

## Irrigation and soil management strategies to improve fruit tree response in limiting soil conditions

Joan Lordan Sanahuja

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## Irrigation and soil management strategies to improve fruit tree response in limiting soil conditions

## DISSERTATION

## to obtain the degree of Doctor by the University of Lleida

## MEMÒRIA DE TESI

per optar al grau de Doctor per la Universitat de Lleida

by

per

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Researcher. Efficient Use of Water Program. IRTA. Spain Substitute Member. The best way to predict the future is to create it. – Peter Druker –

> Als meus pares Al Jaume A la Glòria

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#### Abstracts

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#### Resum

La transformació de terres marginals, juntament amb un augment dels processos de degradació del sòl (p. e. salinització) estan traslladant l'agricultura a sòls més desfavorables, fet que obliga al desenvolupament de noves estratègies de maneig dels cultius. Durant els últims anys s'han introduït noves estratègies de reg, com el reg localitzat enterrat (subsurface drip irrigation, SDI) o el reg deficitari controlat, tot i que resulta necessari avaluar la seva viabilitat i sostenibilitat quan són aplicades en sòls amb propietats físiques limitants. Alhora, l'ús d'esmenes orgàniques i les tècniques d'aeració forçada estan sorgint com a mètodes de recuperació de sòls a baix cost, que podrien millorar potencialment el rendiment dels cultius en situacions desfavorables. És important estudiar els efectes d'aquestes estratègies i tècniques sobre les propietats del sòl, així com en la fisiologia dels cultius i la seva productivitat, tot i que també sobre paràmetres de qualitat d'importància creixent en el sector fructícola, com el potencial de conservació de la fruita. L'objectiu general d'aquesta tesi ha estat el d'avaluar diferents estratègies de maneig del sòl i de reg, i estudiar els seus efectes en plantacions de presseguer i nectarina en sòls amb condicions físiques limitants.

En general, es va observar que el SDI va tenir un millor comportament enfront d'altres sistemes, i va assolir una major eficiència del reg encara que no es va traduir en augments de producció. En aquest sentit, és important recordar que els experiments duts a terme en aquesta tesi es van establir en plantacions en sistema de cavalló – *amb una reduïda àrea infiltració* – en sòls amb problemes encrostament superficial – *amb baixes taxes d'infiltració* – el que significa que un potencial d'infiltració limitat podria ser el principal obstacle a superar per assolir una major eficiència de reg. Avui en dia, el SDI es presenta com un sistema interessant per al sector fructícola, tot i així té algunes limitacions importants (p. e. intrusió radicular) que restringeixen la seva expansió en el sector.

Aquesta tesi estudia l'ús d'esmenes orgàniques per millorar les condicions del sòl mitjançant l'ús de dues alternatives diferents: (i) esmenes superficials, o *mulching*, i (ii) esmenes localitzades, les quals es van aplicar per sota de tubs de reg en les parcel·les experimentals de SDI. El *mulching* va afectar els primers mil·límetres del sòl, mitjançant la prevenció de l'encrostament superficial i el deteriorament de l'estructura superficial del cavalló, el que al seu torn va millorar de forma considerable les propietats hidràuliques del sòl. D'aquesta manera, es va incrementar substancialment l'eficiència del reg, i els arbres sota aquest sistema van presentar un millor estat hídric i nutricional que es va traduir en increments notables de producció. Les esmenes localitzades amb substrats orgànics (p. e. substrat de clofolla d'arròs) van tenir efectes positius sobre el sòl, que al seu torn van millorar el comportament del SDI en aquest tipus de sòls, augmentant de forma notable el rendiment dels cultius.

La injecció d'aire a través del sistema SDI no va tenir cap efecte sobre les propietats del sòl o el rendiment del cultiu. Els assajos experimentals estaven localitzats en parcel·les amb sistema de reg d'alta freqüència en sòls desfavorables, i per tant susceptibles de patir entollament, tot i així no es van observar símptomes d'hipòxia de manera que el sistema en cavalló pot ser suficient per evitar aquest tipus de problemes en condicions de sòl similars.

Les diferents estratègies de maneig dels cultius van ser avaluades no només en base als seus efectes sobre les propietats del sòl o al seu efecte sobre el rendiment productiu, sinó que es va estudiar de la mateixa manera els efectes sobre la fisiologia dels cultius i sobre els paràmetres de qualitat dels fruits. En aquest sentit, es van avaluar paràmetres clàssics de qualitat (p. e. contingut de sòlids solubles, fermesa) i altres de creixent interès en la indústria agroalimentària com el potencial de conservació dels fruits mitjançant l'anàlisi d'activitat de l'enzim polifenol oxidasa (PPO). Els experiments duts a terme en aquesta tesi indiquen que hi ha una estreta relació entre l'estat hídric de la planta i l'activitat de PPO, suggerint que els arbres sota estrès hídric produeixen fruits amb una activitat PPO més alta, i com a conseqüència, amb un potencial de postcollita més baix. Així mateix, l'estat nutritiu de la planta i en particular la fertilització nitrogenada podrien afectar l'activitat de PPO del fruit.

Juntament amb el desenvolupament dels assajos experimentals van ser introduïts i desenvolupats nous mètodes de mesura per tal de facilitar i millorar l'avaluació de la resposta del cultiu. En aquest sentit, es va desenvolupar un mètode d'anàlisi d'imatge a través de l'ús de tècniques de fotografia digital, el qual va resultar ser una eina sensible i fiable per estudiar la resposta del cultiu en diferents condicions o estratègies de gestió.

#### RESUMEN

La transformación de tierras marginales, junto con un aumento de los procesos de degradación del suelo (p. ej. salinización) están trasladando la agricultura a suelos más desfavorables, lo que obliga al desarrollo de nuevas estrategias de manejo de los cultivos. Durante los últimos años se han introducido nuevas estrategias de riego, como el riego localizado enterrado (subsurface drip irrigation, SDI) o el riego deficitario controlado, a pesar de que resulta necesario evaluar su viabilidad y sostenibilidad cuando son aplicadas en suelos con propiedades físicas limitantes. Al mismo tiempo, el uso de enmiendas orgánicas y las técnicas de aireación forzada están surgiendo como métodos de recuperación de suelos a bajo coste, que podrían mejorar potencialmente el rendimiento de los cultivos en situaciones desfavorables. Es de suma importancia el estudiar los efectos de estas estrategias y técnicas sobre las propiedades del suelo, así como en la fisiología de los cultivos y su productividad, aunque también sobre parámetros de calidad de importancia creciente en el sector frutícola, como el potencial de conservación de la fruta. El objetivo general de esta tesis fue el de evaluar diferentes estrategias de manejo de suelo y riego, y estudiar sus efectos en plantaciones de melocotón y nectarina en suelos con condiciones físicas limitantes.

En general, se observó que el SDI tuvo un mejor comportamiento frente a otros sistemas y logró una mayor eficiencia del riego aunque no se traduzco en aumentos de la producción. En este sentido, es importante recordar que los experimentos llevados a cabo en esta tesis se establecieron en plantaciones en sistema de caballón – con una reducida área infiltración – en suelos con problemas encostramiento superficial – con bajas tasas de infiltración – lo que significa que un potencial de infiltración limitado podría ser el principal escollo a superar para lograr una mayor eficiencia del riego. Hoy en día, el SDI se presenta como un sistema interesante para el sector frutícola, aún así tiene algunas limitaciones importantes (p. ej. intrusión radicular) que restringen su expansión en el sector.

Esta tesis estudia el uso de enmiendas orgánicas para mejorar las condiciones del suelo mediante el uso de dos alternativas diferentes: (i) enmiendas superficiales, o *mulching*, y (ii) enmiendas localizadas, las cuales se aplicaron por debajo de la tubería de riego en las parcelas experimentales de SDI. El *mulching* afectó a los primeros milímetros del suelo, mediante la prevención del encostramiento superficial y el deterioro de la estructura superficial del caballón, lo que a su vez mejoró de forma considerable las propiedades hidráulicas del suelo. De esta forma, se incrementó sustancialmente la eficiencia del riego, y los árboles bajo este sistema presentaron un mejor estado hídrico y nutricional que se tradujo en incrementos notables de producción. Las enmiendas localizadas con sustratos orgánicos (p. ej. sustrato de cascarilla de arroz) tuvieron efectos positivos sobre el suelo, que a su vez mejoraron el comportamiento del SDI en este tipo de suelos, aumentando de forma notable del rendimiento de los cultivos.

La inyección de aire a través del sistema SDI no tuvo ningún efecto sobre las propiedades del suelo o el rendimiento del cultivo. Los ensayos experimentales estaban localizados en parcelas con sistema de riego de alta frecuencia en suelos desfavorables y por consiguiente susceptibles de padecer encharcamiento, aún así no se observaron síntomas de hipoxia por lo que el sistema en caballón puede ser suficiente para evitar este tipo de problemas en condiciones de suelo similares.

Las diferentes estrategias de manejo de los cultivos fueron evaluadas no sólo en base a sus efectos sobre las propiedades del suelo o a su efecto sobre el rendimiento productivo sino que se estudió de igual forma los efectos sobre la fisiología de los cultivos y sobre los parámetros de calidad de los frutos. En este sentido, se evaluaron parámetros clásicos de calidad (p. ej. contenido de sólidos solubles, firmeza) y otros de creciente interés en la industria agroalimentaria como el potencial de conservación de los frutos mediante el análisis de actividad del enzima polifenol oxidasa (PPO). Los experimentos llevados a cabo en esta tesis indican que existe una estrecha relación entre el estado hídrico de la planta y la actividad de PPO, sugiriendo que los árboles bajo estrés hídrico producen frutos con una actividad PPO más alta, y como consecuencia, con un potencial de poscosecha más bajo. Asimismo, el estado nutritivo de la planta y en particular la fertilización nitrogenada podrían afectar la actividad de PPO del fruto.

Junto con el desarrollo de los ensayos experimentales fueron introducidos y desarrollados nuevos métodos de medida con el fin de facilitar y mejorar la evaluación de la respuesta del cultivo. En este sentido, se desarrolló un método de análisis de imagen a través del uso de técnicas de fotografía digital, el cual resultó ser una herramienta sensible y fiable para estudiar la respuesta del cultivo en diferentes condiciones o estrategias de gestión.

#### ABSTRACT

Transformation of marginal land along with an increase of soil degradation processes (e.g. salinization) is moving the agriculture into more unfavorable soils, forcing the development of new management strategies. Subsurface drip irrigation (SDI) and deficit irrigation strategies have been widely studied although it is necessary to evaluate their feasibility and sustainability when applied in soils with limiting physical properties. At the same time, organic soil amendments and oxygation techniques are arising as low-cost soil reclamation methods that could potentially improve the crop performance under such situations. It is of paramount importance to study the effects of these strategies and techniques on soil properties as well as on crop physiology and productivity, but also on some quality parameters of growing importance in the fruit sector, such as fruit storability. The general aim of this thesis was to assess various soil management and irrigation strategies and study their effects on peach and nectarine orchards under limiting soil conditions.

Overall, the SDI had a better performance and achieved a higher irrigation efficiency although it was not translated to yield increases. In this sense it is important to remind that the experiments conducted in this thesis were established in orchards planted in ridge systems – *with low infiltration areas* – in soils with soil crusting problems – *with low infiltration rates* – meaning that a limited water infiltration could be the main constraint for the irrigation efficiency. SDI delivers the water needed for the crop beneath the soil surface, so this might be the main reason that would explain a better adaptability of the system under such conditions. Today, SDI is presented as an interesting system for the fruit sector, although it still has some important limitations (e.g. root intrusion) that constraint its expansion in the sector.

This thesis studies the use of organic amendments to ameliorate the soil conditions by using two different approaches: (i) surface amendments, also known as mulch techniques, and (ii) localized amendments, which were set up beneath the drip line in SDI plots. The mulch affected the first millimeters of the soil, by preventing soil crust formation and deterioration of topsoil structure what in turn improved the hydraulic soil properties. Thus, irrigation efficiency was substantially improved, and trees under this system presented a better water and nutrition status that resulted in significant yield increases. Localized soil amendments with organic substrates (i.e. rice husk substrate) had positive effects on soil properties, which in turn improved the performance of SDI in such soils, significantly increasing the crop performance.

Injecting air through the SDI system had no effects on soil properties or crop yields. The experiments were located on plots with a high-frequency irrigation system in an unfavorable soil, and therefore susceptible to waterlogging, yet no symptoms of hypoxia were observed so the ridge tillage system may be sufficient to prevent such problems in similar soil conditions.

#### Abstracts

Crop management strategies were evaluated not only based on their effects on soil properties or crop production, but tree physiology and fruit quality parameters were also studied. In this sense, classic quality parameters (e.g. total soluble solids, firmness) and other of growing interest in the food industry were evaluated, such as the fruit storability by means of the analysis of the activity of polyphenol oxidase (PPO) enzyme. The experiments conducted in this thesis indicate that there exist a close relationship between crop water status and PPO activity, suggesting that trees under water stress produce fruits with higher PPO activities, and consequently, with a lower postharvest potential. Furthermore, the nutritional status of the plant and in particular nitrogen fertilization could affect the fruit PPO activity.

Along with the development of the experiments, new methods of measurement were introduced and developed in order to ease and improve the crop response assessment. In this sense, an image analysis method was developed through the use of digital photography techniques, which resulted in a sensitive and reliable tool to study crop response under different conditions or management strategies.

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## Chapter I. General introduction

# 1. Constraints and future perspectives of fruit tree management in soils with limiting physical factors

# 1.1. The expansion of arable land: soil degradation issues and transformation of marginal lands

According to a FAO report (Alexandratos and Bruinsma, 2012) in the next years, developing countries will need an extra 107 million ha of arable area to maintain a sustainable level of food production; this represents an overall increase of 11%. Globally, there is potential to introduce new cropland, but only a fraction of this extra land is realistically available for agricultural expansion (FAO, 2002) since there exist other technical and socioeconomic constraints. As reported by Alexandratos and Bruinsma (2012), the arable area under irrigation is under continuous expansion, accounting with 16% of the total arable area for 44% of total crop production. Most of this expansion is achieved by converting rainfed lands into irrigated lands although an important part of this irrigation transformation (16.6%) takes place on arid and hyper-arid land which is not suitable for rainfed agriculture, and this figure could increase over the period to year 2050 (Alexandratos and Bruinsma, 2012).

In addition to social and economic impacts derived from land use change, irrigation represents a major driving force that can drastically enhance crop productivity, especially in arid and semiarid areas. However, inappropriate irrigation presents some risks on soil degradation, such as soil erosion, waterlogging and secondary salinization. For instance, it has been reported that half of the world's irrigation systems are influenced by secondary salinization, alkalinization, and/or waterlogging (Pessarakali and Szabolcs, 1999). Secondary salinization has been caused traditionally by flooding irrigation (Vlek et al., 2008) although recently a new form of secondary salinization based on deficient leaching water under localized irrigation systems is calling the attention of the research community (Zhang et al., 2014). New irrigation systems and strategies have to take into account both a better crop and a proper soil management, thus by preventing soil degradation processes and being sustainable over time.

On the other hand, arable land expansion is sometimes at expense of marginal land transformation (Alexandratos and Bruinsma, 2012) what brings a new set of important constraints in terms of soil and irrigation management. Marginal lands present a series of advantages such as better meteorological conditions or lower land prices although are usually located on soils with limiting physical properties (Kang et al., 2013). Such soils are considerably deficient in terms of the infiltration, retention, and transmission of water, and also regarding salinity. Thus, since the physical properties of these soils are often a major constraint to their productivity (Uphoff et al., 2006), it is necessary to restore their productivity by reclamation procedures and soil management techniques (e.g. soil amendments).

Overall, it seems that in the coming decades agriculture will have to deal with unfavorable soils for crop production, either by an increase in soil degradation processes or because of transformation of marginal lands. There is a global need to find and develop new irrigation and soil management strategies to overcome the limiting factors and restore the soil productivity.

# 1.2. Irrigation and soil management strategies to overcome the limiting properties of the soil

Salinity is one of the most important factors affecting the soil structure and thus the crop growth and the fruit yield (Parada and Das, 2006). Soil salinity can affect the crop performance in many ways: (a) by increasing the soil osmotic potential and hence reducing water availability; (b) by increasing concentrations of toxic ions; and (c) by affecting soil structure and thereby reducing both water permeability and soil aeration (Evangelou and McDonald, 1999). Unfortunately, some of these *phenomena* can be exacerbated when the soil is under a humid regime, so a series of soil management strategies need to be adopted in irrigated lands.

Ridge tillage (or permanent raised bed system) is a widely used strategy to overcome salinity and waterlogging issues in many crop systems (Roth et al., 2005). Ridge tillage is the term for any cropping system in which plants are grown on soil formed into raised beds or ridges (Tisdall and Hodgson, 1990) with the purpose to create a better root environment and to enhance the crop performance. When adopted in localized irrigation systems, a proper irrigation management is needed to flush away the excess salts from the root zone, in the outer soil layer of the ridge. Thus, it is important to maintain an appropriate irrigation regime to keep the soluble salts away from the roots. If the irrigation regime is to low, leaching will be no effective; on the other hand if it is too high the water front can resolubilize the leached salts in the soil matrix and salinize again the root zone (Hillel, 2000). Besides the salinity control, the ridge system prevents waterlogging and hypoxic events in the root zone, which is a quite common issue in saline soils (Bakker et al., 2010). As stated in many studies (Tisdall and Hodgson, 1990; West and Black, 1969), the ridge system improves the natural soil drainage and the oxygen flux of the soil, enhancing the aeration status in the rhizosphere and leading the crop to higher yields.

The ridge dimensions can vary depending on the established crop: from small ridges in some horticultural and cereal crops (0.3 m in height and 0.3 in width) to bigger ones in fruit tree orchards (0.8 m in height and 1 m in width). Thus, when ridge systems are set up in fruit tree orchards with drip irrigation systems, the potential infiltration area is drastically reduced. This issue leads to a substantial increase of water runoff what in turn reduces the irrigation efficiency and increases the ridge soil erosion (Gosar and Baričevič, 2011; Liu et al., 2014). These *phenomena* can be solved by providing the irrigation water by a low discharge system (Schwankl and Hanson, 2007), although in soils with extremely low infiltration rates it is not sufficient; or it is not practical regarding the irrigation

scheduling since lengthens considerably the irrigation time and increases the associated costs. Thus, while ridge tillage is an appropriate strategy to apply under limiting soil conditions (Roth et al., 2005), its effectiveness and sustainability is under consideration in fruit tree orchards with drip irrigation systems. In response, other strategies have lately arisen to overcome the ridge system limitations, such as the mulch techniques.

Application of organic amendments and mulch techniques are widely used in crop production in order to ameliorate topsoil physical conditions, especially those related to the hydraulic properties of the soil (e.g. water infiltration)(Cook et al., 2006). The formation of surface crusts or seals on soils, which is commonly attributed to a low organic matter content (Materechera, 2009), can impede water infiltration significantly resulting in increased runoff and erosion (Gicheru et al., 2004). Organic soil amendments have reported beneficial results on the physical properties of the soil by avoiding soil crusting (Gicheru et al., 2004), lowering the soil penetration resistance (Materechera, 2009), improving the hydraulic soil properties (Merwin and Stiles, 1994) or even by decreasing the soil surface water evaporation (Uson and Cook, 1995). The use of organic soil amendments and mulches may help to improve the drip irrigation performance in soils either with low infiltration rates or with low infiltration areas (e.g. ridge systems), reducing or even avoiding, the runoff and erosion. There exist many organic materials that might be used as soil amendments although it is necessary to study their effect on soil properties and their durability under high-frequency irrigation regimes.

Applying irrigation amounts in excess of that needed for evapotranspiration can control the accumulation of soluble salts in the root zone, this irrigation strategy is known as leaching (Hoffman and Shannon, 2007). Drip irrigation can be a good solution to overcome soil salinity if well managed, since leaching is performed continuously and soil salinity is maintained below detrimental levels (Burt and Isbell, 2005). While flooding or furrow irrigation are sometimes inefficient to control salinity, drip irrigation provides a more accurate management by applying lower water volumes in a frequent basis (Hoffman and Shannon, 2007). Overall, since agriculture is under a water scarce scene in many arid and semiarid areas of the world, there exist a global trend to enhance irrigation water use by applying different irrigation strategies. In this sense, several deficit irrigation strategies have been proposed during the last decades, although in general terms it is possible to divide them into sustained deficit irrigation and regulated deficit irrigation strategies. The first one of these strategies implies a reduction of the crop water requirements throughout the growing season (Goldhamer et al., 2005), while the second one applies the water reduction during certain phenological stages (Chalmers et al., 1981). The effects of deficit irrigation strategies on crop productivity and fruit quality have been widely studied during the last years in many fruit crops, providing valuable results (Fereres and Soriano, 2007; Girona and Fereres, 2012; Girona et al., 2005a).

#### Chapter I

However, the sustainability of these strategies in a long-term basis is under review since water deficits lead to substantial decreases in tree growth and in turn reductions in fruit yield (Marsal et al., 2008; Rufat et al., 2010). Furthermore, deficit irrigation strategies present some uncertainties regarding its management with saline water or in salt affected soils, as the leaching fraction is substantially reduced. For instance, Mounzer et al. (2013) indicated that the long-term use of deficit irrigation strategies could considerably affect the sustainability of the agricultural soils when saline reclaimed water was used for irrigation. On the other hand, Aragüés et al. (2014) evaluated the sustainability of several deficit irrigation strategies in a long-term experiment irrigated with saline water and did not found negative effects on crop productivity. Overall, such results proof that there is a global awareness regarding the long-term sustainability of the proposed irrigation strategies. Further research concerning these issues is needed, particularly in soils with limiting physical properties.

At the same time, new drip irrigation systems with interesting features are on their way to professional agriculture, and subsurface drip irrigation (SDI) is the maximum exponent among them. SDI is defined as the application of water below the soil surface by microirrigation low-discharge emitters (Lamm and Camp, 2007). Although surface drip irrigation (DI) is now used more intensively than SDI, microirrigation probably started with water application below the soil surface (Davis, 1974). SDI performance suffered from many problems such as emitter clogging and deficient irrigation uniformity although nowadays, within the arrival of new materials and designs, there is a resurgence of the system (Lamm and Camp, 2007). Since the irrigation water is provided beneath the soil surface, the SDI presents a number of advantages over other systems, such as a reduced water runoff and evaporation and in turn an enhanced irrigation efficiency (Phene et al., 1986). These characteristics could make the SDI an appropriate system to use under limiting soil conditions, such as in soils with low infiltration rates.

On the other hand, SDI presents a series of limitations that could constraint its expansion in the fruit tree sector, such as root intrusion or root pinching. Although these SDI problems have long been recognized, few published, detailed research studies are available (Camp, 1998). Since root intrusion could become a major problem in perennial woody crops, some manufacturing companies have started by re-designing the emitters to prevent the root entrance (e.g. physical barriers or herbicide impregnation). Furthermore, *surface water ponding* have been described by Burt and Styles (2011) as a basic problem that is experienced by many growers in California. This *phenomenon* occurs when water comes up to the soil surface through a "sand chimney", reducing the irrigation uniformity. This issue is commonly associated with high flow rates emitters although it also occurs in soils with low hydraulic conductivities. (Burt and Styles, 2011).

There exist other associated problems with SDI, which are actually common in other localized irrigation systems (e.g. DI), such as temporal waterlogging. Usually, when irrigation is performed in a high-frequency basis in soils with poor

hydraulic properties, the root zone is saturated during sometime after irrigation what might lead the crop to suffer hypoxic events (Bar-Yosef et al., 1989; Pendergast et al., 2013). Along with SDI, other supplementary techniques can be applied to overcome adverse soil factors (e.g. waterlogging), such as forced soil aeration (or oxygation). Recent studies (Abuarab et al., 2013; Bhattarai et al., 2008; Bonachela et al., 2010; Chen et al., 2010; Goorahoo et al., 2002) have reported that SDI can be effectively used for the delivery of aerated water to overcome hypoxia, although there exist many methodologies (e.g. air venturi injectors or hydrogen peroxide injection) that need to be reviewed when used in open-field conditions. Furthermore, while most of these experiments were carried out with herbaceous and horticultural crops (e.g. cotton, pepper, pumpkin, soybean, tomato, watermelon, zucchini or maize), there is no scientific evidence of similar experiments in fruit tree crops so far. However, promising results obtained in previously cited studies encourage further research in fruit tree species concerning this issue.

## 2. Aims and objectives of the thesis

Transformation of marginal land along with an increase of soil degradation processes is moving the agriculture into more unfavorable soils, forcing the development of new management strategies. SDI and deficit irrigation strategies have been widely studied although it is necessary to evaluate their feasibility and sustainability when applied in soils with limiting physical properties. At the same time, organic soil amendments and oxygation techniques are arising as low-cost soil reclamation methods that could potentially improve the crop performance under such situations. It is of paramount importance to study the effects of these strategies and techniques on soil properties as well as on crop physiology and productivity, but also on some quality parameters of growing importance in the fruit sector, such as fruit durability. In this sense, there is a need to find and develop new measurement methodologies to assess the crop response. The general aim of this thesis was to assess various soil management and irrigation strategies and study their effects on peach and nectarine orchards under limiting soil conditions. The interaction between the soil factors and the crop response will provide useful knowledge to evaluate the influence of fruit tree physiology on fruit productivity and quality.

