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PhD school Agricultural Sciences - XXXVI cycle (2020-2021)

PhD Thesis

Growth control and productive aptitude of Olive tree (*Olea europaea* L.)

Candidate:
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Abstract

Optimizing vegetative growth and canopy architecture is crucial for olive tree productivity. The vegetative growth is linked with the root systems, vital for anchorage and nutrient uptake, and in olive it exhibits plasticity and deep exploration strategies against abiotic stress. Leaf structural changes under abiotic stress contributing to olive tree resilience. 1-year-old shoots are the most mainly organs for the production, in fact, in this organ we can find the potentially reproductive buds.

Therefore the 1-year-old shoot growth is crucial for effective management of reproductive growth and fruit production, given the complexity of olive flower and inflorescence development. The latter are influenced by several environmental conditions, biochemicals endogenous signals, cultivar traits, and nutrient availability. After flowering, fruit development happens, and it is a greater sink organ than vegetative growth. Fruit growth dynamics follow a double-sigmoid curve, with water stress during early development impacting final fruit volume, highlighting the importance of irrigation strategies like Regulated Deficit Irrigation (RDI) for optimizing fruit yield and oil production.

Nutrient management is essential for consistent and sustainable olive production, with fertilization supporting vegetative and fruit growth driven by key nutrients like nitrogen, phosphorus, and potassium, as well as micronutrients influencing flowering and fruit development. Fertilization strategies should consider factors like nutrient availability, soil analysis, and leaf diagnosis for optimal tree health and production, while sustainable practices, including the use of wastewater and biological solutions, enhance nutrient availability while minimizing environmental impact.

In the context of olive tree productivity, light availability plays a significant role, with shading potentially reducing photosynthesis early in the season. Anti-insect or anti-hail nets provide beneficial shading while serving multiple purposes in olive cultivation, contributing to overall orchard management and emphasizing the interconnectedness of various factors in optimizing olive production.

In modern olive cultivation, precise irrigation management is essential. Trunk-embedded microtensiometers offer real-time monitoring of trunk water potential, optimizing water usage. Irrigation is not enough to increase vegetative growth and production, so adequate nutrient levels are crucial for plant health and frost tolerance, mitigating vulnerability to intensified frost events due to climate change.

Frost damage can exacerbate susceptibility to diseases like olive knot, caused by bacteria such as *Pseudomonas savastanoi* pv. *savastanoi*. Cultivar susceptibility varies, emphasizing the need for holistic management practices.

Against pests and abiotic stresses, innovative solutions like anti-insect nets can regulate light exposure and enhancing productivity in olive orchards.

1. Introduction

Although in the last years the olive oil consumption is increasing (COI, 2024), in Italy the production is decreasing (AGRISTAT, 2024), also because of climate change.

The global climate is changing, and the average temperatures are increasing (Zandalinas et al., 2021). In Europe, the average temperature between the 1988 and 2002 was 1°C higher than the temperature between 1973 and 1987 (Legave et al., 2009). Comparing the same periods a 7/12 days early flowering date have been reported for the most common fruit tree crops (Legave et al., 2009). Although already in the last years the climate is changing, in the future extreme environmental phenomena can increase (IPCC, 2022). Precipitation could decrease in the Mediterranean basin (Tuel and Eltahir, 2020), especially during summer (Cos et al., 2022). The global warming can also increase the power of winter frosts (Hamouda et al., 2021; Zohner et al., 2020).

Global warming can increase the areas suitable for olive (Olea europaea L.) cultivation in Europe (Tanasijevic et al., 2014), spreading it more north worth. At the same time, without changing the actual agronomical techniques, global warming can increase the yield in some area of the Mediterranean basin like Puglia region in Italy and decrease the yield in others, for example in Andalucia region (Spain) (Fraga et al., 2019; Cabezas et al., 2020). The yield decrease will be caused by different stress factors: frost, heat, drought, and other environmental stresses, especially concerning flowering. For example, in the south of Spain it is possible that, in the future, a lack of chilling for dormancy break requirements associated with heat waves during flowering will cause a decrease of olive yield and a reduction of suitable areas for olive cultivation (Gabaldón-Leal et al., 2017).

Even in the central Italy region, which is about the north border of olive cultivation, climate change and cultural change are modifying the cultivation traditions that need more tools and more precision. In this scenario this thesis was positioned, focusing on the vegetative growth as a potential to produce the following year. The attention to vegetative growth and the reproductive attitude allows the olive grove to be managed to have better and continuous production.

Olive tree cultivation often does not take into account vegetative development, the load of flowers and fruit, nor environmental phenomena. To start considering all these factors it is necessary to know the specific scientific literature. For these reason some general notions are introduced regarding the factors that can affect the vegetative growth and the reproductive attitude of the olive tree.

Hence, the vegetative organs will be introduced to know the period of growth, the management of each organ. Vegetative organs are very related with flowers and fruits production, in fact, also the flowers and fruits cycle development will be presented.

Vegetative and reproductive organs are influenced by the water and nutrient availability, and by the photosynthetic radiation, and the scientific literature information of these factors will be elaborated.

2. Vegetative organs

In the olive tree, unlike other subtropical fruit crops, to produce every year, the olive tree needs to develop new mixed shoots, which bear a majority of flowering buds and a few vegetative buds, every year. Therefore, managing mixed shoot growth and canopy architecture is strategically important for production purposes.

Is possible to differentiate different vegetative parts: roots, leaves, trunk and main branches, and 1-year-old shoots which can be mixed shoots.

2.1.Root

The root system has different functions. Beside the anchorage, it explores the soil niches and absorbs most of the water and nutrients required for the tree vegetative growth and reproduction. It acts as a reserve of nutrients (Mechri et al., 2011).

The olive tree has a marked plasticity that makes it adaptable to different environments, which can also change during the season (Polverigiani et al., 2011).

In six years after planting, the root system can reach the 0.8 m depth in the soil (Chiraz, 2013), the deep exploration of the roots plays an important role against abiotic stress such as high temperatures, water drought, but also involves a large expenditure of energy and nutrients given by the root system, Masmoudi-Charfi et al., (2011) reported that in six-year old trees in a 6m x 6m planting layout, the root system can even reach an overall length of 33 km.

In central Italy, after observation of Polverigiani et al. (2012) the roots below 0.2 mm diameter began to growth already in February, and for all the month they presented the maxim growth rate. In the same observation the roots bigger than 0.2 mm presented the maximum growth rate during May, showing a decrease when the fruits were in active growth (Polverigiani et al., 2012; Chiraz, 2013). The decrease in the growth rate of the roots was also shown by Rosati et al. (2018a), in trees grown in pots with different fruit loads.

It is important to point out that the study of roots is always very difficult, especially in the field, which is why the literature lacks works regarding roots. In the future it is desirable to increase work regarding this apparatus which appears to have dimensions comparable to, if not larger than, the canopy, as well as covering a great importance for the whole entire tree.

2.2.Leaves

Leaves are the main organs deputed to photosynthesis and transpiration. These functions make the leaves very important for both vegetative and reproductive growth.

Leaves can maintain their activity for a maximum of three seasons, usually two seasons (Rapoport et al., 2016) and are renovated with the emission of new shoots. The phyllotaxy is alternate, opposite, and whorled. In suitable conditions, they take about 15 days from the moment of sprout to their maximum expansion (Leon and Bukovac, 1978). Once adult (fully expanded leaves), in the cross section is possible to observe two parts, one formed by three compacted layers of elongated cells interspaced by tricosclereids forming the palisade parenchyma in the upper part, and the second part with only one palisade parenchyma layer in the lower part (Bosabalidis and Kofidis, 2002; Bacelar et al., 2004; Moreno-Alías et al., 2009). Both the layers are in contact with the epidermis (in the up and lower lamina). Furthermore, the upper epidermis is covered by a continuous rough wax layer and a few stellar trichomes, while the lower epidermis is covered by more stellar trichomes whose function is to protect the small and abundant stomata.

Number of cells forming the epidermis, the palisade parenchyma, the spongy parenchyma (which is in the middle of the two palisade layers), and their tissue thickness change in case of growth under water stress as does the number of stomata per leaf area increase in stress conditions (Bosabalidis and Kofidis, 2002; Bacelar et al., 2004). At the same time the dimension of stomata changes in relation, also, to the cultivar (Bosabalidis and Kofidis, 2002; Bacelar et al., 2004).

2.3.Trunk. Branches, and shoots

Trunk and main branches are important as reserve tissues in olive (Bustan et al., 2011). Shoots instead are not only important for the new leaves production, but also to produce the new buds, presented on the nodes, where could develop the inflorescences the following year (Moutier et al., 2004).

The canopy of olive can have different shoots, one of them is the sucker shoot, which presents only vegetative growth and a strong vigour for several years (Neri et al., 2009). On the other hand, in the canopy, it is possible to also individuate the mixed shoots. They can bear the inflorescences in the 1-year-old portion (year n) and show a vegetative growth that will bear inflorescences/fruits in the following year (year n+1) (Neri et al., 2009). These branches are less vigorous than suckers.

