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EFFECTS OF REDUCED AND CONVENTIONAL TILLAGE ON WEED COMMUNITIES: RESULTS OF A LONG-TERM EXPERIMENT IN SOUTHWESTERN SPAIN

Efeito do Cultivo Mínimo e Plantio Convencional em Comunidades de Plantas Daninhas: Resultados de um Experimento de Longo Prazo no Sudoeste da Espanha

ABSTRACT - An important drawback in adopting minimum tillage (MT) and no-tillage (NT) techniques is the frequently observed weed shift promoting adapted species and achieving poorer weed control. These changes can be detected best with long-term experiments, and results might differ depending on soil characteristics and the local flora. The objectives of this work were to evaluate the effect of reduced tillage on weed seed distribution in the soil profile and to identify possible consequences on weed diversity on a long-term experiment maintained during 24 years in Seville (Spain) with three tillage systems: NT, MT and conventional tillage (CT) including moldboard plow on a vertisol. For this purpose, soil seedbanks at 0-8 cm and 8-16 cm depths were enumerated in autumn 2005 and in-field emerged plants in autumn 2005 and winter 2006. Shannon diversity index (H) and evenness (J') were calculated for seedbank and aboveground weed communities. Total weed seed density was highest for NT and lowest for CT. Some big-seeded species, such as *Chrozophora tinctorea* L., showed highest seed density in CT. NT increased the relative density of *Amaranthus blitoides* S. Watson seeds in the seedbank and the abundance of emerged plants of *Malva parviflora* L., *Anagallis arvensis* L. and *Picris echioides* L. Overall, MT led to a less diverse seedbank in the 0-8 cm depth of soil than CT. The frequent drought-induced deep fractures in the expandable clay soil caused natural tillage, which probably resulted in fewer differences in weed seed and seedling densities among tillage treatments compared to what might be expected in other soil types.

Keywords: no-tillage, minimum tillage, moldboard plow, mechanical weed control, seedbank, diversity indexes.

RESUMO - Uma desvantagem importante na adoção de técnicas de cultivo mínimo (MT) e plantio direto (NT) é o deslocamento de plantas daninhas frequentemente observado, promovendo espécies adaptadas e, com isso, um controle de plantas daninhas mais precário. Essas mudanças só podem ser detectadas com experimentos de longo prazo, e os resultados podem diferir, dependendo das características do solo e da flora local. Os objetivos deste trabalho foram avaliar o efeito da lavoura reduzida sobre a distribuição de sementes de plantas daninhas no perfil do solo e identificar possíveis consequências na diversidade de plantas daninhas em um experimento de longa duração mantido durante 24 anos em Sevilha (Espanha) com três sistemas de plantio: NT, MT e preparo convencional (CT), incluindo arado de aiveca em um Vertissolo. Com esse propósito, amostras de solo com 0-8 e 8-16 cm de profundidade foram coletadas no inverno de 2005 e na primavera de

*2006, e as plântulas de plantas daninhas emergentes foram registradas. O índice de diversidade de Shannon (H) e a uniformidade (J') foram calculados para comunidades de banco de sementes e plantas daninhas acima do solo. A densidade total de sementes de plantas daninhas foi maior para NT e menor para CT. Algumas espécies de sementes grandes, como **Chrozophora tinctorea** L., apresentaram maior densidade de sementes no CT. NT aumentou a densidade relativa de sementes de **Amaranthus blitoides S. Watson** no banco de sementes e a presença de plantas emergidas de **Malva parviflora** L., **Anagallis arvensis** L. e **Picris echioides** L. Com relação aos resultados gerais, a TM levou a um banco de sementes menos diversificado na profundidade de 0 a 8 cm do solo do que a CT. As frequentes fraturas profundas na argila expansível do solo provocam um preparo natural, originando provavelmente menos diferenças dos parâmetros analisados do que em outros tipos de solo.*

Palavras-chave: semeadura direta, cultivo mínimo, arado de aiveca, controle mecânico de plantas daninhas, banco de sementes, índices de diversidade.

INTRODUCTION

Production techniques like minimum tillage (MT) and non-tillage (NT) systems are alternatives to reduce soil losses (Brady and Weil, 2002), to maintain organic matter and moisture, and also to provide stabilization of aggregates favoring water infiltration (Liebig et al., 2004; Thierfelder et al., 2015) *versus* conventional tillage (CT). Moreover, conservation agriculture has other benefits such as stimulating beneficial microfauna activity (Zhang et al., 2015), achieving more carbon sequestration than conventional systems (Buchi et al., 2017) and, additionally, may save money and time by reducing tillage operations prior to sowing crops (Chauhan et al., 2012). Nitrogen fertilizer and fuel were found to be the inputs with the highest reduction levels in the USA (Anderson, 2017). However, reduced tillage also involves some disadvantages, such as higher surface compaction that can decrease water infiltration, new weed control problems, and major initial purchases of equipment for direct sowing, spraying weeds, and harvesting crops.

Concerning weed control, fewer management options exist under MT and NT and, therefore, weed problems increase compared to CT for at least two reasons. First, the absence of soil inversion causes recently shed seeds to remain close to the soil surface, in a better position to have a higher probability of establishment (Bàrberi and Lo Cascio, 2001), despite being more exposed to predators like harvester ants (Baraibar et al., 2009). Second, MT and NT allow a higher survival rate of the propagules of perennial species and increase their possibility of sprouting, enabling further development. Thus, MT and NT might promote infestations of species that emerge easily after partial burial or being left on the soil surface. Some examples of these species are grasses like *Bromus diandrus* Roth or *Alopecurus myosuroides* L. and the perennial species *Cirsium arvense* L., *Convolvulus arvensis* L., *Cardaria draba* (L.) Desv., *Sonchus tenerrimus* L. and *Malva parviflora* L. (Mas and Verdú, 2003; Sans et al., 2011; Soane et al., 2012).