The specific objectives of the thesis were:

- To assess the productive response of fruit trees under different irrigation systems (DI vs. SDI) in soils with limiting physical properties.
- To assess the effects of oxygation treatments on fruit tree response under limiting soil conditions.
- To evaluate the use of soil amendments and mulch techniques to ameliorate the soil properties and enhance the fruit tree performance.

- To know the role of the nutrient and the crop water status on essential fruit quality parameters and postharvest durability.
- To assess the feasibility of image analysis techniques as supplementary tools for fruit tree response measurements.

### 3. Outline of the thesis

The thesis consolidates the research findings on the broad three fronts: (i) Soil management and irrigation strategies to overcome limiting soil factors; (ii) the role of tree water status in fruit quality parameters; and (iii) the use of image techniques to assess the fruit tree response. The various studies that address these themes are presented in chapters II-VI.

The following chapter (Chapter II) evaluates the effects of different irrigation strategies (DI & SDI) and soil amelioration techniques (localized amendments & oxygation) on the physical soil properties and the fruit tree response during the first year of the crop establishment. The experiment was carried out in a peach orchard, planted in ridge system under limiting soil conditions.

Chapter III deals with the application of low-cost mulch techniques in soils with limiting physical properties. Soil mulches are of particular interest in fruit tree production since they offer means to improve not only the soil fertility but also the topsoil physical conditions. The three-year experiment studies the effects of the proposed strategies on the irrigation efficiency and the crop productivity.

The relationship between the nutrient and the crop water status on essential fruit quality parameters are treated in Chapter IV. The experiment was carried out in a nectarine orchard and studied the effects of different irrigation strategies on classical quality parameters (e.g. total soluble solids content) and other of growing interest in the fruit sector, such as the fruit durability by means of the polyphenol oxidase (PPO) activity.

The long-term effects of various soil management and irrigation strategies were studied in Chapter V. The three-year experiment was carried out in a peach orchard planted in a ridge system in a soil with limiting physical properties. The study performs a detailed analysis of the treatment effects on the vegetative growth, as well as on the crop production and fruit quality. Furthermore, evidences the relationship between water stress variables and fruit quality parameters.

The use of image techniques as supplementary tools to assess the crop response is widely discussed in Chapter VI. The use of these techniques was previously introduced in the cited experiments, although within this chapter the methodology is extensively reviewed, and compared to other classic measurements.

Finally, chapter VII engages the final discussion of this thesis, with the main conclusions and future perspectives for further research.

## Chapter II. Use of rice husk to enhance peach tree performance in soils with limiting soil conditions

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#### Abstract

This study evaluated the use of rice husk as an amendment in a soil with limiting physical conditions. The research was conducted in a commercial peach orchard planted in 2011 using a ridge planting system. Six soil and water management treatments were evaluated in 18 experimental units, which were set up in the field using a randomized complete block design. The treatments were compared both in terms of soil physical condition effects and crop response. Localized substrate amendment of the soil significantly increased both saturated hydraulic conductivity and aeration porosity by 90-fold and 76%, respectively, compared with unamended treatments. In the case of growth parameters, amended trees presented the highest overall values without affecting crop water status. In terms of canopy ratio (CR), the amended treatment produced the highest overall value (0.56 m<sup>2</sup> m<sup>-3</sup>), a 44% increase compared to control treatment. Localized soil amendment with rice husk contributed to both peach tree performance and residue management.

Keywords: soil amendment, by-product, irrigation, salinity, peach.

## 1. Introduction

According to a FAO report, in the next 30 years, developing countries will need an extra 120 Mha of cropland to maintain a sustainable level of food production; this represents an overall increase of 12.5%. Globally, there is potential to introduce new farmland, but only a fraction of this extra land is realistically available for agricultural expansion (FAO, 2002).

Furthermore, much of the currently uncultivated land presents difficulties for crop conversion and management. Climatology and topography are the two major factors that prevent this, but there are many others linked to the physical conditions of soils that limit farmland expansion.

Factors such as soil texture and soil structure determine crop performance (Anikwe, 2000). Massive structure in the topsoil layer blocks the entry of water and thereby reduces crop water availability. Many studies performed with permanent raised bed systems have reported enhanced soil physical properties resulting in improved performance in many crops (Roth et al., 2005). Similarly, subsurface drip irrigation has been reported as bestowing beneficial effects on crops grown under salinity and limiting soil conditions (Hanson et al., 2008). Soil salinity is another factor that hampers agricultural land expansion.

It has been reported that half of the world's irrigation systems are influenced by secondary salinization, alkalinization, and/or waterlogging (Pessarakali and Szabolcs, 1999). Even so, irrigation systems can double the productivity of rainfed systems and are responsible for one-third of the world's food production (Munns, 2002).

Soil salinity can affect crop performance in many ways: (a) by increasing the osmotic soil potential and hence reducing water availability; (b) by increasing concentrations of toxic ions; and (c) by affecting soil structure and thereby reducing both water permeability and soil aeration (Evangelou and McDonald, 1999). The last of these phenomena can be exacerbated when the soil is under a humid regime or when irrigation is applied continuously. The problems associated with crop production in saline soils can be addressed through the adoption of a series of strategies based on ensuring the application of more appropriate soil and irrigation water management.

Soil substrates have been used to enhance soil physical properties for many years, following good results obtained in greenhouse production. Substrates facilitate root development and root activity, thereby improving crop performance. The use of soil substrates and mulches under open field conditions is currently under research. Mulching may be a good technique for reducing crop evapotranspiration and maintaining soil moisture within the soil profile (Kar and Kumar, 2007). The use of by-products as soil amendments to obtain benefits in terms of crop performance and soil amelioration have been studied in the recent years and good results have been obtained (Garg et al., 2005). Rice (*Oryza sativa* (L.)) husk is the main agro-

industrial residue from the rice milling industry, accounting for about one-fifth of the harvested weight of rice crops (Beagle, 1978). Considering that world rice production is about 685 million Mg per year, world rice husk production may be over 130 million Mg per year. Rice husk dust has been studied as a soil amendment for maize (*Zea mays* (L.)) crops, where it improves both crop performance and soil physical properties (Anikwe, 2000). Soil remediation by traditional means is sometimes expensive, but soil amendments – which mainly use by-products - may improve soil conditions and crop performance at a relatively low cost. It may also offer a useful solution for the management of some of the by-products produced by agro-food industries.

Modifying the soil structure and an appropriate irrigation water management (including air injection) may improve crop performance on marginal land. The hypothesis of this study was that using rice husk as a soil amendment combined with subsurface drip irrigation management could improve both soil conditions (e.g. soil saturated hydraulic conductivity and soil aeration) and crop response (i.e. crop growth and crop water status) in soils with limiting physical properties. The objective of this study was to compare the effects of soil amendment and subsurface drip irrigation management in a first year peach crop (*Prunus persica* (L.) Batsch.) in terms of both soil physical properties and crop response.

## 2. Materials and Methods

#### 2.1. Experimental site

The trial was conducted in a commercial orchard of peach (*Prunus persica* (L.) Batsch. var. *Platycarpa* (Decne.) L. H. Bailey cv. Saturn) grafted onto GF-677 and planted in 2011 in a ridge planting system, spaced 5 x 2.5 m, in the Los Monegros region (Huesca, Spain). The climate is semiarid Mediterranean, with an annual rainfall of 350 mm. Irrigation water comes from the Cinca irrigation canal, has low salinity, sodium and nitrate contents. The soil is a Xeric Torriorthent (Soil Survey Staff, 1999) developed from marls, with a loamy texture (40.3% sand, 39.8% silt and 19.9% clay, USDA). The soil is saline (EC<sub>e</sub> = 6.0 dS m<sup>-1</sup>), with a pH of 8.7 and an organic matter content of 10.0 g kg<sup>-1</sup>.

#### 2.2. Experimental design and treatments applied

The experiment was organized in a randomized complete block design with three replications. Each plot contained five control trees. Six different soil and water management treatments were evaluated: ridge planting with drip irrigation at 100% of the calculated irrigation requirement (R-DI-100); ridge planting with drip irrigation at 70% of the calculated irrigation requirement (R-DI-70); ridge planting with subsurface drip irrigation at 100% of the calculated irrigation at 70% of the calculated irrigation at 100% of the calculated irrigation requirement (R-DI-100); ridge planting with subsurface drip irrigation at 70% of the calculated irrigation at 70% of the calculated irrigation at 100% of the calculated irrigation at 70% of the calculat

70% of the calculated irrigation requirement and air injection through the irrigation system (R-SDI-70+AIR), and; modified ridge planting with subsurface drip irrigation at 70% of the calculated irrigation requirement (MR-SDI-70). The irrigation water requirement was calculated using FAO methodology (Allen et al., 1998). Air injection was performed with a venturi air injector (MAI-A3, Toro Ag. Irrigation, USA).

The ridges were all molded using soil already present in the orchard by the use of a rear v-blade attached to a tractor. The modified ridge treatment included substrate taken from around the dripperline, which included a mix of soil, rice husk and gypsum, at respective proportions of 12:12:1. This allows reducing the application rate to 5.0 Mg ha<sup>-1</sup> of rice husk. The substrate was applied at depths of between 0.2 and 0.4 m depth. Substrate density and porosity were 0.76 Mg m<sup>-3</sup> and 60%, respectively. Ridge dimensions were 1.5 m (bottom) and 1 m (top) wide and 0.8 m high. In subsurface irrigation treatments, the dripperline was buried at a depth of 0.25 - 0.3 m below the surface and was placed at a distance of 0.15 - 0.2 m from each tree trunk.

### 2.3. Measurements

Meteorological data were acquired using an automatic weather station located 7.1 km from the experimental orchard, property of the regional meteorological service. Irrigation water was measured using a pulse water meter (MNK-I-N Zenner GmbH & Co. KG, Saarbrücken, Germany), which was connected to a data logger (EM-50, Decagon Devices Inc., Pullman, WA, USA). One water meter was used per treatment.

physical properties of the ridge were determined through several The measurements taken at the end of the crop season (winter). Soil bulk density was determined by the excavation method (Blake and Hartge, 1986), performing one measurement per plot at two different depths: from surface to a depth of 0.2 m; and from a depth of 0.2 to 0.4 m. Water infiltration rate  $(v_i)$  was measured at the ridge surface, using the single ring infiltrometer method (Pla, 1983). Rings were 0.25 m in diameter, 0.4 high and were inserted 0.15 m deep in the soil in order to prevent lateral seepage loss. Furthermore, a small soil dam was formed around the cylinder. Soil saturated hydraulic conductivity  $(K_s)$  was measured using the single ring method at a depth of 0.2 m. Water infiltration rate and soil saturated hydraulic conductivity were measured once per plot. To determine field capacity moisture content the rings used to measure soil infiltration were covered to prevent evaporation. After 48 hours hours soil samples were collected (one per plot) in order to determine field capacity moisture content (FC). Total porosity was determined by the equation proposed by Danielson and Sutherland (1986) as:  $S_t$ =  $(1 - (\rho_b / \rho_n))$ 

Where  $\rho_b$  is bulk density, determined by the excavation method (Blake and Hartge); and  $\rho_p$  is particle density, which for many mineral soils is 2.65 Mg m<sup>-3</sup>.

Aeration porosity was calculated once per plot, at two different depths: between the surface and a depth of 0.2 m; and from a depth of 0.2 to 0.4 m, and by applying the following formula:  $f_a = S_t - FC$ 

Where:  $f_a$  is the aeration porosity, expressed as a %;  $S_t$  is the total porosity, expressed as a %; FC is the volumetric water content at field capacity, expressed as a %.

Tree growth was assessed using two different methods: 1) trunk diameter measurement at the beginning and end of the season for five control trees per plot, and 2) canopy area measurement, for five control trees per plot at the end of the season, using a digital single-lens reflex camera (Nikon 300s with zoom lens Nikkor 18-105 mm f/3.5-5.6G, Nikon, Tochigi, Japan) and specific software (Photoshop CS5, Adobe Systems, San Jose, CA, USA; ImageJ, NIH, USA). To evaluate tree water status, stem water potential ( $\Psi_{stem}$ ) was determined once, at the end of the season (mid-August), for five control trees per plot. This was done using a pressure chamber (model 3005; Soil Moisture Equipment Corp., Santa Barbara, CA, USA) and following the Shackel method (Shackel et al., 1997). Measurements were taken at 2 p.m. local time. Canopy temperature was also determined at the end of the season (mid-August) on five control trees per plot using a thermal camera and specific software (Ti25 and SmartView 2.1, Fluke Corp., Everett, WA, USA). Canopy ratio (*CR*) was defined as:  $CR = CA / W_i (m^2 m^{-3})$ 

Where: *CA* is canopy area, expressed in  $m^2 ha^{-1}$ ; and  $W_i$  is irrigation water applied, expressed in  $m^3 ha^{-1}$ .

A linear mixed model (Type III sum of squares) for analysis of estimated marginal means (EMM) was built to separate treatment effects. Tukey Honestly Significant Difference (THSD) *post hoc* test was used for comparing treatments regarding soil physical properties and crop response data. Contrast test was used for comparing normal ridge treatments (R) and modified ridge treatment (MR) regarding soil physical properties data. A probability level, P = 0.05, was used for all statistical analyses.

## 3. Results and discussion

#### 3.1. Soil physical properties

Measurements of soil physical properties reported several differences between treatments, and more specifically between the normal ridge and modified ridge treatments (Table II.1). Bulk density within the topsoil layer (0-0.2 m) was similar for all the treatments, with values ranging between 1.30 and 1.45 Mg m<sup>-3</sup>. There were, however, differences between the treatments for depths between 0.2 - 0.4 m. The soil bulk density (0.2-0.4) in the normal ridge treatments was 1.34 Mg m<sup>-3</sup>, while in the modified ridge treatment it was 0.89 Mg m<sup>-3</sup>. Amending the soil with substrate at the mid-layer (0.2 - 0.4 m) reduced the bulk density by 0.45 Mg m<sup>-3</sup>

with respect to the unamended treatments. This reduction may have been due to the low density of the amendment, since the density of the substrate tested in the laboratory was 0.76 Mg m<sup>-3</sup>. As reported in previous studies, it is possible to reduce soil bulk density by applying soil amendments (Anikwe, 2000; Garg et al., 2005).

Table II.1. Soil bulk density ( $\rho_b$ ), soil infiltration rate ( $v_i$ ), soil saturated hydraulic conductivity ( $K_s$ ) and soil aeration porosity ( $f_a$ ) after a one-year trial. The values are means . Values followed by different letters indicate significant differences according to the Tukey HSD test (P <0.05) and contrast test (P<0.05).

R-DI-100	ρ₀ 0-0.2 m	ρ <sub>b</sub> 0.2-0	).4 m	v <sub>i</sub> at surf	ace	K₅ at 0.2 r	n depth	f <sub>a</sub> 0-0.2 m	fa <b>0.2-0</b> .	.4 m
	(Mg m⁻³)	(Mg m⁻³)		(mm h <sup>-1</sup> )		(mm h <sup>-1</sup> )		(%)	(%)	
	1.42	1.33	а	24.80	b	2.15	b	19.27	25.91	b
R-DI-70	1.33	1.25	а	8.86	b	4.03	b	26.76	25.05	b
R-SDI-100	1.37	1.41	а	16.00	b	0.93	b	21.27	18.48	b
R-SDI-70	1.32	1.32	а	22.27	b	3.17	b	25.27	24.64	b
R-SDI-70+AIR	1.45	1.39	а	27.76	b	1.80	b	19.19	22.02	b
MR-SDI-70	1.30	0.89	b	103.67	а	205.00	а	27.72	40.81	а
Prob>F (treatment)	ns	0.0007		0.0070		0.0057		ns	0.0015	
Normal ridge (R)	1.38	1.34	а	19.94	b	2.41	b	22.35	23.22	b
Modified ridge (MR)	1.30	0.89	b	103.67	а	205.00	а	27.72	40.81	а
Prob > F (contrast)	ns	< 0.0001		0.0003		0.0002		ns	< 0.0001	

Soil water dynamics exhibited differences between treatments. While the soil infiltration rate in the normal ridge treatments ranged between 8.9 and 27.8 mm h-1, in the modified ridge treatment, it averaged 103.7 mm h-1. Adding soil amendments produced a r-fold increase in the water infiltration rate at the surface, even when amendment was performed in the mid-layer of the soil. Even greater differences between treatments were found when saturated hydraulic conductivity (Ks) was measured at a depth of 0.2 m. Ks values in the normal ridge treatments ranged between 0.9 and 4.0 mm h-1, while in the modified ridge treatment, they averaged 205.0 mm h-1. The presence of substrate resulted in a 90-fold increase in soil Ks values compared with other treatments. Mbagwu (1992) found that it was possible to increase both the soil infiltration rate and the Ks value in a degraded Ultisol through applying organic amendments. It is crucial to improve water movement through the soil, especially when irrigation is provided by SDI (Ayars et al., 1999).

The aeration porosity in the topsoil layer (surface to 0.2 m) was similar in all the treatments, though there were differences between treatments for the 0.2 to 0.4 m depth. Aeration porosity (0.2-0.4 m) in the modified ridge treatment (40.8%) was twice that registered in normal ridge treatments (23.2%). This difference was due to the presence of substrate in the mid-layer of the soil. Substrate not only eased water movement through the soil profile but also increased potential aeration of the root zone. Soil aeration is one of the major constraints on crop production in

irrigated soils (Bhattarai et al., 2008), since a lack of oxygen reduces plant water and nutrient uptake which, in turn, has a negative impact on crop growth and yield (Jackson et al., 2008).

#### 3.2. Crop response

Differences between treatments were observed for both trunk and canopy growth, following the same pattern (Table II.2). Trees under MR-SDI-70 showed greater vegetative growth, with an average increase in trunk diameter of 28% and in canopy area of 43%, compared to the R-SDI-70 treatment. Subsurface irrigation produced a reduction in the amount of applied water without affecting tree growth. In some cases, SDI improved crop performance, with greater increases in both the canopy area and trunk diameter of the R-SDI-70 trees (0.7 m<sup>2</sup> and 26.1 mm) than in the R-DI-70 trees (0.5 m<sup>2</sup> and 20.7 mm). The trees under the subsurface irrigation treatments (SDI) presented more favorable water status values because the SDI treatments had higher water potential values than the DI treatments. The results provided proof that subsurface irrigation was an appropriate technique to apply under these conditions, as there were no negative effects on crop growth or plant physiology. Similarly, Ayars et al. (1999) reported yield increases in many crops under SDI.

Table II.2. Trunk diameter increase ( $\Delta$ TD), canopy area (CA), midday stem water potential ( $\Psi$ s),
canopy temperature (CT) and canopy ratio (CR) for different treatments. The values are means. Initial
trunk diameter was used as a covariate for the CA test. Values followed by different letters indicate
significant differences according to the Tukey HSD test ( $P < 0.05$ ).

Treatment	ΔTD (mm)	CA (m <sup>2</sup> )	Ψs (MPa)	CT (°C)	CR (m² m-³)
R-DI-100	26.3 bc	0.7 b	- 0.8 b	26.7 a	0.27 c
R-DI-70	20.7 d	0.5 c	- 0.9 b	27.6 a	0.28 c
R-SDI-100	24.2 cd	0.7 bc	-0.6 a	27.2 a	0.25 c
R-SDI-70	26.1 bc	0.7 b	-0.7 a	26.6 a	0.39 b
R-SDI-70+AIR	29.9 ab	0.8 b	-0.7 a	26.7 a	0.41 b
MR-SDI-70	33.3 a	1.1 a	-0.7 a	25.6 b	0.56 a
Prob>F (treatment)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Injecting air through the irrigation system had no effect on crop performance since no significant differences were observed between the R-SDI-70 and R-SDI-70+AIR treatments for any of the plant parameters studied. It seems that there were no oxygen deficiencies in the soil root zone, or – if there were – these were not solved by air injection. Even so, a previous work (Bhattarai et al., 2008) had reported an enhanced crop response in aeration treatments. However, it is important to point out that those earlier experiments were performed with other crop systems and under different edaphoclimatic conditions.

Soil substrate amendment improved crop performance in many ways. In the case of growth parameters, the MR-SDI-70 trees presented the highest overall values

without negatively affecting crop water status ( $\Psi$ s)(Table II.2). The MR-SDI-70 trees also exhibited lower canopy temperature values compared to other treatments; this suggested that the transpiration rate was higher in the trees subjected to this treatment (Sharratt et al., 1983). Soil physical conditions determined crop performance. Substrate amendment reduced soil bulk density, improved water movement and increased potential aeration of the root zone which, in turn, enhanced the crop response. It seems that soil amendment with rice husk substrate improved soil conditions, providing a better soil environment for root activity and thereby resulted in better crop performance.

Canopy ratio (CR) in the SDI treatments with the 70% irrigation water requirement was greater than in the other treatments (Table II.2). Of these, the MR-SDI-70 treatment produced the highest CR value  $(0.56 \text{ m}^2 \text{ m}^{-3})$ ; this was double the CR value associated with the R-DI-100  $(0.27 \text{ m}^2 \text{ m}^{-3})$ , which would be considered standard irrigation practice. Similar results were obtained in a previous work (Pablo et al., 2007) in which SDI turned out to be the most efficient irrigation practice. Canopy ratio (CR) could be a good index to assess irrigation efficiency in multiannual crops. Water efficiency indexes are now becoming important for evaluating crop production since there is a global interest to use irrigation water more efficiently, especially in arid and semiarid areas.

# 4. Conclusions

Soil amendment with rice husk was the most effective technique, since biomass crop production and canopy ratio (CR) were both greater than under alternative systems. In addition, this research demonstrates that SDI makes a positive contribution to crop response, enhancing vegetative growth while it promotes water savings.

The application of these techniques is suitable for mitigating the effects of soils with limiting physical conditions. Localized applications of amendments, as proposed in this paper, imply an important reduction in application rates. It is important to consider an efficient use of by-products since there is a growing interest in industrial and agronomical exploitations.

# Chapter III. Use of organic mulch to enhance peach crop production and water-use efficiency under limiting soil conditions

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#### Abstract

A three-year field experiment was conducted to evaluate the effects of a low-cost organic mulch application in a commercial peach orchard under a ridge planting system. Three treatments were performed in 12 experimental units set up in the field using a randomized complete block design. The orchard was drip-irrigated. Mulch was applied in two treatments, which differed in fertigation (none vs. multi-nutrient fertigation), while the third treatment did not include mulch and served as a control. The treatments were compared in terms of effects on the physical properties of the soil, crop response, and water-use efficiency. Mulch treatment did not alter soil bulk density. However, it significantly improved the topsoil water infiltration rate (2.21 vs. 121.04 mm h<sup>-1</sup>), which in turn enhanced crop growth and fruit yield. The efficiency of irrigation water use in the mulch treatments was higher (42% and 64%) than in the control. On the basis of our results, we propose that mulch techniques should be considered as a beneficial practice to apply in fruit tree production under limiting soil conditions.

Keywords: fruit tree, compost, irrigation water use efficiency, limiting soil.

# 1. Introduction

There is a global need to increase food production while minimizing the amount of irrigation needed. In this regard, the relevance of water-use efficiency (WUE) in arid and semi-arid agricultural areas of world is of particular importance. In recent decades, drip irrigation (DI) systems have allowed a considerable increase in irrigation efficiency. Uniformity is one of the major indicators for the evaluation of DI systems, and soil water distribution homogeneity is the ultimate expression of irrigation uniformity (SongMei et al., 2009). Thus, guaranteeing the correct infiltration of irrigation water and its distribution within the soil is crucial to increase DI efficiency.

Fruit tree production is growing in the Mediterranean Basin, sometimes at expense of the transformation of marginal land. Consequently, limiting soils are now being used for crop production. Such soils are considerably constrained in terms of the infiltration, retention, and transmission of water, and also regarding, salinity, and/or nutrient content. A number of agronomical techniques can be used to improve soil productivity for agricultural purposes; however, many are ineffective or unsustainable. Mulching techniques have emerged in recent years to address these limitations and have given positive results in a variety of scenarios. An early study by Kumar et al. (1985) and other more recent studies (Al-Wahaibi et al., 2007; Diana et al., 2008; Kar and Kumar, 2007; Mubarak et al., 2009; Okonkwo et al., 2011; Zribi, 2013) reported the positive effects of organic mulch and amendments on the physical properties of soil, causing effects such an in increase in water retention and a decrease in evaporation.

Soil mulches are of particular interest for the purposes of organic crop production since they offer a means to improve not only the physical conditions of soil but also its nutrient content. Thus, mulch may serve to ameliorate soils with limiting conditions, thus facilitating crop production. Here we hypothesized that the use of organic compost such as mulch can improve the efficiency of irrigation water use in a fruit crop planted in limiting soils. The objective of this study was to assess the effects of mulch and fertigation in a peach crop (*Prunus persica* (L.) Batsch.) in terms of soil properties and crop response.

