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Influence of soil tillage and *Phoma macdonaldii* on sunflower (*Helianthus annuus* L.) yield and oil quality

Influencia de la labranza del suelo y de *Phoma macdonaldii* sobre el rendimiento y la calidad del aceite de girasol (*Helianthus annuus* L.)

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Abstract. Plant yield and oil content determine sunflower production. Those plant production determinants depend in turn on the plant–environment interaction. In the South West of France, there have been recent advances in soil tillage. To date, 35% of the soil surface dedicated to sunflower is cropped under a reduced tillage system. Major constraints to sunflower cropping are water stress and cryptogamic diseases. The second most important sunflower disease in the South West of France is premature ripening caused by *Phoma macdonaldii*. Aims of this work were to: 1) understand how these factors influence sunflower yield, and 2) quantify the fatty acid quality variation under reduced tillage and *Phoma macdonaldii* infection. Results showed that 1) soil tillage influences sunflower oil fatty acid composition, 2) *Phoma macdonaldii*-induced premature ripening impacts plant nutrition through its effects on organs (leaves, stems, roots), yield and yield components, and 3) the disease influenced oil quality and the balance oleic-linoleic fatty acids.

Keywords: Fatty acids; Oil; Yield; Premature ripening; *Phoma macdonaldii*; Soil tillage.

Resumen. El girasol (*Helianthus annuus* L.) es ampliamente cultivado en Francia. Su aceite se destina principalmente a la alimentación humana y a la industria verde. Su producción es consecuencia del rendimiento de la planta y del contenido de aceite, dependientes estos de la interacción planta–medio ambiente durante el ciclo. En el Sudoeste francés, se ha obtenido un avance reciente en el laboreo del suelo. En la actualidad, el 35% de la superficie dedicada al cultivo de girasol se realiza bajo labranza reducida. Las principales limitantes del cultivo lo constituyen el déficit hídrico y las enfermedades criptogámicas. La infección con *Phoma macdonaldii*, conocida en la región como desecamiento precoz, es la segunda enfermedad en importancia del girasol en el sudoeste francés. Este trabajo tuvo por objetivo: 1) comprender cómo estos factores influyen sobre la producción de girasol, y 2) cuantificar la variación de la calidad de los ácidos grasos frente a las limitaciones impuestas por la reducción de labranzas y la infección con *Phoma macdonaldii*. Los resultados mostraron que: 1) la labranza del suelo influye sobre la composición en ácidos grasos del aceite de girasol, 2) el desecamiento precoz inducido por *Phoma macdonaldii* impacta en la nutrición de la planta por su efecto sobre los órganos (hojas, tallo, raíz), en el rendimiento y en sus componentes, y 3) la enfermedad influyó en la calidad del aceite y en el balance de ácidos grasos oleico *vs*. linoleico.

Palabras clave: Ácidos grasos; Aceite; Rendimiento; Desecamiento precoz; *Phoma macdonaldii*; Labranza del suelo.

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INTRODUCTION

Sunflower (*Helianthus annuus L.*) is the second French crop in oil production, just after oilseed rape (*Brassica napus L.*; CETIOM, 2006a). The Midi Pyrenees region is the second biggest French cropping area. Due to health programs, sunflower oil was mainly used in human feeding during the 70's. Thereafter, the increase of the balance omega-3/omega-6 in human feeding led to its slight decrease during the 90's. In the last decade, in the French context, the strong demand of oleic oil for human feeding and industrial use emphasized the need for high levels of oleic acid content in sunflower crops (Thebaud, 2007).

