOM)

The greatest amount of SOM in the top 5 cm of soil was observed in the case of no tillage technique ($df=2$, $F=25.91$, $P<0.001$). Lower levels of SOM were measured in reduced tillage plots when compared to sod seeding, but these values were higher than in conventional tillage (Table 4). In the underlying layer (5-20 cm), the greatest accumulation of SOM was observed in reduced tillage treatment, with higher statistical significance compared to the conventional tillage ($df=2$, $F=7.28$, $P=0.003$). No differences were observed when crop rotation was considered as a factor for both layers ($df=1$, $F=0.24$, $P=0.629$ for 0-5 class; $F=0.02$, $P=0.887$ for 5-20 class). The interaction tillage system $\times$ crop rotation was not significant ($df=2$, $F=0.05$, $P=0.954$ for 0-5 class; $F=0.34$, $P=0.715$ for 5-20 class).

DISCUSSION

This study highlights a significant influence of tillage system on the development and growth of *Heterodera avenae* populations. As expected, this species was only exceptionally found in plots where durum wheat was cultivated in rotation with faba-bean, regardless of tillage system. In the case of continuous wheat cropping,

Table 4 – Mean percentage ($\pm$ SD) of soil organic matter content in relation to soil management operations and cropping systems.

Agronomic management		Depth	
		0-5 cm	5-20 cm
Conventional tillage	CW	1.87 $\pm$ 0.41 c	1.76 $\pm$ 0.34 b
	LW	1.96 $\pm$ 0.45 bc	1.83 $\pm$ 0.41 b
Reduced tillage	CW	2.36 $\pm$ 0.21 b	2.22 $\pm$ 0.19 a
	LW	2.36 $\pm$ 0.42 b	2.23 $\pm$ 0.39 a
NO-tillage	CW	3.01 $\pm$ 0.49 a	2.03 $\pm$ 0.30 ab
	LW	3.10 $\pm$ 0.59 a	1.91 $\pm$ 0.20 ab

Means in columns followed by different letters are significantly different at $p = 0.05$ according to Fisher's least significant differences test (LSD); rotation treatments are identified by CW (continuous wheat cultivation) and LW (legume-wheat rotation).

significantly greatest infestation levels were instead recorded in conventional tillage systems (90-100% infested plants, on average 112 nematode females per g of root); moderate, but under a growing trend (higher in 2014 than 2013), was the infestation level in reduced tillage plots (from 11 to 66% infested plants, from 11 to 38 females per g of root); no nematodes were recovered in the case of no-tillage. These results are in line with observations conducted by ROVIRA & SIMON (1982) and by ROGET & ROVIRA (1985) on cereal cyst nematodes and by KOENNING *et al.* (1995) on the soybean cyst nematode.

The movement of invertebrate organisms like nematodes through porous spaces, and consequently their damage potential, are closely related to physical and morphological properties of soil (NEHER *et al.*, 1999). ESQUIVEL (1996) observed that proliferation of nematode communities are a combination of an adequate porous space (determined by particles size and inter-particles space) and water content, since water films would allow their movement and therefore the access to food. It is well documented that well drained soils and their macro-porosity favour the development of

higher population densities of plant parasitic nematodes (BOWMAN and ARTS, 2001; AVENDAÑO *et al.*, 2004).

Our experiments were conducted in a light clay texture soil which is poorly suitable for the nematode development. However, in plots where durum wheat was continuously cultivated under conventional tillage, soil physical properties might have been shaped so as to favour a significant growth of nematode populations that take advantage of the continuous availability of their host. This hypothesis is further supported by results of penetrometric observations that showed a higher compaction level (= lower porosity and consequently lower oxygenation) on soil surface layers (within 10 cm depth) in no-tillage plots in comparison to conventional and, at a lower degree, to reduced tillage where compaction was at an intermediate level between sod seeding and conventional tillage (Table 3). These layers were also mainly associated to phytoparasitic nematodes. In accordance with this distribution, YEATES & BONGERS (1999) reported that more than 88% of the total nematode population of the 90 cm of the soil profile was found within the top 0-10 cm layer, rapidly decreasing to 6% in the 10-20 cm layer, and to 0.002% in the deepest layer sampled.

Another factor that may have affected reproduction and diffusion of *H. avenae* is the higher content of organic matter in no-tillage plots compared to conventional and, partially, to reduced tillage. Given that the annual biomass residues were comparable among all plots (around 25-30% of total biomass), detected differences may depend on a greater organic matter mineralization rapidity in tilled soils, as a result of a higher oxygenation degree and of plant residue blending during plowing.

Accumulation of SOM with reduced tillage seems to increase slowly in comparison with no-tillage within the top 5 cm soil profile. In this layer, reduced tillage favour higher oxidation (lower compaction levels) respect to no-tillage practice. Oxidation with reduced tillage is lower compared to conventional tillage and this promotes SOM accumulation year by year. Higher levels of SOM observed in reduced tillage plots respect to conventional tillage in the top 5-20 cm soil layer can be explained by a higher concentration of buried plant residues: only in the first 12-15 cm (disk harrowing) with reduced tillage and 25-30 cm (ploughing) with the conventional tillage. Conversely with no-tillage management, crop residues are not buried (they are concentrated on surface) and this explains the high increase of SOM only in the top 5 cm soil. Besides, in deeper soil layers, SOM does not accumulate significantly, which is in line with the expected slowness of pedological phenomena.

Different studies reported a reduction of plant-parasitic nematode populations and an increase of saprophytic nematode species in soil with a higher organic matter content (BARKER and KOENNING, 1998; Bird, 2000). Both chemical and/or biological mechanisms can explain such effect (WIDMER *et al.*, 2002). For instance, plant-pathogenic nematodes are typically affected by toxic or antagonist compounds released into the soil. On the other side, biological soil dynamics may affect plant pathogenic nematodes through the interactions with increased communities of antagonistic or parasitic microorganisms, including those producing allelochemicals.

In conclusion, the results of the present study emphasize the benefits deriving from wheat/faba-bean rotation to mitigate the action of *H. avenae*. Furthermore, we report the efficiency of no-tillage and partly of reduced tillage in containing nematode populations when rotations are not

convenient for other agronomic or economic reasons. These outcomes provide an important contribution for applying conservation agriculture principles, which promotes the reduction of soil disturbance and crop rotation to limit damages by the cereal cyst nematode and to achieve a sustainable and profitable cropping.

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