The 1-year-old mixed shoot (figure 1), is a proleptic shoot, formed generally from the apical bud of the 2-year-old shoot of the year before. From the lateral buds formed in the shoot that is growing during the season can sprout another shoot, these shoots are called sylleptic shoots, and they also can bear new inflorescences in the axillary buds of the leaves in the following year. The structure and the architectural management of the canopy are important for the vegetative/reproductive equilibrium of the tree. It can be affected by the cultivar and the agronomical techniques (Moutier et al., 2004; Neri et al., 2009).

Figure 1: mixed shoot with fruits in the 1-year-old part, and new vegetative part of the current year of growth.

2.4.Seasonal trend of vegetative growth

Depending on the environmental conditions and the cultivation practices, olive buds sprout about in the end of winter and stop their develop around the middle of autumn (Albarracín et al., 2017). Vegetative buds present an ecodormancy, and the growth arrests of the shoots seems triggered by temperatures and not by photoperiod (López-Bernal et al., 2020). In fact, by placing olive trees from winter temperature to a greenhouse environment (temperatures above 18°C), the bud sprouting should begin in about 2 weeks (López-Bernal et al., 2020).

During the season, the shoot grows, above all, during spring (Strippoli et al., 2013; Marra et al., 2016; Albarracín et al., 2017), with a peak of growth before blooming. Some cultivars, in some climates and conditions, also showed a little peak in summer, during early-summer (Gómez-del-Campo, 2013). Bedbabis et al. (2010) report that in Tunisia is possible to observe a shoot growth at the end of summer.

The trend of vegetative growth is usually sigmoidal, even though several factors can affect it (Aïachi Mezghani et al., 2012; Cherbiy-Hoffmann et al., 2013; Fernandes et al., 2021).

3. Flower and inflorescence

3.1. Architecture of flowers on inflorescences a war for nutrients

An olive tree can produce up to 500,000 flowers during the blooming (Lavee, 1996) and, when the fruit set is adequate, around 2% of them become fruits (Troncoso et al., 1978), although the 1% of fruit forming flowers is sufficient to ensure a good commercial harvest (Griggs et al., 1975).

The flowers are grouped along a paniculate inflorescence in groups of 8-19 (Reale et al. 2006). A flower is normally formed by 4 sepals to form the calic, 4 white petals merged on the base, 2 stamens with large anthers, a pistil composed of a bilobulate stigma, a short style, and a bilocular ovary with two locules, each containing two ovules (King, 1938; Cuevas and Polito, 2004; Martins et al., 2006). In olive, depending on cultivar, environmental and endogenous factors, an inflorescence can contain a mixture of perfect (or hermaphroditic, figure 2 and 3), and imperfect (or staminate, figure 4) flowers (Uriu, 1956; Reale et al., 2006). Staminate flowers have an abnormal pistil that does not develop collapsing, while the stamens grow normally (Uriu, 1956; Rosati et al., 2011). Their presence is usually concentrated in the lateral portion of the inflorescence and less in the apical flowers, even though differences were seen according on the cultivar (Seifi et al., 2008).

The majority of the collapsed pistils was observed by Cuevas et al. (1999) during the phase of their rapid growth, around one month before bloom, when the development of other floral organs is very advanced, during the stage of megaspore mother cell differentiation (Reale et al., 2009). This circumstance suggests the possibility that the management of the number of staminate flowers is regulated by the dominance of some flowers to maintain an equilibrium for nutritional resources. In fact, eliminating the first 4 apical flowers of an inflorescence, the percentage of the hermaphrodite flowers on the base of inflorescence increased (Seifi et al., 2008). In case of low availability of phosphorus, the mass of the pistils is greater in the flowers in an apical position compared to those in the basal position on the inflorescences (Erel et al., 2016). The competition theory is also supported by Rosati et al. (2011), that demonstrated a positive correlation between ovary mass and pistil abortion, correlation supported also by the fruit set (Rosati et al., 2010).

Furthermore, the ratio leaves-to-flowers, and the nutrient and water availability, affect the production of perfect flowers (Uriu, 1956; Perica et al., 2001; Fernandez-Escobar et al., 2008; Rosati et al., 2011). Despite in the past pistil abortion has been attributed mainly to cultivar characteristics (Morettini, 1950), and/or response to water availability during differentiation of the inflorescences (Hartmann and Panetsos, 1961), Fernandez-Escobar et al. (2008) found a tendency to increase pistil abortion with nitrogen deficiency in the leaves, although only for one year in a two-year study. The same study suggested that the nitrogen deficiency can affect the ovary abortion in addition to water stress during the inflorescences differentiation stage. As nitrogen, phosphorus availability can increase the weight of the ovary (Erel et al., 2016).

Other clues that lead to evidence that flowering is driven by competition between flowers along the inflorescence are showed by Reale et al. (2009). In his study the absence of starch grains in the ovary cells of staminate flowers was observed, while on the contrary, in the same cells of hermaphrodite flowers the plastids of the ovary cells contained starch grains.

Dominance effect is present, also in the opening chronology of the flowers along the inflorescence. The perfect flowers tend to anticipate the blooming compared to the staminate ones, and the apical flowers tends to open earlier than those located in the lateral portion of the inflorescence (Cuevas and Polito, 2004; Seifi et al., 2008).

Although a drought stress during winter dormancy does not influence the flower intensity (number of inflorescences per node and number of flowers per inflorescence), a stress during the inflorescence development (March and April according to the environmental conditions) can affect the flower intensity (number of inflorescences per node and number of flowers per inflorescence (Rapoport et al., 2012). A water stress applied during this phase, can also affect the number of perfect flowers, which decreases dramatically, leaving the greater ovaries as showed by Rosati et al. (2010).

Figure 2: Section of a perfect flower of 'arbequina' cultivar.

Figure 3: Perfect flower of 'Rosciola colli Esini' cultivar.

Figure 4: Imperfect flower of 'Rosciola colli Esini' cultivar.

3.2.Architecture of inflorescences on shoot

As already mentioned, the inflorescence of the olive is a paniculate rachis, and it is formed on the axillary buds of the one-year-old portion of the mixed shoots. A shoot can host several inflorescences, depending on the number of the newly formed axillary buds of the shoot as potentially one inflorescence per bud can develop (therefore two per node).

The inflorescences (previously buds) are produced in one-year-old mixed branches grown on both proleptic or sylleptic shoots (Mountier et al., 2004). The preferences about which kind of shoots bears the fruits should be cultivar dependent, in fact, Mountier et al. (2004) showed how ‘Picholine’ cultivar preferred the sylleptics shoots of the proximal part of a one-year-old mixed shoot. While ‘Arbequina’ cultivar confirmed the great aptitude to use all one-year-old mixed shoots. However, there do not appear to be different flowering preferences along the main axis of the mixed one-year shoot between the cultivars (Lodolini et al., 2017).

3.3.Inflorescence development

Even though it has been said that inflorescences can develop only from buds located in the 1-year-old shoots, creating new buds in the previous year is not enough to ensure a good flowering. The fate of the buds (reproductive, or vegetative, or silent) depends on different environmental and physiological factors (Badr et al., 1970; Badr and Hartmann, 1971; Stutte and Martin, 1986b; Rallo and Martin, 1991; Fernandez-Escobar et al., 1992; Baktir et al., 2004; Fabbri and Alerci 1999; Fabbri and Benelli, 2000; Troncoso et al., 2012; Haberman et al., 2017; Salimonti et al., 2021). Among the physiological factors affecting the fate of the buds, two reversible macro-stages can be indicated (induction, initiation) and one irreversible (differentiation). The three stages are discussed hereafter. After these stages the inflorescences and the flowers develop until the full bloom.

Knowing all these stages can be useful to manage the reproductive growth of the olive tree which is in opposition (and dominant) to the vegetative growth (Dag et al., 2010).

3.4.Induction

Induction is a phenological stage that occurs the growth season before the flowering. Generally, it is reported to occur around the pit hardening stage, about 7-8 weeks after full bloom the season before (Sanz-Cortés et al., 2002). However, the time window is very wide. The reproductive bud induction time is the most difficult stage to be detected as it is the most distant phase from flowering and produces no visible morphological differences, but only physiological ones (Hacket and Hartman, 1963;1964;1967; Fabbri and Alerci, 1999; Haberman et al., 2017). Andreini et al. (2008) reported to be able to distinguish the differences between the axillary buds of “on” or “off” trees even in July of the year before the flowering (close to the pit hardening phase), but the technique indicated on the study is not very clear.

Until now, the only method to detect it is to stress some factors and analysed the flowering in the future.

Fernandez-Escobar et al. (1992) found the time of maximum induction around 40-50 days after full bloom (DAFB) (cultivar ‘Manzanillo’ located in California, USA) while Dag et al. (2010) found a no different induction until 120 DAFB (cultivar ‘Coratina’ located in Israel).

It can be assumed that the bud induction is not a fixed physiological step occurring in the same period for all the buds. For example, Haberman et al. (2017) showed that Barnea cultivar in the southern coastal plain of Israel, induced the 13% of reproductive buds already at 22nd of June, while at 1st of September the buds were already inducted.

The factors involved in the induction process are several and the process is not totally known. The fruits (and their embryo) have a negative influence on the induction (Stutte and Martin, 1986a; Dag et al., 2009; Dag et al., 2010;), probably due to the hormones (Fernandez-Escobar et al., 1992) and the phenolic compounds of the fruits (Ryan et al., 2003). As found by Lavee et al. (1986), injecting chlorogenic acid into the scaffolds during winter the flower bud differentiation was reduced by more than 50%.