Over the last twenty years, the advances in farming practices (specialization on crops and field expansion) have contributed to the shortening of crop rotations such as the sunflower-wheat sequence (CETIOM, 2011). The implementation of conservation tillage practices such as no-till (total absence of soil tillage), strip-till (direct sowing, minimum tillage), ridge-till (simple work done on the upper soil layer without deep tillage), and mulch-till [superficial: 15 cm from the soil surface using a field cultivator (for instance); in depth: using a subsoil cultivator] can benefit soils in terms of soil erosion reduction, and increased biological activity and soil water content (Raper et al., 1994; Halvorson et al., 2002; Murillo et al., 2004; Raper, 2005). However, variation of soil compaction attributed to agricultural traffic and/or conservation practices, has been reported in the literature (Lipiec & Stepniewski, 1995; Moreno et al., 1997; Taboada et al., 1998; Raper & Kirby, 2006; Sasal et al., 2006; Taboada & Alvarez, 2008). Increased soil compaction leads to a loss of porosity, water and nutrient availability, and to an increase of soil bulk density and root penetration resistance (Lipiec & Hatano, 2003).

The impact on soil compaction on crop growth is a complex process. Soil mechanical constraints might impair root system's growth; this alteration leads to a decrease in (1) the below and above ground resource acquisition (which leads to a further alteration of the root system), and (2) the efficient use of those resources. This results in a reduction of the above ground plant growth (which also leads to an alteration of the root system), and finally to a global loss of yield (Lipiec & Hatano, 2003; Sadras et al., 2005). Concerning sunflower cropped under soil compaction, literature has reported decreases in (1) leaf area, (2) above ground biomass, (3) plant height, (4) growth rate, (5) rooting depth and (6) final yield (Andrade et al., 1993; Goodman & Ennos, 1999; Bayhan et al., 2002; Botta et al., 2006; Diaz-Zorita, 2004). Even though no interaction has been observed between crop health and tillage practices in France (Lecomte & Quere, 2005), the reduction of traditional soil tillage practices (leaving stubble during winter) has lead to increases of disease infections (Penaud, 1994; Debaeke & Peres, 2003; Taverne, 2005; Seassau et al., 2010c).

Premature death in sunflower may be caused by environmental conditions and several pathogen agents including *Alternaria alternate*, *Macrophomina phaseolina, Verticilium dahliae*, *Fusarium monoliforme* and *Phoma macdonaldii*. Pathogen infections are characterized by (1) a necrotic girdling spot at the stem base and on the stem, (2) early death of the leaf area, and (3) a small head. These symptoms lead to a global yield loss depending on the infection stage (Donald et al., 1987; Seassau, 2010; Seassau et al., 2010b). Studies carried out under controlled and field conditions in Europe and the United States of America have shown the role of *Phoma macdonaldii* as the causal agent of sunflower premature death, the other pathogens acting as opportunists (Donald et al., 1987; Seassau, 2010; Seassau et al., 2010b; Seassau et al., 2010c). *Phoma macdonaldii* Boerema (Telomorph: *Leptosphaeria lindquistii*: McDonald, 1964) is one of the most important diseases in sunflower production, and has been reported worldwide (Gulya et al., 1997). The disease is mainly characterized by the appearance of lesions at the foliar node, and black spots girdling the stem. These are not particularly damaging in terms of yield compared to the infection at the stem base, where leaves become wilted and the stalks turn black, leading to a loss of plant vigor during grain filling; finally, plant death occurs a few weeks before physiological maturity (Donald et al., 1987).

In France, the severity of the disease increased dramatically in the 90's, and the entire French sunflower cropping area is now affected (Peres & Lefol, 1996). It still remains unknown how to limit sunflower premature ripening caused by *Phoma macdonaldii*. There is no real chemical solution (Duroueix, 2005). However, sunflower genetic resistance (Al Fadil, 2006; Alignan, 2006; Al Fadil et al., 2009), crop rotation (Jouffret, 2005), limited water supply after anthesis and additional nitrogen (Carson, 1991; Debaeke & Peres, 2003; Seassau, 2010; Seassau et al., 2010a) can significantly reduce the incidence of *Phoma macdonaldii*, and then premature ripening. The impact of the disease on yield has not been clearly assessed, but yield losses up to 1.3 t/ha have been reported in France (McDonald, 1964). Carson (1985) showed up to 60% yield decreases, with consequences on oil yield. The literature has also reported that plant diseases could act on the fatty acids content, but results on this subject stay unknown and controversial in sunflower.

