

**NUEVAS ESTRATEGIAS EN EL MANEJO SOSTENIBLE DE LA  
ROTACIÓN TRADICIONAL TRIGO DURO-GIRASOL:  
INTRODUCCIÓN DE CULTIVOS INTERCALARES Y CULTIVOS  
ALTERNATIVOS**

New strategies for sustainable management of the  
traditional durum wheat-sunflower rotation:  
Introducing cover crops and alternative crops



**TESIS DOCTORAL**

**Verónica Pedraza Jiménez**

**Directores: Dra. Dña. M<sup>a</sup> Cristina Alcántara y Dr. D. Luis López-Bellido**

**Córdoba 2018**

TITULO: *Nuevas estrategias en el manejo sostenible de la rotación tradicional trigo duro-girasol: introducción de cultivos intercalares y cultivos alternativos*

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**Tesis Doctoral**

**NUEVAS ESTRATEGIAS EN EL MANEJO SOSTENIBLE DE LA  
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New strategies for sustainable management of the traditional  
durum wheat-sunflower rotation:  
Introducing cover crops and alternative crops

Memoria de Tesis realizada para optar al grado de Doctora por la Universidad de  
Córdoba por la Ingeniera Agrónoma Verónica Pedraza Jiménez

Directores: Dra. Dña. M<sup>a</sup> Cristina Alcántara y Dr. D. Luis López-Bellido

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**TÍTULO DE LA TESIS:** *NUEVAS ESTRATEGIAS EN EL MANEJO SOSTENIBLE DE LA ROTACIÓN TRADICIONAL TRIGO DURO-GIRASOL: INTRODUCCIÓN DE CULTIVOS INTERCALARES Y CULTIVOS ALTERNATIVOS*

*New strategies for sustainable management of the traditional durum wheat-sunflower rotation: introducing cover crops and alternative crops*

**DOCTORANDO/A:** VERÓNICA PEDRAZA JIMÉNEZ

**INFORME RAZONADO DE LOS DIRECTORES DE LA TESIS**

(se hará mención a la evolución y desarrollo de la tesis, así como a trabajos y publicaciones derivados de la misma).

La doctoranda Verónica Pedraza Jiménez ha llevado a cabo satisfactoriamente y dentro del plazo establecido, el trabajo de investigación y actividades complementarias necesarias para la presentación de la tesis doctoral titulada “Nuevas estrategias en el manejo sostenible de la rotación tradicional trigo duro-girasol: introducción de cultivos intercalares y cultivos alternativos”. Durante los cinco años de desarrollo de la Tesis ha alcanzado un alto grado de cumplimiento tanto del Plan de Investigación como del de Formación superando en este último caso los requisitos mínimos necesarios.

El cumplimiento del Plan de Investigación ha requerido una duración de cinco años para alcanzar los objetivos propuestos, debido a la propia naturaleza de la tesis desarrollada dentro de la disciplina de Agronomía. Los objetivos han sido: 1.- estudiar la viabilidad de introducir cultivos intercalares en las rotaciones tradicionales y su efecto sobre los contenidos de agua en el suelo, la fertilidad del mismo, los rendimientos, la calidad y sanidad de los cultivos principales y 2.- estudiar la mezcla forrajera *Avena strigosa-Vicia narbonensis* como posible alternativa de cultivo en las rotaciones. El desarrollo de estos objetivos ha requerido ensayos de campo durante 4 años para poder alcanzar conclusiones fiables y también ensayos más dirigidos en condiciones controladas. Esto ha permitido a la doctoranda formarse en un amplio abanico de metodologías y técnicas de análisis que sin duda le serán de mucha utilidad en su futuro. Con esta tesis se hace una importante contribución en el campo de la agronomía ya que proporciona soluciones sostenibles en el manejo de las rotaciones

de cultivos herbáceos necesarias para la conservación y mejora del agro ecosistema así como dar respuesta a la normativa fijada por la PAC.

El trabajo de Verónica Pedraza Jiménez se ha visto complementado con un intenso Plan de Formación que ha incluido la realización de 3 cursos de formación, asistencia a 4 jornadas técnicas, impartición de 2 seminarios, 5 asistencias a Congresos siendo uno de ellos Internacional y 2 artículos en revistas internacionales. Además la realización de una estancia en el centro de investigación North Wyke Rothamsted Research, en Okehampton, Devon (Reino Unido), bajo la tutela del profesor Dr. D. Phil Murray y la Dra. Kate Le Cocq en el departamento “Sustainable soil and grassland systems”, ha permitido a la doctoranda ampliar sus conocimientos en relación a cultivos forrajeros mediante su participación en diferentes estudios relacionados con el incremento de la productividad en mezclas forrajeras.

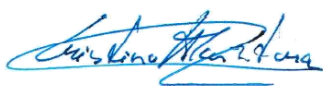
El trabajo realizado por la doctoranda Verónica Pedraza Jiménez queda reflejado en 1 publicación internacional en la revista *Spanish Journal of Agricultural Research*, situada en el tercer cuartil de la base de datos JCR y su participación en otra publicación internacional en la revista *Plant Soil* de primer cuartil. Además, ha colaborado en 3 publicaciones nacionales del Instituto de Investigación y Formación Agraria y Pesquera y realizado 7 comunicaciones a Congresos Nacionales e Internacionales, siendo el resumen de la comunicación presentada en este último publicado en la revista internacional *Procedia of Environmental Sciences*. Estas publicaciones recogen parte del trabajo realizado en la tesis doctoral así como otras colaboraciones.

Consideramos que Verónica Pedraza Jiménez ha alcanzado durante este tiempo de formación, los conocimientos y habilidades necesarias para la obtención del título de doctor, realizando además todas las tareas y trabajos planificados con un grado de cumplimiento muy satisfactorio. Por ello, como directores de la tesis autorizamos la presentación de la tesis doctoral.

Córdoba, 14 de febrero de 2018


Firma de los directores

La directora



Fdo.: Dra. D.ª Cristina Alcántara Braña

El director



Fdo.: Dr. D. Luis López Bellido

## Producción científica derivada de la Tesis Doctoral

Los indicios de calidad que respaldan la calidad científica de esta tesis son:

- **Publicaciones:**

- Pedraza V, Perea F, Saavedra M, Fuentes M, Alcántara C, 2017. *Vicia narbonensis-Avena strigosa* mixture, a viable alternative in rainfed cropping systems under Mediterranean conditions. *Spanish Journal of Agricultural Research* 15 (4): e0905 (aceptado 13/10/17). Journal Citations Report (JCR) 2016: Índice de impacto 0.687; posición 32 y 3º cuartil en el área temática *Agriculture/Multidisciplinary*. <https://doi.org/10.5424/sjar/2017154-10882>
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- **Comunicaciones a congresos nacionales e internacionales:**

- Pedraza V, González-Verdejo CI, Perea F, Saavedra M, Alcántara C, 2015. Reducción de la germinación de jopo del girasol por efecto de restos de *Sinapis alba* en condiciones controladas. En: XV Congreso de Malherbología. Sociedad Española de Malherbología (SEMh), Sevilla (España), 19-23 octubre. pp. 35-41.
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- Alcántara C, Thornton CR, Pérez-de-Luque A, Cocq KL, Pedraza V, Murray PJ, 2015. The free-living rhizosphere fungus *Trichoderma hamatum* GD12 enhances clover productivity in clover-ryegrass mixtures. Plant Soil 398, 165–180. <https://doi.org/10.1007/s11104-015-2646-7>
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La doctoranda



Fdo. Verónica Pedraza Jiménez

Esta Tesis Doctoral ha sido realizada en el Departamento de Ingeniería y Tecnología Agroalimentaria del Instituto de Investigación y Formación Agraria y Pesquera (IFAPA). Los trabajos incluidos en esta tesis han sido financiados por el Fondo Social Europeo (FSE) dentro del Programa Operativo de Andalucía 2007-2013 “Andalucía se mueve con Europa” y el Instituto Nacional de Investigación y Tecnología Agraria (INIA), a través del proyecto RTA2011-00031-00-00 y el subprograma FPI-INIA.



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*Si se siembra la semilla con fe  
y se cuida con perseverancia,  
solo será cuestión de tiempo  
recoger sus frutos*

*-Thomas Carlyle-*



## RESUMEN

La rotación trigo-girasol ha constituido la alternativa tradicional prioritaria en las explotaciones agrarias de cultivos herbáceos de secano del sur de España. Los cada vez más exigentes requerimientos medioambientales de la Política Agraria Común y los nuevos retos de la agricultura del siglo XXI, son aspectos prioritarios que marcan la toma de decisiones de los agricultores respecto al manejo de las explotaciones. Actualmente, la adaptación a estos nuevos desafíos implica llevar a cabo prácticas agronómicas sostenibles así como la diversificación de cultivos en las rotaciones, sin embargo hasta el momento, no ha habido alternativas claras a la rotación tradicional trigo-girasol que superen su rentabilidad.

El objetivo principal de esta tesis ha sido proponer alternativas sostenibles a la rotación trigo duro-girasol en los secanos andaluces, mediante la introducción de los cultivos intercalares mostaza blanca (*Sinapis alba* subsp. *mairei*) y alberjón (*Vicia narbonensis* L.) y nuevos cultivos como la mezcla forrajera alberjón-avena negra (*Vicia narbonensis-Avena strigosa*). Para abordar su estudio se han realizado diferentes ensayos de campo y en condiciones controladas entre los años 2011 y 2016 en varias localidades de la provincia de Córdoba (Córdoba y Santa Cruz) y Sevilla (Carmona).

Este trabajo ha puesto de manifiesto que los cultivos intercalares introducen mejoras respecto a la conservación de suelo, el control de malas hierbas, la incidencia del jopo de girasol (*Orobanche cumana*), la calidad del trigo duro y tiene un efecto positivo en el contenido de humedad y fertilidad del suelo tras su incorporación en la capa más superficial en comparación con sistemas de mínimo laboreo y siembra directa. No obstante, estos cultivos también presentan limitaciones, y precisan de un manejo adecuado que hacen necesarios estudios a más largo plazo para maximizar los beneficios de esta práctica agrícola dentro los sistemas de cultivo de secano, especialmente del girasol. Por su parte, el estudio de la nueva mezcla forrajera ha mostrado su viabilidad como cultivo mixto por el adecuado desarrollo de ambas especies en la mezcla y la obtención de rendimientos superiores a la mezcla estándar veza común-avena común, así como por presentar un similar contenido en proteínas y otros parámetros de calidad. Además, la contribución de nitrógeno realizada al sistema permite considerar este cultivo como una futura alternativa en las rotaciones, obteniéndose los mejores resultados a las dosis de siembra de 49 kg/ha de alberjón y 45.5 kg/ha de avena negra en años secos, y a las dosis de 91 kg/ha combinada con 24.5 kg/ha y 70 kg/ha combinada con 35 kg/ha en años con mayor precipitación acumulada en suelos francos y arcillosos, respectivamente.

Nuestro estudio abre una nueva línea de investigación prometedora para el futuro de los cultivos herbáceos, y pone de manifiesto que tanto los cultivos intercalares como la mezcla forrajera son alternativas económica y medioambientalmente viables en la agricultura de secano de clima mediterráneo.



## SUMMARY

Durum wheat-sunflower rotation is a common practice in rainfed arable areas in southern Spain. However, the increasingly demanding environmental requirements of the Common Agricultural Policy and the new challenges for food and agriculture in the 21st century are important aspects influencing farmers' decision making each growing season. Nowadays, adapting the traditional rotation and the soil management practices to face these goals involves a sustainable management as well as a crop diversification. However, the lack of profitable alternatives makes future adoption difficult for Spanish arable crop farmers.

The present thesis aims to assess the introduction of new crops and cover crops as sustainable alternatives to soil management system in traditional rainfed rotations, by inserting white mustard (*Sinapis alba* subsp. *mairei*) and narbon bean (*Vicia narbonensis* L.) cover crops and the new forage mixture narbon bean-black oat (*Vicia narbonensis-Avena strigosa*). To that end, a range of experiments has been carried out under field and controlled conditions at different locations of the province of Córdoba (Córdoba y Santa Cruz) and Sevilla (Carmona) between 2011-2016.

The short-term results suggested that the tested cover crops may introduce improvements in soil protection, weed and sunflower broomrape control, increased durum wheat quality and higher soil moisture content and fertility after cover crops incorporation into the uppermost layer than other soil management systems such as reduced tillage or no tillage. However, these crops also have limitations and require a proper management that makes necessary further research to analyze the long-term effects and maximize the benefits of this practice in rainfed arable systems, especially in sunflower growth and yields. Results for the forage mixture showed that both species can form a balanced forage mixture for livestock diets, displaying higher dry matter yields and crude protein yield than common vetch-common oat traditional mixture, as well as similar crude protein content and other quality traits. Furthermore, the nitrogen contribution made to the system allows this mixture to be considered as a future alternative in crop rotations, obtaining the best results at a seeding rate of 49 kg/ha for narbon bean and 45.5 for black oat in dry years and at the seeding rates 91 and 24.5 and 70 and 35 kg/ha in rainy years with loamy and clay soils, respectively.

Our results open a path of study for the future of arable crops and show that both cover crops and forage mixtures could be economically and environmentally promising crop alternatives in the traditional rotation under the Mediterranean rainfed conditions of southern Spain.





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## **GLOSARIO DE TÉRMINOS EN ESPAÑOL**

AC: Agricultura de Conservación

C/N: Relación carbono/nitrógeno

CIC: Capacidad de Intercambio Catiónico

CO<sub>2</sub>: Dióxido de carbono

EE.MM.: Estados Miembros

ESYRCE: Encuesta sobre Superficies y Rendimientos de los Cultivos

FAO: Organización de las Naciones Unidas para la Alimentación y la Agricultura

FAPRI: Instituto para la Investigación de la Política Alimentaria y Agraria

K: Potasio

MO: Materia Orgánica

N: Nitrógeno

N<sub>2</sub>: Nitrógeno atmosférico

N<sub>2</sub>O: Óxido nitroso

<sup>15</sup>N: Isótopo estable de N

O<sub>2</sub>: Oxígeno

OCDE: Organización para la Cooperación y el Desarrollo Económico

P: Fósforo

PAC: Política Agraria Común

PIB: Producto Interior Bruto

RAEA: Red Andaluza de Experimentación Agraria

SIE: Superficies de Interés Ecológico

UE: Unión Europea



## **GLOSSARY OF ENGLISH TERMS**

ADF: Acid-Detergent Fibre (Fibra Ácido Detergente)

BS: Bare Soil (Suelo desnudo)

C/N: Carbon to nitrogen ratio (Relación carbono/nitrógeno)

CA: Conservation Agriculture (Agricultura de conservación)

CAP: Common Agricultural Policy (Política Agraria Común)

CC: Cover Crop (Cultivo intercalar o cultivo cubierta)

CCC: Crucifer Cover Crop (Cultivo intercalar crucífera)

CFM: CC above-ground Fresh bioMass (Biomasa fresca de cultivos cubierta)

CP: Crude Protein (Proteína Cruda)

CR: Competition Ratio (Ratio de competición)

CO<sub>2</sub>: Carbon dioxide (Dióxido de carbono)

DAM: Days After Mowing (Días después de la siega)

DAS: Days After Sowing (Días después de la siembra)

DM: Dry Matter (Materia seca)

DDM: Digestible Dry Matter (Digestibilidad)

DW: Durum Wheat (Trigo duro)

GDD: Growing Degree Days (Tiempo térmico)

H: Height (Altura)

HD: Sunflower Head Diameter (Diámetro de capítulo de girasol)

HLW: Specific weight or HectoLitre Weight (Peso específico del grano de trigo)

K: Potassium (Potasio)

LCC: Legume Cover Crop (Cultivo intercalar leguminosa)

N: Nitrogen (Nitrógeno)

N<sub>2</sub>: Atmospheric N (Nitrógeno atmosférico)

N<sub>2</sub>O: Nitrous oxide (Óxido nitroso)

N<sub>dfa</sub>: Nitrogen derived from symbiotic N<sub>2</sub> fixation directly from the atmosphere (Proporción del N atmosférico total derivado de la fijación)

$N_{fixed}$ : Legume N yield derived from  $N_2$  fixation (Cantidad de N cosechado que deriva de la fijación)

$N_{nonsym}$ : Portion of N yield that comes from non-symbiotic sources (Cantidad de N procedente de fuentes no simbióticas)

$N_{sym}$ : Portion of N yield that comes from the symbiotic sources (Cantidad de N fijado simbióticamente)

$N_{transf}$ : Grass N derived by apparent transfer from the legume (Proporción de N en una gramínea derivado de la transferencia aparente desde una leguminosa)

$N_{yield}$ : Amount of N harvested (Cantidad total de N cosechado)

$^{14}N$ : Stable isotope of N (Isótopo estable del N)

$^{15}N$ : Stable isotope of N (Isótopo estable del N)

$\delta^{15}N$ : Stable isotope concentration (Concentración de isótopos estables)

NDF: Neutral-Detergent Fibre (Fibra Neutro Detergente)

NIRS: Near Infra Red Spectroscopy technology (Tecnología de infrarrojos)

NT: No Tillage (Siembra directa o no laboreo)

OC: Sunflower seed Oil Content (Contenido en aceite de la semilla de girasol)

OM: Organic Matter (Materia orgánica)

P: Phosphorus (Fósforo)

PD: Plant Density (Densidad de plantas)

PC: Durum wheat Protein Content (Contenido en proteína del trigo)

QC: Durum wheat Quality group Category (Categorización en grupos de la calidad del trigo duro)

RT: Reduced Tillage (Laboreo mínimo)

SC: Sunflower Cultivar (Cultivar de girasol)

SP: Sunflower Planting date (Fecha de siembra de girasol)

SF: SunFlower (Girasol)

SN: Durum wheat Spike Number (Número de espigas de trigo duro)

V: Durum wheat Vitosity (Vitrosidad del grano de trigo)

WFM: Weed above-ground Fresh BioMass (Biomasa fresca de malas hierbas)



**CAPÍTULO 1**  
**INTRODUCCIÓN GENERAL**



# 1 INTRODUCCIÓN GENERAL

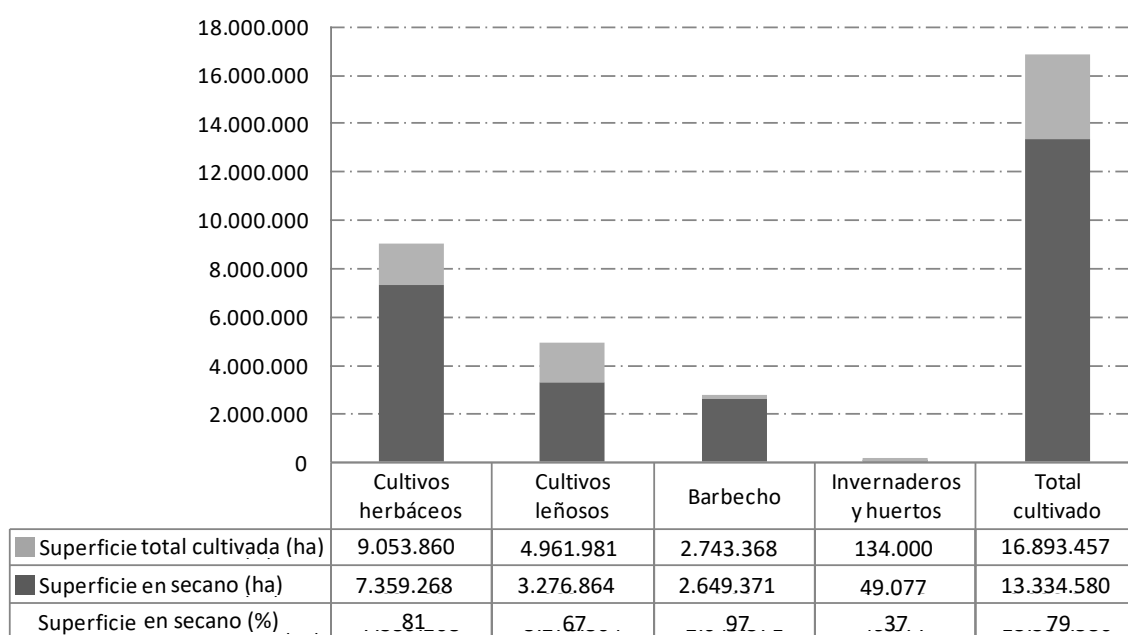
Los cultivos herbáceos extensivos son uno de los principales exponentes de la agricultura de secano mediterránea. En Andalucía, ocupan un parte importante de la superficie agrícola, especialmente las rotaciones trigo duro-girasol. Sin embargo, las particulares condiciones edafoclimáticas en las que se desarrollan han condicionado desde siempre su manejo y rentabilidad. Actualmente, además, existe la necesidad de adaptar estos cultivos a los nuevos retos de la agricultura del siglo XXI y las nuevas exigencias medioambientales. Por ello, esta tesis propone nuevas técnicas de manejo y alternativas de cultivo que permitan esa adaptación. A continuación, se recoge un análisis detallado de los problemas y condicionantes agronómicos, económicos y sociológicos que nos han llevado a la situación actual y a la necesidad de buscar soluciones que permitan la sostenibilidad a largo plazo de estos cultivos.

## 1.1 CONTEXTO ACTUAL DE LOS CULTIVOS HERBÁCEOS EXTENSIVOS

La cuenca Mediterránea es una de las regiones biológicamente más ricas y complejas de la Tierra. Se extiende a lo largo de 3,8 millones de km<sup>2</sup>, y cubre el área que rodea al mar Mediterráneo, incluyendo 24 países de 3 continentes diferentes: sur de Europa, norte de África y la zona más occidental de Asia conocida como “Oriente Próximo” (Sundseth, 2009). Toda la zona goza del que se denomina “clima mediterráneo”, presente también en otras zonas del planeta como Sudáfrica, centro de Chile, gran parte de California o el suroeste de Australia. Sin embargo, debe su nombre a esta región objeto de estudio por constituir más del 50 % del total de las regiones de clima mediterráneo a nivel mundial (Bolle, 2003). Los inviernos son húmedos y templados, y los veranos secos y calurosos, siendo su principal característica la presencia de un periodo de uno o varios meses de sequía seguido de otro de lluvias torrenciales. Por tanto, temperatura y precipitaciones pueden variar mucho en toda la región a lo largo del año (amplitud térmica de > 15°C y lluvias comprendidas entre 100-4.000 mm).

Estas características climáticas, unidas a otro tipo de condicionamientos geográficos, demográficos y culturales, dan lugar a una gran biodiversidad, que unida a la intervención humana se traducen en una gran variedad de actividades rurales, tanto

agrícolas como ganaderas. La agricultura mediterránea es una de ellas, desarrollándose especialmente en 5 países de la cuenca Mediterránea pertenecientes a la Unión Europea (UE): España, Portugal, Francia, Italia y Grecia. Sin embargo en Europa, como ocurre en el resto de las economías avanzadas, la importancia del sector primario es relativamente reducida en comparación con el sector secundario (industria) y terciario (servicios) (CES, 2005). En 2015, el Producto Interior Bruto (PIB) del sector agrario europeo equivalía al 1,6 % del PIB del conjunto de los 28 estados miembros (EE.MM.) de la UE (Eurostat, 2015). En el caso de España, este porcentaje se elevó al 2,3 % del PIB, por encima del promedio comunitario, dando trabajo a cerca de 1 millón de personas (aproximadamente el 4,1 % de la población activa). Además, las 965.000 explotaciones agrarias con las que cuenta y que se extienden a lo largo de casi 17 millones de hectáreas cultivadas (en adelante mill. de ha), hacen que España sea el segundo Estado comunitario en cuanto a extensión agrícola, por detrás de Francia (INE, 2015). Su principal ocupación son los cultivos herbáceos, seguido de cultivos leñosos y las tierras de barbecho (Figura 1).



**Figura 1. Principales cultivos de la agricultura española por superficie total ocupada (ha) y en condiciones de secano (ha y % sobre el total de la superficie cultivada).**

Fuente: Elaboración propia a partir de los datos extraídos de la Encuesta sobre Superficies y Rendimientos de Cultivos del MAPAMA (2015a)

Las condiciones climáticas ya comentadas, unidas a la topografía típica del país, hacen que la agricultura de secano sea predominante a nivel nacional (Figura 1). De hecho, el secano herbáceo representa el 81 % de la superficie total destinada a cultivos herbáceos en España, encontrándose confinada actualmente una gran parte en Andalucía.

### 1.1.1 EL SECANO HERBÁCEO EN ANDALUCÍA

Andalucía se encuentra situada en el sur de España y ocupa el 17 % de la superficie española (87.597 km<sup>2</sup>), convirtiéndose así en la segunda comunidad autónoma en cuanto a extensión y la primera en población, con 8,4 millones de habitantes (96 habitantes por km<sup>2</sup>) (IECA, 2015). Su territorio se considera mayoritariamente rural (90 %). De hecho, más de la mitad de la población (54 %) vive y reside en municipios rurales, por lo que la principal fuente de empleo en estas zonas es la actividad agraria, que en términos económicos representa el 4,8 % del PIB total andaluz y el 28 % del PIB agrario a nivel nacional (INE, 2015).

Con un régimen de precipitación media anual inferior a 500 mm, la superficie andaluza cultivada en 2015 ascendía a 3,6 mill. de ha, de las cuales 2,5 mill. de ha (el 70 %) se desarrollaron en condiciones de secano. Los cultivos más representativos en estas condiciones son los cultivos herbáceos y el olivar, seguido de barbechos, frutales y viñedos (Tabla 1).

**Tabla 1. Principales cultivos de secano en Andalucía por superficie total ocupada (ha) y porcentaje sobre el total (%).**

Cultivo	Superficie cultivada en secano	
	ha	%
<b>Cultivos herbáceos</b>	<b>1.042.348</b>	<b>41,9</b>
Cereales grano	632.995	25,5
Industriales	298.779	12
Leguminosas grano	53.133	2,1
Forrajeras	52.736	2,1
Hortalizas y flores	4.622	0,18
Tubérculos	82	0,02
<b>Cultivos leñosos</b>	<b>1.192.735</b>	<b>47,9</b>
Olivar	980.667	39,5
Frutales	186.053	7,5
Viñedos	22.663	0,9
<b>Barbecho</b>	<b>247.861</b>	<b>9,9</b>
<b>Invernaderos y huertos</b>	<b>2.654</b>	<b>0,1</b>
<i>Total</i>	<i>2.485.598</i>	<i>100</i>

Fuente: Elaboración propia a partir de MAPAMA (2015a)



Además, la importancia de los cultivos herbáceos extensivos de secano en Andalucía es palpable tanto por superficie cultivada (Tabla 1) como por número de explotaciones agrarias dedicadas a dicha actividad. De las 178.640 explotaciones agrícolas registradas, un total de 49.420 se encuentran en condiciones de secano (INE, 2013), y se concentran mayormente en Andalucía Occidental (77 %), concretamente en la depresión del Guadalquivir (Ocaña, 1991). Asimismo, y a pesar de que el tamaño medio de explotación es de 25 ha, el 84 % de la superficie andaluza dedicada a cultivos herbáceos cuenta con un tamaño medio de 75 ha (INE, 2013), lo cual muestra el papel fundamental que siguen teniendo las explotaciones extensivas, especialmente en las campiñas de las provincias de Cádiz, Córdoba y Sevilla.

Gran parte de sus tierras de labor se dedican al cultivo de los cereales trigo duro (287.431 ha) y trigo harinero (118.928 ha), y a los cultivos industriales, donde el girasol ocupa casi el total de la superficie cultivada con 281.552 ha. Le siguen en importancia extensas superficies dejadas en barbecho, y por último, superficies más pequeñas cultivadas con leguminosas grano (especialmente habas, garbanzos y guisantes) y cultivos forrajeros, donde los cereales de invierno (alfalfa, sorgo, trébol, etc.) y la mezcla veza-avena constituyen la principal fuente de producción de forraje para alimentación animal, especialmente cuando es desecado y henificado.

De este modo, se constata la importancia del cultivo del trigo y girasol en Andalucía. De hecho, la rotación trigo-girasol ha sido la alternativa prioritaria en las explotaciones agrarias de campiña durante décadas (RAEA, 2007). El paso de los años ha permitido su adaptación a nuevas técnicas y sistemas de manejo. Sin embargo, las particulares características del clima mediterráneo en la región hacen necesario un desarrollo continuo de técnicas más sostenibles y rentables. Por ello, la rotación trigo-girasol constituye el objetivo central de esta investigación, en un intento de mejorar la situación actual y de modernizarla de cara al futuro.

### **1.1.2 LA ROTACIÓN TRIGO-GIRASOL**

La posibilidad de realizar rotaciones está condicionada principalmente por las precipitaciones y las temperaturas (Sombrero *et al.*, 2013). Estos factores son aún más determinantes en Andalucía, al constituir la disponibilidad de agua y su distribución el principal factor limitante de la producción (López-Bellido *et al.*, 2003). Desde la

antigüedad, los sistemas de producción usados por los agricultores en zonas de secano han sido cultivos anuales, tradicionalmente cereales, siendo la alternativa más extendida el monocultivo de cereal o el cultivo del cereal detrás de barbecho blanco (sin cultivo).

Durante los años cincuenta y sesenta la Revolución Verde permitió mantener la previsión optimista de un progresivo incremento de la producción agraria, frente a un preocupante aumento de la población humana. En consecuencia, se promovió una intensificación de las prácticas agrícolas, de elevados insumos y sin apenas consideraciones a los factores ambientales (Ibáñez, 1997). Sin embargo, a finales de los sesenta (McIntosh, 1986) empezaron a aparecer los problemas derivados de dicha intensificación de la agricultura. Comenzó a observarse una degradación de las tierras de cultivo y el agotamiento del suelo: procesos intensos de erosión, pérdida de elementos fertilizantes solubles, disminución alarmante del contenido de materia orgánica, salinización e implicaciones en procesos contaminantes. Y todo ello propició que los agricultores introdujeran rotaciones de cultivos.

Desde el comienzo de su práctica en la zona, la agricultura herbácea extensiva ha realizado varios tipos de rotaciones. En áreas donde existía una agricultura con ganadería asociada, la rotación se mejoraba introduciendo leguminosas para forraje, como habas, o para pienso, como el guisante. Sin embargo, a principios de los setenta, la superficie dedicada a barbecho blanco y a leguminosas grano se fue reduciendo y sustituyendo por girasol.

La adopción de este cultivo se vio favorecida por varios motivos: sus condiciones favorables de desarrollo y adaptabilidad a la escasez de agua, bajos costes y facilidad de manejo (Connor *et al.*, 2011) y el surgimiento de una política proteccionista con contratos a precios establecidos, que garantizaban al agricultor la venta de la cosecha al igual que el cereal y a diferencia de las leguminosas grano (López-Bellido y López Bellido, 2000). Conjuntamente, la demanda del producto final por parte de la industria extractora y el alto consumo nacional de su aceite, consolidaron poco a poco su importancia dentro de la agricultura española. De este modo, su cultivo se fue intensificando con mayor y más racional uso de abonos, herbicidas y productos fitosanitarios, y su manejo agronómico se fue mejorando con la

aparición de nuevas variedades, maquinaria y técnicas más adaptadas (Gómez-Arnau, 1988; López-Bellido y López Bellido, 2000).

Además, uno de los aspectos más destacables del cultivo del girasol es su impacto positivo en la rotación con el trigo, debido a la excelente complementariedad de ambos cultivos (López-Bellido *et al.*, 1997). Su gran desarrollo radicular le permite extraer agua y nutrientes de capas profundas no explotadas por los cereales. Al mismo tiempo, parte importante del nitrógeno (N) extraído por el cultivo en los perfiles más profundos es restituido por sus residuos en la capa superficial del suelo. Esto favorece la mineralización y el uso eficiente para el posterior trigo en rotación (López-Bellido *et al.*, 2003), evitando también su pérdida por lavado a horizontes no explorados (Osca Lluch y Gómez de Barreda, 2003). Por otro lado, la rotación trigo-girasol facilita la rotura del ciclo de enfermedades y de numerosas especies de malas hierbas, por los diferentes períodos de crecimiento de ambas especies (Lacasta Dutoit y Meco, 2005). Y por último, la cobertura del suelo propiciada por el girasol, cuya estructura aérea lo protege de la acción directa de la lluvia, evita la erosión y en consecuencia, el transporte de sólidos y la pérdida de agua por escorrentía (Ordóñez-Fernández *et al.*, 2007b).

Estas ventajas económicas y ambientales hicieron que la rotación trigo-girasol se convirtiera en el sistema preferido por los agricultores, asegurando rendimientos en condiciones desfavorables para cualquier otro cultivo. Sin embargo, el correcto diseño de la rotación en relación a las especies seleccionadas y adaptadas a nuestra región es tan importante como el sistema de manejo elegido. Tal y como apuntó Connor *et al.* (2011), cada sistema agrícola presenta diferentes tipos de problemas, y por tanto, diferentes soluciones de laboreo. Por ello, en nuestras condiciones, resulta fundamental el cumplimiento de tres aspectos decisivos en el manejo de los cultivos:

1. Todas las intervenciones deben favorecer la infiltración del agua de lluvia de otoño a invierno y reducir la evaporación.
2. Se debe mantener la estructura óptima que permita aprovechar las ventajas del profundo enraizamiento del girasol, evitando la compactación a cualquier profundidad.

3. Es necesario asegurar una buena nascencia de ambos cultivos que sea uniforme en el espacio y el tiempo.

Aunque la emergencia del girasol y el trigo coincide con las épocas húmedas, situaciones de baja uniformidad y efectividad de la lluvia caída así como escasa humedad de los suelos son frecuentes en la zona de campiña, dando lugar a importantes pérdidas por evapotranspiración que imposibilitan la disponibilidad de agua durante posteriores fases de crecimiento en ambos cultivos (Martínez Raya y Francia Martínez, 2006). En consecuencia, los rendimientos y viabilidad económica del sistema se ven completamente influenciados por las condiciones meteorológicas de cada año agrícola (Chapman *et al.*, 1993). Por ello, el intento de mantener las condiciones mínimas necesarias para este sistema de cultivo, ha propiciado una evolución en el manejo y técnicas de cultivo empleadas, dando lugar a una evolución de los métodos de laboreo empleados y momentos de aplicación así como al aumento del empleo de prácticas culturales.

## **1.2 EVOLUCIÓN DE LAS TÉCNICAS DE MANEJO DE SUELO EN LA ROTACIÓN TRIGO-GIRASOL**

Tradicionalmente, el laboreo se ha considerado imprescindible para la implantación y desarrollo de los cultivos herbáceos. En los inicios de la agricultura, el laboreo fue empleado fundamentalmente como un medio para controlar malas hierbas, así como para promover el crecimiento de los cultivos. Su práctica fue adquiriendo una mayor relevancia con la utilización de aperos arrastrados por animales y, con la aparición del tractor, llegó a constituir una de las intervenciones agrícolas más importantes (González, 2003). En cultivos herbáceos de secano, los agricultores han utilizado un laboreo tradicional intensivo durante décadas, consistente en una labor de arado de vertedera tras la quema de los restos del cultivo principal y seguido de varias labores de gradas de discos o cultivador. Estas prácticas alteran el perfil del suelo a profundidades iguales o superiores a 20 cm, favoreciendo todas aquellas propiedades relacionadas con la macroporosidad a corto plazo (permeabilidad, penetrabilidad y aireación). Sin embargo, disminuye la materia orgánica, humedad del suelo y su estabilidad estructural, los elementos asimilables y la actividad biológica a lo largo del tiempo (González, 2003; Lacasta Dutoit, 2005). Este

hecho, unido al fenómeno de la erosión, especialmente grave en los suelos agrícolas andaluces, hizo que los agricultores paulatinamente fueran sustituyendo el uso de la vertedera y aperos de discos por el subsolador o chisel y aperos de dientes. También comenzó el empleo de herbicidas de acción total de presembrado, quedando restringido su uso exclusivamente ante graves problemas de infestaciones de malas hierbas resistentes a herbicidas (Lacasta Dutoit, 2005).

A pesar de las mejoras propuestas por las técnicas minimizadoras del laboreo, sin la disponibilidad de herbicidas adecuados, las malas hierbas se convierten en un factor limitante para el desarrollo de dichos sistemas de laboreo (Fernández-Quintanilla, 1997). Sin embargo, con la aparición de los primeros herbicidas a finales de los cincuenta, ya no se necesita labrar para controlar las malas hierbas, ya que su acción total las elimina sin riesgo para el cultivo posterior, siendo así factible disminuir las labores. De esta forma surge la agricultura de conservación (AC), tanto a nivel nacional como internacional (Fernández-Quintanilla, 1997). Su concepto engloba los sistemas de producción agrícola que llevan a cabo prácticas agronómicas adaptadas a las exigencias de los cultivos y a las condiciones locales de cada región. Para ello, utilizan técnicas de cultivo sostenibles y un manejo de suelo que lo protege de la erosión y degradación, mejora su calidad y biodiversidad, y contribuye a la preservación de los recursos naturales agua y aire, sin menoscabo de los niveles de producción de las explotaciones (AEAC.SV, 2016). Aplicadas a los cultivos herbáceos, los primeros ensayos en España y Andalucía comenzaron en los años ochenta, y las técnicas de AC que se utilizan entre otras son el laboreo mínimo y la siembra directa.

El laboreo mínimo, consiste en una preparación del lecho de siembra sin labor profunda, con una o dos labores superficiales de tipo vertical para romper la costra exterior (sin profundizar más de 20 cm) y dejando además como mínimo el 30 % de los residuos del cultivo anterior sobre el suelo. Las prácticas habituales de los agricultores con cultivos herbáceos de secano consisten en un pase de chisel o de grada de discos para romper y enterrar los restos vegetales anteriores y dos pases de vibrocultivador para evitar problemas de siembra por abundancia de restos en el suelo (González, 2003). En ocasiones, estos últimos se reducen utilizando herbicidas de post-emergencia, ya que uno de los principales problemas del laboreo mínimo es el control de las malas hierbas y los rebrotes, siendo necesaria la aplicación de herbicidas. En la

técnica de manejo de siembra directa o no laboreo, se siembra directamente sobre los restos del cultivo del año anterior, picados y esparcidos con una cosechadora adaptada, sin realizar ninguna labor previa. Desde ese momento, la única alteración que se produce en el suelo es la ocasionada por una sembradora específica, pues no se realiza ninguna labor o intervención en el suelo desde la recolección del cultivo hasta la siembra del siguiente, y el control de las malas hierbas se realiza mediante el uso de herbicidas de bajo impacto ambiental (López-Bellido *et al.*, 1997).

En la actualidad, como veremos en detalle más adelante, las limitaciones en el manejo de suelo contempladas en las últimas normativas medioambientales de la Política Agraria Común, entre otros factores, conducen a un progresivo cambio de tendencia hacia el uso de técnicas de manejo conservacionistas, teniendo el mínimo laboreo y la siembra directa cada vez más importancia frente al laboreo tradicional. Conocidas las particulares condiciones de suelo y clima donde se desarrollan los cultivos herbáceos de secano, es necesario profundizar en los posibles beneficios que las técnicas de agricultura de conservación podrían reportar respecto a los sistemas de manejo tradicional en Andalucía.

### **1.3 ASPECTOS MEDIOAMBIENTALES Y ECONÓMICOS DE LAS DISTINTAS TÉCNICAS DE AGRICULTURA DE CONSERVACIÓN EN CULTIVOS HERBÁCEOS**

El interés en la adopción de técnicas de AC en cultivos herbáceos de secano se debe principalmente al gran número de ventajas medioambientales y beneficios reconocidos derivados de su uso. A continuación se detallan los principales efectos sobre las propiedades físicas y biológicas del suelo.

**1. Sobre las propiedades físicas del suelo.** El manejo del suelo influye directamente en sus propiedades físicas, y con ello, en los principales procesos implicados en la estructura, calidad y capacidad de retención del suelo:

- *Reduce la erosión y la compactación.* La erosión es el principal problema medioambiental de la agricultura tradicional (Louwagie *et al.*, 2011). En la cuenca Mediterránea, una superficie de aproximadamente 1,3 millones de km<sup>2</sup> se encuentra seriamente degradada (Cosentino *et al.*, 2015). Más concretamente, en la Depresión

del Guadalquivir, donde se concentra la zona de cultivos herbáceos de Andalucía, el riesgo de erosión del suelo es de los más altos de España y Europa (Figura 2). Esto se debe a la predominancia de los suelos Vertisoles, cuyo alto contenido en arcillas hace que sean extremadamente duros en seco, y demasiado plásticos en húmedo, presentando especiales problemas para el laboreo y por la compactación (Probert *et al.*, 1987; Coulombe *et al.*, 1996).



**Figura 2. Riesgo de erosión de suelo (en t/ha/año) en los países de la Unión Europea.**

Fuente: PESERA (2015)

Además, la erosión reduce la fertilidad y la capacidad de almacenamiento de agua del suelo y por ello, disminuye la capacidad productiva, siendo necesario aumentar cada año los costes de producción para mantener el mismo nivel productivo. Son muchos los estudios realizados a largo plazo que demuestran la relación entre los sistemas de laboreo más intensivos y profundos y una mayor degradación y erosión de suelo (Kosmas *et al.*, 1997; Cerdan *et al.*, 2010; Cosentino *et al.*, 2015). La AC, al utilizar técnicas conservacionistas (no quema de rastrojos, mínimo o no laboreo y uso de restos de cosecha sobre el suelo), contribuye eficazmente a reducir la erosión, aumentando el volumen y densidad de suelo y consiguiendo una menor compactación (Al-Darby y Lowery, 1986).

- *Aumenta el contenido en materia orgánica*, consiguiendo una mayor estabilidad estructural del suelo. La mayoría de los suelos agrícolas, a lo largo de años de laboreo intensivo, pierden carbono. Esta disminución de materia orgánica deteriora su estructura, estabilidad de agregados, actividad biológica y capacidad de retención

de agua y nutrientes. En consecuencia, a medio y largo plazo se hacen más vulnerables a la erosión, compactación, acidificación, salinización, carencia de nutrientes y sequía. En la agricultura Mediterránea, este hecho se produce de forma muy acusada como consecuencia del bajo contenido en carbono de las áreas dedicadas a cultivos de secano (López-Bellido *et al.*, 1997). Sin embargo, con las técnicas de agricultura de conservación, el contenido en materia orgánica del suelo aumenta a largo plazo. De hecho, existen numerosos estudios que ponen de manifiesto la mayor concentración de carbono orgánico adoptando sistemas de laboreo mínimo o siembra directa que mediante laboreo tradicional en los primeros 10 cm de suelo bajo condiciones de clima mediterráneo (Mazzoncini *et al.*, 2011; Sapkota *et al.*, 2012). La incorporación de dichos residuos en la capa superficial del suelo crea una protección contra la atmósfera y permite una mayor descomposición en condiciones aerobias. Además, la profundización de los residuos a través de las grandes grietas formadas en los suelos Vertisoles durante el verano, permite una mayor estabilización del carbono orgánico en todos los perfiles del suelo (López-Bellido *et al.*, 2010a).

Junto al aumento del contenido en materia orgánica, son conocidos también los cambios a largo plazo en la fertilidad del suelo. Así, un aumento en el contenido de otros nutrientes como N, fósforo (P) o potasio (K) se consigue en la capa más superficial del suelo al utilizar técnicas de agricultura de conservación. Esto es debido a la modificación de la estructura del suelo, que produce cambios en el crecimiento y desarrollo del sistema radicular de los cultivos y aumenta su capacidad de absorción (Cannell y Hawes, 1994; Bravo *et al.*, 2007; Ordóñez-Fernández *et al.*, 2007b; Moussa-Machraoui *et al.*, 2010).

- *Mejora la infiltración del agua y en consecuencia aumenta la humedad del suelo.* El contenido hídrico del perfil del suelo sufre un incremento con las técnicas conservacionistas, acentuado en el laboreo mínimo frente a la siembra directa. Este fenómeno se observa sobre todo en años de baja pluviometría, en el perfil más superficial del suelo, ya que en un clima seco como el de la zona mediterránea, la presencia de un mayor nivel de residuos disminuye la evaporación del agua (Van den Putte *et al.*, 2010). Además, el mínimo laboreo mejora la velocidad de infiltración del agua en el suelo, al romper la costra superficial con la labor realizada, especialmente en los suelos Vertisoles (Potter *et al.*, 1995).



- *Mejora la calidad del agua.* Los restos vegetales que caracterizan a la agricultura de conservación retienen en gran medida los fertilizantes y fitosanitarios en la zona agrícola en la que fueron aplicados. Además, al reducirse la erosión del suelo, se disminuye también el número de sedimentos transportados por el agua de escorrentía hasta las aguas superficiales. En consecuencia, las técnicas de AC también disminuyen este tipo de contaminación del agua y acuíferos con respecto al laboreo tradicional. De hecho, pueden llegar a intervalos de reducción de escorrentía comprendidos entre el 15 y el 89 % según diversos estudios realizados en Europa (Holland, 2004).

- *Reduce las emisiones de dióxido de carbono (CO<sub>2</sub>),* al contribuir significativamente al secuestro de carbono. A escala mundial, el sector agrícola genera cerca de una quinta parte de las emisiones de gases de efecto invernadero del mundo (FAO, 2016a). Concretamente, las prácticas tradicionales han sido una de las causas principales de las emisiones de CO<sub>2</sub> en las áreas cerealistas, ya que en la ruptura del suelo producida por el laboreo se facilita el intercambio de CO<sub>2</sub> y oxígeno (O<sub>2</sub>) entre el suelo y la atmósfera. Las técnicas de AC, al disminuir las labores del suelo y conservar e incorporar residuos del cultivo, absorben y almacenan más carbono, por lo que sintetizan más materia orgánica a largo plazo, aumentando la productividad y disminuyendo el CO<sub>2</sub> liberado a la atmósfera (Carbonell-Bojollo *et al.*, 2015). Estimaciones de Smith *et al.* (1998) indican que la conversión de los sistemas de laboreo a prácticas de manejo mejoradas como el laboreo mínimo y la siembra directa podría permitir una reducción de las emisiones de CO<sub>2</sub> procedentes de la agricultura europea en el futuro, suponiendo así un importante freno contra el calentamiento global y mejorando la salud del suelo, la productividad de los cultivos y la capacidad de adaptación al cambio climático (López Bellido, 2017a).

- *Reduce las emisiones de óxido nitroso (N<sub>2</sub>O).* La agricultura libera a la atmósfera grandes cantidades de CO<sub>2</sub>, metano y N<sub>2</sub>O. El N<sub>2</sub>O es una de las más importantes vías de emisión de gases procedentes de los suelos agrícolas. Los factores más importantes que afectan a las emisiones de N<sub>2</sub>O de los suelos son de carácter ambiental (condiciones climáticas, textura, composición y estructura del suelo) y de manejo de cultivo. Prácticas agrícolas donde se realiza un incremento de la dosis de

aplicación de N por encima del óptimo para el crecimiento de la planta o el rendimiento contribuye a incrementar las emisiones de N<sub>2</sub>O. Por ello, las prácticas agronómicas que incrementan el uso eficiente del N, especialmente si estas son acompañadas por la reducción de la dosis de N (aplicaciones fraccionadas o ajuste de dosis de aplicación de N a las necesidades de los cultivos), son una buena herramienta para ayudar a reducir estas emisiones, así como el uso de cultivos de cobertura que puedan beneficiar al sistema, cuando sea posible, de la fijación biológica del N y en consecuencia incrementen su uso eficiente (López Bellido, 2017c).

**2. Sobre las propiedades biológicas del suelo.** Otra consecuencia medioambiental indirectamente relacionada con el manejo del suelo es su influencia sobre la biodiversidad del suelo y en consecuencia, sobre la de todo el ecosistema creado:

- *Contribuye al aumento de la actividad biológica en el suelo*, muy importante en fenómenos de transformación de nutrientes en el suelo y en la degradación de herbicidas. Se han realizado estudios de sistemas de laboreo a largo plazo en condiciones mediterráneas que han mostrado una biomasa y respiración microbiana del suelo un 71 % y 44 % mayor, respectivamente, en sistemas de mínimo laboreo que de laboreo tradicional (Melero *et al.*, 2009; Sapkota *et al.*, 2012). De hecho, las técnicas conservacionistas han permitido el desarrollo de una estructura del suelo más estratificada, rica y diversa en microfauna, especialmente en microartrópodos (Sapkota *et al.*, 2012). También se ha conseguido un mayor nivel de biodiversidad entre la mesofauna, principalmente lombrices de suelo (Wuest, 2001) y artrópodos como las arañas (Rypstra *et al.*, 1999). En consecuencia, se hacen menos probables las plagas devastadoras y se ayuda a mantener activos los sistemas regulatorios de las poblaciones.

- *Autorregulación del ecosistema.* Los residuos vegetales dejados o incorporados al suelo en las técnicas de AC también proporcionan una fuente de alimento y protección a aves y pequeños animales. Por lo tanto, también contribuyen a la mejora del hábitat de especies muy diferentes y ayudan a conservar las redes tróficas en todos sus niveles (Holland, 2004).

A pesar de todas las ventajas medioambientales enumeradas, los efectos a largo plazo de las prácticas de AC en las rotaciones de cultivos herbáceos no son tan

pronunciados como en el laboreo tradicional. De hecho, existen resultados muy controvertidos en las investigaciones realizadas al respecto. Por ejemplo, son muchos los estudios que no encuentran diferencias entre ambas técnicas en la rotación trigo-girasol (López-Bellido *et al.*, 1997, 2007a; Murillo *et al.*, 1998). Muy numerosas son también las investigaciones que hallan en las prácticas de mínimo laboreo y siembra directa una menor eficiencia en la utilización de nutrientes en ciertas condiciones. Destacan los estudios donde se ha observado una menor mineralización de N, como consecuencia de la mayor presencia de residuos en suelo (Corbeels *et al.*, 2000; López-Bellido y López-Bellido, 2001; Menéndez *et al.*, 2008). Dichos residuos también han propiciado en ocasiones una peor nascencia de los cultivos principales, al crear malas condiciones del lecho de siembra (Moret *et al.*, 2007) o la aparición de un mayor número de plagas en la parcela (Cantero-Martínez *et al.*, 2003). Incluso existen estudios donde el uso de técnicas de AC ha ocasionado una reducción de la producción, siendo muy variadas las razones que explican los resultados agronómicos obtenidos: existencia de propiedades físicas limitantes en la cama de siembra (Hammel, 1995); aumento de patógenos del suelo y malas hierbas (Kirkegaard *et al.*, 1995; Wuest *et al.*, 2000) o una menor eficacia en la utilización de fertilizantes (López-Bellido y López-Bellido, 2001). No obstante, estos factores están directamente relacionados con la localización, características de suelo, clima y especies de la zona evaluada (Van den Putte *et al.*, 2010). Por ello, los aumentos o las pérdidas de los rendimientos agronómicos obtenidos con las técnicas de AC deben ser también evaluados teniendo en cuenta los insumos utilizados (Fox *et al.*, 1991).

En el contexto de agricultura actual, en el que el objetivo “máxima producción” es sustituido por el de “costes mínimos” (Sombrero *et al.*, 2008), los beneficios económicos con respecto al sistema tradicional se deben en muchas ocasiones a la reducción del número de labores en el campo. Esto supone un ahorro de carburante, maquinaria y tiempo de trabajo, y en consecuencia, una disminución de los costes de producción (Allmaras y Dowdy, 1985). Son diversos los estudios económicos comparativos de técnicas de AC frente a laboreo tradicional en rotación de cultivos que muestran cómo los requerimientos de maquinaria específica y gasto adicional de agroquímicos se ven compensados por la reducción de los costes de operación (Sánchez-Girón *et al.*, 2007; Sombrero *et al.*, 2008). Otros revelan cómo la reducción

de carburante y tiempo de trabajo compensan una reducción de los rendimientos obtenidos al usar las técnicas de AC, especialmente bajo la modalidad de siembra directa (Kirkegaard *et al.*, 1995; Gemtos *et al.*, 1998; Murillo *et al.*, 1998).

En España, y en Andalucía más concretamente, la producción de los cultivos bajo sistemas de manejo de agricultura de conservación ha sido en muchos casos similar a la de los tradicionales (López-Bellido y López Bellido, 2000; López-Bellido *et al.*, 2007b). Sin embargo, en muchos otros casos estas producciones también han sido superiores, gracias al uso más eficiente de los recursos agua y suelo (Fernández-Quintanilla, 1997; Cantero-Martínez *et al.*, 2003; Ordóñez-Fernández *et al.*, 2007a; Van den Putte *et al.*, 2010; Melero *et al.*, 2011a). De hecho, las técnicas de AC han permitido la obtención de cosechas aceptables de girasol y trigo en suelos arcillosos y arenosos hasta en años con sequías acusadas (Van den Putte *et al.*, 2010).

A pesar de la existencia de estos estudios y experiencias positivas sobre las técnicas de AC en nuestras condiciones, su implantación en las parcelas de secano está siendo lenta y dificultosa. No obstante, la necesidad de mejorar las producciones en un medio degradado y con fenómenos climáticos tan extremos está propiciando actualmente un leve cambio en su tendencia de uso, como veremos a continuación.

#### **1.4 SITUACIÓN ACTUAL DE LA AGRICULTURA DE CONSERVACIÓN EN ESPAÑA Y ANDALUCÍA**

En conjunto, los resultados de tantos años de estudio en condiciones edafoclimáticas características de la cuenca Mediterránea han demostrado que las técnicas de AC son una alternativa viable al laboreo convencional en todos los cultivos (López-Bellido *et al.*, 2011a).

Sin embargo, según la base de datos AQUASTAT (Tabla 2) elaborada por la Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO) sobre la adopción de agricultura de conservación a nivel mundial (FAO, 2016), los agricultores europeos bajo condiciones de clima mediterráneo seco no contemplan la conservación del suelo y el agua como objetivo prioritario en su toma de decisiones (Cannell y Hawes, 1994; Holland, 2004; Van den Putte *et al.*, 2010). De hecho, se observa un retraso en la implantación de las técnicas de AC en esta zona de Europa

con respecto a zonas similares de otros continentes, como Estados Unidos, Argentina, Australia o Kazajistán (Holland, 2004; Van den Putte *et al.*, 2010; Kassam *et al.*, 2012).

En España y Andalucía, los agricultores siempre se han mostrado escépticos ante la idea de suprimir totalmente las labores agrícolas en sus campos (Fernández-Quintanilla, 1997; López y Arrúe, 1997). En consecuencia, la implantación de nuevas técnicas de cultivo en los herbáceos de secano ha sido escasa hasta la fecha.

**Tabla 2. Adopción de técnicas de agricultura de conservación en países con clima Mediterráneo seco a nivel mundial.**

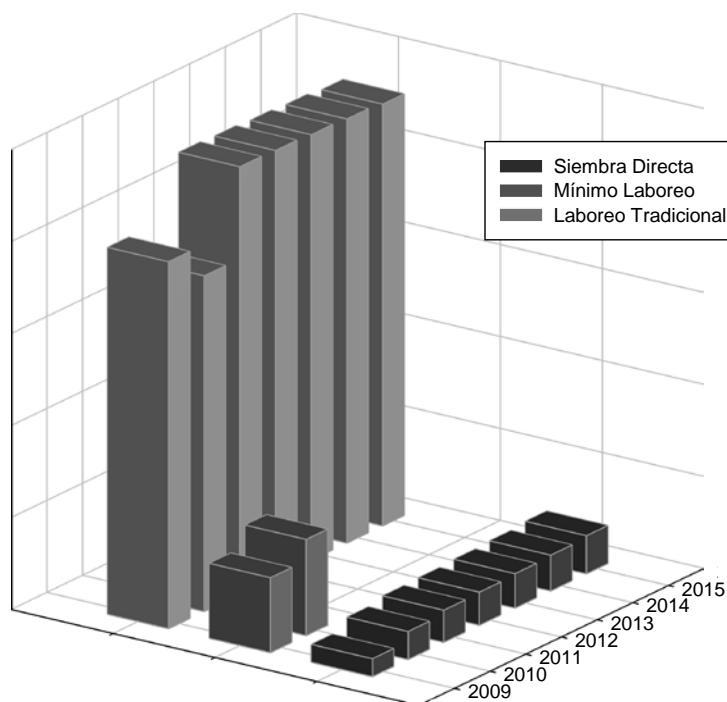
País	Área bajo agricultura de conservación (*10 <sup>3</sup> ha)
Estados Unidos	35.613
Argentina	29.181
Australia	17.695
Kazajistán	2.000
España	792
Italia	380
Sudáfrica	368
Francia	200
Chile	180
Turquía	45
México	41
Portugal	32
República Árabe Siria	30
Grecia	24
Irak	15
Túnez	8
Marruecos	4
Líbano	1,2
<i>Total</i>	86.549

Fuente: Elaboración propia a partir de Aquastat (FAO, 2016) y Kassam *et al.* (2012)

Los resultados de la Encuesta sobre Superficies y Rendimientos de los Cultivos (ESYRCE) elaborada por el Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente (MAPAMA, 2015b), muestra que en cultivos leñosos de secano en España y Andalucía, la principal técnica de mantenimiento de suelo es laboreo mínimo, seguido de la cubierta vegetal espontánea y el laboreo tradicional. Las técnicas de mantenimiento de suelo en las parcelas en barbecho también han sido analizadas por ESYRCE, siendo las técnicas de laboreo tradicional, cubierta vegetal espontánea y laboreo mínimo las más empleadas en estas extensas superficies.

Sin embargo, las estadísticas que desde 2007 se elaboran para cultivos herbáceos solo diferencian siembra directa o laboreo convencional realizado en los cultivos de referencia: cereales grano, girasol, maíz forrajero y otros forrajes. De acuerdo con los resultados de ESYRCE en 2015, la técnica más extendida en los cultivos

herbáceos de referencia en condiciones de secano es laboreo tradicional. De hecho, a nivel nacional representó el 91 % (4,9 mill. de ha) de la superficie total sembrada de trigo y el 97 % (699.893 ha) del total de la girasol. Aunque estos datos muestran la baja aceptación que sigue teniendo actualmente la técnica de siembra directa en cultivos herbáceos, su evolución ha sido muy positiva en los últimos años. De hecho, su superficie se ha duplicado, pasando de representar el 3,9 % de la superficie total de los cultivos de referencia en 2008 al 8,26 % en 2015. Además, los cereales grano y el girasol son los principales cultivos donde se está utilizando, especialmente en Andalucía. Así, la superficie cultivada bajo siembra directa en esta comunidad supuso el 13 % (84.047 ha) del total de la superficie cerealista y el 4 % (11.383 ha) del total sembrado con girasol (MAPAMA, 2015b). Para la técnica de mantenimiento de suelo con laboreo mínimo, los mejores datos de los que se dispone actualmente en España proceden de la experimentación llevada a cabo en ensayos de laboreo de larga duración (Álvaro-Fuentes *et al.*, 2008; López-Bellido *et al.*, 2011a), así como de las estimaciones propias de la Sociedad Española de Agricultura de Conservación-Suelos Vivos (AEAC.SV, 2016). Estos datos sugieren que un 16,3 % (1,2 mill. de ha) del total de la superficie de cultivos herbáceos en el año 2009 y un 20,9 % (1,5 mill. de ha) en el año 2010 utilizaron esta técnica, reduciéndose el porcentaje de laboreo convencional en pro del mínimo laboreo (Figura 3).



**Figura 3. Evolución del porcentaje de la superficie dedicada a cada técnica de mantenimiento de suelo en cultivos herbáceos a nivel nacional durante el período 2009-2015.**

Fuente: Elaboración propia a partir de AEAC.SV (2016) y MAPAMA (2015b)

Estos ligeros cambios de tendencia observados en la adopción de las técnicas de AC nos muestran que la agricultura europea está cambiando. La intervención pública es cada vez mayor, y los agricultores andaluces, hasta ahora poco concienciados y formados sobre las técnicas de AC, se enfrentan a nuevas exigencias que han de cumplir para asegurar la continuidad de sus explotaciones. El cumplimiento de los requisitos medioambientales de la PAC junto a los retos y presiones del mercado constituyen los principales factores determinantes para el futuro del secano herbáceo andaluz. Así, la continuación de la tendencia positiva en la adopción de siembra directa y laboreo mínimo, o la inclusión de cubiertas vegetales como técnicas de mantenimiento alternativas y viables en sus rotaciones, va a depender de las directrices marcadas por estos condicionantes, tal y como se describe a continuación.

### **1.5 FACTORES DETERMINANTES PARA EL FUTURO DE LA AGRICULTURA DE CONSERVACIÓN EN LOS SECANOS HERBÁCEOS ANDALUCES**

En Andalucía, la toma de decisiones de los agricultores se ha visto condicionada por la evolución de las diferentes políticas de la UE, en particular, de la Política Agraria Común (PAC) cada vez más volcada en dar respuesta a las expectativas de la sociedad y

a sus exigencias en cuanto a la protección del medioambiente y a la producción de alimentos sanos y seguros. Por lo tanto, la necesidad de afrontar el futuro sostenible de las rotaciones tradicionales de los cultivos herbáceos de secano ha hecho necesaria la adaptación a los últimos requerimientos de la PAC y a la situación agronómica y comercial de sus cultivos principales a nivel territorial.

### **1.5.1 CONDICIONANTES MEDIOAMBIENTALES DE LA POLÍTICA AGRARIA COMÚN**

En sus inicios (1962-1980), la PAC consideraba la agricultura como una actividad económica unifuncional, que tenía como objetivo primordial la producción de alimentos, la regulación de los mercados y el apoyo a las rentas agrarias. Sin embargo, a finales de los ochenta, con el Acta Única Europea de 1986, se plasmó por primera vez la interacción de la agricultura con el medio ambiente. Desde entonces, la PAC ha sido objeto de numerosos cambios con el fin de adaptarse a la realidad europea de cada momento y se han ido incluyendo medidas para favorecer el desarrollo rural y el respeto por el medio ambiente. Las principales modificaciones se materializaron en las reformas de 1992 (la reforma McSharry), 1999 (Agenda 2000), 2003 (Revisión intermedia) y 2009 (el “Chequeo médico” de la PAC), hasta llegar a la última reforma de 2013 (hacia el Horizonte 2020).

Una de las transformaciones más importantes se produjo con la reforma de la Agenda 2000. En ella la PAC se estructuró en dos pilares donde se contemplaban las preocupaciones ambientales, la política de precios y mercados de apoyo a la renta (pilar 1) y la política ambiental y de desarrollo rural (pilar 2). A partir de ese momento, las exigencias medioambientales comenzaron a regularse a través de tres mecanismos con distintos niveles de exigencia: *ecocondicionalidad*, *buenas prácticas agrarias* y *ayudas agroambientales*.

Con la Reforma de 2003, aunque centrada en el consumo y los contribuyentes, se hizo un mayor hincapié en la *condicionalidad* de las ayudas, y establecía que todo agricultor que recibiera pagos directos debía cumplir una serie de *requisitos legales de gestión* así como unas *buenas prácticas agrarias*. Por ello, se le concedió una importancia primordial al buen manejo de los suelos, evitando la erosión, protegiendo su contenido en materia orgánica y estructura e incluyendo por primera vez entre sus



prácticas las rotaciones de cultivos (artículo 5 del Reglamento (CE) 1782/2003 y Real Decreto 2352/2004). Además en España, las ayudas agroambientales del Real Decreto 172/2004 incluían por primera vez actuaciones relacionadas con la agricultura de conservación, como el mantenimiento de restos de cultivos sobre el suelo, uso de cubiertas vegetales, racionalización del uso de fitosanitarios, aplicación de técnicas de laboreo mínimo y siembra directa así como restricciones en cuanto al volteo de suelo.

Con el “Chequeo médico” de la PAC (2008/2009), los EE.MM. pudieron adoptar por primera vez un marco normativo adaptado a las características específicas de su zona. Por lo tanto, se forzó a los agricultores a orientar sus producciones en función de la demanda de sus mercados. Así en España, se adoptó a partir de 2010 el Programa Nacional de apoyo al sector de cultivos herbáceos (Reglamento (CE) 73/2009). Este incluía el Programa Nacional de ayudas al fomento de la rotación de cultivos en secano. La superficie debía cultivarse de cereales, oleaginosas, proteaginosas y/o leguminosas en las alternativas y dedicar al menos el 20 % de la superficie acogida a las leguminosas, oleaginosas o proteaginosas, para enterrar en verde, producción de grano o aprovechamiento a diente por el ganado.

Finalmente, en el año 2013 se aprobó la última reforma de la PAC. Enmarcada en la estrategia europea denominada *Horizonte 2020*, esta reforma rompe completamente con el modelo de apoyo basado en la producción hasta ahora existente, contemplando un enfoque multifuncional de la agricultura. Por tanto, aborda retos económicos, ambientales y territoriales de forma conjunta, incluyendo un componente “verde” obligatorio en las ayudas (Reglamento (UE) 1307/2013) y simplificando la *condicionalidad*, tal y como veremos a continuación.

**1. El componente “verde” o greening.** Consiste en que el 30 % del Pago Básico a percibir está supeditado al cumplimiento del denominado “pago verde” o “greening” (Real Decreto 1075/2014 y Real Decreto 1076/2014). Las medidas de este componente verde deben proporcionar beneficios medioambientales, considerándose prácticas agrícolas beneficiosas, entre otras, *la diversificación de cultivos y la superficie de interés ecológico*:

- *La diversificación de cultivos.* Si la tierra de cultivo de la explotación cubre entre 10 y 30 ha, se debe realizar la rotación de 2 cultivos. En cambio, si la explotación

posee más de 30 ha debe haber al menos 3 cultivos diferentes. Además, el cultivo principal no puede ocupar más del 75 % de la parcela, y los dos cultivos mayoritarios juntos no podrán suponer más del 95 % de la misma.

- *Contar con Superficies de Interés Ecológico (SIE) en la superficie agraria.* Las explotaciones con más de 15 ha deben asignar el 5 % de la superficie arable a SIE desde el 1 de enero de 2015, y el 7 % en 2017. Hay algunas excepciones, pero la mayoría de los cultivos, entre ellos los cultivos herbáceos, han de cumplir este requisito. Las SIE a introducir para alcanzar los porcentajes fijados son elección de los EE.MM. Entre un total de 10 posibles opciones, las principales SIE elegidas por los países europeos son: cultivos fijadores de N como leguminosas grano y forrajeras (seleccionado en 27 EE.MM, todos excepto Dinamarca), tierras de barbecho (26 EE.MM.), elementos paisajísticos (24 EE.MM.), superficies forestales de rotación corta (20 EE.MM.) y superficies con cultivos cubierta tipo “catch crop” y “green cover” con gramíneas, crucíferas, leguminosas y boragináceas (19 EE.MM., exceptuando Estonia, Grecia, España, Italia, Chipre, Lituania, Malta, Portugal, Finlandia y Reino Unido) (Hart, 2015). En España, las 4 opciones inicialmente elegidas fueron: superficies dedicadas a cultivos fijadores de N, tierras de barbecho, superficies forestadas en el marco de los distintos programas de desarrollo rural y superficies dedicadas a agrosilvicultura que hayan recibido ayudas en el marco de los programas de desarrollo rural (EC, 2017). Sin embargo, a partir de 2018 se admitirá la mezcla de cultivos fijadores de N con otros cultivos (Reglamento Delegado (UE) 2017/1155). No existe una lista de mezclas de cultivos, sólo se exige el requisito de que en esas mezclas haya un predominio del cultivo fijador.

**2. Condicionalidad de las ayudas.** Los agricultores andaluces con cultivos herbáceos de invierno en secano, están supeditados desde el año 2005 a una serie de condiciones de manejo que van dirigidas a la conservación del suelo (antigua Orden de 22 de junio de 2009). Con la nueva PAC, la normativa comunitaria y estatal (Reglamento Delegado (UE) 640/2014 y Real Decreto 1078/2014) regulan ampliamente los aspectos sustantivos de la condicionalidad. Por tanto, la labor de desarrollo de la comunidad autónoma (Orden del 12 de junio de 2015) queda reducida a adaptar las *buenas prácticas agrícolas* de la tierra a las particularidades del territorio andaluz y a

relacionarlas con los *requisitos legales de gestión*. Estas medidas tienen como prioridad actualmente:

- *Evitar la erosión del suelo y mantener su estructura.* Se prohíbe labrar la tierra con una profundidad superior o igual a 20 cm, así como voltear el terreno entre la fecha de recolección de la cosecha y el 1 de septiembre. Hay algunas excepciones donde se autoriza el volteo desde el 15 de mayo por razones agronómicas (como las dobles cosechas), climáticas o de tipología de suelos (endurecimiento de la tierra como consecuencia de las altas temperaturas), pero siempre en terrenos de baja pendiente. Esta práctica también queda justificada para favorecer la implantación de una cubierta vegetal con cultivos herbáceos, para realizar el infiltrado de agua estancada o para incorporar materia orgánica con fines de fertilización y de lucha contra las malas hierbas.

- *Conservar la materia orgánica del suelo.* No podrá quemarse ningún rastrojo, salvo el del arroz. Para el resto de cultivos herbáceos, solamente podrán hacerse quemas autorizadas cuando la administración haga una declaración oficial de zonas afectadas que aconseje la quema por razones fitosanitarias. Cultivos como el girasol o el maíz, en los que tradicionalmente se han quemado los restos de cosecha (cañas), quedan actualmente obligados a realizar su picado e incorporación al suelo, o bien, a su traslado fuera de la parcela.

- *Limitar el uso de la fertilización nitrogenada y fitosanitarios.* Con esta nueva reforma agraria siguen siendo de obligado cumplimiento los *requisitos legales de gestión* correspondientes al tipo de actividad que un agricultor responsable debe poner en práctica en cada región. Por ello, en relación a los cultivos herbáceos de secano, la Directiva 91/676/CEE tiene por objeto la protección de las aguas contra la contaminación producida por nitratos. Esta norma establece la obligación de designar zonas vulnerables a dicha contaminación además de un programa de actuación aplicable por tipo de explotación y cultivo. Contempla por tanto, limitaciones obligatorias a cada clase de fertilizante y recomendaciones en su aplicabilidad (orden del 1 de junio de 2015). En Andalucía, se designaron 22 zonas vulnerables (Real Decreto 36/2008), siendo la más amplia de todas ellas la zona 2 correspondiente al Valle del Guadalquivir, que es la zona ocupada por los cultivos herbáceos de secano. Junto a la limitación en el uso de la fertilización nitrogenada, es muy importante la

reducción de los riesgos y los efectos del uso de fitosanitarios en la salud humana y el medio ambiente. Por ello, la Directiva 2009/128/CE del Parlamento Europeo y del Consejo, y el Real Decreto 1311/2012, establecen el marco de acción para conseguir un uso sostenible de los mismos. Esta reglamentación recoge la obligatoriedad de adoptar una gestión integrada de plagas con bajo consumo de plaguicidas, dando prioridad, cuando sea posible, a los métodos no químicos. Entre los principios generales para la gestión integrada de plagas y relacionados con las prácticas de cultivo, se recogen en el Anexo I del Real Decreto, la rotación de cultivos y la utilización de técnicas como laboreo mínimo y siembra directa.