On the other hand, some weed species may show smaller populations in MT and NT systems, either because their seeds need to be buried at a certain depth to germinate, or because their germination mechanism is activated only if the soil is tilled. Following Navarrete and Fernández-Quintanilla (2003), this is the case of *Veronica hederifolia* L., *Descurainia sophia* (L.) Webb ex Prantl and *Lamium amplexicaule* L. The species *Polygonum* spp. (Froud-Williams et al., 1983), *Fumaria officinalis* L., *Raphanus raphanistrum* L. and *Chenopodium album* L. are also considered to be well adapted to conventional tillage (CT) and their density is expected to decline with reduced tillage. However, some species as *Papaver rhoeas* L. seem to be adapted to both systems (Cirujeda et al., 2003; Navarrete and Fernández-Quintanilla, 2003). Especially herbicide-susceptible species are expected to decrease their density over time in reduced tillage because herbicides are used more intensively, unless they develop resistance.

Changes in weed communities are expected to arise within a few years of implementing MT or NT systems, since weed species better adapted to no-tillage and herbicides will gradually replace other species (Torresen et al., 2003; Primot et al., 2006; Chauhan et al., 2012) and weed pressure can be a serious impediment for establishing the minimum tillage systems (Lee and Thierfelder, 2017). Nevertheless, some other studies indicate that tillage reduction

does not always lead to changes in weed richness, but rather to changes in the relative abundance of weed species (Derksen et al., 1993; McCloskey et al., 1996). In any case, even if a weed shift occurs, it may not be attributable to conservation agriculture alone, because other factors such as crop rotation, climate and long-distance weed propagule movements also may impact. Additionally, soil type probably influences the effects of reducing tillage operations. Vertisols occupy an area of 672,000 ha in Andalusia, Spain (Fernández, 2011), and 260 million ha worldwide (Dudal, 1963), and they are very fertile in years with enough rainfall, although water availability in dry years is a limiting factor. These soils can produce high yields in conservation tillage (Blaise et al., 2015) provided weeds are effectively controlled (Giambalvo et al., 2012). However, few data are available for these types of soils regarding MT.

Biodiversity in agricultural systems generally is accepted as necessary to achieve a high stability and protection against environmental stresses (Altieri, 1999). In this respect, the importance of weed communities, as an integral part of agroecosystems, is increasingly claimed to provide a wide range of ecological functions, such as the ability to respond to some disturbances (Costanza et al., 1997) and pest control (Altieri, 1999). Where MT and NT have been adopted extensively, a few problematic weed species, well adapted to reduced tillage and to herbicides, rapidly increase their population densities and thereby reduce biodiversity. Davis et al. (2005), Torresen et al. (2003) and García et al. (2014) describe some interesting examples of plant species that have played this role in different areas of the world.

Long-term experiments can provide essential information about the tillage effect on weed infestations. In particular, seedbank studies allow a retrospective view of the treatment effects and contribute in achieving a predictive insight into possible future weed problems (Forcella, 1992; Hossain and Begum, 2015). However, long-term trials focusing on weeds are complex and laborious, as evidenced by the few existing examples in the literature showing data for more than 10 years of minimum tillage (Table 1). Unfortunately, the newest research on minimum tillage focuses more on topics other than weeds, even though weed control have been shown to be crucial to adopt minimum tillage (Lee and Thierfelder, 2017). The results of tillage effects on weed communities cannot be extrapolated entirely to other areas with different climates, soils, and floras. Therefore, in order to assess such effects in a particular zone, local experiments are important. Unfortunately, no relevant published data are available for southern Spain. However, such data were collected in the long-term crop and soil management trial described by Ordóñez Fernández et al. (2007), and the results relevant to weed seed banks will be summarized here.

The two objectives of this work were: (1) Evaluate the effect of MT, NT and CT on weed seed distribution in the soil profile and on the emerged weeds analyzing each species individually in the longest crop-free period of a wheat-sunflower-legume rotation. The longest crop-free period was between wheat harvest and sunflower sowing. (2) Identify long-term consequences of the tillage systems on weed diversity after 24 years (1982-2005). The experiment was performed in

Table 1 - Summary of the main studies in long-term experiments focusing on weeds conducted in rainfed conditions

Author	Country	Crop	Period (years)	Soil texture
Sosnoskie et al. (2006)	USA	Continuous corn (*Zea mays* L.), corn-soybean (*Glycine max* L.), corn-oat (*Avena sativa* L.)	35	Silt loam
Hernández-Plaza et al. (2015)	Spain	Wheat (*Triticum aestivum* L. and *T. durum* Desf.)-vetch (*Vicia sativa* L.)	24	Loam
Giambalvo et al. (2012)	Italy	Continuous wheat (*T. aestivum*), wheat-faba bean (*Vicia faba*), wheat-berseem clover (*Trifolium alexandrium*)	18	Clay (vertisol)
Voll et al. (2001)	Brazil	Soybean-wheat	16	70% clay
Dorado and López-Fando (2006)	Spain	Barley (*Hordeum vulgare* L.) -vetch, barley-sunflower (*Helianthus annuus* L.)	16	Sandy loam
Santín-Montanyá et al. (2017)	Spain	Wheat	16	Sandy loam
Carter and Ivany (2006)	Canada	Soybean - barley	14	Sandy loam
Bàrberi and Lo Cascio (2001)	Italy	Wheat (*T. aestivum*) - pigeon bean (*Vicia faba* L. var. *minor*)	12	Sandy loam
Davis et al. (2005)	USA	Corn-soybean-wheat (*T. aestivum*)	12	Silt loam

the Seville region of southern Spain. This site and study represented the rainfed argillic soils in the area, and it was the only second such long-term experiment on heavy clay soils in Europe (Giambalvo et al., 2012).