Plant production consequences of soil tillage have been largely studied in many crops. However, only a few studies have been carried out in sunflower, and none of them include the fatty acid variation (Goodman & Ennos, 1999; Bayhan et al., 2002; Lipiec & Hatano, 2003; Diaz-Zorita, 2004; Sadras et al., 2005; Botta et al., 2006). The symptoms and consequences of *Phoma macdonaldii* have been described by various authors (McDonald, 1964; Donald et al., 1987; Carson, 1991; Peres & Lefol, 1996; Gulya et al., 1997; Pereira et al., 2000; Peres et al., 2000), but the ecophysiological consequences on sunflower oil production under the influence of this disease have not been documented yet.

A study was conducted using naturally prematurely-ripened plants to understand the consequences of soil tillage practices and *Phoma macdonaldii* infection on yield, oil content losses and fatty acid variation. The objectives of this study were: 1) to understand how these factors influence sunflower production, and 2) to quantify the variation of fatty acid quality under this biotic stress.

MATERIALS AND METHODS

Two factors were studied 1) soil tillage and 2) natural infection by *Phoma macdonaldii* leading to either premature ripening (PR) or not premature ripening (NPR). Studies were conducted under a split plot design (four replications, plots of 72 m^2). The tillage factor was characterized by two soil treatments: i) reduced tillage (cover crop followed by soil laboring using a surface subsoiler at 30 cm depth: RT), and ii) soil tillage (cover crop followed by soil laboring using two perpendicular passes with a subsoiler at 50 cm depth: ST). Plants inoculated with *P. macdonaldii* presented black spots girdling the stem base, and were either i) premature ripened (PR), or ii) not premature ripened (green leaves present, NPR) at the harvesting stage.

The trial was set up under farming conditions on the experimental farm of the E.I. Purpan: Ferme de Lamothe (Midi Pyrénées, FRANCE, 43° 30' 11.75" N; 1° 14' 54.53" E; temperate climate, low rainfall). The soil was a drained typic Glossaquaf (Clay: 246.5 g/kg, Silt: 448 g/kg, Sand: 306 g/kg, SOM: 29.9 g/kg, pH: 6.2). The soil was very unstable and hydromorphic, due to a high level of pebbles (over 40%). Soil water storage was consequently low, even in rainy years. The field rotation was wheat / sunflower, with the absence of tillage or minimum tillage (cover crop leaving the stubble outstanding, followed by laboring the soil with one pass of subsoiler at 30 cm depth) on both crops (wheat, sunflower). Even though these practices led to remaining stubble (wheat and sunflower), no particular disease infestations were reported on this field.

Sowing was done on 30 April 2007 (pneumatic drill, gauge 0.6 m, 5.27 plants/m^2) and harvesting on 11 September 2007. No irrigation was applied during crop growth, and fertilization with chicken manure was carried out before sowing. The cultivar used was Melody (half late, Syngenta Seeds SAS, French registration control since 1996), because of its good yield plasticity under abiotic stresses. Its oil content has been qualified as average (CETIOM, 2006b). Melody has been shown to be partially resistant to premature ripening caused by *Phoma macdonaldii*, or other fungal diseases (*Diaporthe helianthi, Sclerotinia sclerotinium*), but can be affected by stem attacks. Growth stages were monitored in each plot on a weekly basis during the cycle (CETIOM, 2004).

During 2007, no symptoms of drought stress were observed in the plants (total rain in the plant cycle: 350 mm). Relative humidity was 84% from sowing to flowering, and 77% from flowering to harvesting. Flowering occurred on 17 July 2007, stage 4.3 [990 growing degree days (GDD); base temperature 4.8 °C from sowing: Hutley-Bull, 1995; Granier & Tardieu, 1998].