# 2. Materials and Methods

## 2.1. Experimental site

The trial was conducted in a commercial orchard of peach (*Prunus persica* (L.) Batsch. cv. Ryan Sun) grafted onto GF-677 and planted in 2010 in a ridge planting system, spaced 5 x 2.5 m, in the region of Los Monegros (Huesca, Spain). The climate is semi-arid Mediterranean, with an annual rainfall of 350 mm. Irrigation water, characterized by a low salt, sodium, and nitrate content, is taken from the Cinca irrigation canal. The soil is a Xeric Torriorthent (Soil Survey Staff, 1999)

developed from marls, with a silt loam texture (USDA; 23.1% sand, 50.7% silt and 26.2% clay). The soil is slightly saline ( $EC_e = 2.46 \text{ dS m}^{-1}$  at 25°C), with a pH of 8.9 and an organic matter content of 7.7 g kg<sup>-1</sup>.

#### 2.2. Experimental design and treatments applied

We evaluated the following three treatments: ridge planting system with no mulch and no fertigation (C); ridge planting system with mulch and no fertigation (M); and ridge planting system with mulch and fertigation (MF). The treatments were applied during 2010 and 2011. In order to study crop recovery, in 2012 (the third and final year of the trial) all the treatments received the same amount of mulch and fertigation. The experiment was organized in a randomized complete block design with four replications and 12 elementary plots. Each elementary plot had six trees. An automated drip fertigation system with auto-compensated emitters was implemented in function of the water requirements of the trees. Drip emitters supplying 0.97 mm h<sup>-1</sup> were spaced 0.33 m apart. Irrigation scheduling was calculated weekly using the FAO methodology (Allen et al., 1998). Meteorological data were acquired using an automatic weather station-property of the regional meteorological service—located 7.5 km from the experimental orchard. Fertigation was supplied during the whole crop cycle by applying a multi-nutrient solution, achieving a dose of 106 kg N ha<sup>-1</sup> year<sup>-1</sup>, 68 kg  $P_2O_5$  ha<sup>-1</sup> year<sup>-1</sup> and 170 kg  $K_2O$  ha<sup>-1</sup> <sup>1</sup> year<sup>-1</sup> at the end of the crop season.

Ridges were molded using soil already present in the orchard by means of a rear vblade attached to a tractor. Ridges were 1.5 m (bottom) and 1 m (top) wide and 0.8 m high. For the mulch treatments (M and MF) organic compost (Table III.1) was applied on the ridge surface at an application dose of 10 Mg ha<sup>-1</sup> year<sup>-1</sup>.

#### 2.3. Measurements

Soil bulk density was determined by the excavation method (Blake and Hartge, 1986), performing one measurement per plot for the C and M treatments, from the surface to a depth of 0.2 m. Water infiltration rate ( $v_i$ ) was measured at the ridge surface using the single ring infiltrometer method (Pla, 1983). Rings 0.25 m in diameter and 0.4 high were inserted into the soil at a depth of 0.15 m in order to prevent lateral seepage loss. Furthermore, a small soil dam was built around the cylinder. Water infiltration rate was measured once per plot. Soil electrical conductivity was measured in a sample taken at a depth of 0.2 m at a range of emitter distances (0, 0.8 and 1 m radius) at the beginning of the irrigation period (mid-May), performing one measurement per plot.

Tree growth was assessed by measuring the trunk diameter of two control trees per plot at the end of the season. To evaluate tree water status, stem water potential  $(\Psi_{stem})$  was determined at midday at the end of development stage III (end of July). For this purpose, we used a pressure chamber (model 3005; Soil Moisture

Equipment Corp., Santa Barbara, CA, USA) and followed the methods described by Shackel (Shackel et al., 1997).

Parameters	Method	Unit	Value
Humidity		%	40.98
Dry weight (d.w.)		%	59.02
pН			7.8
Specific conductivity		dS m <sup>-1</sup>	6.37
Organic matter	Muffle furnace (450 °C)	g kg⁻¹ d.w.	433.4
Organic carbon	-	g kg⁻¹ d.w.	216.7
Total organic nitrogen	Kjeldahl	g kg⁻¹ d.w.	16.7
C/N ratio			12.98
Ammoniacal nitrogen (NH4)		g kg⁻¹ d.w.	3.3
Phosphorus (P2O5)		g kg⁻¹ d.w.	71.5
Potassium (K2O)		g kg⁻¹ d.w.	10.4
Chrome (Cr)	ICP-Plasma	mg kg⁻¹	45.1
Cadmium (Cd)	ICP-Plasma	mg kg⁻¹	0.71
Lead (Pb)	ICP-Plasma	mg kg⁻¹	20.42
Copper (Cu)	ICP-Plasma	mg kg⁻¹	119.03
Zinc (Zn)	ICP-Plasma	mg kg⁻¹	316.16
Mercury (Hg)	ICP-Plasma	mg kg⁻¹	0.66
Nickel (Ni)	ICP-Plasma	mg kg⁻¹	36.46
Plastic materials		% d.w.	0.33
Salmonella sp.	ISO 16140	UFC g <sup>−1</sup>	none
Escherichia coli	UNE-EN ISO 9308-1:2001	UFC g <sup>-2</sup>	none

Table III.1. Main physical and chemical parameters of the compost used in the experiment.

A composite leaf sample was taken randomly from each plot on 19 July. Each sample contained 50 newly but fully developed mid-terminal leaves from current year shoots at a height of 1.5 m in the tree canopy. All samples were cleaned, oven-dried at 65 °C, and ground. Nitrogen (N) concentration was analyzed using the Kjeldahl procedure. Phosphorous (P) and potassium (K) concentrations were determined using an inductively coupled plasma mass spectrograph (IPC – MS; Agilent 7700X, Agilent Technologies, Santa Clara, CA, USA).

To determine yield and quality parameters, the crop from control trees was harvested manually. Concerning yield parameters, production ( $kg tree^{-1}$ ), crop load (CL; number of fruits per tree) and average fruit weight (FW; g fruit<sup>-1</sup>) were determined. Quality parameters such as fruit flesh firmness (FF, in N) and total soluble solids concentration (TSS, in °Brix) were determined in a sample of four fruits per tree using a manual penetrometer (Penefel, Agro Technologie, France) and a thermo-compensated refractometer (Atago Bussan Co., Tokyo, Japan),

respectively. The fruit dry matter concentration (*DMC*, in %) was measured in a sample of two fruits per tree using a forced-draft oven at 68°C.

The agricultural WUE index (García Tejero et al., 2011) was determined in order to assess the effect of the strategies tested. This index is based on the ratio between crop yield (economic yield) and total water applied:

WUEagr (kg m<sup>-3</sup>) = yield (economic) / (irrigation + rain)

A linear mixed model for analysis of estimated marginal means (EMM) was built to separate treatment effects. The Tukey Honestly Significant Difference (THSD) post hoc test was used to compare soil physical properties and crop response data across treatments. The treatment effects on the average fruit weight were evaluated by means of analysis of covariance (ANCOVA) using number of fruits per tree as a covariate. Statistical significance was set at p = 0.05.

# 3. Results

#### 3.1. Physical properties of the soil

Differences in the physical properties of the soil were detected among treatments (Table III.2). Infiltration rate (mm h<sup>-1</sup>) was found to be significantly different in the mulched treatment (P=0.0004, contrast test). We observed that it was 50 times greater than in the control treatment (2.21 vs. 121.04 mm h<sup>-1</sup>). Regarding the soil bulk density (0-0.2 m) we did not find significant differences between treatments, suggesting that the mulch did not alter the soil bulk density (P>0.05, contrast test). The soil bulk density for the treatments ranged from 1.79 Mg m<sup>-3</sup> for the control treatment and 1.66 Mg m<sup>-3</sup> for the mulched treatment.

Table III.2. Soil bulk density ( $\rho$ b), soil infiltration rate (vi), and electrical conductivity at a depth of 0.2 m at various emitter distances (0, 0.8 and 1 m). The values are means. Values followed by different letters indicate significant differences according to the Tukey HSD test (P <0.05).

Treatment	ρ₀ 0-0.2 m	v <sub>i</sub> at surface (mm h <sup>-1</sup> )		<b>EC</b> e	ECe	EC₀ 1 m (dSm <sup>-1</sup> )	
meatment	(Mg m <sup>-3</sup> )			0 m (dSm <sup>-1</sup> )	0.8 m (dSm <sup>-1</sup> )		
С	1.79	2.21	b	0.74	1.6	25.2	
М	1.66	121.04	а	0.80	1.6	30.8	
Prob>F	ns	0.000	)4	ns	ns	ns	

We did not observe significant differences (P>0.05, contrast test) between treatments regarding soil electrical conductivity at any emitter distance (0, 0.8 and 1 m). However, there were significant differences between emitter distances regardless of the applied treatment (P= 0.002). Soil electrical conductivity ranged from 0.74 to 25.2 dS m<sup>-1</sup> in the control treatment and from 0.80 to 30.8 dS m<sup>-1</sup> in the mulched treatment (0 and 1 m emitter distance). Soil salinity increased by more than 30 times within one-meter emitter distance.

#### 3.2. Crop response

The analysis of variance revealed differences among treatments with respect to crop growth, water status, and yield parameters (Table III.3). Differences in trunk diameter growth were observed in all the treatments in 2011 (P<0.0001) and 2012 (P=0.0016) seasons. The control treatment recorded the lowest values of trunk diameter in 2011 (40,5 mm) and 2012 (65.6 mm) seasons while the greatest values were observed in the MF treatment (mulch plus fertigation) for both seasons (57.6 and 81.8 mm in 2011 and 2012 seasons, respectively). Significant differences of trunk diameter growth were observed between M and MF treatments for both seasons (Tukey HSD test).

Table III.3. Trunk diameter in 2011 (TD 2011) and 2012 (TD 2012), midday stem water potential before harvest in 2011 (SWP 2011) and 2012 (SWP 2012), yield in 2011 (Yield 2011) and 2012 (Yield 2012), and WUE in 2012 (WUE 2012). The values are means. Values followed by different letters indicate significant differences according to the Tukey HSD test (P < 0.05).

Treeturent	TD 2	011	TD 20	)12	SWP 2	2011	SWP 2	012	Yield	2011	Yield	2012		r <b>2012</b>
Treatment	(mn	n)	(mn	1)	(MP	a)	(MP	a)	(kg tr	'ee <sup>-1</sup> )	(kg tr	ee <sup>-1</sup> )	(kg r	n-3)
С	40.5	С	65.6	С	-1.21	С	-0.63	С	0.9	b	18.7	b	1.91	b
М	53.1	b	73.9	b	-0.86	а	-0.59	b	1.7	b	26.6	а	2.72	а
MF	57.6	а	81.8	а	-1.05	b	-0.54	а	2.9	а	30.8	а	3.14	а
Prob>F	< 0.0	001	0.00	16	<0.00	01	<0.00	01	0.00	005	0.00	07	0.00	07

There were significant differences between treatments regarding midday stem water potential (SWP) before harvest in 2011 (P<0.0001) and 2012 (P<0.0001). The lowest values of midday SWP were observed in the control treatment for both seasons (-1.21 and -0.63 MPa). Regarding yield, significant differences were observed between treatments in 2011 (P=0.0005) and 2012 (P=0.0007) seasons. While the MF treatment recorded the highest yield in 2011 (2.9 kg tree<sup>-1</sup>) we did not observe significant differences between the control and the M treatment. However, it is important to mention that overall, the yield values, can be considered to be commercially low for all the treatments in 2011 (less than 2 500 kg ha<sup>-1</sup>). In 2012, significant yield differences were observed between the control and the mulched treatments (P=0.0007, Tukey HSD test). The mulched treatments (M and MF) recorded the highest yield values with no significant differences between them regardless the chemical fertigation. The M treatment increased the yield by 40% in average as compared to the control treatment (from 18.7 to 26.6 kg tree<sup>-1</sup>). There were significant differences between treatments regarding the agricultural water use efficiency (P=0.0007). The WUE<sub>agr</sub> in the MF treatment was 64% higher than in the control treatment.

Significant differences between treatments were observed regarding leaf nutrient concentrations (Table III.4). While the MF treatment recorded the highest leaf nutrient (N, P and K) concentrations, the lowest values were observed in the control treatment. Significant differences were observed for N concentration

between M and MF treatments (P = 0.001, Tukey HSD test). There were significant differences for P and K concentrations between the control and both the mulched treatments.

Table III.4. Nutrient concentration in peach leaves at the end of the second experimental year (July 2011). The values are means. Values followed by different letters indicate significant differences according to the Tukey HSD test (P < 0.05).

Nutrient	Treatment						
Nuthent	C	М	MF	Prob > F			
NI (9/ )	1.79	2.17	3.03	0.001			
N (%)	b	b	а	0.001			
D (0()	0.11	0.17	0.19	0.0006			
P (%)	b	а	а	0.0006			
K (0()	1.41	1.96	2.12	0.0005			
K (%)	b	а	а	0.0095			

We observed significant differences between treatments for many quality parameters both in 2011 and 2012 seasons (Table III.5). While dry matter concentration was found to be the highest in the control treatment (P=0.0042, Tukey HSD test) we did not observe significant differences between treatments regarding this parameter in 2012. The MF treatment recorded the lowest values of TSS among all the treatments both in 2011 and 2012 seasons (P<0.0001 and P=0.0002, respectively). In 2011, the control treatment recorded 16 °Brix, 2.5 °Brix more in average than the MF treatment. In 2012 such difference was reduced by 1.2 °Brix. We observed significant differences between the control and the mulched treatments regarding the fruit firmness both in 2011 and 2012. While we observed that the lowest values of FF in 2011 were found in the control treatment, the next season the same treatment recorded the highest FF values (P=0.0003).

Table III.5. Dry matter concentration of the fruit (DMC), total soluble solids content (TSS) and fruit flesh firmness (FF) values for the 2011 and 2012 crop production. The values are means. Values followed by different letters indicate significant differences according to the Tukey HSD test (P <0.05).

Trestment			2011 - TSS		2011 - FF		2012 - DMC	2012 - TSS		2012 - FF	
Treatment	(%	<b>)</b>	(°Br	ix)	(N	)	(%)	(°Br	ix)	(N	)
С	20.4	а	16	а	48.7	b	13.6	13.1	а	58.7	а
М	17.5	b	14.1	b	72.0	а	13.8	12.6	а	54.7	b
MF	16.9	b	13.5	b	71.5	а	13.1	11.9	b	54.6	b
Prob>F	0.00	)42	<0.00	001	<0.0	001	ns	0.00	02	0.00	03

The relationship between the average fruit weight (FW, in g fruit<sup>-1</sup>) and the average number of fruits per tree (CL) for the different treatments can be observed in the Figure III.1 (season 2011) and Figure III.2 (season 2012). By analyzing these pictures it was possible to observe both the distribution of these variables (FW and

CL) within the treatments and the existence of significant differences regarding these variables between the treatments (ANCOVA analysis for FW, ANOVA analysis for CL).

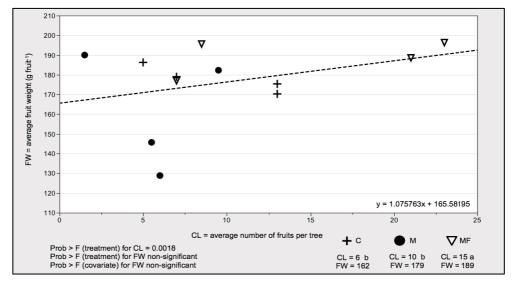


Figure III.1. Average crop load (CL) and average fruit weight (FW) for the 2011 crop production in different plots. The values are means. Values followed by different letters indicate significant differences according to the Tukey HSD test (P<0.05). Average fruit weight (FW) data was evaluated by means of analysis of covariance (ANCOVA) using number of fruits per tree as a covariate.

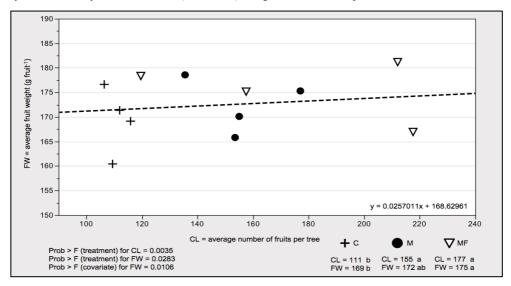


Figure III.2. Figure III.2. Average crop load (CL) and average fruit weight (FW) for 2012 crop production in different plots. The values are means. Values followed by different letters indicate significant differences according to the Tukey HSD test (P < 0.05). Average fruit weight (FW) data was evaluated by means of analysis of covariance (ANCOVA) using number of fruits per tree as a covariate.

#### Chapter III

We observed significant differences between treatments regarding the average fruit weight for both seasons (2011 and 2012). While the MF treatment recorded the greatest values of FW, the control treatment had the lowest values, both in 2011 (P=0.0018, Tukey HSD test) and 2012 season (P=0.0283, Tukey HSD test). There were no significant differences between treatments regarding crop load in 2011. However, crop load differences were significant in 2012 (P=0.0283). Regarding this variable, we observed an increase of 60% of fruits per tree in average when both mulch and chemical fertigation was applied (C vs. MF treatment).

# 4. Discussion

#### 4.1. Physical properties of the soil

While topsoil bulk density (0–0.2 m) was similar among treatments, the water infiltration rate differed. Although the mulch covered the whole ridge surface, it affected only the first millimeters of the soil, preventing soil crust formation and deterioration of topsoil structure. These results indicate that mulch did not affect the soil bulk density, although the water infiltration rate improved considerably (2.21 vs. 121.04 mm h<sup>-1</sup>), thereby pointing to a better water distribution. Similarly, (Merwin and Stiles, 1994) reported significant differences in the hydraulic properties of soil in various groundcover management systems. After a 6-year trial, those authors found that both the cumulative infiltration of soil water and water sorptivity were enhanced after organic mulch treatment, while the soil bulk density remained unaltered. It therefore appears that the hydraulic properties of soil can be modified, depending on factors such as soil type, mulching material, or mulch application dose.

Recent decades have witnessed the conversion of marginal areas into agricultural land in the Mediterranean Basin. This change has brought about drastic changes in crop management and soil tillage techniques, as well as mechanical land conditioning. Soil adequacy techniques lead to a considerable deterioration of the physical properties of soil, which in turn triggers soil degradation and erosion (Nacci, 2001). Here we show that the application of organic mulch impeded both soil crusting and soil sealing, which in turn enhanced the water infiltration rate and consequently reduced surface runoff and ridge soil degradation.

Many studies performed with ridge planting systems have reported enhanced soil physical properties, resulting in improved performance in many crops (Lordan et al., 2013; Roth et al., 2005). We found that soil salinity around the drip-wetted area was considerably lower than that at the outer area of the ridge (in C treatment,  $EC_e$  at 0 m = 0.8 dS m<sup>-1</sup> vs.  $EC_e$  at 0.8 m = 30.8 dS m<sup>-1</sup>) (Table II.2). However, no differences were found between treatments, with a suitable non-saline environment being available in the root zone of all the treatments. Ridge systems provide a better root environment, reduced water logging, and increased irrigation efficiency (Khan et al., 2010). However, they clearly reduce the drip infiltration surface. We

observed that this parameter was reduced by 80 % compared with a non-ridge system (0.33 m<sup>2</sup> dripper<sup>-1</sup> vs. 1.65 m<sup>2</sup> dripper<sup>-1</sup>). This reduction may pose an irrigation management issue when working with drip irrigation systems in which water is applied at high frequency. As Currie (2007) reported, these phenomena may lead to high soil degradation and ultimately to low irrigation efficiency. especially in limiting soil conditions. Ridge planting systems may facilitate crop production under such conditions, although the system itself may reduce the drip infiltration surface, leading to soil degradation problems and low irrigation efficiencies. Under these conditions, mulch techniques may provide an effective remedy since its application causes a decrease in soil crusting and sealing and a notably increase in the hydraulic properties of soil. Crust formation is a major problem in many agricultural soils in arid and semi-arid areas of the Mediterranean Basin. While irrigation is needed to achieve crop production, this practice can lead to soil degradation when applied on a regular basis. Unstable aggregates disintegrate during rainstorms or irrigation periods. Dispersed soil particles fill surface pores and a hard physical crust can develop when the soil dries. Soil crusting causes a drastic reduction in water infiltration, which in turn results in increased runoff and water erosion, and reduced water availability for the crop.

## 4.2. Crop response

Trees under the M treatment showed greater growth compared to the C treatment, with an increase of 37% and 13% in 2011 and 2012, respectively. Trees under the MF treatment achieved the greatest growth among all the treatments, with an increase of 9% and 11% compared to the M treatment in 2011 and 2012, respectively. As shown in the 2011 results, mulch was responsible for the greater part of increase in tree growth, although chemical fertigation contributed, as reflected by a growth difference between the M and MF treatments. According to the leaf nutrition analysis (Table III.4), fertigation led to an increase in N uptake since leaf N concentration in the MF treatment (3.3%) was greater than that of the M and C treatments (2.2 and 1.8%, respectively). N content values below 2.4% in a peach crop may be indicative of N deficiency (Johnson, 2008), while values around 2.5 and above are found to be normal in late peach cultivars under temperate conditions (Rufat and DeJong, 2001). Leaf P and K concentrations were greater in the M and MF treatments than in C. In the latter treatment, these parameters (0.11 and 1.41% for P and K, respectively) were in a deficiency range for a peach crop (Johnson, 2008).

In 2012 all the treatments received the same amount of mulch and fertigation. We observed a tree growth recovery for the C treatment. However, after two years of differential treatment (2010 and 2011), differences were still apparent.

Midday stem water potential (SWP) before harvest is a good indicator of crop water status (Shackel et al., 1997). Both in 2011 and 2012, trees in the M and MF treatments showed a more favorable water status (Table III.3) since they had higher water potential values than those not receiving mulch (C treatment).

Furthermore, of note, the SWP values in 2011 were slightly lower than those in 2012, suggesting that the crop was under a more favorable water status in the last year. The physical properties of ridges may improve progressively, year by year, as a result of periodical organic mulch applications (10 Mg ha<sup>-1</sup> year<sup>-1</sup>), crop root activity, and irrigation management. Similarly, Rubauskis et al. (2004) and Yin et al. (2012) found that mulch application improved tree water status of apple and sweet cherry crops, respectively. Under our experimental conditions, mulch enhanced crop growth and water status.

Similarly, Merwin and Stiles (1994) and Okonkwo et al. (2011) showed that mulch applications improved soil water retention and infiltration, which in turn enhanced crop response. Mulch application at a rate of 10 Mg ha<sup>-1</sup> year<sup>-1</sup> may allow an ammoniacal N supply of 20 kg ha<sup>-1</sup> year<sup>-1</sup> (estimated from Table III.1 data). Since mulch is applied on the soil surface and most of the nitrogen is in an ammoniacal form, much of it may be lost by volatization (Teira Esmatges, 1998). Under these agronomic conditions, crop N requirements might be around 60 and 120 kg N ha<sup>-1</sup> year<sup>-1</sup> (Rufat et al., 2010). Therefore a N supply of 20 kg ha<sup>-1</sup> year<sup>-1</sup>, as best-case scenario, would not cover total crop needs. Mineralization of the compost organic matter may provide an additional supply of this nutrient, although it could be considered a long-term supply since rate of mineralization is slow under semi-arid conditions (Teira Esmatges, 1998). For instance, Merwin and Stiles (1994) demonstrated that organic mulch applications improve tree growth and yield. However, those authors reported that, under their experimental conditions, supplemental fertilizers were required to provide essential elements.

Regarding production parameters (Table III.3), trees in the MF treatment gave the highest yield in 2011. The difference between the yield in the MF treatment with respect to the M and C treatment was attributed to the number of fruits since average fruit weight was similar among treatments (Figure III.1). The relationship between average fruit weight and crop load (number of fruits per tree) is shown in Figure III.1 and Figure III.2 for 2011 and 2012, respectively. Average fruit weight was not affected by average number of fruits per tree since the number of fruits per tree as a covariate was found to be non-significant by ANCOVA. However, it is important to consider that in 2011 the overall fruit production was low for all treatments. In 2012 the recovery in yield attributed to mulch application and fertigation was notable since there were no significant differences between MF and M treatments. However, the lowest yield was obtained in the C treatment. In 2012, vield differences were attributed to the number of fruits per tree and average fruit weight, which were greater in M and MF treatments (Figure III.2). However, in 2012, the average fruit weight was affected by crop load, as it was found to be significant in the ANCOVA test (P = 0.0220). The average fruit weight in 2012 was less variable among treatments (FW<sub>2012</sub> =  $172.4 \pm 5.9$  g fruit<sup>-1</sup> vs. FW<sub>2011</sub> =  $176.3 \pm 24.0$  g fruit<sup>-1</sup>). Crop yield was notably improved by mulch and fertigation.

Fruit quality parameters differed among treatments. Fruit dry matter concentration for the C treatment was greater than that of M and MF treatments, suggesting that

in 2011 the fruits from the C treatment were partially dehydrated (Table III.5). This finding is consistent with other studies that reported increases in peach dry matter content under high water stress conditions (Lopez et al., 2007; Marsal et al., 2006). Furthermore, fruits from the C treatment presented a higher total soluble solids concentration and lower flesh firmness values than fruits treated with mulch in 2011. These lower flesh firmness values could be attributed to water stress (Table III.3), as previous studies have reported accelerated fruit maturation under deficit irrigation (Gelly et al., 2004; Naor, 2006). In addition, high values of total soluble solids under the C treatment could be related to water stress during the final stages of fruit development (stage II and III) or may be caused by a lower individual fruit weight and a higher dry matter content, as proposed by Rufat et al. (2010).