Four hypotheses were tested. (1) Tillage reduction decreases weed biodiversity both in the soil seedbank and in the emerged weed community. (2) Differences in weed seedbank and emergence are expected to occur for the different tillage systems. (3) Changes in the weed flora could happen when reducing tillage intensity, thereby promoting those species adapted to less tillage and to the applied herbicides and decreasing other species adapted to tillage. (4) These changes may cause a total higher seedbank and emerged weed density in MT and NT systems compared to CT.

MATERIAL AND METHODS

Study area

A permanent long-term experiment has been conducted since 1982-1983 at Tomejil, near Seville (Spain), 37°24'07"N and 05°35'10"W, at 97 m above sea level. The site is located in the Andalusian fertile plain called "Campiña," which has a Mediterranean climate. The soil is a fine montmorillonitic Chromic Haploxerert (Soil Survey Staff, 1999) formed on a Miocene marl with more than 700 g kg^{-1} clay in the 0-0.15 m layer and only 1 g kg^{-1} sand (Ordóñez Fernández et al., 2007). The dominate expandable clay of this soil is montmorillonite, which characteristically forms deep fissures during dry periods that facilitate desiccation and probably gives weed seeds the opportunity to enter deeper soil layers naturally, without plowing.

The climate of the experimental site is sub-humid Mediterranean with a mean rainfall of 575 mm (Figure 1, data from the study area at Tomejil, Seville). The dry period is from May to September; low rainfall in May and September is not effective due to high temperatures. The mean air temperature is 15.0 °C in autumn, 11.9 °C in winter, 20.8 °C in spring and 26.3 °C in summer. The average minimum and maximum temperatures are 11.5 °C and 25.5 °C, respectively. Weed emergence begins after the autumn rainfall and generally stops in April.
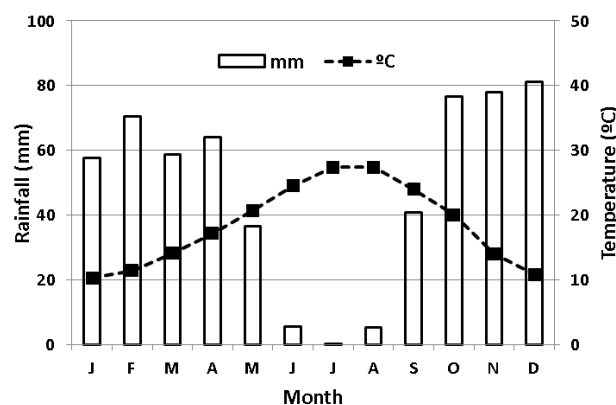

Figure 1 - Ombrothermic diagram of the Tomejil experimental field (Seville, Spain). Average data years 2002 to 2017.

Experimental design

Three tillage systems were compared: NT, MT and CT. The elementary plots measured 180 m x 15 m (2,700 m^2) and were distributed in a randomized complete block design with three replicates for each treatment. In all plots a wheat (*Triticum aestivum* L. and *T. durum* Desf.) – sunflower (*Helianthus annuus* L.) - legume (vetch, *Vicia faba* L. or peas, *Pisum sativum* L.) rotation was performed. Wheat, vetch and peas were sown in December and harvested in May; sunflower was sown in March and harvested in August. The rotation lasts three years, considering one year for each crop. Weed control during the cropping periods was conducted when necessary with authorized herbicides for the respective crop obtaining sufficient control in all 24 years; in many years herbicide use was not necessary in sunflower, a competitive and fast-growing crop. Herbicides were applied with a tractor-mounted field boom sprayer with the following characteristics: speed 6.5 km h^{-1}, pressure 2.5 bar, standard API, Albuz nozzles, spray volume of 200 L ha^{-1} for glyphosate and 300 L ha^{-1} for the other herbicides.

During the 24 years, in the crop-free periods, weed control in CT was conducted mechanically, while herbicides were used in MT and NT (see Table 2). In autumn, before sowing wheat, vetch or peas, the normal treatments were glyphosate + MCPA at 0.6 and 0.5 L ha^{-1}, respectively, in pre-sowing of NT and MT with an additional treatment at higher dosage if needed in wet autumns.

Table 2 - Operations conducted for each tillage system in season 2005-06 at the experimental site

Application date	Tillage system		
	No-tillage	Minimum tillage	Conventional tillage
November	0.7 L ha^{-1} glyphosate 36% + 0.5 L ha^{-1} MCPA 60%	Cultivator[1]	Moldboard plow[2]
January	1 L ha^{-1} glyphosate 36% + 0.7 L ha^{-1} MCPA 60%	Vibro-cultivator[3]	Cultivator
Pre-sowing (March 30th)	2 L ha^{-1} glyphosate 36% + 0.150 L ha^{-1} oxyfluorfen 48%	1.5 L ha^{-1} glyphosate 36% + 1 L ha^{-1} MCPA 60%	Vibro-cultivator

[1] Cultivator: vertical tillage at depth 15-20 cm. [2] Moldboard plow: tillage with soil inversion, at depth 40-45 cm. [3] Vibro-cultivator: vertical tillage at depth less than 15 cm.

During the longer crop-free period previous to sunflower sowing, mechanical treatments were used in MT and more herbicide treatments were needed in NT (Table 2). Thus, the period of time in the trial in which more differences in weed management are expected is the time between the winter crop harvest in May and the sunflower sowing in March, which is exactly the time period considered in this study. As temperatures in summer after wheat harvest are very high and rainfall is very scarce, virtually no weeds emerge until the autumn rainfall.

In the 2005-2006 sampling season the sown crop was sunflower and the ordinary pre-planting operations were conducted for each treatment (Table 2).