Furthermore, fruits, are in competition (and stronger sink) on nutrients availability, creating, in case of high fruit load, a nutrients starvation as Nitrogen, Phosphorus, Potassium, Starch, Sugars, Proteins, etc.

3.5.Initiation and dormancy

Once the buds have gone through the induction phase (considered as the evocation phase) during the summer, they move on to another phase, the initiation and flower bud dormancy. We consider these two physiological steps together because often the literature is confusing.

The beginning of winter rest occurs when shoot stops the growth because of decreasing temperatures (López-Bernal et al., 2020). The end of the rest period is determined when the temperatures are suitable for the bud growth. To have the reproductive budburst a chilling requirement should be satisfied (Rallo and Martin, 1991; Ramos et al., 2018).

For inflorescences buds are recognized endo-dormancy and eco-dormancy. In the first one, buds can sense the growth-promoting signals, both from environment and endogenous, but they do not start to growth until the growth-promoting signals are suitable (Wang et al., 2019). For example, of endo-dormancy there are the chilling requirement or the photoperiod (Lang et al., 2022). After the endodormancy the buds enter in the eco-dormancy, a period in which they do not grow because the environmental conditions are not suitable, the bud exit quickly from this status when the environmental conditions are suitable (Horvath et al., 2003).

So, in endo-dormancy, to release the dormancy, the buds require a period with cold temperatures (De Melo-Abreu et al., 2004). Ramos et al. (2018) determined as suitable temperatures for olive temperatures below 12.5°C although a chilling effect occurred at 15°C. Milak and Perez (2011) used in a trial 18°C exceeding the dormant period in Texas (USA). Controlling the night temperatures permitted to exit to dormancy in two research, one with night temperatures close to 4-8°C (and 18°C during the daytime (Malik and Bradford, 2009) and one with 10°C (Malik and Bradford 2005).

Malik and Perez (2011) showed that, during chilling accumulation, a period of more than 6 days above 26°C can hinders potential flowering.

When the chilling requirements are covered, the buds can potentially start to grow, after that endo-dormancy is released, the buds enter in the eco-dormancy and temperatures over the 12.5°C are enough to start the differentiation (Ramos et al., 2018).

Although the physiology of eco- and endo-dormancy is no-very well know, we can say that without this period with cold temperatures, flowering does not occur (Aybar et al. 2015), although there are extremes examples border line where “stranger” flowering occurs. This is the case of Hawaii, Tenerife Island, and a part of south-west of Andalucia, where flowering window can be very large (also for 4-5 months), or double, but without a lack of production (Miyasaka and Hamasaki, 2016; Medina-Alonso et al., 2020). Is important to consider that in the environments where usually olive is cultivated, around 300-400 hours of cold temperatures are enough to ensure the release from endo-dormancy.

During the dormancy period the genes OeFT1/2 expression in leaves increase with cold temperatures, this increment is linked to the induction times, in fact, in case of high fruit load the increment of these genes’ expression is lower (Haberman et al., 2017).

3.6.Differentiation

This phase of the inflorescences and flowers is characterized by a morphological chance of the meristem, and it is the sole stage compared to induction and initiation that is visible at the microscope. In late winter, the inducted and initiated reproductive buds start to differentiate, the OeAP1-1 genes start to increase its expression already in January, and in February an increment of OeAG-1 can be observed (Haberman et al. 2017). In this period olive tree management is very important and water stress at this time can negatively affect the differentiation (Rapoport et al. 2012; Liu et al. 2019).

4. Fruit growth

Fruit is a stronger sink for nutrients compared to the vegetative growth, leading to a carbon and nutrient starvation of the leaves, buds, shoots, and other tissues when the crop load is high (Bustan et al., 2013; Fernández et al., 2015; Rosati et al., 2018b). The vegetative growth is slowed down in case of high fruit load and low nutrient availability (Fernández et al., 2015).

Olive fruit growth follow, as the stone fruits, a double-sigmoid growth curve (Gucci et al., 2009; Cheng et al., 2017; Fernández et al., 2018). After the fruit set, the ovary enlarges with exponential growth due to the cellular multiplication of what is the fruit, this is the first phase, it ends in summer, about 7-10 weeks after full bloom (Gucci et al., 2009; Fernández et al., 2015). During the first phase, the majority of the volume of the fruit is due to the pit volume. In the second phase the growth of the olive tree slows down or stops, and this is the phase in which the pit hardening occurs. Depending on the environment and the agronomic techniques, pit hardening can start during the first half of summer and last about 50 days (Rapoport et al., 2013). The third phase starts when the pit hardening is concluded, in this time the cells of the mesocarp expand (Gucci et al., 2009), and oil accumulates in the cells.

The growth of fruits is related with the daily VPD often following a hysterical relation dependent on the time of the day Khosravi et al., 2021, 2022).

Water stress during the first phase can affect the final fruit volume, decreasing the cell size and not the cell number of the mesocarp (Rapoport et al., 2004).

Despite the fruit can do the photosynthesis, the dry weight increases as the source/sink ratio (i.e. m^3 of canopy volume per 1000 fruits) increases until at about 2.5 of the ratios (Fernández et al., 2018). The contribution of fruit photosynthesis to the carbon economy is modest, but important for the production of the oil fraction (Sánchez, 1995; Sánchez and Harwood, 2002).

5. Factors affecting the vegetative-reproductive balance

5.1.Irrigation

Olive is a xerophyte species, very tolerant to drought and hot climates (Connor, 2005; Connor and Fereres 2010). In fact, olive can survive in arid conditions (200-300 mm of rainfall per year), while in olive cultivation the production can increase substantially with irrigation (Patumi et al., 1999; Fernández and Moreno, 1999; Moriana et al., 2003; Lodolini et al., 2016).

Olive tree can act different strategies synergically to tolerate the drought. Most important strategies are the regulation of the stomata closure and the transpiration (Moreno et al., 1996; Nogués and Baker, 2000), the regulation of gas exchange (Moriana et al., 2002), a very developed osmotic adjustment (Chartzoulakis et al., 1999), the regulation of the antioxidant system (Bacelar et al., 2007), the appearance of leaf anatomical alterations (Chartzoulakis et al., 1999), the ability of extracting water from the soil due to a deep root system (Fernandez et al., 1997) and to a high water potential gradient between the canopy and the root system (Tombesi et al., 1986).

According to the cohesion theory (Dixon and Joly, 1894; Askenasy, 1895) the water transport follows the SPAC (Soil Plant Atmosphere Continuum) system with water in a metastable phase, from soil to atmosphere throughout roots, trunks, branches, shoots, and leaves or fruits. Negative pressure generated above all by the evaporating surface of the leaves (and/or by the fruits) is the driving force to move the water from the soil to the other organs.

Starting from the system's driving force, we can focus on the leaves (without forgetting the transpiration of the fruits), where the stomata regulate the transpiration (i.e., the driving force). Olive leaves show xerophyte characteristics as thick cuticle of both the surfaces, and protected stomata (they are present only in the lower side of the leaf) by the trichomes (Bacelar et al., 2004), these components act like first shield against the loss of water by dense colonization of stomata and they can reach a density of 500 per mm^{-2} (Bacelar et al., 2004; Sansavini et al., 2012). In fact, water transpires through the stomata, which close in the event of drought and a prolongated drought stress can cause the cavitation effect. The closure of stomata is regulated by the ABA hormone signal, triggered by the low root hydraulic conductance and conductivity (Canales et al., 2021), i.e. when the water potential of the soil is very low, and the VPD is high.

The water transport is supported, anatomically, by the vessels and tracheid (Cruiziat et al., 2002). Compared to other crops, olive tree shows narrow vessels (25-30 µm diameter, depending also by the rootstock (Trifilò et al., 2007) and a high density per surface area (about 440 per mm2) (Trifilò et al., 2007; Dichio et al., 2013) with a major capacity to adapt to drought soils, especially against cavitation (Tyree and Zimmermann, 2002; Trifilò et al., 2007; Canales et al., 2021). A possible adaptation of the olive that can have diameter and density of the vessels negatively correlated with the soil moisture is until now uncertain (López-Bernal et al., 2010; Torres-Ruiz et al., 2013).

This system tolerates, in olive, very low water potential, measured, in shoot, until -7 MPa (Marino et al., 2018), but -2 MPa reduced the fruit size because of reducing of the endocarp growth (Gómez-del-Campo, 2013; Gómez-del-Campo et al., 2014), while the prolongated and severe Stem Water Potential (SWP) around -4 MPa can compromise bud development, and the return of bloom (Gucci et al., 2019).

When the water potential decreases at very negative values, and the tree is under water stress, the stomata are closed (the closure of the stomata is very related with the water potential) (Xyloyannis et al., 2009; Marino et al., 2016). The closure of the stomata allows the system not to lose water but slows down (or stops) transpiration and photosynthesis (Xyloyannis et al., 1999; Guerfel et al., 2009).