Además, en relación a este punto, en Andalucía se cuenta con el Reglamento Específico de Producción Integrada de Cereales de Invierno (Orden de 1 de diciembre de 2015) y de oleaginosas y leguminosas grano (Orden de 6 de abril de 2017). En ellos se recogen todas las limitaciones medioambientales anteriormente descritas y se definen las directrices para una gestión integrada de estos cultivos. Por ello, a través de prácticas obligatorias, prohibidas y recomendadas, este reglamento también fomenta el empleo de técnicas de AC, la inclusión en rotación de cereales y oleaginosas de leguminosas o crucíferas (por el beneficio que su rastrojo deja en el suelo o la biofumigación que producen, respectivamente) y el manejo adecuado de restos orgánicos o enmiendas que permitan alcanzar niveles de materia orgánica deseables en condiciones de secano. Asimismo, a partir de 2018 entrará en vigor una ayuda agroambiental incluida en el Programa de Desarrollo Rural de Andalucía 2014-2020 para sistemas sostenibles de cultivos herbáceos de secano, cuyo objetivo es fomentar la agricultura de conservación en cultivos herbáceos mediante técnicas y manejos del suelo adecuados principalmente en zonas con mayor riesgo, con pendientes superiores al 8%.

### **1.5.2 CONDICIONANTES AGRONÓMICOS Y LIMITACIONES DERIVADAS DE LA BALANZA COMERCIAL TERRITORIAL**

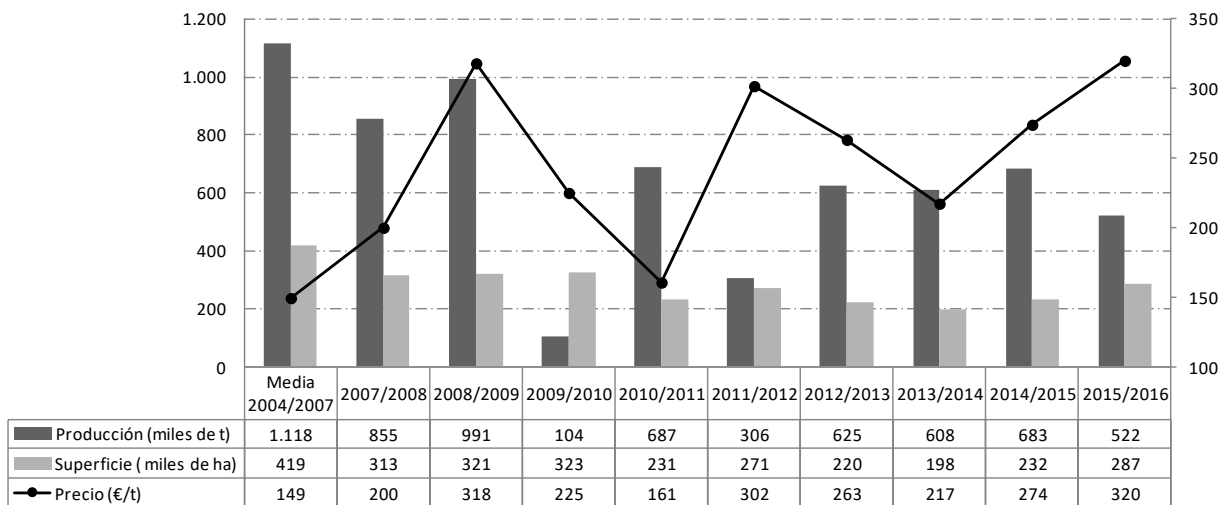
La complejidad de la relación entre agricultura y medio ambiente en la rotación tradicional trigo-girasol, con gran número de exigencias y limitaciones medioambientales, se ve acrecentada por los condicionantes de mercado y problemas agronómicos de sus cultivos principales, los cuales son descritos a continuación.

**1. Condicionantes agronómicos y de mercado del trigo.** Andalucía es la principal productora de trigo duro (*Triticum durum*) de España. Como se apuntaba en el apartado 1.1.1. de este documento, este cultivo ocupa 287.431 ha que suponen el 67 % del total de la superficie dedicada a trigo duro en el país. En el Valle del Guadalquivir, las provincias de Cádiz, Córdoba y Sevilla suponen el 85 % de la superficie cultivada en Andalucía, y aproximadamente el 80 % de la producción total a nivel nacional. Sin embargo, y a pesar de su importancia, en los últimos años ha habido un claro descenso en la superficie cultivada, debido a tres razones fundamentales: el cambio en el sistema de ayudas PAC, las fluctuaciones de precios de los mercados y la falta de variedades adaptadas a nuestras condiciones climatológicas.

- El cambio en el sistema de ayudas de la Política Agraria Común. A comienzos de los años 90 la superficie dedicada al cultivo de trigo duro era de aproximadamente 100.000 ha en España. Impulsada por la PAC, esta superficie llegó rápidamente a las 600.000 ha a través de una ayuda específica para trigo duro, en detrimento de la superficie sembrada con trigo harinero y que solo percibía la ayuda genérica a los cereales. Sin embargo, desde que en la campaña 2005/2006 se aprobó la inclusión del trigo duro en el Régimen de Pago Único y desapareció la ayuda directa a este cultivo, se produjo de nuevo una inversión de la trayectoria de las siembras seguidas por los agricultores andaluces. En este caso, las especies trigo harinero, cebadas y avenas se vieron favorecidas, en detrimento de la superficie de trigo duro. De hecho, en las campañas 2012/2013 y 2013/2014 se alcanzaron las menores superficies de trigo duro de los últimos 20 años (Figura 4). A partir de la campaña 2014/2015 se ha vuelto a romper esta tendencia, siendo la menor incidencia de enfermedades, la calidad de la cosecha y los precios de cotización en los mercados los factores que han explicado el nuevo cambio en esta ocasión.

- Las fluctuaciones de precios de los mercados y la barrera de la rentabilidad. La alta volatilidad de los precios de los cereales en el mercado internacional hace que los agricultores de trigo se enfrenten cada año a una situación de incertidumbre ante la elección de la especie a sembrar. La búsqueda de la disminución de los costes de producción y un enfoque claro hacia la introducción de cultivos de menor riesgo se han convertido durante muchos años en su prioridad. Este hecho se ha visto motivado, en parte, porque el esfuerzo realizado por sembrar trigo duro, con mayores costes

productivos y del que, por norma, se obtienen menos kilos, no se ha visto compensando económicamente durante muchas campañas agrícolas, pagándose al mismo precio que el trigo harinero. Sin embargo, tal y como estimaban las previsiones de la Organización para la Cooperación y el Desarrollo Económico (OCDE), el Instituto para la Investigación de la Política Alimentaria y Agraria (FAPRI) y FAO, un incremento de la producción de trigo duro y una estabilización de los precios (López-Bellido, 2009) se ha producido a partir de la campaña 2014/2015 (Figura 4). La subida al alza de los precios de trigo duro se ha debido principalmente a la alta calidad de las producciones actuales y a la necesidad de cubrir las necesidades nacionales de este cereal. Si se consiguiera dar cobertura de este cereal a nivel nacional, se podrían reducir además las importaciones cada vez mayores realizadas (580 t importadas en la campaña 2014/2015 respecto a la media de 450 t del periodo 2010/2015). En consecuencia, se podrían aumentar sus exportaciones (en 2014/2015 se exportaron 470 t respecto a las 520 t promedio de 2010 a 2015) y ayudar así a satisfacer su demanda en otros zonas del mundo (MAPAMA, 2015c).



**Figura 4. Evolución durante el período 2004-2016 de la superficie (ha), producción (t) y precios (€/t) al inicio de la campaña de comercialización en la Lonja de Sevilla de trigo duro en Andalucía.**

Fuente: Elaboración propia a partir de la Subd. Gral.de Cultivos Herbáceos (MAPAMA, 2016)

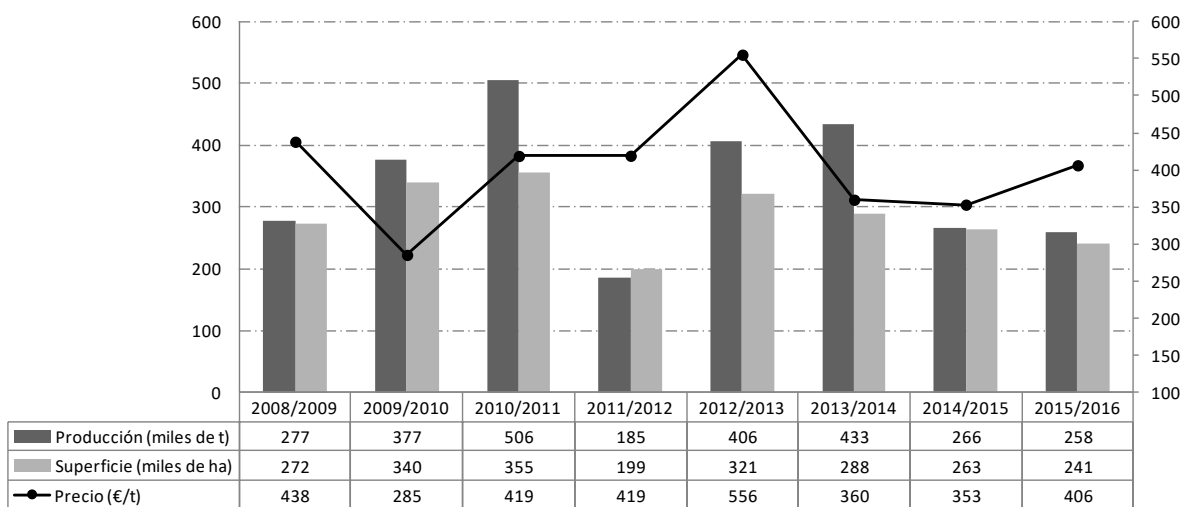
- La falta de variedades adaptadas a nuestras condiciones climatológicas. Las pérdidas provocadas por Oidio (*Blumeria graminis*, f.sp. *Tritici*), Septoria (*Septoria Tritici*) y Roya Parda (*Puccinia Triticina*) supusieron durante muchos años tales pérdidas de cosecha que el control de estas enfermedades se hizo primordial a nivel europeo, nacional y regional. De esta forma, se llevaron a cabo programas de mejora para la

obtención de variedades de trigo duro adaptadas a nuestras condiciones (Domínguez *et al.*, 2007). Desde entonces, cada año se incorporan nuevas variedades de trigos duros al Registro de Variedades, que suelen presentar mejoras respecto de las ya comercializadas. Sin embargo, los elementos positivos que incorporan estas variedades, no siempre se manifiestan, ya que su respuesta depende del tipo de terreno, clima, prácticas culturales, etc. Desafortunadamente, las estimaciones sobre las consecuencias del cambio climático en el Valle del Guadalquivir predicen un aumento de las temperaturas y un descenso de las precipitaciones en las próximas décadas (García-Ruiz *et al.*, 2011). Este hecho afectará aún más a los rendimientos de los cultivos (Ferrara *et al.*, 2009) y, en consecuencia, a la calidad del grano de trigo duro que, a pesar de ser el cereal que mejor se ha adaptado a la comercialización por lotes homogéneos de calidad (Real Decreto 190/2013), en ocasiones no alcanza los requerimientos cada vez más exigentes de la industria semolera en el mercado europeo y mundial.

**2. Condicionantes agronómicos y de mercado del girasol.** Andalucía es la comunidad autónoma con mayor superficie dedicada al cultivo del girasol (*Helianthus annuus*). Alberga 281.550 ha que representan el 39 % del total de la superficie nacional (MAPAMA, 2015a), y como se explicaba en los apartados 1.1.1. y 1.1.2. de este documento, constituye la alternativa más común y adecuada en rotación con trigo duro en sus explotaciones agrarias. Sin embargo, al igual que el trigo duro, el girasol ha experimentado un descenso productivo tanto a nivel autonómico como nacional, principalmente influenciado por la dependencia de los factores meteorológicos y de los precios, la resistencia a nuevas razas de jopo y la importancia de su correcto manejo en rotación.

- La dependencia de la producción de los factores meteorológicos y de los precios. El girasol es un cultivo muy variable en cuanto a rendimientos intercampañas, por cultivarse mayormente en secano y ser muy dependiente de la pluviometría y otros factores meteorológicos. Desde la campaña 2013/2014 la superficie y la producción de girasol se han reducido en Andalucía, al mismo tiempo que han bajado sus precios (Figura 5). El destino mayoritario de su cosecha es la elaboración de aceite de girasol, aunque también se aprovecha como subproducto tras la molturación una

harina integral que sirve a los ganaderos para la alimentación de los animales. A partir de la campaña 2015/2016, las cotizaciones del aceite y la harina de girasol han provocado una subida de los precios. Sin embargo, la escasez de lluvias y los periodos de sequía registrados en las principales provincias productoras, principalmente en Sevilla, que alberga más del 50 % del total de la producción cosechada, han mermado aún más la producción. Por ello, y dada la incertidumbre de las condiciones que influyen en el precio final del girasol, la rentabilidad del girasol actualmente está supeditada a la correcta elección varietal que permita obtener la máxima calidad y rendimientos.



**Figura 5. Evolución de la superficie (ha), producción (t) y precios (€/t) al inicio de la campaña de comercialización en la Lonja de Sevilla de trigo duro en Andalucía.**

Fuente: Elaboración propia a partir del Observatorio de Precios y Mercados (CAPDR, 2016)

- La resistencia a nuevas razas de jopo. Las especiales características de clima y suelo del sur de España han propiciado siempre una buena adaptación del girasol. De hecho, ha sido reducida la presencia de enfermedades comunes y graves de girasol en otros países de Europa tales como Mildiu (*Pasmopara halstedii* (Farl.) Berl. y de Toni), Roya (*Puccinia helianthi* Schw.), Cancro del tallo (*Phomopsis helianthi* Munt.) o Podredumbre húmeda (*Sclerotinia sclerotiorum*). Sin embargo, el jopo del girasol (*Orobanche cumana*) ha puesto en peligro la supervivencia de los cultivos de girasol en la zona andaluza desde la década de los noventa. Las pérdidas que ocasiona esta planta parásita en el cultivo varían según la severidad de la infección, la cantidad de semillas activas que se encuentren en el suelo (Joel *et al.*, 2011) y el nivel de



susceptibilidad o resistencia genética de la variedad cultivada. A pesar de existir variedades híbridas de girasol resistentes a jopo en Andalucía (García Ruíz, 2013), dicha resistencia no asegura a los agricultores que estas variedades no puedan ser atacadas, y continuamente hay que desarrollar resistencia genética por la aparición de nuevas razas (García Ruíz *et al.*, 2007). Las variedades IMI, resistentes a las imidazolinonas producen un retraso considerable en la implantación de jopo en las raíces de girasol, además de controlar determinadas malas hierbas. El uso de este material vegetal resistente de origen no transgénico aporta una nueva posibilidad de lucha contra el jopo del girasol, pero además, las técnicas de manejo del suelo juegan un papel fundamental con el fin de limitar en lo posible su impacto negativo.

**3. Condicionantes en la rotación trigo-girasol.** Uno de los principales problemas de la rotación es el manejo de herbicidas en el trigo previo, que pueden afectar seriamente al cultivo de girasol. En la campaña de cultivo 2014/2015 se observaron por primera vez daños graves de fitotoxicidad en los primeros estadios del girasol sembrado a finales de invierno o principios de primavera en Andalucía y otras comunidades como Castilla La Mancha o Castilla León (Saavedra *et al.*, 2015a). Los daños observados fueron desde muy graves (con pérdidas de casi la totalidad de la cosecha) a leves (los cultivos lograron llegar a maduración), con signos y síntomas en las plantas que apuntaban a residuos de herbicidas a base de sulfonilureas aplicados de 12 a 14 meses antes en el cultivo del cereal. A pesar de existir estudios e informes previos apuntando los posibles riesgos que los residuos de herbicidas utilizados en cereal podrían tener sobre el girasol en rotación (EFSA, 2015, 2016; Serim y Maden, 2014), la inexistencia de casos documentados en el Valle del Guadalquivir no ha hecho posible que este problema se haya estudiado antes en la zona. No obstante, en la manifestación de daños concurren varios factores: técnicas de laboreo utilizadas, fecha de siembra, variedad de girasol elegida, producto comercial utilizado y su modo de aplicación, etc. Por ello, la realización de estudios más profundos a medio y largo plazo son necesarios para intentar paliar este problema que puede afectar gravemente al futuro del cultivo del girasol, y en consecuencia, a la sostenibilidad ambiental de las rotaciones en los sistemas agrícolas tradicionales de secano.

Esta situación de continuo cambio e incertidumbre en relación a las producciones pueden motivar nuevos cambios en el campo andaluz de cara al futuro. Se podría reavivar, por ejemplo, el interés por introducir otras variedades distintas al trigo duro o al girasol, o la sustitución de la rotación por otros cultivos como el olivar, que favorezca aún más su expansión por las campiñas tradicionalmente ocupadas por los cultivos herbáceos. Sin embargo, uno de los retos más importantes de la agricultura actual es satisfacer la demanda de alimentos a nivel mundial. Según las estimaciones de la FAO, la población mundial alcanzará los 9.200 millones de personas en 2050 y será necesario el abastecimiento de productos básicos agrícolas como los cereales (FAO, 2009), base principal de la dieta humana y animal, por lo que la reducción de este cultivo, garantía de seguridad alimentaria, no es una opción viable, siendo necesario iniciar la búsqueda de alternativas o prácticas de cultivo que mejoren sus características cualitativas, ayuden a mejorar su productividad y/o compensen su pérdida de rentabilidad.

## **1.6 ALTERNATIVAS PROPUESTAS PARA LA ADOPCIÓN DE ROTACIONES VIABLES Y SOSTENIBLES EN ANDALUCÍA**

Tal y como se ha sido descrito hasta el momento, los agricultores andaluces con rotaciones tradicionales de secano se encuentran inmersos en un escenario de cambio constante. Cada campaña agrícola la toma de decisiones se ve directamente influenciada por las exigencias medioambientales y de mercados existentes, los principales problemas agronómicos acontecidos y las limitaciones en las prácticas agrícolas. Además, la diversificación de su actividad agraria ha de cumplir con los nuevos requerimientos normativos, teniendo en cuenta los retos de seguridad y calidad alimentaria, mitigación del cambio climático, nutrición y salud animal así como la mejora de la competitividad y la productividad agraria. Por todo ello, la creación de un sistema de manejo de las rotaciones más diversificado y sostenible, que permita mejorar las prácticas de manejo del suelo y el agua al mismo tiempo que se alcanzan rendimientos crecientes en la actividad agrícola, especialmente en la producción de cereal, se hace imprescindible para contrarrestar los efectos del cambio climático (López Bellido, 2017b) y satisfacer las demandas de una población en aumento en número y en exigencias (Lacasta Dutoit y Meco, 2005).

Sin embargo, para la diversificación de las explotaciones agrarias andaluzas no existen actualmente alternativas claras que superen la rentabilidad de la rotación trigo duro-girasol. Ante esta situación, la elección de una correcta estrategia de manejo de suelo que mantenga una filosofía conservacionista en línea con la AC, se puede convertir en la principal medida de actuación a tomar. Más aún tras conocer que el 84 % de la superficie andaluza dedicada a cultivos herbáceos tiene un tamaño medio de explotación de 75 ha (apartado 1.1.1. de este documento) y donde por tanto es necesario realizar una rotación de 3 cultivos de acuerdo con los actuales condicionantes de la PAC (apartado 1.5.1.). Por ello, en un intento de hacer un cambio trascendental de cara al futuro de la agricultura europea y de cumplir adecuadamente con todos los requisitos y condicionantes comentados, la introducción de cultivos intercalares o cultivos cubierta así como la inclusión de cultivos alternativos pueden constituir una buena solución.

Dentro de las técnicas de AC, los cultivos intercalares o cultivos cubierta constituyen la estrategia de agricultura sostenible que promueve mayores niveles de biodiversidad, siendo compatible con técnicas de laboreo y no laboreo con aplicación de herbicidas. En la rotación trigo duro-girasol, la propuesta que hacemos es la introducción de estos cultivos durante el período de tiempo en el que el suelo queda en barbecho entre los dos cultivos principales, es decir, de octubre a marzo aproximadamente antes del cultivo de girasol. Posteriormente, su incorporación al suelo como abono verde se llevaría a cabo justo antes de la siembra del girasol.

Los beneficios que la cubierta vegetal viva podría reportar en el sistema de cultivo objeto de estudio de esta investigación son muchos y muy variados:

- Ayudar a mantener o mejorar el contenido en materia orgánica y N en suelo (Weinert *et al.*, 2002; Kolota y Adamezewska-Sowinska, 2013).
- Mitigar las emisiones de gases de efecto de invernadero e incrementar el secuestro de carbono atmosférico al aumentar el aporte de residuos de cultivo al suelo (López Bellido, 2017a).
- Disminuir la erosión del suelo e incrementar su capacidad de almacenamiento de agua (Clark, 2000; Hartwig y Ammon, 2002).
- Reducir en algunos casos los problemas de patógenos y enfermedades del suelo (Liebman y Mohler, 2001; Kwiatkowski *et al.*, 2016)

- Ayudar a controlar malas hierbas (Ngouajio *et al.*, 2003; Haramoto y Gallandt, 2004).

Es indudable que no existe un cultivo intercalar o cubierta que consiga todas las propiedades arriba mencionadas. La elección de la especie es esencial para alcanzar el objetivo que se persigue con su uso, identificando especialmente las deficiencias a corregir y las particularidades del sistema de cultivo en el que se introducen. No obstante, cualquier cultivo que reportara alguno de los beneficios enumerados anteriormente, permitiría que las producciones obtenidas fueran más sostenibles en el tiempo, contribuyendo a la conservación del medio ambiente y la adaptación al cambio climático. Su introducción en grandes explotaciones serviría para cumplir con el requisito de la SIE de la PACen aquellos países de la UE que seleccionaron los cultivos cubierta como opción (apartado 1.5.1. de este documento), que no es el caso de España pero que podría serlo en un futuro.

Hasta el momento, no existen referencias a otras investigaciones previas en las que se hayan introducido cultivos intercalares en las rotaciones tradicionales de Andalucía. En otros países con condiciones climatológicas similares, se han utilizado ampliamente en rotaciones de cultivos (Brennan y Boyd, 2012a, 2012b; Flower *et al.*, 2012; Brennan *et al.*, 2013; Plaza-Bonilla *et al.*, 2016). Sin embargo, a nivel nacional, los estudios de cultivos cubierta con cultivos herbáceos se han centrado en otros cultivos principales, principalmente maíz (Salmerón *et al.*, 2010; García-González *et al.*, 2016), con condiciones edafoclimáticas y objetivos muy diferentes a los perseguidos en este estudio.

En España, la mayor parte de los cultivos cubierta se utilizan en cultivos leñosos. De hecho, constituye la segunda técnica de mantenimiento de sus suelos en España, por detrás de mínimo laboreo, tal y como vimos en el apartado 1.4. de este documento. Consecuentemente, el mayor número de investigaciones sobre estos cultivos a nivel nacional se realiza en cítricos (Aguilar-Fenollosa y Jacas, 2013; Gómez-Marco *et al.*, 2016), viñedos (Marques *et al.*, 2010; Barrio *et al.*, 2012; Peregrina, 2016) y sobre todo, olivar (Alcántara *et al.*, 2011a, 2011b; Espejo-Pérez *et al.*, 2013; López-Vicente *et al.*, 2016). Por lo tanto, conocida la importancia y extensión del olivar en Andalucía, la mayor parte de los estudios de cultivos cubierta se han realizado en esta zona. En consecuencia, en nuestra comunidad autónoma existe un gran conocimiento

y desarrollo de especies seleccionadas entre la flora autóctona, adaptados a las distintas tipologías de olivar y a las condiciones en las que se desarrollan. La elección de especies como cultivos intercalares para introducirlas en rotación con cultivos herbáceos de secano debe buscarse entre aquellas familias y especies de las que disponemos un mayor conocimiento de su ciclo de desarrollo así como del manejo apropiado para obtener los máximos beneficios. Estas familias son gramíneas, crucíferas y leguminosas:

**1. Gramíneas.** Las especies de esta familia son capaces de producir abundante biomasa y cubrir eficazmente el suelo, por ello se utilizan sobre todo para un control eficaz de la erosión. Aunque la erosión es un grave problema generalizado en nuestras condiciones, el riesgo y la incidencia es mayor en cultivos leñosos, especialmente en olivar, ya que ocupa mayormente suelos más sueltos y con pendiente. Por ello, a priori, no son las especies óptimas para incluirlas en rotación, y al ser además de la misma familia que el trigo duro, dificultan el control de enfermedades y malas hierbas. A pesar de ello, hay especies gramíneas que pueden ser empleadas como cultivo intercalar o cultivo cubierta por su ciclo corto y producción de biomasa, como es el caso de la avena negra (*Avena strigosa* Schreb.), tal y como se constata en numerosos trabajos realizados en zonas similares a la nuestra (Reeves y Price, 2005; Zibilske y Makus, 2009; Flower *et al.*, 2012).

**2. Crucíferas.** Además de la formación de biomasa para el control de la erosión, las crucíferas presentan dos importantes características que las hacen especies apropiadas como cubiertas vegetales a incluir en las rotaciones de cultivos herbáceos. Por un lado, presentan raíces pivotantes (Wolfe, 2000) que facilitan la descompactación del suelo y el aumento de la infiltración. Por otro lado, es conocido su efecto biofumigante contra patógenos de suelo (Angus *et al.*, 1994), al liberarse por la descomposición de la materia orgánica diferentes compuestos volátiles muy eficaces (fundamentalmente glucosinolatos e isotiocianatos derivados de su hidrólisis). Por tanto, ha sido demostrada su acción como fungicida (Mayton *et al.*, 1996), nematocida (Mojtahedi *et al.*, 1991) y herbicida (Alcántara *et al.*, 2011b). Entre las especies ensayadas para olivar, mostaza blanca (*Sinapis alba* subsp. *mairei* cv. Albendín) destacó por su fácil instalación, formación de cobertura y biomasa (Alcántara *et al.*, 2009). Esto unido al hecho de que la mostaza blanca es una especie común en la rotación trigo

duro-girasol como mala hierba, y que ha sido utilizada como cultivo cubierta en otras zonas del planeta en condiciones similares (Smith *et al.*, 2004; Norsworthy *et al.*, 2011; Björkman *et al.*, 2015), ha despertado el interés de su estudio como cultivo intercalar en la rotación tradicional.

**3. Leguminosas.** La capacidad de las leguminosas de fijar N atmosférico las hace una especie muy adecuada como cultivo intercalar en rotación de cultivos herbáceos, especialmente en la campiña, zona catalogada como zona vulnerable por contaminación de nitratos (Alcántara *et al.*, 2007). Entre las especies con las características más adecuadas para su utilización, destaca el alberjón (*Vicia narbonensis* L.), ya que tiene un rápido crecimiento y buena producción de biomasa con pocos o nulos requerimientos de cultivo (Durutan *et al.*, 1990; Siddique *et al.*, 1996). Además, existen variedades resistentes a jopo (*Orobanche crenata*) (Nadal *et al.*, 2007; Nadal y Moreno, 2007), lo que resulta de gran importancia al ser esta planta parásita la principal limitación del cultivo de leguminosas en nuestras condiciones. Este hecho hace también interesante su estudio sobre el control de malas hierbas y enfermedades, al ser conocido el efecto positivo de otras especies leguminosas que han sido utilizadas como cultivo cobertura (Fisk *et al.*, 2001; Fernández-Aparicio *et al.*, 2008; Isik *et al.*, 2009; Brust *et al.*, 2011).

La falta de alternativas rentables a la rotación tradicional se debe, en parte, a los problemas agronómicos no resueltos en cultivos adaptados a la zona, como ocurre con las proteaginosas. La alta incidencia del jopo (*Orobanche crenata*) y la falta de herbicidas registrados en el cultivo, han hecho que especies muy utilizadas como alternativa, por ejemplo habas (*Vicia faba* L.) y guisantes (*Vicia sativa* L.), hayan sufrido una gran regresión en las rotaciones de campiña. Por lo tanto, el estudio de estas especies aptas como cultivos intercalares como posibles cultivos alternativos en la rotación con distintos aprovechamientos (grano o forraje), pueden ser útiles y rentables para el agricultor. Entre los cultivos alternativos posibles, las mezclas de leguminosas y gramíneas pueden resultar una buena opción para la producción de forraje de calidad con un mayor contenido en proteína (Lithourgidis *et al.*, 2007) y buena producción de biomasa (Ibrahim *et al.*, 2012) con pocos costes de producción. Además, la leguminosa forrajera podría representar una manera económica de

disponer de N para el sistema suelo-planta-animal (Valles de la Mora y Cadisch, 2010). Algunas de las investigaciones consultadas sobre el alberjón lo consideran un cultivo prometedor en rotación con el trigo, para la producción de grano (Durutan *et al.*, 1990) o forraje (Van der Veen, 1960; Jacques *et al.*, 1994). Del mismo modo, la avena negra, ha sido utilizada en numerosos estudios como componente de mezclas forrajeras (Bremm *et al.*, 2005; Salgado *et al.*, 2013). Por consiguiente, la evaluación de la viabilidad de estas dos especies como mezcla forrajera podría ser también de gran interés para su empleo como nuevo cultivo en rotación con el trigo duro. Su introducción en grandes explotaciones serviría para cumplir con el requisito de la SIE de la PAC que permite la mezcla de cultivos fijadores de N con otros cultivos en España a partir de 2018 (apartado 1.5.1. de este documento). Además, esta posibilidad ayudaría al agricultor a producir fuentes de aprovisionamiento de calidad para la alimentación animal manteniendo o mejorando los niveles de N en el suelo, de gran importancia entre los requerimientos de la PAC, y permitiría potenciar la creación de sinergias entre la actividad agrícola y ganadera.

Por todo lo expuesto anteriormente, esta investigación persigue el estudio de la viabilidad de introducir cultivos intercalares y nuevos cultivos alternativos rentables en la rotación trigo duro-girasol de la campiña andaluza. Como se ha explicado a lo largo de este capítulo, las condiciones edafoclimáticas de la campiña andaluza tienden a reducir la capacidad productiva del trigo duro y girasol. Este hecho hace especialmente importante la determinación de las posibles pérdidas por competencia con los cultivos principales, especialmente la capacidad de competir por agua con el cultivo siguiente en rotación y el efecto de estas prácticas y alternativas en los rendimientos obtenidos. Los resultados de su estudio permitirán por tanto determinar las posibilidades reales de estos cultivos intercalares y nuevos cultivos así como las estrategias que será necesario diseñar si llegaran a implantarse, para evitar sus posibles perjuicios y obtener de ellas los máximos beneficios.

## **1.7 OBJETIVOS**

El actual marco de desarrollo y necesidades del sector de cultivos herbáceos en condiciones de secano, nos ha llevado a plantear una serie de preguntas: ¿Es posible introducir cultivos intercalares de ciclo corto en la rotación trigo duro-girasol?

¿Reducen los cultivos intercalares el contenido de agua en el suelo? ¿Su introducción mejora la fertilidad del suelo a lo largo de la rotación? ¿Tienen algún efecto sobre la sanidad, rendimientos y calidad de los cultivos principales? ¿Presenta esta técnica de AC mayores beneficios agronómicos que los sistemas de laboreo mínimo y siembra directa? ¿Es viable como alternativa de cultivo en las rotaciones una mezcla forrajera gramínea-leguminosa en base a su nivel productivo y de calidad del forraje? ¿Ejerce además esta mezcla algún efecto en la economía del N en la rotación?

Para responder a estas preguntas, en esta tesis se ha planteado como objetivo general **proporcionar alternativas sostenibles a la rotación tradicional trigo duro-girasol en los secanos andaluces, mediante la introducción de cultivos intercalares y nuevos cultivos**. Para la consecución de este objetivo general, y para responder a las preguntas planteadas anteriormente, se han establecido dos objetivos específicos:

Objetivo específico 1.- Evaluar la viabilidad de la introducción de mostaza blanca (*Sinapis alba* subsp. *mairei*) y alberjón (*Vicia narbonensis* L.) como cultivos intercalares en la rotación trigo duro-girasol sobre el desarrollo, la sanidad, los rendimientos y calidad de los cultivos principales, la disponibilidad de agua y fertilidad del suelo en la rotación.

Dada su complejidad, su estudio se ha llevado a cabo mediante cuatro subobjetivos:

- *Subobjetivo 1.1.-* Evaluar el desarrollo de los cultivos intercalares y su capacidad competitiva y de control sobre las principales malas hierbas de la rotación.
- *Subobjetivo 1.2.-* Evaluar la evolución del contenido de humedad y la fertilidad en el suelo a lo largo de la rotación.
- *Subobjetivo 1.3.-* Determinar los rendimientos, la calidad del trigo duro y del girasol en el sistema propuesto frente a los sistemas de mínimo laboreo y siembra directa de la rotación.
- *Subobjetivo 1.4.-* Determinar el efecto de los cultivos intercalares en el control de jopo de girasol (*Orobanche cumana*).



Objetivo específico 2.- Determinar la viabilidad de la mezcla alberjón-avena negra (*Vicia narbonensis*-*Avena strigosa*) como nuevo cultivo de aprovechamiento forrajero para su inclusión en las rotaciones de cultivos herbáceos de secano y su aportación de N al sistema.

Dos son los subobjetivos que permiten abordar las determinaciones del objetivo 2:

- *Subobjetivo 2.1.-* Evaluar la capacidad de alberjón y avena negra de formar una mezcla equilibrada para aprovechamiento forrajero, estableciendo la dosis óptima de siembra y su influencia en los rendimientos y la calidad nutricional.
- *Subobjetivo 2.2.-* Estudiar la capacidad de *Vicia narbonensis* de fijar N atmosférico en mezcla con *Avena strigosa* a distintas dosis de siembra, y la posible transferencia del mismo a *Avena strigosa* que permita conocer el aporte final de N<sub>2</sub> fijado simbióticamente al sistema.

Es importante resaltar que esta investigación propone el estudio de alternativas que puedan dar algún tipo de respuesta positiva a la difícil situación del sector de los cultivos herbáceos de secano en la campiña andaluza. Sin embargo, los efectos de los ensayos de rotación de cultivos solo son observables a largo plazo. Este proyecto de tesis, por su propia duración y naturaleza, recoge investigaciones finalistas que a corto plazo podrían suponer una buena aproximación al problema desde el punto de vista ecológico, medioambiental y productivo, pero no es objeto de esta tesis el realizar un estudio de la rentabilidad económica de las alternativas de cultivo propuestas. Por tanto, este documento supone sólo el comienzo de una línea de investigación, siendo necesario un estudio a largo plazo para alcanzar conclusiones sólidas en relación a aspectos medioambientales y económicos en ensayos de campo como los contemplados en esta tesis. A pesar de ello, se ha generado importante información científica y técnica que puede ser aplicable en un futuro próximo, así como una serie de directrices útiles para las investigaciones futuras que se realicen al respecto.

**CAPÍTULO 2**  
**METODOLOGÍA GENERAL**



## 2 METODOLOGÍA GENERAL

### 2.1 ENFOQUE METODOLÓGICO Y FASES DE LA INVESTIGACIÓN

Cualquier intento de adaptación de los cultivos herbáceos de secano a los nuevos retos y exigencias de la agricultura a nivel mundial precisa de la realización de ensayos experimentales a largo plazo. Por tanto, y dada la naturaleza del tema de estudio de esta tesis, que consiste en valorar la viabilidad de modificar el manejo de suelo con la introducción de cultivos cubierta y la inclusión de cultivos alternativos en la rotación tradicional de secano, se requieren ensayos experimentales a largo plazo para llegar a resultados concluyentes. Aunque estos ensayos sobrepasan el tiempo de duración normal de una tesis doctoral, su seguimiento durante tres años nos llevará sin duda a una aproximación bastante clara de hacia dónde apuntan los resultados obtenidos.

Con el fin de abordar los objetivos definidos en el capítulo anterior, ha sido necesario realizar ensayos de campo y en condiciones controladas. Para facilitar la comprensión de las distintas fases metodológicas llevadas a cabo, presentamos a continuación un esquema explicativo en el que se exponen los ensayos experimentales que se han realizado para alcanzar cada uno de los objetivos propuestos (Figura 6).

En la primera fase de este estudio (**capítulo 3**) se ha evaluado **la introducción de mostaza blanca (*Sinapis alba* subsp. *mairei*) y alberjón (*Vicia narbonensis* L.) como CC en la rotación trigo duro-girasol (objetivo específico 1)**. Para determinar la viabilidad de estas especies como cultivos intercalares, ha sido necesario realizar 2 ensayos experimentales (experimentos A.1 y A.2), con los que se ha intentado dar respuesta a 3 subobjetivos:

- *Subobjetivo 1.1.*- Evaluar el desarrollo de los cultivos intercalares y su capacidad competitiva y de control sobre las principales malas hierbas de la rotación.
- *Subobjetivo 1.2.*- Evaluar la evolución del contenido de humedad y la fertilidad en el suelo a lo largo de la rotación.
- *Subobjetivo 1.3.*- Determinar los rendimientos, la calidad del trigo duro y del girasol en el sistema propuesto frente a los sistemas de mínimo laboreo y siembra directa de la rotación.

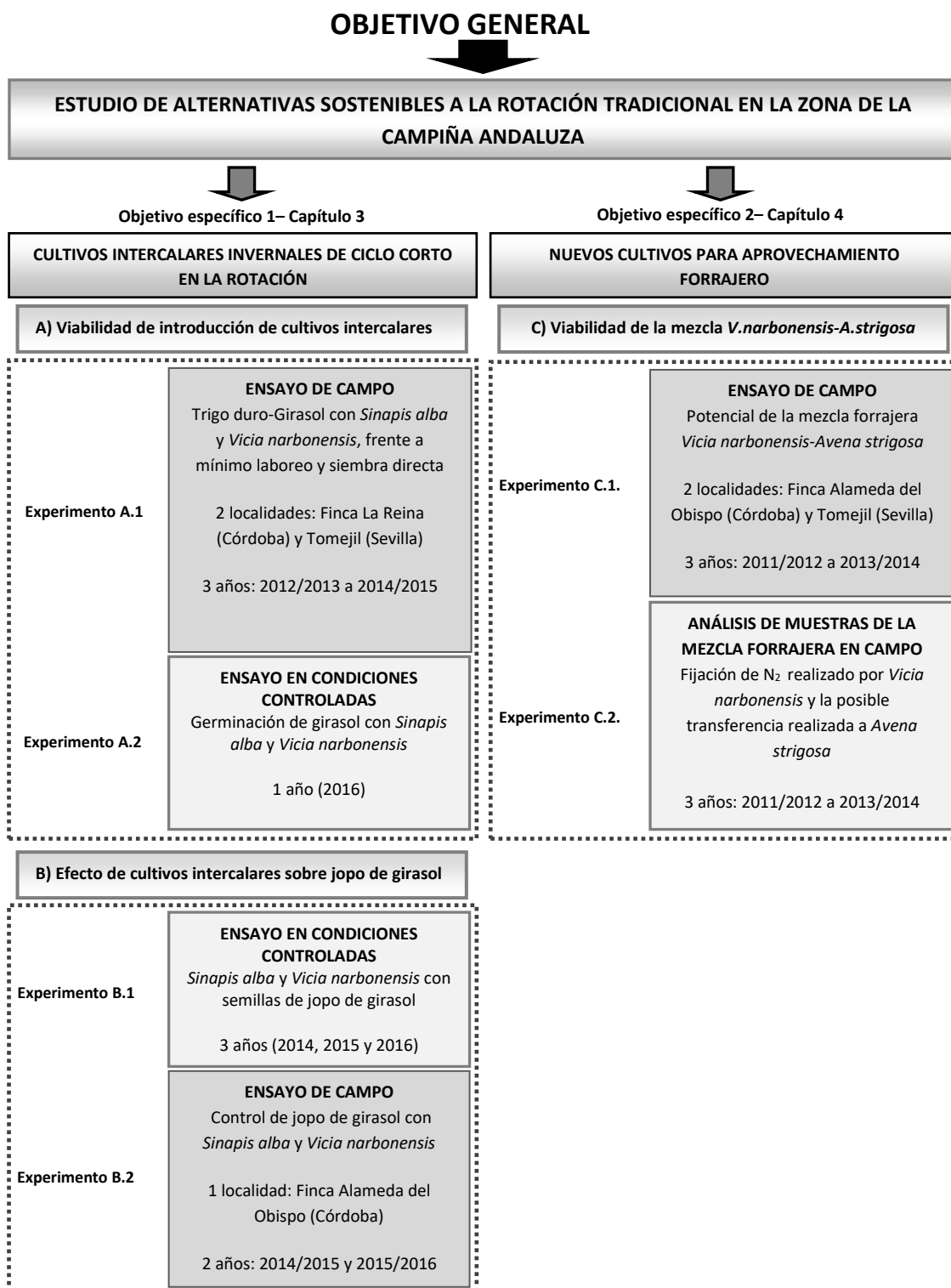


Figura 6. Fases metodológicas de la investigación y relación entre los ensayos experimentales realizados y los distintos objetivos propuestos.

Fuente: Elaboración propia

Adicionalmente, se ha estudiado el efecto de los cultivos intercalares sobre el jopo de girasol (*Orobanche cumana*), realizando otros 2 ensayos experimentales (experimento B.1 y B.2) que dan respuesta al subobjetivo 1.4.:

- **Subobjetivo 1.4.-** Determinar el efecto de los cultivos intercalares en el control de jopo de girasol (*Orobanche cumana*).

A continuación se exponen las principales características de los ensayos de campo y en condiciones controladas realizados para la consecución del objetivo específico 1 (Figura 6):

**Experimento A.1. Ensayo de campo trigo duro-girasol de larga duración.** Este ensayo de campo ha permitido estudiar los subobjetivos 1.1., 1.2. y 1.3. En él se ha implantado y evaluado un nuevo sistema de manejo de la rotación trigo duro-girasol durante 3 años consecutivos (campañas agrícolas 2012/2013, 2013/2014 y 2014/2015). La introducción de los cultivos intercalares se ha llevado a cabo tras el cultivo del trigo y hasta la siembra del girasol, en el período de tiempo en que el suelo queda libre (de octubre a marzo, aproximadamente) y que constituye el período de máxima precipitación y mayores riesgos de erosión (Figura 7). Los cultivos intercalares empleados en el ensayo han sido una crucífera y una leguminosa de ciclo corto y desarrollo invernal rápido: las especies mostaza blanca (*Sinapis alba* subsp. *mairei* cv. Albendín) y alberjón (*Vicia narbonensis* L.), respectivamente.



Figure 7. Parcelas con trigo duro (a), cultivos intercalares de mostaza blanca (b) y alberjón (c), y girasol (d) en el ensayo de campo de larga duración.

**Experimento A.2. Ensayo en condiciones controladas con girasol.** Para completar la investigación del subobjetivo 1.3. ha sido necesario realizar también un ensayo en condiciones controladas. Este se ha efectuado durante el año 2016, tras considerar necesaria su ejecución para completar los resultados obtenidos con girasol en el ensayo de campo de largo duración. Para ello, en primer lugar se ha evaluado el porcentaje de germinación de las semillas de girasol en contacto con las enmiendas de

mostaza blanca y alberjón en placas Petri (Figura 8.a). Posteriormente, el ensayo se realizó en macetas, para ver el efecto de la incorporación de los restos de estos dos cultivos sobre la emergencia y desarrollo del girasol en sus primeros estadios de crecimiento y la duración de dicho efecto (Figura 8.b y c).



Figura 8. Disposición de las placas (a) y macetas de girasol recién emergidas (b) y en estado de plántula en la cámara de crecimiento (c).

**Experimento B.1. Ensayo en condiciones controladas de cultivos intercalares con semillas de jopo de girasol.** Este estudio ha permitido abordar el subobjetivo 1.4., con el fin de determinar si los restos vegetales de mostaza blanca y alberjón pueden ser una medida de control cultural del jopo de girasol (*Orobanche cumana*). Tal y como se describió en el apartado 1.6. del capítulo 1, es conocida la acción biofumigante de las crucíferas contra patógenos y enfermedades de suelo y el efecto positivo de algunas especies leguminosas sobre el control de malas hierbas y enfermedades cuando actúan como cultivos cubierta. En consecuencia, se ha considerado interesante evaluar si existe algún efecto sobre la germinación de las semillas de jopo de girasol, bien por inhibición de la germinación o por la estimulación de la misma favoreciendo una germinación suicida. Para ello, se ha llevado a cabo un ensayo durante 3 años (2014, 2015 y 2016) en condiciones controladas (Figura 9).



Figura 9. Preparación del ensayo con mostaza blanca y alberjón en condiciones controladas.

**Experimento B.2. Ensayo de campo de cultivos intercalares en girasol y su efecto sobre la germinación de jopo.** Este ensayo experimental completa al ensayo anterior para la consecución del subobjetivo 1.4. De este modo, ha sido posible

comprobar si el efecto *in vitro* de los restos vegetales de mostaza blanca y alberjón sobre la germinación del jopo se asemeja al observado en condiciones reales de campo (Figura 10), sin estimulantes de la germinación y con multitud de factores bióticos interactuando simultáneamente. Este estudio se ha realizado durante 2 años consecutivos (campañas agrícolas 2014/2015 y 2015/2016).



Figura 10. Ensayo de campo con los cultivos intercalares creciendo (a) y posteriormente con el cultivo de girasol en estado de floración sobre dichas parcelas (b).

La explicación concisa y detallada de cada uno de estos ensayos, así como la metodología específica y determinaciones llevadas a cabo en cada uno de ellos han sido incluidas en el capítulo 3 de esta tesis doctoral.

En la segunda fase de la investigación (**capítulo 4**) se estudió **la viabilidad de la mezcla alberjón-avena negra (*Vicia narbonensis*-*Avena strigosa*) como nuevo cultivo de aprovechamiento forrajero para su inclusión en las rotaciones de cultivos herbáceos de secano y su aportación de N al sistema (objetivo específico 2)**. Este estudio fue abordado mediante dos subobjetivos:

- *Subobjetivo 2.1.*- Evaluar la capacidad de alberjón y avena negra de formar una mezcla equilibrada para aprovechamiento forrajero, estableciendo la dosis óptima de siembra y su influencia en los rendimientos y la calidad nutricional.
- *Subobjetivo 2.2.*- Estudiar la capacidad de *Vicia narbonensis* de fijar N atmosférico en mezcla con *Avena strigosa* a distintas dosis de siembra, y la posible transferencia del mismo a *Avena strigosa* que permita conocer el aporte final de N<sub>2</sub> fijado simbióticamente al sistema.

Su análisis se ha llevado a cabo mediante la realización de un ensayo experimental en condiciones de campo (experimento C) (Figura 6):

**Experimento C. Ensayo de campo de la mezcla forrajera *Vicia narbonensis*-*Avena strigosa*.** Se ha realizado un ensayo de campo (experimento C.1.) durante 3



años consecutivos (campañas agrícolas 2011/2012, 2012/2013 y 2013/2014). En él se ha determinado el potencial como cultivo forrajero de una nueva mezcla leguminosa-gramínea en rotación con trigo duro, la mezcla forrajera alberjón (*V. narbonensis*)-avena negra (*A. strigosa*) (Figura 11). Al suponer una novedad en la campiña andaluza, ha sido necesario determinar la dosis de siembra y proporción óptima de cada especie en la mezcla forrajera. Su estimación resulta de gran importancia, ya que de la proporción de ambas especies en el cultivo mixto va a depender la obtención de una mezcla equilibrada que permita obtener altos rendimientos y contenido en proteínas.



**Figura 11. Vista general del ensayo de campo de la mezcla forrajera en distintos momentos de desarrollo del cultivo.**

Adicionalmente, como continuación del estudio y con el fin de responder al subobjetivo 2.2., se ha considerado importante estimar el aporte de N realizado al sistema, mediante la cuantificación del  $N_2$  que ha sido fijado simbióticamente por la leguminosa y el posible aporte de N realizado a la gramínea. Para ello, se ha realizado un análisis de muestras del ensayo de campo (experimento C.2.) según el método de evaluación de la fijación de N por abundancia natural del isótopo estable  $^{15}N$  (López-Bellido *et al.*, 2006). El análisis se ha llevado a cabo en el Laboratorio de Isótopos Estables y Espectrometría de Masas (LISEEM) del centro IFAPA Alameda del Obispo (Córdoba). Cada especie fue procesada individualmente para cada una de las 5 dosis de siembra alberjón-avena negra (Figura 12).



**Figura 12. Preparación del ensayo de muestras para su análisis mediante el método de abundancia natural del isótopo estable  $^{15}\text{N}$  en el espectrómetro de masas.**

Una vez finalizada esta fase de la investigación, la justificación de la viabilidad de la mezcla forrajera se ha podido realizar en base a los rendimientos y calidad nutricional (subobjetivo 2.1.), así como desde un punto de vista medioambiental (subobjetivo 2.2.).

Los detalles de la metodología usada en este experimento C, así como los resultados obtenidos, han sido desarrollados en el capítulo 4. Entre los distintos ensayos realizados para la consecución de los objetivos específicos propuestos (capítulo 3 y 4), existen aspectos metodológicos comunes como las áreas y años de estudio y especies utilizadas, por ello, se ha considerado útil realizar una explicación detallada y conjunta de los mismos, tal y como veremos a continuación.

## 2.2 ÁREAS Y AÑOS DE ESTUDIO

Todos los ensayos de campo abordados en esta tesis doctoral se realizaron en el área de secano de la zona Mediterránea del sur de España, concretamente en el Valle del Guadalquivir. Las tres localidades en las que se instalaron los ensayos pertenecen a las provincias de Córdoba y Sevilla. Específicamente se llevaron a cabo en las fincas de experimentación agraria IFAPA Alameda del Obispo (Córdoba), finca IFAPA Tomejil (Carmona, Sevilla) y la finca privada La Reina (Santa Cruz, Córdoba) (Figura 13).

Los suelos de las fincas localizadas en el área de estudio de la provincia de Córdoba están clasificados dentro del Orden *Entisol*, Grupo *Xerofluvent* y Sub-grupo *Típico*. Por otro lado, el suelo correspondiente a la finca del área de estudio de Sevilla es del Orden *Vertisol*, Grupo *Haploxerert* y Sub-grupo *Crómico* (USDA, 2014). En la Tabla 3 se recogen algunas de las principales características de los suelos en cada una de las localidades.

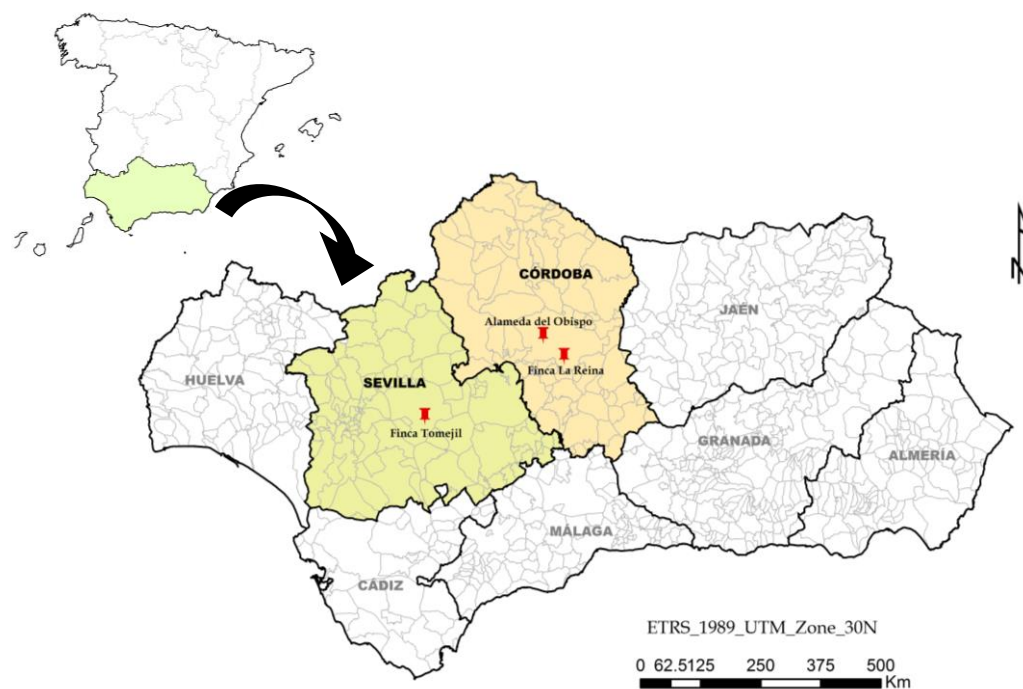


Figura 13. Localización geográfica de las áreas de estudio abordadas.

Fuente: Elaboración propia

Tabla 3. Características principales de las localidades estudiadas.

	ÁREAS DE ESTUDIO		
	Finca IFAPA Alameda del Obispo (Córdoba)	Finca IFAPA Tomejil (Carmona, Sevilla)	Finca La Reina (Santa Cruz, Córdoba)
<b>LOCALIZACIÓN GEOGRÁFICA</b>	37°51'42"N 04°48'00"O	37°24'07"N 05°35'10"O	37°45'03"N 04°39'22"O
<b>TEXTURA DEL SUELO</b>	Franca	Arcilla	Franca
<b>CARACTERIZACIÓN DEL SUELO</b>			
<i>pH</i>	7,61	7,90	7,63
<i>MO (%)<sup>2</sup></i>	1,15	1,67	1,13
<i>P Olsen (ppm)</i>	12,20	24,0	11,98
<i>K asimilable. (ppm)</i>	289	634	270
<i>CIC (cmol/kg)<sup>3</sup></i>	14,01	53,91	33,31
<i>Carbonatos (ppm)</i>	13,85	25,39	23,62
<i>Caliza activa (%)</i>	2,88	5,06	7,00
<i>N orgánico (%)</i>	0,09	0,07	0,08

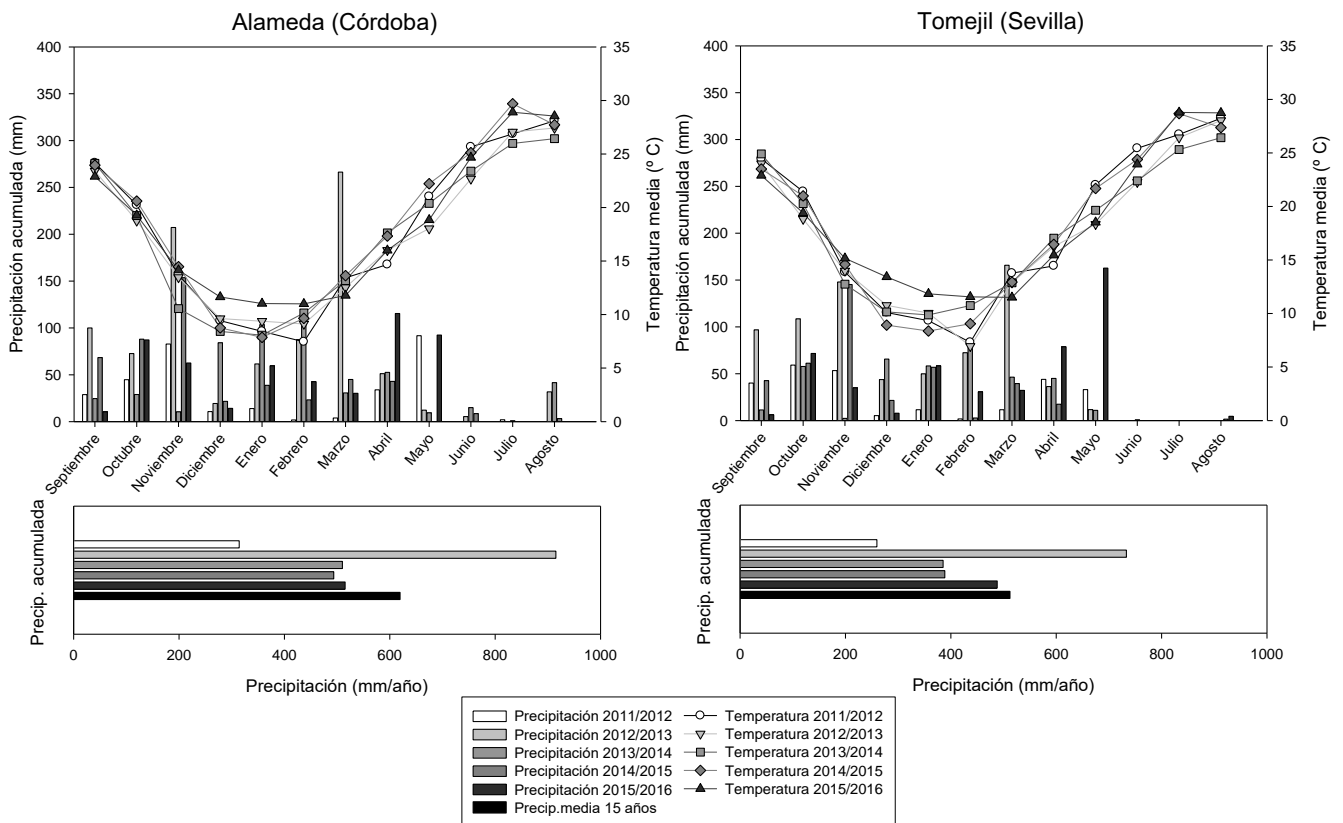
<sup>1</sup>La caracterización de suelo se realizó en las parcelas experimentales al inicio del estudio

<sup>2</sup>MO: Materia Orgánica

<sup>3</sup>CIC: Capacidad de Intercambio Catiónico

Las fincas experimentales pertenecientes al IFAPA, tanto Alameda del Obispo como Tomejil (en adelante Alameda y Tomejil, respectivamente), cuentan con estaciones agroclimáticas situadas en un radio de 500 m alrededor de las ensayos

realizados. La finca La Reina de Santa Cruz (en adelante Santa Cruz) dista menos de 24 km de la finca Alameda de Córdoba. Por ello, los parámetros meteorológicos presentados en esta investigación se corresponden con los datos registrados en dichas estaciones agroclimáticas (Alameda y Tomejil). El período de estudio de dichas parámetros se corresponde con las campañas agrícolas comprendidas entre los años 2011-2016 y han sido recogidos en la Figura 14. La pluviometría registrada en cada localidad se muestra como precipitación acumulada mensual y a lo largo de cada campaña agrícola (desde septiembre a agosto). La temperatura media se ha obtenido como promedio entre las temperaturas máximas y mínimas registradas mensualmente en cada localidad.



**Figura 14.** Distribución de la pluviometría y temperatura media mensual registrada en Alameda (Córdoba) y Tomejil (Sevilla) a lo largo de las campañas agrícolas comprendidas entre 2011 y 2016. Para cada campaña agrícola, la pluviometría se muestra como precipitación acumulada mensualmente y a lo largo de toda la campaña agrícola (desde septiembre a agosto) respecto a la precipitación media de los últimos 15 años (2001-2016).

La precipitación acumulada en los años objeto de estudio con respecto al promedio registrado en los últimos 15 años (media histórica 2001-2016), refleja la gran variabilidad interanual de precipitaciones que puede darse en esta zona. La

precipitación media anual ha sido de 620 mm en Alameda del Obispo y de 512 mm en Tomejil. No obstante, se han registrado años anormalmente secos con lluvias en torno a 300 mm (año agrícola 2011/2012) y otros extraordinariamente húmedos con más de 700 mm (año agrícola 2012/2013). Los años agrícolas 2013/2014 y 2014/2015 también han sido más secos que el promedio histórico, pero las diferencias han sido menos pronunciadas (en torno a 120 mm menos registrados) en ambas localidades. En cambio, la precipitación acumulada durante el año agrícola 2015/2016 ha sido la más parecida a la media histórica, con 515 mm en Alameda y 488 mm en Tomejil.

En cuanto a las temperaturas, las medias oscilan entre los 9 °C en invierno y los 27-28 °C en verano. Las temperaturas medias más bajas, en torno a los 7 °C, han sido registradas en el mes de febrero durante los años agrícolas 2011/2012 y 2012/2013. En cambio, el año agrícola 2015/2016 registró el invierno más caluroso de todo el período de estudio en ambas localidades, con temperaturas en torno a los 11 °C en Alameda y superior a los 12 °C en Tomejil. Del mismo modo, la campaña 2015/2016 mostró los veranos más calurosos de todo el período, con temperaturas medias en torno a los 29 °C, especialmente en Tomejil, lo cual implicó un mayor número de días con temperaturas máximas igual o superior a los 40 °C en dicha zona.

## 2.3 ESPECIES

Como se ha ido avanzado hasta el momento, esta investigación está centrada en la búsqueda de alternativas sostenibles a la rotación trigo duro-girasol, sistema de cultivo prioritario en la campiña andaluza. Por ello, se describen a continuación las características más destacadas de las especies utilizadas como cultivos principales en la rotación, así como aquellas empleadas como cultivos intercalares y como nuevos cultivos alternativos.

### 2.3.1 CULTIVOS PRINCIPALES DE LA ROTACIÓN TRIGO DURO-GIRASOL

Seguidamente se describen las principales características de las especies y variedades empleadas como cultivos principales en los diferentes ensayos realizados.

#### 1. Trigo duro [Familia Poaceae (=Gramineae)]:

- *Triticum durum* cv. Amilcar. Es una de las variedades certificadas más sembradas en España y Andalucía. De espiga aristada, blanca y con barbas negras

(Figura 15), está perfectamente adaptada a la zona Occidental y Oriental de Andalucía. De hecho, es una de las variedades testigo nacional utilizada como tal en todos los ensayos oficiales realizados por la Red Andaluza de Experimentación Agraria (RAEA).



**Figura 15. Trigo duro (*Triticum durum* cv. Amilcar) en distintos momentos del desarrollo del cultivo, espigado (a) y madurez completa (b), en los ensayos de campo realizados.**

Se trata de una variedad de ciclo medio en cuanto a espigado y de ciclo corto en cuanto a maduración. Posee un alto vigor de nascencia y buen ahijamiento. Además, es un trigo de muy alta rusticidad, lo que le proporciona seguridad en muy distintas situaciones. El grano es de muy buena calidad, de tamaño medio, destacando su elevado peso específico, alta vitrosidad y alto contenido en proteínas. Por ello, según los ensayos de la RAEA, es una variedad normalmente clasificada en los grupo 1 y 2 de calidad según Real Decreto 190/2003 (Castilla *et al.*, 2013, 2014; Canseco *et al.*, 2016). En cuanto a enfermedades, es muy tolerante a Roya Parda (*Puccinia Triticina*), Septoria (*Septoria Tritici*) y Oidio (*Blumeria graminis*, f.sp. Tritici) y en dichos ensayos experimentales realizados por RAEA siempre han presentado altas producciones y una alta estabilidad en rendimiento.

## **2. Girasol [Familia Compositae (= Asteraceae)]:**

- *Helianthus annuus* cv. Transol. Es la variedad más vendida de España y la más productiva en Andalucía (Figura 16.a). Los rendimientos conseguidos año tras año la sitúan como líder del mercado convencional (linoleica). De hecho, al igual que la variedad Amilcar de trigo duro, es una de las variedades testigo nacional en todos los ensayos oficiales realizados para girasol en la RAEA, consiguiendo la máxima producción en todo tipo de suelos y condiciones climatológicas en los últimos años



(García Ruíz, 2011, 2012, 2013, 2016; García Ruíz y García López, 2015, 2016). Tiene un ciclo muy precoz, a floración y a maduración y un excelente vigor de nascencia. Además, se caracteriza por poseer una gran rusticidad y resistencia a la raza “F” de jopo de girasol (*Orobanche cumana*).

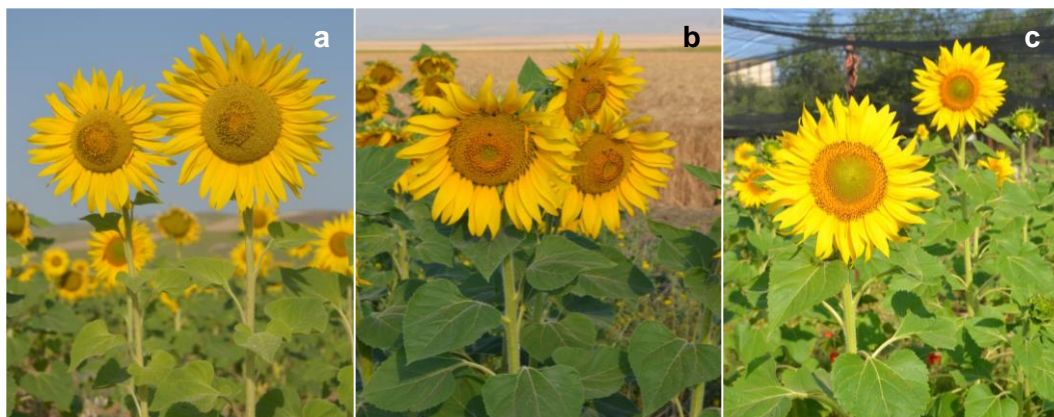


Figura 16. Variedades de girasol (*Helianthus annuus*) utilizadas en los distintos ensayos experimentales realizados: variedad Transol (a), variedad ExpressSun TM P64LE29 (b) y variedad Sambro (c).

- *Helianthus annuus* cv. P64LE29. A partir del año 2013 se incorporaron a los ensayos experimentales variedades con tecnología denominada ExpressSun™, como la variedad P64LE29. Es una variedad de girasol (linoleico) de ciclo muy corto en floración y maduración y buen vigor de nascencia (Figura 16.b). Se trata de un híbrido que lleva incorporado genéticamente la tolerancia a un herbicida de la familia de las sulfonilureas, el “tribenurón-metil”, que ofrece un amplio espectro de control sobre malas hierbas de hoja ancha en post-emergencia de las mismas. Además, presenta un buen nivel de protección ante las principales enfermedades del girasol: resistencia a jopo, máxima resistencia a Mildiu (*Pasmopara halstedii* (Farl.) Berl. y de Toni) y alta tolerancia a Verticilosis (*Verticillium dahliae* Kleb). Este hecho, unido a su gran tolerancia a la sequía, hace que sea muy estable ante todo tipo de situaciones en campo. Además, los ensayos oficiales realizados con esta variedad han mostrado unos niveles de producción y contenido en aceite aceptables (García Ruíz y García López, 2014, 2015).

- *Helianthus annuus* cv. Sambro MR. Sambro es una variedad de girasol híbrido simple con un ciclo corto a floración y ultra corto a recolección. Se seleccionó para la realización del ensayo de campo del efecto de cultivos intercalares sobre el

jopo de girasol, ya que es resistente a las razas A, B, C, D y E pero susceptible a la raza "F" de jopo (García Ruíz, 2006). Tiene un excelente vigor de nascencia en secano, gran resistencia al estrés y gran rusticidad. También posee una gran sanidad, con tallos que se mantienen verdes hasta la cosecha y un buen potencial de producción en todas las condiciones (Figura 16.c).

### 2.3.2 CULTIVOS INTERCALARES Y NUEVOS CULTIVOS EN LA ROTACIÓN

Todas las especies utilizadas en esta investigación como cultivos intercalares o nuevos cultivos pertenecen a las familias crucíferas, leguminosas y gramíneas, tal y como se explicó previamente en el apartado 1.6. del capítulo 1. La selección de las mismas se basó en conseguir ventajas adicionales en la rotación y diferentes aprovechamientos rentables para el agricultor. A continuación se exponen las principales características y posible utilidad de cada una de ellas.

#### 1. Familia Cruciferae (= Brassicaceae):

- *Mostaza blanca* (*Sinapis alba* subsp. *mairei* cv. Albendín). Se trata de una especie anual, dicotiledónea, que presenta características muy adecuadas para emplearla como cubierta vegetal en cultivos leñosos, como cultivo intercalar en cultivos herbáceos y como biofumigante en condiciones de secano (Saavedra *et al.*, 2016b). En Andalucía, la variedad de mostaza blanca registrada es el cultivar "Albendín", al estar adaptado a las condiciones del sur (patente 29205-INIA-IFAPA) (Figura 17).

Con un ciclo vegetativo medio-corto, emerge y se desarrolla rápidamente con las primeras lluvias (40-50 mm), presentando floración desde final de invierno a principios de primavera. Es útil por tanto para formar una cubierta vegetal de gran tamaño en poco tiempo, reduciendo así los riesgos de erosión y degradación del suelo (De Baets *et al.*, 2011; Björkman *et al.*, 2015). Simultáneamente, la elevada formación de biomasa producida ayuda a descompactar el suelo con la raíz pivotante que posee la especie, reteniendo y evitando el lavado de nutrientes, especialmente el N (Alcántara *et al.*, 2009). Además, presenta otras características que facilitan su manejo, como una elevada producción de semilla, que favorece su instalación al año siguiente, y una alta capacidad de competición con la hierba, que puede conducir a una reducción en el uso de herbicidas necesarios (Alcántara *et al.*, 2011a). Por otro lado, la



cubierta vegetal se puede emplear para biofumigar el suelo, al ser una especie grande (de 50 a 120 cm de altura) y contener importantes cantidades de glucosinolatos (Angus *et al.*, 1994). Cuando se incorpora al suelo, estas sustancias se hidrolizan dando lugar a sustancias que son tóxicas para los patógenos del suelo, resultando una desinfección del mismo *in situ* y a bajo coste de cultivo (Alcántara *et al.*, 2011b).



Figura 17. Características de la mostaza blanca (*Sinapis alba* subsp. *mairei* cv. Albendín).

## 2. Familia Leguminosae (= Fabaceae):

- *Alberjón* (*Vicia narbonensis* L.). El alberjón o haba loca es una especie herbácea originaria del noroeste de Asia, desde donde se distribuyó su cultivo a la Cuenca Mediterránea. Es una especie muy parecida a las habas (*Vicia faba* L.) salvo por los zarcillos presentes en sus hojas (compuestas por 2-6 pares de folíolos), por sus vainas cilíndricas y granos redondeados, más parecidos a los de los guisantes (Figura 18). Además, es una leguminosa de crecimiento invernal rápido frente a otras especies como la veza o las habas y supera fácilmente un metro de altura. Su sistema radicular está muy desarrollado, con raíces profundas y bien ramificadas, lo que le proporciona una buena adaptación a condiciones de secano (Nadal *et al.*, 2012). De hecho, sus necesidades hídricas se sitúan a partir de 250-300 mm. A pesar de la gran diversidad de material vegetal existente en la especie, son muy escasas las variedades vegetales comerciales disponibles en nuestro país. Inscritas en los Registros de Variedades Protegidas y Comerciales hay solo tres variedades, Nera, Ylera y María (patente 050202-IFAPA), esta última con cierta tolerancia al jopo (*Orobanche crenata* Forsk.) (Nadal *et al.*, 2007). En esta investigación se ha utilizado una línea híbrida mejorada

(línea 6) del Banco de Germoplasma de leguminosas del centro IFAPA Alameda del Obispo.



Figura 18. Características de la planta de alberjón (*Vicia narbonensis* L.).

El alberjón ha sido cultivado para diversos aprovechamientos y usos: como leguminosa grano para alimentación animal, por el alto contenido proteico de sus granos y sus importantes producciones en condiciones de cultivo de mínimos insumos (Siddique *et al.*, 1996); como planta productora de forraje, por su gran producción de materia seca en climas cálidos y secos (Abd El Moneim, 1992; Larbia *et al.*, 2010); como abono verde, aumentando la materia orgánica del suelo; como donadora de N en sistemas de cultivo mixto (Mofrad *et al.*, 2010) y como cultivo trampa para “limpiar” el banco de semillas de jopo (*Orobancha crenata*) del suelo (Nadal y Moreno, 2007).