Soil, seed and seedling sampling and identification

Soil samples were taken in the central 600 m² of each plot. A 5 cm diameter cylindrical soil sampler of 8 cm depth was used (Eijkelkamp, sample ring kit with open ring holder, model 07.53.SA, Giesbeek Netherlands) maintaining each soil sample in a metal cylinder. Soil cylinders were dried at 50 ºC during 2 days, later extracted from the metal container, soil placed in plastic bags and kept at -25 ºC until processing. The pattern followed for sampling was a fixed grid (Ambrosio et al., 2004) consisting of 7 rows 10 m apart and 3 columns 5 m apart. Two sub-samples were extracted at each of the 21 crossing points of this grid: one sub-sample was of the top 8 cm of soil, and the other of depth 8-16 cm. Thus a total of 378 soil cores were taken. Sampling was conducted in November 2005 when soil preparation for sunflower seeding started.

Weed seeds were separated from the soil following the protocol of Carretero (1977) mixing 200 mL of water with each 100 mL of soil sample, adding 10 g of dispersants (i.e. sodium hexametaphosphate and 5 g of sodium bicarbonate). The liquid sample was shaken during 90 minutes at 11 r.p.m in a shaker (Heidolph model Reax 20, Schwabach, Germany). The solutions including the dispersed seeds were poured through a tower of three sieves of 1, 0.5 and 0.25 mm mesh. The solid fractions were dried at 35-40 ºC during 1 hour and later seeds identified by consulting images available on the internet (HYPPA, 2006). For seedling identification of plants emerging in the field, Spanish weed flora by Villarías (2000) was used. Unidentified plants at the seedling stage were transplanted to pots and grown in the greenhouses of the Faculty of Agricultural Engineers (EUITA), Seville, until the flowering stage and then identified using Carretero (2004).

Under the local conditions, normally no seedling emergence occurs during the dry summer period; it starts only after autumn rains begin. Therefore emerged weeds were counted before soil sample extraction in November 2005 (immediately before seedbed preparation) and afterwards in February 2006, shortly before sowing the sunflower crop, in order to compare the number of seeds in the upper soil profile of depth 8 cm with the emerged weed flora. Flora sampling in November documented the emerged weeds during the whole period after harvesting the previous wheat crop until autumn. Sampling in February documented the surviving plants after tillage operations and herbicide applications in November and described newly emerged weeds, thus to characterize the weed infestation short before sowing the sunflower. No counts were conducted during the crop because weed management in that period was the same for all three tillage systems. Moreover, no herbicide was used during the sunflower in the sampling year because low weed infestation was found, which demonstrated the great competitive capacity of this crop.

Thus, counts in February reflect the main plant community prior to sunflower sowing that would continue growing in the crop in NT if herbicides were not effective and in MT and CT if mechanical treatments were ineffective.

The basic sampling area of the vegetal community was a circular quadrat with a 25 cm diameter, and 21 quadrats were sampled per plot. The center of each quadrat coincided with one of the 21 soil sampling points in each plot. In the February counts, recently emerged seedlings and older plants with more than 4 true leaves were recorded separately.

Data analysis and diversity measurement

The means of weed and seed densities of the 21 samples per plot were calculated and tested for normality and variance homogeneity. Seedbank data of both depths and diversity indexes in February required ln (x+1) transformation to fulfill these requirements. ANOVAs were conducted for each species separately and for the total weed and seed densities considering the tillage factor in a completely randomized design. For the seedbank samples each depth was analyzed separately in all cases. Afterwards Tukey mean separation tests were performed. Boxplots were constructed for weed species that did not show significant differences despite having very different means among treatments due to data dispersal.

Means of the raw seed and seedling numbers per plot were used to calculate the Shannon–Weaver diversity indices (H) of each repetition of the three tillage systems according to the expression (Magurran, 2004):

$$H = -\sum_{i=1}^{S} p_i \ln p_i$$

where S is the number of species or species richness, $p_i = N_i/N$, with N_i being the number of individuals of the species i and N the total number of individuals. Also, the Shannon evenness index (J') was calculated as:

'J' $= H/H_{max}$

where $H_{max} = \ln S$. High J' implies greater uniformity of species' abundance (Magurran, 2004). Data of 21 cores and data of 21 quadrats from each elementary plot were combined in order to compute two diversity indices. Other calculated parameters were plant density (plants m^{-2}), number of weed species per plot (richness), and the number of samples with weeds per plot (being adult plants or seedlings).

The calculated data were analyzed statistically according to the experimental design using the program SPSS 15.0.1 (SPSS, 2006).

RESULTS AND DISCUSSION

Tillage effect on weed seed density

Soil depth of 0-8 cm

The overall mean weed seed density of all species combined in the first 8 cm was significantly higher under NT compared to CT being approximately twice as high, whereas that for MT was intermediate (Table 3), 24 years after applying the different tillage systems, confirming one of the hypotheses of this work. These results affirmed the observations of Torresen et al. (2003) and Conn (2006). When studying the species separately, *Chrozophora tinctoria* L. was the only species with significantly higher mean seed density in CT than in the other two tillage systems, and *Polygonum aviculare* L. was the only species that had its highest seed density in MT. No single species had significantly higher seed densities in NT than in MT or CT.

The results for seed banks are a consequence of the combination of the tillage system and associated weed control methods, and effects of these two management variables cannot be