In 2012 there were differences in total soluble solids and fruit firmness, although dry matter content was similar among all the treatments, suggesting that water status did not show great differences between treatments. However, the soil in the C treatment still had a worse water status than that of the other treatments (Table III.3). This may have affected the total soluble solids in the fruit. The fruit firmness registered in the C treatment was higher than that of fruit from the M and MF treatment, although the values of this parameter varied in a narrow range, from 58.7 N (C treatment) to 54.7 and 54.6 N (M and MF treatments). We conclude that in 2012 all the treatments were under mild water stress (Table III.3), which may have affected various quality parameters (TSS and FF), although it did not affect fruit dry matter content.

Regarding irrigation efficiency, mulch treatments (M and MF) showed the highest agricultural WUE values (kg m<sup>-3</sup>). These values were significantly greater than those obtained in the C treatment (WUE<sub>agr</sub> C = 1.91 vs. WUE<sub>agr</sub> M = 2.72 vs. WUE<sub>agr</sub> MF = 3.14). Agricultural WUE was calculated for the third experimental year since it was considered to be the first year at full fruit production. Differences were evident in spite of the three treatments receiving the same amount of fertilizer and mulch in the third year. These differences in WUE could be attributed to the fact that during the two differential years the fruit yield potential differed between treatments, consequently affecting fruit yield and WUE in the third year. However, our results demonstrate that mulch enhanced both fruit yield and irrigation efficiency, as reported in other crops (Mukherjee et al., 2012; Zhao et al., 2012).

Our findings support the use of the ridge planting system for crop production under limiting soil conditions. Many producers in the Mediterranean area are shifting to this system. However, ridge planting techniques reduce the drip infiltration potential. On the basis of our results, we propose that mulching techniques can provide an effective solution to this drawback. Such techniques were observed to significantly improve the physical properties of the topsoil, which in turn enhanced the hydraulic properties of the matrix and improved crop response. Mulch improved crop growth, fruit yield, and also WUE. Moreover, mulch had a greater effect on crop response than fertigation. This observation highlights the relevance of soil factors on crop performance. In addition, our results reveal the importance

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of an early application of mulch techniques, preferably during crop establishment. As shown in this study, mulch techniques enhanced crop precocity, as mulched trees reached maturity earlier.

This work advocate that there is a need to find more efficient and soil sustainable crop production techniques to apply under limiting soil conditions and encourages further research concerning this issue.

# 5. Supplementary material



Figure III.3. Tree from M treatment (left) and tree from C treatment (right) on April 2010 (J.M. Villar).

# **Chapter IV.** Effect of tree water status and nitrogen nutrition on polyphenol oxidase activity in "Extrem Sunny" nectarine

Paper submitted to HortScience by Lordan J., Pascual M., Villar J.M., Fonseca F., Falguera V., Rufat J.

#### Abstract

This work studied the relationship between nitrogen and water status, and fruit quality parameters in a commercial nectarine orchard (*Prunus persica* (L.) Batsch. var. nectarine cv. Extreme Sunny). Deficit irrigation treatments were established in order to assess the crop response in terms of tree water status and yield parameters. Polyphenol oxidase (PPO) fruit activity was analyzed along different dates before and after harvest. It was found that tree water status was highly linked to PPO activity, and consequently to fruit quality parameters. Results provided evidence that water stress enhanced polyphenol oxidase activity in "Extreme Sunny" nectarines. Tree nitrogen content was also linked to PPO activity, as leaf nitrogen content was highly correlated with this enzyme activity. It was possible to build a reliable prediction model for PPO activity by using water status and nitrogen content variables.

**Keywords**: water stress; peach; fruit quality; subsurface drip irrigation; nutrition status.

# 1. Introduction

Water is becoming an increasingly scarce resource for horticultural production. Besides the need for irrigating new agricultural land, climate variability is resulting in severe drought episodes in many regions of the world (Thakur and Singh, 2012). Regulated deficit irrigation (RDI) strategies are being optimized to help solving this problem. RDI imposes a moderate water stress to reduce vegetative growth and reduce water use at a given crop stage without affecting fruit yield (Girona et al., 2005a).

Micro irrigation systems have extended all over the world in the last decades, among them, drip irrigation is currently leading the market due to its high efficiency, management simplicity and high adaptability to a wide range of conditions and crop systems (Bryla et al., 2003). However, surface drip irrigation presents important constraints in farmlands with limiting soil conditions. Solutions to overcome these handicaps include different irrigation and soil management strategies. On the one hand, subsurface drip irrigation (SDI) improves the efficiency of water use and nutrient uptake and reduces water loss due to surface evaporation, weed germination and the possibility of interference between drip tubing and cultural operations (Ayars et al., 1999; Diamantopoulos and Elmaloglou, 2012). On the other hand, when soil physical properties are the most important problem for crop growth and development, the use of amendments allows reducing crop evapotranspiration, maintaining soil moisture and improving root activity (Lordan et al., 2013). Soil amendments may also be used together with SDI, achieving good results for both crop productivity and water saving (Al-Busaidi et al., 2011).

In certain soils, burying the pipes used for SDI may also result in other problems. Since emitters are deeply placed, sustained wetting fronts may appear and cause hypoxia of the rhizosphere (Bhattarai et al., 2008). This issue is particularly important in salt affected soils, where sodicity may reduce soil porosity and oxygen solubility. Root hypoxia leads to ethylene accumulation, and bacterial anaerobic metabolites can impair the metabolism processes associated with root membrane structure and function (Bhattarai and Midmore, 2009). The use of aerated irrigation water (or oxygation) with SDI can avoid hypoxia either by injecting air by means of the Venturi principle or by supplying hydrogen peroxide to the root zone (Chen et al., 2010).

The effects of RDI on peach fruit quality depend on several factors such as the cultivar, the deficit level or the moment at which it is applied. Lopez et al. (2011) found that severe levels of water stress before harvest reduced "Ryan's Sun" peach sensorial quality, while consumer liking was improved if no deficit was applied just before harvest. Vallverdu et al. (2012) reported a better consumer acceptance of "Tardibelle" peaches in which RDI had been applied during stage II of fruit development when compared with full irrigation. However, these authors

concluded that this preference might have been due to a more advanced maturity. From a commercial standpoint, it is essential to know if this more advanced maturity also entails lower durability.

In spite of the fact that several authors have stated this influence of water stress on the ripening process, there are no references reporting the effects of different water status on essential biochemical parameters such as polyphenol oxidase (PPO) activity in peach or nectarine. PPO activity is a major limitation to fruit handling and processing, and therefore one of the main factors that reduce postharvest durability (Falguera et al., 2012b). As stated by Pascual et al. (2013) pre-harvest factors such as fertilization can affect the PPO activity so it is crucial to understand the relationship between these pre-harvest factors and the postharvest abilities of the fruits. The aim of this study was to find the relationship between water and nutrition status with the PPO activity in nectarine. For this purpose, an experiment was set up in a nectarine orchard (cv. Extreme Sunny) with different irrigation and nutrition levels and its effects to the tree physiology and the fruit quality were studied.

# 2. Materials and Methods

## 2.1. Experimental site

The trial was conducted during 2012 in a commercial orchard of peach (Prunus persica (L.) Batsch. var. nectarine cv. Extreme Sunny) grafted onto GF-677 rootstock and planted in 2009 in a ridge system, spaced 4.5 x 2.5 m, in Aitona (Lleida, Spain). The climate is semiarid Mediterranean, with an annual rainfall of 360 mm. Irrigation water that comes from the Aragón and Catalunya irrigation canal, has low salinity, sodium and nitrate contents. The soil, moderately deep and calcareous, is slightly saline ( $EC_e = 3.2 \text{ dS m}^{-1}$ ), has a silty loam texture, a pH of 8.7 and an organic matter content of 21.0 mg kg<sup>-1</sup>.

### 2.2. Experimental design and applied treatments

The experiment was organized in a randomized complete block design with four replications. Each plot contained eight trees, from which the four central ones were used for measurements. Six soil and irrigation management treatments were evaluated: ridge planting with drip irrigation at 100% of the calculated irrigation requirement, as the control treatment (R-100); ridge planting with drip irrigation at 70% of the calculated irrigation requirement (R-DI-70); ridge planting with subsurface drip irrigation at 70% of the calculated irrigation at 70% of the calculated irrigation requirement (R-SDI-70); ridge planting with subsurface drip irrigation at 70% of the calculated irrigation requirement and air injection through the irrigation system (R-SDI-70+AIR); ridge planting with subsurface drip irrigation at 70% of the calculated irrigation system (R-SDI-70+HP); and modified ridge planting with subsurface drip

irrigation at 70% of the calculated irrigation requirement (MR-SDI-70). All the treatments were continuously fertigated (in every irrigation cycle) by using a Hoagland solution (Hoagland and Arnon, 1950) with nutrient concentrations (mg  $L^{-1}$ ) N 145, Ca 1.4, Mg 17.9, P 9.9, K 172.2, S 25.1, Cu 0.23, B 0.08, Fe 5.60, Mn 0.60, Mo 0.02 and Zn 0.23, what means that irrigation treatment with 70% dose received 70% of fertilization at the end of the fertigation season.

The irrigation water requirement was calculated using the FAO methodology (Allen et al., 1998). Air injection (for R-SDI-70+AIR) was performed with a venturi air injector (MAI-A3, Toro Ag. Irrigation, CA, USA) at a rate of 15 % v/v. Hydrogen peroxide injection was performed with an electromagnetic dosing pump (Dositec-MP, ITC S.L., Spain) at a rate of 0.01% v/v. The ridges were all molded using soil already present in the field by the use of a rear v-blade attached to a tractor. The modified ridge treatment (for MR-SDI-70) included a 1:1 mix of soil and rice husk applied at depths of between 0.2 and 0.4 m. Substrate density and porosity were 0.77 Mg m<sup>-3</sup> and 60%, respectively. Ridge dimensions were 1.3 m (bottom) and 1 m (top) wide and 0.7 m high. In subsurface irrigation treatments, the dripperline was buried at a depth of 0.25 – 0.3 m below the surface and was placed at a distance of 0.15 – 0.2 m from each tree trunk.

### 2.3. Meteorological, growth and water status measurements

Meteorological data were acquired using an automatic weather station located at the experimental orchard (Decagon Devices Inc., Pullman, WA, USA). Irrigation water was measured by means of a pulse water meter (MNK-I-N Zenner GmbH & Co. KG, Saarbrücken, Germany), which was connected to a data logger (EM-50, Decagon Devices Inc., Pullman, WA, USA). One water meter was used per treatment.

To evaluate tree water status, midday stem water potential (SWP) was determined. This was done using a pressure chamber (model 3005; Soil Moisture Equipment Corp., Santa Barbara, CA, USA) following the Shackel method (Shackel et al., 1997). Measurements were taken at midday on July 19<sup>th</sup>. Canopy temperature (CT) was also determined at the same time on July 19<sup>th</sup> using a thermal camera and specific software (Ti25 and SmartView 2.1, Fluke Corp., Everett, WA, USA). Water status measurements were performed the same day in which the leaf and fruit samples for nutrient analysis were collected, and about two weeks before the estimated harvest date.

## 2.4. Leaf and fruit sampling and nutrient analysis

A leaf sample was taken randomly from each plot on July 19<sup>th</sup>. Each leaf sample contained 50 newly fully developed mid-terminal leaves from current year shoots at 1.5 m high in the tree canopy. A sample of five fruits per plot was taken at harvest time. All samples were cleaned, oven-dried at 60 °C, and ground. Leaf nitrogen (N) concentration was analysed by a Kjeldahl procedure. Leaf

phosphorous (P), leaf potassium (K), leaf calcium (Ca), fruit nitrogen (N) and fruit calcium (Ca) concentration were extracted from dry-ashed samples in ammonium acetate, and concentrations on a dry weight basis were determined by using an inductively coupled plasma mass spectrograph (Agilent 7700X, Agilent Technologies, Santa Clara, CA, USA).

#### 2.5. Physical, chemical and enzymatic fruit analyses

Four control trees per plot were manually harvested on August 7<sup>th</sup> in order to determine both yield and quality parameters. Concerning yield parameters, three commercial yield categories were established based upon fruit size: yield 1, with fruit diameters equal or greater than 70 mm (in kg ha<sup>-1</sup>); yield 2, with fruit diameters smaller than 70 mm and equal or greater than 65 mm (in kg ha<sup>-1</sup>); yield 3, with fruit diameters smaller than 65 mm (in kg ha<sup>-1</sup>). Fruit quality parameters were determined in a fruit sample (5 fruits per plot) taken at the harvest date. Mesocarp firmness (FF) was determined with a manual penetrometer (Copa-Technologie, Ctifl, France). Fruit weight (FW) was also measured. Soluble solids (SS) concentration (°Brix) was measured using a thermocompensated refractometer (Atago Bussan Co., Tokyo, Japan). Skin color was measured with a Chroma Meter CR-400 tristimulus colorimeter (Konica Minolta Sensing, Inc., Japan) in the CIELab color space. Parameters a\*, b\* and L\* were determined. Fruit consistency was measured by using a Bostwick consistometer (Central Scientific Co., Alexandria, VA) in centimeters per flow of fruit purée per 30 seconds. Polyphenol oxidase (PPO) activity was determined in the fruit samples (five per plot) that were taken on July 12<sup>th</sup>, July 24<sup>th</sup>, July 30<sup>th</sup> and August 7<sup>th</sup> (harvest date), 2012. Moreover, an additional set of samples was stored one week at 0 °C, and therefore analyzed on August 14th. PPO activity was measured as described by Falguera et al. (2012b), using 4-methylcatechol as substrate and recording the absorbance at 420 nm by means of a Helios Omega spectrophotometer (Thermo Fisher Scientific Inc., Waltham, USA) and its software. One unit (U) of PPO was defined as the amount of enzyme that caused the increase of one absorbance unit (AU) in 1 min.

#### 2.6. Statistical analysis

Analysis of variance and stepwise regression models were carried out using the SAS-STAT package v. 9.2 (SAS®, SAS Institute Inc., Cary, NC, 1989-2009). In addition, multivariate projection techniques based on Principal Component Analysis were performed by means of The Unscrambler v. 10.1 (Camo Process AS, Oslo, Norway). The PCA analysis contained several variables with different scale values, which were standardized by means of dividing each variable for its standard deviation The data were centered before the PC projection of the information and the PCA model was validated using the Full Cross Validation method.

# 3. Results and discussion

## 3.1. Water status and polyphenol oxidase activity

The variability generated by means of the six treatments resulted in different levels of water stress. Stem water potential ranged from -1.3 MPa in the modified ridge plots to -1.8 MPa in the samples with SDI-70 with hydrogen peroxide (Table IV.1). Lordan et al. (2013) found that differences of 0.3 MPa in stem water potential between samples (half of the maximum differences that have been found in this study) significantly affected tree growth measured as trunk diameter increase and canopy area. Likewise, water status could also be assessed by means of canopy temperature, since the less stressed trees had higher transpiration rates and therefore lower temperatures (Sharratt et al., 1983). In this case, the samples in which the lowest SWP was measured had indeed the highest temperature (36.5 °C). while the plants with the best status had a temperature 3.4 °C lower in average. The only difference between the behaviors of both parameters was found in the samples that received full irrigation (R-DI-100), which had also low temperature (statistically equal to that of the less stressed plants) but intermediate values of SWP.

Table IV.1. ANOVA results for stem water potential (SWP), canopy temperature (CT) and nitrogen leaf content (Leaf N) three weeks before harvest fruit nitrogen content (Fruit N) and polyphenol oxidase activity at harvest (08/07) and after one week of cold storage (08/14).

	SWP 07/19 (MPa)	CT 07/19 (°C)	Leaf N (ppm)	Fruit N (ppm)	PPO 08/07 (U/mL)	PPO 08/14 (U/mL)
R-DI-70	-1.5 <sup>ab</sup>	35.5 <sup>ab</sup>	3.2 <sup>a</sup>	1.3	0.404 a	0.433 <sup>a</sup>
R-SDI-70+HP	-1.8 <sup>b</sup>	36.5ª	3.2 <sup>.a</sup>	0.9	0.403 a	0.408 <sup>a</sup>
R-100	-1.5 <sup>ab</sup>	34.0 °	3.1 ª	1.2	0.400 <sup>a</sup>	0.397 <sup>a</sup>
R-SDI-70+AIR	-1.5 <sup>ab</sup>	35.6 <sup>ab</sup>	3.0 <sup>a</sup>	0.9	0.380 ª	0.381 ª
R-SDI-70	-1.5 <sup>ab</sup>	35.2 <sup>b</sup>	3.1 ª	1.0	0.360 ª	0.408 a
MR-SDI-70	-1.3ª	33.1 °	2.4 <sup>b</sup>	1.0	0.285 <sup>b</sup>	0.298 <sup>b</sup>
Treatment (p>F)	0.0072	0.0003	0.0008	n.s.	0.0365	0.0106
Block (p>F)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Leaf nitrogen content was found to be lower in MR-SDI-70 (2.4 %) as compared to other treatments (3.0-3.2 %). However, nitrogen leaf contents around 2.5 are found to be normal in late peach cultivars under temperate conditions (Rufat and DeJong, 2001). Furthermore, fruit nitrogen content was found to be similar among treatments, suggesting no nitrogen crop deficiencies. As far as polyphenol oxidase activity is concerned, the analysis of variance for the samples taken on July 12<sup>th</sup> showed that at this date there were no significant differences among treatments (which appeared for the first time on July 24<sup>th</sup>; data not shown). The lowest values at harvest (August 7<sup>th</sup>) were found in MR-SDI-70 trees that were significantly less stressed in terms of stem water potential and canopy temperature (the same behavior was detected after one week of cold storage, on August 14<sup>th</sup>). Meanwhile, the other treatments were not found to be different. Since the analysis of variance

#### Chapter IV

for skin color parameters a\*, b\* and L\* and soluble solids content at harvest resulted in non-significant models (Table IV.2), this fact leads to the conclusion that water stress enhanced polyphenol oxidase activity in "Extreme Sunny" nectarines. Parimala and Muthuchelian (2010) also described an increase in PPO activity of cotton cultivars subjected to short-term drought stress, arguing that stress conditions fuelled the synthesis of antioxidant enzymes to cope with an increased concentration of reactive oxygen species (ROS). PPO increase due to salt stress has been reported in different species such as flax (Emam and Helal, 2008), soybean (Weisany et al., 2012) or ginseng (Sabir et al., 2012), while waterlogging stress has also been shown to cause the same effect in pigeon pea (Bansal and Srivastava, 2012). In general, when plants have to face stressful conditions, morphological, physiological and biochemical responses are activated to increase their ability to survive, including different mechanisms such as certain solutes accumulation or enhanced antioxidant enzymes activity (Yin et al., 2005). And plant PPO intervenes in several processes such as oxygen scavenging (Queiroz et al., 2008) and defense mechanisms against herbivores (Constabel et al., 2000) and viruses (Li and Steffens, 2002).

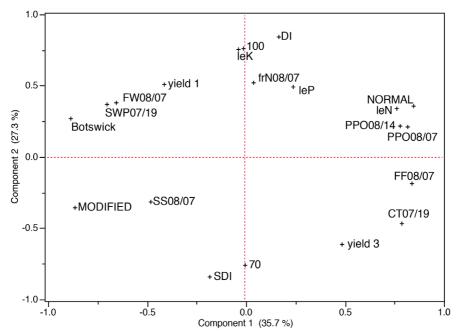
Table IV.2. ANOVA results for soluble solids (SS), skin CIELab parameters, fruit firmness (FF) and
fruit weight (FW) at harvest (08/07).

	SS 08/07	Skin L* 08/07	Skin a* 08/07	Skin b* 08/07	FF 08/07	FW 08/07
7	°Brix	-	-	-	kg/cm <sup>2</sup>	g
R-DI-70	17.9	49.5	17.3	30.5	4.5 ab	177.0
R-SDI-70+HP	18.0	46.6	16.4	27.1	5.2ª	157.7
R-100	17.2	48.8	19.9	29.2	3.9 <sup>ab</sup>	169.1
R-SDI-70+AIR	17.0	49.6	17.1	30.4	5.0 ª	174.3
R-SDI-70	17.4	50.4	19.1	31.1	4.4 <sup>ab</sup>	176.5
MR-SDI-70	20.4	51.1	17.6	33.7	2.6 <sup>b</sup>	182.8
Treatment (P>F)	n.s.	n.s.	n.s.	n.s.	0.0097	n.s.
Block (P>F)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Different superscripts within each column indicate significant differences (p<0.05).

#### 3.2. Relationships among variables: Principal Component Analysis

A Principal Component Analysis was carried out in order to check the relationship of stress parameters and PPO activity between them and among the other measured variables. Overall, the PCA analysis contained 20 variables with different scale values, which were standardized by means of dividing each variable for its standard deviation. The first Principal Component (PC 1) explained the 35.7% of the overall variance and the second one (PC 2) the 27.3%, which means that more than 63% of the overall variance was explained by two components. Other quality variables, such as CIELab parameters, and other treatment descriptors (e.g. oxygation) were not introduced in the final PCA analysis since they had a negligible weight as observed in previous PCA analysis that were carried out before. The loading plot of this analysis (Figure IV.1) and the correlation matrix (Table IV.3) showed that PPO activity at harvest date was indeed positively



correlated with canopy temperature (0.5059) and negatively correlated with SWP (-0.4540), both of them measured three weeks before harvest.

Figure IV.1. Loadings plot representing the two first Principal Components (PCA analysis). Abbreviations for variable names are defined in the *Materials and Methods* section.

The simplified correlation matrix (Table IV.3) showed that both SWP and canopy temperature were indeed highly correlated with the PPO at postharvest (PPO 08/14). These facts reinforce the idea that water status measurements could act as good indicators of PPO activity. In addition, leaf nitrogen content (also measured three weeks before harvest) also appeared highly correlated (0.7345) with PPO 08/14. Similarly, Olivos et al. (2012) stated that critical contents of leaf nitrogen were related to nectarine (cv. Grand Pearl) flesh browning due to PPO activity. PPO activity causes fruit flesh browning, which in turn can be triggered by a combination of factors such as soil fertilization and irrigation management (Crisosto et al., 1999). Falguera et al. (2012b) found that nitrogen fertilization enhances PPO activity in peach, but without analyzing nitrogen content in fruits or leaves. Pascual et al. (2013) found a positive correlation between PPO activity and nitrogen content in flat peach fruits. Bostwick's index appeared negatively correlated with PPO activity at harvest (-0.6105), as it was also reported by Falguera et al. (2012b). On the other hand, the PCA plot also showed that low values of canopy temperature and high values of SWP (i.e. a better water status) led to a higher average FW at harvest and a higher yield 1 category (kg of fruits of more than 70 mm), while yield 3 category (kg of fruits of less than 65 mm) appeared diametrically opposed. Such results are in accordance with previous studies (Berman and DeJong, 1997), where even mild water deficits induced fresh fruit weights reductions.

Table IV.3. Correlation matrix for PPO variables (PCA analysis).

Variable	PPO08/07	PPO08/14
100	0.2221	0.0482
70	-0.2221	-0.0482
DI	0.3699	0.4005
SDI	-0.3699	-0.4005
NORMAL	0.7011	0.7098
MODIFIED	-0.7011	-0.7098
SS08/07	-0.4841	-0.3087
FW08/07	-0.6013	-0.3407
FF08/07	0.6366	0.5567
PPO08/07	1.0000	0.5824
PPO08/14	0.5824	1.0000
yield 1	-0.2974	-0.3245
yield 3	0.3861	0.1994
SWP07/19	-0.4540	-0.4994
CT07/19	0.5059	0.4856
frN08/07	0.1982	0.1031
leK	0.0094	0.1595
leN	0.6078	0.7345
leP	0.3596	0.3350
Botswick	-0.6105	-0.6301

## 3.3. Prediction of polyphenol oxidase activity

The previously discussed results lead to the conclusion that PPO activity after one week of cold storage may be predicted from the values of some parameters measured up to three weeks before harvest. On the basis of the correlations found by means of the Principal Component Analysis, SWP, canopy temperature and leaf N content measured on July 19<sup>th</sup> could be the most useful predictive variables. Therefore, a stepwise regression screening was carried out on these three variables and their binary and tertiary interactions as independent variables vs. PPO 08/14 as dependent variable, using the minimum Bayesian Information Criterion (BIC) to define the parameters to be included in the model. As a result, the only two significant variables were found to be stem water potential (with [p>F]=0.0461) and leaf N content (with [P>F]=0.0033) (without any interaction). The resulting equation to predict PPO activity after one week of cold storage was:

 $PPO = -0.0609 - 0.0908 \cdot SWP + 0.1042 \cdot leafN$ 

with PPO in (U/mL), SWP in MPa and leafN in %. The determination coefficient was 0.6370, with a RMSE of 0.038 and a [P>F] of the overall model of 0.0001.

## 4. Conclusions

In "Extreme Sunny" nectarines, polyphenol oxidase activity of the fruit is enhanced by water stress. Significant differences among treatments with different water status appeared two weeks before harvest, and these differences were also found after one week of cold storage. Plant water status could also be assessed by means of canopy temperature. In addition, besides stem water potential, nitrogen leaf content was also found to be correlated with PPO activity. This preliminary work demonstrates the important role of pre-harvest factors such as soil and irrigation management on tree water status and in turn on quality parameters and postharvest life and encourages further research in this area.