- *Veza común* (*Vicia sativa* cv. Vaguada). La veza es una especie herbácea originaria del centro y sur de Europa y del área Mediterránea. Sus hojas son paripinnadas con 1-8 pares de foliolos, los superiores con un zarcillo ramificado (Figura 19). Es una planta muy cultivada en todo el planeta, al estar adaptada a ambientes mediterráneos y templados (Lithourgidis *et al.*, 2007). De hecho, resiste altas temperaturas pero necesita de precipitaciones superiores a los 350 mm anuales. A diferencia del alberjón, su crecimiento invernal es lento y su altura no suele superar los 80 cm, produciendo menor cantidad de biomasa.

El aprovechamiento principal de esta planta radica en su uso como abono verde o como forraje, aportando una cantidad importante de proteína (Velázquez-Beltrán *et al.*, 2002). Por lo tanto, puede establecerse como cultivo monófito, pero se aconseja sembrarlo con un cereal o una gramínea pratense para beneficiarse de la fijación de N

atmosférico que esta leguminosa realiza debido a la existencia de nódulos radicales por las bacterias del género *Rhizobium*. Así, la veza común junto a cereales de invierno y verano, dátilo, festuca o raigrás es una de las mezclas más utilizadas para la obtención de forrajes conservados (Muslera y Ratera, 1991). Por las especiales características secas de nuestra zona, el henificado de hierba es el sistema más generalizado y la mezcla con avena blanca (*Avena sativa* L.) la más utilizada (Erol *et al.*, 2009), de ahí su establecimiento como mezcla forrajera testigo en el capítulo 4 de esta investigación.



Figura 19. Características de la planta de veza común (*Vicia sativa* cv. Vaguada) y de su cultivo como mezcla forrajera con avena común (*Avena sativa* cv. Chimene) en los ensayos de campo realizados.

### 3. Familia Gramineae (=Poaceae):

- *Avena negra* (*Avena strigosa* Schreb.). Se trata de una gramínea forrajera originaria de Europa del Norte. Con una semilla muy pequeña, su hábito de crecimiento es erecto y puede llegar a alcanzar un metro de altura (Figura 20). Su ciclo es muy corto, con buen crecimiento inicial y floración temprana. De hecho, es considerada la más precoz y rústica de las avenas. Es una avena poco exigente, que tiene un buen comportamiento en suelos pobres en nutrientes y es tolerante al frío y resistente a la sequía.

Su uso principal es la obtención de biomasa para alimentación animal, bien como componente de mezclas forrajeras (Bremm *et al.*, 2005; Salgado *et al.*, 2013) o como cultivo de cubierta mixto (Zibilske y Makus, 2009; Doneda *et al.*, 2012; Flower *et al.*, 2012), demostrándose en todos ellos una buena producción de biomasa y una menor densidad de malas hierbas presentes cuando esta especie forma parte de la

mezcla. Además, resultados previos en ensayos para la formación de biomasa en las campiñas de secano (Perea, comunicación personal) indicaron un mejor comportamiento de esta especie de ciclo corto en nuestro clima, de ahí su inclusión en nuestro estudio.



Figura 20. Características de la gramínea avena negra (*Avena strigosa* Schreb.) y su cultivo como mezcla forrajera con alberjón (*Vicia narbonensis* L.) en los ensayos de campo realizados.

- *Avena blanca* (*Avena sativa* cv. Chimene). Se trata de una gramínea ampliamente utilizada como cultivo forrajero en muchas zonas de España (Lloveras-Vilamanya, 1987; Castro *et al.*, 2000), especialmente en zonas con bajo riesgo de heladas intensas. Con un ciclo de desarrollo más largo que la avena negra, también es de porte alto y erecto. Morfológicamente, son especies muy parecidas. Avena negra tiene el ápice del lema bífido, con aristas de 3-8 mm y de 3 hasta 6 flores por espiguilla; en cambio, avena blanca tiene el lema con dos pequeños dientes, raza vez aristado y presenta de 2-3 flores por espiguilla totalmente cubiertas por las glumas (Figura 21).

Su interés principal es forrajero, ya que aporta muy buenos rendimientos cuando se dan condiciones hídricas adecuadas. De hecho, en España suele sembrarse mezclado con alguna leguminosa de ciclo similar, especialmente con veza común, tal y como se citó anteriormente, para la obtención de forraje henificado. Este es muy apetecible y de gran valor nutritivo, aunque de bajo contenido proteico. Su grano también puede aprovecharse para alimentación animal, teniendo esta variedad una gran aptitud para el doble aprovechamiento. Sin embargo, esta especie suele estar peor adaptada a condiciones de baja pluviometría, prefiriendo los climas frescos y húmedos o en nuestro caso, ambientes mediterráneos con suficientes lluvias primaverales.



**Figura 21.** Características de la gramínea avena blanca (*Avena sativa* cv. Chimene) y su cultivo como mezcla forrajera con veza común (*Vicia sativa* cv. Vaguada) en los ensayos de campo realizados.

Las áreas y años de estudio así como las especies utilizadas forman parte de metodología general de nuestro estudio. El resto de variables y determinaciones realizadas se han considerado metodología específica de cada uno de los ensayos. Por tanto, una descripción detallada de los materiales y métodos utilizados en cada uno de ellos ha sido incluida en los capítulos 3 y 4, que responden a los objetivos específicos y subobjetivos perseguidos en este trabajo.

## **CHAPTER 3**

# **INTRODUCTION OF WINTER COVER CROPS IN A TRADITIONAL DURUM WHEAT-SUNFLOWER ROTATION**



### 3 INTRODUCTION OF WINTER COVER CROPS AS SUSTAINABLE ALTERNATIVE TO SOIL MANAGEMENT SYSTEM OF A TRADITIONAL DURUM WHEAT-SUNFLOWER ROTATION

#### 3.1 SUMMARY

Inserting cover crops (CC) in the durum wheat-sunflower rotation of southern Spain could provide multiple ecological services in line with the Common Agricultural Policy (CAP) environmental requirements, but it must be ensured that their management does not imply any negative effect on the subsequent crops under rainfed Mediterranean conditions. A range of experiments (a long-term field trial with a 3-year study period, a short-term field trial, a pot experiment and three bioassays) were conducted at different locations between 2012-2016 to study 1) the biomass production and weed control as well as the residue quality of CC and their effect on 2) soil moisture, soil nitrogen and organic matter content, 3) durum wheat and sunflower growth, yield and quality and 4) sunflower broomrape control. Treatments were white mustard (CCC) and narbon bean (LCC) cover crops compared with a bare soil managed by reduced tillage (RT) and no tillage (NT). Results suggested that CCC and LCC residue retention did not have a large effect on soil improvement but produced greater ground cover than RT or NT, and therefore greater soil protection, especially LCC. Both CC reduced weed infestation with respect to RT and NT systems. Regarding water availability, the most limiting factor in the Mediterranean region, the results suggested that both CC had a limited impact on total soil moisture content and soil nitrogen (N) and organic matter (OM) contents and they could be used as an alternative to RT in different soil types with changing rainfall patterns. However, cover crops could only be an alternative to NT in years with higher rainfall than the average precipitation for the area. Their introduction in years with low rainfall could be done if CC management is improved with early sowing and early mowing (early-mid March). Moreover, a slightly higher storage of soil N and OM contents were observed in the top-soil after CC cultivation as well as a higher contribution to maintain soil fertility in subsequent crops with respect to the bare soil, particularly in LCC plots. According to the effects on main crops, CC could be an alternative to RT and NT, even improving durum wheat quality compared to NT. However, the variable and poor sunflower results did not allow elucidating the CC effects. Additional bioassays with both CC species showed a lower sunflower seed germination than the control treatment. Conversely, sunflower seedlings in pots with CC incorporation improved their growth. Moreover, other additional bioassays showed a great inhibitory effect of both CC species on broomrape germination and a limited reduced effect compared to RT under field conditions. Our results open a path of study for the winter cover crops as a promising alternative with a wide variety of environmental benefits for farmers. Further research is required to improve the system (seeding rate, N fertilization, sowing and mowing date) and investigate the long-term effects of these cover cropping systems in the traditional durum wheat-sunflower rotation.

**Keywords:** Durum wheat-sunflower rotation, crucifer and legume cover crops, soil protection, soil water content, soil fertility, weed control, main crops productivity



### 3.2 INTRODUCTION

Durum wheat (*Triticum durum*)-sunflower (*Helianthus annuus*) rotation is a common practice in rainfed arable areas in southern Spain. However, this rotation requires further modifications in the current crop management due to the agronomical difficulties, the agricultural market uncertainty and the increase in the variability of weather conditions. Nowadays, there is a continuing need to adapt the durum wheat and sunflower breeding varieties to market demands and resistance to diseases, such as septoria tritici blotch and wheat leaf rust and new sunflower broomrape races, respectively. In addition, their market prices are mainly influenced by the dependence on meteorological factors, being water scarcity and adverse weather patterns the major problem in the Mediterranean region (Cantero-Martínez *et al.*, 2007; López-Bellido *et al.*, 2007a; Campiglia *et al.*, 2015). In fact, irregular rainfall distribution and high temperatures during the growing seasons are frequent phenomena which are predicted to further increase as a result of global warming (Jacobsen *et al.*, 2012). Moreover, soils in the region typically have low organic contents and weak structure, resulting in low infiltration rates (Bilalis *et al.*, 2003), environmental degradation and soil fertility loss. Therefore, it is necessary to replenish the nutrients extracted by the crops or lost in erosion processes (Repullo-Ruibérriz de Torres *et al.*, 2012). These problems strongly influence durum wheat and sunflower responses to agronomical practices and inputs such as soil tillage, fertilizers and herbicides leading to insufficient yield stability and consequently, continuous market fluctuations.

In this context, the Common Agricultural Policy (CAP) environmental programmes have been aimed at improving and achieving more balanced and sustainable crop rotations, in addition to more diversified agricultural activities. A proper cropping system design must take into account the effects on soil quality and fertility, natural resources conservation and agricultural productivity through the accomplishment of the new environmental requirements and cross-compliance principles. Therefore, there is a need for the adoption of conservation agriculture (CA), involving minimum mechanical soil disturbance, permanent soil cover, and diverse crop species grown in sequences and/or associations (Kassam *et al.*, 2012).

Conservation tillage is an essential component of CA (Van den Putte *et al.*, 2010). These techniques minimize the disruption of the soil's structure, composition

and natural biodiversity, thereby reducing erosion and degradation, but also water loss (Holland, 2004; Lyon *et al.*, 2004). For that reason, they are commonplace in areas where preservation of soil moisture because of low rainfall is the objective, being particularly effective at sustaining crop production in semi-arid rainfed regions of America, Europe, North Africa or Australia (Halvorson *et al.*, 1999; Holland, 2004; Kassam *et al.*, 2012; Siddique *et al.*, 2012). In rainfed arable crops with Mediterranean climate conditions, it mainly encompasses no tillage (NT) and reduced tillage (RT) (López-Bellido *et al.*, 1998, 2002; Álvaro-Fuentes *et al.*, 2008; Menéndez *et al.*, 2008; Melero *et al.*, 2011a). NT occurs with no soil movement at all and chemical weed control and RT involved ploughless tillage at 10-15 cm depth, both of them leaving durum wheat straw and sunflower stalks on the soil surface after harvesting (minimum of 30 % soil cover). However, despite the many benefits of these techniques, and numerous advances over the last decades, adoption rates by farmers in rainfed arable farms of Spain are low. Recent estimates show that *ca.* 8 % and 20 % of arable land are now cultivated using NT and RT, respectively (MAPAMA, 2015b; AEAC.SV, 2016).

The unwillingness of farmers to adopt RT and NT implies that the serious water availability and soil erosion problems are slightly perceived (Cosentino *et al.*, 2015). Moreover, there can be other significant constraints affecting adoption, such as the interest in more profitable techniques (Dorn *et al.*, 2013). There are well-documented studies which indicate that these systems, especially NT, can cause some difficulties for weed management (perennials control by shallow tillage or resistant weeds by herbicides overuse), soil workability (excessive residues, soil crust formation or heavy runoff) and crop development (growth and yield) (Alcántara *et al.*, 2009; López-Garrido *et al.*, 2012; Ruehlemann and Schmidtke, 2016). In connection with undertaking involving the mentioned issue, the new CAP requirements have to apply for increasing environmental and sustainable protection, limiting the use of chemicals and obliging agricultural producers to maintain and increase the soil productivity. For these reasons, it would be desirable that farmers looking for more sustainable benefits can seek to introduce other modalities of CA for maintaining the soil fertility and quality.

A promising strategy is to grow cover crops (CC) when the main crop is absent (Kruidhof *et al.*, 2008). CC are grown for various reasons, providing multiple benefits to the agroecosystem (Hartwig and Ammon, 2002). Plant cover protects soil against

erosion by wind and water (Alcántara *et al.*, 2009; Cosentino *et al.*, 2015; Ramírez-García *et al.*, 2015a), increase water infiltration and preserve soil moisture (Clark, 2000; Alcántara *et al.*, 2011a). Consequently, they improve soil structure, soil organic matter (OM) (Kuo *et al.*, 1997; Skoufogianni *et al.*, 2013) and soil nitrogen (N) (Perdigao *et al.*, 2012; Gabriel *et al.*, 2013). Another important property of CC is the effective control of weeds (Kruidhof *et al.*, 2008; Isik *et al.*, 2009), soil borne diseases and pests in different crop systems (Altieri, 1999; Siddique *et al.*, 2012).

These benefits will only be achieved efficiently if the selected CC species are adapted to local environmental conditions and appropriate for the defined agro-ecological target. In addition, these benefits are not usually required as a whole, so farmers should first determine the primary benefits desired (Thorup-Kristensen *et al.*, 2003). In rainfed arable crop rotations in Europe, CC have been primarily used to provide surface cover as well as to improve soil fertility and suppress weeds (Mazzoncini *et al.*, 2011; Alonso-Ayuso *et al.*, 2014; Cosentino *et al.*, 2015; Plaza-Bonilla *et al.*, 2016). However, as far as we know, CC introduction in the durum wheat-sunflower rotation of southern Spain has not been assessed. This may in part be due to the strongly seasonal climate conditions, which could make difficult to maintain ground cover and plant growth. In addition, the traditional rotation incorporates durum wheat growth from autumn to spring, with stubbles retained but no crop production during hot dry summer periods. During the sunflower season, this crop can grow from spring to summer due to its deep-rooted system extracting water from below root zones of other crops (Halvorson *et al.*, 1999; López-Bellido *et al.*, 2003). For that reason, CC replacing the winter bare fallow and being killed and incorporated by mowing before the sunflower planting (from approximately October to March) could reduce erosion without diminishing water and nutrients for the subsequent main crop.

The CC species selection constitutes a crucial component of such an approach. A number of species, including gramineous, leguminous and cruciferous species have been used as CC in olive orchards growing with similar climatic conditions to those of rainfed arable crops. However, grasses are difficult to establish in dryland compacted soils and their development is insufficient to improve soil quality, decomposing and releasing nutrients slowly due to their high carbon to nitrogen (C/N) ratio (Alcántara *et al.*, 2011a).

Several *Brassicaceae* species are being introduced due to their tap root that makes them highly promising for relieving soil compaction (Wolfe, 2000). Moreover, they have a high potential for controlling soil-borne diseases (Angus *et al.*, 1994; Mayton *et al.*, 1996), weeds (Kruidhof *et al.*, 2008; Isik *et al.*, 2009) and nematodes (Mojtahedi *et al.*, 1991; Potter *et al.*, 1998) due to their high glucosinolate contents (Alcántara *et al.*, 2011b). Especially white mustard (*Sinapis alba* subsp. *mairei* cv. Albendín) is cultivated as cruciferous cover crop (CCC), due to their rapid growth and high biomass production under rainfed conditions (Alcántara *et al.*, 2009; Björkman *et al.*, 2015).

Members of the *Leguminosae* are being used increasingly as CC all over the world (Perdigao *et al.*, 2012; Ramírez-García *et al.*, 2015b). The main advantage of legume cover crops (LCC) is their ability to fix atmospheric nitrogen, which can be transferred to the main crop or remain available for the subsequent crop (Perdigao *et al.*, 2012; Tosti *et al.*, 2014). Additionally, the low C/N ratio in legume plant material can be beneficial to use for fast nutrients release on the soil surface (Hasegawa *et al.*, 2000). Furthermore, there are many other positive effects derived from LCC, such as weed control (Isik *et al.*, 2009; Brust *et al.*, 2014) and diseases and pests reduction (Trenbath, 1993; Fernández-Aparicio *et al.*, 2008). Narbon bean (*Vicia narbonensis* L.) is considered to be a promising new LCC due to its low water and nutrients demands (Durutan *et al.*, 1990; Nadal and Moreno, 2007). It is a high-yielding annual legume with rapid growth and high level of ground cover (Nadal *et al.*, 2012). In addition, there is a resistant inbred line to broomrape (*Orobanche crenata* Forsk.), one of the most serious legume pests, which can be used in broomrape-infested areas under rainfed Mediterranean conditions (Nadal *et al.*, 2007).

Given the multiple ecological services and well-proven effects of each species, CCC and LCC are likely to be a good alternative for improving diversity and physical and biological soil properties with respect to the RT or NT systems with bare fallow period in the traditional rotation. Their successful introduction requires a careful study of their growth and effect on the main crops, not only in the season of their presence in the field but in crop rotation throughout the subsequent years (Kolota and Adamezewska-Sowinska, 2013).

In this research we investigated the use of cover crops, grown solely to increase ground cover and not harvested for grain or biomass, in the traditional durum wheat-sunflower rotation of southern Spain. To do so, this chapter reports a series of experiments, which examine the effect of white mustard and narbon bean effect on different elements affecting main crops under Mediterranean conditions. Specifically, the objectives of this research were:

- 1) Evaluate the suitability of white mustard (CCC) and narbon bean (LCC) cultivation as cover crops for biomass production and weed suppression.
- 2) Determine the effect of CC residue retention on soil moisture content and soil fertility compared with a bare soil managed by RT and NT systems.
- 3) Assess the durum wheat and sunflower growth, yield and quality responses to crop rotation and cover cropping systems.
- 4) Evaluate the effect of CCC and LCC on sunflower weedy broomrape (*Orobanche cumana*) control.

### **3.3 MATERIALS AND METHODS**

#### **3.3.1 EXPERIMENTAL DESIGNS AND ASSESSMENTS**

In order to achieve the proposed objectives, different field trials, bioassays and pot experiments were carried out under Mediterranean climate conditions from 2012 to 2016. Comprehensive trial details and general aspects have been described in Chapter 2. For clarity, experimental designs and assessments are divided into the four different experiments of the work, while statistical analyses are reported together.

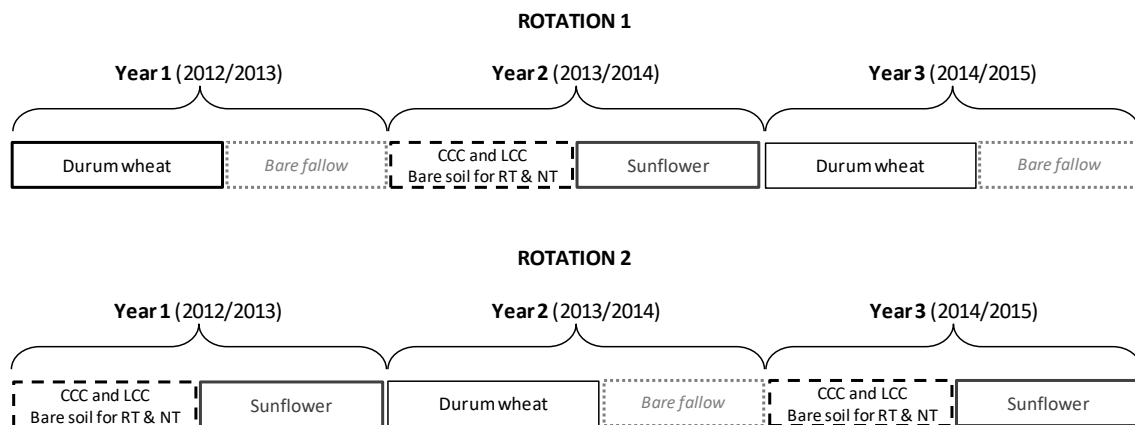
#### **A. AGRONOMIC VIABILITY OF THE INTRODUCTION OF COVER CROPS**

##### **Experiment A.1. Winter cover crops introduction in a long-term durum wheat-sunflower rotation**

This experiment was part of a larger long-term experiment initiated in 2012 and designed to determine the CC effects on durum wheat-sunflower crop rotation. The field study was conducted during the 2012/2013, 2013/2014 and 2014/2015 growing seasons (hereafter year 1, 2 and 3, respectively) at two different locations under rainfed conditions in southern Spain, Tomejil and Santa Cruz. Site characteristics of soil

properties were included in Table 3 (Chapter 2). The locations represent a typical Mediterranean climate. Mean monthly temperature and rainfall were obtained from the Weather Station at the experimental farms. A summary of the weather conditions throughout the entire research is shown in Figure 14 (Chapter 2).

Different CA systems were evaluated in a randomized complete block design. The treatments were 4 soil management systems with 4 replications: CCC and LCC compared with a bare soil managed by RT and NT. To ensure that results were achieved during 2 years for durum wheat and sunflower, two sets 3-year rotation were performed with each growing cycle from 2012 to 2015: rotation 1 (durum wheat-sunflower-durum wheat) and rotation 2 (sunflower-durum wheat-sunflower) (Figure 22). First year the previous crop was durum wheat harvested in late June at both locations. Each plot was 6 m × 30 m (180 m<sup>2</sup>), including a 2.5 m wide buffer along each side of the plots, and providing a 4 m guard between blocks and rotation sets.



**Figure 22.** Durum wheat and sunflower sequences of the two sets 3-year rotation from 2012 to 2015 (rotation 1 and rotation 2). Main crops are shown in continuous line rectangles. White mustard (CCC) and legume (LCC) cover crops and bare soil periods for reduced tillage (RT) and no tillage (NT) are shown in dashed line rectangles.

Cover crops species studied were white mustard (*Sinapis alba* subsp. *mairei* cv. Albendín) and narbon bean (*Vicia narbonensis* L., an inbred line from the IFAPA Germplasm Collection). For the first year, all the plots were cultivated under a *Triticum durum* cv. Amilcar-*Helianthus annuus* cv. Transol crop rotation. From the second year onwards, sunflower was replaced by the hibridum P64LE29 cultivar. Details of cultivars can be found in Chapter 2 (section 2.3.1.).

In the durum wheat season, durum wheat was sown in late October/November at a rate of 360 seeds·m<sup>2</sup> (188 kg/ha). N fertilizer was applied according to the planned

rate in two split applications of 50 % rate each. 200 kg/ha diammonium phosphate (DAP; N-P-K grade 18-46-0) was applied before sowing and the remaining N was applied as granular urea (46 % N) top dressing at tillering and stem elongation stages, that is, stage 21 and 31, respectively, according to the BBCH phenological scale described by Lancashire *et al.* (1991). CCC, LCC and RT plots were sown with a Solá seed drill with 3 m of wheel internal separation. Prior to sowing, RT consisted of one vibro-cultivator operation at 10-15 cm depth followed by a disc harrowing of 5 cm depth to prepare a proper seedbed. NT plots were sown with a no-till drill (Solá Super 395) and received a pre-sowing herbicide treatment (glyphosate at a rate of 2.5 l/ha). All plots received a post-emergence herbicide control of dicotyledonous weeds (Biplay-33 at a rate of 45 gr/ha) or a compound mixture for dicotyledonous and monocotyledonous control (Biplay + Traxos + Isomex + Herbenuron at a rate of 45 gr/ha + 200 cc/ha+ 25 gr/ha+ 13 gr/ha). In the latter stage, all plots were also supplied with Lovit fungicide at a rate of 1 l/ha every year. Wheat was harvested in June/early July each year, using a 1.5 m wide Wintersteiger classic plot combine.

In the sunflower season, CCC and LCC were sown on late October/November with a Solá seed drill with 3 m of wheel internal separation. Cover crops were sown at the recommended seeding rates in the area, at 15 and 110 kg/ha, respectively. The field procedures in these plots consisted of RT management followed by a roller pass after cover crops sowing. The CC were allowed to grow during winter and until the first half of April at late flowering stage (stage 63-67 of oilseed rape and faba bean BBCH scale, respectively). Both CC were mowed at ground level and immediately incorporated into the soil with a brush cutter as well as disc harrowing in Santa Cruz and with a milling machine in Tomejil. No fertilizer was applied to the CC treatments. No herbicides were used during the cover crops growing period (November-March) in any treatment. Sunflower was generally planted in late March/early April at a rate of about 69-70 thousand seeds/ha in 10 lines with 0.70 cm row spacing. CCC, LCC and RT treatments were sown with a single-seed drill with a previous RT soil preparation. In the NT plots aerial-sprayed glyphosate + oxifluorfen were applied at a rate of 2.5 + 0.1 l/ha and subsequently, they were sown with a Solá mechanical seed drill (Trisem 294 EPS). Sunflower was not fertilized. All plots were hand harvested between mid-July and the beginning of September in 5 lines by qualified staff. In both main crops, all the

treatments were soil covered with previous residues (*i.e.* durum wheat straws and sunflower stalks plus weeds). Dates of seeding, killing of the CC and main crops harvest are shown in Table 4. Furthermore, detail information on crop management dates, phenological stages and inputs applied during the different growing seasons is provided in Appendix 1 (rotation 1) and Appendix 2 (rotation 2).

**Table 4. Date of different crops sowing, white mustard (CCC) and narbon bean (LCC) mowing and durum wheat (DW) and sunflower (SF) harvest at Santa Cruz and Tomejil from 2012 to 2015.**

Crop sequence	2012/2013		2013/2014		2014/2015	
	Sowing	Kill/harvest	Sowing	Kill/harvest	Sowing	Kill/harvest
<b>Santa Cruz · Rotation 1</b>						
DW	29/11	01/07	-	-	21/11	03/06
CCC and LCC	-	-	08/11	01/04	-	-
SF	-	-	15/04	25/08	-	-
<b>Tomejil · Rotation 1</b>						
DW	07/12	04/07	-	-	12/12	04/06
CCC and LCC	-	-	06/11	26/03	-	-
SF	-	-	27/03	21/08	-	-
<b>Santa Cruz · Rotation 2</b>						
DW	-	-	27/11	17/06	-	-
CCC and LCC	15/11	01/04	-	-	23/10	10/03
SF	22/04	20/08	-	-	18/03	23/07
<b>Tomejil · Rotation 2</b>						
DW	-	-	21/11	04/06	-	-
CCC and LCC	27/11	10/04	-	-	20/10	11/03
SF	24/04	22/08	-	-	16/03	20/07

The different field assessments were grouped into three groups:

- Cover crops evaluation: plant density, ground cover, height, biomass production and weed suppression. From sowing onwards, phenological growth stages of CC based on environmental conditions were evaluated (Trudgill *et al.*, 2005). Growing degree days (GDD) were calculated based on the maximum and minimum daily temperatures with a 0°C baseline temperature for white mustard (Björkman *et al.*, 2015) and narbon bean (Mwanamwenge *et al.*, 1999). Additionally, phenological development stages of plants were monitoring based on the BBCH scale (Lancashire *et al.*, 1991) of oilseed rape and faba bean, respectively, on a time scale counted in ‘days after sowing’ (DAS) for all events of this research. Plant density (PD) was estimated counting the established CCC and LCC plants in 20 randomly selected 0.1 m<sup>2</sup> area of each plot. Plants with the first pair of true leaves were considered established plants.

Ground cover was evaluated for each cropping system treatment (CCC, LCC, RT and NT). RT and NT systems included the soil covered with previous crop residues. CCC



and LCC combined white mustard or narbon bean ground coverage, respectively, with previous main crop residues. Total weed soil coverage was separately estimated per treatment. Ground cover was determined photographically based on the methodology described by Laflen *et al.* (1981). Ten random photographs per plot were taken with a camera at the height of 1.5 m above the frame during 5 dates from January to April over the cover each year. Total percentage ground cover was determined by counting the different types of coverage in each treatment using a digital grid with 100 crossing points. The template points coinciding with green parts from each specific soil cover came to their percent coverage in each photo.

Plant height (H) was calculated at mowing time as the mean of 20 random measurements by species in each plot. Cover crop above-ground fresh biomass (CFM) for CCC and LCC treatments was evaluated by measuring the fresh weight of the above-ground parts of the plants. Four biomass samples were randomly collected from a 0.5 m<sup>2</sup> area of each plot by cutting at ground level with a sickle. At the same time and in the same area the weed above-ground fresh biomass (WFM) was cut at ground level and collected. Cover crops species were separated by hand from all other plants (total weeds) and immediately fresh weighted. In the RT and NT treatments only weed biomass was collected and fresh weighted. Then, weed species were separately identified and individually fresh weighted at laboratory.

For determining the residue quality, the previous harvested material (CC species and weeds) was dried for 48 h in a forced air oven at 70 °C. A 50 g subsample from the oven-dried samples (CC species and total weeds) was used for C/N ratio determination. The C/N ratio was calculated by dividing the organic carbon concentration by the organic N concentration. The organic carbon was determined by weight loss-on-ignition method (Davies, 1974) and the organic N was obtained by Kjeldahl method (Kjeldahl, 1883).

- Soil analyses. Soil moisture contents were measured by gravimetric analysis. The measurement period started in October 2012 and ended in July 2014. The moisture content was determined at 10 cm intervals to a depth of 60 cm, which was the zone most markedly influenced by the treatments and with the highest root activity. Data were acquired collecting two soil samples per plot at three depths: 0-10, 10-30 and 30-

60 cm. Soil samples were taken with a stainless steel core having an interior diameter of 2 cm and were immediately deposited into glass tubes prevented contamination between depths. Gravimetric soil water content (g/g) was determined by drying soil samples at 105°C for 48 h. Samples were collected at monthly intervals, with the exception of the durum wheat season from the second year onwards, when soil measurements were collected in 4 different moments: before durum wheat sowing, end of tillering-beginning of durum wheat stem elongation (BBCH 29-30), heading (BBCH 55) and early-medium milk (BBCH 73-75).

Additionally, at the beginning of the experiment three soil samples were extracted from each horizon at 0–10 and 10–30 cm depth intervals per plot for soil physical characterization. Thereafter, further soil samples were extracted at CC sowing and killing and at durum wheat sowing and harvest for soil organic N and soil OM content determination. One bulk sample was obtained from each horizon per plot, which consisted of three sub-samples each from 3 positions located in a fixed pattern across each plot. The organic N was determined by Kjeldahl method (Kjeldahl, 1883) and oxidizable OM was determined by the Walkley and Black method (Nelson and Sommers, 1982).

- Durum wheat and sunflower establishment, yield and quality. Phenological growth stages and development of main crops were assessed as the GDD accumulated with a 0°C baseline temperature for wheat (Bauer *et al.*, 1984) and a 7°C baseline temperature for sunflower (Qadir and Malik, 2007) in addition to the BBCH scale of cereals and sunflower, respectively, throughout their lifecycle.

Durum wheat plant density (PD) was scored by counting the plants contained in 1 m of 20 random rows per plot, considering established plants those with two leaves unfolded. Wheat-grain yield was determined on the basis of the harvested plot each year. The number of spikes (SN) and height (H) were counted after the end of flowering from ten 1 m long adjacent rows, corresponding to a 1 m<sup>2</sup> area per plot. Above ground biomass of wheat was harvested at wheat maturity (BBCH 89). Wheat grain was separated from all other vegetal material and dry matter weights were recorded separately. Wheat-grain quality category (QC) was determined according to the Royal Decree on Domestic Wheat Quality Standardization (Royal Decree 190/2013

of March 15, 2013). A 50 g destructive sampling per treatment was analysed at the laboratory. Grain protein content (PC) was determined by means of a Kjeldhal nitrogen analysis ( $N \times 5.7$ ) and was expressed as the percentage on a dry weight basis (BOE, 1977). Specific weight or hectolitre weight (HLW) was assessed according to the method given by ISO (2009). The vitrosity (V), expressed as a percentage, was determined by counting the number of vitreous kernels from a total of 50 grains sliced with a Pohl grain cutter according to the method given by AENOR (2008).

Sunflower plant density (PD) was calculated by counting the seedlings emerged in 4 of the central rows per plot. Sunflower-seed yield was determined on the basis of the harvested plot each year. Sunflower head diameter (HD) and height (H) were determined before harvesting by counting 20 random sunflower head diameters contained in 4 of the central rows per plot. Sunflower head was harvested by hand-cutting at maturity (BBCH 92-97). Sunflower seed was separated from all other vegetal material and dry matter weights were recorded separately. Seed oil content (OC) of sunflower was assessed by analysing a 5 g nondestructive sampling per plot by nuclear magnetic resonance spectroscopy (Benchtop NMR MQR Analyser, Oxford instrument).

#### **Experiment A.2. Effect of white mustard and narbon bean on sunflower seed germination and seedling growth.**

This study was carried out in growth chambers belonging to the Agrarian and Fishing Technologies Research and Education Institute (IFAPA-Alameda del Obispo) located at Córdoba and was divided into two parts.

The first experiment consisted of a germination bioassay conducted in April 2016 for determining the effect that white mustard and narbon bean residues could have on the sunflower germination. For the germination test, 10 sunflower seeds from each cultivar (*Helianthus annuus* cv. Transol and cv. P64LE29) were placed in a 10-cm Petri dish on one piece of filter paper and an artificial perlite based substratum. CCC and LCC plants were collected from Tomejil long-term experiment A.1 to achieve comparable results to those obtained under field conditions. The above-ground parts of white mustard and narbon bean samples were harvested from field trials the same day of the test beginning, at late flowering stage (stage 67-69 of oilseed rape and narbon bean BBCH scale, respectively), timed to coincide with the CC field

incorporation each year (mid-March/early April). As soon as possible, whole plants were cut into 1-2 cm pieces and disinfected in 400 mL of distilled water and 100 mL of 2 % NaOCl (20% commercial bleach) added, then rinsed three times with sterile distilled water. Immediately, water-soluble extracts of white mustard and narbon bean were prepared to simulate the CC incorporation effect, especially to favour glucosinolate release on CCC. Using an Ultra-Turrax high-performance dispersing machine, aqueous extracts of both CC were calculated on the basis of field experimental biomass data: 15 g with 25 mL of distilled water per CCC plate and 29 g with 50 mL of distilled water per LCC plate. The different liquid extracts (15 mL) were placed in the Petri dishes wetting the filter paper with sunflower seeds in addition to a sterile distilled water treatment included as a negative control (without CC extracts). The experiment was arranged as a factorial combination of two cover crops treatments (CCC, LCC) in addition to the control without CC and two sunflower cultivars (cv. Transol and cv. P64LE29) with ten replicate discs. The Petri dishes were sealed with Parafilm, enclosed in aluminium foil and incubated at 24°C for 15 days. To avoid effects of volatile degradation products apart from water-soluble compounds that might be present in the tissues, each treatment was separated in enclosed bioassay chambers. Seed germination was checked every 3 days and seeds were considered as germinated when the radicle was visible through the seed coat.

Based on the results from the bioassay, CC effect on sunflower was further characterized in a pot experiment. This experiment started in April 2016 in order to determine if CCC and LCC residues mixed with soil could have some effect on sunflower seedling emergence and early growth. The two sunflowers cultivars were planted at four different dates after CC incorporation: 0, 7, 15 and 30 days after mowing (DAM). The date 0 corresponds to the day of incorporation; additional dates were 7, 15 and 30 days after amendment incorporation. Pots had a size of 8 cm by 8 cm and a height of 11 cm to allow adequate sunflower root growth. Soil, white mustard and narbon bean plants at late flowering stage were randomly collected from Tomejil long-term experiment A.1. Each plastic pot was filled with 650 g of a mixture of soil-perlite (3:1 v/v). The above-ground CCC and LCC plants were cut into 1-2 cm pieces, weighed on the basis of experimental biomass data (6.55 g of white mustard per CCC pot and 12.56 g of narbon bean per LCC pot) and immediately incorporated and mixed

into each soil. A control treatment with no cover crop was also established. Two sunflower seeds (cv. Transol and cv. P64LE29) were sown at 1 cm depth in a growth chamber at 20°C with a 12 h photoperiod and thinned to one seedling per pot upon emergence (emergence percentage). A total of 144 pots were obtained in a split-split plot design with three CC treatments as main plot, four sunflower planting dates after incorporation as the split plot and two sunflower cultivars as the split-split plot with six replications. Pots were watered to field capacity every 3 days and several variables were evaluated based on those assessed by Saavedra *et al.* (2015a) under similar conditions. All sunflower seedlings were maintained beyond the unfolding of the third pair of leaves (BBCH 16) in each treatment. In all treatments, seedlings were regularly checked for stage of development and leaf length and width were measured at the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> pair of leaves at the widest point using a ruler. Because of the different sowing dates, sunflower seedlings were gradually harvested and smaller seedlings were left for the next sampling date. Each crop cycle lasted 40-45 days. At each harvest, the seedlings were divided into shoots and roots, and shoot height, root depth, fresh and dry matter of above-ground and underground parts were determined. Roots were obtained by washing carefully to reduce the likelihood of loss of fine roots. Total dry weight was determined after drying the component parts for 48 h at 70°C in an oven.

## **B. EFFECT OF COVER CROPS ON SUNFLOWER BROOMRAPE CONTROL**

### **Experiment B.1. Effect of white mustard and narbon bean as organic amendment on sunflower broomrape (*Orobanche cumana*) control**

In this study, we determined the *in vitro* effects of CCC and LCC on the control of sunflower broomrape in order to reduce the sunflower major limitation by cultural control methods. A bioassay was carried out with white mustard and narbon bean vegetal material based on the ability of certain cruciferous and leguminous crops to reduce weeds and soil-borne diseases and pests by inhibiting or stimulating seed germination.

The experiment was performed three times from 2014 to 2016 in laboratories belonging to the IFAPA-Alameda del Obispo. It was prepared according to the

methodology tested by other authors for biofumigant volatile fungicides (Richardson and Munnecke, 1964; Zurera *et al.*, 2007; Perniola *et al.*, 2012). *O. cumana* seeds were collected from flowering spikes parasitizing sunflower in Tomejil the years prior to carry out the bioassays (2013, 2014 and 2015). It was due to the *Orobanchae* seeds need a period of dormancy (after-ripening period) of a few weeks to ensure germination in the next season (Joel *et al.*, 2007). The viability of *O. cumana* seeds and their surface-sterilization were assessed according to the viability test and disinfection protocol for germination assays proposed by González-Verdejo *et al.* (2005), respectively. Approximately 1000 *O. cumana* seeds (0.15 g) were sown on each 6-cm Petri dish containing a previously autoclaved glass-fibre filter paper (Whatman) wetted with 1 mL of sterile deionized water.

Plant material of the CCC and LCC was collected each year from the cover crops grown in the field experiment A.1 at late flowering stage. They were prepared and disinfected as in previous experiment A.2. Known the possible volatile glucosinolate release by CCC degradation, two types of amendment application were made: 1) plant residues based on previous experimental biomass estimations (15 gr per CCC plate and 29 gr per LCC plate), placed in a 15-cm Petri dish with the *O. cumana* Petri dish at the centre of these plant residues in each plate; and 2) water-soluble extracts of both CC prepared as in previous experiment A.2 and applying one aliquot of 1 mL from each CCC and LCC liquid extracts to each of the 6-cm Petri dishes, wetting the filter paper with *O. cumana* seeds. Additionally, a treatment without CC plant residues or liquid extracts (just sterile deionized water) was included, respectively, as a negative control. The different Petri dishes treatments were sealed with Parafilm, enclosed in aluminium foil and conditioned in the dark at 24°C for 10 days. Moreover, an artificial germination stimulant GR24, the synthetic strigolactone stimulant in *Orobanchae* research (Johnson *et al.*, 1976, 1981), was used as a positive control. After the conditioning period, GR24 (1 mL 10<sup>-7</sup> M) was added to the half of the *O. cumana* dishes per treatment. In order to allow valid comparisons, the other half was wetted with additional 1 mL of sterile deionized water. The Petri dishes were again sealed with Parafilm, wrapped with aluminium foil to provide absolute darkness and incubated in controlled growth chambers at the temperature of 24°C for 7 days. To avoid effects of volatile

degradation products apart from water-soluble compounds that might be present in the tissues, each treatment were separated in enclosed bioassay chambers.

The experiment design for each CC treatment (CCC and LCC) was a factorial design with ten replicate discs. The factors studied were: 1) two types of amendment application (plant residues and liquid extracts), in addition to the control without CC; and 2) two different germination processes (with or without synthetic germination stimulant GR24). Percentage of seed germination was scored using a Nikon SMZ 1000 stereomicroscope, by dividing each plate into four sectors and counting four times 100 seeds per plate. Seeds were considered as germinated when the radicle was visible through the seed coat. Photographs were recorded with a Nikon DS-Fi1 digital microscope camera.

### **Experiment B.2. Effect of white mustard and narbon bean cover crops on sunflower broomrape control under field conditions**

This study complements that of the preceding experiment B.1. Based on the results from the bioassay, this field trial was carried out during the growing seasons 2014/2015 and 2015/2016 in order to evaluate the effect of CCC and LCC applied in sunflower susceptible to broomrape (*O. cumana*). The experiment was conducted at the 'Alameda' experimental farm in Córdoba. Site characteristics of soil properties were included in Table 1 (Chapter 2). The average monthly rainfall and temperature from September to July of both years research is shown in Figure 14 (Chapter 2).

In October field experiments were established using a randomized complete block design with four replicates. The treatments consisted of the two different cover crops treatments (CCC and LCC) and one control treatment without cover crop (RT). Total plot size was 6 m<sup>2</sup> (1.5 × 4 m), including a 1 m wide buffer along each side of the plots. Soil preparation consisted of a superficial tillage at a depth of 10-15 cm that was done with a vibro-cultivator and followed by a disc harrowing of a 5 cm depth. CCC and LCC were sown at the same seeding rate in experiment A.1 using a Wintersteiger TC2700 plot seed drill on October 27, 2014 and October 30, 2015. Soil was not fertilized and herbicides were not applied to any plots. To avoid further soil disturbance and maintain a similar disturbance regime to the CC plots, weeds were controlled in fallow plots by hoeing. On March 09, 2014 and 2015, CCC and LCC were

mowed at late flowering with a flail mower, and their residues were incorporated to a depth of 15 cm with 2 passes of a rotary tiller.

In both years, the preceding crop was sunflower and the soil was naturally infected by sunflower broomrape of the race F. For that reason, the sunflower cultivar (*Helianthus annuus* cv. Sambro) susceptible to race F was used to ensure accurately data collection. Sunflower was hand planted in mid-March at a rate of 69-70 thousand seeds/ha in 2 rows spacing 90 cm and 30 cm space between plants by qualified staff. Three sunflower seeds were then sown directly into each seedbed at 7-10 cm depth. Two weeks after emergence, seedlings were thinned to one, *i.e.* 20 plants per row. Blocks were bordered with extra susceptible sunflower plants to ensure the pest infestation. Each plot was equally divided into 2 sub-areas, one of them was used for sunflower development and yield and the other one was used for sunflower broomrape data collection during its lifecycle stages (destructive sampling). The variables evaluated have been divided into three groups:

- Cover crops biomass production and height. Cover crop above-ground fresh biomass (CFM) for CCC and LCC treatments was evaluated at mowing time by measuring the fresh weight of the above-ground parts of the plants. One biomass sample was randomly collected from a 0.5 m<sup>2</sup> area of each plot by cutting at ground level with a sickle. Cover crops species were separated by hand from all other vegetal and immediately fresh weighted. Simultaneously, plant height (H) was calculated as the mean of 4 random measurements by CC species in each plot.

- Sunflower broomrape. Total number of broomrape attachments per host plant and the number of infested plants were visually evaluated per treatment. Plant infestation was evaluated at the appearance of the sunflower BBCH stage 15 (third pair of leaves), BBCH 30 (stem elongation), BBCH 50 (inflorescence emergence), BBCH 65 (full flowering) and BBCH 89 (fully ripe). Simultaneously, the total number of broomrape attachments per host plant was counted and classified according its developmental stage as previously defined by ter Borg *et al.*(1994): stage 1 (tubercles smaller than 2 mm); stage 2 (tubercles greater than 2 mm, without rots development); stage 3 (tubercles with crown roots, without shoot formation); stage 4 (shoot formation, remaining underground); stage 5 (shoot emergence); stage 6 (flowering) and stage 7 (setting of seeds). For the subterranean broomrapes stages, two sunflower



plants were removed per sub-area dedicated to *O. cumana* data collection. Roots were carefully washed in water and broomrapes were counted at laboratory.

- Sunflower characterization. Phenological events based on the BBCH scale for sunflower, mean height (H), head diameter (HD), sunflower yield and seed oil content (OC) were calculated as in experiment A.1. From each sub-area used for sunflower development, sunflower plants were maintained until the harvest (BBCH 92-95) and they were monitoring and harvested concurrently to allow a final growth comparison of the treatments.

### 3.3.2 STATISTICAL ANALYSES

The analysis of variance (ANOVA) was performed using the STATISTIX program (9<sup>th</sup> edition) in order to test each dependent variable based on the assessments previously explained affecting the treatments. To verify the requirements for statistical analysis, the Shapiro-Will-Test for normality and the Levene-test for homogeneity of variance were used. If required, percentage data were arcsine  $(x/100)^{1/2}$  transformed to achieve the statistical assumptions.

The comparison of mean values was estimated using the experimental design of each individual experiment. One-way ANOVA was done with the different cropping systems as the treatments in each growing season by the set of 3-year rotation cycle for experiment A.1. Additionally, a factorial ANOVA was done to test possible above-ground weed fresh biomass differences between treatments from the beginning to end of the rotation 2 (year 1 vs. year 3). This analysis was also performed for the different variables evaluated for durum wheat and sunflower main crops in the rotation 1 and 2, respectively. Another one-way ANOVA test was done for field experiment B.2 with the different CC amendments as the treatments in each growing season. Afterwards, multiple comparison tests were conducted with the Tukey test at a 5 % significant level ( $P \leq 0.05$ ). The bioassay experiment A.2 was performed using a two-way factorial design (2 CC treatments in addition to the control  $\times$  2 sunflower cultivars). A split-split-plot design was adopted for the pot experiment A.2 (2 CC treatments in addition to the control  $\times$  4 sunflower planting dates after CC incorporation  $\times$  2 sunflower cultivars) and a two-way factorial design was used for the bioassay experiment B.1 (2 amendment applications in addition to the control  $\times$  2

germination processes). The main effect and interaction means were compared using the Tukey test and results were considered significant at the 5 % level.

### **3.4 RESULTS AND DISCUSSION**

#### **A. AGRONOMIC VIABILITY OF THE INTRODUCTION OF COVER CROPS**

##### **3.4.1 COVER CROPS DEVELOPMENT AND WEED SUPPRESSION CAPACITY**

###### ***Cover crops installation and development***

With the aim of assessing the effects of cover crops living plants together with dry residues left on the soil surface, results of CC plant density, height, fresh biomass and ground cover for each growing season were shown in Table 5 and Figures 23-25. There were consistent differences in cover crops growth and development among the 3-year period, due to different weather conditions and especially regarding rainfall regime along the growing season (Figure 14 in Chapter 2).

During the wet year 1 both CCC and LCC treatments achieved similar final levels of PD, ground cover and CFM, without significant differences between them (Table 5 and Figure 23). The high rainfall registered in November 2012 (207 and 148 mm in Santa Cruz and Tomejil, respectively) affected the final installation of white mustard plants, especially in Santa Cruz (from 85 to 56 plant/m<sup>2</sup> at 26-42 DAS). However, while CCC and LCC reached 51 and 49 % of ground coverage and produced substantial CFM amounts (9350 and 11148 kg/ha, respectively) in Santa Cruz, both crops slowed down their growth from the stem elongation stage in Tomejil (after 92 DAS and 823 GDD). It was probably due to the frost period recorded on February 2013 (nine consecutive days with  $\leq 0^{\circ}\text{C}$ ) together with the high rainfall occurred from February to March 2013 (238 mm), resulting in temporary waterlogging conditions on heavy clay soils with high water-holding capacity that affected the final ground cover (30 % for CCC and 33 % for LCC) and CFM values (2364 and 2103 kg/ha, respectively). According to Saavedra *et al.* (2016b), white mustard plants growing in waterlogged soils produced significantly lower growth and many seedlings dead. In addition, mustards are frost-sensitive (Haramoto and Gallandt, 2004) and cool weather limited plant growth and development. Cold temperatures are best tolerated by narbon bean plants (Siddique *et al.*, 2001), but they are also affected by waterlogging (Nadal *et al.*, 2012). In fact,

several studies in similar conditions showed that mustards and legumes were strongly affected by differences in weather conditions (Haramoto and Gallandt, 2005; Björkman *et al.*, 2015; Ramírez-García *et al.*, 2015b). However, even though weather conditions were more favorable for narbon bean than white mustard, CCC always reached significantly higher height values than LCC, since white mustard is a higher plant which normally reaches from 50 to 120 cm (Saavedra *et al.*, 2015b) and narbon bean achieves 40-90 cm (Nadal *et al.*, 2012). In spite of the considerable meteorological damages this year 1, the ground coverage produced by both CC was around 30%, the minimum threshold recommended by conservation practices to protect soil from erosion (AEAC.SV, 2016). Therefore, the inclusion of both CC have a significant effect on final ground cover with respect to the residue quantity of NT and RT treatments, which had significant lower ground coverage percentages in Santa Cruz (23 % for NT and 0.13 % for RT) and Tomejil (11 and 3 %, respectively)(Figure 23).

During the year 2, CCC showed a lower PD after 100 DAS in Santa Cruz and Tomejil (16 and 31 plant/m<sup>2</sup>, respectively) (Table 5). This fact, coupled with the greater precipitation and frosts recorded from the stem elongation stage (97 DAS and 844 GDD) could have influenced their lower ground cover results (Figure 24), especially into the heavy clay soil of Tomejil (30 % for CCC ground cover). Similar results about the negative effect of high rainfall and cold temperatures on cover crop ground coverage have been shown by Björkman *et al.* (2015). By contrast, LCC plots showed a great development, especially from the beginning of the side shoots at both locations (103-106 DAS). Consequently, total LCC ground coverage ranged from 70 to 72 % at 140-138 DAS (1340-1353 GDD) in Santa Cruz and Tomejil, respectively.

**Table 5. Winter cover crops characterization: plant density (PD in plant/m<sup>2</sup>) at 2 different dates, white mustard cover crop (CCC) and narbon bean cover crop (LCC) mean height (H in m), CCC and LCC above-ground fresh biomass (CFM expressed as kg/ha) and carbon-to-nitrogen ratio (C/N) in addition to weed above-ground fresh biomass (WFM expressed as kg/ha) and weed C/N ratio in each location and treatment, including reduced tillage (RT) and no tillage (NT), during 2012/2013, 2013/2014 and 2014/2015.**

2012/2013							
Soil management	PD 1	PD 2	H	CFM	C/N ratio	WFM	C/N ratio
	plant/m <sup>2</sup>	plant/m <sup>2</sup>	m	kg/ha	Unit	kg/ha	Unit
<b>Santa Cruz</b>							
	26 DAS	42 DAS	126 DAS	126 DAS		126 DAS	
	271 GDD	434 GDD	1262 GDD	1262 GDD		1262 GDD	
CCC	85.2 a <sup>1</sup>	55.5 a	0.95 a	9350 a	28.25 a	1970 a	21.05a
LCC	47.9 b	48.2 a	0.47 b	11148 a	18.45 a	3387 a	21.23a
RT						3407 a	18.58a
NT							
<b>Tomejil</b>							
	30 DAS	49 DAS	133 DAS	133 DAS		133 DAS	
	287 GDD	464 GDD	1345 GDD	1345 GDD		1345 GDD	
CCC	25.8 a	35.5 a	0.79 a	2364 a	36.37 a	2501 c	42.38a
LCC	42.6 a	43.1 a	0.35 b	2103 a	19.85 a	3462 bc	44.88a
RT						6781 a	42.28a
NT						5757 ab	35.20a
<b>2013/2014</b>							
<b>Santa Cruz</b>							
	67 DAS	103 DAS	143 DAS	143 DAS		143 DAS	
	553 GDD	879 GDD	1379 GDD	1379 GDD		1379 GDD	
CCC	15.4 a	16.3 b	0.73 a	5804 b	19.70 a	2541 a	19.85a
LCC	16.5 a	61.3 a	0.53 a	10733 a	18.75 a	2767 a	20.18a
RT						3023 a	32.45a
NT							
<b>Tomejil</b>							
	65 DAS	97 DAS	139 DAS	139 DAS		139 DAS	
	551 GDD	844 GDD	1366 GDD	1366 GDD		1366 GDD	
CCC	46.5 a	31.3 b	0.72 a	2390 b	37.30 a	3598 a	21.30a
LCC	14.4 b	55.0 a	0.65 a	8892 a	18.58 b	3542 a	19.25ab
RT						4709 a	18.73ab
NT						3362 a	14.23b
<b>2014/2015</b>							
<b>Santa Cruz</b>							
	28 DAS	50 DAS	132 DAS	132 DAS		132 DAS	
	440 GDD	698 GDD	1434 GDD	1434 GDD		1434 GDD	
CCC	14.9 b	13.5 b	0.79 a	13541 a	18.85 a	2266 c	19.85a
LCC	37.3 a	35.3 a	0.43 b	10982 a	17.13 a	2566 c	20.18a
RT						7002 b	32.45a
NT						11936 a	33.17a
<b>Tomejil</b>							
	32 DAS	52 DAS	133 DAS	133 DAS		133 DAS	
	542 GDD	771 GDD	1487 GDD	1487 GDD		1487 GDD	
CCC	36.4 b	27.2 b	1.09 a	17754 b	30.73 a	2298 b	19.05a
LCC	53.3 a	45.9 a	0.90 a	33065 a	15.95 b	757 c	17.55a
RT						4249 a	15.93a
NT						3129 ab	17.80a

<sup>1</sup> Different small letters within each location per column indicate that the differences between treatments were statistically significant (Tukey's,  $p \leq 0.05$ )

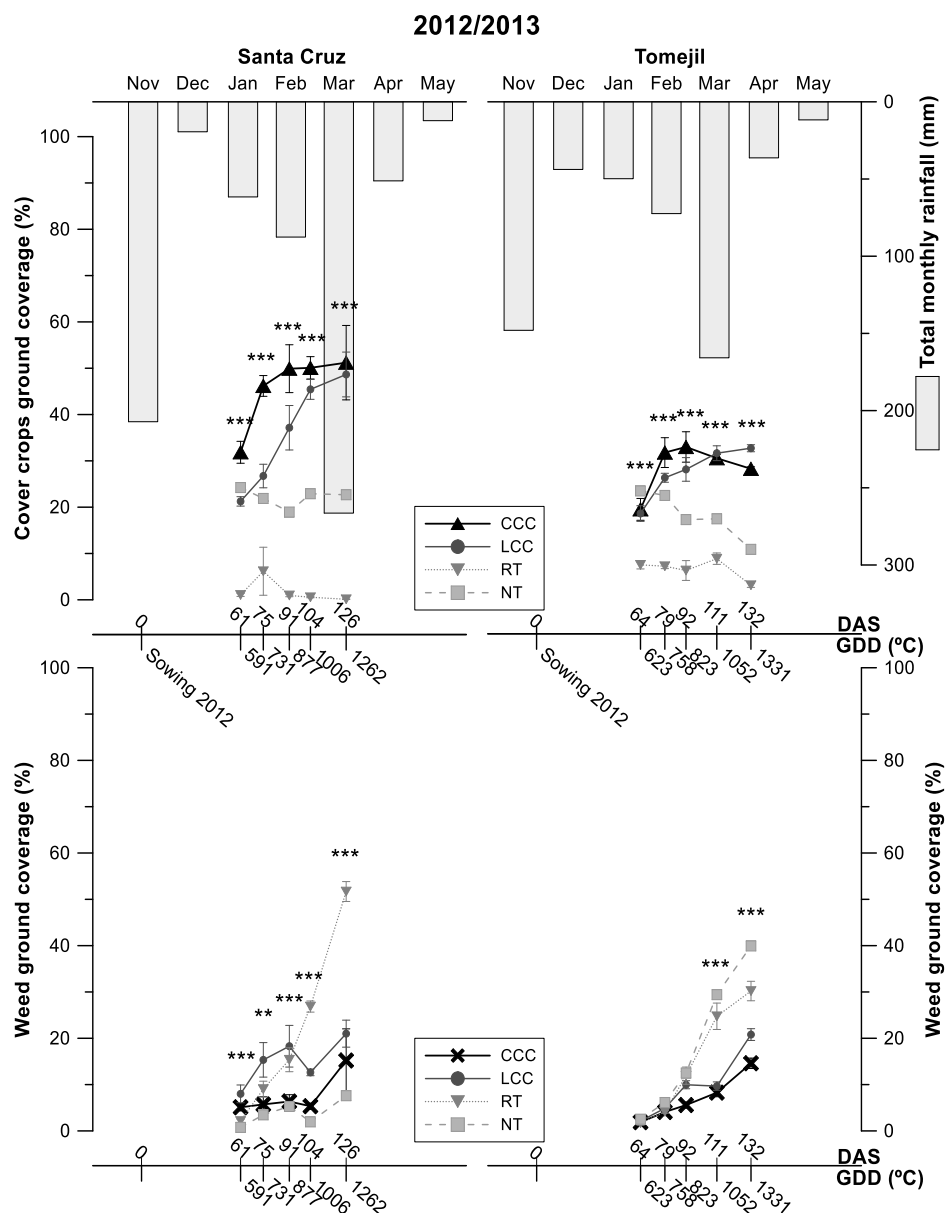


Figure 23. Temporal evolution of the monthly cumulative rainfall, the ground cover mean values of the white mustard and narbon bean cover crops + dry residues (CCC, LCC) compared with the stubble residues of the preceding crops on the reduced tillage (RT) and no tillage (NT) ground in addition to the weed incidence of the different treatments at both locations during 2012/2013. Temporal scale was expressed as the growing degree days (GDD) accumulated in five different dates (61-64, 75-79, 91-92, 104-111 and 126-132 DAS) each year and location with the last interval corresponding to the mowing date. Only significant differences are shown: \*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ .

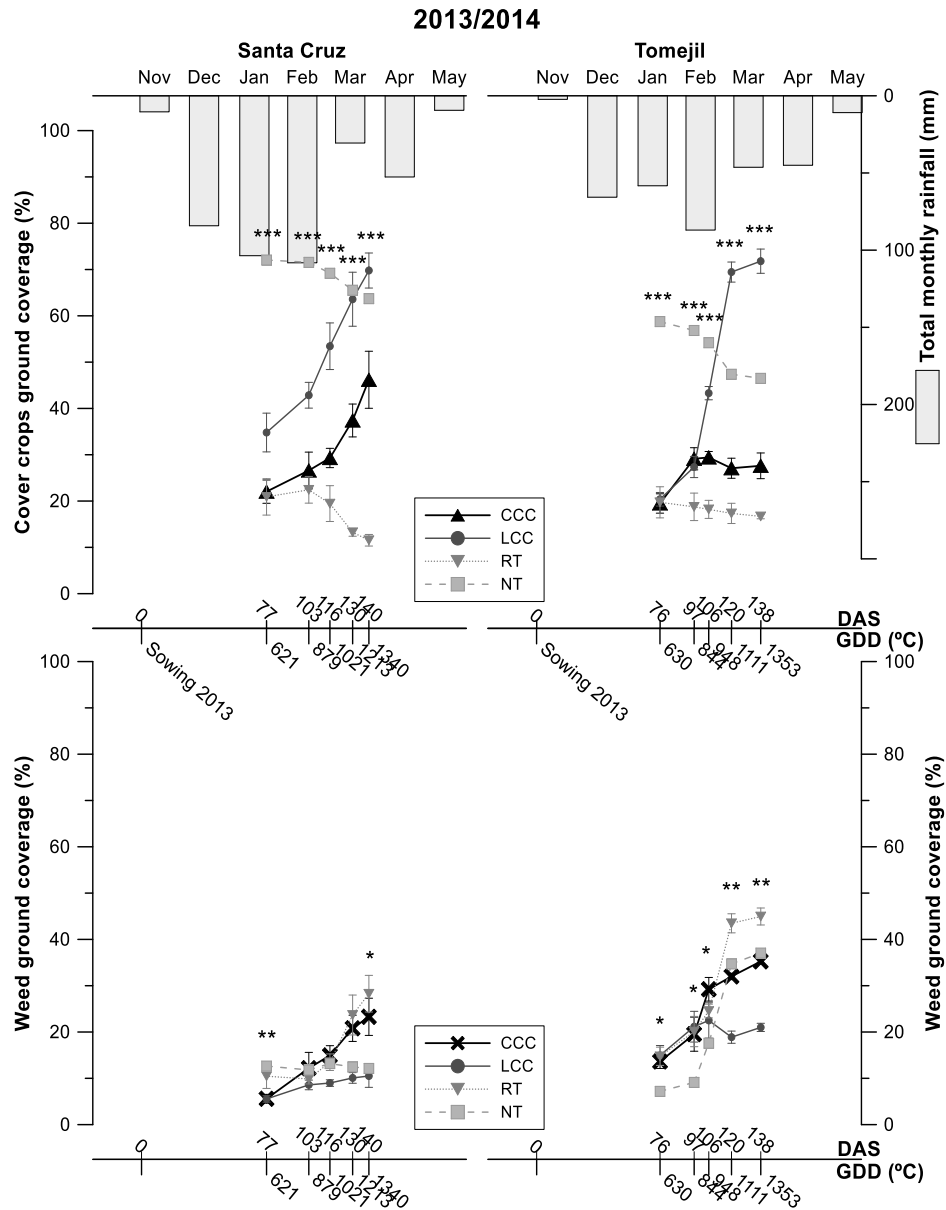


Figure 24. Temporal evolution of the monthly cumulative rainfall, the ground cover mean values of the white mustard and narbon bean cover crops + dry residues (CCC, LCC) compared with the stubble residues of the preceding crops on the reduced tillage (RT) and no tillage (NT) ground in addition to the weed incidence of the different treatments at both locations during 2013/2014. Temporal scale was expressed as the growing degree days (GDD) accumulated in five different dates (76-77, 97-103, 106-116, 120-130 and 138-140 DAS) each year and location with the last interval corresponding to the mowing date. Only significant differences are shown: \*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ .

This better development was also reflected in the narbon bean CFM (10733 and 8892 kg/ha in Santa Cruz and Tomejil, respectively), showing significant differences with respect to those obtained by white mustard (5804 and 2390 kg/ha, respectively) (Table 5). It should be noted the high initial ground cover shown by NT plots (72 and 59 % at 77-76 DAS in Santa Cruz and Tomejil), declining to final NT

ground cover values of 70 and 47 % after 140-139 DAS, respectively. These latter values were similar to those in LCC plots in Santa Cruz (in descending order LCC= NT> CCC> RT) and higher than CCC plots in Tomejil (LCC> NT> CCC> RT). The RT plots always showed the lowest values of soil cover (11 % and 16 % in Santa Cruz and Tomejil, respectively) despite having the highest initial RT stubble residues of the study period (20-21 %). It was probably due to the lower precipitation together with higher temperature from March, which directly influenced decomposition rates as was also reported by Tanaka (1989) and Ordóñez-Fernández *et al.* (2007b).

During the year 3, both CC treatments performed similarly in Santa Cruz. Despite the lowest CCC plant density level (13.5 plant/m<sup>2</sup>), there were no significant differences between treatments on final ground coverage (around 45-46 % after 132 DAS) (Figure 25) and CFM production (13541 and 10982 kg/ha, respectively) (Table 5). In Tomejil, CCC and LCC treatments reached better PD values (27 and 46 plant/m<sup>2</sup>, respectively) and the highest H, ground cover and CFM of the 3-year study. It was probably due to Tomejil did not register frosts during the winter period (Santa Cruz recorded eleven consecutive days with  $\leq 0^{\circ}\text{C}$ ) and the rainfall was favourable for both CC establishment and growth. In addition, the vigorous CC development could have been influenced by the earlier sowing date at both locations (20-23th October 2014) (more information in Appendix 2), because it shortened the period of implantation prior to the cold winter temperatures (Restovich *et al.*, 2012). Similar results were shown by Perdigao *et al.* (2012) and Odhiambo and Bomke (2001), which concluded that early planted cover crops accumulated higher biomass compared with late-planted cover crops. However, significant differences existed between CC, since the degree of LCC soil cover was maintained at above 90 % vs. 63 % obtained by CCC plots and narbon bean CFM (33065 kg/ha) was double the amount produced by white mustard (17754 kg/ha). The NT plots showed the highest initial ground cover percentages in Santa Cruz (36 %), while LCC significantly exceeded it in Tomejil (51 vs. 43 %). The RT plots always had the lowest values (11 and 15 % in Santa Cruz and Tomejil, respectively). Over time, a gradual decline was produced in the cover provided by NT and RT treatments due to the stubble desiccation, with the soil showing 23 and 7 % coverage, respectively, after 132-133 DAS.

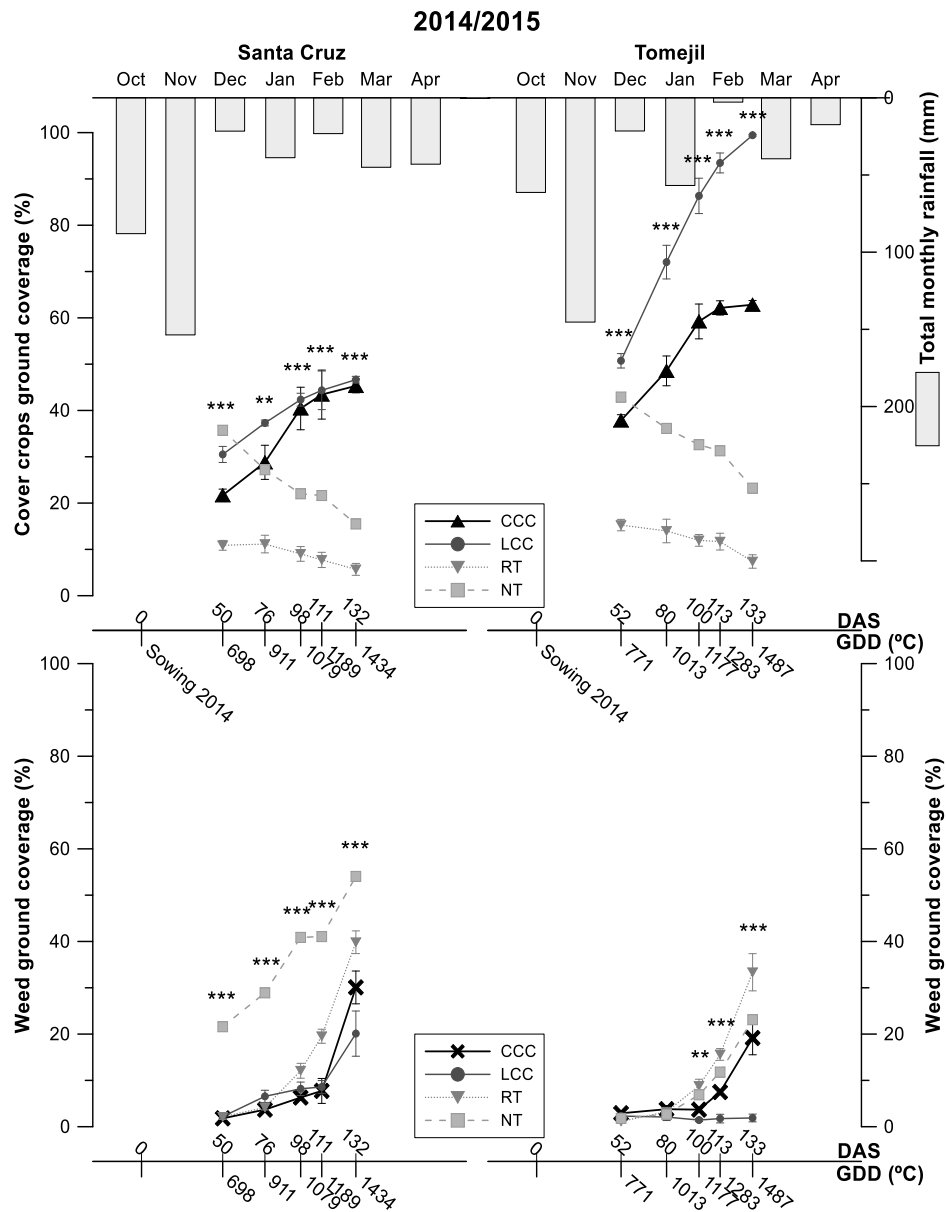


Figure 25. Temporal evolution of the monthly cumulative rainfall, the ground cover mean values of the white mustard and narbon bean cover crops + dry residues (CCC, LCC) compared with the stubble residues of the preceding crops on the reduced tillage (RT) and no tillage (NT) ground in addition to the weed incidence of the different treatments at both locations during 2014/2015. Temporal scale was expressed as the growing degree days (GDD) accumulated in five different dates (50-52, 76-80, 98-100, 111-113 and 132-133 DAS) each year and location with the last interval corresponding to the mowing date. Only significant differences are shown: \*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ .

### Cover crops residue quality: C/N ratio

In our study, as seen above, the climatic differences between years affected the growth and biomass production of white mustard and narbon bean crops and subsequently the residue quality (C/N ratio) (Table 5-7). This biochemical attribute is directly related to the decomposition of biomass (Ordóñez-Fernández *et al.*, 2007b),



which is faster for crop residues with a low C/N ratio, such as narbon bean (values averaging 17.13-18.75 and 15.95-19.85 in Santa Cruz and Tomejil, respectively).

The above-ground narbon bean plants were rich in N and could supply nutrients during the early stages of the subsequent crop, as was previously observed in several studies (Kumar and Goh, 2002; Perdigao *et al.*, 2012). By contrast, the CCC green wastes quality at mowing had higher and wider C/N ratio ranged from 18.85 to 28.25 and 30.73-37.30 in Santa Cruz and Tomejil, respectively. Consequently, white mustard residues were poor in N, decomposing and releasing nutrients slowly, especially in Tomejil. In spite of the CCC residue quality variations, significant differences between CCC and LCC treatments were only noticed the year 2 (37.30 vs. 18.58) and year 3 in Tomejil (30.73 vs. 15.95, respectively). Moreover, according to Chaves *et al.* (2004) the threshold for C/N ratio from various horticultural crucifers and green manures was 36.6. Therefore, all the CC evaluated would have shown adequate C/N ratio to improve soils. Similar results were obtained by Restovich *et al.* (2012) with different cover crops species in a maize-soybean rotation, whose C/N ratio varied as follows: grasses  $\geq$  crucifers  $\geq$  legume and mixtures. Conversely, these results contradict those obtained by Ramírez-García *et al.* (2015b), who got similar C/N ratio for vetch (values between 15 and 18) but lower values for mustard ( $< 13$ ), which probably was affected by different plant nutrition status and phenological stage, as previously was reported by Plaza-Bonilla *et al.*, 2016.