In case of short or prolongated water shortage, olive tree can adopt the osmotic adjustment, one of the processes involved in the adaptation to drought of the trees (Chaves et al., 2003). In a period of water deficit, plants can synthesize several molecules to accumulate like amino acids (e.g., proline and aspartic acid), proteins, sugars (e.g., sucrose, glucose and mannitol), methylated quaternary ammonium compounds (e.g., glycine betaine and alanine betaine) and organic acids (Ingram and Bartels, 1996). The increase of these solute compounds decreases the osmotic potential allowing the movement of the water into the cells. In this way the turgor pressure is stabilized increasing the tissue tolerance to low soil water potential (Tyree and Jarvis, 1982, Bray, 1993).

The sensitivity to low WP depends also on the phenological stage of the tree; The shoot growth and flowering are affected to water-limited conditions, and in some areas of the world supplemental irrigation can help flowering or shoot growth (Moriana et al., 2003; Pérez-López et al., 2007). After the flowering, and that the growth of the endocarp ends, the number of fruits is constant and hardening of the stone occurs, sensitivity to water stress is reduced (Goldhamer, 1999; Moriana et al., 2003; Fernández et al., 2013; Girón et al., 2015; Ahumada-Orellana et al., 2017; Corell et al., 2020). In this period, SWP up to -5 MPa slightly reduces the year's production as long as it is adequately recovery before harvest (Moriana et al., 2003; Fernández et al., 2013; Ahumada-Orellana et al., 2017). In the end of the season (when usually, in the Mediterranean climate conditions, rains occur), oil accumulation can support moderate water stress without substantially reduce the oil content in the fruit (Ben-Gal et al., 2021). The SWP threshold below which oil accumulation could be reduced is -2 MPa (Hueso et al., 2019).

All these situations led to define the Regulated Deficit Irrigation (RDI), which is the most refined system for reducing the quantities of irrigation water. In fact, it is not necessary to provide the complete water requirement of the tree, is possible to reduce the water applying without the loss of production of the orchard (Moriana et al., 2003). RDI is not only a reduction of a percentage of the ET to apply but is an adjustment of the percentage of the ET based on the phenological stage.

RDI strategy reduces the irrigation doses in some periods of the season without affecting the final fruit growth and the tree fruit yield (Chalmers et al., 1981; Goldhamer, 1999; Moriana et al., 2003; Fereres and Soriano, 2007). For example, in olive, Fernandez et al (2013) used RDI with high water amount from the inflorescence formation until the pit hardening, low water level during pit hardening, and high-water level during oil accumulation in the fruit, avoiding the loss of production. Siakou et al. (2022) reduced to half of the sustainable deficit irrigation (SDI) during the fast shoot elongation and the end of the shoot elongation, during the maximum rate of pit hardening, and during the oil accumulation without significant differences in fruit production compared to the farmer irrigation. A study of Gómez-del-Campo (2013) found that with a reduction of 70% of the summer irrigation the loss of production is lesser during July than the same reduction in August, while reducing in both months 50% of the water apply the water saved can be 27%, with a 15 % decrease in the production. No irrigation in the two months following harvest time did not show differences in follow flowering and fruit production in the following season with the irrigation during this period (Agüero Alcaras et al., 2021).

To increase the vegetative growth and the fruit production is possible considers an irrigation program that starts usually in spring, after the fruit set stage, despite also in the end of winter a drought event can decrease the amount of flowering and its quality. In case of end of winter with drought, during the differentiation period a drought event can happen, and the irrigation can increase the inflorescences amount, and the quality of flowers (Rapoport et al., 2011; Liu et al., 2019). The irrigation at maximum levels can continue until the pit hardening, to support the fruit and shoot growth. The irrigation during the first fruit growth phase can increase the number of cells in the mesocarp of the fruit, tissue where the oil content is higher (Rapoport et al., 2004; Gucci et al., 2009; Liu et al., 2019). During pit hardening the fruit and vegetative growth are slow down, for this reason the irrigation management can reduce the amount of water. The production of the oil happens during the third phase of fruit growth (from complete pit hardening to harvest) but the beginning of this phase can change according to the environmental conditions (Rosecrance et al., 2015; Navas-Lopez et al., 2019). In central Italy this period can start after the mid-august and is possible to reduce the amount of water, or null the irrigation in case of rain (Palese et al., 2010), to have the good compromise between oil content and oil quality, in fact the moisture of the soil during this period can influence the quality of oil (Gómez-Rico et al., 2007; Dag et al., 2008; Stefanoudaki et al., 2009).

Gucci et al. (2019) demonstrated as, in central Italy, to close the irrigation during the first and the second phase of fruit growth can negative influence the return bloom the following year respect a full irrigation for all the fruit growth phases with 2277 m^3 of water applied (510 tree/ha). In the same location, similar results were showed by Caruso et al. (2011), with irrigation that, with a severe drought reached the 2100 m^3/ha.

As can be seen from what has been said, to manage irrigation more accurately it is important to know as precisely as possible the phenological phases and the water status of the plant, moving towards what is defined as precision agriculture (Scalisi et al., 2017; Scalisi et al., 2020; Marino et al., 2021; Caruso et al., 2022).

5.2.Nutrition

Nutrients and elements concentration into the tree changes according with tissue of the tree, period, phenological stage, nutrient availability, presence of new vegetation and fruit, and environment (Fernández-Escobar et al., 1999; Fernández-Escobar et al., 2004).

Fertilization is an important aspect to maintain constant and economically sustainable the production every year, and which supports both fruit and vegetative growth.

The root system is the most important system for the assimilation of most of the elements necessary for the tree, the absorption of the elements is operated by the apex of the root, and, in olive, most of the work is operated in the first millimetres of it (Mancuso et al., 2000; Papeschi et al., 2000). The absorption of nutrients involves the movement of ions, that are close to the root hair in the soil, throughout the root surfaces into the xylem (Pallardy, 2007). The movement of the ions from soil to root cells occurs by passive movement through the apoplast. Then the active absorption through the plasmalemmas of epidermal and cortical cells happens, to transport the ions in all the tree.

The root uptake is influenced by environmental factors such as aeration and temperatures that affect metabolism (Lawrence and Oechel, 1983), anoxia of the soil (Rosen and Carlson, 1984; Fisher and Stone, 1990), salinity (Loupassaki et al., 2002), pH, and other characteristics of the soil.

Although I do not want to discuss the management of the olive grove soil here, it is important to say that, given the factors that influence the absorption of nutrients, not only the olive tree must be managed, but also the soil in which it grows and its environment.

Instead, regarding plant nutrition, for olive, like for the other crops, the three most important elements are Nitrogen, Phosphorus, and Potassium (Zipori et al., 2020). These, with the other microelements, build the tree, and the availability of the elements, with also water and other factors, are related with the production of fruit, and the vegetative growth.

5.2.1. Nitrogen

Nitrogen availability affects the performance of the tree. Its low availability can reduce the vegetative growth, leaf area, flowering, and fruit set, while high availability leads to too vegetative growth, and can compromise fruit set and oil yield (Erel et al., 2008; 2013; 2023; Haberman et al., 2019b; Saidana et al., 2009). Nitrogen deficiency can also affect the chlorophyll concentration in the leaves, and consequently the photosynthetic rate (Boussadia et al., 2010; 2015). The form of nitrogen can affect the shoot and root development (Leskovar and Othman, 2019), and the applying of the fertilizer must be very accurate according to the necessity of the farmer (and for the olive) that have different tools to support the decisions, one of them is the levels of leaves element concentration. In the leaves the concentration of N is alternated annually, opposite to the production of fruits (Fernández-Escobar et al., 2004) and different works tried to give a right nitrogen leaves concentration. Fernández-Escobar et al. (2009) found the good level around 1.2-1.3% and was more accurate in Molina-Soria and Fernandez-Escobar (2010) suggesting the threshold, for mature olives, in a range from 1.22% to 1.35% (in dry matter). In 2008, Erel et al. (2008), found a good flowering intensity, fruit set, and number of fruits per tree with a N concentrate on leaves in about 1.2%, while (Ali, 2023) found an optimal leaf concentration in 1.54%. These values can change viewing the optimal range for the photosynthesis rate as found by Boussadia et al. (2015), where they suggested level between 1.7% and 2.5%. Haberman et al. (2019b) working in a different amount of N fertilize trial found a good production with 75-150 kg of N/Ha, these amounts led to have a concentration of N in the leaves about between 1.4% to 1.7%. On the other hand, Fernández-Escobar et al. (2009) showed how a 0 dose of N didn’t lead to a reduction of production in 13 years-long trial.

A deficient concentration of N in the leaves led a high resistance to late frost (April), with a decrease of the responds when the concentration decreases (Fernández-Escobar et al, 2011)

For these reasons the nutrient input in the soil can follow general guidelines but is important to build a fertilize program considering all the parameters in the choices because the system is very complex and doesn’t exist a univocal solution.

5.2.2. Phosphorus

Phosphorus deficiency is rare in olive and in the soil, for this reason P fertilization is not usually in olive cultivation (Fernandez-Escobar, 2010). Furthermore, the pruning and fruits don’t remove high amounts of P (Fernández-Escobar et al., 2015).