# Chapter V. Soil management and irrigation strategies to improve limiting soil conditions and enhance peach tree production and fruit quality

#### Abstract

#### Background and aims

Transformation of marginal land along with an increase of soil degradation processes is moving the agriculture into more unfavorable soils, forcing the development of new management strategies. This study evaluated the effects of various irrigation and soil management strategies on soil properties and fruit tree response under limiting soil conditions. The experiment was carried out during three years in a peach orchard planted in 2011 under a ridge planting system.

#### Methods

Six soil and water management treatments were evaluated in 18 experimental units, which were set up in the field using a randomized complete block design. The treatments were evaluated in terms of soil physical properties, crop growth and productivity, and fruit quality. Data were analysed post hoc by using ANOVA and MANOVA approaches. The Principal Component analysis was performed by means of Unscrambler v.10.1.

#### Results

Soil amendment with rice husk substrate substantially improved the hydraulic properties of the soil and the rhizosphere conditions, as aeration porosity was doubled in amended trees. Overall, it was translated to a better crop performance, since crop growth was increased and commercial yield rose by 52%. In contrast, forced soil aeration, by means of a venturi injector, had no effects on soil properties or on crop response. Fruit quality parameters were analyzed, and among them polyphenol oxidase (PPO) activity, one of the most important parameters determining fruit post-harvest stability, resulted triggered by crop water stress.

#### Conclusions

The results of this study highlight the importance of understanding the effects of irrigation and soil management techniques on crop growth and production and also on other fruit quality parameters of growing interest such as fruit storability.

**Keywords**: Subsurface drip irrigation, soil amendment, deficit irrigation, rice husk, oxygation.

# 1. Introduction

In many areas of the world, increases in agricultural productivity are achieved mostly through the extended use of irrigation systems, sometimes at the expense of the transformation of marginal land. However, the soils of newly irrigated areas often have limiting physical properties that constraint crop production (Anikwe, 2000; García-Ruiz, 2010). Soil and irrigation management techniques—ridge planting systems being a good example—are consistently implemented to overcome these limitations.

Ridge tillage is the term for any cropping system in which plants are grown on soil formed into raised beds or ridges (Tisdall and Hodgson, 1990) with the objective to create a better root environment and to enhance crop performance. Ridge systems are reported to increase crop productivity in soils degraded by high salinity/sodicity and waterlogging (Roth et al., 2005). However, such systems reduce the water infiltration area, thus posing a management issue when working with high-frequency irrigation systems or with soils with considerable constraints in terms of water infiltration and transmission. Mulch techniques and subsurface drip irrigation (SDI) systems have emerged in recent years. These systems provide a wide range of applications and solutions for many agricultural issues.

Recent studies (Mubarak et al., 2009; Okonkwo et al., 2011) report the beneficial effects of organic mulch and soil amendments on the physical properties of soil (i.e. hydraulic and aeration attributes), which in turn enhance crop response. Many organic substrates can be used as soil amendments, although they confer distinct attributes. Rice husk substrates have been used widely (Anikwe, 2000; Ogbodo, 2010; Okonkwo et al., 2011), yielding positive effects both on soil properties and crop performance. Since rice is a major commodity of the food industry, rice husk is extensively produced in many areas of the world. This low-cost by-product has a number of interesting physical properties, including low density and long-term stability (Kaboosi et al., 2012), thus making it a perfect candidate for soil amendment purposes.

On the other hand, newly implemented irrigation systems and strategies, such as SDI and regulated deficit irrigation (RDI), broaden the management possibilities of crops under a range of conditions. Both strategies represent a breakthrough in terms of water-use efficiency, especially in arid and semi-arid areas (Ayars et al., 1999; Girona and Fereres, 2012). However, the use of high-frequency irrigation strategies in limiting soil conditions may lead to constraints, such as root hypoxia and derived issues (Friedman and Naftaliev, 2012). Thus, forced aeration techniques (i.e. oxygation) have been implemented to overcome soil oxygen depletion and improve root conditions, producing promising results in many horticultural and herbaceous crops (Bhattarai et al., 2006; Bhattarai et al., 2008; Bonachela et al., 2010; Goorahoo et al., 2002).

Since marginal land is increasingly being used for agricultural purpose, there is a call for the development and evaluation of new crop management strategies. Although SDI and RDI strategies have been widely studied, it is necessary to evaluate them under limiting soil conditions. Similarly, soil amendments and oxygation treatments have yielded promising results in many cropping systems, although little is known about their effect on fruit trees. The aim of this study was to assess the effects of various irrigation and soil management techniques on soil properties and fruit tree response and to discuss the technical feasibility of such techniques when applied under limiting soil conditions. For this purpose, several treatments were set up in a peach orchard planted in ridge system and their effects on soil physical properties, tree growth, fruit yield, and fruit quality were studied.

# 2. Materials and Methods

## 2.1. Experimental site

The trial was conducted in a commercial orchard of peach (*Prunus persica* (L.) Batsch. var. *Platycarpa* (Decne.) L. H. Bailey cv. Saturn) grafted onto GF-677 and planted in 2011 in a ridge system, spaced 5 x 2.5 m, in the region of Los Monegros (Huesca, Spain). The climate is semi-arid Mediterranean, with an annual rainfall of 350 mm. Irrigation water, characterized by a low salt, sodium, and nitrate content, is taken from the Cinca irrigation canal. The soil is a Xeric Torriorthent (Soil Survey Staff, 1999) developed from marls, with a silt loam texture (USDA; 40.3% sand, 39.8% silt and 19.9% clay). The soil is saline (EC<sub>e</sub> = 6.0 dS m<sup>-1</sup> at 25°C), with a pH of 8.7 and an organic matter content of 10.0 g kg<sup>-1</sup>.

### 2.2. Experimental design and treatments applied

We evaluated the following six ridge systems: drip irrigation (DI) at 100% of the calculated irrigation requirement (R-DI-100); DI at 70% of the calculated irrigation requirement (R-DI-70); subsurface drip irrigation (SDI) at 100% of the calculated irrigation requirement (R-SDI-100); SDI at 70% of the calculated irrigation requirement (R-SDI-70); SDI at 70% of the calculated irrigation requirement plus air injection (15% by volume of air) through the irrigation system (R-SDI-70+AIR); and a modified ridge system with SDI at 70% of the calculated irrigation requirement (MR-SDI-70). The treatments were applied during 2011, 2012 and 2013. The experiment was organized in a randomized complete block design with three replications and 18 elementary plots. Each elementary plot consisted in seven trees, in which the five central trees were monitored. An automated drip fertigation system with auto-compensated emitters was implemented in function of the water requirements of the trees. Drip emitters supplying 1 mm h<sup>-1</sup> were spaced 0.33 m apart. Irrigation scheduling was calculated weekly using the FAO methodology (Allen et al., 1998). Fertigation was supplied during the whole crop cycle by applying a multi-nutrient solution, achieving a dose of 110 kg N ha<sup>-1</sup> year<sup>-1</sup>, 60 kg  $P_2O_5$  ha<sup>-1</sup> year<sup>-1</sup> and 170 kg K<sub>2</sub>O ha<sup>-1</sup> year<sup>-1</sup> at the end of the crop season. Air injection was performed with a venturi air injector (MAI-A3, Toro Ag. Irrigation, USA).

All ridges were molded using soil already present in the orchard by means of a rear v-blade attached to a tractor. The modified ridge treatment included substrate taken from around the dripperline, which included a mix of soil, rice husk and gypsum, at respective proportions of 12:12:1. This approach allowed the reduction of the application rate to 5.0 Mg ha<sup>-1</sup> of rice husk. The substrate was applied at depths of between 0.2 and 0.4 m. Substrate density and porosity were 0.76 Mg m<sup>-3</sup> and 60%, respectively. Ridges were 1.5 m (bottom) and 1 m (top) wide and 0.8 m high.

#### 2.3. Measurements

Meteorological data were collected using an automatic weather station— property of the regional meteorological service—located 7.1 km from the experimental orchard. Irrigation water was measured using a pulse water meter (MNK-I-N Zenner GmbH & Co. KG, Saarbrücken, Germany), which was connected to a data logger (EM-50, Decagon Devices Inc., Pullman, WA, USA). One water meter was used per treatment.

The physical properties of the ridge were determined through several measurements taken at the end of the first season (February 2012). Soil bulk density was determined by the excavation method (Blake and Hartge, 1986), performing one measurement per plot at two depths: from surface to a depth of 0.2 m and from a depth of 0.2 to 0.4 m. Water infiltration rate ( $v_i$ ) was measured at the ridge surface using the single ring infiltrometer method (Pla, 1983). Rings 0.25 m in diameter and 0.4 high were inserted into the soil at a depth of 0.15 m in order to prevent lateral seepage loss. Furthermore, a small soil dam was built around the cylinder. Soil saturated hydraulic conductivity ( $K_s$ ) was measured at a depth of 0.2 m using the single ring method. The parameters  $v_i$  and  $K_s$  were measured once per plot. To determine field capacity moisture content, the rings used to measure soil infiltration were covered to prevent evaporation. After 48 h, soil samples were collected (one per plot) in order to determine field capacity moisture content (FC). Total porosity ( $S_t$ ) was determined by the equation proposed by Danielson and Sutherland (1986) as:  $S_t = (1 - (\rho_b / \rho_p))$ 

Where  $\rho_b$  is bulk density, expressed in Mg m<sup>-3</sup> and determined by the excavation method (Blake and Hartge, 1986), and  $\rho_p$  is particle density, which for many mineral soils is 2.65 Mg m<sup>-3</sup>.

Aeration porosity was calculated once per plot and at two depths: between the surface and a depth of 0.2 m, and from a depth of 0.2 to 0.4 m, and by applying the following formula:  $f_a = S_t - FC$ 

Where:  $f_a$  is the aeration porosity, expressed as a %,  $S_t$  is the total porosity, expressed as a %, and FC is the volumetric water content at field capacity, expressed as a percentage.

Crop growth was analyzed through orthoimage canopy area (OCA) analysis (2012 and 2013 season) and trunk diameter (TD) increase (2011, 2012 and 2013 season). In the OCA method, photographs were taken using a digital single-lens reflex camera (D5100, Nikon, Japan) with a wide-angle zoom lens (SP AF 10-24mm F/3,5-4,5 Di II LD ASL IF, Tamron, Japan) placed in a gyroscope at the top of an arch with height of 3.5 m. The photographs were transferred to a computer and processed to grayscale images (8-bit) in order to calculate image area (IA, in pixels<sup>2</sup>) using specific free software (ImageJ, NIH, USA). An OCA value was obtained for every tree measurement (five trees per plot) by applying the following equation:

 $OCA = IA / S^2$ 

Where OCA is in  $m^2$ , IA is the image area in  $px^2$ , and S is the previous calculated image scale in px/m.

TD measurements were carried out in five trees per plot using a digital caliper (Digimatic, Mitutoyo America Corporation, Chicago, IL, USA).

A composite leaf sample was taken randomly from each plot in mid July 2013. Each sample comprised 50 newly but fully developed mid-terminal leaves from current year shoots taken from a height of 1.5 m in the tree canopy. All samples were cleaned, oven-dried at 65 °C, and ground. Nitrogen (N) concentration was analyzed using the Kjeldahl procedure.

To evaluate tree water status, on 13 July 2013, we determined stem water potential (SWP, in - MPa) in five control trees per plot. This was done using a pressure chamber (model 3005; Soil Moisture Equipment Corp., Santa Barbara, CA, USA) and following the method described by Shackel et al. (1997). Measurements were taken at 2 p.m. local time. Canopy temperature (CT, in °C) was determined in five control trees per plot on 19 July 2013 using a thermal infrared camera and specific software (Ti25 and SmartView 2.1, Fluke Corp., Everett, WA, USA). Stomatal conductance (GS, in mmol s<sup>-1</sup>) was measured in five control trees per plot on 8 July 2013 using a SC-1 leaf porometer (Decagon Devices Inc., Pullman, WA, USA).

On 23 July 2013, five trees per plot were manually harvested. The total yield (in kg ha<sup>-1</sup>) was divided into two categories: yield 1, fruits with a diameter equal to or greater than 70 mm; yield 2, fruits with a diameter lower than 70 mm. Quality parameters were determined in a sample of six fruits per plot on 12 July, 18 July, and 23 July (harvest). Fruit weight (FW, in g fruit<sup>-1</sup>) was measured with a precision weighing balance. Mesocarp firmness (FF, in N) was determined with a manual penetrometer (Copa-Technologie, Ctifl, France). Total soluble solids concentration (TSS, in °Brix) was measured using a thermocompensated refractometer (Atago Bussan Co., Tokyo, Japan). Fruit purée consistency (BI, in cm) was measured in 50

centimeters of flow per 30 sec using a Bostwick consistometer (Central Scientific Co., Alexandria, VA, USA). Skin color was measured with a Chroma Meter CR-400 tristimulus colorimeter (Konica Minolta Sensing, Inc., Japan) in the CIELab color space. Parameters a\*, b\* and L\* were determined. Polyphenol oxidase activity (PPO) was measured as described by Falguera et al. (2012) using 4 methylcatechol as substrate and recording the absorbance at 420 nm for 2 min by means of a Helios Omega spectrophotometer (Thermo Fisher Scientific Inc., Waltham, USA) and its specific software. In order to analyze nitrogen content (frN, in %), fruits were oven-dried at 60 °C to a constant weight and milled to fine powder. Nitrogen content was determined by using the Kjeldahl procedure.

The main statistical analyses were performed by using the JMP package (SAS Institute Inc., Cary, NC, USA). A repeated-measures MANOVA was used to analyze TD and OCA evolution along the seasons. A linear mixed model for analysis of estimated marginal means (EMM) was built to separate treatment effects. The Tukey Honestly Significant Difference (THSD) post hoc test was used to compare soil physical properties, yield, and quality parameters. Statistical significance was set at p = 0.05. In addition, multivariate projection techniques based on Principal Component Analysis were performed by means of Unscrambler v. 10.1 (Camo Process AS, Oslo, Norway).

# 3. Results

## 3.1. Physical properties of the soil

Physical measurements of the soil were taken after the first crop season. Major differences were observed between treatments, and more specifically between the normal ridge and the modified ridge treatment (Table V.1). Topsoil bulk density (from surface to 0.2 m depth) ranged from 1.30 to 1.45 Mg m<sup>-3</sup>, with non-significant differences between treatments or ridge type (normal vs. modified). Bulk density for the consecutive soil layer (from 0.2 to 0.4 m depth) ranged from 0.89 to 1.41 Mg m<sup>-3</sup>, with significant differences between treatments (P=0.0007) and ridge type (P<0.0001). The plots in the MR-SDI-70 treatment presented the lowest soil bulk density values (0.89 Mg m<sup>-3</sup>) compared to other treatments (mean value of 1.34 Mg m<sup>-3</sup>). The rest of the treatments presented similar values, ranging from 1.25 to 1.39 Mg m<sup>-3</sup>.

Water infiltration rate ( $v_i$ , expressed in mm h<sup>-1</sup>) at the soil surface and saturated hydraulic conductivity ( $K_s$ , expressed in mm h<sup>-1</sup>) at a depth of 0.2 m showed considerable differences between treatments. The plots in the MR-SDI-70 treatment presented higher water infiltration rates (103.67 mm h<sup>-1</sup>) than the other treatments (P=0.007). The water infiltration rate for normal ridge treatments ranged from 8.9 to 27.8 mm h<sup>-1</sup>, with non-significant differences between them. Similarly, saturated hydraulic conductivity at a depth of 0.2 m was higher in the modified ridge treatment than in the other ones (P=0.0057). The plots in this treatment averaged a  $K_s$  of 205.0 mm h<sup>-1</sup> while the other ridge treatments recorded a mean value of 2.4 mm h<sup>-1</sup>.

Regarding soil aeration porosity, we found similar results within the first top layer of the soil (from surface to a depth of 0.2 m). Non-significant differences were detected between treatments or ridge types, with average treatment values ranging from 19.2 to 27.7%. However, significant differences between treatments (P=0.0015) were observed when studying the consecutive soil layer (from 0.2 to 0.4 m in depth). While non-modified ridge treatments presented an average aeration porosity of 23.2%, the modified ridge treatment registered 40.8% (P<0.0001).

Table V.1. Soil bulk density ( $\rho_b$ ), soil infiltration rate ( $v_i$ ), soil saturated hydraulic conductivity ( $K_s$ ), and soil aeration porosity ( $f_a$ ) at the end of 2011. The values are means. Values followed by different letters indicate significant differences according to the Tukey HSD test (P <0.05) and contrast test (P<0.05).

Treatment	ρ <sub>b</sub> 0-0.2 m	ρ <sub>b</sub> 0.2-0.4 m	v <sub>i</sub> at surface	K₅ at 0.2 m	f <sub>a</sub> 0-0.2 m	f <sub>a</sub> 0.2-0.4 m
rreatment	(Mg m⁻³)	(Mg m⁻³)	(mm h <sup>-1</sup> )	depth (mm h <sup>-1</sup> )	(%)	(%)
R-DI-100	1.42	1.33 a	24.80 b	2.15 b	19.27	25.91 b
R-DI-70	1.33	1.25 a	8.86 b	4.03 b	26.76	25.05 b
R-SDI-100	1.37	1.41 a	16.00 b	0.93 b	21.27	18.48 b
R-SDI-70	1.32	1.32 a	22.27 b	3.17 b	25.27	24.64 b
R-SDI-70+AIR	1.45	1.39 a	27.76 b	1.80 b	19.19	22.02 b
MR-SDI-70	1.30	0.89 b	103.67 a	205.00 a	27.72	40.81 a
Prob>F (treatment)	ns	0.0007	0.007	0.0057	ns	0.0015
Normal ridge (R)	1.38	1.34 a	19.94 b	2.41 b	22.35	23.22 b
Modified ridge (MR)	1.30	0.89 b	103.67 a	205.00 a	27.72	40.81 a
Prob > F (contrast)	ns	< 0.0001	0.0003	0.0002	ns	< 0.0001

### 3.2. Crop growth, yield, and quality parameters

Figure V.1 shows the evolution of TD for the treatments during the growing seasons of 2011, 2012 and 2013 (top) and the evolution of OCA for the treatments during the growing seasons of 2012 and 2013 (bottom). MANOVA test results indicate that the treatments influenced TD (Figure V.2). In addition, the MANOVA TD analysis (Figure V.1) presented both a time effect and time-treatment interaction effect since both factors were significant (P<0.0001). This observation implies that TD increased over time for all the treatments, although this parameter showed distinct behavior over time. MANOVA analyses for OCA were performed for two separate growing seasons (2012 and 2013) since this parameter is not a cumulative variable; leaf canopy area is created and destroyed every season. The MANOVA test results revealed significant differences between treatments for this variable (Figure V.3). Furthermore, both a time effect and a time-treatment effect for the two growing seasons were detected (P<0.0001 in all the cases) (Figure V.1). This finding suggests that the OCA changed over time, although its evolution differed between treatments.

Figure V.2 presents the analysis of TD evolution for various treatment descriptors (drip system, irrigation dose, ridge type and aeration). Trees under the SDI system 52

reached higher overall TD since DI factor was significant (P=0.0089). The timedrip system interaction was also found to be significant (P<0.0001), suggesting distinct behavior over time. Irrigation dose was significant (P=0.0286) when TD was analyzed. Trees at full irrigation (100) reached higher TD than those under deficit irrigation (70). Time and time-irrigation dose interaction were also significant (both P<0.0001). Trees under the modified ridge treatment registered greater TD than unamended trees (P=0.0454). The time-ridge type interaction was non-significant. Trees under forced aeration showed similar TD to those in nonaerated treatments, as indicated by non-significant differences.

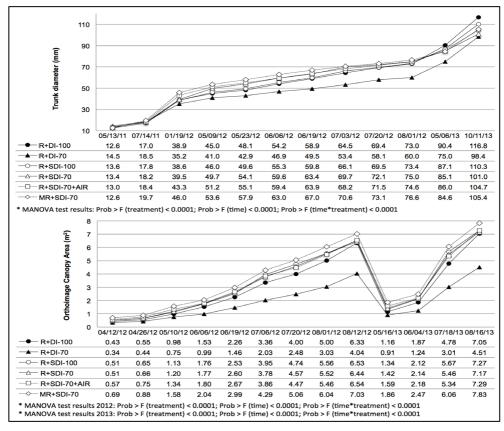


Figure V.1. Trunk diameter (TD) evolution for the treatments (upper) during the growing seasons of 2011, 2012 and 2013. Orthoimage canopy area (OCA) evolution for the treatments (lower) during the growing seasons of 2012 and 2013. Expressed values are the calculated treatment means for selected dates.

Figure V.3 presents the analysis of OCA evolution for various treatment descriptors (drip system, irrigation dose, ridge type, and aeration). Trees under the SDI system reached greater TD values than those receiving surface DI in the growing seasons of both 2012 (P=0.0003) and 2013 (P=0.0005). Time and time–drip system interaction was significant in the growing seasons of both 2012 and

2013. Irrigation dose was significant both in 2012 (P=0.0177) and 2013 (P=0.0130). Trees under the full irrigation regime registered higher OCA values than those receiving deficit irrigation.

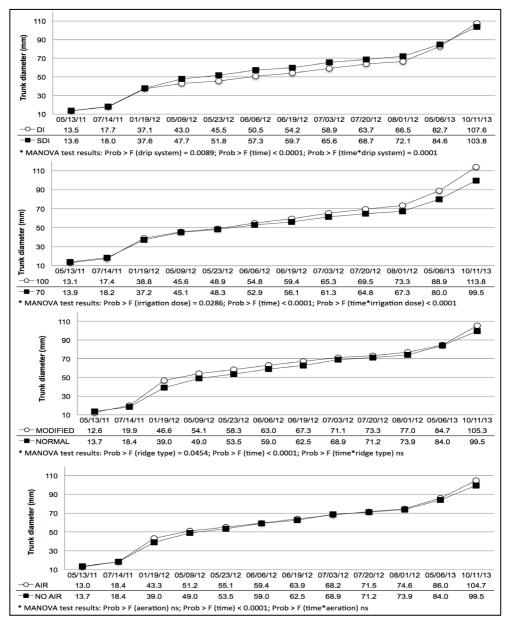


Figure V.2. Trunk diameter (TD) evolution for several treatment descriptors (from top to bottom: drip system, irrigation dose, ridge type and aeration) during the growing seasons of 2011, 2012 and 2013. Expressed values are the calculated treatment means for selected dates.

The irrigation dose-irrigation interaction was significant for both growing seasons. Trees under the modified ridge treatment recorded higher OCA values than those of unamended trees, since ridge type was significant in the growing seasons of both 2012 (P=0.0275) and 2013 (P=0.0439). We did not find significant differences between aerated and non-aerated treatments in any growing season when OCA was analyzed.

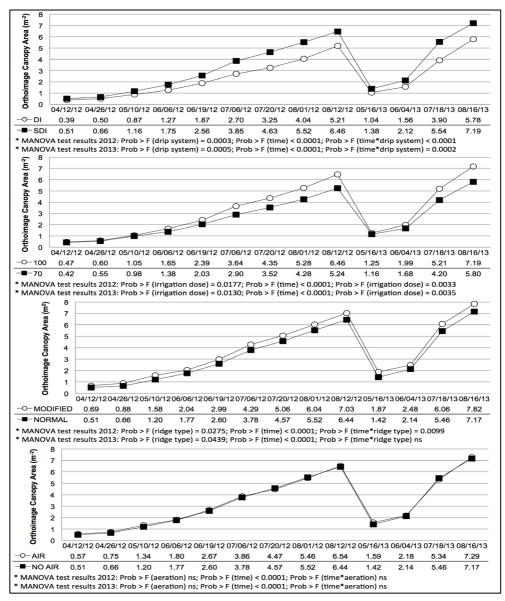


Figure V.3. Orthoimage canopy area (OCA) evolution for several treatment descriptors (from top to bottom: drip system, irrigation dose, ridge type and aeration) during the growing seasons of 2012 and 2013. Expressed values are the calculated treatment means for selected dates.

#### Chapter V

Figure V.4 shows the yield distribution (total yield, yield 1, and yield 2) within the treatments (top) and within treatment descriptors, such as irrigation system, irrigation dose, ridge type, and forced aeration (bottom). Treatments showed significant differences with respect to commercial yield (yield 1) (P<0.0001), non-commercial yield (yield 2) (P<0.0001) and overall production (total yield) (P<0.0001). The DI system with full irrigation (R-DI-100) reached the highest yield, with 24 923 kg ha<sup>-1</sup> on average (total yield), with non-significant differences with the following treatment, R-SDI-100, which registered 22 978 kg ha<sup>-1</sup> on average. The lowest yields (total yield) were observed in SDI treatments with deficit irrigation (SDI-70), which presented average values lower than 15 500 kg ha<sup>-1</sup>. Regarding the commercial yield, non-significant differences were detected between treatments at full irrigation and the MR-SDI-70 treatment, all of them with average yields above 11 900 kg ha<sup>-1</sup>. Lower values of commercial yield were obtained in the rest of the deficit treatments (70).