Based on our observations, the highest C/N ratios were shown by white mustard plants producing the lowest fresh biomass the year 1 and 3 in Tomejil. For that reason, we speculate that other factors leading to variation in white mustard C/N ratio could include waterlogging from heavy rainfall and frosts, lack of N fertilization or variation in residual fertility for the previous crop (Björkman *et al.*, 2015). To maximize benefits from their cultivation it is important to consider the particular requirements of each plant species. Further research focusing on N fertilization and early sowing date of CC could reduce C/N variations and improve the results obtained, as was pointed out by several authors in similar conditions (Alcántara *et al.*, 2011a; Mazzoncini *et al.*, 2011; Restovich *et al.*, 2012; Saavedra *et al.*, 2016b).

### **Ground coverage, biomass and C/N ratio of weeds**

The weeds ground coverage, identification and biomass in the different cover crop sequences were measured from 2012 to 2015 (Table 5 and Figures 23-25). Weeds evolution and identification were included in Appendix 3.

During the wet year 1, the emergence of natural weeds in Santa Cruz was characterized by large variation, with wild oat (*Avena sterilis*) and goosegrass (*Galium aparine*) in all treatments (Appendix 3). In this case, weed ground cover installed in the RT plots was significantly higher than the rest of treatments (52 vs. 8-21 %) (Figure 23). By contrast, the predominant species in Tomejil was bristly oxtongue (*Picris echioides*), which represented almost all of the emerged weeds and was present in all plots, especially in the RT and NT plots (more information in Appendix 3). The contribution of other species to the total count was negligible. In keeping with the foregoing, NT followed by RT plots presented the greatest weed ground cover with respect to CC treatments (40 and 30 % vs. 15-21 %, respectively). The WFM results showed RT plots displaying the highest values at both locations (Table 5), with greater incidence in Tomejil than Santa Cruz (6781 and 3407 kg/ha, respectively). The CCC plots (1970-2500 kg/ha) followed by LCC plots (3387-3462 kg/ha) produced the lowest WFM but the C/N ratio for weeds did not show significant differences at any site.

During the year 2, main weeds in Santa Cruz consisted of durum wheat volunteers, goosegrass (*Galium aparine*), field bindweed (*Convolvulus arvensis*) and blackspot hornpoppy (*Glaucium corniculatum*), while a high incidence of bristly oxtongue (*Picris echioides*) and durum wheat volunteers for all treatments were observed in Tomejil (Appendix 3). The RT plots showed higher weed ground cover and WFM than the rest of treatments in absolute terms at both sites (Figure 24 and Table 5). However, significant differences were only detected for weed ground coverage values in Santa Cruz (in descending order RT > CCC > NT = LCC). In Tomejil, LCC plots showed the lowest significant weed coverage (21 %) followed by similar values for RT, NT and CCC treatments. Weeds biomass production performed similarly in all treatments (values ranging from 2541 to 3023 kg/ha in Santa Cruz and 3362-4709 kg/ha in Tomejil) meanwhile the C/N ratio displayed the highest values for CCC treatments (21.30), followed by LCC and RT treatments (19). This fact showed the low N uptake by these weeds compared to that obtained in NT plots (14.23).

Finally, during the year 3, sand mustard (*Diplotaxis virgata*) and durum wheat volunteers were the main weeds observed in Santa Cruz, whilst bristly oxtongue (*Picris echinoides*) remained as the major weed in Tomejil, followed by durum wheat volunteers with a high biomass in all treatments (Appendix 3). The highest above-ground weed cover and WFM were observed for NT plots (54 % cover and 11936 kg/ha, respectively) in Santa Cruz (Figure 25 and Table 5). The lesser weed incidence occurred in the CC plots, without significant differences in the C/N ratio (42-45) or WFM (2266-2566 kg/ha). In Tomejil, the largest weeds were observed in the RT plots (33 % and 4249 kg/ha), followed by NT (23 % cover and 3129 kg/ha). Moreover, soil coverage of weeds in LCC plots was around 90 % lower than NT and RT plots, while in CCC plots it was about 17-42 % lower. According to Teasdale *et al.* (2007), the ability of CC to suppress weeds is proportional to the amount of cover crop canopy produced, and a more vigorous cover crop would reduce the growth of weeds (Hiltbrunner *et al.*, 2007). Based on the results of our study this hypothesis could be confirmed, since treatments of narbon bean accumulating more biomass and ground cover resulted in poor weed-soil contact.

It should be noted that weed numbers increased from year 1 to 3 for NT and RT treatments and the opposite occurred for CCC and LCC at both locations in the crop rotation 2. In fact, the analysis of variance showed the interaction between treatments at both locations and between years in Tomejil, with significantly lower WFM values the year 3 compared with the year 1 and 2 (ANOVA Table in Appendix 3). These results could be attributable to the CCC and LCC plant installation, suppressing weed populations due to the dense canopy produced which makes them more competitive than weeds for light, moisture, and nutrients compared to plots where no cover crops were sown. Similar results were obtained by Kruidhof *et al.* (2008) and Dorn *et al.* (2013), which found that cover crops could both prevent weed seed production and reduce weed establishment in subsequent crops. Additionally, white mustard (Haramoto and Gallandt, 2004) and some legume species (Teasdale, 1996; Fujii, 2001) have been shown to release weed suppressing allelochemicals, which could also have a suppressive effect throughout rotation 2. Given the different weed species observed in Santa Cruz and Tomejil, the weed suppressive potential of CC might be evaluated depending on the field site and the dominant weed species presence.

The present study suggested that both winter cover crops incorporation by mowing in spring (mid March-early April) could constitute a better practice for soil conservation and weed control than RT or NT bare soil management systems, which only leave a residue cover on soil surface. This fact is crucial in semiarid regions where vegetation cover is not frequent but necessary for erosion control. Although our study presented no data on soil erosion under the treatments conditions, soil protection was assumed based on the coverage values and both species provided an effective winter ground cover with values  $\geq 30\%$ , the minimum recommended by conservation practices. However, narbon bean presented higher establishment values, ground coverage and biomass than white mustard, especially in late October (earlier sowing date) compared with late November (late planting date). Both cover crops showed similar C/N ratios but legume was more suitable for green manure because it provided the system with N (low C/N ratio). Moreover, CCC results suggested that cooler early-season conditions and heavy winter rainfall reduce growth, especially on clay soils. Moreover, the marked effect of the CC on weed control can be seen by comparing crop rotation 1 and the different years in the rotation 2, showing a reduced weed infestation when CC were growing on the soil surface. This fact demonstrated that substantial changes in arable cropping systems can be readily achieved by farmers with relatively simple changes in management practices. Nevertheless, crop establishment is the result of a large number of interacting processes, each of them being influenced by climatic conditions and field specific factors. Therefore, the species characterization proposed in this study provides the basis for further developments towards a higher understanding of cover crops in rainfed crop rotation in southern Spain. For this purpose, additional data from a wider range of soil and annual weather conditions should be included in this approach and further parameters related to other agro-environmental targets should be defined, *e.g.* for rooting pattern, N uptake, weed seed production after cover crops incorporation with different seeding rates and sowing dates. The availability of comprehensive quantitative parameter sets of cover crops characteristics will be an important contribution to management optimization and improved farmers decision support.

### 3.4.2 EFFECT OF WHITE MUSTARD AND NARBON BEAN COVER CROPS ON SOIL MOISTURE CONTENT AND SOIL FERTILITY

#### *Soil moisture content*

In this part of the research, we examined the different soil management treatments on soil moisture content throughout the growing season in each rotation sequence. Figure 26 shows the moisture contents in each soil profile (0-10, 10-30 and 30-60 cm) in Santa Cruz and Tomejil, as measured on each sampling date for treatment (CCC, LCC, RT and NT) during the first set of 3-year rotation (rotation 1).

Soil moisture contents did not show significant differences between treatments at any layer or location during the year 1 due to the high rainfall recorded from November to April (693 and 516 mm in Santa Cruz and Tomejil, respectively), which correspond to 76 and 70 % of the annual rainfall in Santa Cruz and Tomejil, respectively. In that period, the soil moisture contents for the first profile ranged from 19 to 31 % in Santa Cruz, while these values averaged from 23 to 44 % in Tomejil where a Vertisol soil with higher soil water holding capacity exists. Moreover, this abundant rainfall favoured the increase of the soil water reserves in the deep profiles, with a scarce impact of the treatments that displayed similar moisture profiles. Thus, the soil moisture contents in the deeper layers 10-30 and 30-60 cm varied between 24-39 and 29-38 % in Santa Cruz and 29-43 and 46-61 % in Tomejil, respectively. For similar clay soils in the region, water retention at field capacity and the wilting point were near 39 and 24 %, respectively (García-Tejero *et al.*, 2011). Meanwhile, for Entisol soils such as those in Santa Cruz, these latter values were 31 and 17.3 %, respectively. Field capacity is hard to fulfil in Mediterranean regions as a result of the long dry spells throughout the year. However, the heavy rainfall period during the year 1 contributed to getting soil moisture content at field capacity in the second and third profiles at both locations. Nevertheless, topsoil layer drying was very fast during spring due to the high potential evaporation and the low rainfall that typically occur in this season (Ward *et al.*, 2012). Consequently, low soil moisture contents were observed in the first profile at both locations during the durum wheat grain filling period, but without significant differences between treatments. In Santa Cruz, variations in soil moisture

content ranged between 7-12 % in May and 2-4 % in June, while the second and third profiles retained from 12 to 19 % and from 19 to 26 %, respectively.

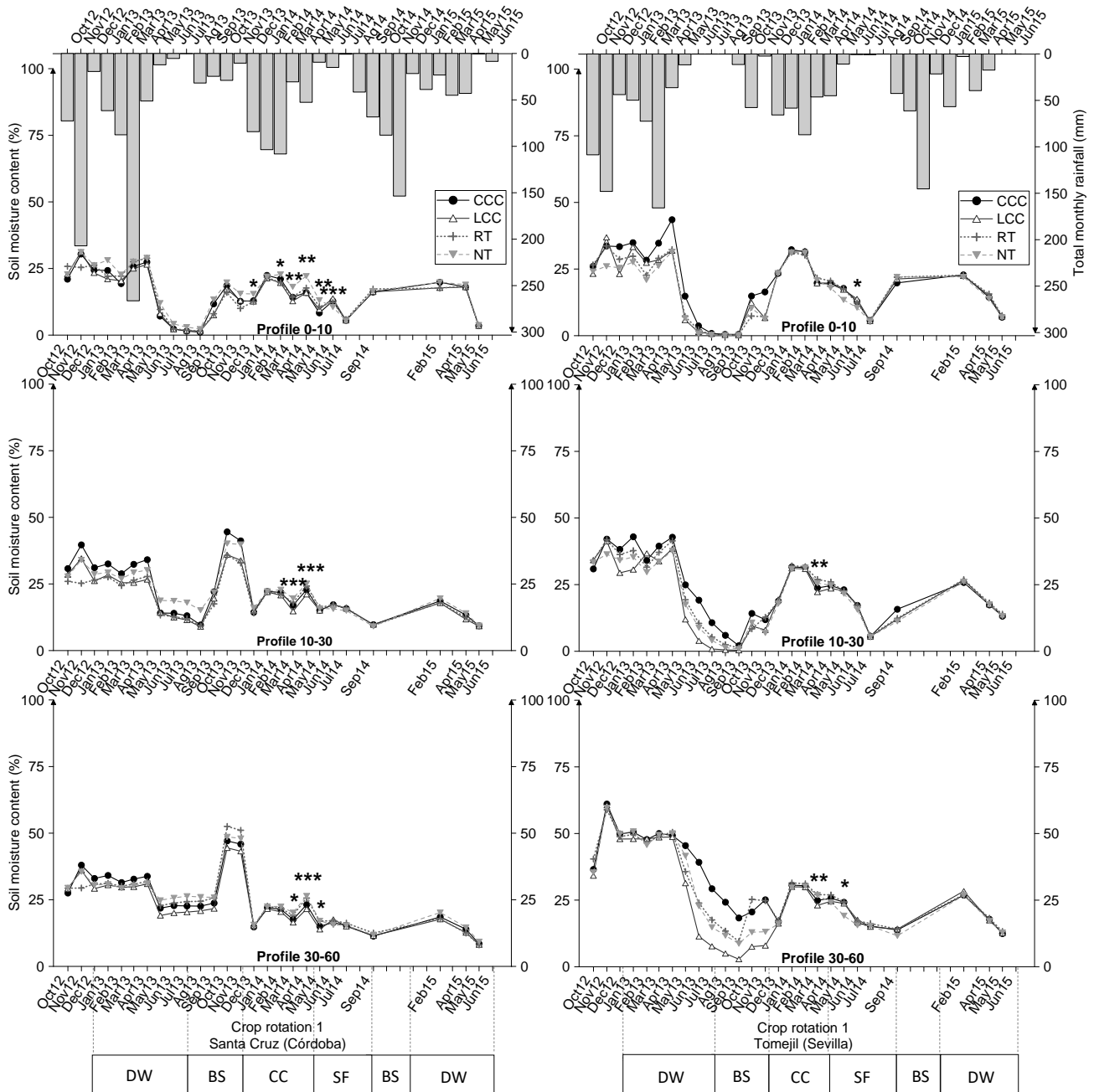


Figure 26. Temporal evolution of the monthly cumulative rainfall and soil moisture content in the three profiles (0-10,10-30 and 30-60 cm) for white mustard cover crop, narbon bean cover crop, reduced tillage and no tillage treatments (CCC, LCC, RT and NT) in Santa Cruz and Tomejil during the growing seasons 2012/2013, 2013/2014 and 2014/2015 according to the rotation 1: durum wheat-bare soil-cover crops-sunflower-bare soil-durum wheat (DW-BS-CC-SF-BS-DW). Differences between treatments were determined by Tukey's test. Only significant differences are shown: \*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ .

These results varied greatly in Tomejil. The topsoil showed the lowest soil moisture content, with 6-15 % in May and 1-4 % in June. Meanwhile, the second

profile ranged between 12-25 % in May and 4-20 % in June and the third profile averaged about 31-45 % and 11-39 %, respectively. These larger variations could be due to the deep and wide cracks opened in this kind of clay soils, which dried out quickly due to the changes in soil volume under such conditions (Richard *et al.*, 1995).

The year 2 of the rotation 1, differences between both CC and RT were not noticed all over the sunflower cropping season at both locations (Figure 26). However, NT treatments in Santa Cruz showed higher moisture content than CC from December to May, being more pronounced from March to May in all layers (between 27-57, 5-33 and 14-23 % higher in the 0-10, 10-30 and 30-60 cm profiles, respectively). It could be due to the highest stubble retention observed at the surface of the NT plots that year (ground cover values averaging 70-72 % throughout the period vs. values increasing from 22 and 35 % to 46 and 70 % for CCC and LCC, respectively) (Figure 24 in the previous section 3.4.1.), which contributed towards the maintenance of soil moisture levels by limiting the evaporation (Unger *et al.*, 1997). Conversely, the lowest moisture contents in CC plots were probably due to the increased water demand of cover crops from the stem elongation stage to full flowering. In May, after CC killing and sunflower germination, the soil moisture contents were also higher in NT than CC plots, but only in the first profile. However, during the sunflower emergence occurred in June, LCC (14 %) followed by CCC and RT treatments (both 12 %) showed the highest soil moisture content in the topsoil layer without significant differences in deeper layers. It could be due to the highest CC residues incorporated into the soil from the high ground coverage levels (70 % and 46 %, respectively) (Figure 24) and great biomass produced (10700 and 5800 kg/ha, respectively) (Table 5 in the previous section 3.4.1.). In Tomejil, differences between treatments were only observed at isolated moments. In the first profile, LCC plots also had the highest moisture contents in June (14 %) and were consistent with the greater ground cover (72% cover) and aerial biomass (8892 kg/ha) incorporated into the soil (Figure 24 and Table 5). In the deep layers, significant differences were only observed in March before CC killing, with the highest values reached by RT (27 %) and NT (26 %) due to the higher demand of CC at full flowering, especially in the LCC plots (22%) (Figure 26). However, two months later (May 2014), CC and RT stored more soil moisture contents than NT in the third profile. These differences could be due to the greater CC residues retention previously reported and

the minimum tillage performed for the sunflower planting in RT plots, reducing weed infestation (45 % weed cover) and consequently, the water use by them (Figure 24). These results are in agreement with those in Aboudrare *et al.* (2006), who explained that bare soil evaporation and weed transpiration were responsible for high water losses affecting the soil moisture profile at sunflower planting with different fallow management, especially in deeper soil layers (notably below 40 cm).

Finally, the year 3 of the rotation 1 also showed no significant differences between treatments or locations at any sampling date or layer. From the beginning of the durum wheat-growing cycle to the end of the winter season, soil moisture contents averaged around 17-20 % and 19-22 % in the first profile of Santa Cruz and Tomejil, respectively (Figure 26). By contrast, soil moisture reserves in the deeper layers increased from September to February due to the winter rainfall recorded (370 and 327 mm in Santa Cruz and Tomejil, respectively) (Figure 14 in Chapter 2). Nevertheless, these values varied similarly in both profiles of each location (soil moisture content ranged between 18-20 % in Santa Cruz and 26-28 % in Tomejil). These results showed again the greater soil water holding capacity of the Vertisol soils. From April onwards, as was observed during the year 1, topsoil moisture contents decreased more sharply (from 18-19 and 14-15 % to 3-4 % and 6-8 % in Santa Cruz and Tomejil, respectively) than both second and third profiles (from 12-15 and 26-27 % to 8-9 and 12-13 % in Alameda and Tomejil, respectively). This period corresponded to the durum wheat grain filling period but significant differences between treatments were not observed.

Soil moisture contents during the second set of 3-year rotation (rotation 2) are shown in Figure 27 for each profile and treatment in Santa Cruz and Tomejil. During the wet period of the year 1 (from November to April), there was no clear effect of CC in the topsoil layer at both locations, due to the high rainfall recorded in spite of the higher water demand of the CC (values ranged from 18 to 27 % in Santa Cruz and from 30 to 50 % in Tomejil). The second and third profiles showed higher soil moisture contents than the first one at both locations (values > 42 and > 51 % in Santa Cruz and > 60 and > 82 % in Tomejil, respectively) and significant differences between treatments were only observed in the 10-30 cm layer. In fact, LCC showed the highest



results from November to May in Santa Cruz (values ranged from 59 to 64 %), with NT plots holding the lowest moisture contents (averaging between 40-45 %). Conversely, in Tomejil, there were no appreciable differences between treatments, except for CCC that even registered higher values than the rest of treatments in May. These results indicated that soil moisture storage was not influenced by water consumption of the CC in rainy years. In addition, as was also reported by Dabney *et al.* (2001), the CC ground cover and roots probably increased water infiltration into the soil, while NT plots provided less favorable soil physical conditions (Tormena *et al.*, 2002) and led to higher soil compaction (Silva *et al.*, 2000). From May onwards, soil moisture contents were promptly reduced because of the scarce rainfall recorded, as also occurred in rotation 1. In the the 0-10 cm layer, values dropped to 2-3 % in Santa Cruz and 0.1-1 % in Tomejil. Nevertheless, significant differences were only shown in August and September in Tomejil, with the highest moisture reserves for CCC and LCC treatments. In deeper layers, there were no significant differences between treatments but CCC and LCC plots always showed higher soil moisture contents in absolute terms than NT plots at both locations, although particularly pronounced in Tomejil. Thus, in the second and third profiles, both CC obtained values > 19 and > 35 % in Santa Cruz and > 10 and > 33 % in Tomejil, respectively, while results for NT dropped to 1 % and 6 % in Tomejil.

This reduction of NT moisture values was probably due to the reduction of water infiltration into the soil after no-till management during the dry period after the sunflower emergence (June 2013). Lower water infiltration into the bare soil compared to areas sown with CC has previously been reported in olive orchards by Castro *et al.* (2006). Aboudrare *et al.* (2006) affirmed that sunflower water use is maximal from sowing to star bud stage, compared with the two other periods (star bud-flowering and flowering-maturity) being related to initial soil moisture stored. Conversely, Richard *et al.* (1995) declared that a severe soil-water deficit around flowering is especially critical, leading to substantial yield loss. In any case, deeper rooting offers sunflower the possibility to reach deeply placed soil water (Leach *et al.*, 1986), especially from the 30-60 cm layer on which the CC introduction had a positive effect, although not significant, toward an increased soil water reserves with respect to NT.

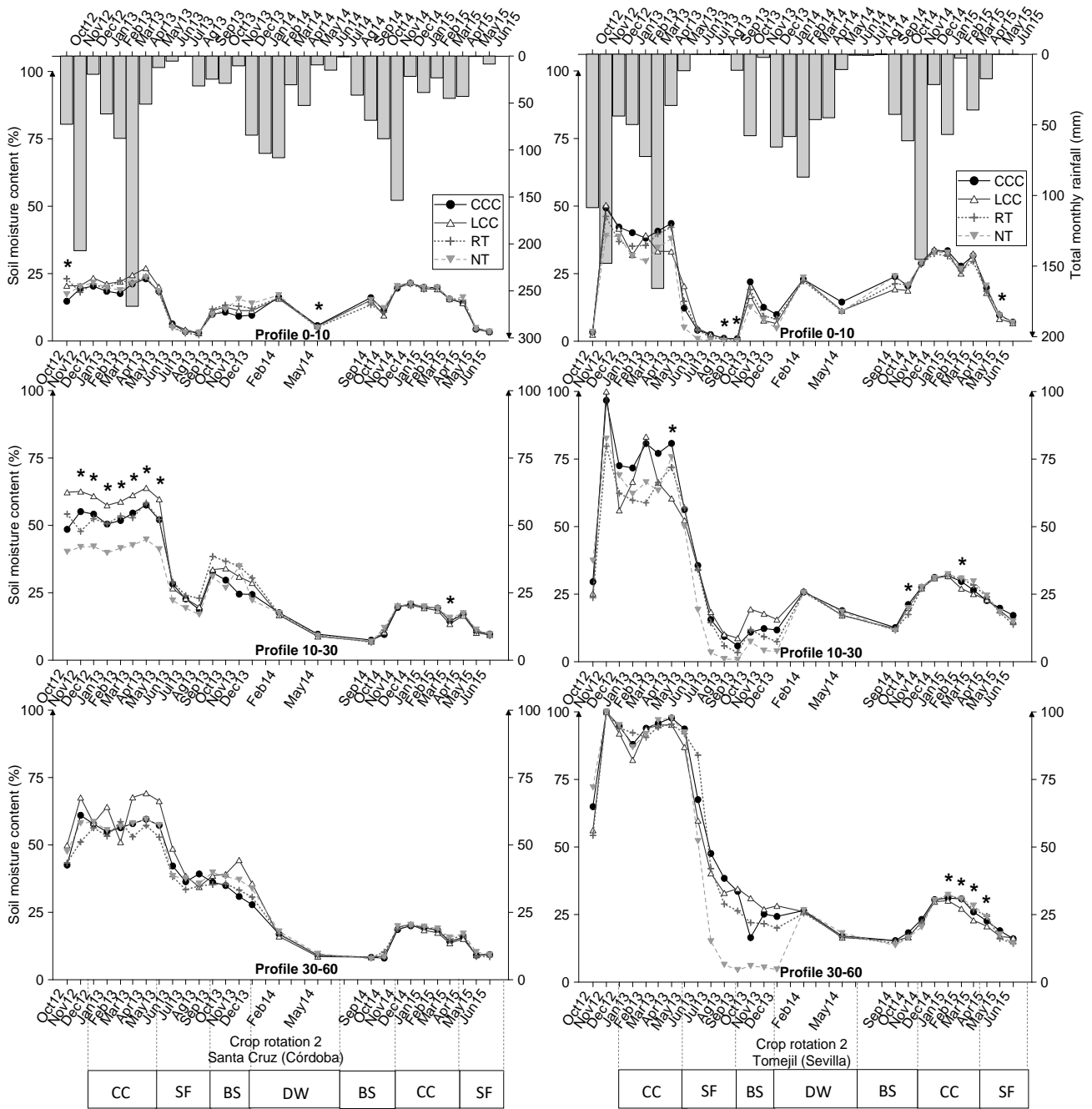


Figure 27. Temporal evolution of the monthly cumulative rainfall and soil moisture content in the three profiles (0-10,10-30 and 30-60 cm) for white mustard cover crop, narbon bean cover crop, reduced tillage and no tillage treatments (CCC, LCC, RT and NT) in Santa Cruz during the growing seasons 2012/2013, 2013/2014 and 2014/2015 according to the rotation 2: cover crops-sunflower-bare soil-durum wheat-bare soil-cover crops-sunflower (CC-SF-BS-DW-BS-CC-SF). Differences between treatments were determined by Tukey's test. Only significant differences are shown: \*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ .

During the dry year 2, significant differences between treatments were no observed all over the durum wheat growing season, except for May 2014 in Santa Cruz, when CCC and LCC treatments had slightly higher soil moisture contents than RT

and NT in the topsoil (by 14-16 % and 9-11 % higher, respectively) at the durum wheat ripening stage. It could be due to both CC residues retention with respect to RT and NT plots, which created a protective effect that reduced water evaporation from soil (Gregory *et al.*, 2000; Bolliger *et al.*, 2006). Finally, during the sunflower crop sequence of the year 3, significant differences between treatments were observed in March 2015 in Santa Cruz, when the NT (16 %) followed by RT plots (15 %) showed higher moisture content than CCC (14 %) and LCC (13 %) treatments in the second profile. This fact was probably due to the high water demand of both CC at their inflorescence emergence (Figure 27). In Tomejil, the high LCC ground cover development (99% cover) and subsequent biomass production (33000 kg/ha) (Figure 25 and Table 5 in the previous section 3.4.1.) resulted in the significantly lower soil water contents observed at isolated moments in all profiles. This effect was particularly noticeable from January to April in the 30-60 cm layer (values decreasing by 3-14 % with respect to CCC plots and by 7-24 % with regard to both RT and NT treatments). Nevertheless, from May onwards there were no significant differences in the second and third profiles. Thus, CC introduction did not affect the sunflower water reserve at its most advanced stages of development. Therefore, early mowing date of both CC should be done in order to avoid these negative soil moisture effects as was previously cited by Saavedra *et al.* (2016b), as a good management practice to use in periods of higher temperatures and evaporation rates of the cover crops.

As expected, soil moisture content depended on the soil management systems used, year conditions, soil depth and site-specific soil properties. However, our study showed a limited short-term impact on the water content stabilization during the durum wheat crop periods after a previous year with CC installation. Similar results about the limited impact of CC on water balance in dryland grain production systems in regions with a Mediterranean climate has been shown by Ward *et al.* (2012), where after a 3-y study at two different locations of south-western Australia, CC and residue retention were found to have a limited impact on total evaporation. During the sunflower crop cycle, there was occasional evidence of changes in soil moisture contents during CC growth stages. At isolated moments, CC plots significantly increased the soil moisture content after cover crops incorporation and sunflower planting at 0-10 cm depth. These results suggest that CCC and LCC can be used as an

alternative to RT in different soil types with changing rainfall patterns. However, CC could be an alternative to NT in years with higher precipitation than the average rainfall of the area, or in years with low rainfall ( $\leq$  average annual rainfall of the area) if CC are properly managed to avoid water competition, maximizing soil protection but minimizing soil water use by the cover crop. Impacts of cover cropping practices on water balance components have been demonstrated in many locations around the world with cereals crops (Tanaka, 1989; Krueger *et al.*, 2011; Murungu *et al.*, 2011; Qi *et al.*, 2011). In many regions and climatic zones, CC and residue retention have been shown to reduce evaporation from the soil surface (Gregory *et al.*, 2000; Bolliger *et al.*, 2006; Salado-Navarro and Sinclair, 2009). However, under Mediterranean conditions in southern Spain, CC have only been used in olive orchards with positive impacts in soil water content (Richard *et al.*, 1995; Alcántara *et al.*, 2011a). Further research will be needed to determine key management factors for CC such as sowing and mowing dates. Early sowing date could anticipate the CC full flowering stage to early spring (around 15<sup>th</sup> October or even before) and an early mowing date (Mid March or even before) could minimize water uptake by the CC, improving soil protection together with moisture reserves for the subsequent crop. If these aspects are improved, they would be the closest thing to the ideal cover crops, making use only of water that the crop would not normally use, producing sufficient biomass, protecting soil surface against erosion and enhancing infiltration and stored soil water by appropriate management during the long period of bare soil preceding sunflower planting.

### ***Soil fertility***

#### ***Soil organic nitrogen content***

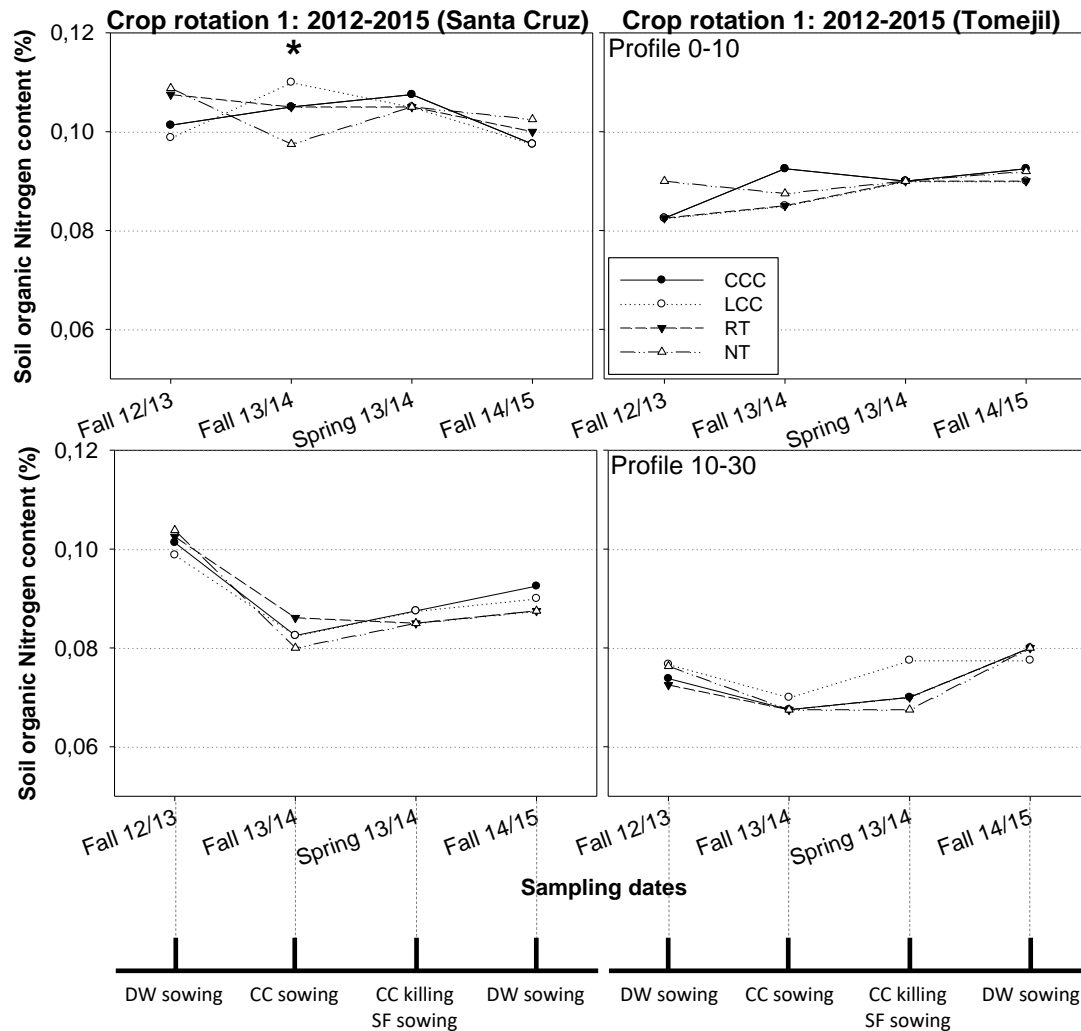
N availability is other of the most crucial aspects of the conservation cropping systems implementation. In general, the uppermost layer shows higher organic N contents than the deeper ones due to the contact with the crops residue cover and stubbles left on the soil surface (Murillo *et al.*, 1998; Ordóñez-Fernández *et al.*, 2007a). In fact, some authors have shown that the greatest N and OM accumulation of conservation tillage systems observed in the shallow layers (0-15 cm) disappears in deeper layers (Baker *et al.*, 2007; Blanco-Canqui and Lal, 2008). Consequently, the study of soil fertility was focused on the 0-10 and 10-30 cm profiles and the first profile

displayed values 15-23 % higher than the second one for all treatments and sampling dates (Figure 28 and 29). However, the evolution of organic N contents was different in each location during the 3-years study period. This fact corroborates the important role of soil types and weather conditions regarding the residue decomposition and their effects on soil fertility (Kriauciuniene *et al.*, 2012).

Similar organic N values were observed for all the treatments during the fall 12/13 in the crop rotation 1 (Figure 28), since all of them had the same soil management prior to the field trials beginning. During the fall 13/14 (year 2) before CC sowing, significant differences were observed in Santa Cruz. It was probably due to the soil management techniques used (tillage vs. no tillage). In fact, they only occurred in the first profile, the area most influenced by tillage. Therefore, LCC, CCC and RT treatments showed higher values than NT plots (by 7-11 % higher) due to the greater wheat straw decomposition (Flower *et al.*, 2012). Results for all the treatments and locations did not show significant differences during the spring 13/14 after CC killing. However, organic N contents recorded by CC treatments were the highest in absolute terms in the second profile, especially LCC treatment in Tomejil (by 11-12 % higher than RT and NT plots). It was probably due to the further breakdown of the previous carbon bean residues after a high biomass production (8892 kg/ha) with a significant lowest C/N ratio (18.58) that made them easily decomposable (Table 5 in the previous section 3.4.1). Similar results were obtained by Russell and Fillery (1996, 1999), which concluded that the primary value of legume stubble in the N economy of rotations is to replenish the soil organic N reserve at depth due to their below-ground-biomass-N fixation. Consequently, CC growing during periods when the soil might otherwise be fallow contributed on soil N content throughout the rotation in our climate conditions, especially legume cover crops (Dabney *et al.*, 2001).

During the fall 14/15, results did not show significant differences between treatments at any profile or location but final soil organic N values showed an improvement related to the presence of both CC treatments throughout the rotation 1 at both locations (Figure 28). In Santa Cruz, final organic N contents slightly decreased from the beginning to the end of the study period but the N contents in absolute terms declined less for the CC treatments than for RT and NT plots in the first (by 1-4 vs 6-7 % lower) and second profile (9 vs. 15 % lower). By contrast, final results improved in the

first and second profiles of Tomejil (by 1-12 % higher) and the CC treatments showed the greatest improvement (by 10-12 % higher) with respect to RT and NT (9 and 2 % higher, respectively) in the first layer.



**Figure 28.** Soil organic nitrogen content in the two profiles (0-10 and 10-30 cm) for white mustard cover crop, narbon bean cover crop, reduced tillage and no tillage treatments (CCC, LCC, RT and NT) in Santa Cruz and Tomejil according to the soil sequence in the rotation 1 (2012/2015): durum wheat-cover crops-sunflower-durum wheat (DW-CC-SF-DW). Differences between treatments were determined by Tukey's test. Only significant differences are shown: \*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ .

Results from crop rotation 2 showed similar organic N contents for all the treatment during the fall 12/13 (year 1), with a high variability of results observed in the first profile due to the soil variability existing in both sites prior to the trials installation (Figure 29). The high rainfall occurred during the year 1 diminished both CC ground covers and biomass productions and increased their C/N ratios (Figure 23 and Table 5). Therefore, there was an analogous breakdown of CCC and LCC residues this

wet year 1, which produced similar ground covers, biomass productions and C/N ratios. Results for the first profile did not show significant differences between treatments during the spring 12/13 after CC incorporation but CCC treatments showed significantly higher N contents (0.09 %) than the rest of treatments (around 0.08 %) in the second profile of Santa Cruz. The organic N contents for all the treatments during the fall 13/14 (year 2) and fall 14/15 (year 3) did not show significant differences at any profile or location. However, the weather conditions occurred during the year 3 led to a great development and subsequent biomass production of LCC plots (Figure 25 and Table 5). Consequently, LCC treatments showed the greatest N values, especially in Tomejil, where significant differences with respect to the rest of the treatments were observed in the first profile (0.113 % of N content vs. 0.09-0.10 %, respectively). This large increase observed in LCC plots of Tomejil was also noticeable in the second profile (13 % higher than the rest of treatments) but significant differences between them were not found. This absence of differences could be related to the soil type studied. Vertisol soils contain expandable mineral clays that shrink upon drying, forming wide cracks into which topsoil and organic materials tend to fall. It probably allowed a mixing of organic materials and soil particles which reduced concentration gradients typically found in conservation tillage systems (Ordóñez-Fernández *et al.*, 2007b; Melero *et al.*, 2011a).

The organic N contents of all the treatments in the crop rotation 2 also showed an improvement from the initial to the final values for the first profile at both locations (Figure 29). However, whereas in Santa Cruz NT treatment increased by 20 % followed by CCC and LCC plots (10 and 9 %, respectively), in Tomejil LCC increased by 36 % followed by RT and CCC plots (13 and 12 %, respectively). Results for the second profile showed that organic N contents for LCC were the only ones that improved from the beginning to the end of the rotation 2 in Tomejil (8 % higher), while the RT plots decreased by 4 % and CCC jointly with NT treatments remained unchanged (0 %). Conversely, final N contents were lower than the initial ones for all treatments in Santa Cruz but the treatments with lower losses were CCC and LCC compared to RT and NT (7 and 9 % of N losses vs. 10 and 14 %, respectively).

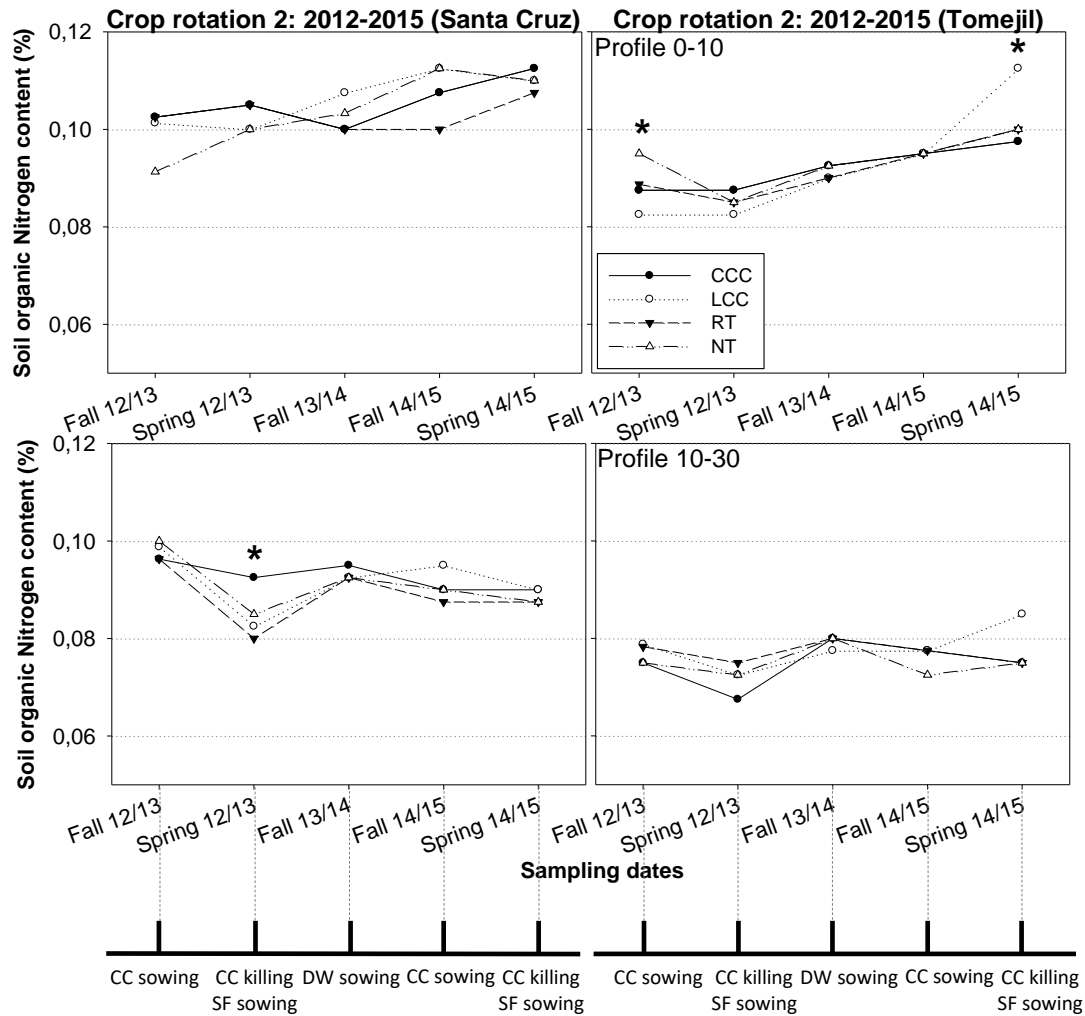


Figure 29. Soil organic nitrogen content (%) in the three profiles (0-10, 10-30 and 30-60 cm) for white mustard cover crop, narbon bean cover crop, reduced tillage and no tillage treatments (CCC, LCC, RT and NT) in Santa Cruz and Tomejil according to the soil sequence in the rotation 2 (2012/2015): cover crops-sunflower-durum wheat-cover crops-sunflower (CC-SF-DW-CC-SF). Differences between treatments were determined by Tukey's test. Only significant differences are shown: \*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ .

Results from both rotations showed greater soil organic N contents occurring in the top-soil after the CCC and LCC killing and incorporation into the soil in addition to a higher contribution to maintain soil nitrogen fertility in subsequent crops after CC cultivation with respect to the bare soil during the fallow period (Dabney *et al.*, 2001). In this regard, Frede *et al.* (1994) reported similar results with the change from traditional tillage methods to CA systems, with significant differences being isolated cases. Additionally, LCC plots slightly improved their N values from the beginning to the end of the study period in most of the sampling dates. It could be related to the high input of legume crop residues in continuous cover cropping compared to



rotations with a bare fallow period and their capacity to fix biologically N into the soil. These results are in agreement with Perdigao *et al.* (2012), who affirmed that leguminous green manure species can be used as a N source for the following crops because they can fix atmospheric N biologically and improve the soil's N fertility. López-Bellido *et al.* (2010a) and Melero *et al.* (2011a) also reported that crop rotations that include a leguminous species showed positive effects on enhancing soil fertility because they had the greatest N inputs to the soil. However, the changes in N content occur slowly (Mazzoncini *et al.*, 2011) and more time than 3-year study is necessary for an effective soil N content knowledge under southern Spain conditions (Murillo *et al.*, 1998).

### ***Soil organic matter content***

The overall fertility of an agricultural soil has always been related to its content in OM. In fact, the maintenance of adequate levels of this parameter in the soil is of great agronomic importance, since it intervenes in all the processes connected to the structure dynamics, the plant growth and the macro and microbial life sustaining it (Ordóñez-Fernández *et al.*, 2007b). Among factors that may affect changes in soil oxidizable OM content, weather conditions, crop plant species and the level of organic and mineral fertilization are mentioned most frequently (Harasim *et al.*, 2016). The consequent beneficial effect of crop residues primarily depends on their nutrient release.

In our study, the value of this trait was not affected by treatments, soil layers or sampling dates in any location during the rotation 1 (Figure 30). However, slightly greater OM contents were found in the uppermost layer (0-10 cm) than in the second one, and this difference was 12-22 % and 17-22 % higher in Santa Cruz and Tomejil, respectively. These results basically corroborated those in the literature, existing greater OM contents near the soil surface due to the surface placement of crop residues (Dick, 1983; Unger *et al.*, 1997; Ordóñez-Fernández *et al.*, 2007a). In fact, this increase was even greater in absolute terms during the spring 13/14 (year 2) in Santa Cruz, with 5 and 12 % higher OM contents than at the initial crop sequence for CCC and LCC treatments, respectively. Moreover, this improvement observed in LCC plots was maintained until the end of rotation 1 (by 12 %), while the rest of treatments

decreased their OM contents from 4 to 13 %. By contrast, all treatments improved from the beginning to the end of the rotation 1 in the first profile of Tomejil (values between 4-6 % higher). In the second profile of Santa Cruz, the OM contents decreased in all treatments, but LCC treatment was less affected than the rest of treatments (10 % vs. 13-24 % lower, respectively). A similar trend was also noted in the second profile of Tomejil, with final OM contents slightly lower than those at the beginning for all treatments (between 5 and 13% lower) except for the RT plots (values around 0.46 % higher). However, significant differences were not observed.

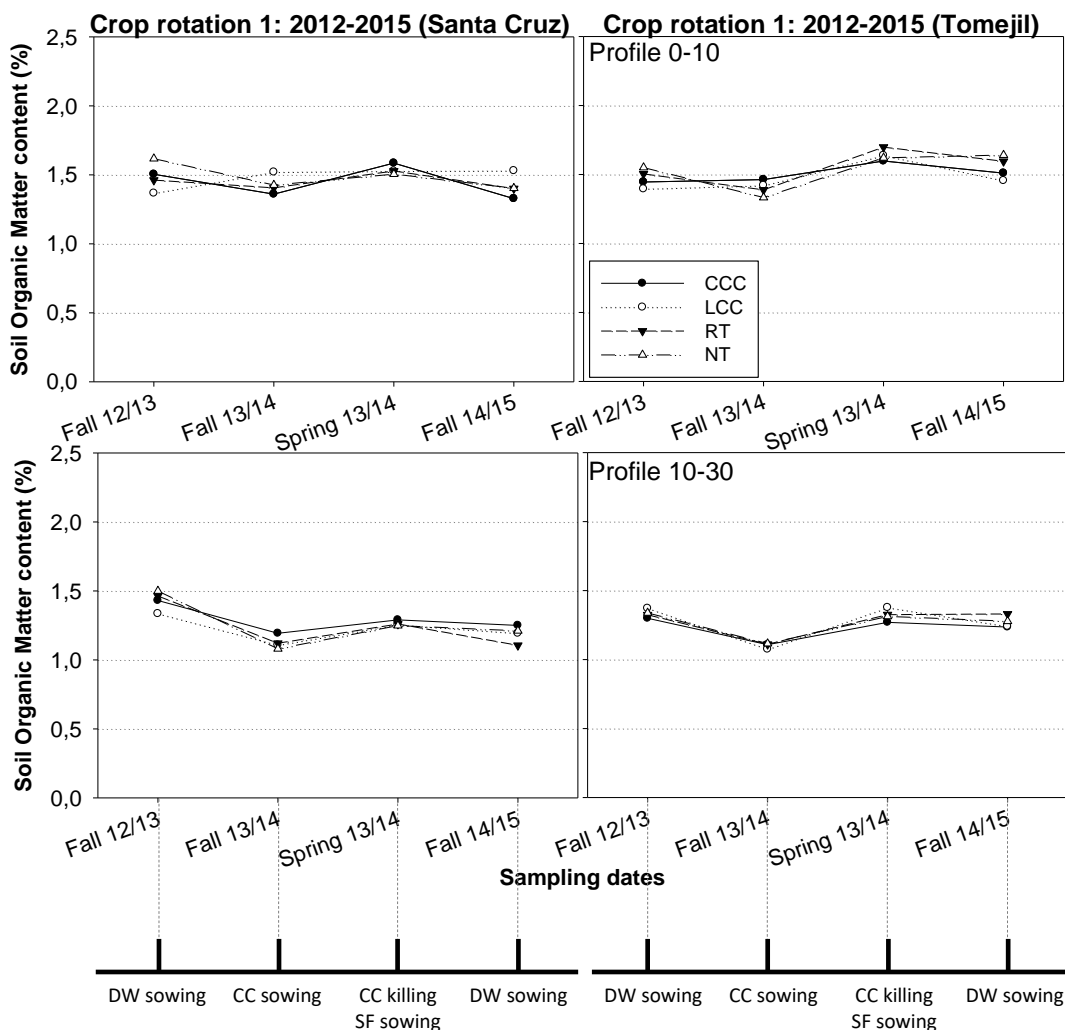


Figure 30. Soil organic matter content (%) in the three profiles (0-10, 10-30 and 30-60 cm) for white mustard cover crop, narbon bean cover crop, reduced tillage and no tillage treatments (CCC, LCC, RT and NT) in Santa Cruz and Tomejil according to the soil sequence in the rotation 1 (2012/2015): durum wheat-cover crops-sunflower-durum wheat (DW-CC-SF-DW). Differences between treatments were determined by Tukey's test. Only significant differences are shown: \*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ .

In the rotation 2 the trend was similar to the rotation 1 (Figure 31). The OM contents in the topsoil layer slightly increased from the beginning to the end of the rotation for LCC and NT treatments in Santa Cruz (0.5 % and 5 % respectively), although without significant differences between treatments. In Tomejil, the initial soil variability showed NT treatments with the highest initial OM content (1.70 %) in the uppermost layer, followed by CCC treatment (1.54 %) and LCC/RT plots (1.49 %). However, LCC treatment was improved throughout the rotation 2, reaching the highest OM content (1.94 %) after CC incorporation during the spring 14/15 (year 3). LCC treatment had the highest ground cover and biomass production that year (Figure 25 and Table 5), which contributed not only to the highest soil organic N contents (Figure 29) but also to the highest OM contents due to the great biomass produced. Therefore, its significant low C/N ratio (15.95) decomposed quickly (Hasegawa *et al.*, 2000) and had a positive effect on soil organic matter content (by 16 % higher than the rest of treatments) in the topsoil layer. Similar short-term experiments also indicated a significant positive effect on soil OM content as a result of the use of cover cropping systems (Kulig *et al.*, 2004; Harasim *et al.*, 2016).

Moreover, a similar trend in the decrease of the OM contents was observed in the second profile of Santa Cruz and Tomejil, showing lower values than the topsoil at both locations (around 15-24 and 20-28 % lower, respectively). In addition, once again the OM results were reduced from the beginning to the end of the rotation 2 in the second profile, but both CC treatments showed the lowest reduction in Tomejil (5 % vs. 13-14 % lower OM contents observed in RT and NT plots).

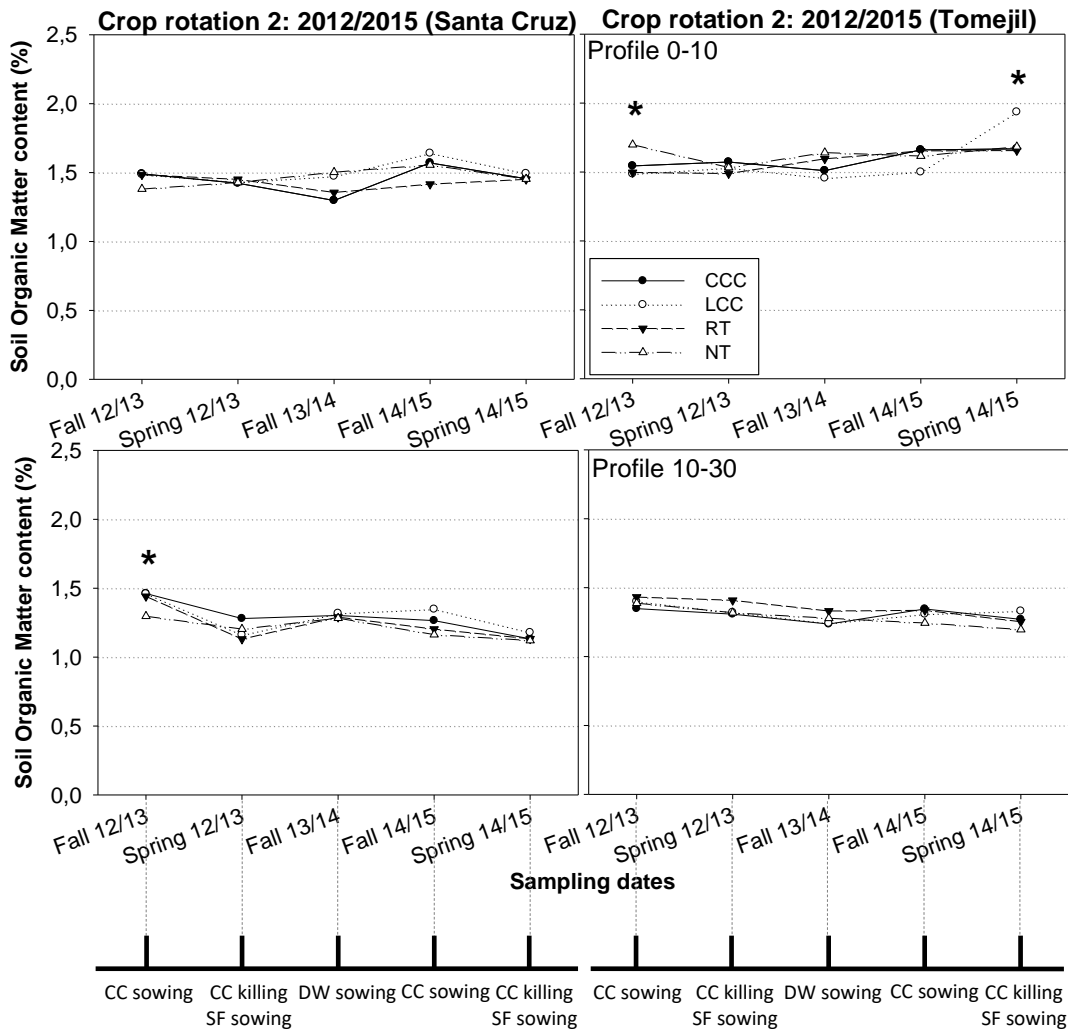


Figure 31. Soil organic matter content (%) in the three profiles (0-10, 10-30 and 30-60 cm) for white mustard cover crop, narbon bean cover crop, reduced tillage and no tillage treatments (CCC, LCC, RT and NT) in Santa Cruz and Tomejil according to the soil sequence in the rotation 2 (2012/2015): cover crops-sunflower-durum wheat-cover crops-sunflower (CC-SF-DW-CC-SF). Differences between treatments were determined by Tukey's test. Only significant differences are shown: \*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ .

As occurred with soil organic N contents, the different conservation cropping systems did not have a great influence in the short-term soil OM contents. However, the increase OM trend of the CC treatments in the uppermost layer, especially the LCC plots, and the lowest reduction of soil OM contents for both CC treatments in the deeper layers could lead to a larger storage of soil OM contents in the long-term. In general, our results are in agreement with similar studies on conservation tillage techniques (Mazzoncini *et al.*, 2011; Melero *et al.*, 2011a; Harasim *et al.*, 2016). However, results can vary due to soil type, crop rotation sequence and the quantity and quality of plant residues returned to the soil (Ordóñez-Fernández *et al.*, 2007b). In

this regard, one of the most important factors affecting the residue quality is the C/N ratio. Our results showed legume cover crops with lower C/N ratio than non-leguminous ones (such as white mustard, durum wheat and sunflower), which slightly accelerated the residue decomposition into the soil (Melero *et al.*, 2011a). Nevertheless, changes in both N and OM content occur very slowly and are greatly influenced by weather conditions (Melero *et al.*, 2011b). If the improvement of the N and OM levels by the use of winter cover crops, especially legume cover crops, are confirmed in the long-term, these benefits could impact on other agroecosystem processes further than protecting the soil from erosion or improving the physicochemical properties of the soil. The contribution of cover crops residues to the soil could mitigate greenhouse gas emissions on nitrous oxide (N<sub>2</sub>O) due to the biological N fixation (López Bellido, 2017c) and soil carbon dioxide (CO<sub>2</sub>) due to the greater soil carbon sequestration (Repullo-Ruibérriz de Torres *et al.*, 2012). Therefore, long-term studies are necessary to determine the magnitude of the effects of cover cropping factor on noticeable N and OM storage with respect to other CA techniques such as NT or RT and their potential to improve sustainability in cropping systems under rainfed Mediterranean conditions.

### **3.4.3 EFFECT OF WHITE MUSTARD AND NARBON BEAN ON DURUM WHEAT AND SUNFLOWER GROWTH, YIELD AND QUALITY**

#### ***Durum wheat growth, yield and quality***

With the aim of assessing the durum wheat response to the different cover cropping systems and the crop rotation effect, growth, yield and quality parameters were shown in Tables 8-10 and Figure 32. Durum wheat varied in terms of the cover crops treatments and years in most variables evaluated (Table 6). However, there was no interaction between treatments×year for any variable (except for HLW in Tomejil). The results were analyzed for both locations and years separately.

Plant density, mean H and SN values during the years 1 and 3 of the rotation 1 did not show significant differences between treatments in Santa Cruz (Table 7). Similar results were observed in Tomejil, except for PD values obtained by LCC plots the year 1, followed by CCC and RT, which were significantly higher than NT

treatments in Tomejil (Table 7). Since it was the first year of the crop rotation sequence, CC treatments had no effect on the PD results, but could there be an effect of the management system applied on the Vertisol soil, obtaining a higher PD in minimum tillage plots (CCC, LCC and RT) compared with no-tilled plots (NT). Similar results under Mediterranean conditions were obtained by López-Garrido *et al.* (2014), who found worse emergence values in a wheat-sunflower-fodder pea crop rotation under NT technique than RT. Nevertheless, conversely to the negative effect of this latter research on its height and quality values, our results did not show significant differences for these parameters. Durum wheat characterization for rotation 2 (year 2) displayed significant differences between treatments for PD in Santa Cruz, with significant highest values obtained by the NT treatments in this *Typical Xerofluvent* soil. Subsequently, it resulted in a higher SN than the rest of treatments. However, in this rotation 2 there were no significant differences between treatments for any of the variables studied in Tomejil.

**Table 6. Summary of ANOVAs for testing the effect of treatments, year and their interaction on the different variables evaluated for durum wheat in Santa Cruz and Tomejil.**

Durum wheat ( <i>Triticum durum</i> cv. Amilcar)	Santa Cruz			Tomejil		
	Treatments <sup>1</sup> (TR)	Year <sup>2</sup> (Y)	TR × Y	Treatments <sup>1</sup> (TR)	Year <sup>2</sup> (Y)	TR × Y
	F			F		
PD	1.30 n.s. <sup>3</sup>	1.77 n.s.	0.54 n.s.	1.80 n.s.	82.72***	1.62 n.s.
H	2.06 n.s.	29.98***	1.18 n.s.	1.10 n.s.	97.05***	0.96 n.s.
SN	2.60 n.s.	42.14***	1.35 n.s.	0.44 n.s.	100.10***	1.24 n.s.
Yield	0.75 n.s.	11.95***	0.65 n.s.	1.44 n.s.	127.14***	1.22 n.s.
PC	0.67 n.s.	20.58***	0.75 n.s.	8.19**	47.39***	1.12 n.s.
HLW	0.85 n.s.	9.07**	0.66 n.s.	2.90*	91.43***	5.71**
V	0.43 n.s.	98.85***	0.55 n.s.	4.85**	220.01***	1.77 n.s.

<sup>1</sup>Treatments: CCC, LCC, RT, NT

<sup>2</sup>Year: year 1 (2012/2013), year 2 (2013/2014), year 3 (2014/2015)

<sup>3</sup> $p < 0.05^*$ ,  $p < 0.01^{**}$ ,  $p < 0.001^{***}$ , n.s.: not significant

The contradictory PD results showed that durum wheat crop growth responded to tillage systems, but with differences between locations, as was previously reported by Cantero-Martínez *et al.* (2007) at three different sites with a wide variability of results. It should be noted the best PD and SN values obtained during year 3 with respect to year 1 in Tomejil and the highest H results during the year 2 at both locations. The low rainfall and high temperature conditions during the year 3 and 2

compared to the wet year 1 could have promoted wheat growth and development. In fact, higher GDD were accumulated the year 3 and 2 compared to year 1 by the plants from the stem elongation stage (2301 and 2177 GDD after 174 DAS, respectively). The crop rotation effect together with the most favourable weather conditions could have improved the growth and subsequent development in all treatments, without significant differences. Therefore, no residual effect of the different cover cropping treatments (CCC and LCC) was observed in the short-term evaluation of the durum wheat growth parameters.

**Table 7. Durum wheat characterization: plant density (PD as plant/m<sup>2</sup>), mean height (H as m) and spikes number (SN as spikes/m<sup>2</sup>) in the white mustard cover crop, narbon bean cover crop, reduced tillage and no tillage treatments (CCC, LCC, RT and NT) during the different growing seasons (2012/2013 and 2014/2015 for rotation 1 and 2013/2014 for rotation 2) in Santa Cruz.**

Santa Cruz									
	Rotation 1 (DW-SF-DW)						Rotation 2 (SF-DW-SF)		
	Year 1 (2012/2013)			Year 3 (2014/2015)			Year 2 (2013/2014)		
	PD	H	SN	PD	H	SN	PD	H	SN
	plant/m <sup>2</sup>	m	spikes/m <sup>2</sup>	plant/m <sup>2</sup>	m	spikes/m <sup>2</sup>	plant/m <sup>2</sup>	m	spikes/m <sup>2</sup>
	28 DAS	160 DAS		21 DAS	174 DAS		43 DAS	174 DAS	
	<b>254 GDD</b>	<b>1847 GDD</b>		<b>242 GDD</b>	<b>2177 GDD</b>		<b>293 GDD</b>	<b>2301 GDD</b>	
CCC	308.9 a <sup>1</sup>	0.84 a	362.2 a	317.9 a	0.72 a	300.0 a	282.8 b	0.80 a	209.7 b
LCC	325.5 a	0.81 a	348.6 a	310.6 a	0.74 a	326.1 a	309.3 ab	0.81 a	220.9 b
RT	324.8 a	0.84 a	323.3 a	309.6 a	0.69 a	293.9 a	312.5 ab	0.83 a	239.7 b
NT	335.4 a	0.86 a	351.5 a	316.4 a	0.78 a	336.5 a	320.3 a	0.82 a	278.1 a

Tomejil												
	20 DAS			153 DAS			27 DAS			152 DAS		
	207 GDD			1814 GDD			297 GDD			1855 GDD		
	44 DAS	186 DAS										
	333 GDD	2301 GDD										
	301.0 a	0.92 a	293.6 a	429.6 a	0.78 a	366.1 a	312.1 a	0.94 a	326.9 a	430.9 a	0.76 a	355.3 a
CCC	360.9 ab <sup>1</sup>	0.71 a	202.9 a	416.7 a	0.73 a	386.4 a	301.0 a	0.92 a	293.6 a	429.6 a	0.78 a	366.1 a
LCC	392.4 a	0.70 a	203.6 a	429.6 a	0.78 a	366.1 a	312.1 a	0.94 a	326.9 a	430.9 a	0.76 a	355.3 a
RT	349.3 ab	0.77 a	211.8 a	430.9 a	0.76 a	355.3 a	299.1 a	0.93 a	333.1 a	426.6 a	0.75 a	379.9 a
NT	325.5 b	0.71 a	236.3 a	426.6 a	0.75 a	379.9 a	314.1 a	0.94 a	310.7 a			

<sup>1</sup> Different small letters within each year per column indicate that the differences between treatments were statistically significant (Tukey's,  $p \leq 0.05$ )

Grain yield results also showed that durum wheat crop varied significantly among years (Figure 32), but with differences between locations, as was also observed by other authors in similar studies under Mediterranean conditions (Cantero-Martínez *et al.*, 2007). In fact, the year 1 in Santa Cruz, the wettest of the 3-years study gave a higher grain yield than the other two in absolute terms. Similar results were obtained by López-Bellido *et al.* (1998), revealing a direct relationship between grain yield and the amount of rainfall during the vegetative period of durum wheat. Additionally, there were no significant differences either between grain yields produced or between

treatments the year 1 and 3 of the rotation 1 in Santa Cruz (values averaging 3887-4100 and 2732-3710 kg/ha, respectively). They only differed significantly with the results obtained the year 2 (around 2166 and 2521 kg/ha), which were the lowest of the 3-years study period, without significant differences between treatments. These results are in agreement with those recorded by the national agricultural statistics (MAPAMA, 2017), with a lower production than the previous years and a mean grain yield of 2553 kg/ha for farmers in the area (province of Córdoba).

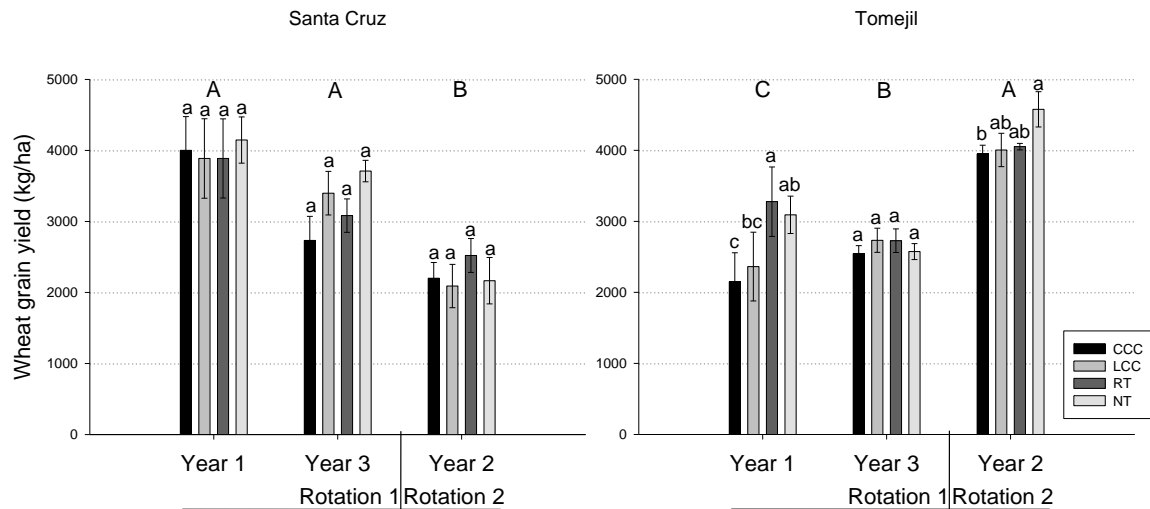


Figure 32. Wheat grain yield (kg/ha) in the white mustard cover crop, narbon bean cover crop, reduced tillage and no tillage treatments (CCC, LCC, RT and NT) during the different growing seasons (2012/2013 and 2014/2015 for rotation 1 and 2013/2014 for rotation 2) in Santa Cruz and Tomejil. Significant differences between treatments are shown as different small letters and significant differences between years as different capital letters per column (Tukey's,  $p \leq 0.05$ ).

By contrast, the heavy rainfall occurred from February to March 2013 (238 mm) on the Vertisol soil of Tomejil absorbed so much water that produced temporary waterlogging conditions which probably affected the final grain yield results. Therefore, year 1 showed the lowest grain yield of the study period (values averaging 2150-3277 kg/ha), thus highlighting how harmful excessive rainfall may be on Vertisols. Similar grain yield results were observed by López-Bellido *et al.* (2001) for this kind of soils during a very wet year. Moreover, differences between treatments were significant, with higher grain yields obtained in RT followed by NT treatments compared to CC treatments. As noted above, year 1 was the beginning of the crop rotation 1 and CC treatments could not have any effect on the grain yield results. For that reason, there appears to be no ready explanation for these grain yield results.



Following with the rotation 1, year 3 produced higher grain yields than year 1 (values averaging 2545-2727 kg/ha, respectively) without significant differences between treatments. However, the highest grain yield was obtained during the year 2 (around 3949-4577 kg/ha), with significantly higher values obtained by NT plots, followed by RT and LCC and the lowest was the CCC treatment. According to MAPAMA (2017), similar average productions were recorded by farmers in the area (province of Seville) during the study period, with the lowest average yields observed the year 1 (3027 kg/ha) and the highest the year 2 (3479 kg/ha).

After the 3-year study period, we have concluded that treatments had no significant effect on grain yield for the 3 years as a whole (Table 6). Differences, however, were significant in the wet year 1 and dry year 2 in Tomejil; yields were higher with RT in the former and with NT in the latter case (Figure 32). The treatment $\times$ year interaction was not significant in any location (Table 6). Moreover, there was also no residual effect of the cover crops treatments, since the previous year with the different cropping systems in addition to sunflower main crops did not affect the final durum wheat yield. With respect to this, Plaza-Bonilla *et al.* (2016) observed that the incorporation of cover crops did not reduce the grain yield of the different cash crops in grain legume-based rotations managed under conventional tillage. Therefore, we concluded that the use of cover crops could be efficient to improve different soil features affecting the long-term cropping system level without reducing durum wheat productivity under Mediterranean conditions.

The effect of the cropping system treatments and crop rotation on the quality of durum wheat was also studied (Table 8). The grain quality of durum wheat is influenced by the PC, HLW and V, and indeed governs its final quality group category according to the Royal Decree 190/2013 of March 15, 2013 (more information in Appendix 4). The PC differed significantly between years (Table 8). It obtained the highest values during the year 3 (from 11.9 to 13.4 %) and the lowest ones during the year 1 at both locations (from 10.1 to 11.1 %), with intermediate results observed during the year 2 (11.5-13.6 %). According to López-Bellido *et al.* (1998), the grain PC varies significantly and inversely with the amount of rainfall. Rainfall from April to May (durum wheat at stem elongation-grain filling) (Appendix 1 and 2) could strongly influence PC in Santa Cruz and Tomejil, which were the greatest during the year 1 with

330 and 214 mm, respectively, and decreased thereafter (93-12 mm the year 2 and 88-56 mm the year 3, respectively) (Figure 14, chapter 2). Moreover, low temperatures have a negative effect on PC (Rao *et al.*, 1993; Tonitto *et al.*, 2006). Temperature always recorded high values under Mediterranean conditions, especially during the grain filling stage. Nevertheless, the lower GDD accumulation occurred during the year 1 in Santa Cruz and Tomejil (1847 and 1814 GDD accumulated, respectively) could also diminish the PC results compared to the warmest years 3 and 2 (1855-2177 and 2301 GDD accumulated, respectively).