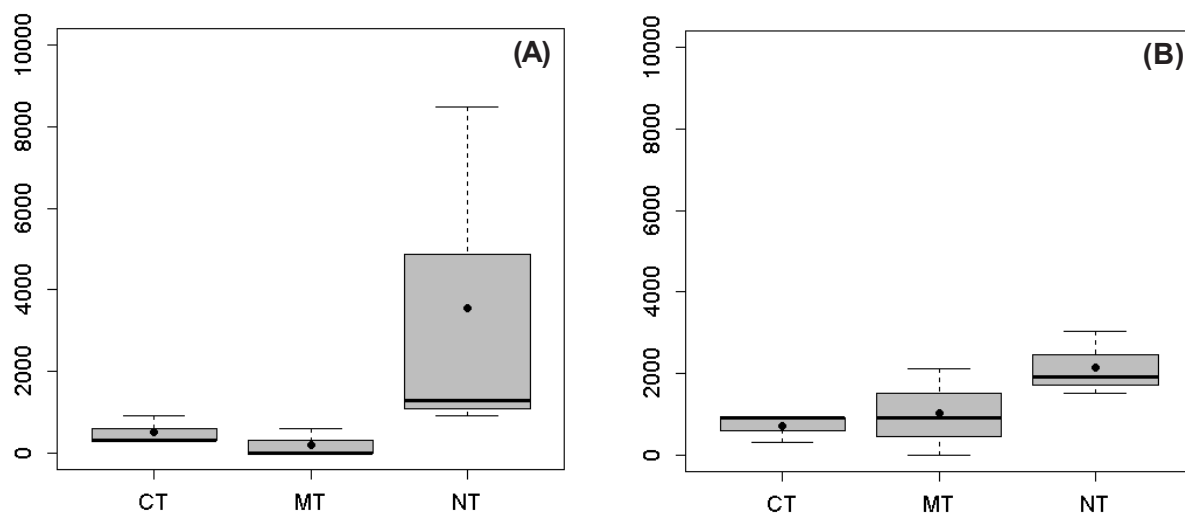***Table 3*** - Mean seed density (seeds m^{-3}) found in the 0-8 cm depth of soil sampled in November 2005

	CT	MT	NT	Seed size (mm)
Amaranthus blitoides S. Watson	506 a	202 a	3,596 a	1.2-1.7[2]
Anagallis arvensis L.	707 a	1,010 a	2,156 a	0.9-1.4 x 0.6-1.0[2]
Chamaesyce canescens L.	505 a	202 a	616 a	1.0-1.5 x 0.6-0.9[2]
Chenopodium vulvaria L.	0 a	0 a	103 a	1.0-1.5[2]
Chrozophora tinctoria L.	505 a	101 b	103 b	4.0-5.0 x 3.5-4.0[2]
Convolvulus tricolor L.	202 a	0 a	0 a	4.0-5.0 x 3.6-4.3[2]
Brassicaceae	0 a	0 a	103 a	
Poaceae other than *P. paradoxa*	0 a	101 a	103 a	
Kickxia spuria L.	0 a	910 a	822 a	0.7-1.0 x 0.5-0.8[2]
Linaria latifolia Mill.	101 a	101 a	0 a	1.6-2.4 x 1.5-2.0[2]
Papaver rhoeas L.	0 a	101 a	205 a	0.7-0.9[1]
Phalaris paradoxa L.	0 a	0 a	103 a	1.7-1.8[1]
Picris echioides L.	1,112 a	505 a	719 a	0.9-1.0 x 2.5-3.0[1]
Polygonum aviculare L.	707 b	2,122 a	719b	2.0-3.0[2]
Total	4,345 b	5,355 ab	9,348 a	

CT: Conventional tillage, MT: Minimum tillage, NT: No-tillage. Mean seed number in rows followed by the same letters are not significantly different according to the Tukey test (*P*<0.05). [1] HYPPA (2006), [2] Castroviejo (1986-2012).

separated. Thus, when more seeds of a certain species are found in CT this is due to the combination of moldboard ploughing and no herbicide use. In contrast, when more seeds are found in NT, the reason is lack of tillage and adaptation of the plants to the herbicides used.

Most of the results obtained in other long-term studies comparing tillage effects on weed seed distribution found more weed seeds in the upper soil layer in NT compared to CT (Table 3), confirming the results of the present work (Dorado and López-Fando, 2006; Torresen et al., 2003; Carter and Ivany, 2006; Sosnoskie et al., 2006). However, Bàrberi and Lo Cascio (2001) did not find any differences in weed seed content in the upper soil part due to tillage systems and, surprisingly Swanton et al. (2000) and Ruisi et al. (2015) found the opposite results. This heterogeneity is probably caused by the different soil types as well as by the dominating weed species behaving differently. In fact, weed seed size is one of the relevant variables explaining why some species decrease and other increase with NT. Large seeds probably survive a shorter time in MT and NT as there is less contact between seeds and solid soil particles protecting them (Terpstra, 1990; Reuss et al., 2001) and also due to seed predation. This would explain significantly higher mean density in CT of the big seeds of *C. tinctoria* and the same tendency of *Convolvulus tricolor* L. and *P. echioides* in the present trial compared to the other tillage systems (Table 3). Small-seeded and perennial weeds are generally more abundant in conservation agriculture (Bajwa, 2014) although Hernández-Plaza et al. (2015) showed in their long-term experiment that CT did not strictly allow only big seeds to survive but offered a greater variability in seed size than MT and NT. However, species shift processes are complex and cannot be attributed to seed size, only.

Seeds of some species, i.e. *Amaranthus blitoides* S. Watson., *Anagallis arvensis* and *P. rhoeas,* tended to be more abundant under NT although variability of data did not allow statistical differences (Figure 2). However, mean density of *A. blitoides* seeds under NT was 7 times greater than under CT and almost 18 times greater than under MT (Figure 2); moreover, the parameters relative density, relative frequency and relative abundance of seeds differed significantly being again higher for NT compared to the other two systems (data not shown, p<0.05). A higher mean density of *A. blitoides* under NT has been observed by other authors, too (Zawieja and Kordas, 2003), even though in other cases this species is supposed to be favored by tillage (Masiunas et al., 1997; Derksen et al., 1993). A possible explanation of such contradictory results is that small seeds may have a stronger interaction with soil particles which may influence seed germination (Reuss et al., 2001) so that species like *A. blitoides* may increase or decrease in importance depending on the soil properties. Additionally, irregular seed distribution in the soil is very common so that experimental results may be also conditioned by this fact and differences may be smaller than expected in some cases (see in this work Figures 2, Table 3).

CT: Conventional tillage, MT: Minimum tillage, NT: No-tillage. Points indicate mean value; thick horizontal line shows the median.

Figure 2 - Box plot showing seed density (seeds m^{-3}) of *Amaranthus blitoides* (A) and *Anagallis arvensis* (B) found in the 0-8 cm depth of soil sampled in November 2005.