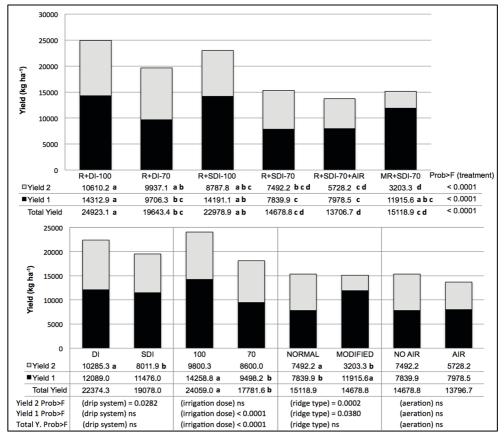


Figure V.4. Yield distribution for treatments (upper) and treatment descriptors (drip system, irrigation dose, ridge type and aeration)(lower). Yield is divided into commercial yield (Yield 1), non-commercial yield (Yield 2), and total yield (Total yield). Mean values followed by different letters indicate significant differences according to the Tukey HSD test (P < 0.05).

Close inspection of the yield distribution through treatment descriptors allowed a detailed analysis of the experiment. Non-significant differences were found for the commercial yield and for the overall production regarding the irrigation system implemented. However, trees under full irrigation achieved higher commercial productions than those under deficit irrigation (P<0.0001). Also, non-commercial yield was similar between irrigation doses since non-significant differences were observed. Overall, full irrigation treatments presented overall higher production than deficit irrigation ones (P<0.0001). Trees amended with rice husk showed higher commercial yields than non-amended ones (P=0.0380). In contrast, non-commercial yield was greater for trees in the unamended treatments (P=0.0002), although overall production was similar since there were non-significant differences between them. Forced aeration did not affect crop production as attested by the lack of significant differences for any of the yield categories.

Figure V.5 shows the evolution of fruit weight (FW) for the treatments. From the first collection of samples, about two weeks before the harvest (07/12/2013), the modified ridge treatment registered higher FW than other treatments, with significant differences at harvest (07/23/2013) with the other deficit irrigation treatments (P<0.0001). However, no significant differences were detected at harvest time between the modified ridge treatment and full irrigation treatments.

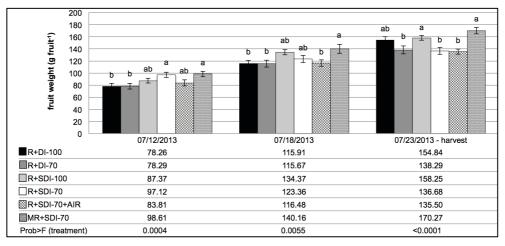


Figure V.5. Figure V.5. Fruit fresh weight evolution for treatments until harvest date. Expressed values are the treatment means. Different letters indicate significant differences according to the Tukey HSD test (P < 0.05).

Significant differences between treatment effects on fruit quality were observed (Table V.2). The average FW was higher in full irrigation treatments and in MR-SDI-70 than in the other treatments (P<0.0001). R-SDI-70+AIR registered higher TSS values (P=0.0011) compared to the other treatments, with no differences with R-SDI-70. The MR-SDI-70 treatment, along with R-SDI-100, registered lower TSS contents, below 12° Brix. Regarding fruit firmness (FF), there were no significant differences were found between treatments. We observed significant

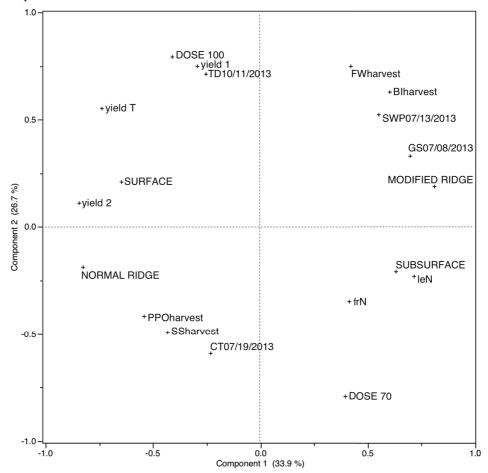
differences between treatments (P<0.0001) for the Bostwick Index (BI). While the modified ridge treatment registered higher values (5.50 cm), the remaining deficit irrigation treatments showed values below 4 cm. CIELab color values (L\*, a\*, and b\*) for fruit skin were also affected by the treatments. The modified ridge treatment presented lower values on average than the other treatments, although significant differences were also found when compared to R-DI-100 (P=0.0039). Regarding the a\*/b\* ratio, MR-SDI-70 presented higher values compared to the rest of the treatments (P=0.0002). Polyphenol oxidase activity (PPO) levels differed in the fruits harvested from the different treatments (P<0.0001). Fruits under MR-SDI-70 had lower PPO activity than those grown under the other treatments.

Table V.2. Fruit fresh weight (FW, in g fruit<sup>-1</sup>), total soluble solids content (TSS, in °Brix), fruit firmness (FF, in N), the Bostwick Index (BI, in cm), fruit skin color in CIELab parameters (L\*, a\*/b\* ratio), and polyphenol oxidase activity (PPO<sub>ha</sub>, in U mL<sup>-1</sup>) of fruits harvested from the treatments. The values are means. Values followed by different letters indicate significant differences according to the Tukey HSD test (P <0.05).

Treatment	FW	TSS	FF	BI	L*	a*/b*	<b>PPO</b> <sub>ha</sub>
R+DI-100	154.84 ab	12.63 ab	63.24	4.25 bc	48.39 a	0.62 b	0.5013 a
R+DI-70	138.29 b	12.20 ab	56.48	2.75 e	41.32 ab	0.89 b	0.4897 ab
R+SDI-100	158.25 a	11.81 b	59.98	4.71 b	44.53 ab	0.90 b	0.4591 b
R+SDI-70	136.68 b	12.89 ab	55.99	3.75 cd	44.03 ab	0.86 b	0.5181 a
R+SDI-70+AIR	135.50 b	13.21 a	62.69	3.25 de	42.54 ab	0.86 b	0.5106 a
MR+SDI-70	170.27 a	11.67 b	56.91	5.50 a	37.89 b	1.47 a	0.4097 c
Prob>F	<0.0001	0.0011	ns	<0.0001	0.0039	0.0002	<0.0001

### 3.3. Relationships among variables: PCA

A global Principal Component Analysis (PCA) model was carried out over all the data in order to determine the variables that made the greatest contribution to the overall variance (Figure V.6). The PCA analysis contained 19 variables with different scale values, which were standardized by means of dividing each variable by its standard deviation. The variables were the following: codified treatment descriptors (ridge type, irrigation dose and drip system); soluble solids content at harvest (SSharvest); polyphenol oxidase activity at harvest (PPOharvest); fruit weight at harvest (FWharvest); the Bostwick Index at harvest (BIharvest); stem water potential at midday a few days before harvest (expressed in negative values, SWP07/13/2013); leaf stomatal conductance a few days before harvest (GS07/08/2013); canopy temperature a few days before harvest (CTharvest); various yield categories (yield 1, yield 2 and yield T); tree trunk diameter at the end of the experiment (TD10/11/2013); leaf nitrogen content mid July 2013 (leN); and fruit nitrogen content at harvest (frN). Moreover, the data were centered before the principal component (PC) projection of the information, and the PCA model was validated using the Full Cross Validation method. The first PC (PC 1) explained 33.9% of the overall variance and the second one (PC 2) 26.7%, thus



implying that more than 60% of the overall variance was explained by two components.

Figure V.6. Variable loadings represented in the two first Principal Component space (PCA analysis). Abbreviations for variable names are defined in the Materials and Methods section.

# 4. Discussion

### 4.1. Physical properties of the soil

The topsoil layer (surface to 0.2 m depth) was unaltered in this experiment, so no differences in bulk density were found in this soil layer. However, soil amendment reduced the soil bulk density by 34% in the mid-layer (0.2 to 0.4 m depth) compared to other treatments. Rice husk substrate–with a measured bulk density of 0.76 Mg m<sup>-3</sup>–was placed below the subsurface dripperline, thus altering the bulk density within this soil layer. Many authors have reported that organic soil amendments lead to changes in soil bulk density in many crops and soil types

(Anikwe, 2000; Garg et al., 2005; Okonkwo et al., 2011). However, such changes are subject to the substrate properties, application doses, and soil conditions. It is important to note that rice crop residues are highly siliceous (rich in  $SiO_2$ ). This property confers rice-based substrates long-term stability in the soil (Mandal et al., 2004). In fact, rice husk envelopes have achieved promising results in drainage systems (Kaboosi et al., 2012).

Soil water dynamics were altered by substrate amendment. The soil infiltration rate ranged from 8.9 to 27.8 mm h<sup>-1</sup> in non-modified ridge treatments, with nonsignificant differences between them. However, at the soil surface, this parameter increased fivefold in the modified ridge treatment, although amendment was in the mid-layer. Thus, the water infiltration rate increased considerably as a result of substrate amendment while topsoil bulk density (from the surface to 0.2 m depth) remained unaltered. Similarly, Merwin and Stiles (1994) reported changes in hydraulic soil properties caused by various groundcover management systems (GMSs) in a fruit tree orchard. After 6 years under various GMSs, soil water characteristics were affected while soil bulk density values were similar in all treatments. Moving forward, saturated hydraulic conductivity (K<sub>s</sub>) at a depth of 0.2 m increased 85 times when the ridge was modified with rice husk substrate. Rice husk amendment altered the soil bulk density in the mid-layer of the soil, which in turn enhanced K<sub>s</sub> in the inner layer. Similarly, Mbagwu (1992) and later Anikwe (2000) reported that the application of various types of organic amendments, among them rice husk substrates, increases K<sub>s</sub> in limiting soils. It is critical to improve water movement through the soil, especially when irrigation water is provided by localized distribution systems (e.g. DI)(Ayars et al., 1999). Thus the hydraulic properties of soil can be improved by ridge modification with rice husk amendments.

Rice husk amendment doubled the aeration porosity in the mid-layer of the soil (0.2-0.4 m depth) with no effects on the soil surface. As aeration porosity is inversely related to the bulk density of soil (Khan et al., 2000), it is comprehensible that aeration porosity changed only in the subsurface stratus since bulk density modifications were manifest only in this layer. In addition, previous studies (Anikwe, 2000; Khan et al., 2000; Ogbodo, 2010; Okonkwo et al., 2011) reported positive effects of rice husk amendments on soil aeration.

Rice husk substrate increased the potential aeration of the root zone, diminished the bulk density of the soil mid-layer, and improved overall water movement through the soil profile. Unexpectedly, R-SDI-70+AIR had no effect on any of the soil physical properties addressed, including aeration porosity. Our results are not consistent with those found by others (Bhattarai et al., 2006; Bhattarai and Midmore, 2009; Bhattarai et al., 2008; Chen et al., 2010), who described that oxygenation treatments (applied by similar means) enhance soil aeration and crop performance. However, in those studies, the experiments were performed in horticultural crops and under distinct soil conditions to those tested in the present manuscript. It is important to note that forced aeration by means of a venturi 60

injector required a high amount of hydraulic energy (between 1.2 and 1.5 bar). In contrast, the local application of the rice husk substrate did not involve any loss of hydraulic energy, although it had an associated application cost.

#### 4.2. Crop growth, yield, and quality parameters

Both TD and canopy area were affected by the treatments. Trees in the MR-SDI-70 treatment registered the highest TD and OCA values in every growing season, while those in R-DI-70 recorded the lowest values. At the end of the 2012 growing season, trees under MR-SDI-70 registered 28% higher TD values and 74% higher OCA values on average compared to those grown under R-DI-70. Thus, the application of rice husk substrate and use of a SDI system increased tree growth, while the irrigation dose remained unaltered. The major differences were observed between R-DI-70 and the other treatments (Figure V.1). Differences in tree growth parameters in function of the drip system were more evident in 2012. Trees under SDI were 5.14 mm wider (TD) and 0.78 m<sup>2</sup> greater (OCA) on average than those under DI treatments. In 2013, differences in OCAs were still apparent (according to the MANOVA test), although TDs were similar (on average). On the basis of these observations, we propose that the SDI system is a valuable technique under limiting soil conditions. The experiment was set up in a soil with poor hydraulic properties-these caused by soil crusting and soil sealing. The former process causes a drastic reduction in water infiltration (as shown in this experiment), which in turn results in increased runoff and water erosion and reduced water availability for the crop (Kirkham, 2014). Thus, the application of irrigation water underneath the first soil layer might be an effective strategy to overcome soil crusting and to increase irrigation efficiency. Similarly, Lamm and Camp (2007) reported that SDI systems reduce soil compaction and soil crusting. Those authors highlighted some advantages of SDI with respect to water and soil issues, including greater efficiency of water usage as a result of the reduction (or elimination) of soil water evaporation, surface runoff, and deep percolation. However, other studies (Bryla et al., 2005; Bryla et al., 2003) describe higher irrigation efficiencies under DI and SDI systems when compared to other systems (e.g. microjet or furrow), although no significant differences were found between surface and subsurface systems.

Regarding the irrigation dose, trees under full irrigation registered higher TD and OCA values than those under deficit irrigation. Although these differences were not evident in 2011 and early 2012, they were accentuated over 2012 and 2013, together with an increase in the crop ET and the irrigation demand. In addition, larger differences between irrigation doses were observed for DI than for SDI treatments (Figure V.1). After 3 years of the experiment, the difference in TD for irrigation doses (100 vs. 70) was 18.40 mm on average for DI and only 9.30 mm for SDI. Similarly, OCA differences were 2.54 vs. 0.10 m<sup>2</sup> (DI vs. SDI, respectively). These results suggest that the reduction in irrigation dose (30%) in DI treatments was critical and led to a decrease in crop growth (lower TD and OCA), while in SDI treatments this reduction did not alter this variable. Deficit

irrigation applied in woody trees (e.g. fruit trees) has a considerable effect on vegetative growth (Ruiz-Sanchez et al., 2010). Reductions in trunk cross sectional areas in response to water deficits lead to substantial decreases in tree size and canopy volume (Girona et al., 2005a; Girona et al., 2005b; Marsal et al., 2008).

Concerning the ridge type, amended trees achieved greater TD and higher OCA, thereby suggesting that this system has a positive effect on crop growth. At the end of the third experimental year (2013), TD and OCA values in amended trees were 5.80 mm and 0.65 m<sup>2</sup> greater on average than those of unamended ones. Rice husk substrate enhanced the soil physical properties (i.e. bulk density, hydraulic properties and aeration porosity), which in turn improved irrigation efficiency and the crop performance. These results are consistence with those found in other studies (Anikwe, 2000; Okonkwo et al., 2011), in which the use of rice husk amendments resulted in an increase in biomass production in herbaceous crops (e.g. maize, rice and peanut crop). However, there is no scientific evidence of similar experiments conducted in fruit trees or woody crops to date. However, organic mulch applications (on soil surface) have been tested in many other experiments (Baldi et al., 2014; Bravo et al., 2012; López et al., 2014; Merwin and Stiles, 1994), providing positive results regarding tree growth and fruit yield.

Air injection through the irrigation system did not affect fruit tree growth since TD and OCA were unaltered by aeration treatment. Since the 1960s (Grable, 1966), soil aeration has been considered one of the major factors limiting crop productivity. However, the first positive results of forced aeration in open-field crop experiments were not obtained until many decades later (Bhattarai et al., 2010; Bonachela et al., 2010; Goorahoo et al., 2002). Those findings contrast with our results; however, we should stress that those experiments were conducted on different species (e.g. cotton, pepper, pumpkin, soybean, tomato, watermelon, and zucchini), in different soil conditions—most of them in heavy clay soils— and using distinct soil/irrigation management systems to those addressed in the present study. Indeed, after extensive research with more than 35 commercial, drip-irrigated orchards, Friedman and Naftaliev (2012) concluded that oxygen demand is highly affected by factors such as soil attributes, irrigation management, and even tree age, and that oxygen requirements may vary from one crop to another.

We observed that fruit tree production was affected by the treatments applied. The highest yields were obtained in full irrigation treatments; however, regarding the commercial yield, the modified ridge treatment registered similar values. Nevertheless, leaving the individual results of treatments aside, the yield analysis by treatment descriptors provides a more detailed appreciation of the effects of the irrigation systems implemented, the doses of irrigation applied, ridge modification, and forced aeration. Commercial yield (fruits above 70 mm) was not affected by the irrigation system, with average fruit production between 11 400 and 12 000 kg ha<sup>-1</sup>. Overall, the total yield was not influenced by the drip system implemented, although vegetative growth was affected considerably. Therefore, although higher irrigation efficiencies were observed in SDI treatments, they did not yield increases 62

in production. It is important to note that the experiment was set up in a commercial peach orchard, where trees were dormant pruned every year by trained operators to regulate fruit production. Canopy differences between treatments were reduced after pruning since one of the common objectives of this agricultural practice is to control tree size and shape and to achieve greater orchard uniformity (Marini and Sowers, 2000). Such practices would explain the absence of higher yields in SDI treatments despite increased vegetative growth. For instance, after a 3-year study in a peach orchard, Bryla et al. (2003) did not observe significant differences in fruit yield between DI and SDI systems.

The irrigation dose was one of the main factors affecting crop production. While trees under deficit irrigation barely achieved 10 t ha<sup>-1</sup> of commercial fruit yield, full irrigation increased this parameter by 50%. Full irrigation did not affect the average fruit weight (g fruit<sup>-1</sup>), which was similar between irrigation doses, but did increase commercial fruit yield (fruit above 70 mm) per tree (data not shown). Tree growth was not affected by irrigation dose during early crop stages (2011 and early 2012), although it was later on in 2012 and in 2013 (first year of fruit production). Thus, while the water deficit applied was not critical during initial stages of tree growth, it did determine a decrease in yield. The water deficit used was set at 70% of ET<sub>c</sub> and distributed uniformly over the whole crop cycle, an approach known as sustained deficit irrigation (Goldhamer et al., 2005). The effects of deficit irrigation strategies on fruit yield depend not only on the level of deficit applied but also on the phases in which it is applied. While some authors have found that sustained strategies are the most effective in terms of crop yields (Goldhamer et al., 2005), others (Faci et al., 2014; Rufat et al., 2010) uphold that it is more effective to apply the irrigation deficit in a certain crop phase (i.e. pit hardening phase in peach). However, in the long-term, deficit strategies tend to decrease crop yield since vegetative growth is reduced and tree canopies become smaller (Girona et al., 2005a). Overall, deficit irrigation strategies are proposed as alternatives to those involving full irrigation in areas with limited water resources (Pérez-Pastor et al., 2014).

Regarding the ridge type, while rice husk amendment did not affect the total crop production, it did influence the distribution of the production. While the commercial yield in unamended trees was about 53% of the total, in the amended ones it reached 79%. Overall, rice husk amendment increased the commercial fruit yield by 52% (P=0.0380). Ridge modification affected the number of fruits per tree (data not shown) but also the average fruit weight (Figure V.5). Of all the treatments, the modified ridge treatment recorded higher fruit weights from the first sample collection (07/12/2013) until harvest time (07/23/2013). Fruit weight in amended trees increased by 25% at harvest compared to unamended ones (P<0.0001), with non-significant differences with the production of trees subjected to full irrigation. Thus, rice husk enhanced both fruit growth and crop load, an effect that may be attributable to an increase in irrigation efficiency. Many studies (Merwin and Stiles, 1994; Nielsen et al., 2004) have reported beneficial effects of

organic amendments on crop production, particularly in limiting soil conditions where such amendments substantially improve the hydraulic properties of the soil (Anikwe, 2000; Okonkwo et al., 2011).

We did not observe any effect of forced aeration on crop growth or crop production. Both the crop load (data not shown) and the average fruit weight (Figure V.5) for the aeration treatment were similar to those achieved in the non-aerated one, thereby suggesting that forced aeration did not affect fruit setting or fruit growth. In contrast, forced aeration has been reported to have beneficial yield effects on many horticultural (Bhattarai et al., 2006; Bonachela et al., 2010; Goorahoo et al., 2002) and herbaceous (Bhattarai and Midmore, 2009) crops. Thus, while active aeration techniques (e.g. oxygenation) are commonly used in some horticultural crops and crop systems (Urrestarazu and Mazuela, 2005), there is little evidence of their effects on fruit tree crops. For instance, forced aeration achieved positive results in open field experiments when tested in poorly drained soils (Bhattarai et al., 2010) or with hypoxic irrigation water (Maestre-Valero and Martínez-Alvarez, 2010).

We observed substantial effects of treatment on fruit quality. In this regard, the average fruit weight was considerably influenced by the treatment applied. Lower fruit weight (FW) was observed in the deficit irrigation treatments (below 140 g fruit<sup>-1</sup>), except for the modified ridge treatment (MR-SDI-70), which showed the highest average values among all the treatments (170.27 g fruit<sup>-1</sup>). Results for TSS content were the inverse of those observed for FW. Thus, lower TSS values (below 12 °Brix) were observed in R-SDI-100 and MR-SDI-70. While FF was not affected, the results for BI differed between treatments. Again, we distinguished two groups: full irrigation treatments and MR-SDI-70, with BI values above 4.25 cm; and the rest of the deficit irrigation treatments, with BI values below 3.75 cm. On the basis of these results, we conclude that trees under deficit irrigation, except for those in MR-SDI-70, experienced a sustained mild water deficit, which led to smaller fruits with higher TSS contents and low BI. Even mild water stress can induce fresh fruit weight reductions in peach (Berman and DeJong, 1997). Similarly, higher TSS contents may be attributable to water stress in late stages of fruit development (Crisosto et al., 1994) or may be caused by a lower individual fruit weight (Rufat et al., 2010). Likewise, low BI values found in the deficit irrigation treatments could be attributed to water stress during fruit enlargement stages, which causes decreases in fruit viscosity (Patanè and Cosentino, 2010). However, water stress may delay fruit maturity in peach crops (Lopez et al., 2011), which would explain the lower purée consistencies detected since BI diminishes as peach maturity advances (Falguera et al., 2012a). Peach skin color was also affected by the treatments. Fruits under the modified ridge treatment had higher  $a^*/b^*$  and lower L\* values on average than those in the other treatments, although significant differences with the rest of the treatments were found only for a\*/b\* values (P=0.0002). Peach skin color during fruit ripening changes along with CIELab coordinates (Delwiche and Baumgardener, 1985). As peach ripening advances, the skin becomes darker and less green, decreasing the L\* and increasing the a\* coordinates. The color results suggest that fruits under the modified ridge treatment were at a more advanced stage of maturity at the harvest date. However, other fruit parameters, like fruit firmness, indicated that all the treatments were at a similar stage of maturity (Valero et al., 2007). Thus, assuming that treatments were at similar maturity stages at harvest, fruits under MR-SDI-70 presented higher a\*/b\* ratios, as shown by greater intensity of red. The intensity of peach color is directly associated with appearance and affects consumer acceptance (Iglesias and Echeverría, 2009). Overall, fruits under MR-SDI-70 had a better appearance for consumer purposes, not only because of the greater size but also because of a more attractive skin color.

Enzymatic results showed differences in PPO activity between treatments (P<0.0001). Fruits under the MR-SDI-70 treatment presented lower PPO activities, suggesting better fruit storability since the activity of this enzyme is one of the most important factors determining post-harvest durability (Falguera et al., 2012b; Jiang et al., 2004). The PPO activity for the remaining deficit irrigation treatments (70) was 23% higher than in the modified ridge treatment. This observation may indicate that PPO activity is affected by soil or irrigation management techniques. For instance, this parameter has been reported to be modified by several agricultural practices, such as fertilization (Pascual et al., 2013).

#### 4.3. Relation between variables: PCA

The PCA analysis included crop production variables (yield 1.2,T), fruit quality variables (FWharvest, SSharvest, BIharvest, PPOharvest), physiological variables (SWP07/08/2013. CT07/19/2013, GS07/08/2013), growth variables (TD10/11/2013), and nutrient status variables (leN, frN). These variables are related between each other and between the treatment descriptors, namely irrigation dose (dose 100, dose 70), drip system (surface, subsurface), and ridge type (normal ridge, modified ridge). PC 1, which explained 33.9% of the overall variance, was clearly identified with the irrigation dose. Within this component it was possible to observe a strong and positive correlation between full irrigation and TD at the end of the experiment, thus suggesting that the main factor influencing crop growth was the irrigation dose rather than the ridge type or drip system. A strong and positive correlation was also detected between TD and the full irrigation dose with commercial fruit yield. Thus, full irrigation treatments produced larger trees with greater commercial yields. This finding was already revealed in the ANOVA analysis for the yield categories (Figure V.4). PC 2, which explained 26.7% of the overall variance, was clearly related to the ridge type. Within this component, it was possible to observe strong relationships between water status variables and fruit quality parameters. We noted a strong correlation between the water stress variables. Thus, the loading plot showed that high values of stomatal conductance (GS) led to lower canopy temperatures (CT) and higher stem water potential (SWP; i.e. a better water status). The relationship between these variables is widely documented in fruit tree physiology (Girona and Fereres, 2012; Jones, 1999); however, our PCA further strengthens those findings. Quality parameters such as FW and TSS content are affected by crop water status. Therefore trees with a better water status (higher SWP and GS, and lower CT) produced larger fruits with lower TSS content. Thus, peach trees under some treatments experienced mild water stress, which diminished FW but increased the TSS content, a valuable parameter for peach quality and consumer acceptance (Iglesias and Echeverría, 2009). Our results are in accordance with those reported in other studies in peach crops (Berman and DeJong, 1997; Crisosto et al., 1994).