**Table 8. Durum wheat quality parameters according to the Spanish Wheat Quality Standardization System: protein content (PC as %), specific weight (HLW as kg/hl) and vitreosity (V as %) establishing the final quality group category (QC) for the white mustard cover crop, narbon bean cover crop, reduced tillage and no tillage treatments (CCC, LCC, RT and NT) during the different growing seasons (2012/2013 and 2014/2015 for rotation 1 and 2013/2014 for rotation 2) in Santa Cruz and Tomejil.**

Santa Cruz												
Rotation 1 (DW-SF-DW)								Rotation 2 (SF-DW-SF)				
Year 1 (2012/2013)				Year 3 (2014/2015)				Year 2 (2013/2014)				
	PC	HLW	V	QC	PC	HLW	V	QC	PC	HLW	V	QC
	%	kg/hl	%	-	%	kg/hl	%	-	%	kg/hl	%	-
CCC	10.8 a <sup>1</sup>	85.1 a	76.5 a	4	13.3 a	84.5 a	95.5 a	1	11.6 a	83.3 ab	95.0 a	3
LCC	10.5 a	84.7 a	70.5 a	4	13.1 a	84.9 a	95.0 a	1	11.7 a	83.5 ab	94.5 a	3
RT	10.6 a	83.9 a	69.0 a	4	13.4 a	84.2 a	96.5 a	1	11.5 a	83.7 a	94.0 a	3
NT	10.1 a	84.6 a	69.5 a	4	11.9 a	84.2 a	94 a	2	12.0 a	82.8 b	95.5 a	2
Tomejil												
CCC	11.1 a <sup>1</sup>	85.6 a	79.5 a	3	13.0 ab	82.3 bc	96.0 a	1	12.9 a	81.8 ab	97.5 a	2
LCC	11.1 a	84.9 ab	77.5 a	3	13.6 a	82.2 c	97.5 a	1	13.6 a	81.2 b	98.0 a	1
RT	10.6 a	84.6 ab	78.5 a	4	12.2 b	83.4 a	94.0 a	2	12.9 a	81.6 b	98.0 a	2
NT	10.1 a	84.2 b	68.0 a	4	12.4 ab	83.3 ab	95.0 a	2	12.6 a	83.2 a	96.5 a	2

<sup>1</sup> Different small letters within each year per column indicate that the differences between treatments were statistically significant (Tukey's,  $p \leq 0.05$ )

Significant differences between treatments were not observed (Table 8), except for Tomejil the year 3, where LCC treatments reached the highest PC values followed by CCC and NT. It was probably due to the high biomass produced by LCC plots during the year 2 (8892 kg/ha, Table 5 of the section 3.4.1.). They slightly improved the N values from the beginning to the end of the rotation 1 in the uppermost layer (Figure 28 of the previous section 3.4.2.). Therefore, in a system with all treatments receive similar N fertilizer rate, this additional residual N present in the LCC plots contributed to improving the PC results obtained by the subsequent durum wheat rotation. Some studies have analyzed wheat grain PC as a function of tillage system, reporting no significant differences (Cox and Shelton, 1992; Campbell *et al.*, 1998). López-Bellido *et*

*al.* (1998) in contrast, reported higher grain PC for conventional tillage techniques than for no tillage, showing that rotations including a legume crop prompt an increase in PC due to the legume's ability to fix atmospheric nitrogen (N<sub>2</sub>), thereby increasing residual soil N, as probably occurred in our results. HLW showed significant treatment×year interaction (Table 6), with similar results the year 1 and 3 in Santa Cruz (values averaging 83.9-85.1 kg/hl) but higher the year 1 than year 3 in Tomejil (values around 84.2-85.6 vs. 82.2-83.3 kg/hl) (Table 8). The year 2 showed the lowest values at both locations (81.2-83.7 kg/hl). Treatments had no effect on this index the year 1 and 3 in Santa Cruz but it did in the rest of cases, varying widely without following any fixed pattern. Results for V differed between years, without significant differences between year 3 and 2 at both locations (values around 94 and 98 %) but with the lowest values the year 1 (from 68 to 79.5 %). With regard to the different treatments, there were no significant differences between them.

The set of PC, HLW and V values obtained at the end of the rotation 1 (year 3) resulted in an improvement of the final durum wheat QC with respect to year 1 and also to that obtained during year 2 in all the treatments at both locations. Overall, in our study, crop rotation had a significant influence on grain quality. Durum wheat was classified in the lower QC the year 1, with all treatments belonging to group 4 in Santa Cruz and group 3 and 4 for CCC-LCC and RT-NT treatments, respectively in Tomejil. By contrast, CCC and LCC were ranked in group 1 the year 3 at both locations. RT plots also belonged to group 1 in Santa Cruz and NT fell under group 2, while both treatments were clustered in group 2 in Tomejil. Intermediate durum wheat QC was observed during year 2 at both locations, with a lower classification for CCC, LCC and RT (group 3) plots than NT (group 2) in Santa Cruz. However, Tomejil showed a greater QC for LCC plots (group 1) than the rest of treatments (group 2). According to Debaeke *et al.* (1996), the effect of the preceding crop is a key factor in wheat quality. In our results, in addition to the wheat quality improvement from year 1 to 3 in all treatments, cover crops seemed to have a clear beneficial influence on winter wheat quality. Thus, treatments with minimum tillage (CCC, LCC and RT) had better QC than NT plots in Santa Cruz at the end of rotation 1. In Tomejil this trend was only observed in CC treatments (CCC and LCC plots) during year 3 and in LCC plots the year 2. Therefore, quality parameters were probably improved by the inclusion of cover crops

jointly with minimum tillage management in preceding sunflower crop sequence, with subsequent higher values for the final category index.

It is very characteristic in Mediterranean conditions that differences in growing season precipitation contribute to durum wheat development. Many studies have reported how crop growth, grain yield and quality responded to tillage systems, with differences between locations and at locations between years (López-Bellido *et al.*, 1996, 1998; Mrabet, 2000; Cantero-Martínez *et al.*, 2007). In our results, these parameters vary depending on weather conditions in the different growing seasons. However, no relationship was found between yield depending on the total rainfall of the year and the treatments at the end of the rotation 1 and the year 2. Therefore, no residual effect of the cover crops treatments cultivated during the previous year was observed on durum wheat. By contrast, treatments, tillage management and weather conditions greatly affected durum wheat quality. The effect of the preceding crop was a crucial factor affecting wheat quality parameters. The wheat cultivated after a preceding crop with minimum tillage system had a higher quality compared to NT treatments. These results even enhanced when wheat was grown in rotations including cover crops, especially in Vertisol soils, and particularly legume species. Although this study is related to a single 3-yr crop rotation and specific crop management techniques, they provide useful information on the behaviour of durum wheat under different crop management system in the preceding crop and under various weather conditions over time. Nevertheless, long-term studies are required to investigate the long-term effects of these cover cropping systems on durum wheat yield and quality. These studies could also determine if, after cover crops cultivation, the subsequent wheat crop could reduce mineral fertilizer rate required and improves the phytosanitary condition of durum wheat.

### ***Sunflower growth, yield and quality***

Sunflower response after the different cropping system treatments in the crop rotation was shown in Tables 11-12 and Figure 33. Treatments had significant effects on all variables, except for HD in Santa Cruz and H and OC in Tomejil (Table 9). Moreover, all parameters studied, except OC in Tomejil, were significantly influenced by year. Moreover, statistical analysis showed an interaction between treatments and

years in most variables evaluated, except for HD in Santa Cruz and for H and HD in Tomejil, with a similar effect of treatments each year.

**Table 9. Summary of ANOVAs for testing the effect of treatments, year and their interaction on the different variables evaluated for sunflower in Santa Cruz and Tomejil.**

Sunflower ( <i>Helianthus annuus</i> ) Variables evaluated	Santa Cruz			Tomejil		
	Treatments <sup>1</sup> (TR)	Year <sup>2</sup> (Y)	TR × Y	Treatments (TR)	Year (Y)	TR × Y
	F			F		
PD	0.38 n.s. <sup>3</sup>	46.67***	2.73*	5.17**	47.07***	5.21**
H	5.09**	45.08***	4.80**	1.66 n.s.	6.58**	1.23 n.s.
HD	1.20 n.s.	20.66***	1.54 n.s.	8.08**	67.18***	0.67 n.s.
Seed yield	3.91*	15.48***	3.22*	9.45***	4.40*	4.28**
Seed OC	5.09**	283.32***	4.29**	0.49 n.s.	2.53 n.s.	5.08**

<sup>1</sup>Treatments: CCC, LCC, RT, NT

<sup>2</sup>Year: year 1 (2012/2013), year 2 (2013/2014), year 3 (2014/2015)

<sup>3</sup> $p < 0.05^*$ ,  $p < 0.01^{**}$ ,  $p < 0.001^{***}$ , n.s.: not significant

There were consistent differences in crop development among the three years studied. Plant density showed significantly higher values the year 3 at both locations (Table 10), with significant differences between treatments in Tomejil. The greatest installation was observed in CCC and RT plots (7 plant/m<sup>2</sup>), followed by LCC (4.35 plant/m<sup>2</sup>) and, finally, NT treatments (2.70 plant/m<sup>2</sup>). The lowest sunflower PD observed in the LCC treatments could be due to the greater biomass produced by these cover crops during the year 3 (33000 kg/ha), whose incorporation to the soil could negatively affect the sunflower emergence as well as the largest weed biomass killed in NT plots (3129 kg/ha). Results for the rest of years varied according to locations, being greater during the year 1 than 2 in Santa Cruz and the opposite in Tomejil. Both sites only showed significant differences between treatments the year 1, with higher installation in NT plots than the rest of treatments. These differences between treatments were not due to lower soil moisture content since the wet year 1 showed the highest soil water content due to high rainfall recorded, and the soil moisture storage was not influenced by treatments. In fact, the only exception was NT holding the lowest moisture contents in the 10-30 cm layer from November to May in Santa Cruz. By contrast, the best PD values in the RT and CCC plots during the dry year 3 in Tomejil coincided with the highest moisture content recorded in the uppermost layer on May (65 DAS and 666 GDD) (Figure 27 in the previous section 3.4.2.). The

influence of water availability on sunflower growth and production has previously been reported by Halvorson *et al.* (2000) and López-Bellido *et al.* (2002), limiting them especially by severe water stress in dry years.

**Table 10. Sunflower characterization: plant density (PD as plant/m<sup>2</sup>), mean height (H as m), sunflower head diameter (HD as mm) and seed oil content (OC as %) in the white mustard cover crop, narbon bean cover crop, reduced tillage and no tillage treatments (CCC, LCC, RT and NT) during the different growing seasons (2012/2013 and 2014/2015 for rotation 2 and 2013/2014 for rotation 1) in Santa Cruz and Tomejil.**

Santa Cruz															
Rotation 2 (SF-DW-SF)								Rotation 1 (DW-SF-DW)							
Year 1 (2012/2013)				Year 3 (2014/2015)				Year 2 (2013/2014)							
PD	H	HD	OC	PD	H	HD	OC	PD	H	HD	OC				
plant/m <sup>2</sup>	m	mm	%	plant/m <sup>2</sup>	m	mm	%	plant/m <sup>2</sup>	m	mm	%				
<b>32 DAS</b>		<b>120 DAS</b>		<b>50 DAS</b>		<b>124 DAS</b>		<b>41 DAS</b>		<b>132 DAS</b>					
<b>328 GDD</b>		<b>1925 GDD</b>		<b>500 GDD</b>		<b>1943 GDD</b>		<b>510 GDD</b>		<b>2132 GDD</b>					
CCC	3.1 b <sup>1</sup>	1.3 a	129.6 a	51.6 a	4.67 a	0.88 b	103.8 a	45.9 a	2.1 a	0.9 bc	128.7 a	40.8 a			
LCC	3.8 ab	1.2 a	129.0 a	51.9 a	4.6 a	0.89 b	104.3 a	45.6 ab	2.0 a	0.9 c	124.8 a	40.5 a			
RT	3.7 ab	1.2 a	117.4 a	52.0 a	4.8 a	0.90 b	101.8 a	45.3 ab	2.0 a	1.1 ab	139.1 a	40.6 a			
NT	4.3 a	1.2 a	116.1 a	48.5 b	3.6 a	1.01 a	111.8 a	43.0 b	2.7 a	1.2 a	150.7 a	41.9 a			
Tomejil															
<b>48 DAS</b>				<b>120 DAS</b>				<b>65 DAS</b>		<b>126 DAS</b>		<b>54 DAS</b>		<b>147 DAS</b>	
<b>538 GDD</b>				<b>1937 GDD</b>				<b>666 GDD</b>		<b>1785 GDD</b>		<b>595 GDD</b>		<b>2140 GDD</b>	
CCC	1.3 ab <sup>1</sup>	1.2 a	215.1 ab	41.7 ab	7.2 a	0.96 a	137.8 a	43.2 ab	1.94 a	1.2 ab	155.8 a	41.9 a			
LCC	0.9 b	1.2 a	242.6 a	39.8 b	4.4 ab	0.8 a	141.0 a	44.4 a	2.29 a	1.2 a	165.4 a	40.8 a			
RT	1.8 ab	1.3 a	205.6 ab	41.9 ab	7.1 a	1.0 a	117.0 a	42.0 ab	3.19 a	1.2 ab	144.9 a	41.9 a			
NT	1.9 a	1.2 a	196.0 b	43.2 a	2.7 b	0.7 a	81.4 a	40.8 b	2.63 a	1.0 b	134.6 a	42.4 a			

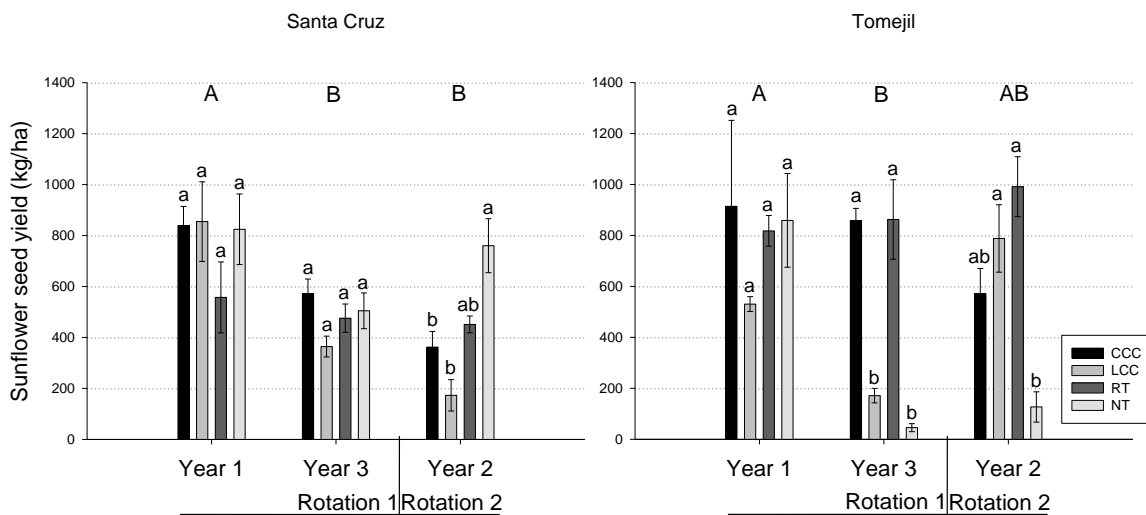
<sup>1</sup> Different small letters within each year per column indicate that the differences between treatments were statistically significant (Tukey's,  $p \leq 0.05$ )

After sunflower development (from 120 to 147 DAS), plants showed the highest H the year 1, without significant differences with year 2 in Tomejil, and the lowest H values the year 3 at both locations. The wet year 1 did not show significant differences between treatments at both locations (values averaging 1.16-1.25 m). In Santa Cruz, sunflower plant H was always better in NT treatments than the rest of cases during the dry years 2 (1.01 m) and 3 (1.19 m). However, year 2 in Tomejil showed the highest H values for LCC (1.23 m) and the lowest for NT (0.99 m), without significant differences between treatments the year 3. The highest soil moisture contents observed by NT

plots from March to April the year 2 in Santa Cruz were probably the cause of their greater H results (Figure 26 in the previous section 3.4.2.). On the contrary, similar soil moisture (Figure 27), N (Figure 29) and OM (Figure 31) contents were observed in Santa Cruz the year 3, so there appears to be no ready explanation for the highest H results in NT plots. In Tomejil, soil moisture in May (30-60 cm layer) and June (0-10 cm layer) during the year 2 (Figure 26) in addition to the N (Figure 28) and OM (Figure 30) contents were also higher for LCC plots, influencing on the final plant development. The same trend was followed by HD in Tomejil, with the highest results obtained the year 1 (196-243 mm), followed by year 2 (135-165 mm) and the lowest results also obtained the year 3 (81-141 mm). However, HD was greater the year 2 in Santa Cruz (values from 125 to 151 mm), followed by year 1 (116-130 mm) and finally year 3 (102-112 mm). Significant differences were not found between treatments in any case, except for year 1 in Tomejil where LCC, followed by CCC and RT obtained the highest values.

The better early growth of the plants during year 1 was reflected in increased values of seed yield at both locations (Figure 33), whilst dry years 2 and 3 obtained very low results. In view of these results, it could be considered the influence of the different sunflower cultivars used the year 1 (cv. Transol) with respect to the year 2 and 3 (cv. P64LE29 cultivar) (Chapter 2). However, García Ruíz and García López (2014, 2015) showed similar results for both cultivars in similar conditions these same years. Sunflower seed yield in the most productive year 1 was over 557-855 kg/ha in Santa Cruz and 531-914 kg/ha in Tomejil, without significant differences between treatments. The dry years 2 and 3 did not show significant differences in Santa Cruz. However, greater results were observed in NT plots (760 kg/ha), followed by RT (451 kg/ha) and finally both CC treatments (173-362 kg/ha) the year 2. Results did not show significant differences between treatments the year 3 (values averaging 364-571 kg/ha). Therefore, this low-productivity was probably due to the marked effect of the weather conditions (López-Bellido *et al.*, 2002) since farmers of the province of Córdoba also obtained low sunflower yields averaging 849 kg/ha during the dry year 3 (MAPAMA, 2017). In Tomejil, the year 2 was more productive than year 3, when the low rainfall jointly with high temperatures (2140 GDD) produced the worst results of the study period. These results are in agreement with those obtained by farmers in the

area (province of Seville), with a lower production than the previous years and a mean grain yield of 1053 kg/ha (MAPAMA, 2017). Seed yield obtained the year 2 by RT and LCC treatments (991 and 788 kg/ha, respectively) improved with respect to those obtained by CCC (571 kg/ha) and NT plots (117 kg/ha), probably due to the greater soil moisture stored in these treatments from May (0-10 cm layer) to June (30-60 cm layer) (Figure 26). By contrast, LCC and NT treatments obtained very low productivity during the year 3 (171 and 46 kg/ha, respectively), while CCC and RT produced over 858 and 862 kg/ha, respectively. The lowest yields obtained by LCC and NT treatments could also have been diminished by the lower sunflower emergence detected in both treatments. Therefore, the impact of cover crops on subsequent crop yields is also affected by the amount of biomass return to the soil (Clark, 2000; Tonitto *et al.*, 2006; Blanco-Canqui *et al.*, 2012).



**Figure 33. Sunflower seed yield (kg/ha) in the white mustard cover crop, narbon bean cover crop, reduced tillage and no tillage treatments (CCC, LCC, RT and NT) during the different growing seasons (2012/2013 and 2014/2015 for rotation 1 and 2013/2014 for rotation 2) in Santa Cruz and Tomejil. Significant differences between treatments are shown as different small letters and significant differences between years as different capital letters per column (Tukey's,  $p \leq 0.05$ ).**

Sunflower quality results, measured as OC, were also affected by variations in growth and seed yield. Year 1 showed higher OC (values over 48-52 %) than years 3 (43-46 %) and 2 (40-42 %) in Santa Cruz. Significant differences between years were not found in Tomejil (values averaging 40-44 %). Nevertheless, all results were considered high OC content according to Martínez-Force *et al.* (2007) by taking values  $\geq 40$  %. NT treatments reached significantly higher OC than the rest of treatments during year 1 at both locations, whilst the opposite was occurred during the year 3,



with the highest OC values observed in CCC and LCC plots in Santa Cruz and Tomejil, respectively. By contrast, there were no significant differences between treatments the year 2 at any location.

Despite the similar plant density results between treatments the years 2 and 3 (with the exception of the year 3 in Tomejil), the sunflower yield had a very low productivity for both years as a whole. Furthermore, results from all the treatments were prohibitive for farmers during the year 3 in Santa Cruz, whilst LCC and NT plots were the worst in Tomejil. Nevertheless, the low sunflower seed yield produced by all treatments during year 3 (including NT and RT plots) suggested that it was not a consequence of the preceding cover crops introduction and other factors could be involved. In fact, the best seed yields obtained by CCC that year in Tomejil jointly with the variable results between treatments the year 2 at both locations prove it. Many factors can restrict the production of sunflower crops, including weather conditions, diseases, insects, and weeds, as well as herbicidal residue in the soil (Serim and Maden, 2014). In fact, according to Saavedra *et al.* (2015a) and Saavedra *et al.* (2016a), severe phytotoxicity effect on sunflower plants was observed in southern Spain during the growing season 2014/2015 and some farmers in the area stated that the same symptoms were presented in previous growing seasons. Their results demonstrated that the phytotoxicity could be due to residues of sulphonylurea herbicides, applied from 12 to 14 months before the sunflower sowing on the cereal sequence. Among these herbicides are those based on the active substance metsulfuron-methyl, such as Biplay-33 used in the durum wheat sequence of our experimental trials. Moreover, a prior review of the European Food Safety Authority (EFSA, 2015) highlighted the possible risk derived for its use in cereals and rotational crops and proposed a reduction of application rate, since metsulfuron-methyl is not readily biodegradable and their effects are not sufficiently evaluated. These results also suggested that our climatic conditions do not favour the degradation of these active substances. Thus, the low rainfall occurred during years 2 and 3 jointly with high temperatures (2132-2140 GDD accumulated) probably increased the possible negative effect derived from the wheat straw of the preceding durum wheat sequence, justifying the low yields obtained on our sunflower plots.

In view of all these results, further research about the risks of using these herbicides is needed on durum wheat-sunflower crop rotations. Nevertheless, in addition to the durum wheat straw residues, it must be taken into account the cover crop residues preceding the sunflower sowing in the crop rotation. Members of *Brassicaceae* (Haramoto and Gallandt, 2004) and *Leguminosae* family (Korolen, 2007; Rice, 2012) have been frequently cited as allelopathic crops. However, there are no previous studies on *V. narbonensis* species or *S. alba* effects on sunflower crops. In an attempt to exclude any additional biochemical effect of white mustard and narbon bean cover crops on sunflower seed germination and seedling growth, two trials under controlled conditions were performed for each cultivar (experiment A.2.). These results have been included in Appendix 5.

Preliminary results derived from the germination test showed that CCC and LCC liquid extracts could have some effect on sunflower seeds emergence, since the germination percentage was reduced by 59-75 % and 29-50 %, respectively, with respect to the control without CC extracts. Based on these results, CC effect on sunflower was studied in a pot experiment with four different sunflower planting dates after CC mowing (0, 7, 15 and 30 DAM). Statistical analysis showed there was an interaction treatment×planting date in all variables evaluated except for dry matter of sunflower above-ground part that all sampling dates showed the lowest values in the control treatment (Appendix 5). However, there was no interaction between treatment×cultivar, planting dates×cultivar or treatment×planting dates×cultivar in most of the variables. There were also no differences between cultivars, except for the length of the 1<sup>st</sup> of leaves. Seedling emergence did not show significant differences between treatments at the different planting dates and cultivars, except for cv. Transol at 0 DAM when control showed higher values than CCC followed by LCC. Hypocotyl length presented higher values at 0 and 7 DAM than after 15 and 30 DAM, while root length showed shorter roots in sunflowers seedling planted at 0 DAM compared to the rest of planting dates, but this effect was observed in all treatments. Moreover, this latter variable only showed significant differences between treatments at isolated moments, in which the CC treatments always had better or similar behaviour than the control in both cultivars. Similar results were obtained by dry matter values (above-ground and underground parts), with a similar or greater dry matter production in CCC

and LCC treatments compared to the control in both cultivars. In addition, leaf length and width at the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> pair of leaves always showed longer values for CC treatments than the control in both cultivars, without following any fixed pattern between planting dates.

Overall, our results demonstrated that white mustard and narbon bean had no negative effect on sunflower seedling growth in both cultivars, unlike the results obtained in similar sunflower pot experiments with other *Brassica* species (Jafariehyazdi and Javidfar, 2011). In fact, better growth of sunflower seedling through surface-retained white mustard and narbon bean cover crops could be primarily related to the positive effect of the plant material (OM and nutrients) with respect to the control treatment. Therefore, cover crops residue phytotoxicity is not a key mechanism of growth reduction in these experiments. However, given the large number of factors influencing sunflower development and production under field conditions, after the 3-year study of sunflower crop sequence and the additional trials under controlled conditions, no definitive conclusions can be drawn with regard to the effect of white mustard and narbon bean on sunflower crop under field conditions. To encourage the introduction of cover crops by farmers, it is necessary to show them not only their positive effect on soil but also on crop yields and quality, which ultimately determine their monetary benefits in the traditional wheat-sunflower rotation. Therefore, further research is required with long-term follow up, in order to verify real causes affecting sunflower growth and production and establishing control measures under the Mediterranean rainfed conditions of southern Spain.

## **B. EFFECT OF COVER CROPS ON SUNFLOWER BROOMRAPE CONTROL**

### **3.4.4 EFFECT OF WHITE MUSTARD AND NARBON BEAN ORGANIC AMENDMENT ON SUNFLOWER BROOMRAPE (*Orobanche cumana*) CONTROL**

Statistical analysis for each cover crops amendment (CCC and LCC) in the experiment B.1 showed there was an interaction between the factors *amendment application* and *germination processes*, except for the CCC treatment the first and second years of study (2014 and 2015) when all the *O. cumana* seeds with synthetic

strigolactone stimulant GR24 showed the highest germination percentage results. The means of the different amendment applications results have been represented by germination process separately in the Figures 35 and 36.

First results showed that GR24 induced high germination of *O. cumana* in the positive control treatment (76 % in 2014, 38 % in 2015 and 65 % in 2016). The germination percentage varied between years of study since in an attempt to simulate field conditions, each year we used different *O. cumana* seeds which vary in their ability to germinate (Elzein and Kroschel, 2003). Some spontaneous germination (12-16 %) was observed in the negative control. Similar studies have obtained percentages of spontaneous germination in other *Orobanchae* species ranging from 0.1 to 10 % (Goldwasser and Yoder, 2001; Fernández-Aparicio *et al.*, 2008; Plakhine *et al.*, 2012). In addition, Plakhine *et al.* (2012) revealed that the spontaneous germination is not the result of seed treatments or microscope light in the lab, but is an expression of a lethal phenotype which is only genetically controllable. Consequently, the percentages of spontaneous germination observed depend on the population of *O. cumana* obtained from the field. A large number of studies showed the variability of *O. cumana* populations (Castejon *et al.*, 1989; Gagne *et al.*, 1998). Within Spanish populations, three distant gene pools occur, Cuenca province, Castilla-León area and Guadalquivir Valley in Andalusia, apparently deriving from separate introduction events (Pineda-Martos *et al.*, 2013). Different races occurred within each gene pool and still continue developing new races so different germination percentages will continue to exist due to the complex germination control mechanism of the *Orobanchae* seeds. Despite the variability in the population genetics of weedy broomrapes, all experiments with CCC and LCC amendments showed similar and consistent response under controlled conditions. The differences between non-germinated and germinated seeds were easily observed under the stereomicroscope in all case studies (Figure 34).

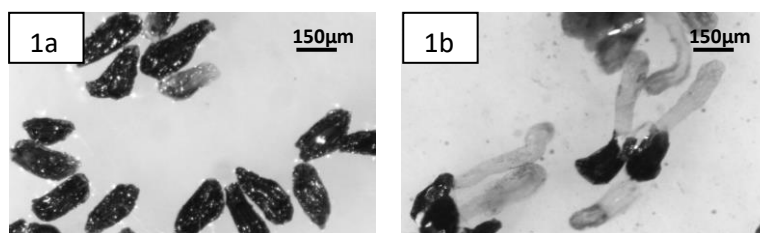


Figure 34. Non-germinated (a) and germinated *O. cumana* seeds with visible radicle through the seed coat (b).

The effect of the *S. alba* amendment on *O. cumana* seeds germination is shown in Figure 35. *S. alba* inhibited *O. cumana* germination, whatever their amendment application (residues or plant extract), with significant differences with the control treatment in all the cases. Results indicated that CCC amendments even inhibited the percentage of spontaneous germination since the different *S. alba* applications without synthetic stimulant showed practically zero germination percentages (from 0 to 3 % with plant residues around *O. cumana* Petri dishes and 0-1.1 % with liquid extracts wetting the *O. cumana* seeds).

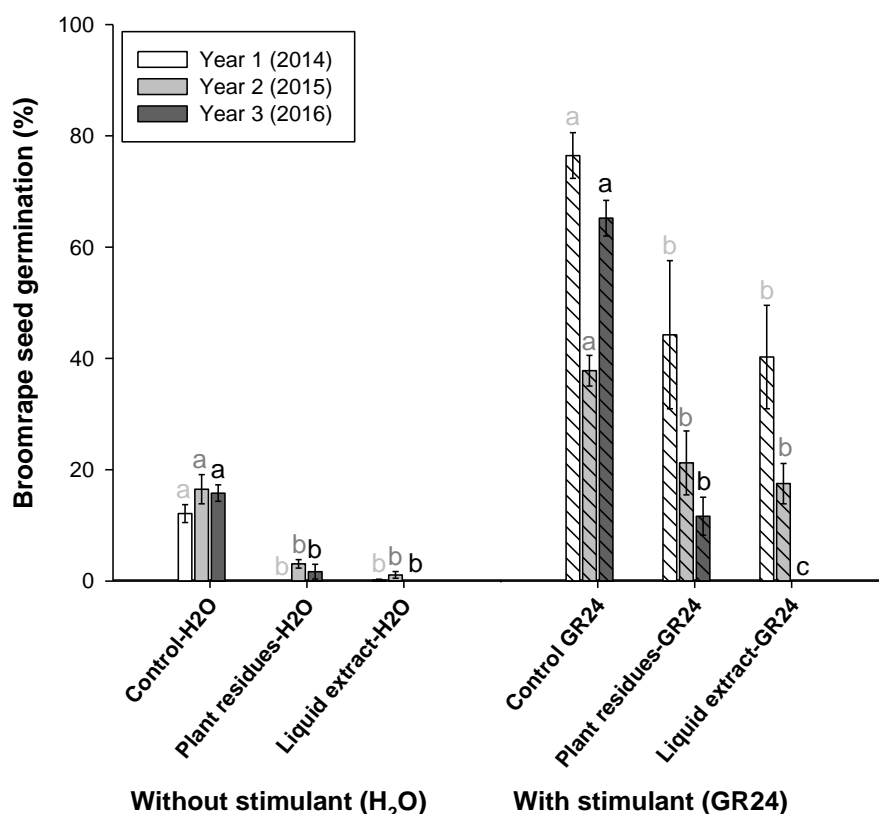


Figure 35. Percentage of *O. cumana* seed germination obtained without and with synthetic germination stimulant GR24 according to two types of *S. alba* amendment application (plant residues or liquid extracts) in addition to the negative and positive control without amendment from 2014 to 2016. Significant differences between the amendment applications are shown as different small letters compared to control within each germination process per column (Tukey's,  $p \leq 0.05$ ).

The analysis of variance for germination percentage of seeds after exposure to GR24 showed that there was a highly significant difference between the two types of CCC amendment applications and control (Figure 35). There were no significant differences between plant residues-liquid extracts in 2014 and 2015 bioassays, with a reduction of germination rate ranging from 42-47 % in 2014 to 44-54 % in 2015,

respectively. *S. alba* plant residues highly reduced germination rate the third year 2016 (82 %) but liquid extracts had a more reducing effect with a total inhibition of *O. cumana* seed germination (100 %). The *in vitro* inhibitory effect of members of *Brassicaceae* family has been widely studied worldwide. These species contain glucosinolates that are hydrolyzed by the enzyme myrosinase to form isothiocyanates and other glucosinolate derivatives (Chan and Close, 1987; Haramoto and Gallandt, 2004). Laboratory studies suggest that brassica residues and extracts containing these compounds are toxic to weeds (Bialy *et al.*, 1990; Brown and Morra, 1996), nematodes (Potter *et al.*, 1998) and pathogens that cause soil borne diseases and plant pests (Angus *et al.*, 1994; Norsworthy, 2003; Zurera *et al.*, 2007). Thus, volatile compounds from various black (*Brassica nigra* L.) and brown mustard (*Brassica juncea* L.) caused 89 % and 100 % inhibition of lettuce seed germination, respectively (Oleszek, 1987). Additionally, water-soluble extracts from white and brown mustard also showed suppressive growth effect on *Fusarium graminearum* growing less than 50 % of control (Perniola *et al.*, 2012).

The biofumigation potential of the plants used for bioassays will depend on several factors influencing the glucosinolate profile, such as environmental growth conditions and soil where they were collected, although these effects have not yet been quantified (Saavedra *et al.*, 2016b). The phenological stage of the brassica species is another important factor (Clossais-Besnard and Larher, 1991). White mustard was collected at late flowering according to the CCC field incorporation each year varying between full flowering and flowering declining (BBCH 65-67) (Ríos *et al.*, 2016b). This stage was selected for incorporation because it typically contains maximum glucosinolate content (Fieldsend and Milford, 1994; Ríos *et al.*, 2016a). However, the type and concentration of glucosinolates have been found to vary not only between *Brassica* species and cultivars (Kirkegaard and Sarwar, 1998) but also between different tissues of the same plant (Bellostas *et al.*, 2004). Therefore, differences in the biofumigant effect produced by *S. alba* between years can be explained by the different plant material harvested each growing season despite being collected in the same phenological stage.

The sunflower broomrape control with LCC amendment is shown in Figure 36. Similar to *S. alba* effects, the different *V. narbonensis* applications (plant residues and

liquid extracts) reduced *O. cumana* germination existing significant differences with the control treatment throughout the trials. The spontaneous germination rate of *O. cumana* seeds showed significant differences in the different amendment applications of *V. narbonensis*. The application of LCC plant residues highly reduced *O. cumana* germination rate from 57 % in 2014 to 76 % in 2015 and 2016. Nevertheless, liquid extracts were found to totally inhibit *O. cumana* seed germination. Germination percentages of *O. cumana* seeds after treatment with GR24 also displayed a significant reduction with respect to the control treatment. The contact between *O. cumana* seeds and plant residues reduced germination by 90-97 % and liquid extract by 82-100 %, without significant differences between them.

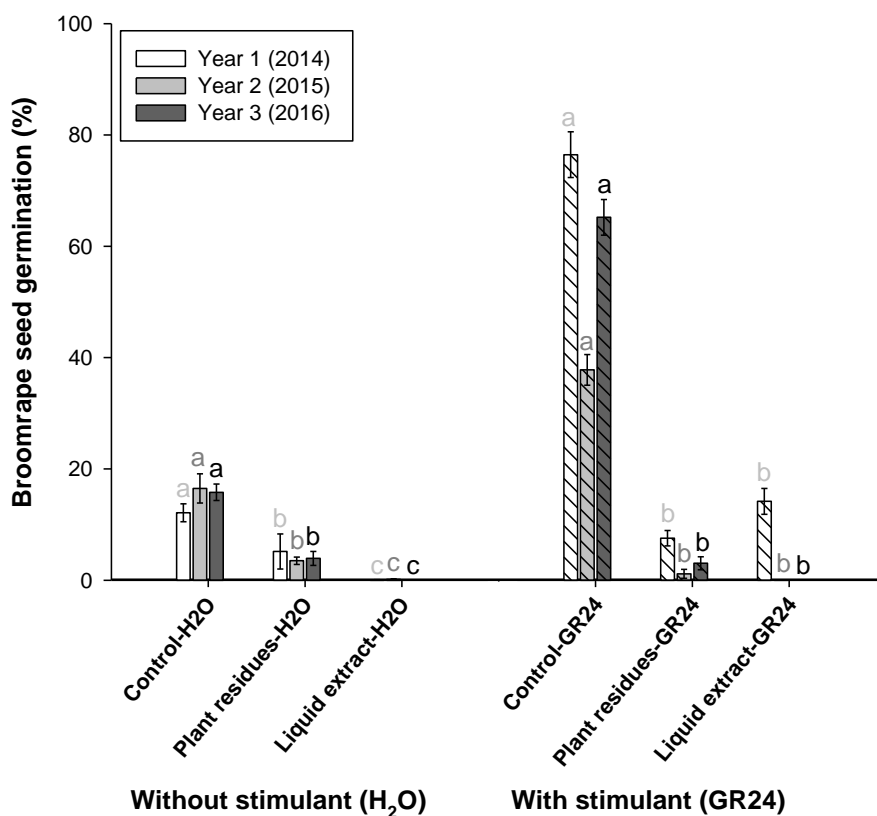


Figure 36. Percentage of *O. cumana* seed germination obtained without and with synthetic germination stimulant GR24 according to two types of *V. narbonensis* amendment application (plant residues or liquid extracts) in addition to the negative and positive control without amendment from 2014 to 2016. Significant differences between the amendment applications are shown as different small letters compared to control within each germination process per column (Tukey's,  $p \leq 0.05$ ).

The bioassays results suggested that *V. narbonensis* plants residues and extracts can also be used for controlling sunflower broomrape by its inhibitory effect of *O. cumana* germination. Many researchers have shown that many species within

the *Leguminosae* family contain secondary plant products and water-soluble phytotoxic compounds that have this allelopathic potential, finding them in plant parts in differing concentrations (Chung and Miller, 1995). Results of lettuce bioassays indicated the presence of phytotoxic substances in decomposition products of vetch (*Vicia* sp.) extracts (Patrick *et al.*, 1963) and aqueous extracts of red clover (*Trifolium pratense* L.) and ladino clover (*T. repens* L.) were reported to reduce germination and growth of other forage crops by its isoflavonoids compounds (Rice, 2012). Moreover, the allelopathic effects of alfalfa (*Medicago sativa* L.) and bird vetch (*Vicia cracca* L.) leaf and roots extracts were observed on several weeds species inhibiting seed germination from 25 to 100 % (Korolen, 2007). Other research with hairy vetch (*Vicia villosa* L.) and crimson clover (*Trifolium incarnatum* L.) extracts and residues also demonstrated the potential for these species to inhibit several weed and crop species (White *et al.*, 1989). However, there are no previous studies on *V. narbonensis* species. Therefore, we cannot confirm if the reduced germination of *O. cumana* was caused by these secondary plant products. It would be necessary to identify if these compounds are present in the extracts and determine their profile, but the potential to inhibit germination was present in all LCC amendment applications tested.

Additionally, obligate root-parasitic plants of the *Orobanchaceae* evolve extremely specialized germination control mechanisms (Fernández-Aparicio *et al.*, 2009). Despite the fact that there is much published information on the genetic variability of *O. cumana*, its genetic resistance mechanisms, germination stimulants and biochemical control worldwide, scarce information exists about the cultural control of sunflower broomrape. Some bioassays have studied the potential of 'trap crops' for stimulating *O. cumana* seed germination. Species identified include hybrid maize lines and soybean cultivars due to their ability to produce strigolactones (Ma *et al.*, 2013; Zhang *et al.*, 2013). However, the control of *Orobanche* spp. is difficult and only partially effective due to the complex relationship between parasite and host at on-farm level. The effective inhibition of *O. cumana* seed germination by crucifers and legumes amendments under controlled conditions makes necessary the evaluation of CCC and LCC effects on the *Orobanche* spp. seed bank under field conditions.



Field results each growing season were shown in Table 11. Sunflower broomrape incidence and severity showed no significant differences between treatments any growing season. Nevertheless, the percentage of plants with emerged broomrapes and the average number of broomrapes per infected sunflower plant tends to be reduced both years in plots where CCC and LCC were previously incorporated.

**Table 11.** Crop sequence characterization: white mustard cover crop (CCC) and narbon bean cover crop (LCC) above-ground fresh biomass (CFM expressed as kg/ha) and height (H in m); sunflower broomrape incidence (percentage of plants with emerged broomrapes) and broomrape severity (number of emerged *O. cumana* shoots per host plant) in addition to host mean height (host H in m), head diameter (HD in mm), host seed yield (kg/ha) and seed oil content (OC expressed as %) in each treatment during 2014/2015 and 2015/2016.

	CFM	H	Plants with emerged broomrapes	Number of emerged broomrape per host plant	Host H	HD	Host seed yield	OC
	kg/ha	m	%	Unit	m	mm	kg/ha	%
<b>2014/2015</b>								
	<b>130 DAS</b>		<b>97 DAS</b>		<b>97 DAS</b>			
	<b>1419 GDD</b>		<b>1197 GDD</b>		<b>1197 GDD</b>			
<b>CCC</b>	30849	1.28	13 a <sup>1</sup>	3 a	1.69 a	130 a	-	-
<b>LCC</b>	30493	0.73	13 a	3 a	1.72 a	127 a	-	-
<b>RT</b>			23 a	4 a	1.70 a	130 a	-	-
<b>2015/2016</b>								
	<b>124 DAS</b>		<b>96 DAS</b>		<b>96 DAS</b>		<b>128 DAS</b>	
	<b>1552 GDD</b>		<b>1046 GDD</b>		<b>1046 GDD</b>		<b>1711 GDD</b>	
<b>CCC</b>	32600	1.82	56 a	5 a	1.60 b	130 a	1027 a	45 a
<b>LCC</b>	33525	0.81	62 a	5 a	1.80 a	155 a	1503 a	44 a
<b>RT</b>			67 a	7 a	1.78 a	161 a	1358 a	45 a

<sup>1</sup>Different small letters within each growing season per column indicate that the different between treatments were statistically significant (Tukey's,  $p \leq 0.05$ )

Both cover crops showed a good behaviour during the growing season 2014/2015, with high H (1.28 m CCC and 0.72 m LCC) and CFM production (30849 and 30493 kg/ha, respectively) at the mowing date (130 DAS, 1419 GDD). The broomrape incidence was 13 % in the CCC and LCC treatments compared to the 23 % of the RT treatment. During the growing season 2015/2016, CCC and LCC also presented a good development 124 DAS (1552 GDD), with higher H (1.82 m CCC and 0.81 m LCC) and CFM production (32600 and 33500 kg/ha, respectively) than the previous year. This latter values could be due to the weather conditions (Figure 14 in Chapter 2), with a warmer and rainier winter during 2015/2016 than that of 2014/2015, especially from December to February (11°C vs. 8-9°C average monthly temperature and 116 mm vs.

84 mm cumulative rainfall, respectively) which probably favoured the higher CC biomass production. In addition, this year the broomrape incidence and severity were higher than the previous one but without significant differences between treatments. A similar trend in the decrease of the parasitic weed presence was observed in the CCC and LCC treatments (broomrape incidence of 57-62 % with a mean number of 5 broomrapes per plant compared to an incidence of 67 % with 7 broomrapes per plant in the RT treatment).

The evolution of the broomrape attachments per plant showed the lowest incidence in the CC treatments during all the sunflower-growing cycle both years, with significant differences between CC and RT only at 63 DAS (sunflower stem elongation, BBCH 30) during the year 2014/2015 (Figure 37). The sunflower broomrape incidence during the field season 2014/2015 was lesser than 2015/2016 in all counting dates.

Seeds of *O. cumana* only germinate when a host root is nearby (Parker and Riches, 1993) but also require a moist environment (for several days) together with suitable temperatures, with an optimum of 20°C (Foy *et al.*, 1991). The highest rainfall jointly with mild temperatures recorded during April and May during 2015/2016 may have influenced a greater connection between *O. cumana* seeds and the host root on sunflower plants compared to 2014/2015 (115.4 and 95.4 mm vs. 43 and 0.4 mm, respectively) (Figure 14 in Chapter 2). However, the *O. cumana* development was faster during 2014/2015 than 2015/2016. Both years the first tubercles began to appear from the second counting date (63 DAS). These results agree with Duca *et al.* (2013), who found that depending on environmental conditions the establishment and underground phase of the life-cycle of *O. cumana* ranges from 30 to over 100 days. According to our results, the weather conditions not only seem to influence the underground development of broomrape but also in its above-ground part. Mean monthly temperatures from March to May during 2014/2015 (14°C, 17°C and 22°C, respectively) were higher than during 2015/2016 (12°C, 16°C and 19°C, respectively) (Figure 14 in Chapter 2), with mean maximum temperatures reaching up to 6°C more compared to 2015/2016. Consequently, the parasitic weeds reached the flowering (S6) and setting of seeds (S7) stages 63 DAS during 2014/2015, at the sunflower inflorescence emergence stage (BBCH 50), while the S6 stage was shown in some attachments 63 DAS during 2015/2016, but no setting of seeds (S7) was observed until

the next counting date 92 DAS (sunflower full flowering, BBCH 65). Moreover, sunflower GDD accumulated from the second to the fourth counting dates during 2014/2015 were higher than in 2015/2016 (657, 916 and 1083 GDD vs. 537, 790 and 989 GDD) and this fact could explain the faster sunflower broomrape development, ripening and drying.

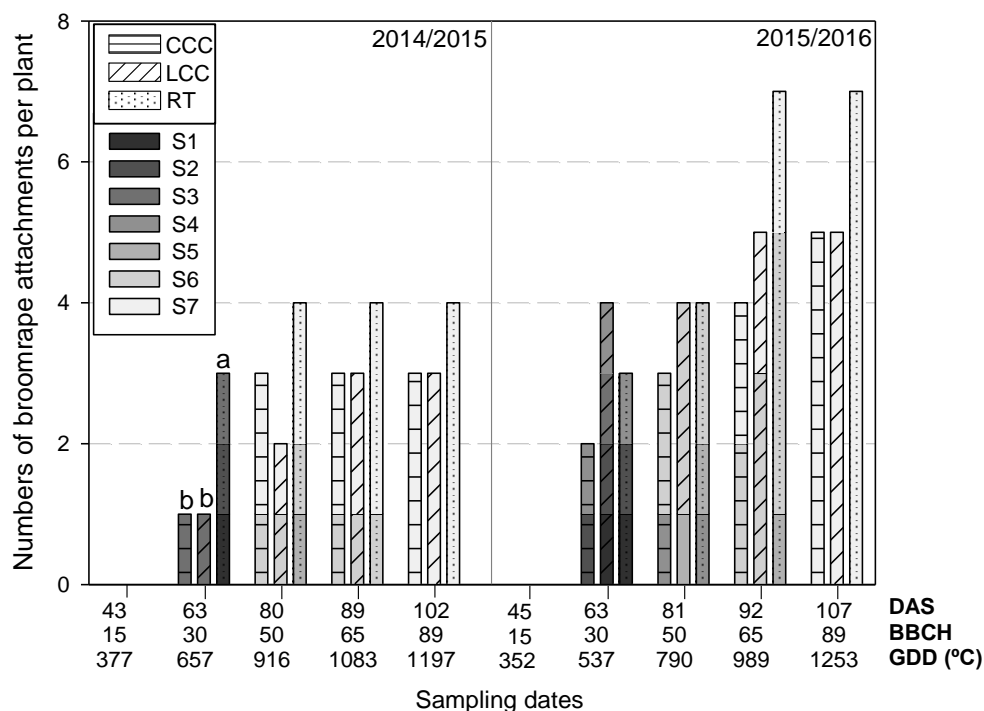


Figure 37. Evolution of the sunflower broomrape (*O. cumana*) attachments (stages of development according to ter Borg *et al.* (1994) where S1=tubercles smaller than 2 mm; S2=tubercles greater than 2 mm, without roots development; S3=tubercles with crown roots, without shoot formation; S4=shoot formation, remaining underground; S5=shoot emergence; S6=flowering; and S7=setting of seeds) per host plant at different sunflower phenological stages (BBCH 15, third pair of leaves; BBCH 30, stem elongation; BBCH 50, inflorescence emergence; BBCH 65, full flowering and BBCH 89, fully ripe of the BBCH scale), expressed as days after sowing (DAS) and growing degree days (GDD) accumulated, during the field season 2014/2015 and 2015/2016. Significant differences between treatments are shown as different small letters within each counting date per column (Tukey's,  $p \leq 0.05$ ).

Sunflower development was not affected by treatments during 2014/2015, with mean H and HD values without significant differences (Table 11). However, CCC showed significantly lower height than LCC and RT, both with no differences between them in spite of the highest H displayed by all LCC treatments probably due to the N fixation performed by the previous legume cover crop. In the second year under field conditions, HD showed no differences between any treatments but values were greater than the first year for LCC and RT (varying from 127-130 to 155-161 mm,

respectively). Regarding sunflower seed yield and quality, during the field season 2014/2015 severe bird attacks prevented us from obtaining these results. However, results from the growing season 2015/2016 showed that there were no significant differences between treatments, with seed yields varying between 1000-1500 kg/ha and OC of 44-45 %. Parker and Riches (1993) cited that the success of cultural measures becomes evident only in the long-term and will not improve yields in the present crop, because of the long underground developmental phase as well as the high seed production and longevity.

According to the present results from field trials, there were no significant differences in the sunflower broomrape reduction by incorporating cover crops during the growing seasons 2014/2015 and 2015/2016. Nevertheless, the trend shown both years of study was clearly promising, revealing that all CCC and LCC treatments reduced broomrape incidence and severity in absolute terms and these results are in line with those obtained under controlled conditions. The effective control of weeds and soil borne diseases and pest by crucifer and legume cover crops have been widely studied under field conditions, either through direct competition from cover crop biomass (Kruidhof *et al.*, 2008; Isik *et al.*, 2009) or through allelopathy (Gardiner *et al.*, 1999; Fernández-Aparicio *et al.*, 2008; Barrau *et al.*, 2009). For example, *S. alba* has been introduced in olive groves of southern Spain, reducing the inoculum of *Verticillium dahliae* by incorporating cover crop residues into the soil in spring after mowing under rainfed conditions (Cabeza-Fernández and Bejarano-Alcázar, 2008). However, previous references to the control of *Orobanche* spp. with cover crops have not been found in the literature worldwide.

Intercropping cereals with legumes and other crops is a common practice in Africa that influences other parasitic weeds of the family *Scrophulariaceae* (Elzein and Kroschel, 2003). Previous studies showed that intercropping maize with cowpea and sweet potato can significantly reduce the emergence of *Striga* (witchweed) in Kenya (Oswald *et al.*, 2001). In addition, the inhibition of *Striga hermonthica* was significantly greater in maize-silverleaf [*Desmodium uncinatum* (Jacq.) DC.] intercrop than that observed with other legumes, *e.g.* sun hemp (*Crotalaria* spp.), soybean or cowpea due to the allelopathic effect of *Desmodium* species inhibiting the development of haustoria of *Striga* (Khan *et al.*, 2000). Compared with other parasitic weeds, the

control of *Orobanche* spp. was proved to be exceptionally difficult. The ability of the parasite to produce a tremendously high number of seeds, which remained viable in the soil for so long, and their intimate physiological interaction with their host plants, are the main difficulties that limit the development of successful control measures that can be accepted and used by farmers (Elzein and Kroschel, 2003). Only trap crops have been proposed as a control measure of *Orobanche* species. Maize and soybean trap crops have been reported to induce *O. cumana* germination under field conditions (Ma *et al.*, 2013; Zhang *et al.*, 2013). For *Striga* spp., crop rotation with trap and catch crops has also been studied, using cotton and soybean to induce the germination (Kroschel, 2001) and sudan grass (*Sorghum sudanense* L.) for *S. hermonthica* control (Oswald *et al.*, 1999). Nevertheless, these two methods (trap and catch crops) only had a reasonable effect in areas where the parasite infestation level of the soil was very low and even after four years of continuous cropping with cowpea or cotton in western Kenya, damaging levels of *Striga* still remained in the soil (Odhiambo and Ransom, 1996).

Against this background, and given that sunflower resistance varieties need continuous breeding and selection effort to keep pace with the development and spread of new races (Louarn *et al.*, 2016), no single control method is both effective and economically feasible for farmers (Ma *et al.*, 2013). For that reason, our results open a path of study for the control of sunflower broomrape through the use of *S. alba* and *V. narbonensis* cover crops. Long-term experiments including CCC and LCC with sunflower resistant varieties could give more exploitation for developing reliable cover cropping strategies. If the findings are corroborated after several years of study, *S. alba* and *V. narbonensis* could represent an alternative method of cultural control for one of the most problematic weeds in arable rainfed fields in southern Spain.

### 3.5 CONCLUSIONS

This chapter shows that white mustard and narbon bean could be successfully established in durum wheat-sunflower rotations of southern Spain. The inclusion of CC did not have a large effect on soil fertility improvement, but produced greater soil protection with a higher percentage ground cover than RT or NT bare soil management systems, especially LCC at early sowing date as a result of higher establishment and

biomass values than CCC, which was affected by waterlogged and frost conditions. Moreover, both cover crops were very effective in controlling weeds, reducing infestation with respect to bare soil management systems. Both CC residues retention were found to have limited impact on total moisture content storage during the durum wheat crop seasons. During the sunflower crop sequences, both CC showed occasional positive short-term impacts on the soil moisture contents compared to RT in the uppermost layer. Therefore, these cover cropping systems may be an alternative to RT in different soil types with changing rainfall patterns; whereas their introduction with respect to NT could be used in years with higher rainfall than the average precipitation of the area or in years with low rainfall if the CC are properly managed (probably early sowing and early mowing). Similarly, the different cropping systems did not have a great influence on the short-term soil fertility, but a slightly higher storage of soil N and OM contents were observed in the top-soil after CCC and LCC incorporation in addition to a higher contribution to maintain soil fertility in subsequent crops after CC cultivation with respect to the bare soil during the fallow period. Changes in N content occur slowly but the greatest N inputs made by legume species jointly with their easily decomposable residues (low C/N) made LCC more suitable for green manure than CCC treatments. This fact was also reflected in main crops yield and quality results. Durum wheat growth and production were influenced by the weather conditions and the enhancer effect of crop rotation in all treatments, but the effect of the preceding crop was crucial for wheat quality parameters with similar CC behaviour than RT but better than NT plots. By contrast, no definitive conclusions can be drawn with regard to the effect of CCC and LCC on sunflower crop rotation, due to the poor development of all treatments without following an established pattern. Complementary experiments to the long-term field trial showed that both CC reduced sunflower seed germination; whereas sunflower seedlings in pots not only were not affected by CC species but also improved their growth under controlled conditions. Moreover, other additional bioassays showed a great inhibitory effect of both species on broomrape germination, one of the major sunflower limitations. Under field conditions, CCC and LCC displayed a slight effect in the control of broomrape compared to RT, reducing broomrape incidence and severity just in absolute terms.

Our results provide positive indications for the use of winter cover crops in the durum wheat-sunflower rotation under southern Spain conditions and form a basis for further research on the optimization of this system. However, their effects on soil water storage, soil quality and fertility are limited in the short-term and results from more time than 3-year study are required to developing reliable cover cropping strategies. This long-term study is very important from the environmental point of view since the cover crops incorporation into crop rotations could help not only to reduce soil erosion or improve soil structure and fertility in the long-term, but also to mitigate climate change. The modifications introduced by these CA techniques on the N content (biological N fixation by LCC) and carbon dynamics in the soil (greater soil OM obtained by both CC with respect to RT and NT treatments and subsequent soil carbon sequestration improvement) could reduce N<sub>2</sub>O and CO<sub>2</sub> emissions in the long-term and thus contribute to mitigating global warming. Moreover, if weed and sunflower weedy broomrape control are corroborated after several years of study, *S. alba* and *V. narbonensis* could represent an alternative method of cultural control in arable rainfed fields in southern Spain. Key management factors for CC as seeding rate, N fertilization, sowing and mowing dates must be determined to improve CC growth over the winter, weed control and water storage, especially for *S. alba* cover crop on clay soils.

The inclusion of cover crops in farming systems in regions with a Mediterranean climate is unlikely to have major impacts on the water balance, but could still increase overall sustainability of the farming system. To this end, long-term research on cover crop is needed to design optimal rotations, determining their effect on main crops yield and quality, especially on sunflower installation and development. This is our sticking point, and elucidating it would represent the major breakthrough in the cover crops introduction by farmers. If these goals are achieved and specific benefits derived from *S. alba* and *V. narbonensis* cover crops are clarified, rainfed crop rotations in southern Spain would be improved under different climatic conditions and field specific factors. The proper cover crop and main crop management, together with crop residues and tillage system control would effectively ensure a cropping system providing environmental advantages according to the main goals desired by farmers.

**CHAPTER 4**  
**NEW CROPS INTRODUCTION**





## 4 NEW CROPS INTRODUCTION: *Vicia narbonensis*-*Avena strigosa* MIXTURE

The portion of this chapter included from section 4.2 to 4.7. (Experiment C.1.) has been previously published as '*Vicia narbonensis*-*Avena strigosa* mixture, a viable alternative in rainfed cropping systems under Mediterranean conditions' (Pedraza *et al.*, 2017), and has been reproduced with permission. Copyright is held by Spanish Journal of Agricultural Research. Additionally, a complementary study on N<sub>2</sub>-fixation and N transfer in the mixture *Vicia narbonensis*-*Avena strigosa* at different seeding ratios has been presented from section 4.8. to 4.12. (Experiment C.2.).

### 4.1 SUMMARY

The inclusion of forage mixtures in low-input cropping systems would be a sustainable alternative crop in line with the new agricultural trends, such as the new CAP requirements and livestock needs. Therefore, the viability as new forage crop of the *Vicia narbonensis*-*Avena strigosa* mixture at three different seeding ratios (65:35, 50:50 and 35:65) were compared with their monocrops and the standard mixture *Avena sativa*-*Vicia sativa* (65:35) for three growing seasons under comparable soils and Mediterranean climatic conditions. Several variables were determined to evaluate the intercropping systems development and analyze the interrelationships between mixture components on forage yield, forage quality and soil fertility through acquisition and transformation of nitrogen (N) resources using the natural abundance method. Forage mixtures growth, yield and N contribution varied according to the environmental conditions with a negative influence of a dry year 1 for legumes (< 300 mm) and a wet year 2 for oats (> 630 mm). Higher dry matter yield than control mixture in addition to similar crude protein, acid-detergent fibre, neutral-detergent fibre and digestible dry matter values, were produced at 35:65 in dry years and 65:35 and 50:50 in rainy years with loamy and clay soils, respectively. However, competition ratio, biological N<sub>2</sub> fixation made by *V. narbonensis* and N transfer to *A. strigosa* indicated the positive mixing effect of both species without significant differences between mixed treatments. Intermediate ground coverage values, dry matter yield and crude protein were observed with respect to pure stands, as well as a similar transformation of N into biomass compared to narbon bean monoculture. We conclude that the use of narbon bean-black oat mixture could substantially contribute to resource-efficient and sustainable agricultural cropping systems over a wide range of productive and environmental levels, including increased residual N resource for subsequent crops in the long-term. Thus, it would be a promising option to be considered as a forage mixture for livestock diets and crop rotation systems under Mediterranean environments.

**Keywords:** Black oat, narbon bean, forage mixture rates, dry matter yield, forage quality, symbiotic N<sub>2</sub>-fixation, N transfer

## C. 1. *Vicia narbonensis*-*Avena strigosa* MIXTURE, A VIABLE ALTERNATIVE IN RAINFED CROPPING SYSTEMS UNDER MEDITERRANEAN CONDITIONS

### 4.2 ABSTRACT

The demand of vegetable protein for animal feed and the need to diversify the crop rotation in rainfed Mediterranean climates has led to study the viability as new forage crop of the *Vicia narbonensis*-*Avena strigosa* mixture. Therefore, a 3-year field trial was conducted at two different and representative locations of the area to evaluate the capacity of both species to form a balanced mixture and to define its potential for high yield and forage quality. Different seeding ratios (65:35, 50:50 and 35:65) were compared with their pure stands and the standard mixture *Avena sativa*-*Vicia sativa* (65:35). Forage mixtures establishment and growth varied according to the environmental conditions with a negative influence of a dry year 1 for legumes (< 300 mm) and a wet year 2 for oats (> 630 mm). However, competition ratio indicated that there were not significant differences between mixed treatments, displaying intermediate ground coverage values, dry matter yield and crude protein regarding pure stands. Higher dry matter yield than control mixture in addition to similar crude protein, acid-detergent fibre, neutral-detergent fibre and digestible dry matter values, were produced at 35:65 in dry years and 65:35 and 50:50 in rainy years with loamy and clay soils, respectively. The appropriate development of both species in the mixture at different soil and rainfall conditions, as well as a good yield often higher than control mixture and a great forage quality, confirm to the narbon bean-black oat mixture as a viable and profitable crop alternative in rainfed cropping systems under Mediterranean conditions.

**Keywords:** Black oat, narbon bean, mixture rates, intercropping, dry matter yield, forage quality

### 4.3 INTRODUCTION

Rainfed arable areas occupy over 28 million hectares in southern Europe, which are mainly devoted to cereal-industrial crop rotation (Eurostat, 2015). Nowadays, this traditional practise is facing the urge to modify the current crop management and to diversify the crop production. They are especially influenced by the stagnating products price, the need to improve and achieve more balanced agro-ecosystem through the crop rotation (Flower *et al.*, 2012), as well as the accomplishment of the new Common Agricultural Policy (CAP) greening measures. The most noteworthy measure included is the 'crop diversification', where the farm holdings with an arable land of 10-30 ha have to cultivate at least two crops, and farms exceeding 30 ha must have at least three crops, with no more than 75 % of the cultivated area assigned to one crop. Moreover, Southern Europe is also a great livestock region with an

important number of heads of cattle (over 14 million) and sheep (over 24 million) next to the rainfed arable crops areas. The major constraint of the livestock development in these regions is the dependence of imported powerful food sources due to the limited on-farm production of forage, especially in the dry years of Mediterranean countries (Sadeghpour *et al.*, 2014). Therefore, it is necessary to satisfy the increasing demands for livestock (Alizadeh and da Silva, 2013) and there is the possibility of obtaining supplementary forage sown with arable crops in Southern Europe. Intercropping of winter grasses with annual legumes is extensively used in Mediterranean areas for forage production under rainfed conditions (Lithourgidis *et al.*, 2006). This system increases fodder yield concerning dry weight thanks to the grasses (Hashemi *et al.*, 2013) and improves feed quality owing to the higher crude protein concentration of legumes (Umunna *et al.*, 1995).

The environmental conditions (Gierus *et al.*, 2012) in addition to the seeding ratios of the selected species (Caballero *et al.*, 1995) are crucial factors affecting yield and quality of intercropping systems. The vetch (*Vicia sativa* L.)-oat (*Avena sativa* L.) mixture is the most traditionally used in Southern Europe and many studies have reported that these species are the most appropriate for mixture in Mediterranean regions (Lithourgidis *et al.*, 2006). Nevertheless, common vetch is described as susceptible to broomrape (*Orobancha crenata* Forsk.) (Gil *et al.*, 1987), one of the most serious legume pests in Mediterranean areas. However, findings of Nadal and Moreno (2007) revealed that narbon bean (*Vicia narbonensis* L.) is a high-yielding annual legume with an inbred line resistant to broomrape. It would be a good alternative for cropping in broomrape-infested areas under rainfed Mediterranean conditions. In these areas it is also known for being a promising crop in rotation with wheat (Durutan *et al.*, 1990) and a good source of protein fodder for cattle (Van der Veen, 1960) and sheep (Jacques *et al.*, 1994). On the other hand, Flower *et al.* (2012) showed that black oat (*Avena strigosa* Schreb.) is a winter cereal crop with a more rapid growth and higher biomass production than other oats, widely used in Australia (Flower *et al.*, 2012) and USA (Reeves and Price 2005). It has been used not only for grain production and as a green cover crop due to its weed control potential (Santos *et al.*, 2010), but also to obtain forage for dairy cows feed (Salgado *et al.*, 2013).

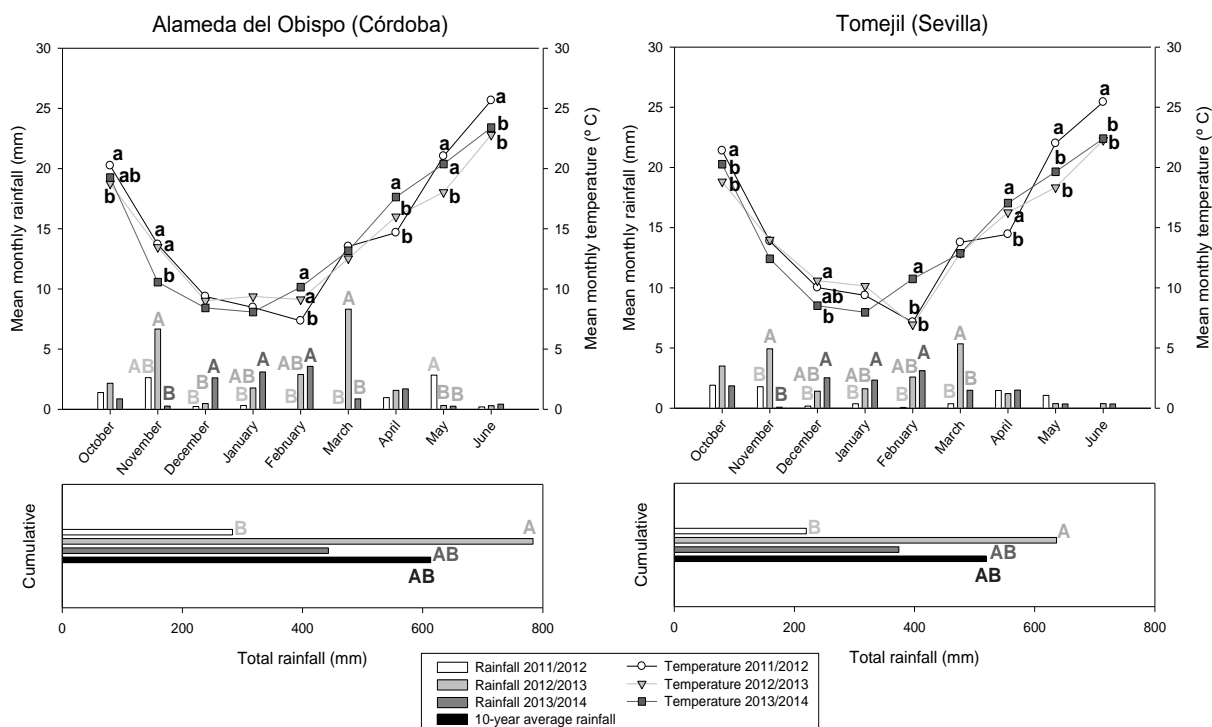
Given the capacity for high dry matter and protein yield documented in rainfed conditions of each species separately, mixtures of narbon bean with black oat could be a promising alternative for increased forage production in these areas. However, despite the fact that there is much published information on the forage yield and quality of legume-grass intercropping system worldwide, no information exists on the adaptation of intercropping black oat-narbon bean, seeding ratios, forage yield and quality both in Mediterranean regions and overseas.

In order to provide a new viable rotation crop alternative based on the narbon bean-black oat mixture, two main objectives were considered in this study: 1) determine if a stable forage mixture for balanced growth of the two species can be formed by narbon bean and black oat at different seeding ratios (65:35, 50:50 and 35:65) compared with the pure stands and the common vetch-common oat mixture (65:35), and 2) define if the studied mixture can achieve a high forage yield and quality production under rainfed Mediterranean conditions.

## **4.4 MATERIALS AND METHODS**

### **4.4.1 EXPERIMENTAL DESIGN AND FIELD PROCEDURES**

Field studies were conducted during the 2011/2012, 2012/2013 and 2013/2014 growing seasons (hereafter year 1, 2 and 3, respectively) at two different locations representing the typical soils occupied by rainfed arable crops in Southern Spain. The experiments were established on a Vertisol clay soil (*Chromic Haploxerert*) at the 'Las Torres-Tomejil' experimental farm (hereafter Tomejil) in Carmona (Sevilla) (37°24'07''N, 05°35'10''W) and an Entisol loam soil (*Typical Xerofluvent*) at the 'Alameda del Obispo' experimental farm (hereafter Alameda) located in Córdoba (37°51'42''N, 04°48'00''W). Soil samples were collected at 20 cm depth and analysed showing that Tomejil soils have higher pH (7.9 vs. 7.61), organic matter content (1.67 vs. 1.15 %), P (Olsen) (24 vs. 12.2 ppm) and K (634 vs. 289 ppm) than those at Alameda. Mean monthly temperature and rainfall were monitored by the Weather Station located *in situ* at each location (Figure 38), being obtained the mean daily temperature by averaging minimum and maximum air temperatures.



**Figure 38.** Mean monthly temperature and rainfall in addition to total rainfall during all the growing seasons and the cumulative 10-year average rainfall from October to June. Only significant differences between years are shown as different small letters within each monthly temperature per column and different capital letters within each monthly and cumulative rainfall per column (Tukey's,  $p \leq 0.05$ ).

Cumulative rainfall during the 3-year study in addition to the cumulative 10-year average rainfall from October to June was included. The highest rainfall amounts were recorded in year 2 (784 mm and 636 mm in Alameda and Tomejil, respectively) and the lowest values in year 1 (283 mm and 220 mm, respectively). The year 3 showed intermediate values (443 mm and 374 mm) without significant differences with the 10-year average rainfall (613 mm and 519 mm).

Species studied were narbon bean (*Vicia narbonensis* L., an inbreed line from the IFAPA Germplasm Collection) and black oat (*Avena strigosa* cv. Saia) besides the traditional common vetch (*Vicia sativa* cv. Vaguada)-common oat (*Avena sativa* cv. Chimene) used as control mixture. Narbon bean: black oat seeding ratios were 100:0 (D1), 65:35 (D2), 50:50 (D3), 35:65 (D4) and 0:100 (D5). They were established creating a proportional mixture according to the most common seeding rates of the pure stands of each species in the area and increasing and/or decreasing respectably in 35, 50 and 65 %. Seeding rates for each treatment are given in Table 12. Common vetch: common oat seeding ratio was 65:35 (C) based on the traditional sowing rate in the

study area. The experimental layout consisted of a randomized complete block design with six treatments and four replicates. The total number of elementary plots was 24 with a size of 24 m<sup>2</sup> (2 m × 12 m).

**Table 12. Seeding rates and 1000 seed weight for pure stands and mixtures in each legume-grass seeding ratio treatment.**

Species	Seeding rates (kg/ha)					Species	C
	D1 100:0	D2 65:35	D3 50:50	D4 35:65	D5 0:100		
<i>V. narbonensis</i>	140	91	70	49	0	<i>V. sativa</i>	91
<i>A. strigosa</i>	0	24.5	35	45.5	70	<i>A. sativa</i>	49
1000 seed weight (g)							
<i>V. narbonensis</i>	226.5					<i>V. sativa</i>	72.70
<i>A. strigosa</i>	19.43					<i>A. sativa</i>	25.25
Seeding rates (seeds/m <sup>2</sup> )							
<i>V. narbonensis</i>	61	40	30	21	0	<i>V. sativa</i>	125
<i>A. strigosa</i>	0	126	180	234	360	<i>A. sativa</i>	194

The previous crop was durum wheat harvested in mid-June each growing season except the year 1 when a fallow land preceded the trial in Alameda. Before sowing, the land was ploughed and harrowed, and the seedbed was prepared with a vibro-cultivator pass. All plots were hand sown in 10 lines spacing 200 mm and the seed uniformly distributed by qualified staff. Soil was not fertilized and weeds were controlled by hoeing. Sowings were performed after the first autumn rains on mid-November in 2011 and 2012 and early November in 2013 in both locations.

#### 4.4.2 ASSESSMENTS

Plant density and ground cover were evaluated for each component of the mixtures separately. Plant density was estimated counting the established plants in each seeding rate treatment 22 and 32 days after sowing (DAS) in 12 randomly selected 0.1 m<sup>2</sup> area of each plot during the three years. Plants with the first pair of true leaves were considered established plants. Plant ground coverage was determined photographically based on the methodology described by Laflen *et al.* (1981). Five random photographs per plot were taken with a camera at the height of 1.5 m above the frame during 5 dates from January to April over the cover (from 60-65, 85-90, 105-115, 130-135 to 145-160 DAS). Total percentage ground cover was determined by counting the different species coverage in each treatment using a

digital grid with 100 crossing points. The template points coinciding with green parts of each species came to their percent coverage in each photo.