Disease observations were conducted at the same time as crop phenology, and were performed starting the stage 4.3. Premature ripening due to *Phoma macdonaldii* was assessed when the stem base presented circling black spots and the plant was completely dry. Because of the occurrence of natural inoculation with *P. macdonaldii* and PR plants at physiological maturity, PR and NPR plants were harvested separately on the same plot.

Yield components, oil and plant biomass data from PR and NPR plants were obtained from sunflower head samples on eight consecutive meters (seed and oil components), or from three consecutive plants (yield, yield components and plant biomass) randomly extracted in each sub plot (PR and NPR). At harvesting, various plant organs were obtained. They were cleaned, separated and characterized. The dry matter from kernels, leaves, stems and roots was obtained after drying at 65 °C during 72 hours. Measurements on the vegetative and root systems, and the weight of 1000 grains were obtained directly. The number of grains per head was obtained thereafter from previous data.

The oil quality data were obtained from milled sunflower seed samples (20g, three sub-samples per plot), by near infrared spectroscopy (Ayerdi Gotor et al., 2007; Niewitetzki et al., 2007; Haddadi et al., 2010). The FOSS NIR System 6200 was used with this purpose. For each sample the reflectance value was measured from 400 nm to 6200 nm at an interval of 2 nm.

Data from both harvests were analyzed separately using analysis of variance (ANOVA). Each variable was compared under the two treatments and their interaction: 1) soil tillage: reduced tillage *vs.* soil tillage; and 2) *P. macdonaldii* inoculation: PR and NPR using Rgui (2.12.0). A Student *t* test was carried out when significant differences appeared at $p<0.05$.

RESULTS AND DISCUSSION

Tillage impact on plant growth and oil quality. No interactions were observed between soil tillage and premature ripening. An increase ($p<0.05$) of 8.5% of oleic acid, and consequently a decrease ($p<0.05$) of 2.8% of linoleic acid, were observed under reduced tillage (Table 1). Except for the fatty acid variation, no relevant results were obtained from the plant vegetative system under tillage treatments. This can be attributed to the lack of discrimination between the techniques of tillage used at both tillage levels and the climatic conditions (e.g., Thebaud et al., 2008). The main source of fatty acid variations among all environmental factors is temperature. Management practices (e.g., date of sowing) and other environmental factors (intercepted solar radiation, nitrogen

Table 1. Impact of *P. macdonaldii*: premature ripening (PR); non-premature ripening (NPR); reduced tillage (RT), and soil tillage (ST) on various seed and oil components. Each value is the mean ± 1 S.E. of n=24.
Tabla 1. Efecto de *P. macdonaldii*: desecamiento precoz (PR); ausencia de desecamiento precoz (NPR); labranza reducida (RT), y suelo laboreado (ST) en varios componentes de la semilla y del aceite. Cada valor es el promedio ± 1 E.E. de n=24.

Seed and oil components	Impact of *P. macdonaldii*			Impact of Tillage		
	PR	NPR	Average of treatments	RT	ST	Average of treatments
Kernel Dry Matter (%)	95.6 a** ± 0.2[c]	95.7 b**± 0.2	95.7	95.7 ± 0.2	95.6 ± 0.2	95.7
Kernel Protein content (%)	16.0 a* ± 0.9	14.7 b*± 1.7	15.4	15.6 ± 1.6	15.0 ± 1.1	15.3
Oil (%)	48.1 ± 2.0	47.9 ± 2.1	48.0	47.9 ± 2.0	48.0 ± 2.1	48.0
Palmitic acid (% of oil)	6.9 a***± 0.1	6.7 b***± 0.1	6.8	6.7 b* ± 0.2	6.9 a* ± 0.2	6.8
Stearic acid (% of oil)	3.0 a**± 0.3	2.6 b**± 0.4	2.8	2.8 ± 0.5	2.8 ± 0.3	2.8
Oleic acid (% of oil)	20.4 a***± 1.6	23.2 b***± 2.1	21.8	23.1 a* ± 2.5	21.3 b* ± 2.0	22.2
Linoleic acid (% of oil)	70.0 a*** ± 1.6	67.3 b*** ± 2.2	68.7	68.0 b* ± 2.3	69.9 a* ± 1.6	69.0