Low P availability negatively affected the reproductive growth and yield in a trial with potted olive trees (Erel et al., 2016). An increasing of P application increased the fruit set in young olive trees (Erel et al., 2008), also the percentage of perfect flowers and fruit set increase with the P application (Erel et al., 2013; Erel et al., 2016). Erel et al. (2016) showed an increment of the performance was observed until a P level of about 0.10% of the dry leaf weight. Foliar application of phosphorus on young plantlets grown in high P-content soil didn’t affect vegetative growth (Jiménez-Moreno and Fernández-Escobar, 2017), while in two different pot trials the results on shoot growth were opposite (Erel et al., 2013; Jiménez-Moreno and Fernández-Escobar, 2016).

In leaves, the content of P decreases during the summer season, to recovers during Autumn (Fernández-Escobar et al., 1999), in other cases the negative peak can be recognized at the end of spring (Stateras and Moustakas, 2018) for the use of phosphorus by the inflorescences. Moreover, seems that during the autumn following the “off-summer” the accumulation increases, finding the highest levels of P in leaves in on-year.

Although during the season the concentration of P in leaves changes, for the fertilize management Fernández-Escobar (2018) indicated 0.1-0.3% as an adequate concentration of P in leaves. Considering the effect on flowering performance Erel et al. (2013) showed the levels between 0.10% and 0.15% as the concentration with the maximum performance, levels that ensure a good vegetative growth (Jiménez-Moreno and Fernández-Escobar 2016). Zipori et al. (2020) suggested 0,12% for Israel grower, as confirmed by Haberman et al. (2021).

5.2.3. Potassium

Potassium is an important element involved in plant water management and osmoregulation, as well as it is involved to tolerance of many abiotic stresses (Cakmak, 2005), and also in the osmotic uptake of water by roots. For example, in case of drought stress, K starvation can increase stomatal conductance, improving the performance (Arquero et al., 2006). In a long-term period, a too high concentration of potassium in the leaves could decrease the concentration of other nutrient as N, Na and P (Haberman et al., 2019a), while in the short-term study didn’t influence the concentration of N, P, and B in the leaves (Erel et al., 2008; Vishekaii et al., 2023).

Vegetative growth was not affected by a less K deficiencies (Erel et al., 2008), while a long-term K deficiency can reduce the productivity (Haberman et al., 2019a). Potassium influences, instead, the intensity of flowering (Erel et al., 2008; Erel et al., 2013). Furthermore, potassium can reduce bearing effect (Haberman et al., 2019a) despite this element does not fluctuate in leave according to fruit load (Fernández-Escobar et al.,1999).

A foliar application of K during summer can improve the freeze tolerance during winter (Saadati et al., 2021).

The suggested levels of adequate concentration of K in the leaves was more than 0,8% (Fernández-Escobar, 2018), the good level could be more than 1% without exceeding levels of 1.2% (Haberman et al., 2019a). Another study indicates that the number of inflorescences per branches can increase when the concentration of K in leaves increases, also until 0.3 mol/kg (i.e. about 1.17 %) (Erel et al., 2008).

The K fertilisation has to take in account different parameters, as said before, drought stress can decrease the K uptake (Arquero et al., 2006), as well as salinity of soil can decrease the concentration of K in leaves (Loupassaki et al., 2002). Moreover, not only drought, but also the temperatures of the soil can affect the K adsorption (Benlloch-González et al., 2017). In case of foliar application, the uptake is higher in young than in mature leaves (Restrepo-Díaz et al., 2009).

5.2.4. Micronutrients

Calcium is not directly involved in the vegetative growth but is involved in the fruit growth and maturation (Morales-Sillero et al., 2021; Lodolini et al., 2023). In inflorescences the content of Ca (as the content of Mg) increases during the phase of development of flowers, from 5 µm gDW-1 to almost 15 µm gDW-1 (Bouranis et al., 2010). Instead, in the leaves, Ca is higher in the old ones, but does not show differences between "on" and "off" year in the normal soil, with concentration about 1% on dry matter (Fernández-Escobar et al., 1999), that is an adequate level for Fernandez-Escobar (2018). In a saline soil, olive can show an alternate concentration according with fruit load (Bustan et al., 2013).

One of the most important microelements in the literature of olive is boron (B). Boron is involved in flowering, and for this reason the foliar fertilization in pre-blooming period is suggested (Perica et al., 2001). The B concentration is higher in the reproductive organs as buds and fruits, and the foliar application can induce boron translocation from leaves to buds and fruits (Hegazi et al., 2018). (Vishekaii et al., 2019) showed how the B application (in boric acid or chelate) also influenced the vegetative growth, the uptake of other macro or micronutrients, and the content of carbohydrates in the tissues, as confirmed by Hegazi et al. (2018). The effect of B application is influenced on several factors, one of them is the cultivar of olive (Delıboran et al., 2022).

5.2.5. Fertilize management

The cultivation of olive trees requires, in most cases, a fertilization program which, agronomically, can maintain a constant and high production to satisfy the farmer's demand for income (Erel et al., 2018).

The fertilization at the beginning of the season is common in the traditional cultivation, but in the modern, and precision agriculture the nutrients have be available for absorption when the trees need the elements (Zipori et al., 2020). For example, during winter, the cold temperatures can decrease the nutrient uptake or, as well as viewed for K, with high temperatures during summer (Benlloch-González et al., 2017). Moreover, during winter the nutrients more instable like N can leach, while in summer could create a salt layer in the soil.

Fertilization regime should be based on several considerations:

A) the possibility of considering dividing the quantity of nutrients to be administered to the field during the season into several applications, starting from before the development of the flower, until the beginning of winter. Divide the doses can improve the loss of nutrients by leaching, is useful to regulate the plant phenology, is possible to reduce or increase the nutrients application according with fruit load (Bustan et al., 2013), or according with vegetative growth, or also to direct the quality of the oil (Dag et al. 2009; Erel, et al. 2013; Hagagg et al., 2013; Tekaya et al., 2013; Morales-Sillero et al., 2021).

B) use the soil analysis.

C) use the leaves to diagnose the nutritional status directly on the tree (Fernández-Escobar et al. 2009). Using the values of the nutrient concentration in the leaves not always easy to recognize an excess of some elements, such as nitrogen, via the leaves (Erel et al. 2018). In fact, it does not accumulate in the leaves, but either stimulates vegetative growth, or accumulates in the fruits, or in the branches (Bustan et al., 2013). An analysis of some elements in the fruits, and a check of vegetative growth could be excellent diagnostic tools. Furthermore, the leaf concentration of the elements, often change according to the season, ant according to the cultivar (Fernández-Escobar et al., 1999; Iqbal et al., 2022).

On the other hand, is important to pay attention to pollution, greenhouse gas production, and the welfare of society, to meet the compromise between these almost opposite factors. (i.e. production and sustainability).

Often the precise vision of certain situations leads to not having exhaustive answers and only a broad-spectrum vision can resolve certain problems mentioned above. In this way, both for irrigation and fertilization in Israel, the use of wastewater was studied (Segal et al., 2011), increasing what we call the circular economy. In other cases, the vegetation waters from the mills themselves were used (Mekki et al., 2013), or the digestate (Pittarello et al., 2024).

The use of residual pruning or the olive mill pomace waste in orchard require a particular attention, in fact olive suffer of the allelopathic phenomena (Giorgi et al. 2007; Endeshaw et al., 2015).

From these examples it can be seen that there are many situations from which water and minerals can be recovered. Furthermore, today various biological solutions are also being studied to increase the availability, capture and absorption of water and minerals, such as the use of mycorrhizae and bacteria (Briccoli et al., 2015).

5.3.Photosynthetic radiation

The productivity of the tree is directly correlated with the photosynthetic capacity of the leaves, the mainly photosynthetic organs. The irradiance, absorption and utilization of photon energy are factors that affected the net photosynthetic rate (PN). The saturation irradiance of olive is low compared with other species (Bongi and Long, 1987; Higgins et al., 1992), and the shade can reduce leaf PN, especially when shading is practiced from the beginning of the season (Gregoriou et al., 2007). In fact, the leaves that develop in a shaded environment show a greater area and thylakoids (expressed as the percentage of the chloroplast area), lesser thickness, and lesser stomatal and trichome density. Despite the negative effect of the shade was observed by several studies, it seems that a slight shade (no more than 30% of light subtraction) during summer does not affect the net photosynthesis (Gregoriou et al., 2007), and in some cases the net photosynthesis can increase (Lodolini et al., 2018). During summer, the irradiation is in the maximum levels, and high intensity decreases the efficiency of photosynthesis in olive, in fact the saturation point in olive is around 500-800 $\mu molm^{-2}s^{-1}$ (Bongi and Long, 1987; Sofo et al., 2009; Proietti et al., 2012). The explication of this phenomenon can be found in the dissipated energy through nonphotochemical processes such as thermal dissipation (Demmig-Adams et al., 1995, Maxwell and Johnson, 2000). Furthermore, the shadow can be useful against damage to the photosynthetic apparatus in case of drought, making the plant recover from stress more quickly when it is re-irrigated (Sofo et al., 2009).

In another study, Cherbiy-Hoffman et al. (2013) shaded the trees at levels between 40% and 70% of PAR (Photosynthetic Active Radiation) the canopy, for a period that started from a few weeks after the pit hardening and ended at 7 months later. In this study no significant differences in the oil content, fruit dry weight, and the no-bearing shoots elongation were found (Cherbiy-Hoffmann et al., 2013). Whereas a high reduction of light (around 80%) can lead to a lesser flowering induction and differentiation (Tombesi and Cartechini, 1986; Trentacoste et al., 2022a), whereas a 50% reduction of the radiation didn't show differences in number of inflorescences per node and the number of flowers per inflorescence compared to the non-shaded trees (Trentacoste et al., 2022b).