Phenological events for species were determined based on the maximum and minimum daily temperatures to calculate growing degree days (GDD) with a 0°C baseline temperature for narbon bean (Mwanamwenge *et al.*, 1999) and black oat (Bauer *et al.*, 1984) and based on the BBCH scale (Lancashire *et al.*, 1991) of faba bean and cereals.

Dry matter (DM) yield for the different mixed treatments was also assessed at the time of harvesting. All plants were harvested on the same day in mid-April each year, on the closest stage to the optimum phenological development in both species according to Muslera and Ratera (1991), at the flat pods stage for legumes (71-78 stage in the BBCH-scale) and early milk stage for grasses (71-73 stage). Two biomass samples were randomly collected from a 0.5 m<sup>2</sup> area of each plot by cutting at 5-7 cm above ground level with a sickle. Species were separated by hand, and the weight after drying for 48 h in a forced air oven at 70 °C was determined.

The effect of competition between the two species used in the mixture was calculated using the competition ratio (CR) which indicates the degree to which one species competes with the other in an intercrop. The CR is calculated according to the following formula (Lithourgidis *et al.*, 2011):

$$CR_{nbean} = \left( \frac{Y_{nbeani}/Y_{nbean}}{Y_{boati}/Y_{boat}} \right) \left( \frac{Z_{boati}}{Z_{nbeani}} \right)$$

$$CR_{boat} = \left( \frac{Y_{boati}/Y_{boat}}{Y_{nbeani}/Y_{nbean}} \right) \left( \frac{Z_{nbeani}}{Z_{boati}} \right)$$

where  $Y_{nbean}$  and  $Y_{boat}$  are the yields of narbon bean and black oat, respectively, as monocrops and  $Y_{nbeani}$  and  $Y_{boati}$  are the yields of the species as intercrops.  $Z_{nbeani}$  and  $Z_{boati}$  are the sown proportion of intercropped narbon bean and oat respectively.

For determining the forage quality, 50 g from the oven-dried samples were used for the control and mixed treatments. Crude protein (CP) content and CP yield according to AOAC (1990) were determined. Besides, the study included acid-detergent fibre (ADF) and neutral-detergent fibre (NDF) values according to Goering



and Van Soest (1970) and digestible dry matter (DDM) content according to the Tilley and Terry method modified by Alexander and McGowan (1966).

Statistical analysis was done by combined analysis of variance (McIntosh, 1983). The comparison of mean values was estimated using a randomized complete block experimental design for each dependent measured variable influenced by the treatments (fixed factor) at the two field locations and the three years (random factors). The fixed factor 'treatment' represented the different crop mixtures (D1, D2, D3, D4, D5 and C). The random factors 'location' and 'year' represented the effect of the environmental variables 'geographical location and soil features' (Alameda and Tomejil) and 'rainfall and temperature each year' (year 1, 2 and 3). Differences between treatments were submitted to contrast analysis. The overall variance was partitioned by orthogonal contrasts to assess differences between pure stands-intercrops treatments and between control-mixed treatments. Contrast coefficients are shown in each row of Table 13. Differences between means were compared using the Tukey test at the 0.05 probability test including in the STATISTIX 9 program.

**Table 13. Application of orthogonal constrasts to the forage mixtures experiment.**

Contrast	Treatment					
	D1 100:0	D2 65:35	D3 50:50	D4 35:65	D5 0:100	C 65:35
Pure <i>A. strigosa</i> vs. Narbon bean-black oat mixtures	0	-1	-1	-1	3	0
Pure <i>V. narbonensis</i> vs. Narbon bean-black oat mixtures	3	-1	-1	-1	0	0
Control vs. Narbon bean-black oat mixtures	0	-1	-1	-1	0	3

## 4.5 RESULTS

### 4.5.1 EFFECT OF ENVIRONMENTAL CONDITIONS

Statistical analysis showed an interaction between the factors year and location in all variables evaluated, except for *Avena* plant density and the DDM that all years showed the highest values in Alameda and Tomejil, respectively (Table 14). However, there was no interaction between treatments-year or treatments-location for any variable (except for *Avena* plant density and CP content that showed treatments-year interaction). The results have been represented by each factor (year, location and treatment) separately in the tables and figures.

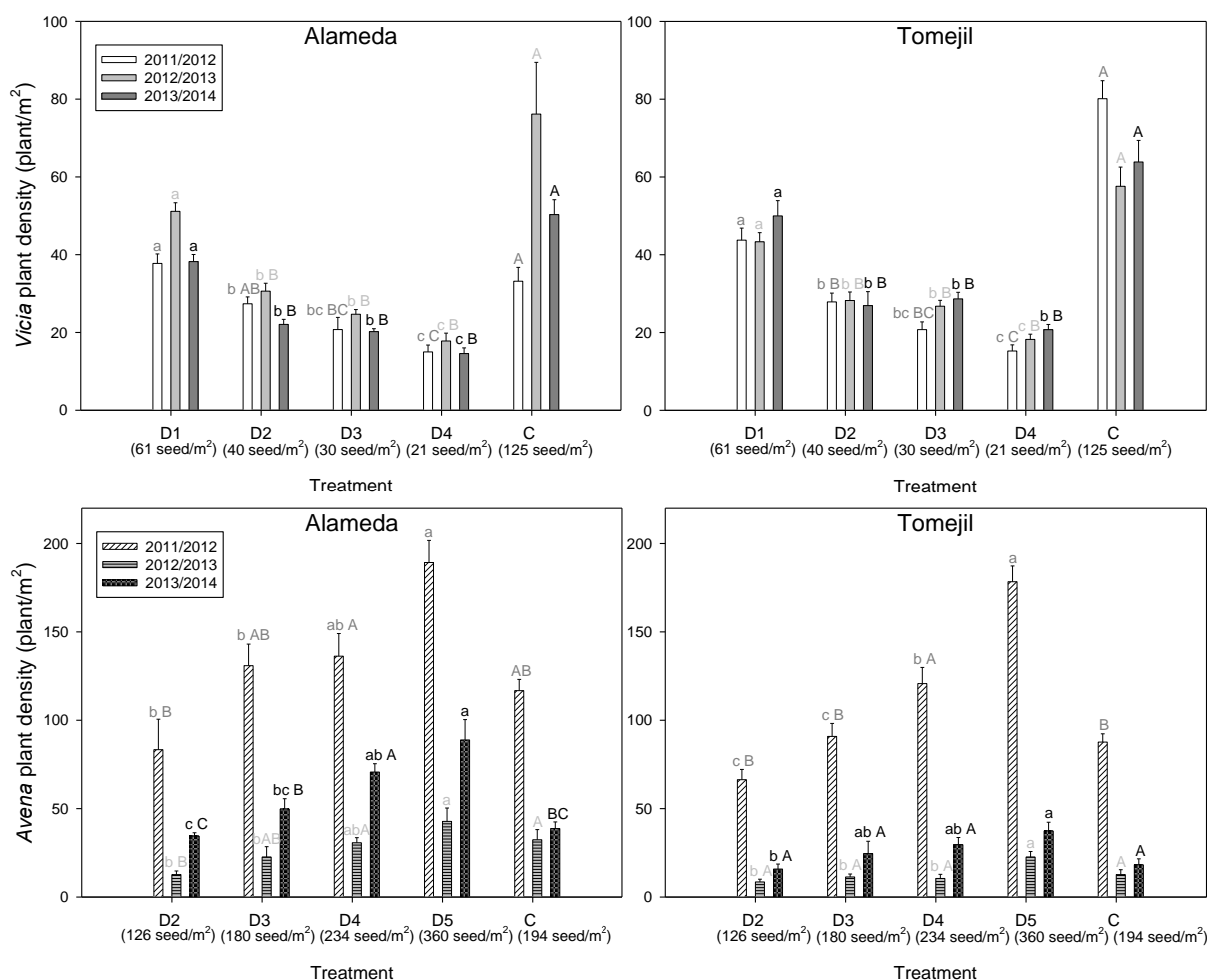
**Table 14. Summary of ANOVAs for testing the effect of treatments, year, location and interactions between factors on the different variables evaluated.**

Variables evaluated	Treatments <sup>1</sup> (TR)	Year <sup>2</sup> (Y)	Location <sup>3</sup> (L)	TR × Y	TR × L	Y × L
	<i>F</i>					
<b>Plant density</b>						
<i>Vicia</i>	20.20*** <sup>4</sup>	3.79*	13.87**	0.10 n.s.	0.66 n.s.	12.81***
<i>Avena</i>	35.53***	364.35***	52.40***	8.28**	0.64 n.s.	2.20 n.s.
<b>Ground cover</b>						
<i>Vicia</i>	70.62***	367.84***	102.78**	1.76 n.s.	1.24 n.s.	119.44***
<i>Avena</i>	10.59***	155.81***	122.01***	0.23 n.s.	0.16 n.s.	60.44***
<b>CR</b>						
<i>Vicia</i>	1.14 n.s.	10.79***	11.69**	0.74 n.s.	1.14 n.s.	37.86***
<i>Avena</i>	0.47 n.s.	10.79***	1.75 n.s.	0.44 n.s.	0.85 n.s.	4.39*
<b>DM yield</b>	0.69 n.s.	141.61***	185.90***	2.68 n.s.	0.32 n.s.	155.76***
<b>CP content</b>	59.91***	20.64***	0.27 n.s.	8.08**	1.22 n.s.	18.56***
<b>CP yield</b>	3.14 n.s.	35.26***	125.54***	1.52 n.s.	0.50 n.s.	141.85***
<b>ADF</b>	1.69 n.s.	427.69***	43.10***	0.70 n.s.	0.02 n.s.	29.64***
<b>NDF</b>	5.14*	175.84***	23.95***	1.23 n.s.	0.11 n.s.	13.89***
<b>DDM</b>	7.39*	248.57***	20.88***	0.73 n.s.	0.05 n.s.	1.49 n.s.

<sup>1</sup>Treatments: D1, D2, D3, D4, D5, C<sup>2</sup> Year: year 1 (2011/2012), year 2 (2012/2013), year 3 (2013/2014)<sup>3</sup> Location: Tomejil, Alameda<sup>4</sup>  $p < 0.05^*$ ,  $p < 0.01^{**}$ ,  $p < 0.001^{***}$ , n.s.: not significant

#### 4.5.2 STUDY OF SPECIES INTERACTION IN THE MIXTURE

The success of the mixture installation depended on the success of each species separately, which varied in relation to the narbon bean or black oat proportion in the mixture and the weather conditions in each location (Figure 39). *Vicia* plant density results indicated the highest values in Alameda year 2 (ranged from 17 to 25 % higher) and more similar results every year in Tomejil. However, *Avena* plant density showed the highest values the year 1 (about 56-82 and 79-89 % higher in Alameda and Tomejil, respectively). *Vicia* plant density values of C were greater than those in the proposed mixture treatments all years and locations. However, *Avena* plant densities for D4 and D3 were higher or similar to C in most of the cases.

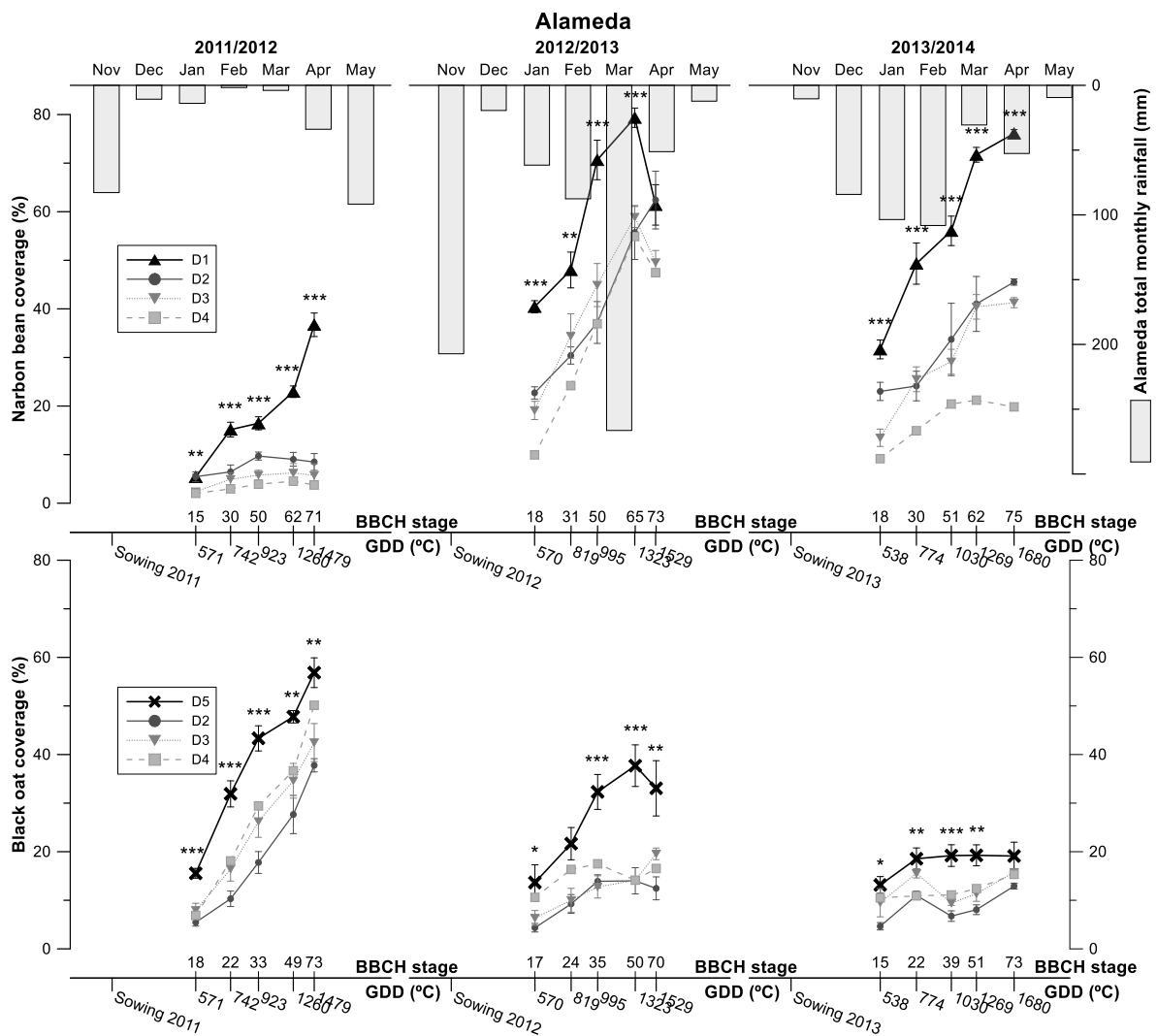


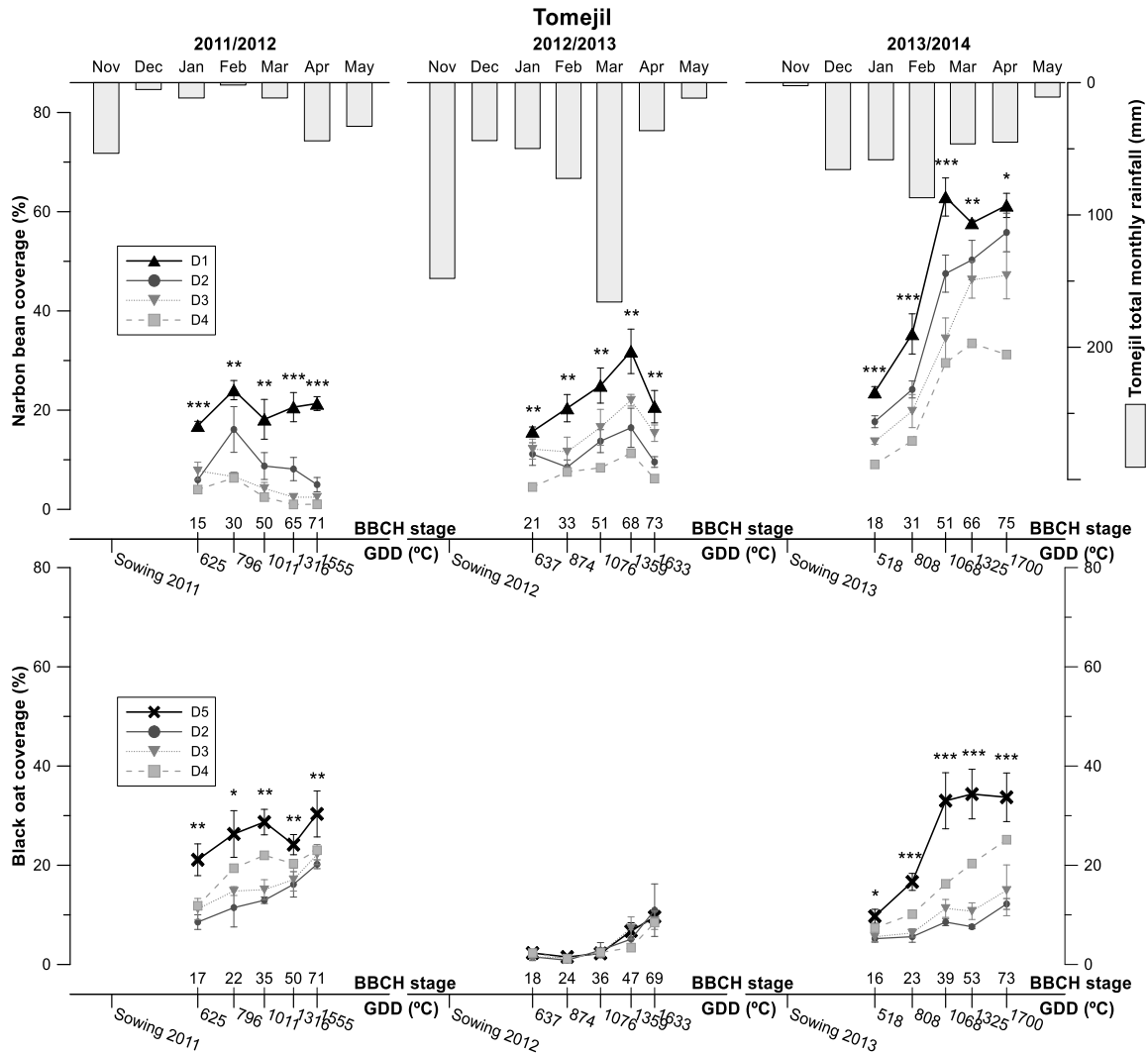
**Figure 39.** Plant density (plant/m<sup>2</sup>) of the component species of the mixture (D2, D3, D4) compared to pure stands (D1 and D5) and the control mixture (C) in each treatment and location during all the growing seasons. Significant differences between species in mixture are shown as different small letters compared to pure stands and different capital letters compared to control within each treatment per column (Tukey's,  $p \leq 0.05$ ).

The temporal evolution of the ground coverage sole crops registered the highest values compared to the mixed treatments, with significant differences on most of the sampling dates (Figure 40). Results for year 1 showed that ground coverage of narbon bean-mixtures decreased (6 % in Alameda and 3 % in Tomejil) from the 50 stage in Alameda and an early 30 stage (after 796 GDD accumulated) in Tomejil. Meanwhile, black oat increased (38-57 % in Alameda and 21 % in Tomejil) coinciding with an extended late winter-early spring drought (5.8 and 12.8 mm accumulated from February to March, respectively).

By contrast, the narbon bean ground cover increased as black oat mixes scarcely evolved from the 35-39 stage (995-1076 GDD) during the year 2 in both locations. It was probably due to the high rainfall recorded from February to March

(354 and 239 mm in Alameda and Tomejil, respectively). A similar trend occurred the year 3 in Alameda, with 139 mm recorded from February to March. During year 3 in Tomejil (374 mm accumulated), both species registered the highest growth stages of the study period at the time of harvest (73 black oat stage and 75 narbon bean stage with 1700 GDD). The lowest growth stage at both locations occurred the year 1 (219-283 mm) for narbon bean (71 stage with 1479-1555 GDD) and the year 2 (636-784 mm) for black oat (69-70 stage with 1529-1633 GDD). Narbon bean species grown at D2 and D3 and black oat species at D4 showed the lower decrease compared to their pure stands in all case studies.





**Figure 40.** Temporal evolution of the monthly cumulative rainfall, phenological growth stage and ground cover mean values of the component species in mixture (D2, D3, D4) compared with pure stands (D1 and D5) in both locations all the growing seasons. Temporal scale was expressed as the growing degree days (GDD) accumulated in five different dates (60-65, 85-90, 105-115, 130-135 and 145-160 DAS) with the last interval corresponding to the harvest time. The phenological growth stage was based on the BBCH-scale for faba bean and cereals (1, leaf development; 2, side shoots/tillering; 3, stem elongation; 5, inflorescence emergence; 6, flowering; 7, development of fruit). Differences between treatments were determined by contrasts. Only significant differences are shown: \*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ .

#### 4.5.3 FORAGE YIELD AND QUALITY

All narbon bean-black oat intercrops resulted in intermediate DM yields between pure stands each year and location except for the year 3 when D2 and D3 in Alameda and D4 in Tomejil produced the highest DM yield (Table 15). Moreover, intercropping treatments were not affected by seeding ratio. Differences with C were

only shown in Alameda the year 1 and 3, with the highest DM yields produced by D4 the first year and D2-D3 the third one.

**Table 15. Dry matter (DM) yield, crude protein (CP) content and CP yield of the different treatments during 2011/2012, 2012/2013 and 2013/2014 in Alameda and Tomejil.**

Legume-oat seeding ratio	2011/2012			2012/2013			2013/2014		
	DM yield	CP	CP yield	DM yield	CP	CP yield	DM yield	CP	CP yield
	kg/ha	g/kg of DM	kg/ha	kg/ha	g/kg of DM	kg/ha	kg/ha	g/kg of DM	kg/ha
<b>Alameda</b>									
<b>100:0 (D1)</b>	5003 b <sup>1</sup>	158 a	793 a	5870 a	167 a	981 a	2849a	108 a	313 a
<b>65:35 (D2)</b>	8668 aA <sup>2</sup>	89 bAB	766 aA	4549 aA	158 aA	716 aA	3020aA	88 abA	261 abA
<b>50:50 (D3)</b>	9452 aA	74 bcB	700 aA	4430 aA	148 aA	667 aA	2941aA	82 abcA	247 abA
<b>35:65 (D4)</b>	10385 aA	77 bcB	807 aA	4374 aA	142 aA	614 aA	2461aAB	79 bcA	187 abA
<b>0:100 (D5)</b>	11279 a	68 c	766 a	1639 b	55 b	90 b	1693a	61 cA	99 b
<b>65:35 (C)</b>	5724 B	119 A	686 A	5354 A	157 A	838 A	1296B	105 A	145 A
<b>Tomejil</b>									
<b>100:0 (D1)</b>	1807 b	149 a	278 a	1892 a	139 a	347 a	4153 a	133 a	572 a
<b>65:35 (D2)</b>	2928 abA	95 bB	282 aAB	929 abA	121 aA	113 abA	4696 aA	122abA	573 aA
<b>50:50 (D3)</b>	3343 abA	99 bAB	320 aAB	1407abA	122 aA	179 aA	4046 aA	124 aA	499 aAB
<b>35:65 (D4)</b>	3476 abA	79 bB	272 aB	556 abA	110 aA	61 abA	4753 aA	108 abA	496 aAB
<b>0:100 (D5)</b>	4436 a	75 b	328 a	193 b	50 b	10 b	3742 a	81 b	293 a
<b>65:35 (C)</b>	3884 A	128 A	496 A	707 A	125 A	89 A	3419 A	109 A	363 B

<sup>1</sup> Different small letters within each location per column indicate that the differences between narbon bean:black oat mixtures-pure stands were statistically significant (Tukey's,  $p \leq 0.05$ )

<sup>2</sup> Different capital letters within each location per column indicate that the differences between narbon bean:black oat mixtures-control were statistically significant (Tukey's,  $p \leq 0.05$ )

Competition ratio results of both narbon bean and black oat species showed no significant differences between the seeding ratio treatments neither location nor year (Table 16). The CR was lower than unity in all the intercropped narbon bean cases except for year 2 in Alameda and year 3 in Tomejil. Conversely, CR for the intercropped black oat was significantly greater than unity in all cases except for Alameda the year 2 and Tomejil the year 3.

Crude protein content results showed that all mixed forages were enhanced by increasing the proportion of legume seeding ratio (Table 15). Mixed treatments were not affected by seeding ratio the year 1 and year 2, and this second year there were no significant differences with sole legume (D1) (from 142 to 167 g/kg of DM in Alameda and 110-139 g/kg of DM in Tomejil). During the year 3, the highest CP contents were produced by D2 in Alameda (88 g/kg of DM) and D3 and D1 in Tomejil (124 and 133 g/kg of DM, respectively), without significant differences between them. Differences between C and intercrops were only shown during the year 1. C registered the highest CP contents (119 g/kg of DM in Alameda and 128 g/kg of DM in Tomejil), but with a lower difference with D2 in Alameda (89 g/kg of DM) and D3 in Tomejil (99 g/kg of

DM). Following the same previous trend, the CP yield improved as narbon bean seeding proportion increased (Table 16). All mixed treatments showed similar values to pure narbon bean. Significant differences were only found in Tomejil the year 2 (D1 got 347 kg/ha vs. 61 and 179 kg/ha of D4 and D3, respectively) and in Alameda the year 3 (D1 produced 313 kg/ha against 187-261 kg/ha of the mixtures). Significant differences between intercrops and C were only noted in Tomejil the years 1 (C achieved 496 kg/ha vs. 282 and 320 kg/ha of D2 and D3) and year 3 (when intercrops reached 496-573 kg/ha and C showed 363 kg/ha).

**Table 16. Competition ratio (CR) index for narbon bean: black oat mixtures in three seeding ratios (65:35, 50:50 and 35:65) during 2011/2012, 2012/2013 and 2013/2014 in Alameda and Tomejil.**

Seeding ratio	2011/2012		2012/2013		2013/2014	
	CR <sub>nbean</sub>	CR <sub>boat</sub>	CR <sub>nbean</sub>	CR <sub>boat</sub>	CR <sub>nbean</sub>	CR <sub>boat</sub>
<b>Alameda</b>						
<b>65:35 (D2)</b>	0.20 a <sup>1</sup>	5.77 a	2.28 a	0.51 a	0.38 a	1.64 a
<b>50:50 (D3)</b>	0.17 a	8.96 a	1.80 a	0.61 a	0.34 a	2.87 a
<b>35:65 (D4)</b>	0.16 a	7.69 a	2.38 a	0.43 a	0.69 a	1.48 a
<b>Tomejil</b>						
<b>65:35 (D2)</b>	0.26 a	6.93 a	0.36 a	2.93 a	1.15 a	0.71 a
<b>50:50 (D3)</b>	0.22 a	6.17 a	0.57 a	2.31 a	1.66 a	0.69 a
<b>35:65 (D4)</b>	0.09 a	8.95 a	0.88 a	1.59 a	1.60 a	0.56 a

<sup>1</sup>Different small letters within each location per column indicate that the differences between mixtures were statistically significant (Tukey's,  $p \leq 0.05$ )

The ADF values (Table 17) displayed significant differences between mixing ratios in Alameda during year 2 and Tomejil the year 1, with the lowest ADF contents produced by D3 and D4 (361 and 220 g/kg of DM, respectively). However, C showed lower ADF values in absolute terms all locations and years, although significant differences were only noticed in Alameda the year 1 and Tomejil the years 1 and 3. NDF contents for intercrops were not affected by seeding ratio, except for year 3 when D4 in Alameda (593 g/kg of DM) and D2 in Tomejil (552 g/kg of DM) showed the lowest values. Nevertheless, C showed lower NDF values than intercropping treatments the years 1 and 3. The highest DDM contents were shown by C all locations and years, except for D4 in Tomejil the year 1 (821 g/kg of DM) and D2 in Alameda the year 2 (640 g/kg of DM), which registered the highest values.

**Table 17. Acid-detergent fibre (ADF), neutral-detergent fibre (NDF) and digestible dry matter (DDM) of mixed stands and control treatments during 2011/2012, 2012/2013 and 2013/2014 in Alameda and Tomejil.**

Seeding ratio	2011/2012			2012/2013			2013/2014		
	ADF	NDF	DDM	ADF	NDF	DDM	ADF	NDF	DDM
g/kg of DM									
<b>Alameda</b>									
<b>65:35 (D2)</b>	298 a <sup>1</sup>	485 a	746 ab	400 ab	498 a	602 a	397 a	604 a	559 b
<b>50:50 (D3)</b>	297 a	507 a	743 ab	361 b	491 a	640 a	401 a	610 a	546 b
<b>35:65 (D4)</b>	319 a	534 a	720 b	377 ab	478 a	634 a	385 a	593 ab	577 b
<b>65:35 (C)</b>	228 b	384 b	831 a	412 a	514 a	639 a	368 a	529 b	668a
<b>Tomejil</b>									
<b>65:35 (D2)</b>	241 a	440 a	772 b	353 a	526 a	645 ab	415 a	552 ab	573 b
<b>50:50 (D3)</b>	228 ab	438 a	812 a	346 a	510 a	653 ab	420 a	566 a	578 b
<b>35:65 (D4)</b>	220 b	434 a	821 a	372 a	508 a	627 b	411 a	569 a	586 b
<b>65:35 (C)</b>	217 b	393 b	819 a	333 a	480 a	708 a	368 b	508 b	700 a

<sup>1</sup>Different small letters within each location per column indicate that the differences between mixtures-control were statistically significant (Tukey's,  $p \leq 0.05$ )

#### 4.6 DISCUSSION

The study of the species and their interaction in the mixture disclosed that it is possible to get a balanced forage mixture with narbon bean and black oat in rainfed Mediterranean conditions. However, the variables studied in order to characterize the intercropped narbon bean and black oat species (plant density and ground cover evolution) displayed the importance of the 'year' effect on the establishment and development of the species in mixture, such as was previously reported by Gierus *et al.* (2012). Although variation due to year was expected, differences of rainfall recorded were so different over the three experimental years that a negative influence of the drier year 1 for legumes and the wet year 2 for grasses was verified. Narbon bean plants were affected by drought conditions (< 300 mm) and winter frost period registered from the stem elongation/inflorescence emergence stage, as also occurred in white clover-mixtures studied by Annicchiarico and Tomasoni (2010). Conversely, black oat showed lower development and growth during year 2, when total rainfall values were significantly higher than the 10-year average (784 mm and 636 mm in Alameda and Tomejil, respectively). The amount of rainfall accumulated, especially in November, February and March led to waterlogging during critical growth stage period as emergence, heading and flowering which reduced root growth and penetration and therefore production of tillers and fertile heads as was also reported by Watson *et al.*



(1976). Taken together to the highest water retention capacity of the Vertisol clay soil, the decrease of oats plant density in Tomejil was high due to the adverse effects of excess moisture, as was previously reported by Urban *et al.* (2015). However, total rainfall amounts the year 3 (443 mm and 374 mm in Alameda and Tomejil, respectively) were statistically similar to the 10-year average rainfall. Both species showed a positive ground cover evolution accompanied by the highest phenological growth stage at the time of harvest and an accumulation of 1700 GDD, which was similar to the requirements shown by Mwanamwenge *et al.* (1999) in other Mediterranean climate zones.

Besides the 'year' effect, our results also showed the 'seeding ratio' important effect on the final mixture due to the individual species growth and the interaction between them (Caballero *et al.*, 1995; Lithourgidis *et al.*, 2006). In spite of the highest plant density and ground coverage reached by sole cropping treatments, the 65:35 and 50:50 seeding ratios showed the most similar trend to pure stand for the intercropped narbon bean. Moreover, the 50:50 and 35:65 displayed the greatest establishment and growth for black oat in mixture, without significant differences with the control during year 2 (even higher) and year 3. Our results also highlighted that a lower seeding rate and plant density of the proposed mixture compared to the control produced similar forage yield. This fact implies lower installation costs for farmers and an additional advantage of this mixture to include in the cropping systems since the economic feasibility of intercropping systems is ultimately determined by their monetary benefits, as was pointed out by Lithourgidis *et al.* (2011).

The absence of significant differences in CR in both components in the mixed treatments also evidenced their suitability to grass-legume mixture binary forage. The  $CR_{\text{boat}}$  was significantly higher than unity, showing the greatest competitive ability of black oat to exploit resources in association with narbon bean in all the case studies (Lithourgidis *et al.*, 2011) except year 2 in Alameda and year 3 in Tomejil, when  $CR_{\text{nbean}}$  was higher than unity in all intercrops. In all other cases, CR results for both species were positive but lower than unity, indicating that there was a positive benefit and these species can be grown in an intercrop (Dhima *et al.*, 2013). Indeed, all different narbon bean-black oat mixtures (D2, D3 and D4) showed good DM yields with similar or higher values than control mixture.

However, the fact that the species in the mixture displayed a different behaviour influenced by the weather conditions each study year, affected not only their growth and development, but also to subsequent forage yield and quality production (Caballero *et al.*, 1995). The different mixtures reached DM yields between black oat and narbon bean pure stands although similar to black oat in monoculture during year 1 when the weather conditions favoured the oats growth (Vandermeer, 1984). In contrast, during the year 2 with the highest rainfall occurring from the emergence to the flowering black oat stage, the intercrops DM yields were similar to narbon bean sole crop, especially noteworthy in Alameda. However, the intercropping system improved black oat yields the year 3 and year 2, increasing the DM yields from 20-44 % to 62-79 %, respectively, and this latter range displayed significant differences with the sole grass, as was reported by Dhima *et al.* (2013). The greatest DM yields for the intercropping narbon bean-black oat were provided by 35:65 in both locations during the drier year 1, by 65:35 the other study years in Alameda and by 50:50 and 35:65 in Tomejil. These average DM yields produced by the new mixture proposed were the highest in absolute terms. This fact could provide greater monetary advantages if farmers decide to cultivate intercropping systems, as were previously reported by Lithourgidis *et al.* (2011).

Regarding forage quality, increasing on-farm protein production it is also desirable to achieve more economically feasible farming systems (Sadeghpour *et al.*, 2014). The CP content is often considered as the most important component of forage quality (Lithourgidis *et al.*, 2007; Cazzato *et al.*, 2012), and CP yield (based on forage CP content and total DM produced) is a valuable measure of the total protein that can be harvested for livestock enterprises (Caballero *et al.*, 1995; Cazzato *et al.*, 2013). Our results showed that CP values enhanced with the seeding proportion of narbon bean in the intercrops (Sadeghpour *et al.*, 2014). The greatest CP contents were reached by the narbon bean sole crops but existing mixed treatments with similar CP values the year 2 (all mixed treatments in both locations) and year 3 (50:50 seeding ratio in Tomejil). In addition, CP contents achieved by 65:35 in Alameda the year 2 and by 50:50 in Tomejil the year 3 were higher than control mixture in absolute terms. Previous studies reported the highest CP content recorded when the legume was grown as a sole crop or formed a high proportion in intercrops with grasses

(Lithourgidis *et al.*, 2007; Dhima *et al.*, 2013). Moreover, our findings showed that 65:35 and 50:50 achieved CP contents ranged from 80 to 160 g/kg of DM all years and locations, which comply with the CP content requirement for good quality forage content (Leng, 1990). The total CP yields observed in narbon bean-black oat intercrops resulted in intermediate values between monocrops, without significant differences between intercrops, except for CP yield produced the year 2 in Tomejil and year 3 in Alameda due to their lower DM forage yield which is based for measurement (Dhima *et al.*, 2013). Similar results without significant differences in CP yield were reported by Lithourgidis *et al.* (2011) for faba bean-oat intercrops in Greece. Nonetheless, 65:35 in Alameda and 50:50 in Tomejil gave the more analogous results all the years, that is, the mixing ratios with higher legume proportion in the mixture, as was reported in many other studies and constitutes the reason for including vetches in an intercropping system (Lithourgidis *et al.*, 2006; Sadeghpour *et al.*, 2014).

Important criteria for evaluating forage quality are also ADF and NDF, being ADF a better efficient predictor of forage digestibility (Caballero *et al.*, 1995; Lithourgidis *et al.*, 2007; Corleto *et al.*, 2009; Sadeghpour *et al.*, 2014). In fact, as the ADF percentage decrease, quality and digestibility of forage usually increase. Our results showed all the ADF values (ranging from 220 to 419 g/kg of DM) were in agreement with the high quality forage levels (Van Saun, 2015). In addition, ADF contents at 50:50 in Alameda and 35:65 in Tomejil were lower or similar, respectively, in some cases to those obtained by the control, all of them values consistent with those presented in previous studies for Southern Europe (Caballero *et al.*, 1995; Lithourgidis *et al.*, 2007). On the other hand, the NDF content is a good measure of voluntary intake of the feed and it is inversely related to its digestibility or energy density (Sayar *et al.*, 2014). All NDF values obtained ranged between 485-600 g/kg of DM, forming reasonably good quality forage. The lowest NDF levels were found at 35:65 in almost all the cases, without significant differences with the control treatment the year 2. Lithourgidis *et al.* (2006) reported similar results, decreasing % NDF ratio while vetch ratio declined in a mixture. Finally, high DDM contents are required by ruminants to digest the greatest portion of the dry matter in a feed intake (Van Saun, 2015). The highest DDM contents were found at 50:50 during the rainiest year 2 (639-652 g/kg of DM). Meanwhile, the control mixture showed the highest values during the

years 1 and year 3, quite similar to the digestibility levels described by Caballero *et al.* (1995) but closely followed by 35:65 (from 577 to 821 g/kg of DM) in both locations.

#### 4.7 CONCLUSIONS

The study of narbon bean and black oat species at different seeding ratios showed good ground cover evolution and adequate phenological growth according to the GDD accumulation despite the influence of environmental fluctuations. Plant density for the intercropped narbon bean and black oat species was lower than sole cropping and control mixture, but the CR of all intercrops did not show differences between mixed treatments and both species in monoculture. It confirms that both species despite becoming dominant under different environmental conditions can be grown successfully as an intercrop. Furthermore, narbon bean-black oat mixtures reached higher DM yields and CP yield than common vetch-common oat traditional mixture and all quality traits (CP content, ADF, NDF and DDM) complied with the high-quality forage requirements. Higher dry matter yields and similar quality values than control mixture were displayed at 35:65 with the scarcity of rain and 65:35 and 50:50 with increased rainfall recorded in loamy and clay soils, respectively. The fact that it is possible to form a balanced mixture between narbon bean-black oat and to get a forage of high quality, jointly to their adaptation capacity at different soil and rainfall conditions, makes the mixture *V. narbonensis*- *A. strigosa* an economically and environmentally promising option to be considered as a forage mixture for livestock diets in Mediterranean environments. Moreover, it is a viable rotation crop alternative to be implemented by farmers in the CAP greening framework for all Southern Europe countries. In order to improve the emergence and subsequent development of both species, especially in difficult environmental conditions (poor and waterlogging soils, frosty periods or drought), further research should be focused on studying the water balance and N role in the intercropping system. Under rainfed Mediterranean conditions, it would be useful to define the water use efficiency for the studied mixture as well as to determine the seeding ratio that maximizes the N<sub>2</sub> atmospheric fixation for improving forage production and the residual N resource for subsequent crops in low-inputs cropping systems.

## C. 2. NITROGEN FIXATION AND NITROGEN TRANSFER IN THE MIXTURE *Vicia narbonensis*-*Avena strigosa* AT DIFFERENT SEEDING RATIOS

### 4.8 INTRODUCTION

Agriculture in the twenty-first century faces multiple challenges, including food and fibre production to feed a growing population (FAO, 2009). For that purpose, more efficient and sustainable methods need to be used, reducing the overuse of inputs such as mineral fertilizers (FAO, 2011). Foremost among these is the N fertilizer, the major limiting factor for worldwide plant growth (Fustec *et al.*, 2010). In fact, during the past few decades, the concern about possible environmental problems associated with high N fertilizer use has rekindled in using pasture legumes as a source of biologically-fixed N<sub>2</sub> (Ledgard and Steele, 1992) to improve soil fertility in crop rotation systems (Rasmussen *et al.*, 2012).

N<sub>2</sub>-fixation formed between soil bacteria of the genera *Rhizobium* and legumes could range roughly from 100 to 200 million tonnes of nitrogen annually (Biswas and Gresshoff, 2014), so the N<sub>2</sub>-fixation has been described to contribute, at least, 16 % of the global N supply (Liu *et al.*, 2010). However, additional studies have shown a higher contribution of legumes as a forage component when they grow associated with grasses. This is because they can get the benefit of the symbiotically fixed legume either via N sparing (Kumar and Goh, 2000) or through direct and indirect N transfer (Tessema and Baars, 2006; He *et al.*, 2009; Valles de la Mora and Cadisch, 2010). According to estimates, the amount of N transferred can vary from 0 to 65 % of the total biological nitrogen fixation (Ledgard and Steele, 1992; Nyfeler *et al.*, 2011). For subsequent crops, forage legumes contribute N both directly from forage legume residues and indirectly through mineralization of soil N pools build up during the entire sward phase (Rasmussen *et al.*, 2012). Thus, their accurate quantification could be important for improving forage and following crops quality and yields (Peoples *et al.*, 2009b; Valles de la Mora and Cadisch, 2010).

The amounts of N fixed from atmospheric N<sub>2</sub> in legume/grass pastures throughout the world can range from 13 to 682 kg N/ha/yr (Ledgard and Steele, 1992; Herridge *et al.*, 2008). Many relevant factors account for these changes on biological

nitrogen fixation: legume persistence and production, soil N status, environmental conditions, pests and diseases and competition with the associated grass, including the grass species and the dynamic relationship established between legume and grasses (Ledgard and Steele, 1992). Despite the importance of these factors, the impact on the N contribution of grass-legume mixtures at different seeding ratios has rarely been investigated under comparable soil and climatic conditions (Nyfeler *et al.*, 2011; Rasmussen *et al.*, 2012; Suter *et al.*, 2015), let alone the currently proposed narbon bean (*Vicia narbonensis* L.)-black oat (*Avena strigosa* cv. Saia) mixture for which there was no previous information to Pedraza *et al.* (2017). Nevertheless, results from this latter lead to think in its possibilities as a profitable alternative to get an efficient N contribution apart from forage yield and quality for livestock feed in low-input cropping systems under Mediterranean conditions. Moreover, the responses to the new CAP greening measures include crop diversification and Ecological Focus Area (EFAs) covered by N-fixing crops and mixtures of these with other crops for all Southern Europe countries (Hart, 2015), so the study of the new legume species fixation is essential to optimize possible cropping systems for the future and contribute to the mitigation of N<sub>2</sub>O emissions in the long-term (López Bellido, 2017c).

There are different methods to determine the N symbiotically fixed by legumes, all of them with some errors inherent to the measures (Valles de la Mora and Cadisch, 2010). However, over the past 20 years, the technological advances in isotope ratio mass spectrometry have turned the nitrogen-15 isotope of N (<sup>15</sup>N) natural abundance method into a precise technique which detects small natural variations in the abundance of <sup>15</sup>N in soil and plant materials without adding fertilizer (López-Bellido *et al.*, 2006). The natural <sup>15</sup>N abundance method is based on small differences between atmospheric N<sub>2</sub> (0.3663 % atoms <sup>15</sup>N) and the soil N. Considering that soil N is generally more abundant in <sup>15</sup>N than the atmospheric N<sub>2</sub>, it would be expected that the non-fixing plants in which the primary N source is that derived from the soil, would have more <sup>15</sup>N than the fixing plants which take N<sub>2</sub> from the atmosphere and the soil. Variations in natural abundance signatures likely result from discrimination against the heavier <sup>15</sup>N over the lighter nitrogen-14 isotope (<sup>14</sup>N) during N uptake and transfer from *Rhizobium* to host plants. The differences between isotopic compositions of the source and the sink (plant or soil) reflect the magnitude of naturally occurring <sup>15</sup>N

variation during N translocation, which could thus form a basis for tracing N transfer between plants (He *et al.*, 2009).

Following the previous study about the viability of the *Vicia narbonensis*-*Avena strigosa* mixture in rain-fed cropping systems under Mediterranean conditions (Pedraza *et al.*, 2017) (section C.1. of this Chapter 4), this study aims at evaluating the nitrogen role in the narbon bean- black oat mixture at the different seeding ratios (65:35, 50:50 and 35:65). The quantification of the N<sub>2</sub>-fixation made by the legume *V. narbonensis* and the N transfer to the accompanied grass *A. strigosa* at each seeding ratio could define the proper mixture rate for improving forage production and the residual N resource for subsequent crops in low-inputs cropping systems.

## 4.9 MATERIAL AND METHODS

### 4.9.1 PLANT ANALYSIS

The plant material used belongs to the field studies conducted during the 2011/2012, 2012/2013 and 2013/2014 growing seasons (year 1, 2 and 3, respectively) at the 'Tomejil' experimental farm in Carmona (Sevilla) and the 'Alameda' experimental farm located in Córdoba under rainfed conditions. Species studied were the same that the previous section (section C.1. of this Chapter 4), that is, narbon bean (*Vicia narbonensis* L.): black oat (*Avena strigosa* cv. Saia) at the seeding ratios 100:0 (D1), 65:35 (D2), 50:50 (D3), 35:65 (D4) and 0:100 (D5). In November all forage mixtures were sown every year. No additional fertilizer was applied at any plot (0 N). The biomass samples collected from the harvest at the development of fruit stage (mid-April) were the basis for the measurements of total N in the forage mixtures. Narbon bean and black oat were separated by hand from the 0.5 m<sup>2</sup> area of each plot. Plant samples were dried at 70<sup>o</sup> C for 48 hours in a forced-air oven and ball-milled for one hour before being subsampled, weighed and packed into tin capsules (8x5 mm, pressed) with a range between 1.500-2000 µg for legumes and from 2.800 to 3.500 µg for grasses using a high-precision balance (XP6, METTLER TOLEDO). These weights were optimized in order to reach a nitrogen signal between 2000-4000 mV.

Legume N<sub>2</sub>-fixation was determined by the natural <sup>15</sup>N abundance method (López-Bellido *et al.*, 2006; Nyfeler *et al.*, 2011). Total N in the dry matter samples and the δ<sup>15</sup>N were determined using a Delta V Advantage Isotope Ratio Mass Spectrometer

from Thermo Fisher Scientific (Bremen, Germany). This system was equipped with a ConFlo IV Universal Interface for continuous flow analysis and a Flash 2000 HT elemental analyzer. A molecular sieve packed column (5 Å, 1 m×1/8"×2 mm) from Thermo Scientific (Bremen, Germany) was used for chromatographic separation. Isodat Gas Isotope Ratio MS Software (version 3.0 from Thermo Scientific, Bremen, Germany) was also used to acquire and process the signal obtained by IRMS analysis.

#### 4.9.2 CALCULATIONS AND STATISTICAL METHODS

##### Nitrogen fixation

The percentage of N derived from N<sub>2</sub>-fixation (% N<sub>dfa</sub>) by legumes was calculated using the equation proposed by Ledgard and Peoples (1988):

$$\% N_{dfa} = 100 \times \left( \frac{\delta^{15}N_{ref} - \delta^{15}N_{leg}}{\delta^{15}N_{ref} - B} \right)$$

where  $\delta^{15}N$  legume is the atom percent (‰) excess  $^{15}N$  relative to atmospheric N in each treatment and the  $\delta^{15}N_{ref}$  (‰) is the  $\delta^{15}N$  units for reference plant include. The subscript 'leg' (legume) represents *V. narbonensis*, and the subscript 'ref' (reference) represents *A. strigosa*, grown into the same soil and conditions that the fixing legumes, at the 0:100 (D5) seeding ratio in the same plot that served as a reference plant (Boller and Nosberger, 1988). These plants provide a measure of the  $\delta^{15}N$  of soil mineral N available to the legume. The value B is the  $\delta^{15}N$  of a nodule legume grown in N-free media, with atmospheric N<sub>2</sub> as the only N source. Due to the similarity between the narbon bean and faba bean phenological growth stage and development, the B value used correspond to the faba bean value studied by López-Bellido *et al.* (2010b), B = - 1.7 ‰.

The narbon bean N yield derived from atmospheric N<sub>2</sub>-fixation (N<sub>fixed</sub>) (Kg of N/ha) was calculated from the total N in the legume above-ground biomass and the % N<sub>dfa</sub>.

##### Nitrogen transfer

In addition to the legume N derived from symbiotic N<sub>2</sub>-fixation directly from the atmosphere (N<sub>dfa</sub>), the  $\delta^{15}N$  technology also quantified the grass N derived from transfer (N<sub>transf</sub>) of the N<sub>dfa</sub> (He *et al.*, 2009; Nyfeler *et al.*, 2011):



$$\% N_{transf} = \left( 1 - \frac{\delta^{15}N_{grassmix}}{\delta^{15}N_{grassmono}} \right) \times 100$$

where  $\delta^{15}N$  'grassmix' is the  $\delta^{15}N$  units for grasses grown in mixture and 'grassmono' the grasses grown in monocultures (D5) next to the mixture plots (at the same N level), that is, these are the reference plants for grasses grown in mixtures with legume (Zanetti *et al.*, 1997). According to Nyfeler *et al.* (2011), since a possible different mineralization rate of organic matter under the mixture as compared to the grass monoculture could result in an overestimation of  $N_{transf}$ , we called the values derived from  $\% N_{transf}$  'apparent transfer'. The  $\% N_{transf}$  was multiplied by the shoot N accumulation of the black oat species to receive the amount of  $N_{transf}$  (Kg of N/ha).

#### **Total nitrogen yield and nitrogen from symbiotic and non-symbiotic sources**

The total amount of N (total  $N_{yield}$ ) (Kg of N/ha) harvested from each forage mixture treatment involves legume ( $N_{Legume\ yield}$ ) and grass yield ( $N_{Grass\ yield}$ ). They were calculated using the N concentration from each distinct fraction in species and their respective harvested dry matter (Rasmussen *et al.*, 2012; Thilakarathna *et al.*, 2012):

$$Total\ N_{yield} = N_{Legume\ yield} + N_{Grass\ yield}$$

In addition, both terms include a portion of N yield that comes from the symbiotic sources ( $N_{sym}$ ) previously described,  $N_{fixed}$  and  $N_{transf}$  (Nyfeler *et al.*, 2011):

$$N_{sym} = N_{fixed} + N_{transf}$$

Finally, N from non-symbiotic sources ( $N_{nonsym}$ ) was calculated as the *total*  $N_{yield}$  minus  $N_{sym}$ , according to Nyfeler *et al.* (2011). It comprises just the N derived from the soil organic matter, since no N mineral fertilizer was added any year or location.

$$N_{nonsym} = Total\ N_{yield} - N_{sym}$$

#### **Statistical analysis**

Statistical analysis was done by combined analysis of variance (McIntosh, 1983). A randomized complete block experimental design was used for each distinct fraction ( $\% N_{dfa}$ ,  $N_{Legume\ yield}$ ,  $\% N_{transf}$ ,  $N_{Grass\ yield}$ ,  $Total\ N_{yield}$ ,  $N_{sym}$  and  $N_{nonsym}$ ) influenced by the treatments (fixed factor) at the two field locations and the three years (random

factors). As in the previous experiment C.1., the random factors ‘location’ and ‘year’ represented the effect of the environmental variables ‘geographical location and soil features’ (Alameda and Tomejil) and ‘rainfall and temperature each year’ (year 1, 2 and 3). The mean %  $N_{dfa}$  and %  $N_{transf}$  parameters were subjected to arcsine  $(x/100)^{1/2}$  transformations to normalize the data and stabilize variance throughout the data range. Differences between means were compared using the Tukey test at the 0.05 probability test. Correlations shown are Pearson correlations of samples means. We computed statistics using the STATISTIX 9 program.

## 4.10 RESULTS

### 4.10.1 EFFECT OF ENVIRONMENTAL CONDITIONS

Statistical analysis showed an interaction between the factors year and location in all variables evaluated (Table 18). However, there was no interaction between treatments-year or treatments-location for any variable. The results have been represented by each factor (year, location and treatment) separately in the tables and figures.

**Table 18. Summary of ANOVAs for testing the effect of treatments, year, location and interactions between factors on the distinct fractions evaluated.**

Variables evaluated	Treatments <sup>1</sup> (TR)	Year <sup>2</sup> (Y)	Location <sup>3</sup> (L)	F		
				TR × Y	TR × L	Y × L
<b>N</b> <i>Legume yield</i>	11.19** <sup>4</sup>	14.81***	34.18***	1.57 n.s.	2.31 n.s.	36.23***
% <b>N</b> <i>dfa</i>	0.19 n.s.	22.15***	0.03 n.s.	0.18 n.s.	0.75 n.s.	5.57*
<b>N</b> <i>Grass yield</i>	4.09 n.s.	95.10***	57.20***	0.79 n.s.	0.48 n.s.	33.49***
% <b>N</b> <i>transf</i>	4.14 n.s.	0.99 n.s.	0.01 n.s.	0.64 n.s.	0.50 n.s.	4.02*
<b>Total N</b> <i>yield</i>	2.95 n.s.	18.71***	80.63***	2.03 n.s.	0.92 n.s.	44.54***
<b>N</b> <i>sym</i>	11.62**	24.59***	39.52***	1.56 n.s.	1.65 n.s.	36.63***
<b>N</b> <i>nonsym</i>	1.09 n.s.	72.23***	76.87***	2.84 n.s.	0.35 n.s.	45.30***

<sup>1</sup>Treatments: D1, D2, D3, D4, D5, C

<sup>2</sup>Year: year 1 (2011/2012), year 2 (2012/2013), year 3 (2013/2014)

<sup>3</sup>Location: Tomejil, Alameda

<sup>4</sup> $p < 0.05^*$ ,  $p < 0.01^{**}$ ,  $p < 0.001^{***}$ , n.s.: not significant

### 4.10.2 NITROGEN YIELD AND SYMBIOTIC NITROGEN FIXATION IN NARBON BEAN

The analysis of variance for narbon bean N yield ( $N_{Legume\ yield}$ ) showed clear differences between locations and the 3-years study weather conditions. Likewise, the interaction year×location was significant (Table 18) and consistent with the narbon

bean DM yield achieved in each case during the study period. In fact,  $N_{Legume\ yield}$  was positively correlated with the legume DM yield at both locations ( $R=0.98^{***}$  in Alameda and  $R=0.70^{***}$  in Tomejil). In Alameda, the highest N legume yields were observed the year 2 (values between 94 and 152 kg N/ha), followed by the  $N_{Legume\ yield}$  obtained the year 3 (from 20 to 54 kg N / Ha) without significant differences between treatments (Table 19).

**Table 19. Narbon bean N yield (kg N/ha) and forage legume N<sub>2</sub>-fixation proportion (% N<sub>dfa</sub>) of narbon bean pure stand (D1) and the different mixtures (D2, D3, D4) in each legume-grass seeding ratio treatment during 2011/2012, 2012/2013 and 2013/2014 in Alameda and Tomejil.**

Legume-oat seeding ratio	2011/2012		2012/2013		2013/2014	
	$N_{Legume\ yield}$	$N_{dfa}$	$N_{Legume\ yield}$	$N_{dfa}$	$N_{Legume\ yield}$	$N_{dfa}$
	(kg N/ha)	(%)	(kg N/ha)	(%)	(kg N/ha)	(%)
<b>Alameda</b>						
<b>100:0 (D1)</b>	133a <sup>1</sup>	36a	152a	56a	54a	72a
<b>65:35 (D2)</b>	30b <sup>2</sup>	56a	110a	57a	28a	70a
<b>50:50 (D3)</b>	16b	46a	102a	57a	23a	71a
<b>35:65 (D4)</b>	9b	53a	94a	58a	20a	68a
<b>Tomejil</b>						
<b>100:0 (D1)</b>	48a	49a	56a	76a	45a	58a
<b>65:35 (D2)</b>	13b	15b	17ab	73a	50a	64a
<b>50:50 (D3)</b>	6b	42a	22ab	75a	49a	69a
<b>35:65 (D4)</b>	2b	35a	9b	68a	81a	73a

<sup>1</sup> Different small letters within each location per column indicate that the differences between narbon bean: black oat mixtures-pure stands were statistically significant (Tukey's,  $p \leq 0.05$ )

The opposite occurred in Tomejil, where the highest values were observed the year 3 (values ranged from 45 to 81 kg N/ha). However, while the year 3 there was no significant differences between treatments, data from year 2, apart from their strong variability, differ from legume sole crop (56 kg N/ha) to mixed treatments (D2 and D3 achieved 17-22 kg N/ha vs. 9 kg N/ha of D4). The lowest  $N_{Legume\ yield}$  values for narbon bean were observed during year 1 in both locations, which had the lowest narbon bean development (Figures 39 and 40 of the previous section C.1). Significant differences between legume sole crop and narbon bean-black oat intercrops were displayed (D1 got 133 kg N/ in Alameda and 47kg N/ha in Tomejil, respectively) but the mixed treatments were not affected by seeding ratio (9-30 kg N/ha in Alameda and 2-13 kg N/ha in Tomejil, respectively).

Narbon bean N fixing capacity (%  $N_{dfa}$ ) was significantly influenced by the year but not by the location although the interaction between year and location was significant (Table 18). The highest values were observed during the year 3 and 2, with proportions from 56 to 76 % of the narbon bean N being derived from biological N fixation. Moreover, there were no significant differences when grown in monoculture or in mixture with grasses (Table 19). The lowest %  $N_{dfa}$  values for narbon bean were also obtained in the year 1 (values averaging 16-55 %) in both locations. Moreover, significant differences were observed in Tomejil, where D2 showed lower %  $N_{dfa}$  (15 %) than legume pure stand and the other seeding ratio treatments (35-49 %).

#### **4.10.3 BLACK OAT NITROGEN YIELD AND NITROGEN TRANSFER FROM NARBON BEAN**

The N yield harvested in the companion black oat of each mixed treatment and in pure stand ( $N_{Grass\ yield}$ ) was significantly affected by the year, location and by the interaction year $\times$ location (Table 18). The highest levels of  $N_{Grass\ yield}$  (kg N/ha) were observed in all cases during the year 1, with the highest values averaging 134-139 kg N/ha in Alameda and without significant differences between treatments (Table 20). In Tomejil, values ranged from 34 to 54 kg N/ha, with the highest results obtained by black oat pure stand (D5) and decreasing as the black oat seeding ratio percentage decreased (42, 41 and 34 kg N/ha at D4, D3 and D2, respectively). During the year 2  $N_{Grass\ yield}$  showed the smallest values at both locations (from 5 to 16 kg N/ha in Alameda and 2-3 kg N/ha in Tomejil), coinciding with the worst growth and development of black oat that year. This was due to the strong correlation between  $N_{Grass\ yield}$  and black oat DM yield at both locations ( $R=0.97^{***}$ ). However, there were no significant differences between grass pure stand and mixed treatments. Finally,  $N_{Grass\ yield}$  ranging between 18 and 40 kg N/ha in Alameda and 18-41 kg N/ha in Tomejil were achieved during year 3, without significant differences any treatment or location.

According to our results, the proportion of N aparent transferred from the narbon bean to the black oat in each seeding ratio (%  $N_{transf}$ ) showed a great variability between samples. Results were not affected by years or locations but their interaction was significant (Table 18). The highest  $N_{transf}$  means in absolute terms were achieved the year 3 in Alameda (values averaging 28-31 %) and year 2 in Tomejil (from 21 to 25

%), without significant differences between treatments (Table 20). For all other cases, the quantity of N in black oat derived from narbon bean apparent transfer varied from 0 to 16 %. In spite of the great variation of the data for %  $N_{transf}$  on the study cases, there were no significant differences between the mixed treatments neither location nor year.

**Table 20. Black oat N yield (kg N/ha) and atmospheric N<sub>2</sub>-fixation transferred from the legume to the grass (%  $N_{transf}$ ) of black oat pure stand (D5) and the different mixtures (D2, D3, D4) in each legume-grass seeding ratio treatment during 2011/2012, 2012/2013 and 2013/2014 in Alameda and Tomejil.**

Legume-oat seeding ratio	2011/2012		2012/2013		2013/2014	
	$N_{Grass\ yield}$	$N_{transf}$	$N_{Grass\ yield}$	$N_{transf}$	$N_{Grass\ yield}$	$N_{transf}$
	(Kg N/ha)	(%)	(Kg N/ha)	(%)	(Kg N/ha)	(%)
<b>Alameda</b>						
<b>65:35 (D2)</b>	103a <sup>1</sup>	6a	5a	0a	18a	30a
<b>50:50 (D3)</b>	118a	1a	10a	11a	27a	28a
<b>35:65 (D4)</b>	139a	5a	9a	7a	24a	31a
<b>0:100 (D5)</b>	133a	-	16a	-	25a	-
<b>Tomejil</b>						
<b>65:35 (D2)</b>	34b	9a	2a	25a	19a	16a
<b>50:50 (D3)</b>	41ab	13a	3a	22a	18a	1a
<b>35:65 (D4)</b>	42ab	6a	2a	21a	31a	4a
<b>0:100 (D5)</b>	54a	-	2a	-	40a	-

<sup>1</sup> Different small letters within each location per column indicate that the differences between narbon bean: black oat mixtures-pure stands were statistically significant (Tukey's,  $p \leq 0.05$ )

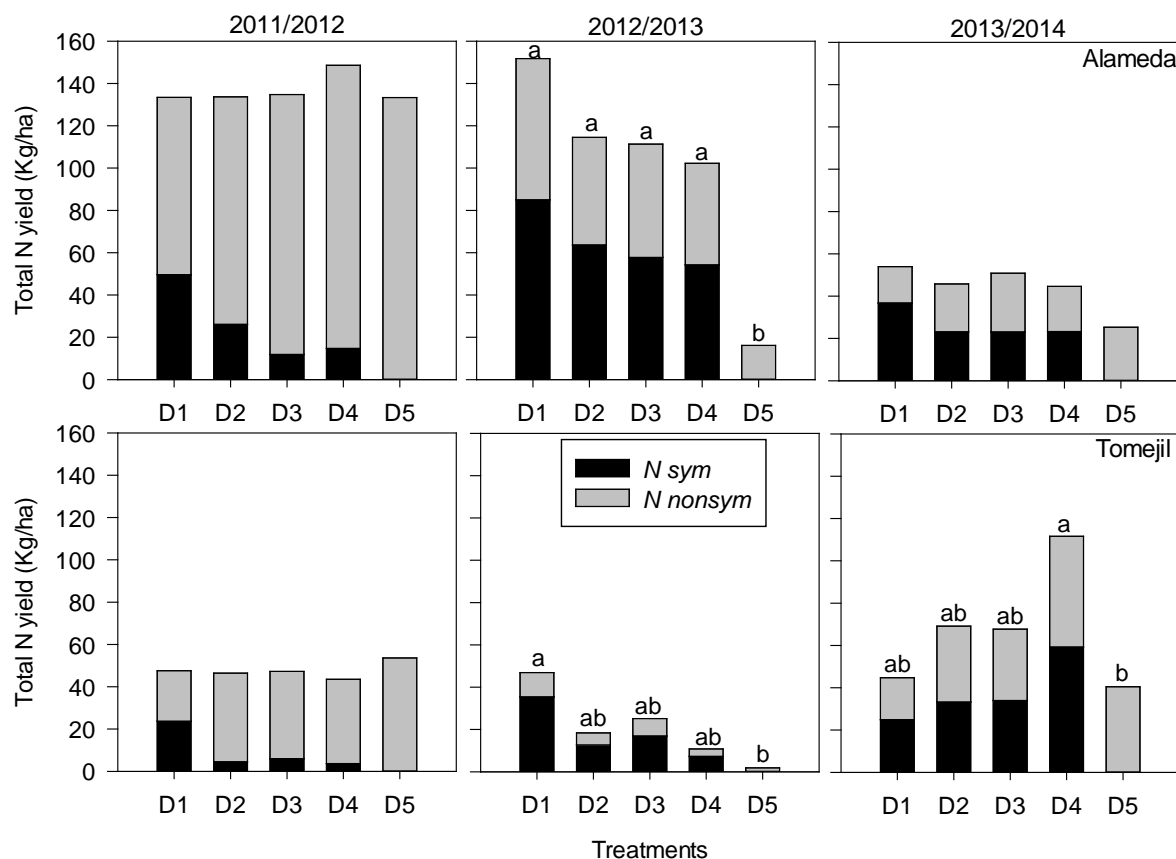
#### 4.10.4 TOTAL NITROGEN YIELD IN FORAGE MIXTURES

As a whole, total N yield (involving legume and grass N yield) was significantly influenced by the year and location and the interaction between both variables was highly significant (Table 18). The total  $N_{yield}$  harvested was expressed as the sum of the portion of N yields coming from  $N_{sym}$  and  $N_{nonsym}$  for each forage mixture treatment in Figure 41. Both variables ( $N_{sym}$  and  $N_{nonsym}$ ) followed a similar trend than total  $N_{yield}$ , with a significant effect of the years, location and the year×location interaction (Table 18).

The highest total  $N_{yield}$  values were obtained in Alameda during the years 1 (values averaging 133-149 kg N/ha) and 2 (from 16 to 152 kg N/ha), following by Tomejil the year 3 (from 40 to 112 kg N/ha) (Figure 41). These specific cases coincide with those of greater DM yield (Table 15 in the previous section C.1), observing a strong correlation between both variables ( $R=0.82^{***}$  in Alameda and  $R=0.70^{***}$  in Tomejil). In the rest of study cases, total  $N_{yield}$  varied from 18 to 56 Kg N/ha.

Nevertheless, significant differences between treatments were only observed in year 2 at both locations and year 3 in Tomejil. During the year 2, total  $N_{yield}$  achieved by narbon bean pure stand and all the mixed treatments significantly differed with those obtained by black oat sole crop in Alameda (from D1 to D4 with values ranging 102-152 kg N/ha vs. 16 kg N/ha of D5, respectively). Meanwhile, all narbon bean-black oat intercrops resulted in intermediate results between monocrops that year 2 in Tomejil, with D3 and D2 producing the highest mixing ratios values (25 y 18 kg N/ha). Total  $N_{yield}$  showed a higher variability the year 3 in Tomejil, with D4 showing the highest values (112 kg N/ha). These results were significantly higher than the rest of mixed treatments (D3 and D2 averaging 68- 69 kg N/ha) and pure stands (45 kg N/ha and 40 kg N/ha for D1 and D5, respectively).

Yield of  $N_{sym}$  (kg N/ha) harvested in the entire forage mixture showed a significant effect of the year, location and the interaction year $\times$ location on the results (Table 18). The highest  $N_{sym}$  values were observed the year 2 in Alameda (values from 54 to 85 kg N/ha) (Figure 41). These values performed roughly 53-56 % of the total N derived from symbiotic sources. High  $N_{sym}$  results occurred the year 3 at both locations (33-59 kg N/ha in Tomejil and 7-35 kg N/ha in Alameda) which represented 45-68 % and 50-56 % of the total  $N_{yield}$ . However, there were no significant differences between  $N_{sym}$  made by narbon bean sole crop (D1) and none of the mixed treatments. The  $N_{sym}$  results were consistent with the amount of N fixed by the legume and consequently, with the  $N_{Legume\ yield}$  of narbon bean in all treatments, whose variables were positively correlated ( $R=0.90^{***}$  in Alameda and  $R=0.96^{***}$  in Tomejil). For this reason,  $N_{sym}$  reached low values the year 2 in Tomejil (values averaging 7-35 kg N/ha). Nevertheless, most of the total  $N_{yield}$  derived from  $N_{sym}$  (portions averaging 67-75 %), without significant differences between D1 and forage mixtures. The lowest  $N_{sym}$  values were observed during the year 1, especially in Tomejil, where significant differences between legume pure stand and the rest of mixed treatments in Tomejil were displayed (24 kg N/ha vs. 4-6 kg N/ha, respectively).



**Figure 41.** Total N yield, total yield of symbiotically fixed nitrogen ( $N_{sym}$ ) and yield of non symbiotically fixed nitrogen ( $N_{nonsym}$ ) expressed as (Kg N/ha) from each pure (D1, D5) and mixed (D2, D3, D4) treatments during 2011/2012, 2012/2013 and 2013/2014 in Alameda and Tomejil. Different small letters per column indicate that the differences between treatments were statistically significant (Tukey's,  $p \leq 0.05$ ).

Yield of  $N_{nonsym}$  harvested also showed significant differences between years, locations as well as a significant year $\times$ location interaction (Table 18). During the year 1 most of the total  $N_{yield}$  derived from non-symbiotic sources in all treatments (values from 84 to 134 kg N/ha in Alameda and from 24 to 54 kg N/ha in Tomejil), correlated with the highest  $N_{Grass\ yield}$  achieved that year ( $R=0.87^{***}$  in Alameda and  $R=0.78^{***}$  in Tomejil). In fact,  $N_{nonsym}$  portions supposed approximately 80-91 % of total  $N_{yield}$  in the mixed treatments at both locations. By contrast, the portion of total  $N_{yield}$  related to  $N_{nonsym}$  was lower during year 2 and 3 at both locations (Figure 41). However, while year 3 did not show significant differences between treatments (values averaging 17-28 kg N/ha in Alameda and 20-52 kg N/ha in Tomejil), year 2 displayed them at both locations. Results from Alameda showed the lowest  $N_{nonsym}$  values for black oat sole crop (16 kg N/ha), followed by D4 and the rest of mixed treatments without significant differences with legume sole crop (values averaging 54-66 kg N/ha). In Tomejil D5 also

displayed the lowest  $N_{nonsym}$  values (2 kg N/ha in Tomejil), followed by all the mixed treatments (4-8 kg N/ha) and finally the highest values obtained for legume pure stand (D1) (12 kg N/ha).

#### 4.11 DISCUSSION

The study of the nitrogen role in the narbon bean-black oat mixture showed the positive mixing effect at the different seeding ratios (65:35, 50:50 and 35:65). All the variables quantified were affected by the 'year' effect, given the different weather conditions occurred during the study period (Pedraza *et al.*, 2017). Therefore, given the correlation between  $N_{yield}$  and DM yield in both species, the results were conditioned by the development of the species each year of study, as previously was reported by other authors (Unkovich *et al.*, 2010; Biswas and Gresshoff, 2014). The negative influence of the dry year 1 for narbon bean caused the lowest  $N_{Legume\ yield}$  of the study period. Narbon bean in the different mixed treatments reached lower  $N_{Legume\ yield}$  than in monoculture, although without significant differences between mixing ratios. However, the highest  $N_{Legume\ yield}$  was reached during the years 2 and 3, with similar values between all the treatments, except for the year 2 in Tomejil when more similar  $N_{Legume\ yield}$  than narbon bean pure stand was displayed by 65:35 and 50:50 seeding ratios. By contrast, the positive influence of the year 1 and the negative effect of the wet year 2 were reflected in the  $N_{Grass\ yield}$  reached by black oat although without differences between pure and mixing ratios.