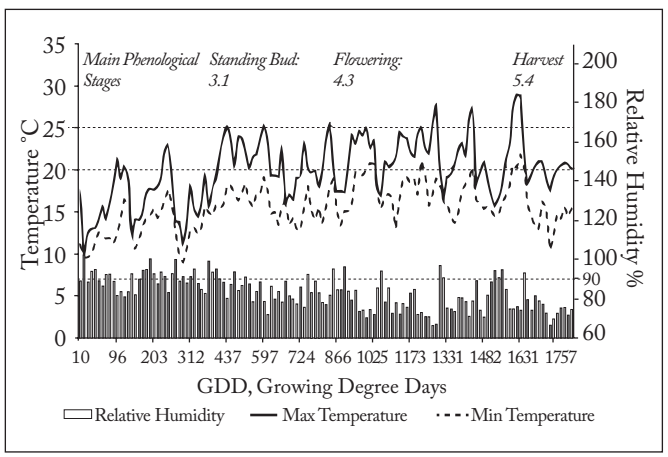
a, b: homogenous group according to Student test; * Significant Probability at 0.05, ** Significant Probability at 0.01, *** Significant Probability at 0.001.
c: standard deviation.
a, b: Grupos de homogeneidad por test de Student; * Significativo a nivel de probabilidad de 0,05, ** Significativo a nivel de probabilidad de 0,01, *** Significativo a nivel de probabilidad de 0,001.
c: desvío standard.

availability and water supply) also act on the fatty acid synthesis pathway, and consequently on the final fatty acid content (Aguirrezabal et al., 2009; Echarte et al., 2010). In parallel, the negative action of soil compaction has been reported on leaf area (and consequently on intercepted solar radiation), nitrogen and plant water availability (Tardieu, 1987; Lipiec & Hatano, 2003; Diaz-Zorita, 2004). Even if no studies of nitrogen and soil water availability were carried out on this field, the authors can indirectly attribute this variation to soil tillage.

Changes of fatty acid content under different water management systems have been reported in literature (Roche et al., 2006; Haddadi et al., 2010). Depending on the situation, oleic acid content has been favored by irrigation (Flagella et al., 2004; Roche et al., 2006; Haddadi et al., 2010) but also by a slight drought stress (Baldini et al., 2002), with a consequent decrease of linoleic acid. There exists a direct relationship between Δ12 desaturase (enzyme involved in the desaturation of oleic acid to linoleic acid: Garces et al., 1989) and water deficit (Roche, 2005). According to Roche (2005; Roche et al., 2006), the regulation of this enzyme could allow the plant to acclimate itself to water scarcity by maintaining membrane function under drought (more saturated fatty acids in membrane lipids lead to sustained membrane fluidity). Depending on the spatial distribution of the root system, if the bulk density and penetration resistance are not strong enough to reduce root exploration and resource acquisition (Tardieu, 1987; Lampurlanes & Cantero Martinez, 2003; Lipiec & Hatano, 2003), reduced tillage should favor the root contact with the soil matrix and consequently water absorption. In the absence of data regarding soil mechanical constraints and water content, further investigations with more discriminating soil tillage treatments are required to test this hypothesis.