The obtained results (Table 3) nevertheless support the hypothesis that there is a species-specific response to reduced tillage in the seedbank.

Polygonum aviculare L. mean seed densities were significantly higher under MT than under the other two systems, and some other species including *Chenopodium vulvaria* L., *Kickxia spuria* L. Dumort. and *Phalaris paradoxa* L. tended to have higher mean seed density under NT (Table 3) with the complete absence of seeds of these species in some tillage treatments; this shows a clear trend either to remain steady or to grow in importance in some tillage systems and to reduce or to disappear in others.

Higher *P. aviculare* mean seed densities were found for MT compared to the other two systems (Table 3). In constrast, Ruisi et al (2015) found *P. aviculare* associated to CT and Dorado and López-Fando (2006) also found higher *P. aviculare* seed density with increasing tillage intensity in an experiment performed in central Spain, although not in all cases. Also in several studies on winter cereal crops (i.e. Froud-Williams et al., 1983; Dorado et al., 1999; Verdú and Mas, 2004), *P. aviculare* has been described as a characteristic species for CT, because higher seedling densities were recorded under this system. However, Bàrberi and Lo Cascio (2001) did not find any differences in the seedbank of this species after 12 years of comparative tillage in 0-45 cm soil depth. Such diverse and contradictory results justified the need of repeating these kind of experiments at different sites. According to Verdú and Mas (2004) the higher density recorded in the MT soil seedbank in the present study could be explained by differences in seedling recruitment, seedling survival and adult plant sizes of emerged *P. aviculare* plants under the three tillage systems (CT, MT and NT). Discovering which of these factors is mainly responsible would require very detailed sampling. In the present trial the heavy clay soil may have prevented some seeds from germinating, and the small seed size of this species could contribute to explaining the higher seed abundance in MT.

Soil depth of 8-16 cm

The same trend found in 0-8 cm was observed for these species in the 8-16 cm depth of soil but in this case significant differences were only found for *P. aviculare* (higher in MT than in CT and NT) with similar amount of seeds compared to 0-8 cm depth. The rest of species maintained the same pattern as in the 0-8 cm soil depth, but with non-significant differences among treatments (data not shown). In the 8-16 cm depth NT tended to have the highest quantity of seeds (7,725 seeds m^{-3}) compared to CT (4,635 seeds m^{-3}) and MT (5,159 seeds m^{-3}) so that differences seemed to be softened in depth. These results are similar to those found by Auskalnienë and Auskalnis (2009) who also described differences in the upper layer (0-10 cm

in that case) with less weed seeds in CT than in NT but no significant differences in the deeper soil layer (10-20 cm). Additionally, these authors also found higher species number in the upper soil layer of NT and MT (at 0-5 cm) while no differences were found at 5-20 cm.

Vertisolic soils develop large fissures during desiccation, which contributed to natural tillage in all treatments in the present experiment. This probably reduced the differences among tillage treatments at 8-16 cm soil depth, similar to what was found by Cardina et al. (2002). In fact, this is probably the reason why weed seeds were found in the 8-16 cm depth of soil in NT even 24 years after the last soil tillage (data not shown), but seeds were not found at this depth in other studies conducted on other soil types. In lighter soils subjected to conservation tillage a large proportion of the weed seedbank generally remains on or close to the soil surface after crop sowing (Chauhan et al., 2012) even if this tillage effect is not found in all studies (e.g., Carter and Ivany. 2006). An effect of soil texture is also that in light soils seed dormancy generally is reduced as there are less soil aggregates and seeds are less protected (Terpstra, 1990) and the long-term tillage effect may be seen earlier as in heavy soils. On the other hand, as discussed by Carter and Ivany (2006), 24 years can be considered a too short period of time to try to understand any differences when studying long-lasting species as *Chenopodim album* L. or others that survive 30-40 years (Holm et al., 1977); *C. album* showed still a survival rate of 28% after 17 years of burial (Burnside et al., 1996).

Seedbank diversity

In the 0-8 cm depth of soil highest mean seedbank diversity and evenness were found for CT and lowest diversity for MT. Concerning evenness, MT presented the lowest species abundance uniformity in the 0-8 cm depth of soil, indicating the existence of more concentrated seedbanks. The same tendency was detected in the 8-16 cm depth of soil for both indices (Table 4). However, it is noteworthy that many species were found at more homogenous densities under CT compared to the other tillage systems and only two species composed more than half of the total weed seeds found under NT and MT demonstrating low diversity (Table 3). The findings of Storkey and Neve (2018) demonstrate that weed competition is lower in a situation with more diverse weed flora; thus, the circumstance under CT found in the present trial would be more desirable from the productive point of view.

Table 4 - Shannon diversity index (H) and Shannon evenness (J') of the soil seedbank for the three tillage systems in the two depths of soil profile

Parameter/depth	0-8 cm			8-16 cm		
	CT	MT	NT	CT	MT	NT
H	1.48 (0.08) a	1.12 (0.42) b	1.31(0.22) ab	1.35 (0.23) a	1.07 (0.54) a	1.13 (0.33) a
J'	0.93 (0.02) a	0.74 (0.17) b	0.81 (0.10) ab	0.77(0.08) a	0.64 (0.20) a	0.67(0.13) a

CT: Conventional tillage MT: Minimum tillage NT: No-tillage. The brackets show standard errors. Means in rows followed by the same letters are not significantly different according to the Tukey test (*P*<0.1).

One possible explanation for finding higher weed seed diversity in CT in the present study is that centuries of widespread use of this weed control technique in the area may have selected for a large number of weed species well-adapted to deep tillage leading to the highest diversity in CT. The use of herbicides, which is the main weed control technique in NT, is relatively recent (starting about 50 years ago), so chemical control can be expected to reduce weed diversity, as herbicide resistance is not yet a problem in the area. In MT chemical and mechanical weed control is combined, causing the lowest diversity, confirming that plants of few species are expected to survive and reproduce under both control methods.