Moreover, the influence of the environmental conditions was also reflected in the  $N_2$ -fixation, as was indicated by Peoples *et al.* (2009a) and López-Bellido *et al.* (2011b). However, despite the large differences due to the year-on-year variable environmental conditions, the proportion of the narbon bean N yield derived from  $N_2$ -fixation did not vary between the different mixed treatments and the legume pure stand, except for the year 1 in Tomejil when the 65:35 seeding ratio showed the lowest values. It could be due to the worst narbon bean development and yield that year in the clay soil, which also occurred across different pedo-climatic conditions in other studies (Rasmussen *et al.*, 2012; Suter *et al.*, 2015). Our results differed from those obtained by Nyfeler *et al.* (2011), who observed that an increasing percentage of grasses in mixtures stimulated the percentage of  $N_2$  fixation in the legume plants.



Carlsson *et al.* (2009) observed that different legumes achieved a higher proportion of their N from N<sub>2</sub>-fixation when grown in mixture with grasses than when they are grown in monoculture. Although it would seem reasonable that N fixation on an area basis would vary widely with the proportion of legume in the sward, our study could not confirm it. In any case, intercropping with grasses had a positive effect, stimulating the N<sub>2</sub>-fixation made by the legume in mixture. Moreover, the proportion of N derived from the atmospheric N varied between 35-56 % the worst year 1 for narbon bean and 56-76 % during the years 2 and 3, similar to those obtained by other legumes under similar weather conditions (López-Bellido *et al.*, 2011b). The percentages of apparent N transfer of symbiotically fixed N from the narbon bean to black oat did not differ between mixed treatments because they showed a great variability. During the first year, our results ranged between 1-13 % but the highest N<sub>transf</sub> values obtained in absolute terms varied between 21-31 % during the year 2 and 3. As apparent N transfer is influenced by climate and management practices, a wide variation in values has previously been reported for grass–clover swards, ranging from zero to 65 % (Nyfeler *et al.*, 2011; Enriquez-Hidalgo *et al.*, 2016) and averaging 11-34 % (Laidlaw, 2009; Thilakarathna *et al.*, 2012). Other studies concluded that increased grass percentage in the sward increased the apparent N in grasses derived from legume N transfer (Nyfeler *et al.*, 2011). Although there were no differences between the different mixed treatments, the important feedback mechanisms exerted by this accompanying grass was observed in our results (Nyfeler *et al.*, 2011). In fact, the lack of differences in the % N<sub>transf</sub> despite having lower or higher black oat proportion in the mixture demonstrated the ability of both narbon bean and black oat to expand their acquisition for N from symbiotic sources when grown in mixtures. To our knowledge, fertilization application could stimulate grass growth, grass yield, increase the amount of N accumulated (N derived from the soil and from fertilizer N) and the N transferred (Pirhofer-Walzl *et al.*, 2012; Suter *et al.*, 2015). Nevertheless, the suppression of legume N fixation by N fertilizer has been widely documented both in relation to proportion and amount of N fixed because fertilizer applications seem to influence mainly the N relocated from legumes to grasses (Biswas and Gresshoff, 2014; López-Bellido *et al.*, 2011b; Rasmussen *et al.*, 2012). Despite this, the amount of N fixed ultimately depends on several factors (narbon bean DM, N<sub>Legume yield</sub> and N<sub>dfa</sub>) (Carlsson

and Huss-Danell, 2003), which together with the values from the accompanying black oat are crucial to firstly determine their N role in the intercropping for subsequent mixture optimization and efficient resource use.

Total  $N_{yield}$  of the forage mixture did not show differences between pure stands and mixed treatments during the year 1 due to the high contribution of the pure grass ( $N_{nonsym}$ ) at both locations. The high correlation shown between total  $N_{yield}$  and DM yield reflected the positive effect of the dry year 1 on the black oat and its negative influence on the narbon bean. By contrast, the increased  $N_{sym}$  portion achieved during years 2 and 3 at both locations made that black oat sole crop always achieved the lowest total  $N_{yield}$  except for the year 3 in Alameda without differences between treatments.

In fact, during the year 2 and 3 more than half of the total  $N_{yield}$  was derived from  $N_{sym}$  in narbon bean monoculture and all mixed treatments. It was especially noteworthy the year 3, since it was statistically similar to the 10-year average and promoted a positive development of both species, especially observed at the 35:65 seeding ratio with the highest total  $N_{yield}$ . This fact highlighted that acquisition of  $N_{sym}$  by the entire mixture was stimulated by grasses and as a result, mixed treatments obtained the same  $N_{sym}$  yield as pure narbon bean stands. A similar strong positive mixing effect was also observed by Nyfeler *et al.* (2011), who even obtained higher values than legume pure stands. The yield of  $N_{nonsym}$  harvested in the forage mixture also showed this significant beneficial effect. During the years 1 and 3 the similar N acquired from non-symbiotic sources was observed in all the treatments but the advantage was even bigger during the year 2, when mixed treatments harvested higher  $N_{nonsym}$  than black oat pure stands. However,  $N_{nonsym}$  was largest at 65:35 and 50:50 seeding ratios in Alameda. Therefore, narbon bean-black oat mixtures stimulated the acquisition of both  $N_{sym}$  and  $N_{nonsym}$ , as was also concluded by Nyfeler *et al.* (2011).

#### 4.12 CONCLUSIONS

In conclusion, this study showed that narbon and black oat, as a new mixture crop alternative in rainfed cropping systems under Mediterranean conditions, combine well from the point of view of the soil N contribution. The quantification of the

biological N<sub>2</sub> fixation made by the legume *V. narbonensis* and the N transfer to the accompanied grass *A. strigosa* at each seeding ratio showed the positive mixing effect of both species. Legume and grass N yields varied greatly within years due to their high correlation with DM yields, but the overall means were not different between pure and mixed treatments except for the year 1, with a negative effect for narbon bean but positive for black oat. Nevertheless, the biological N fixation contribution made by the narbon bean was similar to all treatments and narbon bean also transferred similar fixed N to companion black oat. This effect was the result of mutual grass–legume interactions, which stimulated the acquisition of symbiotic and non-symbiotic N resources and efficient transformation of N into biomass compared to black oat monoculture and similar to narbon bean sole crop even in dry years of poor development for narbon bean. In addition, higher total N yield was only observed at 35:65 with average rainfall patterns in clay soils. Therefore, both species in the different mixing ratios could improve soil fertility through the increase of soil N derived from symbiotic sources.

This fact is very important from an environmental viewpoint since the *V. narbonensis*-*A. strigosa* mixture could contribute to productive and resource efficient agricultural crop systems by reducing the input of N fertilization and thus could help to mitigate greenhouse gas emissions, especially N<sub>2</sub>O emissions in the long-term. Further research should be focused on the residual N resource and buildup obtained by the decomposing residues of plants for the subsequent wheat in the crop rotation system, making a better adjusting of the necessary N fertilization. Moreover, due to the large dependence of N contribution on DM yield, improve the black oat development without decrease the proportion of N derived from narbon bean N<sub>2</sub>-fixation could offer an important advantage for tackling future agricultural challenges. Therefore, additional studies will be needed to identify the effect of different fertilizer N rates on the different mixed treatments production, N<sub>2</sub>-fixation in narbon bean-black oat mixture and narbon bean N transfer to black oat. They could help to optimize composition and management of the mixture and increase their inclusion into low-inputs cropping systems under rainfed Mediterranean conditions as a sustainable alternative in line with the new agricultural trends, such as the new CAP requirements and restrictions and livestock demands and needs.

**CAPÍTULO 5**  
**DISCUSIÓN GENERAL**



## 5 DISCUSIÓN GENERAL

La adaptación de los sistemas agrícolas a nuevas técnicas y prácticas de manejo es fundamental para afrontar los nuevos retos de la agricultura del siglo XXI (Foley *et al.*, 2011). Además, la producción de alimentos para satisfacer la demanda de una población mundial en aumento es uno de los desafíos actuales más importantes (FAO, 2009), ya que de aquí a 2050 los sistemas agrarios tendrán que producir más en una superficie menor de tierra, haciendo un uso más eficiente de los recursos disponibles y con un impacto mínimo sobre el medio ambiente (Hobbs *et al.*, 2008). Por lo tanto, la transformación hacia una agricultura sostenible que lleve a cabo prácticas agronómicas adaptadas a las necesidades de los cultivos y a las condiciones locales de cada región es la única solución para contribuir activamente a la consecución de estos grandes objetivos (Björklund *et al.*, 2012). En el caso de la producción de cereales, el uso de técnicas de cultivo y manejo de suelo que mejoren su calidad y preserven los recursos naturales sin menoscabo de los niveles de producción es esencial para mantener la productividad de la tierra y el ritmo de la demanda, ya que constituyen la base principal de la dieta humana y animal a nivel mundial.

La rotación trigo-girasol constituye la alternativa tradicional prioritaria en las explotaciones agrarias de cultivos herbáceos de secano del sur de España, ocupando una extensión considerable y generando una parte significativa de la producción a nivel nacional y europeo. Dado que la UE avanza hacia una agricultura sostenible (Öhlund *et al.*, 2015), la adaptación de la rotación tradicional a los requerimientos actuales de la PAC y los principios de condicionalidad, tales como la realización de prácticas agrícolas sostenibles y la diversificación de cultivos (EC, 2014) resultan prioritarias. Sin embargo, teniendo en cuenta las circunstancias agronómicas y socioeconómicas locales, y la falta de alternativas que superen la rentabilidad de la rotación, la diversificación de las explotaciones agrarias andaluzas en cuanto a nuevas prácticas de manejo y cultivos, entraña cierta dificultad.

En este contexto, en la presente tesis doctoral se ha evaluado la viabilidad de introducir cultivos intercalares en las rotaciones tradicionales como técnica de manejo, así como proporcionar nuevas alternativas de cultivo sostenibles en los secanos andaluces.

En este trabajo, las especies estudiadas como cultivos cubierta en la rotación fueron mostaza blanca y alberjón debido a que los beneficios de las cubiertas vegetales compuestas por especies de las familias *Brassicaceae* y *Leguminosae* son ampliamente conocidos en distintos sistemas de cultivo en condiciones de secano (Alcántara *et al.*, 2009; Perdigao *et al.*, 2012; Ramírez-García *et al.*, 2015b). Diversos aspectos se han tenido en cuenta para valorar la viabilidad de incluirlos como cultivos intercalares, tales como la cobertura de suelo y la producción de biomasa generada, la influencia en el contenido de humedad del suelo y la fertilidad del mismo así como los efectos que pueden tener sobre el desarrollo, producción y calidad de los cultivos principales, las malas hierbas y el jopo del girasol (*Orobanche cumana*). Dada la fuerte influencia de las condiciones meteorológicas en la agricultura de secano de clima mediterráneo y que el factor más limitante de la producción es la disponibilidad de agua (López-Bellido *et al.*, 2003), las diferentes condiciones meteorológicas acontecidas durante los 3 años de estudio influyeron en el crecimiento de la mostaza blanca y el alberjón, y en su posterior efecto en el contenido de humedad de suelo.

El cultivo intercalar de mostaza blanca alcanzó suficiente cobertura de suelo todos los años ( $\geq 30\%$ ), pero los resultados sugirieron que las condiciones meteorológicas del año 2012/2013, marcado por elevadas precipitaciones y temperaturas más bajas durante el invierno, afectaron a su crecimiento y desarrollo, especialmente en suelos arcillosos como los de Tomejil. La sensibilidad a las heladas y el encharcamiento de algunas especies crucíferas ha sido previamente citada en estudios previos (Haramoto y Gallandt, 2004; Saavedra *et al.*, 2015b, 2016b). Sin embargo, su rápido crecimiento permitió una recuperación de la especie (Alcántara *et al.*, 2009), produciendo una biomasa comprendida entre los 5.800-13.500 kg/ha en suelos francos y entre 2.360-17.750 kg/ha en suelos arcillosos. Los años que la mostaza blanca tuvo un peor desarrollo, se observó una escasa influencia en el contenido de humedad del suelo a corto plazo, bien por el aumento del contenido de agua con las altas lluvias registradas, por la reducida demanda por una menor biomasa producida, por la mayor infiltración a través de las raíces (Dabney *et al.*, 2001) o por todo en su conjunto. En cambio, aquellos años agrícolas y localidades donde hubo una mayor producción de biomasa de mostaza blanca, el contenido de humedad del suelo fue similar al del tratamiento mínimo laboreo, pero más bajo que la siembra directa en los

momentos con una mayor demanda de agua del cultivo (floración), aunque sin afectar a las reservas hídricas disponibles durante el resto del ciclo del cultivo del girasol. Además, el cultivo de mostaza blanca redujo la incidencia de malas hierbas en comparación con la existente en los tratamientos laboreo mínimo y siembra directa en todos los casos estudiados. Por lo tanto, nuestro estudio pone de manifiesto su efectivo control de malas hierbas, no solo por competencia directa con el cultivo (Kruidhof *et al.*, 2008) sino también por su efecto biofumigante al incorporarse en el suelo (Haramoto y Gallandt, 2004). Prueba de ello ha sido su efecto inhibidor en la germinación del jopo de girasol bajo condiciones controladas, y el ligero resultado positivo observado en condiciones de campo.

El estudio del alberjón como cultivo intercalar ha demostrado que esta especie se adapta perfectamente a nuestras condiciones meteorológicas y edáficas, y aunque el encharcamiento es uno de sus limitantes (Nadal *et al.*, 2012), su tolerancia a las bajas temperaturas (Siddique *et al.*, 2001) hace posible alcanzar no sólo el umbral mínimo de cobertura de suelo recomendado, sino niveles > 70 % cuando se realiza una siembra temprana o existen condiciones meteorológicas favorables (años agrícolas 2013/2014 y 2014/2015). A pesar de la mayor producción de biomasa asociada con dichos niveles de cobertura, el contenido de agua del suelo sólo se vio reducido en momentos aislados, especialmente durante el año 2014/2015 en Tomejil por la alta demanda de agua de esta especie (33.000 kg/ha). Sin embargo, también se han observado momentos aislados en los que la cobertura de suelo alcanzada por alberjón ha tenido efectos positivos con respecto a los tratamientos mínimo laboreo y siembra directa, la mayoría de ellos en el primer y segundo perfil de suelo (0-10 y 10-30 cm) durante los años agrícolas 2012/2013 y 2013/2014. Una ventaja adicional es la capacidad de fijación de N de las leguminosas (Perdigao *et al.*, 2012), así como su baja relación carbono/nitrógeno (C/N) y más rápida descomposición y liberación de nutrientes (Hasegawa *et al.*, 2000), que han conducido a una ligera mejora en el contenido de N y materia orgánica en la capa superior del suelo (0-10 cm), sobre todo durante la campaña agrícola 2014/2015 en Tomejil, cuando se produjo la mayor biomasa de alberjón. En el estudio conjunto de la rotación se observó un efecto positivo en los valores de N desde el principio hasta el final del período de estudio y una mayor contribución al mantenimiento de la fertilidad del suelo con respecto a los



tratamientos laboreo mínimo y siembra directa. Asimismo, nuestros resultados demostraron un efecto supresor de malas hierbas, característica que poseen algunas especies leguminosas (Isik *et al.*, 2009; Dorn *et al.*, 2013), así como también un efecto inhibitor en el control de jopo de girasol similar al efecto alelopático observado previamente en otras leguminosas bajo condiciones controladas (Chung y Miller, 1995; Rice, 2012). No obstante, al igual que ocurrió con la enmienda de mostaza blanca, los resultados obtenidos en condiciones de campo solo reflejaron un leve efecto positivo a corto plazo.

Nuestros resultados sugieren que las especies mostaza blanca y alberjón pueden incluirse con éxito en las rotaciones como cultivos intercalares de invierno, proporcionando así una alternativa sostenible de manejo de los cultivos herbáceos de secano. Sin embargo, el reto clave es conseguir la implementación de medidas medioambientales sostenibles que a la vez permitan un manejo agrónomicamente viable y sean económicamente rentables para los agricultores (Lithourgidis *et al.*, 2011). Esto implica que los cultivos principales en la rotación alcancen buenos rendimientos. A este respecto, los tratamientos de laboreo reducido (cultivos intercalares de mostaza blanca y alberjón así como mínimo laboreo) mostraron mejor calidad del trigo duro que en el sistema de siembra directa al final del período de estudio, especialmente los tratamientos con cultivos intercalares de mostaza blanca y alberjón en suelos arcillosos. En cuanto al girasol, los irregulares niveles de crecimiento y producción obtenidos a lo largo del periodo de estudio en todos los tratamientos nos han impedido sacar conclusiones claras en condiciones de campo, aunque el posible efecto negativo de los cultivos intercalares precedentes ha sido descartado basándonos en los resultados obtenidos en el ensayo en macetas realizado en condiciones controladas. Por lo tanto, son necesarios estudios futuros para determinar el posible efecto a largo plazo de la introducción de cultivos intercalares tanto sobre la conservación de recursos naturales en el agroecosistema, como sobre el desarrollo y rendimientos de los cultivos principales de la rotación, así como las causas reales que han podido afectar a la instalación y el desarrollo del girasol, ahondando de manera prioritaria en los posibles daños por fitotoxicidad de los residuos de herbicidas a base de sulfonilureas (Saavedra *et al.* 2015a, 2016a).

El uso generalizado de mezclas forrajeras de leguminosas y gramíneas para la producción de heno en condiciones de secano mediterráneas (Lithourgidis *et al.*, 2006), así como la demanda actual de proteína vegetal para la alimentación animal (Alizadeh y da Silva, 2013), han conducido al estudio de la viabilidad de la mezcla forrajera alberjón-avena negra (*Vicia narbonensis-Avena strigosa*) como alternativa de cultivo. Los resultados mostraron que, a pesar de la influencia de las condiciones meteorológicas en el desarrollo de cada especie, y por tanto de los rendimientos y el contenido en proteínas (Gierus *et al.*, 2012) de la mezcla así como la contribución de N realizada al sistema (Nyfeler *et al.*, 2011), ambas especies pueden constituir con éxito una mezcla forrajera (Nadal y Moreno, 2007; Flower *et al.*, 2012). El desarrollo del alberjón se vio afectado por la sequía acontecida durante el año agrícola 2011/2012 (< 300 mm) mientras que la avena negra fue sensible al encharcamiento (Urban *et al.*, 2015), mostrando un menor desarrollo durante el año 2012/2013. Sin embargo, las precipitaciones acontecidas durante el año 2013/2014 fueron similares al promedio de los últimos 10 años y ambas especies mostraron un buen crecimiento y desarrollo, además de mayores rendimientos de avena negra en cultivo mixto que en dosis pura, comportamiento similar al observado en otros estudios (Dhima *et al.*, 2013; Sadeghpour *et al.*, 2014). Además, la mezcla alberjón-avena negra siempre formó un forraje equilibrado para la dieta de ganado, con mayores rendimientos de materia seca y proteína cruda que los obtenidos por la mezcla estándar veza común-avena común y un mayor rendimiento de N (procedente tanto de fuentes simbióticas como no simbióticas) en comparación con el monocultivo de avena negra y similar al obtenido por la dosis pura de alberjón. Todo ello podría proporcionar ventajas económicas a los agricultores que decidan cultivar este tipo de sistemas de cultivo mixto en sus explotaciones (Lithourgidis *et al.*, 2011). Por otra parte, el forraje obtenido en todos los tratamientos cumplió con los requisitos de alta calidad (Van Saun, 2015) y fue consistente con los resultados obtenidos en otros estudios previos del sur de Europa (Caballero *et al.*, 1995; Lithourgidis *et al.*, 2006, 2007), especialmente las dosis 35:65 en años secos y las dosis 65:35 y 50:50 aquellos años con mayor pluviosidad en suelos francos y arcillosos, respectivamente.

Podemos, por tanto, concluir que este estudio proporciona información útil, desde el punto de vista ecológico y medioambiental, sobre cultivos intercalares y nuevos cultivos a incluir como alternativas sostenibles en la rotación tradicional trigo duro-girasol. Consecuentemente, la introducción de los cultivos intercalares mostaza blanca y alberjón podría ser una de opción viable empleada por el agricultor para mejorar su sistema de cultivo al repercutir en la mejora de la fertilidad y estructura del suelo al mismo tiempo que cumple con los requisitos medioambientales actuales de la PAC (tanto el componente verde como las medidas de condicionalidad) que le permitan percibir el pago directo completo. Por un lado, de acuerdo con el Reglamento (UE) nº 1307/2013, los cultivos intercalares de mostaza blanca y alberjón cultivados durante el período de tiempo en el que el suelo queda libre entre los dos cultivos principales (es decir, de octubre a marzo aproximadamente antes de la siembra del girasol), se podrían utilizar para cumplir con las normas de diversificación de cultivos en fincas > 30 hectáreas, al considerarse los cultivos de invierno y primavera como cultivos diferentes y al pertenecer ambas especies a las familias admisibles como cultivos. Por otro lado, en relación con el requisito de contar con SIE en explotaciones > 15 ha, una de las opciones elegidas por España fue cultivos fijadores de N (EC, 2014), por lo que el alberjón podría utilizarse igualmente para tal fin. Además, la mezcla alberjón-avena negra también puede ser una alternativa prometedora, especialmente en aquellas zonas de cultivo con ganadería asociada. Asimismo, puede ser de utilidad a partir de 2018 para justificar como SIE la mezcla de cultivos fijadores de N con otros cultivos (Reglamento Delegado (UE) 2017/1155). Entre las 10 opciones de SIE disponibles, en un principio España no seleccionó superficies con cultivos cubierta tipo “catch crop” o “green cover” con gramíneas, crucíferas, leguminosas o boragináceas. Sin embargo, es importante mencionar que 19 de los 28 estados miembros de la EU, muchos de ellos situados en el centro y sur de Europa, sí que optaron por incluir esta medida. Esto significa que si los cultivos intercalares probados han mostrado un buen desarrollo y beneficios positivos en el sur de España, es posible que en algunos de estos países también produzcan buenos resultados si se llegan a estudiar en sus condiciones locales. Al mismo tiempo, es importante citar que entre 2015 y 2017 algunos estados miembros han cambiado u ampliado las opciones de SIE inicialmente adoptadas, entre ellas España, al incluir la

mezcla de especies. Consecuentemente, este hecho, unido a las sucesivas reformas conocidas de la PAC desde sus inicios, nos hace pensar que los cultivos intercalares podrían ser una medida a considerar entre las SIE de España en futuras rectificaciones, más aún si nuestros resultados se confirman y se constata una mejora del sistema a largo plazo.

Por todo ello, los resultados obtenidos en este trabajo a corto plazo deben tenerse en cuenta como punto de referencia para futuras investigaciones de más largo alcance. El planteamiento que hemos seguido es especialmente importante ya que la correcta instalación y desarrollo de los cultivos invernales de ciclo corto son resultado de la interacción de un gran número de factores agronómicos, influenciados a su vez por condicionantes edafoclimáticos (Bodner *et al.*, 2010; Brennan y Boyd, 2012a; Ramírez-García *et al.*, 2015a). Además, todas las variables incluidas en nuestra investigación proporcionan una buena caracterización agronómica de las especies estudiadas, por lo que constituyen herramientas adecuadas de evaluación que pueden emplearse en estudios futuros.

Los esfuerzos a largo plazo deben centrarse en determinar la fecha de siembra y siega óptima de mostaza blanca y alberjón, para mejorar su instalación y crecimiento así como su manejo, especialmente en condiciones desfavorables (suelos pobres y encharcados o períodos de heladas o sequía). Este hecho sería especialmente importante en el caso de la mostaza blanca, ya que un adelanto de la fecha de siembra podría reducir los problemas de instalación observados y aumentar así los contenidos de humedad del suelo, sin reducir su efecto supresor de malas hierbas al asegurar el alcance de un estado fenológico final similar al obtenido en nuestro estudio (plena floración). A su vez, con respecto al control de malas hierbas, investigaciones futuras deberían analizar el efecto de los diferentes sistemas de manejo sobre las malas hierbas a lo largo de toda la rotación. Existen estudios experimentales que indican que los cultivos cubierta reducen la densidad de malas hierbas en los cultivos siguientes en rotación (Teasdale, 1996). Sin embargo, factores como las distintas prácticas de laboreo utilizadas ejercen una gran influencia (Brust *et al.*, 2011). Por lo tanto, sería interesante explorar los posibles mecanismos supresores implicados en los cultivos intercalares (competencia y alelopatía) y cuantificar la abundancia de malas hierbas en

trigo duro y girasol para cada uno de los sistemas de laboreo de conservación considerados en esta investigación.

Por otra parte, la determinación de la dosis de siembra más adecuada para los cultivos intercalares, así como el efecto de estrategias de manejo complementarias como la fertilización nitrogenada sobre ellos y la mezcla forrajera estudiada, podrían mejorar los resultados obtenidos. Es cierto que los resultados derivados del estudio del aporte de N realizado por la mezcla forrajera han mostrado que esta podría contribuir a mantener sistemas productivos de uso más eficiente del N al reducir las necesidades de fertilización. Por tanto, dada la relación entre el rendimiento de materia seca y la aportación de N realizada, la mejora del desarrollo de la avena negra en la mezcla pasa por incrementar la disponibilidad de N maximizando la proporción derivada de la fijación simbiótica del alberjón. En este sentido, sería necesario el estudio de la dosis de fertilización nitrogenada mínima que tuviera un efecto positivo sobre el desarrollo de estas especies, su producción de biomasa y el N residual disponible para los cultivos siguientes en rotación.

Con respecto a la mezcla forrajera, además de la fertilización nitrogenada, otros aspectos importantes del manejo del cultivo tales como las necesidades hídricas o el uso de herbicidas deberían también ser exploradas, con el fin de elaborar un itinerario técnico completo para los agricultores que decidan introducir este nuevo cultivo. Asimismo, el interés de esta mezcla forrajera radica en su introducción como alternativa al girasol en rotación con el trigo duro, por lo que las principales variables asociadas con el desarrollo y producción del cultivo principal también deberían ser estudiadas a largo plazo. Por último, la rentabilidad de estas nuevas alternativas en comparación con la de los distintos sistemas de laboreo de conservación utilizados debería ser analizada.

De confirmarse la rentabilidad y viabilidad de los cultivos intercalares y nuevos cultivos a largo plazo, esta investigación ofrecería una contribución importante y prometedora a la agricultura de cultivos herbáceos de secano del sur de España, brindando a los agricultores la posibilidad de integrar nuevas alternativas sostenibles y rentables en sus explotaciones, dando solución a los principales problemas agronómicos del sector al mismo tiempo que se cumplen los requerimientos normativos vigentes. De esta forma, y con todas las mejoras posteriores necesarias,

sería posible avanzar en la implantación de técnicas de agricultura de conservación viables, a la vez que se abordarían los principales problemas ambientales y agronómicos a nivel mundial y regional, contribuyendo a la consecución de los grandes retos actuales de la agricultura de seguridad y calidad alimentaria, mitigación del cambio climático, nutrición y salud animal así como la mejora de la competitividad, sostenibilidad y la productividad agraria.



**CAPÍTULO 6**  
**CONCLUSIONES GENERALES**





## 6 CONCLUSIONES GENERALES

El análisis y discusión de los resultados obtenidos nos permiten extraer las principales conclusiones de este trabajo:

- Los buenos porcentajes de cobertura alcanzados por los cultivos intercalares mostaza blanca (*Sinapis alba* subsp. *mairei*) y alberjón (*Vicia narbonensis* L.), así como la elevada producción de biomasa con una baja relación C/N, concretamente en alberjón, evidencian que ambas especies podrían ser eficaces en el control de la erosión y la conservación del suelo, además de excelentes abonos verdes que proporcionarían un aumento de la fertilidad de los mismos. En consecuencia, la introducción de estos cultivos supone una clara ventaja en relación a la conservación y mejora de los suelos con respecto al mínimo laboreo, pero además proporciona un mejor control de malas hierbas, por lo que podría ser una buena alternativa a la siembra directa sin detrimento de las mejoras alcanzadas por este sistema de manejo de suelo.
- Un correcto manejo de estos cultivos sería de gran importancia para el mantenimiento y conservación del agua en el suelo, especialmente en años de escasa pluviometría, si se quieren alcanzar en el cultivo de girasol contenidos de humedad similares a los obtenidos por los suelos en siembra directa. Por el contrario, con respecto al mínimo laboreo, tanto en años secos como lluviosos, el empleo de cultivos intercalares mejora los contenidos de agua en suelos francos y arcillosos en el cultivo de girasol. En el cultivo de trigo no ha habido efecto significativo de los cultivos intercalares, por lo que serán necesarios más años para detectar si su introducción afecta a los contenidos de agua en el suelo a largo plazo.
- Los suelos con cultivos intercalares, especialmente los de alberjón, tuvieron mayores contenidos de N y materia orgánica en el horizonte más superficial, lo cual pone de manifiesto que esta técnica de manejo de suelo supone una herramienta muy útil en el contexto de una agricultura preocupada por la conservación del agroecosistema, ya que favorecería la protección y mejora de la fertilidad del suelo. La gestión más eficiente de los flujos de N y la mejora del secuestro de carbono orgánico

por el suelo podrían además a largo plazo ayudar a reducir las emisiones de gases de efecto invernadero de CO<sub>2</sub> y N<sub>2</sub>O en el futuro.

- Los buenos rendimientos alcanzados por el trigo duro en los tratamientos en que se emplearon cultivos intercalares (similares a los de laboreo mínimo y siembra directa), unidos a la mejora de la calidad del grano, especialmente con alberjón, muestran que los beneficios medioambientales que aporta la introducción de cultivos intercalares no suponen una pérdida de producción con respecto a otros sistemas de manejo de suelo. No obstante, no hemos obtenido datos concluyentes del efecto de los cultivos intercalares sobre los rendimientos del girasol, aunque ensayos en condiciones controladas han confirmado que no hay una reducción en el crecimiento y desarrollo de las plántulas tras su empleo. El escaso desarrollo y baja producción del girasol se observó en todos los tratamientos, por ello es necesario validar en condiciones de campo los resultados obtenidos, para clarificar si el empleo de cultivos intercalares podría afectar a la producción del girasol y por tanto a la rentabilidad de la rotación.

- Los ensayos en condiciones controladas realizados para determinar el efecto de los cultivos intercalares sobre el control de jopo de girasol (*Orobanche cumana*), mostraron que tanto mostaza blanca como alberjón tienen un efecto inhibitor en la germinación *in vitro* de las semillas de jopo. En condiciones de campo, los resultados no mostraron diferencias con respecto a las parcelas de mínimo laboreo, pero ambos cultivos intercalares disminuyeron la incidencia de jopo en valores absolutos. Esto supone una ventaja frente a otros sistemas que habrá que valorar convenientemente también en términos de rentabilidad.

- El estudio de la viabilidad de la mezcla forrajera alberjón-avena negra (*Vicia narbonensis-Avena strigosa*) ha demostrado que dicha mezcla podría ser un cultivo alternativo prometedor para su inclusión en las rotaciones. Las dosis de siembra óptimas variaron en función de las diferentes condiciones meteorológicas del año y de las características edáficas de las fincas de estudio, pero la mezcla forrajera mostró un rendimiento en materia seca superior a la mezcla estándar veza común-avena común, similar contenido en proteínas y otros parámetros de calidad analizados. Además, mediante el uso del método de abundancia natural del isótopo estable <sup>15</sup>N, se observó

una contribución de N positiva en el sistema agrícola tanto por la fijación simbiótica de N realizada por el alberjón como por las interacciones mutuas alberjón-avena negra que estimularon la adquisición de N<sub>2</sub> fijado.

En síntesis, los cultivos intercalares así como la mezcla forrajera son alternativas viables y aptas para la agricultura de secano en el sur de España. Futuras investigaciones a largo plazo deben realizarse para mejorar su manejo así como su viabilidad económica. La demanda de alimentos seguros y la protección y conservación del medioambiente, así como la preocupación por el cambio climático y su impacto sobre el recurso suelo, exigen cambios urgentes en el manejo de las explotaciones. El empleo de técnicas de manejo de suelo conservacionistas, y la reducción en el uso de fitosanitarios, requiere la introducción de medidas culturales para suplir las carencias existentes y para equilibrar la balanza entre la rentabilidad y la protección al medioambiente. De igual modo, es necesaria la introducción de cultivos alternativos para responder a la necesidad de diversificación de las producciones que propicien tanto el equilibrio de los agroecosistemas como el de los precios y mercados.



**CAPÍTULO 7**  
**REFERENCIAS BIBLIOGRÁFICAS**



## 7 REFERENCIAS BIBLIOGRÁFICAS

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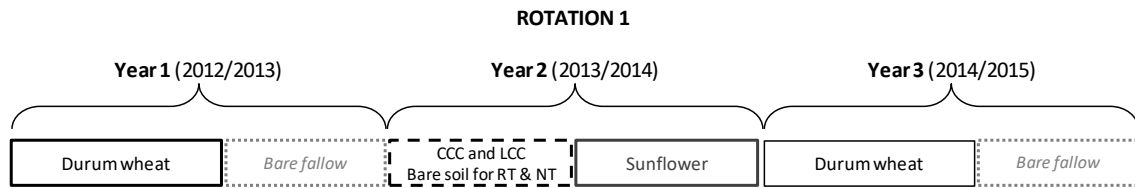
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## **APPENDIX 1**

### **DETAIL INFORMATION OF ROTATION 1**



# APPENDIX 1: DETAIL INFORMATION OF ROTATION 1



## Santa Cruz growing season 2012-2013: durum wheat

Temporal evolution of the durum wheat (*Triticum durum* cv. Amilcar) crop during the first year (2012/2013) of the wheat-sunflower-wheat crop rotation conducted at Santa Cruz under rain-fed Mediterranean conditions in Southern Spain. Temporal scale was expressed as the phenological growth stage and growing degree days (GDD) accumulated in different dates during all the growing cycle (days after sowing, DAS) each year and location with the last interval corresponding to the harvest time.

CROP	DATE	DAS	EVENT	PHENOLOGICAL STAGE	BBCH	GDD <sup>1</sup>
DURUM WHEAT(Durum wheat cv.Amilcar)	27/11/2012		NITROGEN BASE DRESSING DAP (N-P-K grade 18-46-0) 200 kg/ha			
	28/11/2012		HERBICIDE NT PLOTS Glyphosate 2,5 l/ha			
	29/11/2012	0	SOWING cv.Amilcar 360 seed/m <sup>2</sup>			
	20/12/2012	21	EMERGENCE	FIRST LEAF UNFOLDED	11	174,4
	27/12/2012	28	PLANT DENSITY	1 <sup>o</sup> -2 LEAVES UNFOLDED	11,12	254
	17/01/2013	49	FERTILISATION Urea (46% N) 150 kg/ha	BEGINNING OF TILLERING	21	432,1
	29/01/2013	61	CROP MONITORING	4 TILLERS DETECTABLE	24	550,85
	07/02/2013	70	HERBICIDE Biplay-33 45 kg/ha	END OF TILLERING	25,28	639,3
	27/02/2013	90	CROP MONITORING	BEGINNING OF STEM ELONGATION	28,30	826,05
	21/03/2013	112	FERTILISATION Urea (46% N) 100 kg/ha	STEM ELONGATION	32,35,37	1082,05
	11/04/2013	133	FUNGICIDE Lovit 1 l/ha	END OF BOOTING-BEG OF HEADING	49,53	1364,45
	22/04/2013	144	CROP MONITORING	HEADING-FLOWERING	58,61,65	1578,3
	07/05/2013	159	CROP MONITORING	ANTHESIS-WATERY RIPE	69,71,73	1824,7
	08/05/2013	160	WHEAT HEIGHT AND SPIKES NUMBER	ANTHESIS-WATERY RIPE	69,71,73	1847,05
	24/05/2013	176	CROP MONITORING	MEDIUM MILK-LATE MILK	75,77	2130,4
	10/06/2013	193	CROP MONITORING	EARLY DOUGH	83,85	2457,05
	25/06/2013	208	CROP MONITORING	HARD DOUGH-FULLY RIPE	87,89	2813,65
01/07/2013	214	WHEAT PRODUCTION AND GRAIN QUALITY	FULLY RIPE	89	2968,85	

<sup>1</sup> Phenological events were determined based on the maximum and minimum daily temperatures to calculate growing degree days (GDD) with a 0°C baseline temperature for wheat (Bauer *et al.* 1984) and based on the BBCH scale (Lancashire *et al.* 1991) of cereals during the entire trial.

### Santa Cruz growing season 2013-2014: cover crops + sunflower

Temporal evolution of the winter cover crops narbon bean (*Vicia narbonensis*) and white mustard (*Sinapis alba* subsp. *mairei*) preceding the sunflower crop (*Helianthus annuus* L.) during the second year (2013/2014) of the wheat-sunflower-wheat crop rotation conducted at Santa Cruz under rain-fed Mediterranean conditions in Southern Spain. Temporal scale was expressed as the phenological growth stage and growing degree days (GDD) accumulated in different dates during all the growing cycle (days after sowing, DAS) each year and location with the last interval corresponding to the harvest time.

CROP	DATE	DAS	EVENT	PHENOLOGICAL STAGE	BBCHC <sup>1</sup>	BBCHL <sup>2</sup>	GDD <sup>3</sup>
SUNFLOWER ( <i>Helianthus annuus</i> cv. P64LE29)	08/11/2013	0	SOWING CCC (15 kg/ha) and LCC (110 kg/ha)				
	10/12/2013	32	EMERGENCE LCC	PAIR OF SCALE LEAVES VISIBLE	0	9,10	289,4
	14/01/2014	67	EMERGENCE CCC	CCC COTYLEDONS UNFOLDED AND LCC 2-4 LEAVES UNFOLDED	10	12,14	552,95
	14/01/2014	67	PLANT DENSITY 1st date	CCC COTYLEDONS UNFOLDED AND LCC 2-4 LEAVES UNFOLDED	10	12,14	552,95
	21/01/2014	77	GROUND COVER 1st date	LCC 5-7 LEAVES UNFOLDED AND CCC 2-3 LEAVES UNFOLDED	12,13	15,17	621
	19/02/2014	103	PLANT DENSITY 2nd date	CCC 8-9 LEAVES UNFOLDED AND LCC 2-5 VISIBLELY EXTENDED INTERNODES	18,19	32,35	879,4
	19/02/2014	103	GROUND COVER 2nd date	CCC 8-9 LEAVES UNFOLDED AND LCC 2-5 VISIBLELY EXTENDED INTERNODES	18,19	32,35	879,4
	04/03/2014	116	GROUND COVER 3rd date	CCC 3-5 VISIBLY EXTENDED INTERNODES AND LCC FIRST INDIVIDUAL FLOWER BUDS VISIBLE	33,35	55	1021,15
	18/03/2014	130	GROUND COVER 4th date	CCC INDIVIDUAL FLOWER BUDS VISIBLE AND LCC FLOWERS OPEN 3 RACEMES PER PLANT	55,57	63	1213,45
	28/03/2014	140	GROUND COVER 5th date	CCC 30-50% OF FLOWERS ON MAIN RACEME OPEN AND LCC FULL FLOWERING- FLOWERING DECLINING	63,65	65,67	1339,75
	31/03/2014	143	CC HEIGHT, DM YIELD AND WEED IDENTIFICATION	CCC FULL FLOWERING- FLOWERING DECLINING AND LCC FLOWERING DECLINING-END OF FLOWERING	63,65	65,67	1378,95
	31/03/2014	143	HERBICIDE NT PLOTS Glyphosate (2,5 l/ha) + Oxifluorfen (0,1 l/ha)				1378,95
	01/04/2014	144	CC INCORPORATION				1396,2
	15/04/2014	0	SUNFLOWER SOWING cv. P64LE29 69-70·10 <sup>3</sup> plant/ha				
	30/04/2014	15	EMERGENCE	COTYLEDONS- 2 LEAVES UNFOLDED	10,12		171,9
	14/05/2014	29	PLANT DENSITY 1st date	5-8 LEAVES UNFOLDED	15-18		373,15

	<b>26/05/2014</b>	<b>41</b>	PLANT DENSITY 2st date	1-2 VISIBLY EXTENDED INTERNODES	<b>31-32</b>	<b>509,5</b>
	<b>04/06/2014</b>	<b>50</b>	CROP MONITORING	INFLORESCENCE EMERGENCE	<b>51,55</b>	<b>631,55</b>
	<b>25/08/2014</b>	<b>132</b>	SUNFLOWER HEIGHT	OVER RIPE-HARVESTED PRODUCT	<b>92,97</b>	<b>2132,25</b>
	<b>25/08/2014</b>	<b>132</b>	SUNFLOWER HEAD DIAMETER	OVER RIPE-HARVESTED PRODUCT	<b>92,97</b>	<b>2132,25</b>
	<b>25/08/2014</b>	<b>132</b>	SUNFLOWER PRODUCTION	OVER RIPE-HARVESTED PRODUCT	<b>92,97</b>	<b>2132,25</b>

<sup>1</sup> Phenological stage of white mustard cover crop (CCC) based on the BBCH scale (Lancashire *et al.* 1991) of oilseed rape.

<sup>2</sup> Phenological stage of narbon bean cover crop (CCC) based on the BBCH scale (Lancashire *et al.* 1991) of faba bean. After CC incorporation and sunflower sowing, the phenological stage refers to sunflower BBCH scale.

<sup>3</sup> Phenological events were determined based on the maximum and minimum daily temperatures to calculate growing degree days (GDD) with a 0°C baseline temperature for narbon bean (Mwanamwenge *et al.* 1999) and white mustard (Björkman *et al.* 2015), and a 7°C baseline temperature for sunflower (Qadir and Malik, 2016) and based on the BBCH scale (Lancashire *et al.* 1991) of oilseed rape, faba bean and sunflower, respectively, during the entire trial.



### Santa Cruz growing season 2014-2015: durum wheat

Temporal evolution of the durum wheat (*Triticum durum* cv. Amilcar) crop during the third year (2014/2015) of the wheat-sunflower-wheat crop rotation conducted at Santa Cruz under rain-fed Mediterranean conditions in Southern Spain. Temporal scale was expressed as the phenological growth stage and growing degree days (GDD) accumulated in different dates during all the growing cycle (days after sowing, DAS) each year and location with the last interval corresponding to the harvest time.

CROP	DATE	DAS	EVENT	PHENOLOGICAL STAGE	BBCH	GDD <sup>1</sup>
DURUM WHEAT ( <i>Durum wheat</i> cv. Amilcar)	15/10/2014		NITROGEN BASE DRESSING DAP (N-P-K grade 18-46-0) 200 kg/ha			
	17/10/2014		HERBICIDE NT PLOTS Glyphosate 2,5 l/ha			
	21/11/2014	0	SOWING cv. Amilcar 360 seed/m <sup>2</sup>			
	04/12/2014	13	EMERGENCE	FIRST LEAF UNFOLDED	11	182,65
	12/12/2014	21	PLANT DENSITY	1°-2 LEAVES UNFOLDED	12,13	242,05
	07/01/2015	47	CROP MONITORING	BEGINNING OF TILLERING	21,22	455,1
	15/01/2015	55	FERTILISATION Urea (46% N) 150 kg/ha	TILLERING	21,22,23	529,15
	29/01/2015	69	CROP MONITORING	4 TILLERS DETECTABLE	23,24	623,85
	11/02/2015	82	HERBICIDE Biplay + Traxos + Isomex+Herbenuron 45 kg/ha+ 200 cc/ha + 25 gr/ha+13 gr/ha	END OF TILLERING	25,28	733,5
	04/03/2015	103	CROP MONITORING	END OF TILLERING	27,30	978,25
	08/04/2015	138	FERTILISATION Urea (46% N) 100 kg/ha	STEM ELONGATION	32,35	1485,55
	14/04/2015	144	CROP MONITORING	HEADING	55,56	1587,85
	17/04/2015	147	FUNGICIDE Lovit 1 l/ha	HEADING	55,56	1642,25
	07/05/2015	167	CROP MONITORING	MEDIUM MILK-LATE MILK	75,77	2009,55
	14/05/2015	174	WHEAT HEIGHT AND SPIKES NUMBER	LATE MILK-EARLY DOUGH	80,83	2177,05
03/06/2015	194	WHEAT PRODUCTION AND GRAIN QUALITY	FULLY RIPE	89	2627,25	

<sup>1</sup> Phenological events were determined based on the maximum and minimum daily temperatures to calculate growing degree days (GDD) with a 0°C baseline temperature for wheat (Bauer *et al.* 1984) and based on the BBCH scale (Lancashire *et al.* 1991) of cereals during the entire trial.

### Tomejil growing season 2012-2013: durum wheat

Temporal evolution of the durum wheat (*Triticum durum* cv. Amilcar) crop during the first year (2012/2013) of the wheat-sunflower-wheat crop rotation conducted at Tomejil under rain-fed Mediterranean conditions in Southern Spain. Temporal scale was expressed as the phenological growth stage and growing degree days (GDD) accumulated in different dates during all the growing cycle (days after sowing, DAS) each year and location with the last interval corresponding to the harvest time.

CROP	DATE	DAS	EVENT	PHENOLOGICAL STAGE	BBCH	GDD <sup>1</sup>
DURUM WHEAT (Durum wheat cv. Amilcar)	05/12/2012		NITROGEN BASE DRESSING DAP (N-P-K grade 18-46-0) 200 kg·ha <sup>-1</sup>			
	06/12/2012		HERBICIDE NT PLOTS Glyphosate 2,5 l/ha			
	07/12/2012	0	SOWING cv. Amilcar 360 seed·m <sup>-2</sup>			
	20/12/2012	13	EMERGENCE	FIRST LEAF UNFOLDED	11	124,2
	27/12/2012	20	PLANT DENSITY	2-3 LEAVES UNFOLDED	12,13	206,55
	17/01/2013	41	FERTILISATION Urea (46% N) 150 kg·ha <sup>-1</sup>	BEGINNING OF TILLERING	21	406,4
	30/01/2013	54	CROP MONITORING	3-5 TILLERS DETECTABLE	23,25	542,85
	25/02/2013	80	HERBICIDE Biplay-33 45 kg·ha <sup>-1</sup>	END OF TILLERING	29	726,1
	27/02/2013	82	CROP MONITORING	BEGINNING OF STEM ELONGATION	29,3	742,8
	18/03/2013	101	FERTILISATION Urea (46% N) 100 kg·ha <sup>-1</sup>	STEM ELONGATION	35,37	971,45
	08/04/2013	122	FUNGICIDE Lovit 1 l·ha <sup>-1</sup>	BEG OF HEADING	52,57	1251,15
	24/04/2013	138	CROP MONITORING	HEADING-FLOWERING	58,61,65	1547,45
	09/05/2013	153	CROP MONITORING	ANTHESIS-WATERY RIPE	71,73	1813,55
	09/05/2013	153	WHEAT HEIGHT AND SPIKES NUMBER	ANTHESIS-WATERY RIPE	71,73	1813,55
	23/05/2013	167	CROP MONITORING	LATE MILK	77	2061,05
	11/06/2013	186	CROP MONITORING	EARLY DOUGH-SOFT DOUGH	83,85	2421,75
26/06/2013	201	CROP MONITORING	HARD DOUGH-FULLY RIPE	87,89	2774,35	
04/07/2013	209	WHEAT PRODUCTION AND GRAIN QUALITY	FULLY RIPE	89	2980	

<sup>1</sup> Phenological events were determined based on the maximum and minimum daily temperatures to calculate growing degree days (GDD) with a 0°C baseline temperature for wheat (Bauer *et al.* 1984) and based on the BBCH scale (Lancashire *et al.* 1991) of cereals during the entire trial.

### Tomejil growing season 2013-2014: cover crops + sunflower

Temporal evolution of the winter cover crops narbon bean (*Vicia narbonensis*) and white mustard (*Sinapis alba* subsp. *mairei*) preceding the sunflower crop (*Helianthus annuus* L.) during the second year (2013/2014) of the wheat-sunflower-wheat crop rotation conducted at Tomejil under rain-fed Mediterranean conditions in Southern Spain. Temporal scale was expressed as the phenological growth stage and growing degree days (GDD) accumulated in different dates during all the growing cycle (days after sowing, DAS) each year and location with the last interval corresponding to the harvest time.

CROP	DATE	DAS	EVENT	PHENOLOGICAL STAGE	BBCHC <sup>1</sup>	BBCHL <sup>2</sup>	GDD <sup>3</sup>
SUNFLOWER ( <i>Helianthus annuus</i> cv. P64LE29)	06/11/2013	0	SOWING CCC (15 kg/ha) and LCC (110 kg/ha)				
	10/12/2013	34	LCC EMERGENCE	PAIR OF SCALE LEAVES VISIBLE	0	9,12	382,3
	03/01/2014	58	CCC EMERGENCE	CCC COTYLEDONS- FIRST LEAF UNFOLDED AND LCC 2-4 LEAVES UNFOLDED	10,11	12,14	539,25
	10/01/2014	65	PLANT DENSITY 1st date	CCC 2-3 LEAVES UNFOLDED AND LCC 4-5 LEAVES UNFOLDED AND	11, 12,13	14,15	551,3
	21/01/2014	76	GROUND COVER 1st date	CCC 2-7 LEAVES UNFOLDED AND LCC 5-8 LEAVES UNFOLDED- BEGINNING OF SIDE SHOOT DEVELOPMENT	12,15, 17	15,18,21	630,15
	11/02/2014	97	PLANT DENSITY 2nd date	CCC 5-8 LEAVES UNFOLDED AND LCC 2-5 SIDE SHOTS DETECTABLE	15,18	22,25	844,05
	11/02/2014	97	GROUND COVER 2nd date	CCC 5-8 LEAVES UNFOLDED AND LCC 2-5 SIDE SHOTS DETECTABLE	15,18	22,25	844,05
	20/02/2014	106	GROUND COVER 3rd date	CCC FINAL LEAF DEVELOPMENT-FIRST SIDE SHOOTS AND LCC 5-9 SIDE SHOOTS UNFOLDED-STEM ELONGATION	18,21	25,33	947,9
	06/03/2014	120	GROUND COVER 4th date	CCC INFLORESCENCE EMERGENCE AND LCC FLOWER BUDS PRESENT	39,52	50	1111,2
	20/03/2014	134	CROP MONITORING	CCC 30% FLOWERS ON MAIN RACEME AND LCC FLOWERS OPEN 3-5 RACEMES	63	63,65	1303,9
	24/03/2014	138	GROUND COVER 5th date	CCC 30% FLOWERS ON MAIN RACEME AND LCC FLOWERS OPEN 3-5 RACEMES	63	63,65	1353,2
	24/03/2014	138	HERBICIDE NT PLOTS Glyphosate (2,5 l/ha) + Oxifluorfen (0,1 l/ha)				1353,2
	25/03/2014	139	CC HEIGHT, DM YIELD AND WEED IDENTIFICATION	CCC 40% OF FLOWERS ON MAIN RACEME OPEN AND LCC FLOWERS OPEN 3-FULL FLOWERING	65	63,67	1365,7
	26/03/2014	140	CC INCORPORATION				1377

<b>27/03/2014</b>	<b>0</b>	SUNFLOWER SOWING cv.P64LE29 69-70·10 <sup>3</sup> plant/ha			
<b>10/04/2014</b>	<b>14</b>	EMERGENCE	COTYLEDONS, 2 LEAVES UNFOLDED	<b>10,12</b>	<b>114,35</b>
<b>29/04/2014</b>	<b>33</b>	PLANT DENSITY 1st date	4-6 LEAVES UNFOLDED	<b>14-16</b>	<b>304,5</b>
<b>20/05/2014</b>	<b>54</b>	PLANT DENSITY 2st date	1-3 VISIBLY EXTENDED INTERNODES	<b>31-32-33</b>	<b>595,4</b>
<b>17/06/2014</b>	<b>82</b>	CROP MONITORING	FLOWERING	<b>61,65</b>	<b>980</b>
<b>21/08/2014</b>	<b>147</b>	SUNFLOWER HEIGHT	OVER RIPE-HARVESTED PRODUCT	<b>92,97</b>	<b>2140,35</b>
<b>21/08/2014</b>	<b>147</b>	SUNFLOWER HEAD DIAMETER	OVER RIPE-HARVESTED PRODUCT	<b>92,97</b>	<b>2140,35</b>
<b>21/08/2014</b>	<b>147</b>	SUNFLOWER PRODUCTION	OVER RIPE-HARVESTED PRODUCT	<b>92,97</b>	<b>2140,35</b>

<sup>1</sup> Phenological stage of white mustard cover crop (CCC) based on the BBCH scale (Lancashire *et al.* 1991) of oilseed rape.

<sup>2</sup> Phenological stage of narbon bean cover crop (CCC) based on the BBCH scale (Lancashire *et al.* 1991) of faba bean. After CC incorporation and sunflower sowing, the phenological stage refers to sunflower BBCH scale.

<sup>3</sup> Phenological events were determined based on the maximum and minimum daily temperatures to calculate growing degree days (GDD) with a 0°C baseline temperature for narbon bean (Mwanamwenge *et al.* 1999) and white mustard (Björkman *et al.* 2015), and a 7°C baseline temperature for sunflower (Qadir and Malik, 2016) and based on the BBCH scale (Lancashire *et al.* 1991) of oilseed rape, faba bean and sunflower, respectively, during the entire trial.

### Tomejil growing season 2014-2015: durum wheat

Temporal evolution of the durum wheat (*Triticum durum* cv. Amilcar) crop during the third year (2014/2015) of the wheat-sunflower-wheat crop rotation conducted at Tomejil under rain-fed Mediterranean conditions in Southern Spain. Temporal scale was expressed as the phenological growth stage and growing degree days (GDD) accumulated in different dates during all the growing cycle (days after sowing, DAS) each year and location with the last interval corresponding to the harvest time.

CROP	DATE	DAS	EVENT	PHENOLOGICAL STAGE	BBCH	GDD <sup>1</sup>
DURUM WHEAT( <i>Durum wheat</i> cv.Amilcar)	17/11/2014		NITROGEN BASE DRESSING DAP (N-P-K grade 18-46-0) 200 kg/ha			
	18/11/2014		HERBICIDE NT PLOTS Glyphosate 2,5 l/ha			
	19/11/2014		HERBICIDE Glyphosate 2,5 l/ha			
	12/12/2014	0	SOWING cv.Amilcar 360 seed/m <sup>2</sup>			
	02/01/2015	21	EMERGENCE	FIRST LEAF UNFOLDED	11	184,95
	08/01/2015	27	PLANT DENSITY	2-3 LEAVES UNFOLDED	12,13	297,55
	23/01/2015	42	FERTILISATION Urea (46% N) 150 kg/ha	TILLERING	21	358,6
	28/01/2015	47	CROP MONITORING	1-2 TILLERS DETECTABLE	21,22	397,8
	10/02/2015	60	CROP MONITORING	2-4 TILLERS DETECTABLE	22,24	504,55
	26/03/2015	104	HERBICIDE Biplay + Traxos + Biopower 45 g/ha + 200 cc/ha + 600 cc/ha	STEM ELONGATION	32,33	999,35
	10/04/2015	119	FUNGICIDE Lovit 1 l/ha	STEM ELONGATION	35,37	1249,55
	15/04/2015	124	FERTILISATION Urea (46% N) 100 kg/ha	STEM ELONGATION	35,37	1331,25
	05/05/2015	144	CROP MONITORING	HEADING	75,77	1679,05
	13/05/2015	152	WHEAT HEIGHT AND SPIKES NUMBER	ANTHESIS-WATERY RIPE	80,83	1854,7
	04/06/2015	174	WHEAT PRODUCTION AND GRAIN QUALITY	FULLY RIPE	89	2348,5

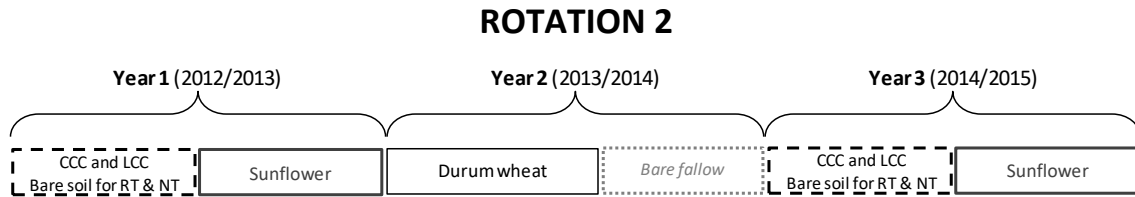
<sup>1</sup> Phenological events were determined based on the maximum and minimum daily temperatures to calculate growing degree days (GDD) with a 0°C baseline temperature for wheat (Bauer *et al.* 1984) and based on the BBCH scale (Lancashire *et al.* 1991) of cereals during the entire trial.

## **APPENDIX 2**

### **DETAIL INFORMATION OF ROTATION 2**



## APPENDIX 2: DETAIL INFORMATION OF ROTATION 2



### Santa Cruz growing season 2012-2013: cover crops + sunflower

Temporal evolution of the winter cover crops narbon bean (*Vicia narbonensis*) and white mustard (*Sinapis alba* subsp. *mairei*) preceding the sunflower crop (*Helianthus annuus* L.) during the first year (2012/2013) of the wheat-sunflower-wheat crop rotation conducted at Santa Cruz under rain-fed Mediterranean conditions in Southern Spain. Temporal scale was expressed as the phenological growth stage and growing degree days (GDD) accumulated in different dates during all the growing cycle (days after sowing, DAS) each year and location with the last interval corresponding to the harvest time.

CROP	DATE	DAS	EVENT	PHENOLOGICAL STAGE	BBCHC <sup>1</sup>	BBCHL <sup>2</sup>	GDD <sup>3</sup>
SUNFLOWER ( <i>Helianthus annuus</i> cv. Transol)	15/11/2012	0	SOWING CCC (15 kg/ha) and LCC (110 kg/ha)				
	01/12/2012	16	EMERGENCE	CCC COTYLEDONS UNFOLDED-FIRST LEAF UNFOLDED AND LCC PAIR OF SCALE LEAVES VISIBLE	11	10	191.3
	11/12/2012	26	PLANT DENSITY 1st date	CCC 1-2 LEAVES UNFOLDED AND LCC 2- 4 LEAVES UNFOLDED	11,12	12,14	270.6
	27/12/2012	42	PLANT DENSITY 2st date	CCC 4-5 LEAVES UNFOLDED AND LCC 5- 8 LEAVES UNFOLDED	14,15	15,18	433.75
	15/01/2013	61	GROUND COVER 1st date	CCC 5-9 LEAVES UNFOLDED AND LCC BEGINNING OF SIDE SHOOTS	15,19	20,22	591.4
	29/01/2013	75	GROUND COVER 2nd date	CCC 2-3 SIDE SHOOTS AND LCC 3-7 SIDE SHOOTS	22,23	23,27	730.6
	14/02/2013	91	GROUND COVER 3rd date	CCC 1-2 VISIBLY EXTENDED INTERNODES AND LCC 2-3 VISIBLY EXTENDED INTERNODES	31,32	32,33	877.25
	27/02/2013	104	GROUND COVER 4th date	CCC INDIVIDUAL FLOWER BUDS AND LCC FIRST INDIVIDUAL FLOWER BUDS VISIBLE	55,57	50,51	1005.8
	21/03/2013	126	GROUND COVER 5th date	CCC 30 ON MAIN RACEME OPEN AND LCC FLOWERS OPEN 3- 5 RACEMES PER PLANT	63	63,65	1261.8
	21/03/2013	126	CC HEIGHT, DM YIELD AND WEED IDENTIFICATION	CCC 30 ON MAIN RACEME OPEN AND LCC FLOWERS OPEN 3- 5 RACEMES PER PLANT	63	63,65	1261.8
	01/04/2013	137	CC INCORPORATION				1412.65



Appendix 2: Detail information of rotation 2

01/04/2013	137	HERBICIDE NT PLOTS Glyphosate (2,5 l/ha) + Oxifluorfen (0,1 l/ha)		1412.65
22/04/2013	158	SUNFLOWER SOWING cv. Transol 69-70·10 <sup>3</sup> plant/ha		
08/05/2013	16	EMERGENCE	COTYLEDONS- 2 LEAVES UNFOLDED	10,12 156.75
13/05/2013	21	PLANT DENSITY 1st date	FIRST PAIR OF LEAVES UNFOLDED	12 227
24/05/2013	32	PLANT DENSITY 2st date	4-5 LEAVES UNFOLDED	14,15 328.1
10/06/2013	49	CROP MONITORING	3-4 VISIBLY EXTENDED INTERNODES	34,35 537.75
14/06/2013	53	CROP MONITORING	6-8 VISIBLY EXTENDED INTERNODES	36,38 607.95
25/06/2013	64	CROP MONITORING	INFLORESCENCE EMERGENCE- BEGINNING OF FLOWERING	59,61 787.35
10/07/2013	79	CROP MONITORING	FLOWERING DECLINING-END OF FLOWERING	67-69 1094.35
24/07/2013	93	CROP MONITORING	DEVELOPMENT OF FRUIT	73-75 1372.95
20/08/2013	120	SUNFLOWER HEIGHT	OVER RIPE- HARVESTED PRODUCT	92,97 1924.55
20/08/2013	120	SUNFLOWER HEAD DIAMETER	OVER RIPE- HARVESTED PRODUCT	92,97 1924.55
20/08/2013	120	SUNFLOWER PRODUCTION	OVER RIPE- HARVESTED PRODUCT	92,97 1924.55

<sup>1</sup> Phenological stage of white mustard cover crop (CCC) based on the BBCH scale (Lancashire *et al.* 1991) of oilseed rape.

<sup>2</sup> Phenological stage of narbon bean cover crop (CCC) based on the BBCH scale (Lancashire *et al.* 1991) of faba bean. After CC incorporation and sunflower sowing, the phenological stage refers to sunflower BBCH scale.

<sup>3</sup> Phenological events were determined based on the maximum and minimum daily temperatures to calculate growing degree days (GDD) with a 0°C baseline temperature for narbon bean (Mwanamwenge *et al.* 1999) and white mustard (Björkman *et al.* 2015), and a 7°C baseline temperature for sunflower (Qadir and Malik, 2016) and based on the BBCH scale (Lancashire *et al.* 1991) of oilseed rape, faba bean and sunflower, respectively, during the entire trial.

### Santa Cruz growing season 2013-2014: durum wheat

Temporal evolution of the durum wheat (*Triticum durum* cv. Amilcar) crop during the second year (2013/2014) of the wheat-sunflower-wheat crop rotation conducted at Santa Cruz under rain-fed Mediterranean conditions in Southern Spain. Temporal scale was expressed as the phenological growth stage and growing degree days (GDD) accumulated in different dates during all the growing cycle (days after sowing, DAS) each year and location with the last interval corresponding to the harvest time.

CROP	DATE	DAS	EVENT	PHENOLOGICAL STAGE	BBCH	GDD <sup>1</sup>
DURUM WHEAT(Durum wheat cv.Amilcar)	25/11/2013		NITROGEN BASE DRESSING DAP (N-P-K grade 18-46-0) 200 kg/ha			
	27/11/2013	0	SOWING cv. Amilcar 360 seed/m <sup>2</sup>			
	14/12/2013	17	EMERGENCE	FIRST LEAF UNFOLDED	11	188,5
	09/01/2014	43	PLANT DENSITY	2-3 LEAVES UNFOLDED	12,13	292,6
	21/01/2014	55	CROP MONITORING	7-9 LEAVES UNFOLDED	17,2	437,3
	24/01/2014	58	FERTILISATION Urea (46% N) 150 kg/ha	BEGINNING OF TILLERING	20,21	468,05
	20/02/2014	85	CROP MONITORING	TILLERING	25,28	755,05
	27/02/2014	92	FERTILISATION Urea (46% N) 100 kg/ha	BEGINNING OF STEM ELONGATION	30	828,55
	04/03/2014	97	HERBICIDE Biplay-33 45 kg/ha	STEM ELONGATION	31,32	891,05
	06/03/2014	99	CROP MONITORING	STEM ELONGATION	31,32	918,35
	15/03/2014	108	FUNGICIDE Lovit 1 l/ha	STEM ELONGATION	32,37	1038,85
	20/03/2014	113	CROP MONITORING	BOOTING	39,4	1111,05
	29/04/2014	153	CROP MONITORING	HEADING	53,56	1729,2
	20/05/2014	174	WHEAT HEIGHT AND SPIKES NUMBER	SOFT DOUGH-HARD DOUGH	85,87	2301,5
	17/06/2014	202	WHEAT PRODUCTION AND GRAIN QUALITY	FULLY RIPE	89	2747,7

<sup>1</sup> Phenological events were determined based on the maximum and minimum daily temperatures to calculate growing degree days (GDD) with a 0°C baseline temperature for wheat (Bauer *et al.* 1984) and based on the BBCH scale (Lancashire *et al.* 1991) of cereals during the entire trial.

### Santa Cruz growing season 2014-2015: cover crops + sunflower

Temporal evolution of the winter cover crops narbon bean (*Vicia narbonensis*) and white mustard (*Sinapis alba* subsp. *mairei*) preceding the sunflower crop (*Helianthus annuus* L.) during the first year (2014/2015) of the wheat-sunflower-wheat crop rotation conducted at Santa Cruz under rain-fed Mediterranean conditions in Southern Spain. Temporal scale was expressed as the phenological growth stage and growing degree days (GDD) accumulated in different dates during all the growing cycle (days after sowing, DAS) each year and location with the last interval corresponding to the harvest time.

CROP	DATE	DAS	EVENT	PHENOLOGICAL STAGE	BBCHC <sup>1</sup>	BBCHL <sup>2</sup>	GDD <sup>3</sup>
SUNFLOWER ( <i>Helianthus annuus</i> cv. P64LE29)	23/10/2014	0	SOWING CCC (15 kg/ha) and LCC (110 kg/ha)				
	02/11/2014	10	EMERGENCE	CCC COTYLEDONS UNFOLDED-FIRST LEAF UNFOLDED AND LCC PAIR OF SCALE LEAVES VISIBLE	11	10	190,55
	20/11/2014	28	PLANT DENSITY 1st date	CCC 1-2 LEAVES UNFOLDED AND LCC 3- 4 LEAVES UNFOLDED	11,12	13,14	440
	12/12/2014	50	PLANT DENSITY 2st date	CCC 9 LEAVES UNFOLDED AND BEGINNING OF SIDE SHOOTS AND LCC BEGINNING OF SIDE SHOOTS	19-21	21	697,65
	12/12/2014	50	GROUND COVER 1st date	CCC 9 LEAVES UNFOLDED AND BEGINNING OF SIDE SHOOTS AND LCC BEGINNING OF SIDE SHOOTS	19-21	21	697,65
	07/01/2015	76	GROUND COVER 2nd date	CCC 2-5 SIDE SHOOTS AND LCC 3-7 SIDE SHOOTS	22-25	23,27	910,7
	29/01/2015	98	GROUND COVER 3rd date	CCC 1 VISIBLY EXTENDED INTERNODES AND CCL 3-5 VISIBLY EXTENDED INTERNODES	31	33-35	1079,45
	11/02/2015	111	GROUND COVER 4th date	CCC INDIVIDUAL FLOWER BUDS AND LCC FIRST INDIVIDUAL FLOWER BUDS VISIBLE	50	50,51	1189,1
	04/03/2015	132	GROUND COVER 5th date	CCC 30% OF FLOWERS ON MAIN RACEME OPEN-FLOWERING DECLINING AND LCC FLOWERS OPEN 3 RACEMES PER PLANT- FULL FLOWERING	63,67	63,65	1433,85
	04/03/2015	132	CC HEIGHT, DM YIELD AND WEED IDENTIFICATION	CCC 30% OF FLOWERS ON MAIN RACEME OPEN-FLOWERING DECLINING AND LCC FLOWERS OPEN 3 RACEMES PER PLANT- FULL FLOWERING	63,67	63,65	1433,85
	10/03/2015	138	CC INCORPORATION				1513,85
	10/03/2015	138	HERBICIDE NT PLOTS Glyphosate (2,5 l/ha) + Oxifluorfen (0,1 l/ha)				1513,85
	18/03/2015	146	SUNFLOWER SOWING cv. P64LE29				

69-70·10 <sup>3</sup> plant/ha						
14/04/2015	27	EMERGENCE	COTYLEDONS-2 LEAVES UNFOLDED	10,12	239,3	
07/05/2015	50	PLANT DENSITY 1st date	4-5 LEAVES UNFOLDED	14,15	500	
14/05/2015	57		1-2 VISIBLY EXTENDED INTERNODES	31,32	618,5	
16/06/2015	90	PLANT DENSITY 2st date	DISC FLORETS IN OUTER THIRD OF INFLORESCENCE IN BLOOM	63	1139,45	
17/06/2015	91		DISC FLORETS IN OUTER THIRD OF INFLORESCENCE IN BLOOM	63	1156	
23/07/2015	127	SUNFLOWER HEIGHT	OVER RIPE- HARVESTED PRODUCT	92,97	1943,05	
23/07/2015	127	SUNFLOWER HEAD DIAMETER	OVER RIPE- HARVESTED PRODUCT	92,97	1943,05	
23/07/2015	127	SUNFLOWER PRODUCTION	OVER RIPE- HARVESTED PRODUCT	92,97	1943,05	

<sup>1</sup> Phenological stage of white mustard cover crop (CCC) based on the BBCH scale (Lancashire *et al.* 1991) of oilseed rape.

<sup>2</sup> Phenological stage of narbon bean cover crop (CCC) based on the BBCH scale (Lancashire *et al.* 1991) of faba bean. After CC incorporation and sunflower sowing, the phenological stage refers to sunflower BBCH scale.

<sup>3</sup> Phenological events were determined based on the maximum and minimum daily temperatures to calculate growing degree days (GDD) with a 0°C baseline temperature for narbon bean (Mwanamwenge *et al.* 1999) and white mustard (Björkman *et al.* 2015), and a 7°C baseline temperature for sunflower (Qadir and Malik, 2016) and based on the BBCH scale (Lancashire *et al.* 1991) of oilseed rape, faba bean and sunflower, respectively, during the entire trial.

### Tomejil growing season 2012-2013: cover crops + sunflower

Temporal evolution of the winter cover crops narbon bean (*Vicia narbonensis*) and white mustard (*Sinapis alba* subsp. *mairei*) preceding the sunflower crop (*Helianthus annuus* L.) during the first year (2012/2013) of the wheat-sunflower-wheat crop rotation conducted at Tomejil under rain-fed Mediterranean conditions in Southern Spain. Temporal scale was expressed as the phenological growth stage and growing degree days (GDD) accumulated in different dates during all the growing cycle (days after sowing, DAS) each year and location with the last interval corresponding to the harvest time.