Premature ripening due to *Phoma macdonaldii* impact on plant production. The *Phoma macdonaldii* infection (pycniospore germination) reaches an optimum between 20 – 25 °C, 95% relative humidity (Roustaee et al., 2000a). The meteorological data showed that the level of relative humidity appropriate for *Phoma macdonaldii* infection occurred 22 days during the pre-flowering period (mean temperature 17.5 °C), and 6 days

Fig. 1. Meteorological conditions during the growing season (from sowing: 30 April 2007, to harvesting: 11 September 2007) recorded at the weather station on the experimental farm (CESBIO). Maximum and minimum temperature (°C), relative air humidity (%), (Growing Degree Days base 4.8 °C from sowing).
Fig. 1. Condiciones meteorológicas durante el ciclo de cultivo (siembra: 30 de abril de 2007, cosecha: 11 de septiembre de 2007), datos de la estación meteorológica en sitio experimental (CESBIO). Temperatura máxima y mínima (°C), humedad relativa del aire (%), (grados día base 4.8 °C desde siembra).



during the post-flowering period (mean temperature 19.5 °C, with 3 days > 25 °C, Fig. 1). Those climatic conditions might have favored fungus infections. *Phoma macdonaldii* symptoms were observed from stage 4.3. However, sunflower is susceptible to infection from the cotyledon stage (Roustaee et al., 2000b; Alignan, 2006). The first symptoms of premature ripening attributed to *Phoma macdonaldii* on the base stem (characteristic spot necrosis) were measured on 24 July 2007 (stage 4.3, 1092 GDD) and scored. At the harvesting day, 68% of all plants had stem base necrosis.

Premature ripening caused by *Phoma macdonaldii* tends to increase with leaf area (Taverne, 2005). The observed loss of biomass did not reflect a decline in the number of total leaves, but early drying due to *Phoma* infection: -10% of the green leaf number ($p<0.05$, data not shown), 29% decrease of leaf biomass ($p<0.01$; Table 2). At that developmental morphology stage, decreases of 21% in stem biomass ($p<0.01$) and 20% in root weight ($p<0.05$) were observed (Table 2). These results that lead to plant premature death, are consistent with previous research carried out worldwide under field and controlled environmental conditions (McDonald, 1964; Donald et al., 1987; Peres & Lefol, 1996; Gulya et al., 1997; Seassau, 2010; Seassau et al., 2010b). At the appropriate time during the plant developmental morphology, resources are mobilized to the sunflower head to accomplish reproduction and lipid genesis (Merrien & Milan, 1992; Aguirrezabal et al., 1996; Marschner, 2003). The stem and basal infections negatively impact upon organs directly linked to the functions of nutrition and reserve: root necrosis (Donald et al., 1987; Gulya et al., 1997; Peres et al., 2000; Al Fadil et al., 2009; Seassau, 2010), internal stem decay (Carson, 1991; Peres et al., 2000; Seassau, 2010), leaf necrosis (McDonald, 1964; Donald et al., 1987; Peres & Lefol, 1996; Gulya et al., 1997; Seassau, 2010; Seassau et al., 2010b), and transport vessel colonization (observations conducted at early or later plant developmental morphology stages under controlled or field conditions, respectively: Al Fadil et al., 2009; Seassau, 2010; Seassau et al., 2010c). The necrosis of organs such as root or leaves (Debaeke & Peres, 2003) leads to a reduction in the amount of required resources. Also, the necrosis of organs such as leaves or stem leads to a reduction in the amount of reserves mobilized for grain filling and lipid genesis. The addition of these indirect actions finally limits the synthesis of the reserves and/or their transport to the grain.

Total field yield was equal to 3750 kg/ha. Grain filling is directly linked to the state of the plant foliar system (Merrien & Milan, 1992). *Phoma macdonaldii* presence leads to early senescence of the plant with the direct result of yield loss (Donald et al., 1987; Carson, 1991; Peres et al., 2000; Seassau, 2010; Seassau et al., 2010b). Premature ripening showed direct consequences on yield components: -17% in the number of grains per head ($p<0.01$), and -25% in the weight of a thousand grains ($p<0.001$, Table 3). Grain yield also presented a significant decrease from non premature ripening to premature ripening (-38%, $p<0.001$, Table 3). These results indicate that plants suffered from an infection of *Phoma macdonaldii* during grain filling. A variation of seed weight and oil content, characteristic of premature ripening, was observed by Donald et al. (1987), Seassau (2010) and Seassau et al. (2010b). The reduction in the number of grains per head suggests that the disease infection started at a stage previous to that determining the number of grains per head (Alignan, 2006), and continued until plant maturity.