The observed differences for evenness can be explained by the weed seed dispersal methods of each tillage system. Thus, in CT the soil is mixed and the seedbank is more or less uniformly dispersed by tillage operations resulting in higher evenness values, while in the MT and NT lightweight seeds, as for example the wind-dispersed *P. echioides* or *Conyza* spp. (Savage et al., 2014) are little buried or not buried at all if the MT operation has been already carried out so that it may be plausible that this kind of seeds are not easily evenly distributed under MT.

Influence of tillage type on the aboveground weed richness

Emergence in autumn (November)

Weeds counted in November belonged to stages 11 to 29, according to the BBCH scale (BBCH, 1997).

More seedlings emerged in autumn with decreasing tillage intensity accounting for 1.7, 7.4 and 14.6 plants m^{-2} for CT, MT and NT, respectively (p<0.001), confirming another of the previously stated hypotheses. This was also observed for two weed species: *Picris echioides* and *K. spuria* where more seedlings were found in NT compared to the other systems (1.0, 4.3 and 8.3 plants m^{-2} in the first species for CT, MT and NT, respectively and with 0, 0.5 and 1.6 plants m^{-2} of the second species for CT, MT and NT, respectively) (p<0.001). Other species with interesting results were *Amaranthu*s sp. with 0, 0 and 2.75 plants m^{-2} for CT, MT and NT, respectively (p=0.3) or *Ridolfia segetum* with 0, 0 and 0.3 plants m^{-2} for CT, MT and NT, respectively (p=0.07). Results of the other species were in all cases tending to show more seedlings in NT or MT compared to CT (data not shown). Mean richness of the emerged species was significantly lower for CT than for the other two systems accounting for 3, 8.3 and 8.0 species per treatment for CT, MT and NT, respectively, suggesting that tillage reduction increases the number of emerged species, despite the fact that the opposite results were found for the seedbank.

Lack of differences in species richness of the vegetal community for the different tillage systems was also found in other long-term trials in Mediterranean conditions (Giambalvo et al., 2012; Santín-Montanyá et al., 2017). However, species richness was much higher in the Italian trial and, opposite to the present results, no significant differences were found among treatments.

Additionally, some species showed significant differences for certain parameters. This is the case of *Malva parviflora* L., with higher mean density in NT (0.035 plants m^{-2}) than in CT (0.0 plants m^{-2}). By contrast, *P. paradoxa* showed a higher mean density under MT (0.08 plants m^{-2}) than under CT (0.0 plants m^{-2}) or NT (0.01 plants m^{-2}), although the mean density was very low in any case.

In the present trial there was an important contribution of the wind-dispersed species *P. echioides* derived from areas surrounding the experimental site. In particular, this species accounted for 1, 4.3 and 8.3 plants m^{-2} at CT, MT and NT, respectively, explaining at least partially that more adult plants were found at NT in February, followed by MT and CT, despite seedbank data do not correlate with these findings. However, as these seeds are wind-dispersed in late summer distribution of this species can be considered as aleatory. Higher density under NT than under CT for *M. parviflora* is a likely behavior for a perennial species as also described by (Torresen, 2003). In fact, seeds were not found in the field at all possibly due to the mainly vegetative reproduction of the plants.

Emergence in winter (February)

Counts of seedlings in stages 11 to 14 according to the BBCH scale (BBCH, 1997) were separated from adult plant assessments. Similar to the observations in autumn, fewer weeds emerged under CT than under MT and NT after the tillage performed in November and January for MT and CT (Tables 5 and 6). No weeds emerged at all under CT. Also the number of samples with weeds per plot and the weed species richness per plot followed the same pattern (Table 5). *Linaria latifolia* Mill. was the only species with a mean density higher for MT compared to CT and NT (Table 5), but unfortunately its seed number was low, so the emergence result could not be supported by the seedbank data.

These results are in agreement with the statements that tillage has been used to control weeds since the origin of agriculture, and a reduction in tillage may dramatically increase weeds (Chauhan et al., 2012). When comparing the emergence in November and adult plants still present in February, which apparently survived the tillage or herbicide operations, CT was the most effective control method (0% survival), followed by MT (19% survival) and by NT (25% survival). Thus, although weed seed number in the soil was greater in NT than in CT (Table 3), the proportion of weeds found in winter in NT was the highest of the three tillage systems, consisting of plants tolerant to the applied herbicide.

Table 5 - Mean seedling density (plants m^{-2}), frequency of samples with weed plants and species richness per plot for the three tillage systems in the winter aboveground sampling

Species	CT	MT	NT
Linaria latifolia Mill.	0.00 b	3.72 a	0.65 b
Picris echioides L.	0.00 a	0.97 a	2.35 a
Anagallis arvensis L.	0.00 b	0.57 ab	1.05 a
Phalaris paradoxa L.	0.00 a	0.49 a	0.40 a
Kickxia spuria L.	0.00 a	0.16 a	0.16 a
Vicia lutea L.	0.00 a	0.08 a	0.00 a
Total	0.00 b	5.99 a	4.61 a
Number of samples with weeds per plot	0.00 b	10.55 a	10.21 a
Number of weed species per plot	0.00 b	4.00 a	4.30 a

CT: Conventional tillage, MT: Minimum tillage, NT: No-tillage. Means in rows followed by the same letters are not significantly different according to the Tukey test (*P*<0.1).