Finally, the slight shadow during some period of the season can help the tree against the high intensity irradiation, this is the case of the anti-insect net installed on olive and other crops, or the anti-hail nets, which can act as multifunctional tools (Sofo et al., 2009; Shahak, 2008; Lodolini et al., 2018; Neri et al., 2021;).

6. Scope of the thesis

There are several problems with olive cultivation in central Italy. Olive cultivation has often been abandoned over the past few decades due to a lack of income. This thesis observed these phenomena with a large scale of view, focusing on different subjects. Increasing knowledge of the vegetative/reproductive equilibrium is fundamental to improving olive cultivation, productivity, and the response to climatic and social changes.

The scope of this thesis is to encourage a different olive production for this region, using new tools (in this case the micro-tensiometer for the water tree status), changing the agriculture practices (fertilization to sustain the water available), observing the physiopathies (late frost, and high irradiance) and the pathologies (olive knot), and the growth pattern to improve the agricultural techniques.

How should fertilization be managed in central Italy, where rainfall can be more intense and frequent, to increase vegetative growth?

In cases where rainfall is insufficient, can a new tool like the micro-tensiometer improve the assistance in acting in precision irrigation management?

In the context of climate change, how do late frosts that damage inflorescences affect olive groves and various cultivars?"

After a frost event, some pathogens (e.g., *Pseudomonas savastanoi* pv *savastanoi*) can increase their manifestation (Valverde et al., 2020), especially in some olive cultivars." In this thesis, we investigate how different cultivars respond to inoculation with *Pseudomonas savastanoi* pv *savastanoi*."

Finally, can the shading provided by an anti-insect net affect the growth of shoots and fruits?"

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Preliminary observations on the use of microtensiometers to continuously measure water potential in a mature olive orchard

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Abstract— The modern cultivation of olive needs to be performant and sustainable. Stem water potential (SWP) is a key indicator to implement precise irrigation and increase system productivity, but it is a manual and labor-intense measurement. New trunk-embedded microtensiometers allow to measure trunk water potential (TWP) continuously, and in this study we report for the first time their performance in olive. TWP values were generally higher than SWP values measured with the pressure chamber. The values of both indicators showed a very clear trend in response to irrigation and similar relative changes. Overall, this new technology looks promising but further studies are needed.

Keywords — precise irrigation, proximate sensing, Olea europaea, tree water status.

I. INTRODUCTION

The modern cultivation of olives needs to be performant and sustainable. Irrigation should be provided in all cultivation areas to maximize yield and water use efficiency. Climate change has increased the frequency of drought events, thus reducing available water for agriculture. In parallel, the global temperature is rising, potentially increasing trees' transpiration rates [1]. Farmers urgently need to increase irrigation efficiency by using plant-based indicators associated with desired physiological responses to stress.

Stem water potential (SWP) is among the most used tree water status indicators. SWP is assessed by measuring the balancing pressure of non-transpiring, equilibrated leaves. Importantly, SWP is a direct measure of the water status of the tree, in particular, of most of the canopy, as opposed to the indirect soil or remote methods, and the localized leaf water potential. The SWP method has been largely used in research, so there are several references on how to efficiently use it for water management; however, it presents some limitations. The time requested for the measurements can limit the number of observations achievable during the day. This can be a constrain both for research trials and large farm operations. Furthermore, the measurement is destructive, limiting its use in young trees with reduced foliage. Finally, it is labour-intensive, the measurements are generally performed in a small time frame in the hottest period of the day (to obtain the 'midday' SWP), and specialized personnel is needed, because of the use of pressurized gas.

Continuous measuring technologies have been widely tested for water management in woody species [2]. They are non-destructive and automatic measurements that allow for remote real-time access. Despite these advantages, there is still a gap between the amount of information developed by researchers and private companies, and the adoption by growers and irrigation managers. The main reasons for this discrepancy have been carefully discussed in Fernández [3] and can be summarized as: difficulty to use and maintain, high price, and complex data interpretation. In addition, most of these technologies do not directly measure tree water status, but some other physiological parameters, such as shrinkage and swelling of organs, or canopy temperatures, that are influenced by tree water status. Environmental parameters and the genotype specific response to drought can affect these processes, having a strong effect on data interpretation [4,5].

Microtensiometers are a new technology that allows continuous information of tree water status. It measures directly the pressure on the trunk (or primary branches) using a mechanical pressure sensor installed directly in the wood. In almond, values obtained using microtensiometers and the pressure chamber were similar [6,7], while some differences, especially during evening, were seen in pear [8,9]. In nectarine, the difference between trunk and stem water potential was around 0.1 MPa in a well wet soil, and about 0.3 MPa in deficit irrigation trials [10] with Ψ trunk<Ψ stem. All the works that used the microtensiometers demonstrated a circadian trend (daily) of trunk water potential (TWP) with a decrease during daylight and an increase during the night.

To our knowledge, there are no official reports on the performance of microtensiometers in olive (*Olea europaea* L.). Considering the key role of SWP as an irrigation tool for this drought-tolerant species [11], we decided to characterize the changes in TWP in response to irrigation with the microtensiometers in a mature commercial olive orchard. We aim to give insights into the pros and cons of this methodology and on its potential to be used to improve water use efficiency in olive.

II. MATERIALS AND METHODS

The experiment was conducted from June to October 2022, in a 17-year-old olive orchard cv Manzanillo planted in a 6.4 x 3.2m spacing and located in Orland (CA, USA). The orchard was drip irrigated with a double drip line and 1.89 l/h emitters spaced 0.91 m, delivering a total of 5.11 l/h per tree. The soil was a sandy loam.

At the beginning of June, a tree was equipped with microtensiometers (FloraPulse, Davis, CA, USA, Fig 1). Two sensors were installed at the base of two primary branches, following the procedure suggested by [6]. Despite sensors being placed in the branches, microtensiometer measurements are referred to as Trunk Water Potential-microtensiometer (TWP-mt) throughout the text for consistency with literature.

The tree was irrigated following the farmers' practices all the season except for the pit hardening stage (from July 1st until August 20th) when water application was reduced by 50%. Grower irrigation was based on ETc, calculated using historical ETo values and the Kc of 0.7 [12]. A flowmeter (Sensus iPEARL, Raleigh, NC, USA) was installed in the irrigation line to continuously monitor grower's water applications, which resulted in 508 mm in the experimental period, 762 mm over the entire season. It was distributed in irrigation cycles of 72-96 hours at 5 days intervals until mid-July, and 2-3 days intervals in the second half of July, August and September.

Stem water potential was measured at midday every 1 or 2 weeks throughout the growing season using a pressure chamber (PMS Instrument Co., Corvallis, OR) on shaded shoots with 2-3 pairs of leaves bagged with myler bags at least 15 minutes prior to the reading. On August 31st and September 21st a daily survey was conducted, with 8 and 6 measurement points, respectively. Pressure chamber measurements are referred to as Stem Water Potential-pc (SWP-pc) throughout the text. The SWP baseline (corresponding to non-stressed values for those specific days of measurements) was calculated using the values of VPD collected by the closest CIMIS (California Irrigation Management Information System) meteorological station # 222 (Gerber), located 30 km from the experimental site and the equation reported by [13].

Data analysis was performed using Sigmaplot 14 (Systat Software, Inc., San Jose, CA, USA).

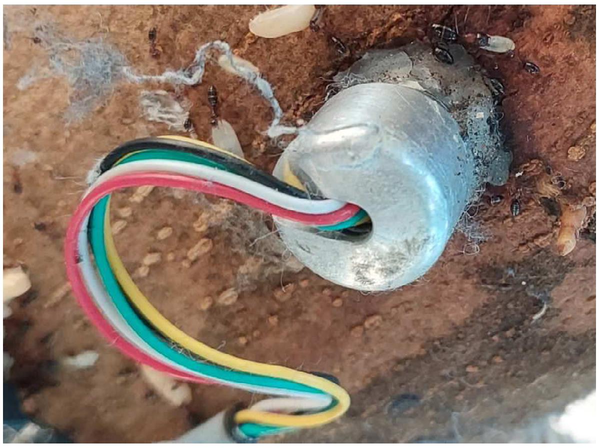

Fig. 1. Microtensiometer installed on the primary branch.

III. RESULTS AND DISCUSSION

The continuous TWP-mt showed a very clear trend in response to irrigation cycles, with an increase of the TWP-mt in response to irrigation (an average of 79 % increase in the daily minimum TWP after watering and 85 % in the maximum daily TWP during each irrigation cycle in the study period) and a decrease when irrigation was withheld (an average of 32 % decrease in the daily minimum TWP in the day before watering and 26 % in the maximum daily TWP during each irrigation cycle in the study period, Fig. 2 and 4). After the reducing irrigation at the beginning of July, WP-mt slightly decreased in one month, showing a response of the tree water status to the deficit irrigation. The midday SWP baseline was close to the daily TWP-mt minimum in June and September, when trees were fully irrigated, and to the daily TWP-mt maximum in July and August, during the deficit irrigation.