Premature ripening due to *Phoma macdonaldii* impact on oil quality. The disease did not significantly affect the oil content. Oil accumulation in seeds is optimal under good climatic conditions (e.g., temperature greater than 25 °C, not exceeding 35 °C, 25 days after anthesis: Rondanini et al., 2003; Rondanini et al., 2006). The cultivar Melody is well known for its good grain yield plasticity, and its oil content is qualified as average by the CETIOM (CETIOM, 2006b). The association between favorable environmental conditions and lipogenesis activity (humid conditions, mid-low temperatures), could help explain the small differences observed on this trait (Fig. 1, Berger et al., 2010).

Table 2. Impact of *P. macdonaldii*: premature ripening (PR); non-premature ripening (NPR); reduced tillage (RT), and soil tillage (ST) on biomass of various plant organs. Each value is the mean ± 1 S.E. of n=24.
Tabla 2. Efecto de *P. macdonaldii*: desecamiento precoz (PR); ausencia de desecamiento precoz (NPR); labranza reducida (RT), y suelo laboreado (ST) en la biomasa de varios órganos vegetales. Cada valor es el promedio ± 1 E.E. de n=24.

Plant organ	Impact of *P. macdonaldii*			Impact of Tillage		
	PR	NPR	Average of treatments	RT	ST	Average of treatments
Leaves (gr/m²)	643.5 a** ± 370.1[c]	985 b** ± 473.9	814.7	825.3 ± 492.8	799.7 ± 384.9	812.5
Stem (gr/m²)	2078.9 a***± 515.1	2858.2 b***± 911.5	2468.6	2439.7 ± 879.6	2541.4 ± 755.2	2490.6
Root (gr/m²)	475.4 a***± 142.9	645.9 b***± 216.7	560.7	559.1 ± 217.7	567.1 ± 171.1	563.1

a, b: Homogenous group according to Student test; * Significant Probability at 0.05, ** Significant Probability at 0.01, *** Significant Probability at 0.001.
c: standard deviation.
a, b: Grupos de homogeneidad por test de Student; * Significativo a nivel de probabilidad de 0,05, ** Significativo a nivel de probabilidad de 0,01, *** Significativo a nivel de probabilidad de 0,001.
c: desvío standard.

Table 3. Impact of *P. macdonaldii*: premature ripening (PR); non-premature ripening (NPR); reduced tillage (RT), and soil tillage (ST) on various yield components. Each value is the mean ± 1 S.E. of n=24.
Tabla 3. Efecto de *P. macdonaldii*: desecamiento precoz (PR); ausencia de desecamiento precoz (NPR); labranza reducida (RT), y suelo laboreado (ST) en varios componentes del rendimiento. Cada valor es el promedio ± 1 E.E. de n=24.

Yield Components	Impact of *P. macdonaldii*			Impact of Tillage		
	PR	NPR	Average of treatments	RT	ST	Average of treatments
Head Diameter (cm)	12.9 a** ± 1.9[c]	14.5 b**± 2.4	13.7	13.7 ± 2.5	13.9 ± 1.9	13.8
Number of grains per head	1065.9 a** ± 354.1	1292.7 b**± 294.5	1179.3	1153.8 ± 367.4	1234.0 ± 287.4	1193.9
Grain weight (gr/m^2)	1620.2 a*** ± 684.3	2706.6 b***± 891.9	2163.4	2092.9 ± 1001.9	2324.0 ± 876.9	2208.5
Thousand grain (gr)	32.1 a*** ± 6.1	41.6 b*** ± 7.0	36.9	36.3 ± 8.8	38.1± 6.8	37.2

a, b: Homogenous group according to Student test; * Significant Probability at 0.05, ** Significant Probability at 0.01, *** Significant Probability at 0.001.
c: standard deviation.
a, b: Grupos de homogeneidad por test de Student; * Significativo a nivel de probabilidad de 0,05, ** Significativo a nivel de probabilidad de 0.01, *** Significativo a nivel de probabilidad de 0,001.
c: desvío standard .