Table 6 - Mean adult plant density (plants m^{-2}), frequency of samples with weed plants and species richness per plot for the three tillage systems in the winter aboveground sampling

Species	CT	MT	NT
Picris echioides L.	0.00 b	0.57 b	1.86 a
Anagallis arvensis L.	0.00 b	0.49 ab	0.81 a
Malva parviflora L.	0.00 b	0.16 ab	0.49 a
Kickxia spuria L.	0.00 a	0.08 a	0.24 a
Convolvulus tricolor L.	0.00 a	0.00 a	0.16 a
Linaria latifolia Mill.	0.00 a	0.08 a	0.08 a
Ridolfia segetum (L.) Moris	0.00 a	0.00 b	0.08 a
Total	0.00 b	1.38 a	3.72 a
Number of samples with weeds per plot	0.00 a	3.66 a	11.33 a
Number of weed species per plot	0.00 a	2.8 a	3.10 a

CT: Conventional tillage, MT: Minimum tillage, NT: No-tillage. Means in rows followed by the same letters are not significantly different according to the Tukey test (*P*<0.1).

Concerning newly-emerged seedlings between November and February, equally high numbers were observed in MT and NT despite a higher seedbank in NT. Thus, superficial tillage with the vibro-cultivator probably stimulated weed emergence in MT. Lack of emergence in CT demonstrated the ability of moldboard tillage to place seeds in non-favorable positions for germination regardless of seed diversity being highest in CT.

Relationship between the different parameters

Concerning winter emergence of particular species, an accordance between the seedbank results and emergence in winter was found for the species *A. arvensis* and *K. spuria* (Tables 3, 5, and 6) as more seeds and also more seedlings and adult plants were found under MT and NT and less under CT. The emergence of more seedlings in plots under MT and NT than under CT in winter is consistent with the expected results because despite detecting a lower weed seed species diversity under MT, more weed seeds were available under this treatment at 0-8 cm depth of soil (Table 3), facilitating emergence when tillage was surficial.

Regarding winter emergence of particular species, the results show that *A. arvensis* and *P. echioides* were favored by NT, as also found by Dorado and López Fando (2006) for the first species. Similarly, *P. echioides* is well-adapted to perennial crops as alfalfa where no tillage is conducted during 5 or more years (Meiss et al., 2010).

With respect to adult plants present in winter after the tillage operations, significantly higher mean densities were found for the overall weed density and for *A. arvensis*, *M. parviflora*, and *P. echioides* densities under NT, decreasing with increasing tillage intensity (Table 6). Number of samples with weeds per plot and weed species richness were again lowest for CT where no emergence occurred. The reason to find a significantly higher mean overall and specific adult

plant density for *A. arvensis*, *M. parviflora* and *P. echioides* is that these species are probably not easily-controlled with glyphosate and MCPA herbicides. Other authors also confirm that perennial species such as *Malva* spp. generally have high mean densities under NT (Davis et al., 2005; Torresen et al., 2003).

Mean seedling emergence of *P. echioides* tended to be higher for plots under NT than under the other systems and the frequency and the relative abundance of this species (data not shown) were statistically significant following the same pattern (higher under NT compared to CT and MT, $p<0.05$). However, at least in the 2005-06 sampling year, no new *P. echioides* seeds were produced in the CT plots, as no seedling nor adult plants were found. Similar events likely occurred in prior years with sunflower. Thus, more *P. echioides* seeds found under CT are most probably wind-distributed and not a consequence of the tillage treatments.

Concerning diversity indexes, no significant differences were found between tillage treatments from aboveground weed communities in autumn (seedlings) (Table 7). Nevertheless, no seedlings or adult plants were found in CT in winter, after soil preparation for sunflower sowing, causing lower diversity than in the other two tillage systems (Table 7).

Table 7 - Shannon diversity index (H) and Shannon evenness (J') obtained in the autumn sampling (seedlings) and in the winter sampling (seedlings or adult plants) for the three tillage systems

Date	Autumn						Winter		
Parameter/weed size	Seedlings			Seedlings			Adult plants		
	CT	MT	NT	CT	MT	NT	CT	MT	NT
H	0.57 a (0.29)	0.79 a (0.39)	1.15 a (0.11)	0.00 b (0)	1.06 a (0.19)	1.21 a (0.04)	0.00 b (0)	1.02 a (0.01)	1.10 a (0.29)
J'	0.52 a (0.27)	0.46 a (0.23)	0.56 a (0.05)	0.00 b (0)	0.81 a (0.10)	0.83 a (0.06)	0.00 b (0)	0.93 a (0.01)	0.88 a (0.05)

CT: Conventional tillage MT: Minimum tillage NT: No-tillage. Means in rows followed by the same letters are not significantly different according to the Tukey test ($P<0.05$). The brackets show standard errors.

In conclusion, the three tillage systems conducted over 24 years had an effect on both the seed bank and on the emerged weed communities, thereby confirming the hypotheses. The main results were that CT, using moldboard plow, caused a seedbank with lower seed density than under NT but with a higher Shannon diversity and Shannon evenness indexes in the first 8 cm of soil compared to MT. NT showed an intermediate level of diversity, but with almost twice as many seeds as the other two systems and with more than half of the seeds belonging to only two species, i.e. *A. blitoides* and *A. arvensis*. Thus, MT and NT increase the overall seedbank and decrease diversity in the top 8 cm of soil. These results are potentially negative for weed control under MT.

Additionally, distinct weed species responded differently to the tillage systems. For instance, *C. tinctorea* seed density in the 0-8 cm depth of soil was highest under CT; *P. aviculare* showed a larger seed number for MT than for CT or NT, and *A. blitoides* had the highest mean density and relative abundance of seeds under NT. Thus, reduction of tillage intensity can cause weed species shifts. These newly- dominating species under MT and NT can cause more or less severe weed control problems depending on the difficulty of controlling them in the main crops in the area.

Concerning emergence, the adult plant density for the species *A. arvensis*, *M. parviflora* and *P. echiodes* were higher for NT, while the highest seedling density was for *L. latifolia* and occurred in MT. Both, weed species richness and number of samples with plants were higher for MT and NT than for CT.

Comparisons with other studies show the need of repeating this kind of long-term study as both consistent and contradictory results were found overall and for certain species. In particular, studies in clay soils would be interesting to compare with the results found here.

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