With respect to PPO activity, the loading plot showed a high and positive correlation between this fruit quality parameter and CT and a negative correlation with SWP and GS—all of these water status parameters were measured some weeks before harvest. Our observations reinforce the notion that water stress triggers PPO. The activity of this enzyme causes fruit flesh browning, which in turn can be influenced by a combination of factors, such as soil fertilization and irrigation management (Crisosto et al., 1995). For instance, nitrogen fertilization affects PPO activity, which in turn influences post-harvest stability (Falguera et al., 2012b; Pascual et al., 2013). In our study, PPO activity was not affected by crop nitrogen status since neither nitrogen fruit content nor nitrogen leaf content were highly related to the activity of this enzyme. This observation does not imply that nitrogen fertilization does not affect PPO activity (as reflected in some studies) but that under certain conditions (e.g. water stress) tree water status is the predominant factor determining this activity. Indeed, while deficit irrigation can improve some quality attributes, it can also significantly reduce post-harvest stability as a result of an increase in PPO activity (Buendía et al., 2008). Similarly, pre-harvest water stress plays a crucial role in determining post-harvest browning potential in avocado fruits (Bower et al., 1989). Analogous results were found in peach and nectarine crops, where nectarines under deficit irrigation treatments showed improved quality parameters but a lower post-harvest durability (Pliakoni and Nanos, 2010). Overall, one of the key processes underlying the decrease peach post-harvest life could be attributed to enhanced PPO activity in water-stressed trees. For instance, Buendía et al. (2008) stated that regulated deficit irrigation strategies should be selected with care as they affect the anti-oxidant content of peaches (e.g. phenolic compounds). For decades, classic quality parameters applied to fruit have been those related to aesthetic properties (e.g. size, color and firmness). Later on, new fruit properties related to consumer acceptance were introduced (e.g. taste and flavor). Other value added characteristics (e.g. antioxidant content and durability) are now increasingly demanded by both consumers and the food industry. Nowadays, fruit durability is a major quality parameter; therefore a full understanding of the factors that affect this parameter is crucial in order to determine the most appropriate management systems (e.g. irrigation) for a given crop.

# 5. Conclusions

Ridge modification with rice husk substrate substantially improved the hydraulic properties of the soil by increasing the infiltration rate and the bulk density. Aeration porosity was doubled in amended trees, thus increasing potential aeration in the root zone in a soil with considerable limiting properties. We propose that rice husk substrate contributes to a better crop performance, as trees under the modified ridge treatment showed greater vegetative growth (both trunk and canopy) and improved commercial yield and fruit quality (larger fruits with a better appearance). In contrast, forced soil aeration, by means of a venturi injector, does not affect the soil properties or the crop response.

The irrigation regime was the main factor influencing crop growth and production. Given that SDI systems showed superior vegetative growth—although no effect was observed on the commercial yield or the fruit quality—these systems should be considered for the management of soil crusting issues and for increasing irrigation efficiency.

Regarding other fruit quality issues, the polyphenol oxidase (PPO) activity appeared to be highly affected by the crop water status. Trees under deficit irrigation showed an increase in the activity of PPO, one of the most important parameters determining fruit post-harvest stability.

The results of this study highlight the importance of understanding the effects of irrigation and soil management techniques on crop growth and production and also on other fruit quality parameters of growing interest.

Chapter VI. An image-based method to study the fruit tree canopy and the biomass production in a peach orchard

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#### Abstract

The feasibility of two non-destructive methods based on image processing techniques was assessed for fruit tree research. The methods were evaluated in a two-vear (2011 and 2012) field experiment, during which various irrigation and soil management treatments were set up in a commercial peach orchard. Canopy image analysis was conducted using two approaches, namely the orthoimage and the lateral image technique. The proposed methods were compared with other classical measurements such as trunk diameter (TD) increase and pruning weight (PW). Orthoimage canopy area (OCA) analysis resulted reliable to study active crop growth along the growing season. The OCA values obtained were highly correlated with TD measurements ( $R^2 = 0.88$ ), thus describing an exponential significant model (y =  $0.0997 \text{ e}^{-0.0521x}$ ). Cumulative crop growth was determined using the virtual pruning (VP) technique. VP estimates were well correlated with fruit tree PWs during 2011 ( $R^2 = 0.86$ ) and 2012 ( $R^2 = 0.80$ ). The non-destructive image-based techniques proved sensitive to crop growth and useful for the study of fruit tree canopies. On the basis of our results, we conclude that the proposed image analysis methods are valuable new approaches with wide applications in fruit tree research

**Key words**: crop growth, photography techniques, leaf area index, pruning, peach.

## 1. Introduction

Tree canopy analysis has been widely studied. In this regard, Ross (1981) stated the importance of such analysis to further our understanding of plant physiology. The determination of the canopy area is an essential aspect when addressing plant productivity (Kvet and Marshall, 1971) since biomass production is closely related to the interception of light (Monteith, 1977), which in turn is directly linked to the leaf area index (LAI) (van Wijk and Williams, 2005). This index is closely associated with processes such as photosynthesis and transpiration and may influence the pattern of crop growth, development, and productivity. Indeed, most crop production strategies focus on maximizing the interception of solar radiation (Loomis et al., 1967; Monteith, 1969). Thus, by studying canopy area, it is possible to evaluate the response of plants to environmental conditions or crop management strategies, e.g. irrigation, fertilization, soil management (Ali and Anjum, 2004).

The quantification of biomass production in multi-annual crop experiments is a challenge since most classical methods are destructive and/or involve laborious assessments. Plant and leaf sampling is an accurate method for this purpose (Evans, 1972), but it is not suitable when measurements are repeated periodically. In these cases, it is appropriate to apply allometric models that relate shape and size of the leaves to the leaf area, which can be defined with great precision. In contrast, these models involve tedious measurements that require long-term sampling, especially during periods of active growth when shape and size of the leaves change rapidly (Baker et al., 1996).

Other common methods to determine fruit tree growth and development include the measurement of trunk diameter and shoot length; however, these two approaches provide only a rough idea of total biomass, and their relationships with total crop growth is influenced by many factors, such as plant competition, fructification, and cultural interventions (Bevacqua et al., 2012; Willaume et al., 2004). Pruning weight is another common method for biomass production study; however, it provides information only on cumulative tree growth and it is not suitable for the analysis of crop development at distinct phenological stages. Other techniques based on the measurement of interception and transmission of solar radiation in leaf canopies (Jonckheere et al., 2004) can be used to evaluate the development of the canopy but lack precision (Bréda, 2003). The most common method to determine the LAI is based on the measurement of photosynthetically active radiation (PAR) both above and below the canopy (Board et al., 1992). This method involves several PAR measurements taken using sensors placed beneath the canopy. However, as an indirect method to estimate the LAI, it requires several measurements to characterize the crop canopy.

Over the last twenty five years, precision agriculture has been introduced in many areas of the world along with the arrival of remote sensors mounted in satellites, aerial vehicles or tractors (Mulla, 2013). Among them, LiDAR technology (light

detection and ranging) has arisen as a precise tool to estimate the canopy volume of fruit trees (Palleja et al., 2010). Although this technology is still very expensive is likely to be adopted in the coming years as the price of the sensors decrease.

During recent decades, traditional photography has evolved to digital photography, leading to the development of a full range of electronic light sensors. Although digital technology was initially costly, it is now highly affordable.

Digital camera sensors convert light into digital information, which can then be modified, treated, and analysed by computer programs. With the availability of digital light sensors, there are now viable alternatives for estimating growth and biomass production in many crops. Digital image analysis has been reported to be a precise method to quantify crop biomass production (Campillo et al., 2011; Campillo et al., 2008; Pforte et al., 2012; Rico-García et al., 2009; Serdar and Demirsoy, 2006). Earlier studies (Diebolt and Mudge, 1988; Lindsey and Bassuk, 1992) found a close relationship between the LAI and image measurements, thus providing additional benefits over traditional methods (Smith et al., 1991).

Image based techniques offer a full range of applications for implementation in fruit tree science, where other methods either lack consistency or are not feasible. In this work we evaluated the use of digital image analysis to study the crop canopy and crop growth in a commercial peach orchard under different irrigation and soil management treatments. The objective was to assess the feasibility of image analysis techniques through digital photography as a supplementary tool for crop response assessment and crop biomass estimation.

# 2. Materials and Methods

### 2.1. Experimental site and applied treatments

The experiment was conducted during 2012 and 2013 in a commercial peach orchard located in the Los Monegros region (Huesca, Spain). The climate is semiarid Mediterranean; with an annual rainfall of 350 mm. Trees were planted in 2011 in a ridge planting system. One-year-old peach trees (Prunus persica (L.) Batsch. cv. Saturn) grafted onto GF-677 were grown with a spacing of 5 m x 2.5 m. Soil texture is loam (40.3% sand, 39.8% silt and 19.9% clay)(United States Department of Agriculture classification, USDA). A randomized complete block design with three replications was set up. Each elementary plot contained seven trees. Plant measurements were made on the five central trees. Six different soil and water management treatments were evaluated (Table VI.1): ridge planting with drip irrigation at 100% of the calculated irrigation requirement (R-DI-100); ridge planting with drip irrigation at 70% of the calculated irrigation requirement (R-DI-70); ridge planting with subsurface drip irrigation at 100% of the calculated irrigation requirement (R-SDI-100); ridge planting with subsurface drip irrigation at 70% of the calculated irrigation requirement (R-SDI-70); ridge planting with subsurface drip irrigation at 70% of the calculated irrigation requirement and air injection through the irrigation system (R-SDI-70+AIR); and modified ridge planting with subsurface drip irrigation at 70% of the calculated irrigation requirement (MR-SDI-70). The irrigation water requirement was calculated using the methodology proposed by the Food and Agriculture Organization of the United Nations (Allen et al., 1998). Air injection was performed with a venturi air injector (MAI-A3, Toro Ag. Irrigation, USA). The modified ridge treatment included substrate taken from around the dripperline, which included a mix of soil, rice husk, and gypsum, at respective proportions of 12:12:1. Ridge dimensions were 1.5 m (bottom) and 1 m (top) wide and 0.8 m high. In subsurface irrigation treatments, the dripperline was buried between 0.25 m and 0.3 m below the surface and was placed at a distance of between 0.15 m and 0.2 m from each tree trunk.

Treatment	Ridge type	Drip system	Irrigation dose	Air injection
R-DI-100	Normal ridge	Surface drip irrigation	100% ETc	-
R-DI-70	Normal ridge	Surface drip irrigation	70% ETc	-
R-SDI-100	Normal ridge	Subsurface drip irrigation	100% ETc	-
R-SDI-70	Normal ridge	Subsurface drip irrigation	70% ETc	-
R-SDI-70+AIR	Normal ridge	Subsurface drip irrigation	70% ETc	12% Air injection (v/v)
MR-SDI-70	Ridge modified with rice husk substrate	Subsurface drip irrigation	70% ETc	-

Table VI.1. Irrigation and soil management treatments applied in the experimental plot.

#### 2.2. Measurements

Crop growth was analysed through canopy image analysis, trunk diameter and pruning weight for five control trees per plot during the 2011 and 2012 growing seasons. Canopy image analysis was conducted by using two approaches, the orthoimage and the lateral image techniques. The orthoimage technique was used to study evolution of the canopy foliage during active tree growth (canopy with leaves) while the lateral image technique was used to study the cumulative shoot/branch growth between growing seasons (canopy without leaves). In the orthoimage technique, the photographs were taken using a digital single-lens reflex camera (D5100, Nikon, Japan) with a wide-angle zoom lens (SP AF 10-24mm F/3,5-4,5 Di II LD ASL IF, Tamron, Japan) placed in a gyroscope on the top of an arch placed at a height of 3.5 m (Figure VI.1.a). The photographs were transferred to a computer and processed using specific software (Photoshop CS5, Adobe Systems, San Jose, CA, USA) to isolate the green pixels of the canopy and thus obtain a simpler image. The photographs were transferred to a computer and processed using commercial software (Photoshop CS5, Adobe Systems, San Jose, CA, USA) to isolate the green pixels of the canopy. Images were processed to grayscale images (8-bit) in order to calculate image area (IA, in pixels) by using open source software (ImageJ, NIH, USA)(Figure VI.2).

#### Chapter VI

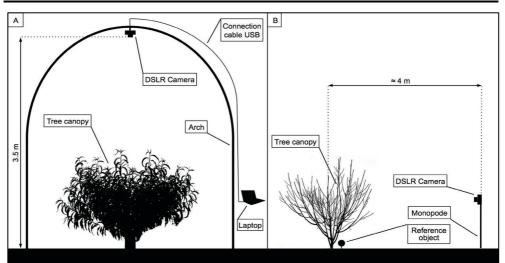


Figure VI.1. Layout of the method used in the orthoimage (A) and the lateral image technique (B).

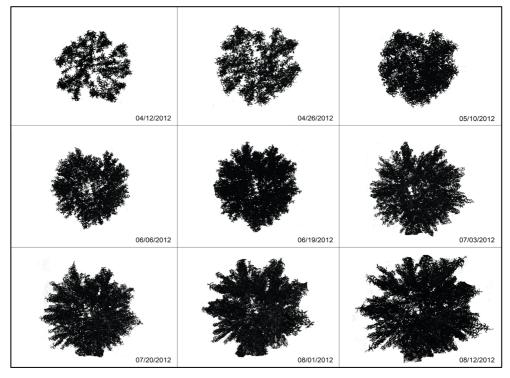


Figure VI.2. Canopy growth evolution of a tree canopy throughout the 2012 growing season obtained by using the orthoimage technique.

The grayscale cut-off point was setup for a few images and the applied for the rest of the images of the same batch (same date). This action of the process required the expertise of an image technician to include all the pixels of the canopy in the pixel counting. An orthoimage canopy area (OCA) value was obtained for each tree measurement by applying the following equation:

 $OCA = IA / S^2$  (Equation 1)

Where OCA is the orthoimage canopy area in  $m^2$ , IA is the image area in  $px^2$ , and S is the previous calculated image scale in pixel/m.

The increase in OCA was also determined by applying the following equation:

 $\Delta OCA_{year} = OCA_{n year} - OCA_{1 year}$  (Equation 2)

Where  $\Delta OCA$  is the season increase in the orthoimage canopy area in m<sup>2</sup> during the growing season,  $OCA_n$  is the last OCA measurement of the season in m<sup>2</sup>, and  $OCA_1$  is the first OCA measurement of the season in m<sup>2</sup>.

In the lateral image technique, the photographs were taken using a digital singlelens reflex camera (D5100, Nikon, Japan) with wide-angle zoom lens (SP AF 10-24mm F/3,5-4,5 Di II LD ASL IF, Tamron, Japan) placed on a monopod at a height of 1.5 m (Figure VI.1.b One picture per tree was taken at a distance of approximately 4 m. The photographs were transferred to a computer and processed using specific software (Photoshop CS5) to isolate the brown pixels of the canopy and thus obtain a simpler image, which was finally processed to a grayscale image (8-bit) using ImageJ software. This image was then converted to binary by defining a grayscale cut-off point and then skeletonized (reduced to single pixel lines) using ImageJ software in order to measure its total length (in pixels; Figure VI.3).

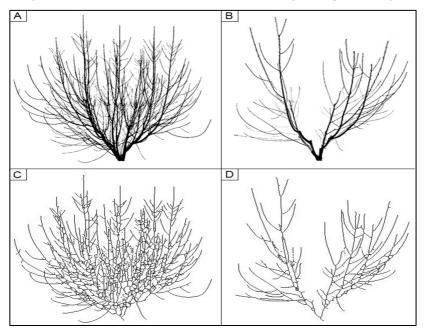


Figure VI.3. Isolated area of the canopy before pruning (A) and after pruning (B). Skeletonized images used to calculate the total length before pruning (C) and after pruning (D).

Image scale (S, in pixels/m) was obtained using ImageJ and a reference object with known dimensions, which appeared in every photograph. Using this technique, it was possible to calculate two variables, namely virtual pruning (VP) and cumulative shoot growth (CSG), as defined by the following equations:

 $VP_{year} = TL_{bp year} - TL_{ap year}$  (Equation 3)

Where  $VP_{year}$  is the virtual pruning in m tree<sup>-1</sup>,  $TL_{bp}$  is the total canopy length in m tree<sup>-1</sup> before pruning, and  $TL_{ap}$  is the total canopy length in m tree<sup>-1</sup> after pruning.

 $TL = TL_{px} / S$  (Equation 4)

Where TL is the total canopy length in m tree<sup>-1</sup>,  $TL_{px}$  is the total canopy length in pixels tree<sup>-1</sup>, and S is the calculated image scale in pixel/m.

 $CSG_{year} = TL_{bp year} - TL_{ap year-1}$  (Equation 5)

Where  $CSG_{year}$  is the cumulative shoot growth in m tree<sup>-1</sup>,  $TL_{bp year}$  is the total canopy length in m tree<sup>-1</sup> before pruning, and  $TL_{ap year-1}$  is the total canopy length in m tree<sup>-1</sup> after pruning the previous year.

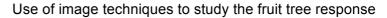
Trunk diameter (TD) measurements were carried out using a digital caliper (Digimatic, Mitutoyo America Corporation, Chicago, IL, USA) placed 0.1 m above the soil. Trees were dormant pruned in the winters of 2011 and 2012, pruning weight (PW, in kg tree<sup>-1</sup>) was determined in five control trees per plot.

A repeated-measures MANOVA was used to analyze TD and OCA evolution over time (Figure VI.3). A linear mixed model (Type III sum of squares) for the analysis of estimated marginal means (EMM) was built to separate treatment effects for PW and VP variables. Tukey's Honestly Significant Difference (THSD) *post hoc* test was used to compare treatments regarding pruning data. A probability level of P = 0.05 was used for all statistical analyses.

### 3. Results and discussion

#### 3.1. Study of the active crop growth

Figure VI.4 shows the evolution of TD for the different treatments along the 2012 growing season with values ranging from 35.2 mm (R+DI-70, in 01/19/12) to 76.6 mm (MR+SDI-70, in 08/01/12). We observed significant differences between treatments (P<0.0001) and also found that the time at which the images were taken significantly affected the measurement (P<0.0001). The interaction between treatment and time was also significant (P<0.0001), suggesting that some treatments showed a different behaviour over time compared to others.



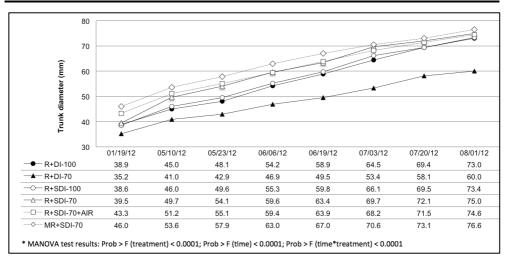


Figure VI.4. Trunk diameter evolution during the 2012 growing season for the different treatments. Expressed values are the calculated treatment means for the selected dates (x-axis).

Figure VI.5 shows the evolution of OCA for the treatments in 2012 growing season with values ranging from 0.34 m<sup>2</sup> (R-DI-70, in 04/12/12) to 7.03 m<sup>2</sup> (MR-SDI-70, in 08/12/12). We observed significant differences between treatments (P<0.0001), as well as a time effect (P<0.0001). The interaction between treatment and time for this variable was found to be significant (P<0.0001).

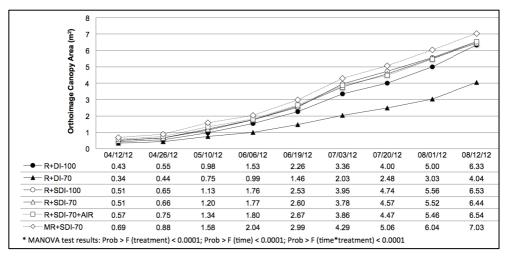


Figure VI.5. Orthoimage canopy area evolution during the 2012 growing season for the different treatments. Expressed values are the calculated treatment means for the selected dates (x-axis).

Both TD and OCA were good treatment descriptors as both were found to be highly significant (P<0.0001) during 2012. However, it is important to compare the overall treatment trend in both figures, as the objective was to evaluate the OCA variable as an indicator of fruit tree growth. Both TD and OCA showed similarities regarding treatment evolution along the season. While the MR+SDI-70 treatment

#### Chapter VI

registered the highest levels for the TD and the OCA data, the R+SDI-70 treatment gave the lowest values for these two variables.

The relationship between TD and OCA variables is shown in Figure 6. A model was constructed using 540 observations from several measurements along the season. The exponential curve fitting was the most suitable method to describe the allometric model between the two variables. The model ( $y = 0.0997 e^{0.0521x}$ ) was significant (P<0.0001) and showed a high coefficient of determination ( $R^2 = 0.8797$ ). Exponential models are commonly observed in allometric studies where total tree biomass is related to TD (Ahmed et al., 2013; Durkaya et al., 2013). However, such studies were conducted in forestry science experiments for uncultivated species, which were not altered, e.g. pruning, thinning. Our experiment was conducted in a commercial tree orchard subjected to crop maintenance practices, such as pruning, fertilization, and irrigation, during the growing season. Cultural practices may disrupt the relationship between the trunk and the canopy, especially those directly affecting the canopy/trunk of the tree, e.g. pruning.

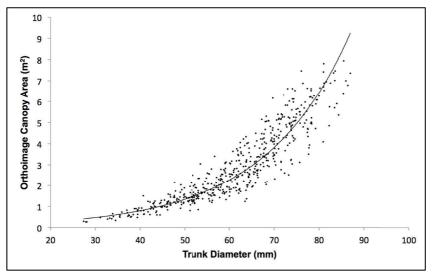


Figure VI.6. Correlation between orthoimage canopy area and trunk diameter. Curve fitting equation:  $y = 0.0997e^{0.0521x}$ . P (model) < 0.0001. Coefficient of determination: 0.87967.

The most notable trend in Figure VI.6 is the large uncertainty of canopy area estimates for larger TD. While canopy area was easily estimated during the early crop stages (lower TD), the relationship between the variables became uncertain at late crop stages (greater TD).

TD and OCA both proved to be useful in the measurement of fruit tree growth, although they provide information on distinct tree parameters. While TD measurements are cumulative between seasons, OCA measurements are cyclic, since leaf biomass is produced and destroyed every season. Continuous

measurement of canopy area provides important information on crop growth, which can be used in understanding crop performance under different cultural strategies. For instance, Fernández-Pacheco et al. (2014) applied image-based methods to estimate crop coefficient ( $K_c$ ) values in horticultural crops. Canopy volume of fruit trees can be well estimated by using LiDAR sensors, as reported in many studies (Palacin et al., 2007; Palleja et al., 2010; Wei and Salyani, 2005). However the price of these sensors is still high so image techniques based on digital photography have been lately introduced in agricultural research. For instance, Pforte et al. (2012) found that 2D canopy images obtained by digital cameras were well correlated with those obtained by LiDAR sensors, suggesting that digital cameras could be use as precise tools to estimate canopy areas.

The resources applied to determine the TD and OCA differed considerably. Each measurement required the acquisition of the TD and the OCA of 96 trees (entire plot). It took two people one hour to measure the TD of the entire plot (equivalent to 2.0 man-hours). On the other hand, it took three people 30 minutes to take the photographs for the OCA measurement. In addition, the OCA measurement took one person one hour to process all the images by computer and to obtain the OCA values (equivalent to 2.5 man-hours). The camera was mounted at 3.5 m at the top of an arch, and required 3 people to perform each OCA measurement. During the last year we developed a jib trolley to mount the digital camera on, which allowed to perform the same process by consuming less resources since the measurement could be executed by a single operator within the same period of time (30 minutes per 96 trees). In this sense, the development of new image sensors with higher resolutions, such as LiDAR sensors, along with the arrival of new technology. such as the UAV (unmanned aerial vehicle) drones, might suppose a remarkable advance in image techniques and fruit tree research. Until then, image analysis via digital photography techniques is presented as a low-cost tool that provides reliable and fast information on crop response. It was necessary to perform the OCA measurements in certain meteorological conditions in order to obtain high quality canopy images. For that purpose the photographs were taken in cloudless conditions so that the illumination and the canopy colours were standardized during the measurements. It was necessary as well, to avoid the measurements in windy conditions so that the canopy shape was not affected during the image process.

### 3.2. Study of the cumulative crop growth

Pruning weight, expressed as kg per tree, has been widely used in fruit tree research as a classical method to evaluate crop growth under different management strategies (Abrisqueta et al., 2010; Bryla et al., 2005; Bryla et al., 2003). However, this technique is highly time-consuming and requires many operators to carry out the measurements when performed in crops with high pruning requirements. Figure VI.7 and Figure VI.8 show the mean distribution of pruning weight (PW, in

#### Chapter VI

kg tree<sup>-1</sup>) and virtual pruning (VP, in m tree<sup>-1</sup>) measurements for the different treatments in 2011 and 2012, respectively.