In the presence of *Phoma macdonaldii*, increases of palmitic acid (+3%, p<0.001), stearic acid (+15.4%, p<0.01) and linoleic Acid (+4%, p<0.001) were observed. Only the oleic acid content decreased under the disease pressure (-12%, p<0.001, Table 1.). As we observed (Tables 2 and 3), an infection at the 4.3 plant developmental morphology stage showed a negative impact on the number of leaves, biomass (vegetative organs are still in the growing phase), stem length and yield components. These resulted in a yield decline, and a decrease in oil quality and content. This was because of the lack of resources required for grain filling and lipid genesis (Berger et al., 2010). Plants direct their assimilates (carbohydrates, proteins) to their products at a lower energetic cost when they are under stress (Merrien & Milan, 1992). In our study, protein contents decreased (p<0.001, Table 1) by 0.9% under infection. Fatty acid content, which has a significant energy cost, decreased under constrained conditions (Table 1).

Studies on changes in fatty acid content due to environmental conditions stay controversial. It is well known that favorable environmental conditions (e.g., high night temperatures) lead to a decrease in the linoleic acid and a subsequent increase of oleic acid (Izquierdo et al., 2002; Roche et al., 2006; Aguirrezabal et al., 2009; Berger et al., 2010). Environmental factors and management practices induce fatty acid content variation (Aguirrezabal et al., 2009; Echarte et al., 2010). A slight drought stress can have a positive effect on oleic acid content (Baldini et al., 2002). However, content of this acid has often been considered to be positively influenced by irrigation (Flagella et al., 2004; Roche et al., 2006; Haddadi et al., 2010). The reverse is true for linoleic acid content (under good temperature conditions). In our study, and under the absence of hydric stress and the occurrence of intermediate temperature, it was not possible to explain the decrease in oleic acid via the environmental conditions only.

Changes in unsaturated and saturated fatty acid composition due to disease infections remain unknown. However, Cahoon et al. (2003) reported that the expression of a gene encoding different forms of Δ12 desaturase could be linked to the presence of *Sclerotinia sclerotinium*. This could not be observed in the present study. Variations in the fatty acid levels obtained under *Phoma macdonaldii* stress were partly explained by the early senescence of plants. Under premature ripening caused by *Phoma macdonaldii*, a sunflower plant also suffers from pre-ripening: early senescence due to the premature shutdown of water and nutrient assimilation. Under these conditions, these plants will be harvested at over-maturity, when the surrounding, unstressed sunflowers plants reach their physiological maturity (CETIOM, 1996). A decrease in the oleic acid content of the grain has been observed under over-maturity (Baldini et al., 2002).

CONCLUSION

Tillage negatively impacted the oleic acid content. This could be the result of the positive effect of reduced tillage on soil water availability for the root system.

Premature ripening attributed to *Phoma macdonaldii* negatively impacted organs linked with plant nutrition and resource storage and allocation (root, stem, leaves and head). This resulted in a decrease of global yield under the disease pressure. The fatty acid variation related with the increase of oleic acid is partly explained by: (1) the indirect consequence of the disease pressure through the nutrition mechanisms, and (2) the disease pressure itself. Since information does not exist about the direct impact of *Phoma macdonaldii* on fatty acid synthesis, further research is required on this regard.

No interaction was observed between soil tillage and premature ripening in this study. However, since the disease pressure can vary under crop management and environmental conditions, and since the recent development of soil conservation practices in sunflower cropping, understanding the behavior of sunflowers under *Phoma macdonaldii* pressure and in absence of traditional tillage remains an important issue.

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