The data of SWP-pc taken with the pressure chamber were lower than the TWP-mt in 32 out of 33 measurements, with a maximum difference of 1.15 MPa on the 14th of June.

Focusing on a daily trend, the circadian trend is appreciable, with a decrease of the TWP-mt during the light period and, opposite, an increase during the night period of the day. This trend was shown also for the cherry and olive fruit growth with fruit shrinkage during the light period when the VPD was maximum and substantial enlargement during the dark period according to a hysteresis pattern [14, 15,16], while in apple Gonzalez Nieto et al. [17] showed the same trend for the fruit diameter and the TWP-mt. Similar trend was shown also by the pressure chamber measurements. The negative peak of SWP-pc was at the same hours as the positive peak of the VPD, while it was anticipated with respect to the peak of TWP-mt on both days (Fig. 4). This fact needs to be more studied, the delay of the negative peak in TWP-mt with respect to SWP-pc and VPD could suggest also why, in pear, during the afternoon was found lower values of SWP-pc with respect to TWP-mt [9].

Differences in the readings of the two sensors installed in two branches of the same tree were assessed by comparing the difference between the maximum and minimum TWP-mt daily values (Δ-mt, Fig. 3). Δ-mt was responsive to the irrigation cycles, decreasing during rewatering and increasing in response to water stress. The north-exposed branch presented the biggest differences Δ-mt, with a gap that increased during the no-irrigation period (Fig. 3). It is possible that the canopy of the branch exposed in this tree to the north part was more sensitive to stress, due to the compartmentalization of the trunk of the olive trees. More studies should explore the effect of installation point on these sensors output.

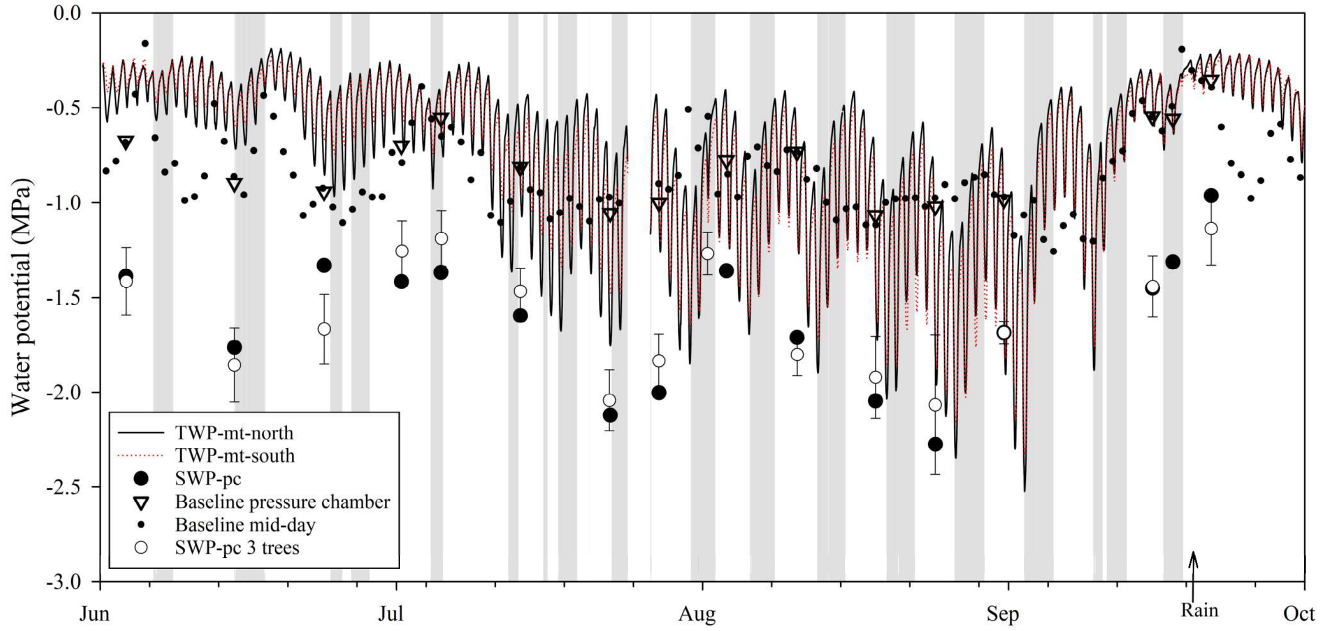

Fig. 2. Seasonal trend of the hourly water potential values measured with the two microtensiometers (TWP-mt) installed on one north-exposed and south-exposed branch (black and red lines, respectively), and the midday stem water potential measured weekly with a pressure chamber (SWP-pc) in the tree where the microtensiometers were installed (black filled circles). The SWP-pc of neighbor trees is shown for comparison (empty circles; mean±st. dev.), and the calculated SWP baseline. The arrow indicates a rain of 21 mm.

Values of TWP-mt and SWP-pc show a linear relationship (R^2:0.7480). While relative changes in WP measured with the two techniques are similar (slope ~1), TWP-mt values tend to be greater (less negative; intercept ~8.3) than SWP-pc for the midday SWP range considered, as also shown in Figure 4.

In nectarines, this linear relationship showed a slope of ~1, but the intercept was smaller than in olive, indicating less difference between the two measurements [10].

In pear, when using the midday measurements the relationship had a slope of ~ 1, while, for the same crop, in the afternoon the difference grew, with SWP-pc showing lower values than TWP-mt [9]. Nectarines, pear, and almonds are species with a different capacity to respond to water stress with respect to olive [13,18], for example, olive tree can reach levels of -7 MPa in some environments [19].

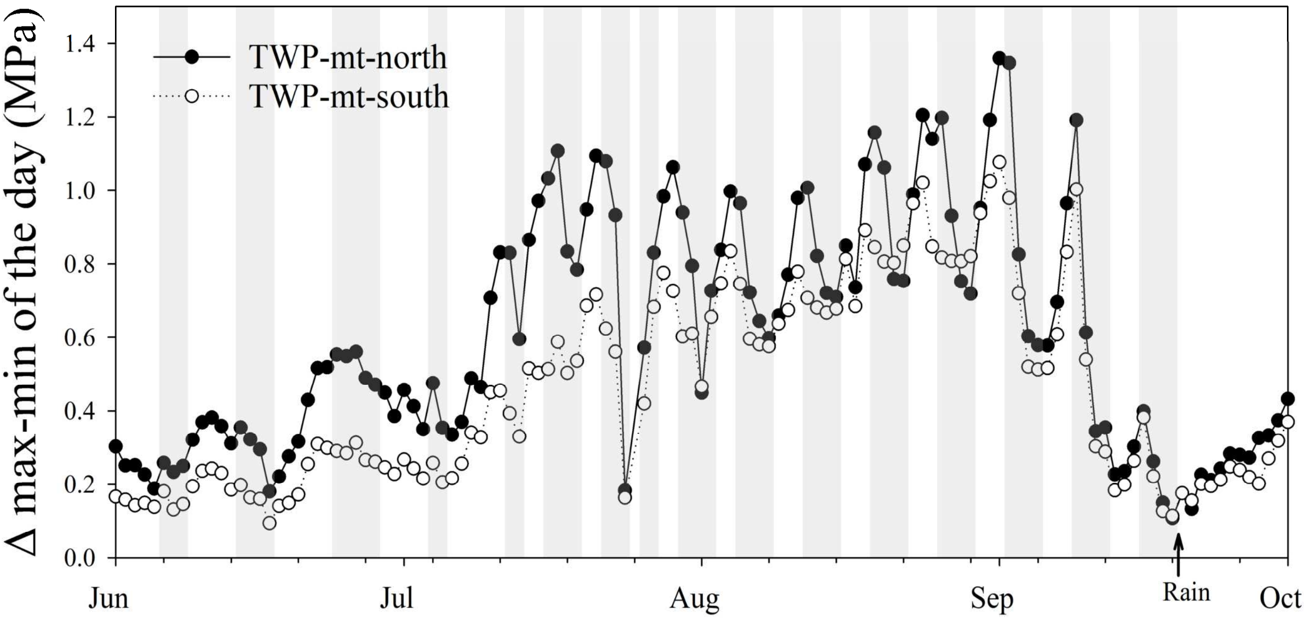

Fig. 3. Difference between maximum and minimum daily TWP of the microtensiometer installed one on the north part of the canopy and another one on the south part. The grey bars indicated the irrigation period. Arrow indicates 28 mm of rain for the 19 of September.