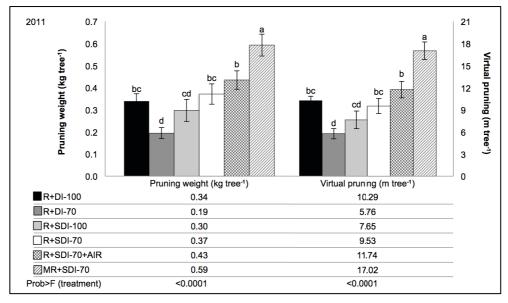


Figure VI.7. Pruning weight (kg tree<sup>-1</sup>) mean and virtual pruning (m tree<sup>-1</sup>) mean values for the different treatments for the 2011 growing season.

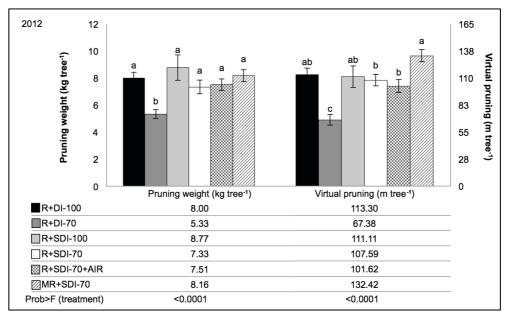


Figure VI.8. Pruning weight (kg tree<sup>-1</sup>) mean and virtual pruning (m tree<sup>-1</sup>) mean values for the different treatments for the 2012 growing season.

The effects of the treatments were significant in both years (P<0.0001), with similar treatment distributions. In 2011, PW and VP reflected the same differences between treatments, as they had exactly the same factor level means as shown by Tukey's HSD test. In 2012, the distribution was quite similar between variables, although VP was more sensitive, as reflected by more factor level means in Tukey's HSD test. Figure VI.10 shows the correlation between PW and VP in 2011 and 2012. The correlation between these two variables can be described by a significant linear model (P<0.0001), which presented a high correlation index ( $R^2_{2011} = 0.86$ ;  $R^2_{2012} = 0.80$ ). Thus, there was a close relationship between PW and VP. On the basis of this observation, we propose that it is possible to determine cumulative tree growth by applying VP method.

The resources applied to determine PW and VP differed considerably. It took three people four hours to measure the PW of the entire plot (equivalent to 12.0 manhours). On the other hand, it took three people 30 minutes to take the photographs needed for the VP measurement (96 trees). In addition, the VP measurement took one person 30 minutes to process all the images by computer and to obtain the VP values (equivalent to 2.0 man-hours). The VP methodology required fewer resources (2.0 vs. 12.0 man-hours) and provided more information about the orchard management (Figure VI.9).

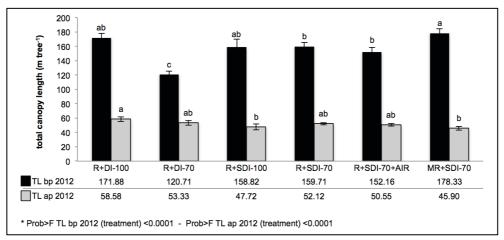


Figure VI.9. Treatment mean values for total canopy length before (TL  $_{bp 2012}$ ) and after (TL  $_{ap 2012}$ ) pruning in the 2012 growing season.

Figure VI.9 shows the mean distribution of TL for the treatments before  $(TL_{bp})$  and after  $(TL_{ap})$  pruning in 2012. There were many differences between treatments before the pruning that could be attributed to treatment effects. After pruning, such differences were reduced since one of the objectives of pruning practices is orchard uniformity. It is important to note that the experimental trial was conducted in a commercial fruit orchard in which company operators performed the pruning manually. Thus, the detection of differences between treatments after pruning could be attributed to the pruning performance of each operator. Therefore, image

techniques allowed us not only to study the treatment effects but the overall pruning performance, which is valuable information for researchers and field technicians alike.

Figure VI.10 shows the relation between the increase in OCA ( $\Delta$ OCA<sub>2012</sub>) and cumulative shoot growth (CSG<sub>2012</sub>) in 2012 growing season. A linear correlation was detected between these two variables (y = 0.0481x;  $R^2 = 0.5737$ ), both representing the cumulative plant growth during 2012 growing season. While CSG integrated the tree growth from shoots and branches,  $\Delta$ OCA integrated the tree growth from leaf biomass. By analysing both variables, we were able to get a rough idea of the total aboveground biomass produced in a single season (2012).

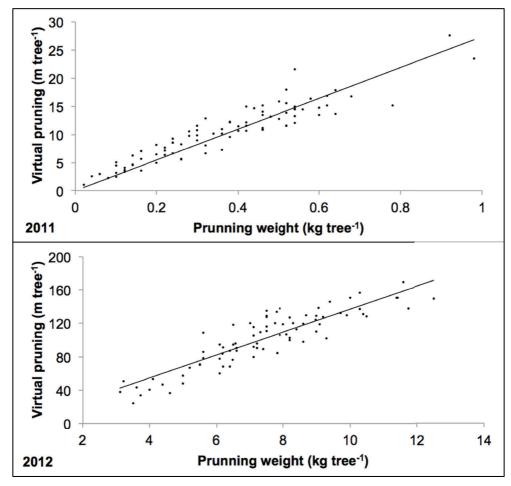


Figure VI.10. Virtual pruning and pruning weight correlation in 2011 (upper image) and 2012 (lower image) growing seasons. P(model) in 2011 < 0.0001. Coefficient of correlation in 2011: 0.86107. P(model) in 2012 < 0.0001. Coefficient of correlation in 2012: 0.80393.

In addition, there was a positive correlation between these two variables, so the more shoots/branches a tree generated, the more leaf biomass it was able to produce, as both GSG and  $\Delta$ OCA increased linearly (Figure VI.11).

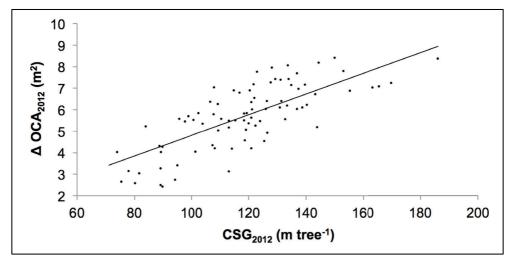


Figure VI.11. Correlation between cumulative shoot growth (CSG<sub>2012</sub>) and orthoimage canopy area increase ( $\Delta OCA_{2012}$ ) in the 2012 season growing. Curve fitting equation: y = 0.0481x. P (model) < 0.0001. Coefficient of correlation: 0.5737.

# 4. Conclusions

The orthoimage canopy area (OCA, expressed in  $m^2$ ) method proved reliable to study active crop growth along the season. The OCA was closely related to trunk diameter (TD, expressed in mm) measurements, with a high degree of correlation during early growing stages. We consider these two techniques suitable to study active fruit tree growth since they are non-destructive and growth-sensitive approaches. However, although related, these methods measure different tree growth parameters.

Cumulative crop growth was determined by the virtual pruning (VP, expressed in m tree<sup>-1</sup>) method and was well correlated with fruit tree pruning weight (PW, in kg tree<sup>-1</sup>). We propose that VP is a good estimator of PW since it proved to be a reliable technique and required fewer resources than other classical techniques (i.e. PW). Furthermore, by analysing the total length (TL) of a tree before and after pruning, we gathered information on pruning performance. Cumulative shoot growth (CSG) and increase in OCA ( $\Delta$ OCA) provided information on aboveground biomass production and were positively correlated.

Given the sensitivity to tree growth and the non-destructive nature of these image techniques, we consider them novel and highly suitable tools for research into active fruit tree growth.

**Chapter VII. General discussion** 

# 1. Irrigation strategies in fruit tree production under limiting soil conditions

SDI and DI systems were evaluated in soils with limiting physical properties in peach and nectarine orchards. Simultaneously, the experiments compared the application of different irrigation regimes (100 vs. 70%  $\text{ET}_{c}$ ) within the systems and studied the effects on crop growth and fruit production. The reduction in the amount of applied water (-30%) by the SDI system did not affect negatively the fruit tree growth, since trees had a similar vegetative development than those under DI or SDI at full irrigation. However, when such reduction was applied by the DI system, fruit trees achieved lower trunk diameters and canopy volumes. Such differences were evidenced during the first year of the orchards, crop establishment, although were maintained until the end of the experiments, at fruit production stages.

In the same way, as observed in Chapter II, trees under SDI presented a better water status (lower CT and higher SWP) compared to those under DI when the water reduction was applied. Overall, it could be observed that under limiting soil conditions the SDI had a better performance and achieved a higher irrigation efficiency. Similarly, Pablo et al. (2007) reported that plots under SDI presented higher irrigation efficiencies, although those experiments were carried out in sandy loam soils, with no infiltration issues. In this sense it is important to remind that the experiments conducted in this thesis were established in orchards planted in ridge systems - with low infiltration areas - in soils with soil crusting problems - with low infiltration rates – meaning that a limited water infiltration could be the main constraint for the irrigation efficiency. As pointed out in Chapter V, SDI delivers the water needed for the crop beneath the soil surface, so this might be the main reason that would explain a better adaptability of the system under such conditions. For instance, as reported by Lamm and Camp (2007), SDI systems increase the irrigation efficiency as a result of the reduction (or elimination) of soil water evaporation, surface runoff, and deep percolation.

On the other hand, in Chapter V it was observed that indistinctly of the applied regime, trees under SDI had a better vegetative development than those under DI but it was not translated into fruit yield increases. It is important to note that the experiments were set up in commercial fruit orchards, which were dormant pruned every season. Such practices attenuated the differences in canopy volumes among treatments (as observed in Chapter VI), what could reduce the potential fruit setting, and consequently could explain the absence of higher yields in SDI treatments. For instance, after a 3-year study in a peach orchard, Bryla et al. (2003) did not observe significant differences in fruit yields between DI and SDI systems, and indicated that plots under SDI system suffered from *water ponding*, a SDI limitation *phenomenon* that has actually been described by Burt and Styles (2011). By analyzing the results in this thesis, it can be stated that by SDI it is possible to

reduce the irrigation dose by -30% during initial crop stages (1-2 years) without affecting the crop development. However, the imposed deficit could be too high to apply during production stages.

Overall, the irrigation dose appeared to be the main factor affecting the crop production (Chapter V). Thus, while the irrigation deficit (-30%) was not critical during initial crop stages, it determined significant yield reductions when applied on production stages. When sustained deficit irrigation is applied in a moderate level, it allows the plant to adapt progressively to the water deficit so that stress does not affect significantly the crop, but when stress increases severely the production is affected (Fereres and Soriano, 2007). For instance, Goldhamer et al. (2005) proved that in almond trees sustained deficit irrigation was the least detrimental to yield when compared to other regulated deficit irrigation (RDI) strategies. However, Fereres and Soriano (2007) carried out a long-term experiment in a peach orchard and found that RDI was more advantageous than sustained deficit irrigation for the same amount of water reduction. Among them, RDI during Stage II of peach growth appeared to be the most effective in terms of fruit production and water productivity. When the water deficit is applied at the period of rapid expansion of fruit growth (Stage III), the fruit yield is considerably reduced (Marsal et al., 2006; Rufat et al., 2010). Thus, it is crucial to elucidate the effects of water stress on crop response at different phenological stages in order to determine the best strategy in accordance to different scenarios and production objectives. Overall, deficit irrigation strategies are proposed as alternatives to those involving full irrigation in areas with limited water resources (Pérez-Pastor et al., 2014) although its long-term sustainability needs to be reviewed in different case scenarios (e.g. under limiting soil conditions).

Air injection through the SDI system did not affect the crop performance since no effects were observed in any of the studied variables. Neither the vegetative growth nor the physiological status resulted affected by the oxygation treatments. Similarly, none of the production parameters or the fruit quality attributes were affected by the forced aeration treatments. Our results are not consistent with those found in other studies (Bhattarai et al., 2006; Bhattarai and Midmore, 2009; Bhattarai et al., 2008; Chen et al., 2010), which described that oxygenation treatments (applied by similar means) enhanced soil aeration and crop performance. However, in the cited studies, the experiments were performed in other crop species and under particular soil conditions (i.e. heavy clay soils), prone to hypoxia. It is important to stress this last statement, since oxygation was developed with the purpose to overcome hypoxic events (Goorahoo et al., 2002), so no beneficial effects from these techniques are expected if hypoxia is not suffered by the crop. The experiments of this thesis were set up in salt affected soils under high-frequency irrigation systems, which were susceptible to suffer waterlogging events. Thus, no effects from oxygation treatments could be described although no symptoms of hypoxia could be observed either. In this sense, it is important to remind that the experiments were carried out in orchards planted in ridge system, which is an appropriate system to enhance the soil aeration an avoid waterlogging events (Roth et al., 2005; Tisdall and Hodgson, 1990).

## 2. Use of soil amendments and mulch techniques to ameliorate the soil conditions

In this thesis we proposed the use of organic amendments to ameliorate the soil conditions by using two different approaches: (i) surface amendments, also known as mulch techniques, and (ii) localized amendments, which were set up beneath the drip line in SDI plots.

Regarding the use of organic amendments as mulch techniques we observed beneficial effects on the topsoil hydraulic properties what in turn improved the crop response. The organic mulch application did not affect the soil bulk density, although the water infiltration rate was considerably improved. Similar results were described by Merwin and Stiles (1994), who found that after organic mulch applications both cumulative infiltration and water sorptivity were enhanced, while soil bulk density remained unaltered. Thus, the mulch affected only the first millimeters of the soil, by preventing soil crust formation and deterioration of topsoil structure what in turn improved the hydraulic soil properties. The irrigation efficiency was considerably improved by mulch application since trees were in a more favorable water status, which was translated into fruit yield increases. Similar results were found in apple (Rubauskis et al., 2004) and cherry crops (Yin et al., 2012), were organic mulch applications improved considerably the water status and the crop production. Thus, as previously pointed by Yin et al. (2012) and Zribi (2013) mulch techniques can reduce irrigation water use and increase the protection of the soil from water erosion, which can be enhanced by DI systems. However, it is important to point out that organic mulch applications may not cover the whole nutrient requirements, especially if high yields are expected or the mulch fertility is low (as in this study). For instance, Nielsen et al. (2003) reported the need of supplemental fertilizers to provide essential elements (i.e. micronutrients) when organic mulch applications were performed.

As stated by Schwankl and Hanson (2007), the emitter discharge (in mm  $h^{-1}$ ) needs to match the infiltration soil rate (in mm  $h^{-1}$ ) in order to prevent runoff and provide a proper water distribution within the soil profile. The infiltration area is limited on ridge systems (widths < 1 m) so the infiltration soil rate must be appropriate to prevent runoff problems when irrigation is provided by DI systems. In such cases, organic mulches improve considerably the potential infiltration of the ridge, enhancing the irrigation efficiency and avoiding the ridge erosion.

Localized soil amendments with organic substrates (i.e. rice husk substrate), as proposed in this thesis, resulted in beneficial soil effects, which in turn enhanced the crop performance. The rice husk amendment reduced the mid-layer (0.2 - 0.4 m) soil bulk density, increased the aeration porosity, and improved both the soil

infiltration rate and the saturated hydraulic conductivity. Similar results were obtained by Anikwe (2000) and Okonkwo et al. (2011) who reported beneficial effects on soil properties by the use of rice husk substrates. However, such amendments were performed on soil surface unlike the ones in this thesis, which proposes the application of soil amendments in the mid-layer of the soil. It is important to note that the soil amendment was performed in SDI treatments, so the rice husk substrate was placed beneath the drip line in order to improve the hydraulic properties in the influence area of the emitter.

As observed in Chapter II, the rice husk amendment improved the vegetative crop growth and the crop water status during initial crop stages. These differences were maintained until production stages (Chapter V), in which amended trees reached higher trunk diameters and canopy volumes compared to unamended ones. The rice husk amendment enhanced both the fruit growth and the crop load what was translated to an increase of 52% of the commercial fruit yield. It seems that rice husk substrate improved soil properties, providing a better soil environment for root activity and in turn a better crop performance. Many studies (Merwin and Stiles, 1994; Nielsen et al., 2004) have reported beneficial effects of organic amendments on crop production, particularly in soils with limiting physical conditions where such amendments substantially improved the hydraulic properties of the soil (Anikwe, 2000; Okonkwo et al., 2011). The application of rice husk substrate improved the SDI performance in soils with limiting physical properties, what might be an alternative to consider in such conditions.

It is important to note that rice husks are highly siliceous (rich in SiO<sub>2</sub>), have a high C:N ratio, and high content of cellulose and lignins, which confer rice-based substrates a long-term stability in the soil (Correa et al., 2009; Mandal et al., 2004). Rice husk occupies a preeminent position in the by-product industry, not only in terms of its amount produced worldwide, but also because of its unique chemistry-related features (Soltani et al., 2014). Localized applications of amendments, as proposed in this thesis, imply an important reduction in application rates. It is crucial to consider an efficient use of by-products since there is a growing interest in industrial and agronomical exploitations.

# 3. The relationship between the crop water status and the fruit quality

During the last decades several deficit irrigation strategies have been proposed with the main objective of increasing the water use efficiency. Thus, a wide range of studies have been carried out in many fruit tree species, providing valuable results regarding crop water stress and crop production variables (Girona and Fereres, 2012). In this thesis, the use of more advanced statistical techniques, as the multivariate analysis (Chapter IV & V), evidenced the existing relationship between the crop water status and some fruit quality parameters.

In this sense, it was observed that some quality variables like fruit weight (FW) and total soluble solids (TSS) content were close related to plant water status. Thus, trees with better water status (higher SWP and GS, and lower CT) produced bigger fruits with lower TSS contents as compared to stressed ones. These relations were previously described by Berman and DeJong (1997) and Crisosto et al. (1994) in a series of experiments carried out in peach orchards. In Chapter III, it was observed how trees under high water stress conditions induced the partial dehydration of peach fruits, thus substantially increasing the fruit dry matter content and decreasing the flesh firmness. These findings are in accordance with those described in peach by other authors (Gelly et al., 2004; Lopez et al., 2007; Marsal et al., 2006), who reported the acceleration of the fruit ripening process when plants were subjected to water stress in late stages of fruit development.

This thesis studied the relationship between the crop water status and the PPO activity (Chapter IV & V), which is one of the most influencing factors on fruit stability and durability. Crisosto et al. (1995) reported that pre-harvest factors and cultural practices (e.g. fertilization or irrigation) could influence postharvest fruit quality and performance. For instance, Falguera et al. (2012b) and Pascual et al. (2013) indicated that nitrogen fertilization affected the PPO activity, and in turn the peach postharvest stability. The experiments carried out in this thesis indicated that water stress triggers PPO activity in peach and nectarine crops, thus reducing the postharvest life of the fruits. Similar results were found by other authors, who described an enhanced PPO activity (Buendía et al., 2008) and a lower postharvest stability (Pliakoni and Nanos, 2010) of peach fruits under deficit irrigation treatments.

It is basic to know the relationship between water stress and quality parameters since they provide useful information on how to manage the irrigation strategies. Actually, deficit irrigation strategies are being used in a commercial scale in a wide variety of crops (e.g. tomato, vineyard) to increase some specific quality parameters (e.g. TSS content, phenolic compounds) and increase the consumer acceptance of the final products (Chen et al., 2014; Romero et al., 2015). Nowadays, fruit durability is a major quality parameter; therefore a full understanding of the factors that affect this parameter is crucial in order to determine the most appropriate strategy to enhance this value-added attribute.

#### 4. Image analysis: a new tool for fruit tree research

This thesis evaluated the use of image techniques to study the fruit tree performance by using two different approaches: (i) Study of the canopy active growth, and (ii) study of the cumulative crop growth. The former used the orthoimage canopy area (OCA) technique, in which the canopy area was measured at different dates along the crop growth season and later analyzed by computer means. The OCA values were compared to trunk diameter (TD) increases within the same dates, describing similar trends among treatments. Both variables (OCA)

& TD) were sensitive to crop growth and provided useful information to assess the differential tree development in the experiments. In addition, it was observed a close relationship between TD and OCA, although the relationship between these variables became uncertain at late crop stages. Continuous measurement of canopy area provides important information on crop performance, which can be used in understanding crop response under different cultural strategies or environmental conditions (Ali and Anjum, 2004). For instance, Fernández-Pacheco et al. (2014) developed an image-based method to estimate the crop coefficient ( $K_c$ ) of lettuce crops and hence adjust water crop requirements. In this thesis, the non-destructive methodology was used to evaluate the crop response under different applied treatments (Chapters II, IV & V).

Pruning weight, expressed as kg per tree, has been widely used in fruit tree research as a classical method to evaluate cumulative crop growth under different management strategies (Abrisqueta et al., 2010; Bryla et al., 2005; Bryla et al., 2003). In chapter VI, a new methodology to estimate the pruning weight (PW) was introduced, namely virtual pruning (VP), which was highly correlated with the classic variable (i.e. PW). Thus, on the basis of these results, the VP methodology was proposed as a reliable technique to study the cumulative tree growth, which provided additional and useful information (e.g. about pruning performance), and consumed fewer resources, especially when the crop had high pruning requirements. Other authors (Campillo et al., 2011; Campillo et al., 2008; Rico-García et al., 2009; Serdar and Demirsoy, 2006) have applied similar methodologies to estimate the crop biomass of horticultural and fruit tree crops obtaining promising results. The quantification of biomass production in multiannual crop experiments is a challenge since most classical methods are destructive and/or involve laborious assessments. There exist other image-based techniques, such as LiDAR technology (light detection and ranging), which is a precise tool that offers a wide range of applications in fruit tree research. Although this technology is still very expensive is likely to be adopted in the coming years as the price of the sensors decrease. For instance, Pforte et al. (2012) compared LiDAR and digital image methodologies in a plum tree orchard and concluded that the 2D image analysis could be a comparable and cost-effective alternative option to the LiDAR system. Overall, image analysis through the use of digital photography techniques is presented as a sensitive and reliable tool to be used in fruit tree research, which can be applied to study the crop performance under different conditions or management strategies, and to estimate the cumulative biomass production by non-destructive means.

### 5. Future research

Future work should take into account the long-term sustainability of irrigation and soil management developed strategies. In this sense, there is a need to establish long-term experiments and modeling studies, and assess the effects on both the environment and the crop productivity. These statements are especially meaningful 92

when crops are established in soils with limiting conditions, which are more susceptible to the loss of soil productivity. One of the major issues that agriculture will face over the next years is soil salinity and its side effects. In this sense, during last years many studies have focused in the assessment of the risk of salinization by irrigation strategies (Geerts and Raes, 2009; Hsiao et al., 2007; Kaman et al., 2006; Raine et al., 2007; Sarwar and Bastiaanssen, 2001). Nowadays, deficit irrigation strategies are being expanded in many areas of the world although it is uncertain if these strategies are sustainable in arid and semiarid areas, where saline water is used in many cases (Aragüés et al., 2014). Similarly, SDI technology is more often used in a wide range of crops as higher irrigation uniformities and efficiencies are expected by the use of this system. However, its durability is under review especially when used in fruit tree orchards, where root intrusion and pinching is a risk that many growers do not want to take.

This thesis proposed different alternatives to overcome the limiting soil factors, ranging from different irrigation strategies (e.g. SDI, and deficit irrigation) to low-cost soil amendments. To that purpose, the experiments evaluated the effects of the different strategies on soil properties but also on crop growth, productivity and fruit quality. In this sense, we evaluated a new quality parameter of growing importance in the fruit sector, such as fruit durability and stability. We believe that this fruit quality attribute should be taken into consideration in further research when evaluating new management strategies. Similarly, new tools to evaluate the crop performance were introduced, such as the image-based analysis by means of photography techniques. In next future, new optical sensors and technologies, such as LiDAR, would be probably more accessible and widely used in fruit tree research, offering new ways to assess the crop performance under different conditions.

The use of by-products as soil amendments is a practice that it is widespread in many agricultural areas all around the world. In many cases, these materials are cost-attractive and present some interesting properties (e.g. long-term stability, or porosity) what makes them perfect candidates to be used as soil amendments. However, it is essential to analyze their effects on the soil properties and avoid the use of these products if represent any environmental risks.

It is of paramount importance to develop and assess new irrigation strategies in order to enhance the water use efficiency in crop systems and assess its sustainability in a long-term basis.

#### 6. Conclusions

- i. Subsurface drip irrigation was a valuable system that might be used in soils with infiltration issues. Under the experiment conditions, it allowed to reduce the irrigation dose by -30% without affecting crop growth and water status during first crop stages.
- ii. Forced aeration by means of air injection through the SDI system did not cause any effects on crop performance or crop productivity under the experiment conditions.
- iii. Surface soil amendments (i.e. organic mulch) improved the topsoil physical properties and hence enhanced the crop growth, productivity and water status. This low-cost technique represents a good solution to apply in soils with limiting physical properties.
- iv. Water infiltration was improved by the use of mulch techniques in a ridge system.
- v. Localized soil amendment by the use of rice husk substrate improved the physical conditions in the mid-layer of the soil what in turn improved the SDI efficiency and hence the crop performance.
- vi. Water stress triggers the polyphenol oxidase (PPO) activity in nectarine and peach fruits, an enzyme that is highly related with the fruit postharvest stability.
- vii. Image analysis by means of digital photography is presented as a valuable and reliable tool to study the fruit tree performance and offers a wide range of applications to be used in fruit tree research.

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The Nation that destroys its soil destroys itself. Franklin D. Roosevelt.