CROP	DATE	DAS	EVENT	PHENOLOGICAL STAGE	BBCHC <sup>1</sup>	BBCHL <sup>2</sup>	GDD <sup>3</sup>
SUNFLOWER ( <i>Helianthus annuus</i> cv. Transol)	27/11/2012	0	SOWING CCC (15 kg/ha) and LCC (110 kg/ha)				
	20/12/2012	23	EMERGENCE	CCC COTYLEDONS UNFOLDED-FIRST LEAF UNFOLDED AND LCC PAIR OF SCALE LEAVES VISIBLE	11	10	204.4
	27/12/2012	30	PLANT DENSITY 1st date	CCC 1-2 LEAVES UNFOLDED AND LCC 2- 4 LEAVES UNFOLDED	11,12	12,14	286.75
	09/01/2013	43	CROP MONITORING	CCC 3 LEAVES UNFOLDED AND LCC 4- 5 LEAVES UNFOLDED	13	14,15	405.85
	15/01/2013	49	PLANT DENSITY 2st date	CCC 5-7 LEAVES UNFOLDED AND LCC 5- 8 LEAVES UNFOLDED	15,17	15,18	464.15
	30/01/2013	64	GROUND COVER 1st date	CCC 5-9 LEAVES UNFOLDED AND LCC BEGINNING OF SIDE SHOOTS	15,19	20,21	623.05
	14/02/2013	79	GROUND COVER 2nd date	CCC FROM NO TO 2 SIDE SHOOTS AND LCC 2-7 SIDE SHOOTS	20,22	22,27	757.7
	27/02/2013	92	GROUND COVER 3rd date	CCC 1 VISIBLY EXTENDED INTERNODES AND LCC 2-3 VISIBLY EXTENDED INTERNODES	31	32,33	823
	18/03/2013	111	GROUND COVER 4th date	CCC FIRST PETALS VISIBLE AND LCC FIRST FLOWERS OPEN	59,61	59,60	1051.65
	08/04/2013	132	GROUND COVER 5th date	CCC 30% FLOWERS ON MAIN RACEME AND LCC FLOWERS OPEN 3 RACEMES-FULL FLOWERING	63	63,65	1331.35
	09/04/2013	133	CC HEIGHT, DM YIELD AND WEED IDENTIFICATION	CCC 30% FLOWERS ON MAIN RACEME AND LCC FLOWERS OPEN 3 RACEMES-FULL FLOWERING	63	63,65	1345.4
	10/04/2013	134	HERBICIDE NT PLOTS Glyphosate (2,5 l/ha) + Oxifluorfen (0,1 l/ha)				1360.5
	10/04/2013	134	CC INCORPORATION				1360.5
	24/04/2013	148	SUNFLOWER SOWING cv. Transol 69-70·10 <sup>3</sup> plant/ha				
	09/05/2013	15	EMERGENCE	FIRST PAIR OF LEAVES UNFOLDED		10,12	160,6
23/05/2013	29	PLANT DENSITY 1st date	3-4 LEAVES UNFOLDED		13,14	310,1	
11/06/2013	48	PLANT DENSITY 2st date	9 LEAVES UNFOLDED- BEGINNING OF STEM		19,3	537,8	

ELONGATION						
<b>26/06/2013</b>	<b>63</b>	CROP MONITORING	INFLORESCENCE EMERGENCE- BEGINNING OF FLOWERING	<b>51</b>	<b>785,4</b>	
<b>25/07/2013</b>	<b>92</b>	CROP MONITORING	DEVELOPMENT OF FRUIT	<b>71</b>	<b>1349,75</b>	
<b>22/08/2013</b>	<b>120</b>	SUNFLOWER HEIGHT	FLOWERING DECLINING-END OF FLOWERING	<b>92,97</b>	<b>1937,1</b>	
<b>22/08/2013</b>	<b>120</b>	SUNFLOWER HEAD DIAMETER	OVER RIPE- HARVESTED PRODUCT	<b>92,97</b>	<b>1937,1</b>	
<b>22/08/2013</b>	<b>120</b>	SUNFLOWER PRODUCTION	OVER RIPE- HARVESTED PRODUCT	<b>92,97</b>	<b>1937,1</b>	

<sup>1</sup> Phenological stage of white mustard cover crop (CCC) based on the BBCH scale (Lancashire *et al.* 1991) of oilseed rape.

<sup>2</sup> Phenological stage of narbon bean cover crop (CCC) based on the BBCH scale (Lancashire *et al.* 1991) of faba bean. After CC incorporation and sunflower sowing, the phenological stage refers to sunflower BBCH scale.

<sup>3</sup> Phenological events were determined based on the maximum and minimum daily temperatures to calculate growing degree days (GDD) with a 0°C baseline temperature for narbon bean (Mwanamwenge *et al.* 1999) and white mustard (Björkman *et al.* 2015), and a 7°C baseline temperature for sunflower (Qadir and Malik, 2016) and based on the BBCH scale (Lancashire *et al.* 1991) of oilseed rape, faba bean and sunflower, respectively, during the entire trial.

### Tomejil growing season 2013-2014: durum wheat

Temporal evolution of the durum wheat (*Triticum durum* cv. Amilcar) crop during the second year (2013/2014) of the wheat-sunflower-wheat crop rotation conducted at Tomejil under rain-fed Mediterranean conditions in Southern Spain. Temporal scale was expressed as the phenological growth stage and growing degree days (GDD) accumulated in different dates during all the growing cycle (days after sowing, DAS) each year and location with the last interval corresponding to the harvest time.

CROP	DATE	DAS	EVENT	PHENOLOGICAL STAGE	BBCH	GDD <sup>1</sup>
DURUM WHEAT(Durum wheat cv.Amilcar)	19/11/2013		NITROGEN BASE DRESSING DAP (N-P-K grade 18-46-0) 200 kg/ha			
	21/11/2013	0	SOWING cv. Amilcar 360 seed/m <sup>2</sup>			
	14/12/2013	23	EMERGENCE	FIRST LEAF UNFOLDED	11	188
	04/01/2014	44	PLANT DENSITY	2 LEAVES UNFOLDED	12	332,65
	14/01/2014	54	CROP MONITORING	3-7 LEAVES UNFOLDED	13,17	413,5
	17/01/2014	57	FERTILISATION Urea (46% N) 150 kg/ha	BEGINNING OF TILLERING	19, 20	421,7
	19/02/2014	90	CROP MONITORING	TILLERING	21, 23	739,95
	04/03/2014	103	CROP MONITORING	END OF TILLERING	27,29	881,7
	07/03/2014	106	FERTILISATION Urea (46% N) 100 kg/ha	BEGINNING OF STEM ELONGATION	30	924,3
	18/03/2014	117	CROP MONITORING	STEM ELONGATION	32,35	1074
	19/03/2014	118	FUNGICIDE Lovit 1 l/ha	STEM ELONGATION	32,35	1090,15
	24/03/2014	123	HERBICIDE Biplay-33 45 kg/ha	BOOTING	39,4	1156,35
	14/04/2014	144	CROP MONITORING	HEADING	53,54	1488,35
	14/05/2014	174	CROP MONITORING	MEDIUM MILK-LATE MILK	75,77	2081,15
	26/05/2014	186	WHEAT HEIGHT AND SPIKES NUMBER	SOFT DOUGH-HARD DOUGH	85,87	2301,5
	04/06/2014	195	WHEAT PRODUCTION AND GRAIN QUALITY	FULLY RIPE	89	2486,55

<sup>1</sup> Phenological events were determined based on the maximum and minimum daily temperatures to calculate growing degree days (GDD) with a 0°C baseline temperature for wheat (Bauer *et al.* 1984) and based on the BBCH scale (Lancashire *et al.* 1991) of cereals during the entire trial.

### Tomejil growing season 2014-2015: cover crops + sunflower

Temporal evolution of the winter cover crops narbon bean (*Vicia narbonensis*) and white mustard (*Sinapis alba* subsp. *mairei*) preceding the sunflower crop (*Helianthus annuus* L.) during the third year (2014/2015) of the wheat-sunflower-wheat crop rotation conducted at Tomejil under rain-fed Mediterranean conditions in Southern Spain. Temporal scale was expressed as the phenological growth stage and growing degree days (GDD) accumulated in different dates during all the growing cycle (days after sowing, DAS) each year and location with the last interval corresponding to the harvest time.

CROP	DATE	DAS	EVENT	PHENOLOGICAL STAGE	BBCHC <sup>1</sup>	BBCHL <sup>2</sup>	GDD <sup>3</sup>
SUNFLOWER ( <i>Helianthus annuus</i> cv. P64LE29)	20/10/2014	0	SOWING CCC (15 kg/ha) and LCC (110 kg/ha)				
	10/11/2014	21	EMERGENCE	CCC COTYLEDONS UNFOLDED-FIRST LEAF UNFOLDED AND LCC PAIR OF SCALE LEAVES VISIBLE	11	10	375.5
	21/11/2014	32	PLANT DENSITY 1st date	CCC 2 LEAVES UNFOLDED AND LCC 7-9 LEAVES UNFOLDED	12	17-19	541.75
	11/12/2014	52	PLANT DENSITY 2st date	CCC BEGINNING OF SIDE SHOOTS AND LCC BEGINNING OF SIDE SHOOTS	21	21-23	771.3
	11/12/2014	52	GROUND COVER 1st date	CCC BEGINNING OF SIDE SHOOTS AND LCC BEGINNING OF SIDE SHOOTS	21	21-23	771.3
	08/01/2015	80	GROUND COVER 2nd date	CCC 2-5 SIDE SHOOTS AND LCC 3-7 SIDE SHOOTS	22,25	23,27	1013.4
	28/01/2015	100	GROUND COVER 3rd date	CCC 1-2 VISIBLY EXTENDED INTERNODES AND LCC 3-5 VISIBLY EXTENDED INTERNODES	31,32	33-35	1176.65
	10/02/2015	113	GROUND COVER 4th date	CCC INDIVIDUAL FLOWER BUDS AND LCC FIRST INDIVIDUAL FLOWER BUDS VISIBLE	39,5	50,51	1283.4
	02/03/2015	133	GROUND COVER 5th date	CCC 30 % OF FLOWERS ON MAIN RACEME OPEN- FLOWERING DECLINING AND LCC FLOWERS FULL FLOWERING- FLOWERING DECLINING	63,67	65,67	1486.85
	02/03/2015	133	CC HEIGHT, DM YIELD AND WEED IDENTIFICATION	CCC 30% OF FLOWERS ON MAIN RACEME OPEN AND LCC FLOWERS FULL FLOWERING- FLOWERING DECLINING	63,67	65,67	1486.85
	11/03/2015	142	HERBICIDE NT PLOTS Glyphosate (2,5 l/ha) + Oxifluorfen (0,1 l/ha)				1611.35
	11/03/2015	142	CC INCORPORATION				1611.35
	16/03/2015	147	SUNFLOWER SOWING cv.P64LE29 69-70·10 <sup>3</sup> plant/ha				
	13/04/2015	175 (28)	BIRD ATTACKS SUNFLOWER RESOWING cv.P64LE29 69-70·10 <sup>3</sup> plant/ha				210.25



Appendix 2: Detail information of rotation 2

	05/05/2015	50		COTYLEDONS (RESOWING) AND 5 LEAVES UNFOLDED (SOWING)	10,15	439.25
			EMERGENCE			
	13/05/2015	58		3-7 LEAVES UNFOLDED	13-17	558.9
			CROP MONITORING			
	20/05/2015	65	PLANT DENSITY 1st date	FROM 4 LEAVES UNFOLDED TO 1 VISIBLY EXTENDED INTERNODES	14-15,31	665.5
	22/06/2015	98	PLANT DENSITY 2st date	FROM INFLORESCENCE TO DISC FLORETS IN OUTER THIRD OF INFLORESCENCE IN BLOOM	51,63	1190.95
	20/07/2015	126	SUNFLOWER HEIGHT	OVER RIPE-HARVESTED PRODUCT	92,97	1785.2
	20/07/2015	126	SUNFLOWER HEAD DIAMETER	OVER RIPE-HARVESTED PRODUCT	92,97	1785.2
	20/07/2015	126	SUNFLOWER PRODUCTION	OVER RIPE-HARVESTED PRODUCT	92,97	1830.3

<sup>1</sup> Phenological stage of white mustard cover crop (CCC) based on the BBCH scale (Lancashire *et al.* 1991) of oilseed rape.

<sup>2</sup> Phenological stage of narbon bean cover crop (CCC) based on the BBCH scale (Lancashire *et al.* 1991) of faba bean. After CC incorporation and sunflower sowing, the phenological stage refers to sunflower BBCH scale.

<sup>3</sup> Phenological events were determined based on the maximum and minimum daily temperatures to calculate growing degree days (GDD) with a 0°C baseline temperature for narbon bean (Mwanamwenge *et al.* 1999) and white mustard (Björkman *et al.* 2015), and a 7°C baseline temperature for sunflower (Qadir and Malik, 2016) and based on the BBCH scale (Lancashire *et al.* 1991) of oilseed rape, faba bean and sunflower, respectively, during the entire trial.

## **APPENDIX 3**

# **WEEDS EVOLUTION AND IDENTIFICATION**



## APPENDIX 3: WEEDS EVOLUTION AND IDENTIFICATION

### ABOVE-GROUND WEED FRESH BIOMASS EVOLUTION IN THE ROTATION 2

ANOVA table and general, treatments and year means for above-ground weed fresh biomass (WFM, Kg/ha) in Santa Cruz and Tomejil.

	WFM (kg/ha)	
	Santa Cruz	Tomejil
<b>ANOVA table</b>		
<b>Treatments<sup>1</sup> (TR)</b>	3.52*	6.63**
<b>Year<sup>2</sup> (Y)</b>	1.10 n.s.	5.95**
<b>TR × Y</b>	1.74 n.s.	1.36 n.s.
<b>General means</b>	3.214,5	3.678,7
<b>TR means</b>		
CCC	2.259,1 b	2.799 b
LCC	2.906,8 ab	2.587 b
RT	4.477,6 a	5.246,1 a
NT	-	4.082,4 ab
<b>Year means</b>		
<b>Year 1</b>	2.921,2 a	4.625,1 a
<b>Year 2</b>	2.777,4 a	3.802,9 ab
<b>Year 3</b>	3.944,9 a	2.608,0 b

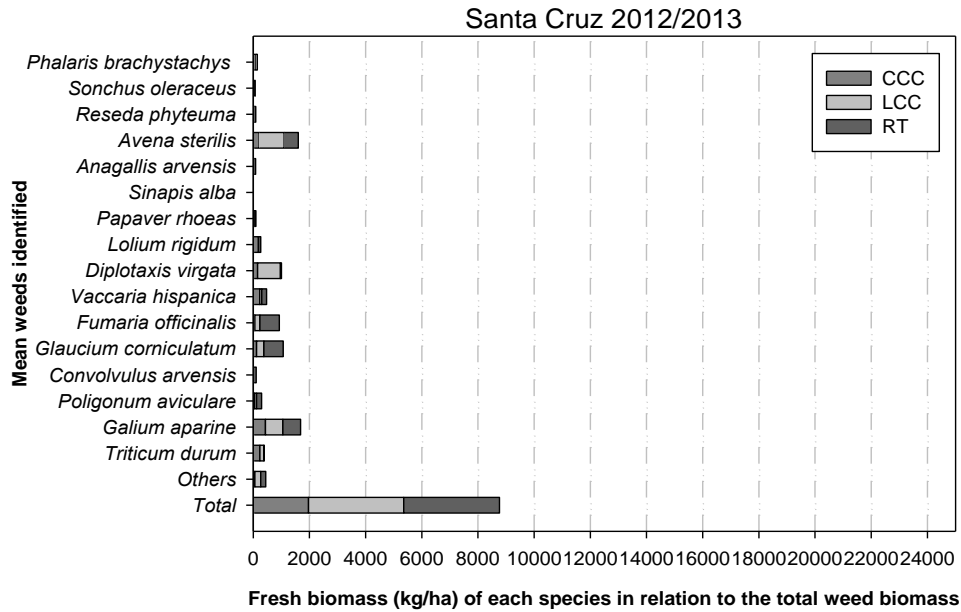
<sup>1</sup>Treatments: CCC, LCC and RT in Santa Cruz and CCC, LCC RT and NT in Tomejil

<sup>2</sup>Year: year 1 (2012/2013), year 2 (2013/2014), year 3 (2014/2015)

<sup>3</sup> $p < 0.05^*$ ,  $p < 0.01^{**}$ ,  $p < 0.001^{***}$ , n.s.: not significant

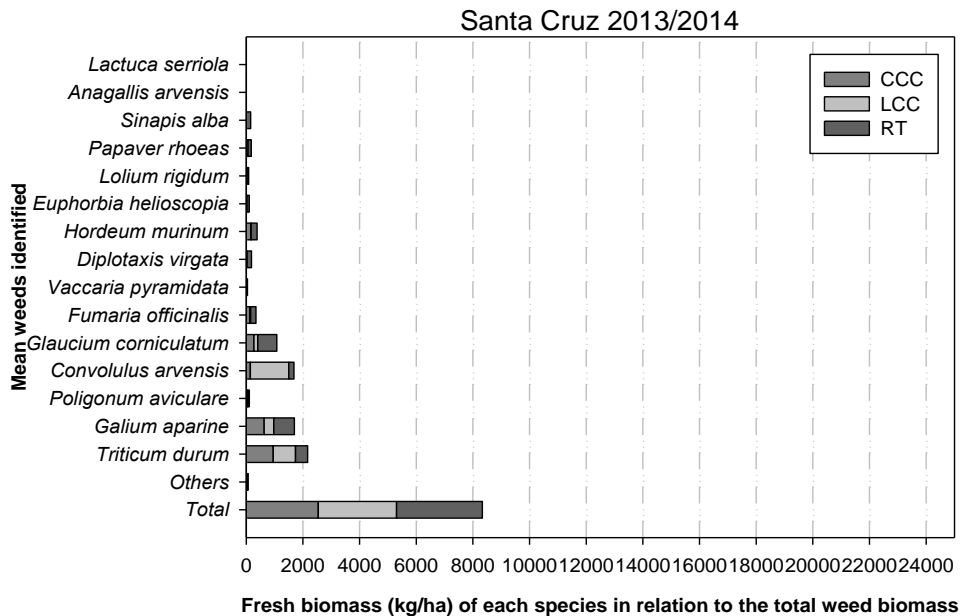
## WEED IDENTIFICATION AND CATALOGUING IN SANTA CRUZ

### Growing season 2012-2013 (Rotation 2)



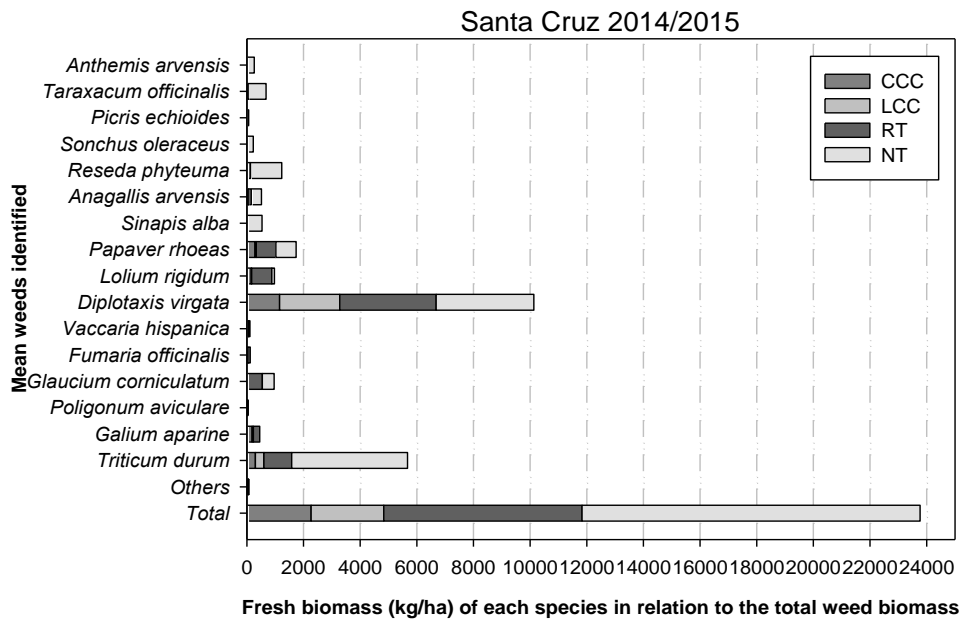
Mean weeds identified expressed as fresh biomass (kg/ha) of each species in relation to the total weed biomass per treatment in Santa Cruz during 2012/2013 (rotation 2).

### Growing season 2013-2014 (Rotation 1)



Mean weeds identified expressed as fresh biomass (kg/ha) of each species in relation to the total weed biomass per treatment in Santa Cruz during 2013/2014 (rotation 1).

**Growing season 2014-2015 (Rotation 2)**



Mean weeds identified expressed as fresh biomass (kg/ha) of each species in relation to the total weed biomass per treatment in Santa Cruz during 2014/2015 (rotation 2).

CATALOGUING WEEDS IN SANTA CRUZ (CÓRDOBA)				
Year of occurrence	Family and gender	Species	Biological type	Common name
<b>PAPAVERACEAE</b>				
1,2,3	Papaver	rhoeas	Te-An	Amapola (common poppy, red poppy)
1,2,3	Glaucium	corniculatum	Te-An	Hierba lagartera, amapola cornuda, amapola loca (blackspot hornpoppy or red horned-poppy)
<b>FUMARIACEAE</b>				
1,2,3	Fumaria	officinalis	Te-An	Zapatitos, conejitos, fumaria (common fumitory, drug fumitory, earth smoke)
<b>CARIOPHYLLACEAE</b>				
1,2,3	Vaccaria	pyramidata	Te-An	Collejón (cowherb, cowcockle, cow basil)
<b>POLYGONACEAE</b>				
1,2,3	Polygonum	aviculare	Te-Tcr-An	Ciennudos (common knotgrass, lowgrass)
<b>BRASSICACEAE = CRUCIFERAE</b>				
1,2,3	Diploaxis	virgata	Te-An	Jaramago (sand mustard)
1,2,3	Sinapis	alba	Te-An	Mostaza blanca (white mustard)
<b>RESEDACEAE</b>				
1,3	Reseda	phyteuma	Te-He-An	Sosieganiños (rampion mignonette)
<b>PRIMULACEAE</b>				
1,2,3	Anagallis	arvensis	Te-An	Murajes (Scarlet pimpernel)
<b>EUPHORBIACEAE</b>				
2	Euphorbia	helioscopia	Te-An	Lechetreznas (sun spurge)
<b>CONVOLVULACEAE</b>				
1,2	Convolvulus	arvensis	Gr-Per	Corregüela, campanitas (field bindweed)
<b>RUBIACEAE</b>				
1,2,3	Galium	aparine	Tcr-Te-An	Amor del hortelano (goosegrass)
<b>ASTERACEAE = COMPOSITAE</b>				
3	Anthemis	arvensis	Te-An	Manzanilla bastarda, manzanilla silvestre (corn chamomile, field chamomile)
2	Lactuca	serriola	Te-Hbi-An-Bi	Lechuga silvestre, lechugueta (prickly lettuce, milk thistle)
1,3	Sonchus	oleraceus	Te-An	Cerraja (common sowthistle)
3	Taraxacum	officinalis	Hro-Per	Diente de león (common dandelion)
3	Picris	echioides	Te-Hbi-An-Bi	Raspasayos, rompesayos (bristly oxtongue)
<b>POACEAE</b>				
1,2,3	Lolium	rigidum	Te-An	Vallico, ray-grass (annual ryegrass)
1	Avena	sterilis	Te-An	Avena loca (wild oat, sterile oat)
1	Phalaris	brachystachys	Te-An	Alpiste, triguera (confused canary-grass)
2	Hordeum	murinum	Te-An	Cebadilla (wall barley, false barley)
1,2,3	Triticum	durum	Te-An	Trigo duro (durum wheat)

Source: own elaboration based on Saavedra and Pastor (2002)

\*Te: Erect therophyte; Tcr: creeping therophyte

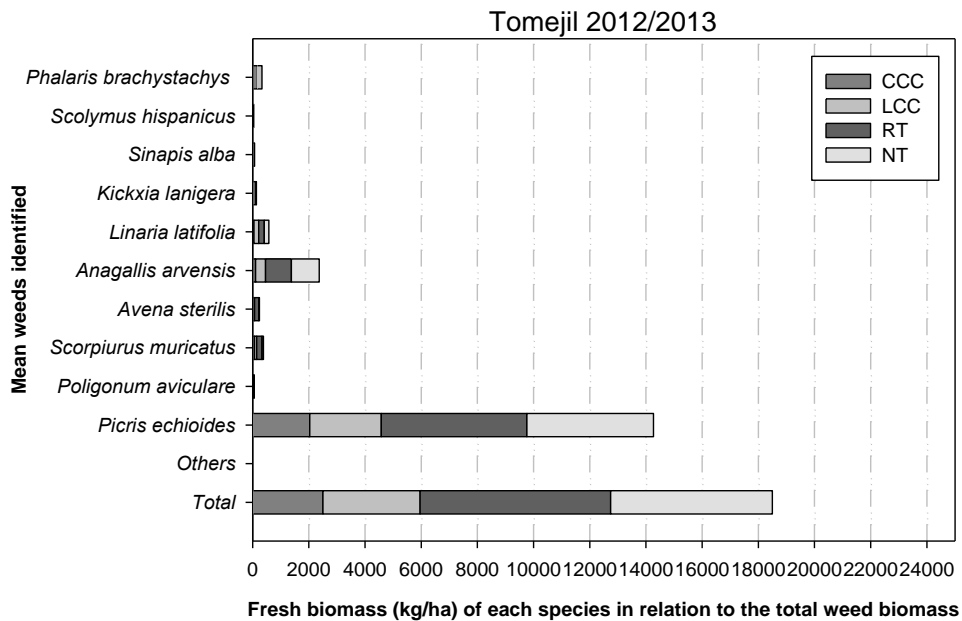
\*He: Erect hemicryptophyte; Hbi: Biannual hemicryptophyte; Hro: Basal rosette hemicryptophyte

\*Gr: Geophyte with rhizome

\*Plant duration. An: Annual; Per: Perennial plant; Bi: Biannual

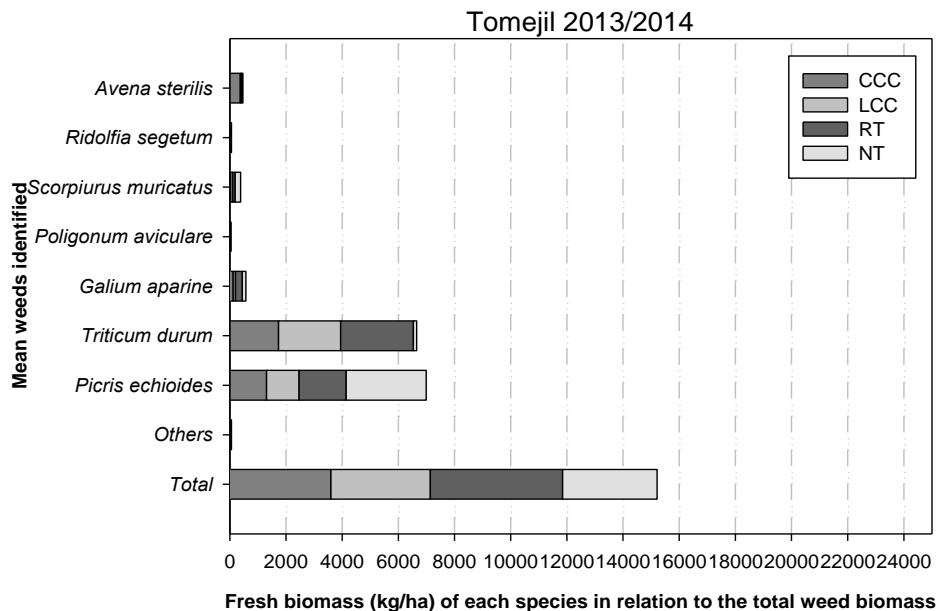
## WEED IDENTIFICATION AND CATALOGUING IN TOMEJIL

### Growing season 2012-2013 (Rotation 2)



Mean weeds identified expressed as fresh biomass (kg/ha) of each species in relation to the total weed biomass per treatment in Tomejil during 2012/2013 (rotation 2).

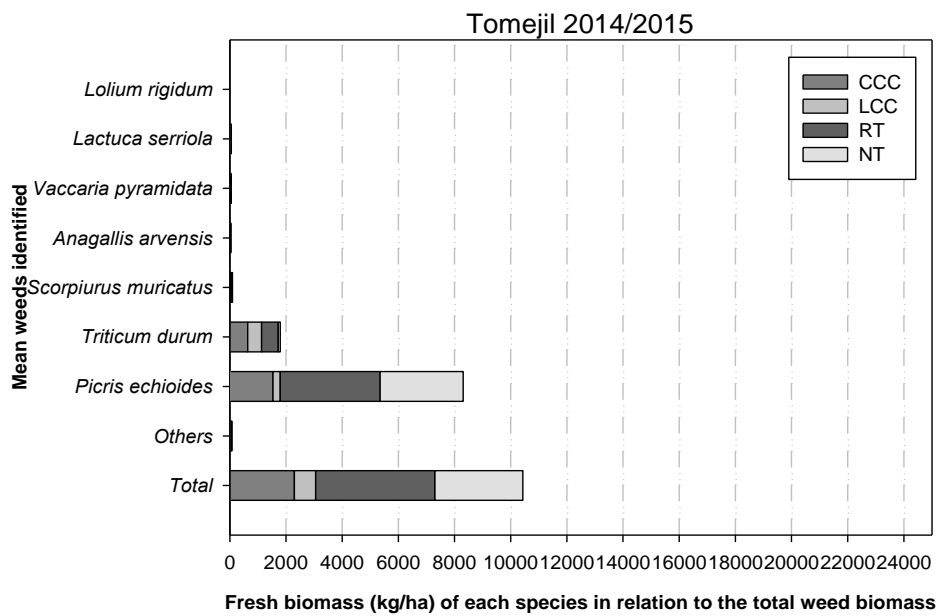
### Growing season 2013-2014 (Rotation 1)



Mean weeds identified expressed as fresh biomass (kg/ha) of each species in relation to the total weed biomass per treatment in Tomejil during 2013/2014 (rotation 1).



**Growing season 2014-2015 (rotation 2)**



Mean weeds identified expressed as fresh biomass (kg/ha) of each species in relation to the total weed biomass per treatment in Tomejil during 2014/2015 (rotation 2).

CATALOGUING WEEDS IN TOMEJIL (SEVILLA)				
Year of occurrence	Family and gender	Species	Biological type	Common name
<b>CARIOPHYLACEAE</b>				
3	Vaccaria	pyramidata	Te-An	Collección (cowherb, cowcockle, cow basil)
<b>POLYGONACEAE</b>				
1,2	Polygonum	aviculare	Te-Tcr-An	Ciennudos (common knotgrass, lowgrass)
<b>BRASSICACEAE = CRUCIFERAE</b>				
1	Sinapis	alba	Te-An	Mostaza blanca (white mustard)
<b>PRIMULACEAE</b>				
1,3	Anagallis	arvensis	Te-An	Murajes (Scarlet pimpernel)
<b>FABACEAE = LEGUMINOSAE</b>				
1,2,3	Scorpiurus	muricatus	Tcr-An	Lengua de oveja (scorpion's-tails)
<b>APIACEAE = UMBELLIFERAE</b>				
2	Ridolfia	segetum	Te-An	Nerdo (false fennel, corn parsley, false caraway)
<b>SCROPHULARIACEAE</b>				
1	Linaria	latifolia	Te-An	Conejitos, gallos (toadflax)
1	Kickxia	lanigera	Tcr-An	- (Cancerworts, fluellins)
<b>RUBIACEAE</b>				
2	Galium	aparine	Tcr-Te-An	Amor del hortelano, rabula (goosegrass)
<b>ASTERACEAE = COMPOSITAE</b>				
3	Lactuca	serriola	Te-Hbi-An-Bi	Lechuga silvestre, lechugueta (prickly lettuce, milk thistle)
1,2,3	Picris	echioides	Te-Hbi-An-Bi	Raspasayos, rompesayos (bristly oxtongue)
1	Scolymus	hispanicus	Hbi-Bi-Per	Cardillos (common golden thistle, Spanish oyster thistle)
<b>POACEAE</b>				
3	Lolium	rigidum	Te-An	Vallico, ray-grass (annual ryegrass)
1,2	Avena	sterilis	Te-An	Avena loca (wild oat, sterile oat)
1	Phalaris	brachystachys	Te-An	Alpiste, triguera (confused canary-grass)
2,3	Triticum	durum	Te-An	Trigo duro (durum wheat)

Source: own elaboration based on Saavedra and Pastor (2002)

\*Te: Erect therophyte; Tcr: creeping therophyte

\*He: Erect hemicryptophyte; Hbi: Biannual hemicryptophyte; Hro: Basal rosette hemicryptophyte

\*Gr: Geophyte with rhizome

\*Plant duration. An: Annual; Per: Perennial plant; Bi: Biannual



**APPENDIX 4**  
**DURUM WHEAT GRAIN QUALITY**



## APPENDIX 4: DURUM WHEAT GRAIN QUALITY

The Regulation on Domestic Wheat Quality Standardization was developed with the aim to improve farmers' competitiveness through standardization. The Ministry of Agriculture and Fisheries, Food and Environment (MAPAMA) along with other agents within the grain sector identified the lack of standardization as a critical factor that was discouraging the use of wheat varieties more adapted to the processing industry requirements, certified seeds and other agricultural inputs and technology that would improve the quality of domestic wheat production.

Unlike some other European Member States, Spain is a net importer of food and feed-quality wheat. Most of Spain's main suppliers, including France, the United Kingdom and the United States, use similar classification standards that facilitate a more transparent and efficient trade. Foreign countries' classification systems have been considered as a reference to create Spain's wheat standardization.

The ultimate goal of the quality standardization of Spanish wheat is that objective typified differences in quality result in price differences that, driven by supply and demand, would be an advantage to farmers in terms of added value of their production. The Royal Decree on Wheat Quality Standardization is mandatory, but no fines are imposed in the event of non-compliance.

Protein content (PC), specific weight (HLW) and vitreosity (V) are the parameters considered to classify durum wheat into four categories (refer to the table above) according to the Royal Decree 190/2013 of March 15, 2013, that modifies the Royal Decree 1615/2010 of December 7, 2011, on Domestic Wheat Quality Implementation. The Royal Decree 190/2013 was published in the Official Spanish Gazette on March 15, 2013 and included the Global Quality Index (GQI) as a theoretical potential classification under the standardization system linked to the durum wheat variety. Producers, buyers and distributors should consider that the genetics of the wheat variety planted can only partially determine the final quality of the grain. Final quality characteristics will strongly depend on the agro-climatic conditions under which the crop is grown. Gluten quality index (GI) and yellow pigment content (YC), are optional parameters to determine the durum wheat high quality subgroup (high gluten quality and high color) within categories 1 and 2. The determination of all the physico-

chemical durum wheat parameters was determined by Near Infra Red Spectroscopy (NIRS) technology.

**Durum wheat quality group categories according to the protein content (PC), specific weight (HLW) and vitreosity (V) quality ranges includes in the Royal Decree 190/2013 of March 15, 2013.**

Groups	PC	HLW	V	GI	YC	GQI *
	%	kg/hl	%	Unit	Unit	
1	≥ 13	≥ 80	> 80	≥ 75	≥ 19	100 ≤ GQI < 105
2	≥ 12	≥ 78	> 75	≥ 75	≥ 19	
3	≥ 11	≥ 77	> 60			
4	Other than 1,2 or 3					

Source: Royal Decree 190/2013

\* GQI theoretical classification. GQI was calculated as a percentage of the mean GQI for the control cultivars (Amilcar, Avispa, Gallareta and Simeto) equaled to 100 with the formula: (PC×40%) + (GI ×30%) + (YI×20%) + (HLW×10%)

### DURUM WHEAT QUALITY IN SANTA CRUZ

Durum wheat final quality group category (QC) according to the protein content (PC), specific weight (HLW) and vitreosity (V) quality ranges includes in the Royal Decree 190/2013 of March 15, 2013, for each treatment during the different growing seasons (2012/2013 and 2014/2015 for rotation 1 and 2013/2014 for rotation 2) in Santa Cruz.

	Santa Cruz											
	Rotation 1 (DW-SF-DW)								Rotation 2 (SF-DW-SF)			
	Year 1 (2012/2013)				Year 3 (2014/2015)				Year 2 (2013/2014)			
	PC	HLW	V	QC	PC	HLW	V	QC	PC	HLW	V	QC
CCC	4	1	2	4	1	1	1	1	3	1	1	3
LCC	4	1	3	4	1	1	1	1	3	1	1	3
RT	4	1	3	4	1	1	1	1	3	1	1	3
NT	4	1	3	4	2	1	1	2	2	1	1	2

### DURUM WHEAT QUALITY IN TOMEJIL

Durum wheat final quality group category (QC) according to the protein content (PC), specific weight (HLW) and vitreosity (V) quality ranges includes in the Royal Decree 190/2013 of March 15, 2013, for each treatment during the different growing seasons (2012/2013 and 2014/2015 for rotation 1 and 2013/2014 for rotation 2) in Tomejil.

	Santa Cruz											
	Rotation 1 (DW-SF-DW)								Rotation 2 (SF-DW-SF)			
	Year 1 (2012/2013)				Year 3 (2014/2015)				Year 2 (2013/2014)			
	PC	HLW	V	QC	PC	HLW	V	QC	PC	HLW	V	QC
CCC	3	1	2	3	1	1	1	1	2	1	1	2
LCC	3	1	2	3	1	1	1	1	1	1	1	1
RT	4	1	2	4	2	1	1	2	2	1	1	2
NT	4	1	4	4	2	1	1	2	2	1	1	2

## **APPENDIX 5**

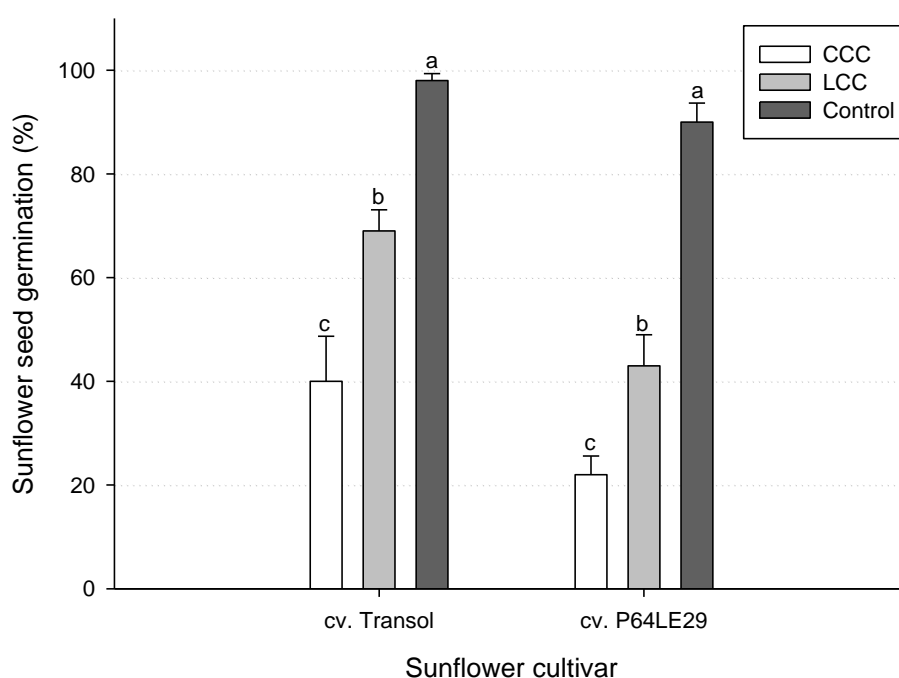
# **EFFECT OF COVER CROPS ON SUNFLOWER SEED GERMINATION AND SEEDLING GROWTH (EXPERIMENT A.2)**





## APPENDIX 5: EFFECT OF COVER CROPS ON SUNFLOWER SEED GERMINATION AND SEEDLING GROWTH (EXPERIMENT A.2.)

### EFFECT OF WHITE MUSTARD AND NARBON BEAN ON SUNFLOWER SEED GERMINATION



Percentage of seed germination of each sunflower cultivar (cv. Transol and cv. P64LE29) obtained with water-soluble extracts of white mustard (CCC) and narbon bean (LCC) compared with a control without cover crop. Different small letters within each cultivar per column indicate that the differences between treatments were statistically significant (Tukey's,  $p \leq 0.05$ ).

## EFFECT OF WHITE MUSTARD AND NARBON BEAN ON SUNFLOWER SEEDLING GROWTH

Summary of ANOVAs for testing the effect of treatments, sunflower planting dates, cultivars and their interaction on the different variables evaluated.

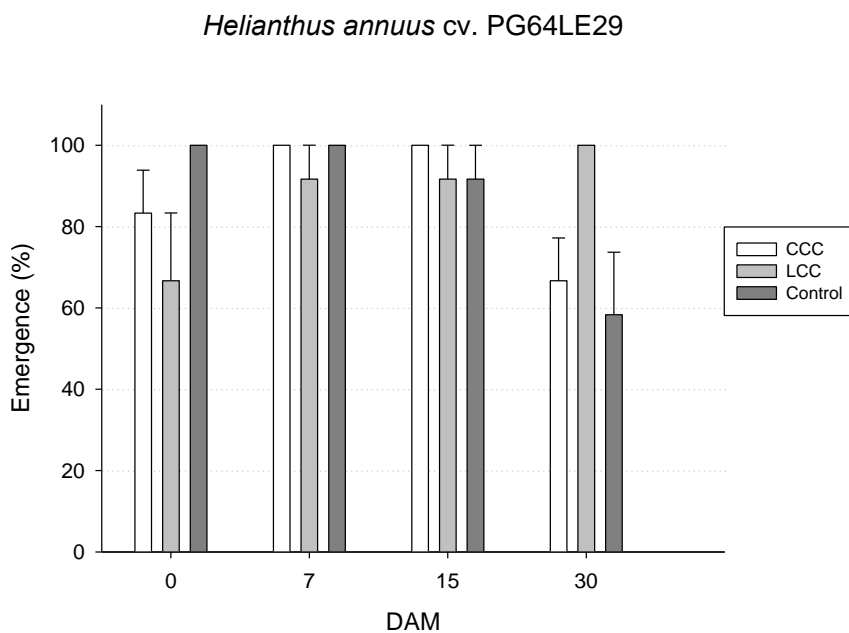
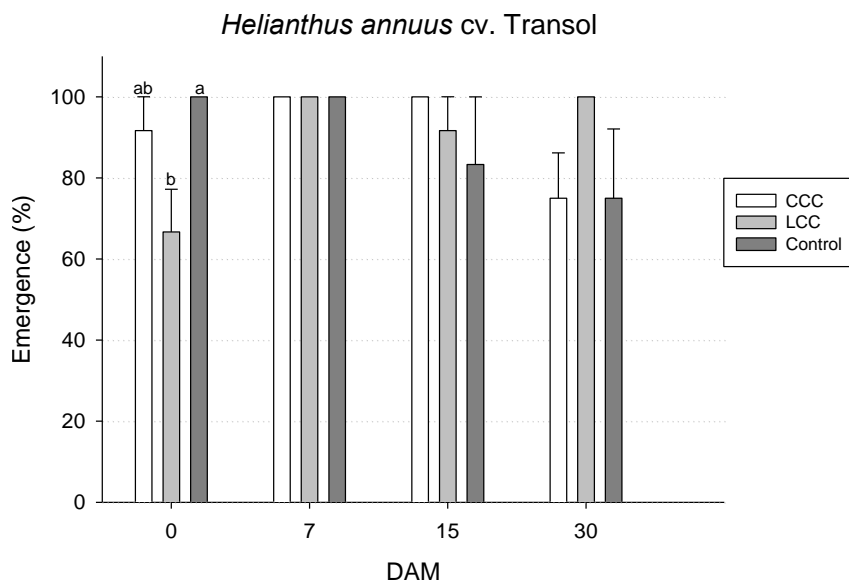
Variables evaluated	Treatments <sup>1</sup> (TR)	Planting dates <sup>2</sup> (SP)	Cultivar <sup>3</sup> (SC)	TR × SP	TR × SC	SP × SC	TR × SP × SC
Emergence	0.05 n.s. <sup>4</sup>	5.55**	0.59 n.s.	5.44***	0.04 n.s.	0.39 n.s.	0.28 n.s.
Hypocotyl length	12.77**	20.95***	0.08 n.s.	3.97**	0.41 n.s.	0.32 n.s.	1.90 n.s.
Root length	1.28 n.s.	54.48***	2.72 n.s.	3.42**	0.15 n.s.	2.35 n.s.	0.59 n.s.
Dry matter							
Above-ground part	102.24***	7.53***	0.59 n.s.	1.4 n.s.	0.03 n.s.	5.79**	2.96*
Underground part	23.31***	4.63**	0.12 n.s.	10.72***	4.39*	1.53 n.s.	0.62 n.s.
Leaf length							
1 <sup>st</sup> pair	19.64***	25.42***	5.12**	4.46**	0.63 n.s.	4.54**	1.53 n.s.
2 <sup>nd</sup> pair	147.70***	66.10***	0.17 n.s.	17.35***	0.47 n.s.	5.75**	2.81*
3 <sup>rd</sup> pair	325.87***	71.91***	3.55 n.s.	4.15**	1.53 n.s.	2.55 n.s.	0.92 n.s.
Leaf width							
1 <sup>st</sup> pair	24.21***	17.26***	3.04 n.s.	4.39**	1.60 n.s.	3.35*	1.62 n.s.
2 <sup>nd</sup> pair	111.30***	86.99***	2.55 n.s.	27.43***	1.55 n.s.	7.72***	0.81 n.s.
3 <sup>rd</sup> pair	285.90***	29.22***	2.15 n.s.	2.39*	1.32 n.s.	1.16 n.s.	1.15 n.s.

<sup>1</sup>Treatments: CCC, LCC, Control

<sup>2</sup>Planting dates: 0, 7, 15, 30 days after mowing (DAM)

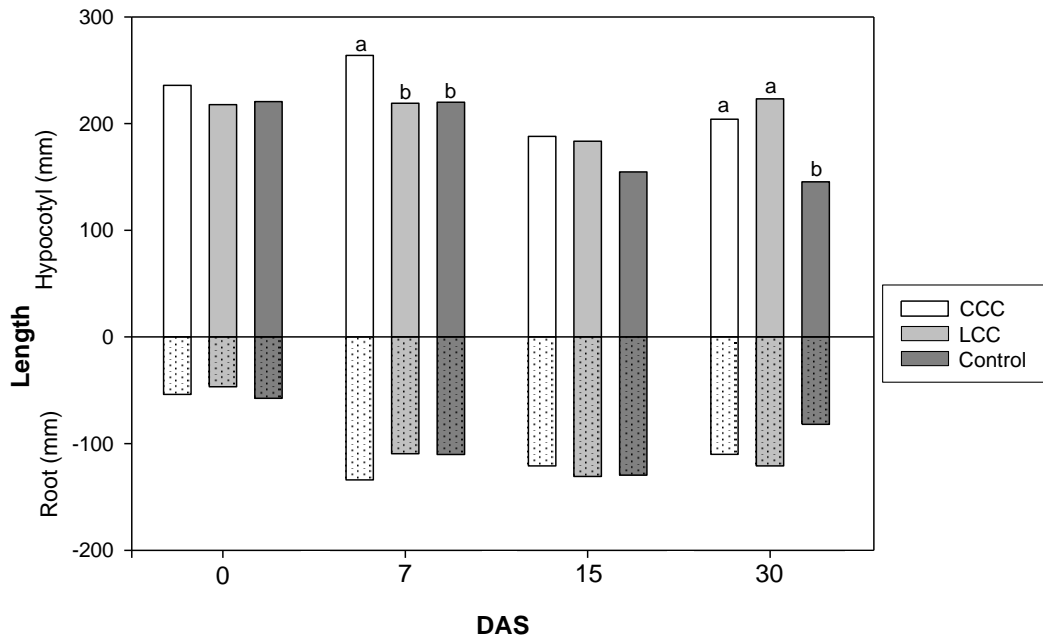
<sup>3</sup> Cultivar: cv. Transol, cv. P64LE29

<sup>4</sup> $p < 0.05^*$ ,  $p < 0.01^{**}$ ,  $p < 0.001^{***}$ , n.s.: not significant

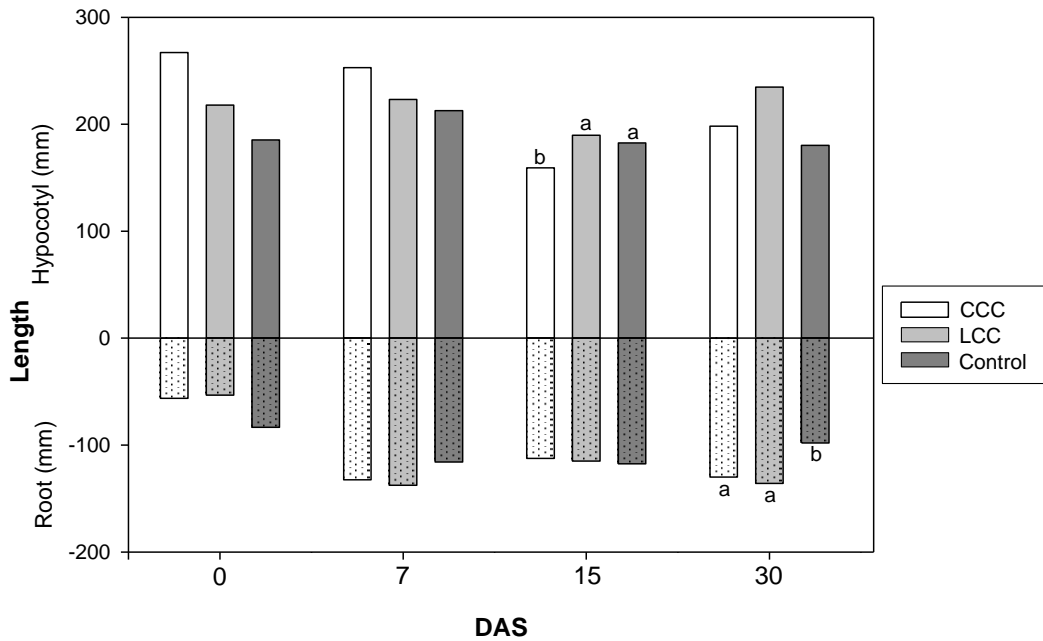


**Emergence (%) of each sunflower cultivar (cv. Transol and cv. P64LE29) planted in pots with white mustard (CCC) and narbon bean (LCC) residues mixed with soil compared with a control without cover crop in four sunflower planting dates after CC incorporation: 0, 7, 15 and 30 days after mowing (DAM). Different small letters within each planting date per column indicate that the differences between treatments were statistically significant (Tukey's,  $p \leq 0.05$ ).**

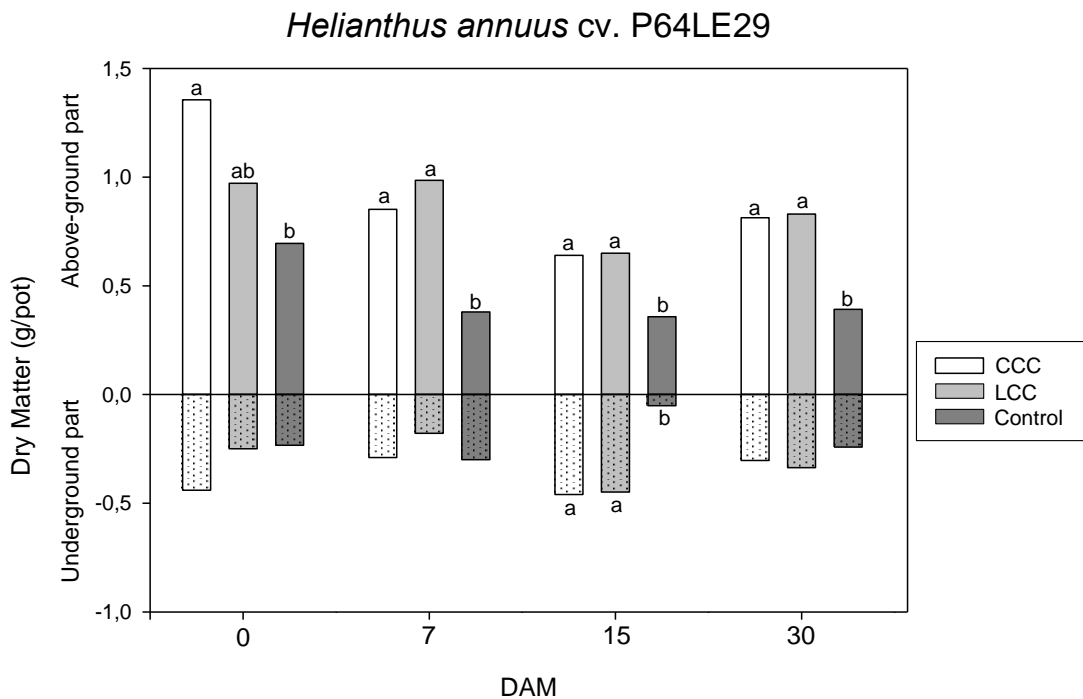
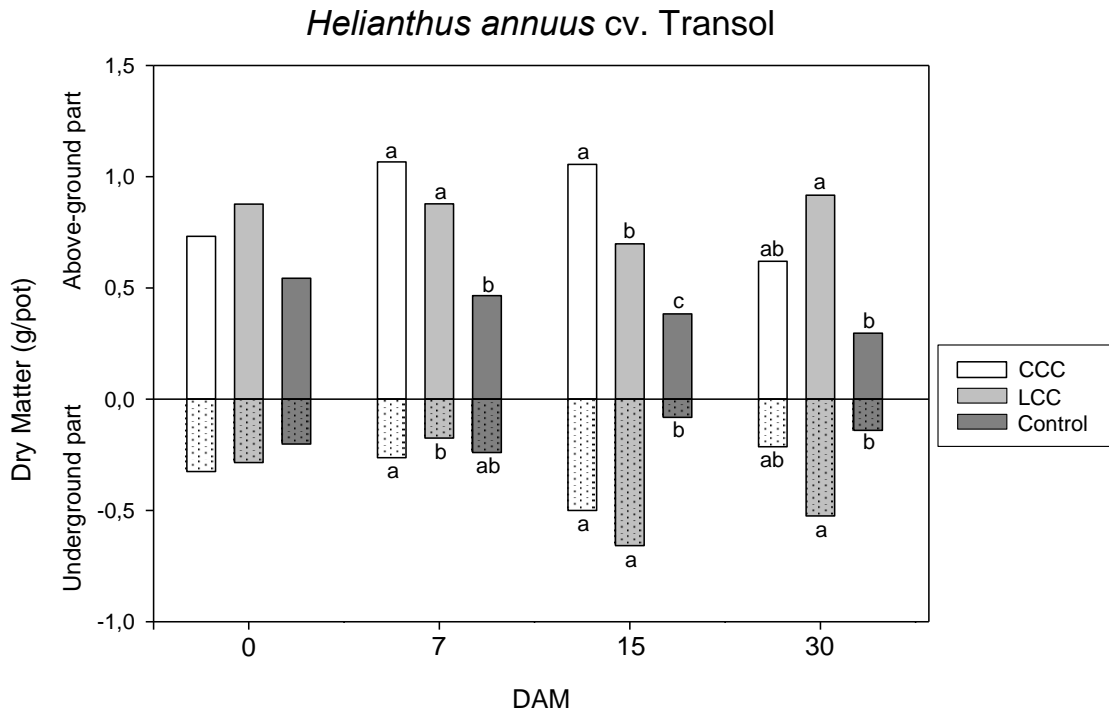
*Helianthus annuus* cv. Transol



*Helianthus annuus* cv. P64LE29

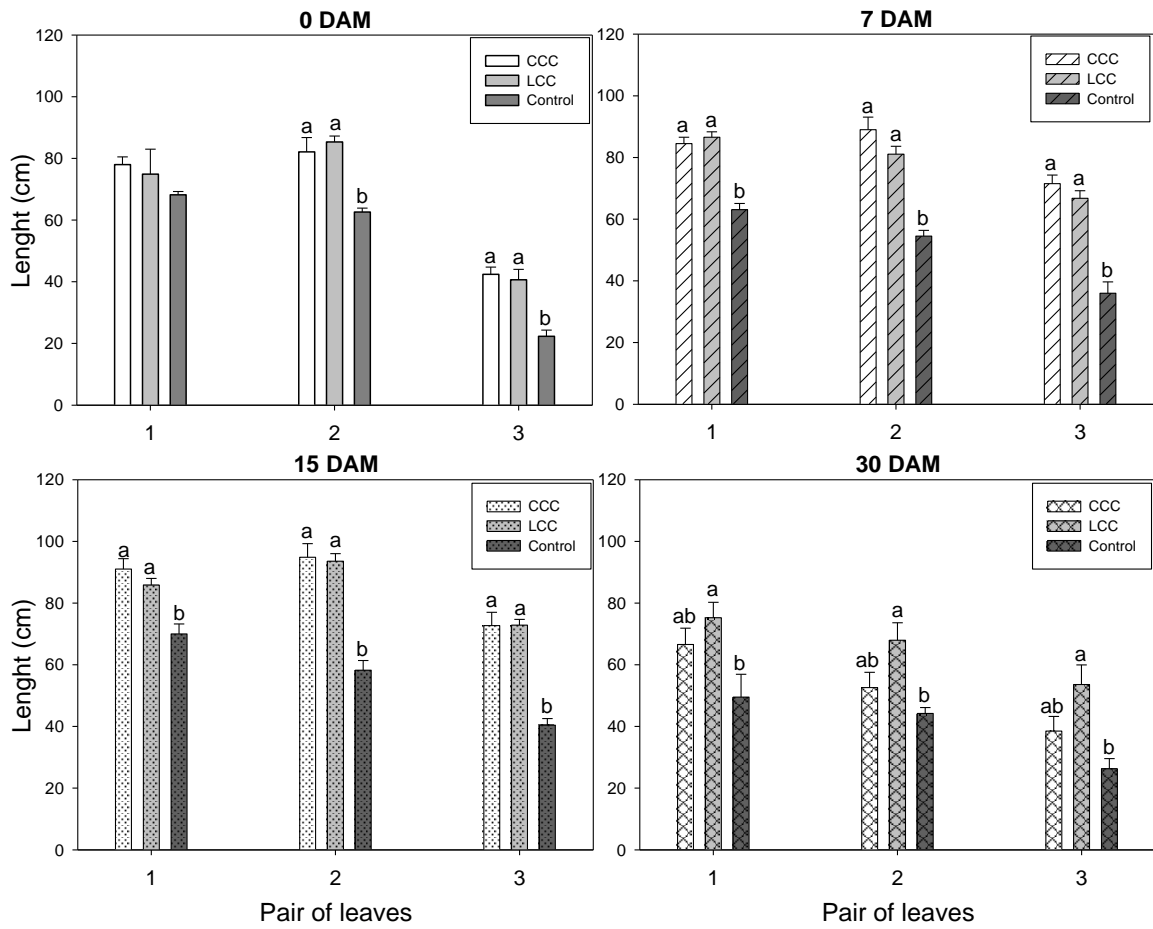


Hypocotyl and root length (mm) of each sunflower cultivar (cv. Transol and cv. P64LE29) planted in pots with white mustard (CCC) and narbon bean (LCC) residues mixed with soil compared with a control without cover crop in four sunflower planting dates after CC incorporation: 0, 7, 15 and 30 days after mowing (DAM). Different small letters within each variable and planting date per column indicate that the differences between treatments were statistically significant (Tukey's,  $p \leq 0.05$ ).



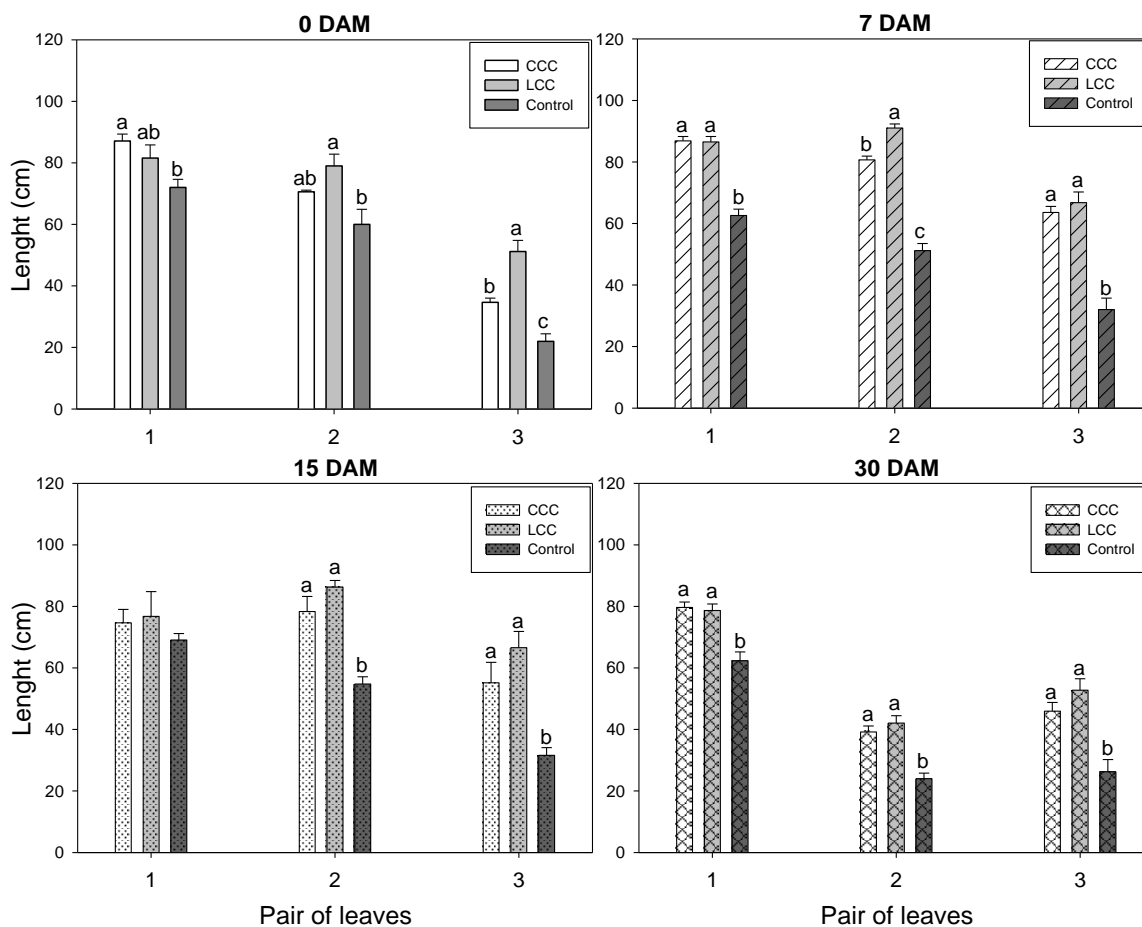
Dry matter (g/pot) of above-ground and underground parts of each sunflower cultivar (cv. Transol and cv. P64LE29) planted in pots with white mustard (CCC) and narbon bean (LCC) residues mixed with soil compared with a control without cover crop in four sunflower planting dates after CC incorporation: 0, 7, 15 and 30 days after mowing (DAM). Different small letters within each variable and planting date per column indicate that the differences between treatments were statistically significant (Tukey's,  $p \leq 0.05$ ).

*Helianthus annuus* cv. Transol



Leaf length at the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> pair of leaves of sunflower cv. Transol planted in pots with white mustard (CCC) and narbon bean (LCC) residues mixed with soil compared with a control without cover crop in four sunflower planting dates after CC incorporation: 0, 7, 15 and 30 days after mowing (DAM). Different small letters within each pair of leaves per column indicate that the differences between treatments were statistically significant (Tukey's,  $p \leq 0.05$ ).

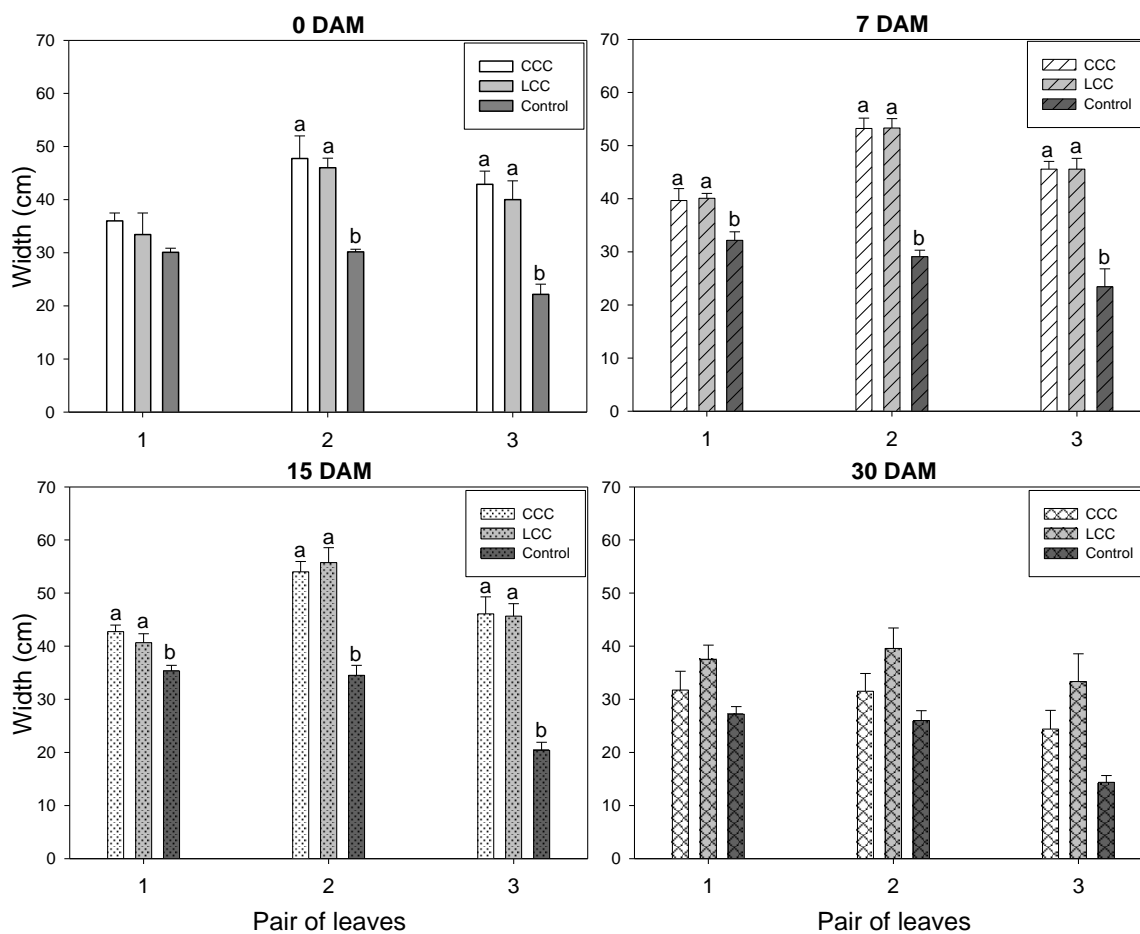
*Helianthus annuus* cv. P64LE29



Leaf length at the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> pair of leaves of sunflower cv. P64LE29 planted in pots with white mustard (CCC) and narbon bean (LCC) residues mixed with soil compared with a control without cover crop in four sunflower planting dates after CC incorporation: 0, 7, 15 and 30 days after mowing (DAM). Different small letters within each pair of leaves per column indicate that the differences between treatments were statistically significant (Tukey's,  $p \leq 0.05$ ).

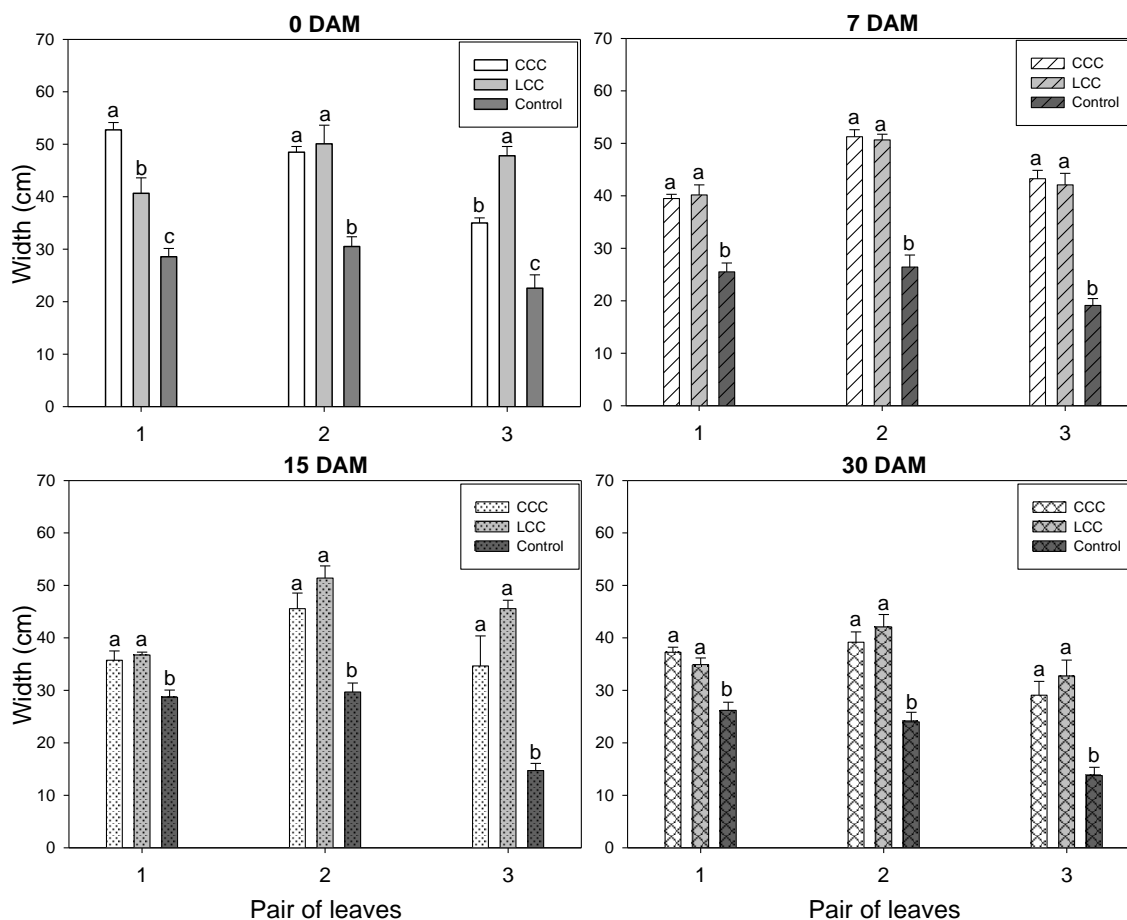


*Helianthus annuus* cv. Transol



Leaf width at the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> pair of leaves of sunflower cv. Transol planted in pots with white mustard (CCC) and narbon bean (LCC) residues mixed with soil compared with a control without cover crop in four sunflower planting dates after CC incorporation: 0, 7, 15 and 30 days after mowing (DAM). Different small letters within each pair of leaves per column indicate that the differences between treatments were statistically significant (Tukey's,  $p \leq 0.05$ ).

*Helianthus annuus* cv. Transol



Leaf width at the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> pair of leaves of sunflower cv. P64LE29 planted in pots with white mustard (CCC) and narbon bean (LCC) residues mixed with soil compared with a control without cover crop in four sunflower planting dates after CC incorporation: 0, 7, 15 and 30 days after mowing (DAM). Different small letters within each pair of leaves per column indicate that the differences between treatments were statistically significant (Tukey's,  $p \leq 0.05$ ).