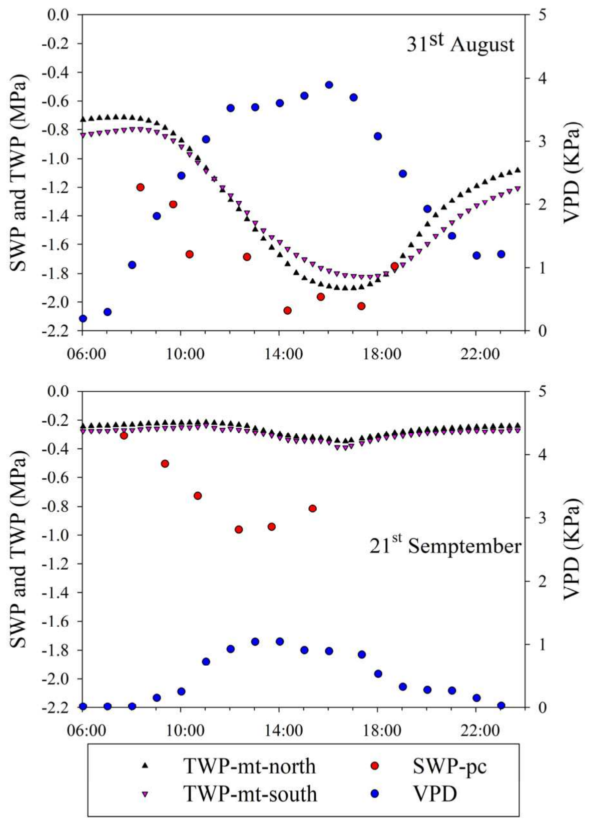

Fig. 4. TWP taken by microtensiometers, SWP taken by pressure chamber, VPD.

IV. CONCLUSION

The microtensiometers were effective in tracking tree water status in olive; nonetheless, they are expensive, and it is not possible for a farmer to set up a fleet of them to have a representative status in heterogeneous fields. These arguments make it important to supplement microtensiometers with mapping tools (ET stations, satellite maps, drones, etc.). In addition, future studies should shed light on the effect of installation point within the tree and on the relation of absolute values of TWP-mt with manual measurements of SWP-pc since we didn't find complete agreements between the two. This study demonstrates that having continuous information of water stress allows to extrapolate multiple parameters that can give larger information on water stress than a single timepoint, which can be key to implement more efficient irrigation in the future.

Further studies are necessary to understand the impact of physiological and environmental factors on microtensiometer readings, and to develop irrigation guidelines based on those readings. .

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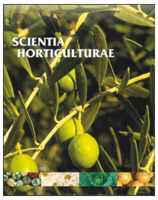

Shoot growth pattern and return bloom of six olive varieties in response to cultivation practices stimulating or limiting the vegetative growth in young trees

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ABSTRACT

Olive trees show a very marked alternate bearing behavior. Generally, this behavior is regulated by the balance between growth of vegetative and reproductive organs. A high fruit load can negatively affect the elongation of 1-year-old mixed shoots and lead to decreased production of new potential reproductive buds in the following year. Applied nutrients and water availability can also affect new shoot growth, yet, the best period for vegetative growth and bud induction is unclear. In this study we investigated growth pattern of the vegetative portion of 1-year-old mixed shoots (year n) and the return bloom in the following year ($n + 1$) in six olive cultivars under "strong" and "weak" vegetative growth practices. Results found that in the following year the number of inflorescences on 1-year-old mixed shoots was affected as a consequence of the different level of vegetative growth. In addition to the greater vegetative growth of 1-year-old mixed shoots, the number of sylleptic shoots also seemed to be influenced by cultivation practices. Moreover, sylleptic shoots had more inflorescences per bud than the 1-year-old shoots. The nodes of the 1-year-old mixed shoots that grew during June and July produced the majority of inflorescences per bud compared to the nodes that grew during spring and late summer.

1. Introduction

Olive trees (*Olea europaea* L.) show marked alternate bearing behavior (Lavee, 2007) with "on" and "off" years. Alternate bearing could be an effective evolutionary strategy for the conservation of a single olive tree during its exceptionally long life cycle. Trees manage resources, synchronizing seed production with root and canopy development (Lodolini and Neri, 2012; Smith and Samach, 2013). Therefore, the vegetative and reproductive growth are in competition for the resources, with flowers and fruits prevailing, generating alternate bearing patterns over the seasons (Obeso, 2002). Endogenous and exogenous factors of alternate bearing can be genetic, environmental, nutritional, hormonal, agronomic (Baktir et al., 2004; Fernández-Escobar et al., 2004; Lavee, 2007; Yanik et al., 2013) and may interact at general (whole tree) or local levels (single shoot) (Fichtner and Lovatt, 2018; Rosati et al., 2018a,b). Locally, the buds formed on new mixed (vegetative and reproductive) 1-year-old shoots may change to being reproductive or vegetative.

Furthermore, the parent shoot relative growth rate can affect the axillary buds to develop into sylleptic shoots (Costes et al., 2006) and the presence of fruits can influence the number of sylleptic shoots during the growth of the 1-year-old shoots (Castillo-Llanque and Rapoport, 2011).

It is well understood that the balance among sink tissues (fruit development vs. bud formation and shoot elongation) for metabolites, such as carbohydrates (Seyyednejad et al., 2001; Salimonti et al., 2021) and polyamines (Pritsa and Voyiatzis, 2004), plays an important role in regulating the level of alternate bearing and the fluctuation of fruit production over years (Rosati et al., 2018a). Hence, a high fruit load can negatively affect mixed shoot elongation (Proietti, and Tombesi, 1996; Dag et al., 2010; Fernández et al., 2015) and lead to lower production of new potential reproductive buds in the following year. Moreover, applied nutrients and water availability can affect new shoot growth (Fernandez-Escobar et al., 1996).

Furthermore, new buds must endure several stages in response to

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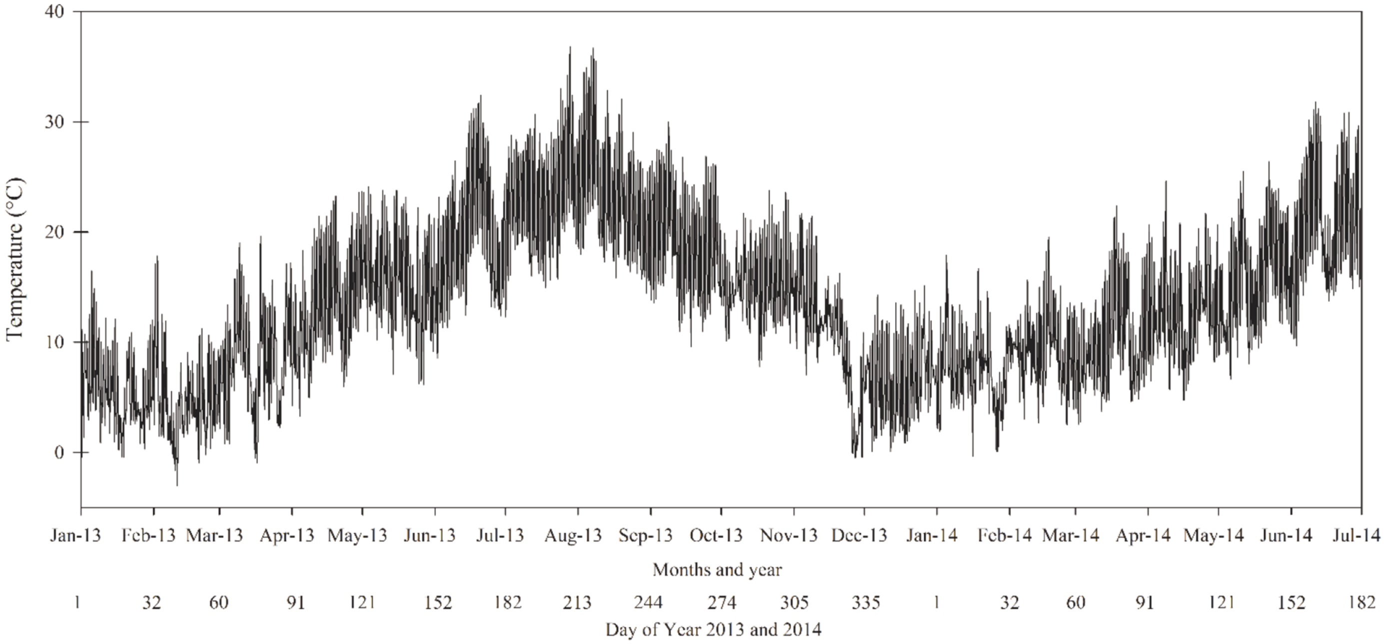

Fig. 1. Hourly mean temperatures (°C) from January 2013 to July 2014 (Protezione Civile Marche, Regione Marche).

different environmental and physiological factors to ensure a flowering fate (Badr et al., 1970; Badr and Hartmann, 1971; Stutte and Martin, 1986; Rallo and Martin, 1991; Fernandez-Escobar et al., 1992; Fabbri and Alerci, 1999; Fabbri and Benelli, 2000; Baktir et al., 2004; Troncoso et al., 2012; Haberman et al., 2017; Salimonti et al., 2021).

Three stages are reported for the bud development: induction, initiation, and differentiation. All are essential in achieving an "on" year, yet in short vegetative growth climatic conditions, as is the case in central Italy, the moment of induction to form flowers is most crucial as this period coincides with the completion of pit hardening of the fruit in the current year crop (year n). In other climatic conditions, bud induction for the early-formed buds can start immediately after fruit set and continue after pit hardening is completed for some cultivars (Fernandez-Escobar et al., 1992; Dag et al., 2010; Haberman et al., 2017). In some areas it can occur even after harvest for buds formed in late summer or autumn.

Generally, bud induction is negatively influenced by the disequilibrium caused by the carbon starvation of the different organs (Bustan et al., 2011; Rosati et al., 2018a), and by gibberellins (Stutte and Martin, 1986; Fernandez-Escobar et al., 1992). Both are dramatically influenced

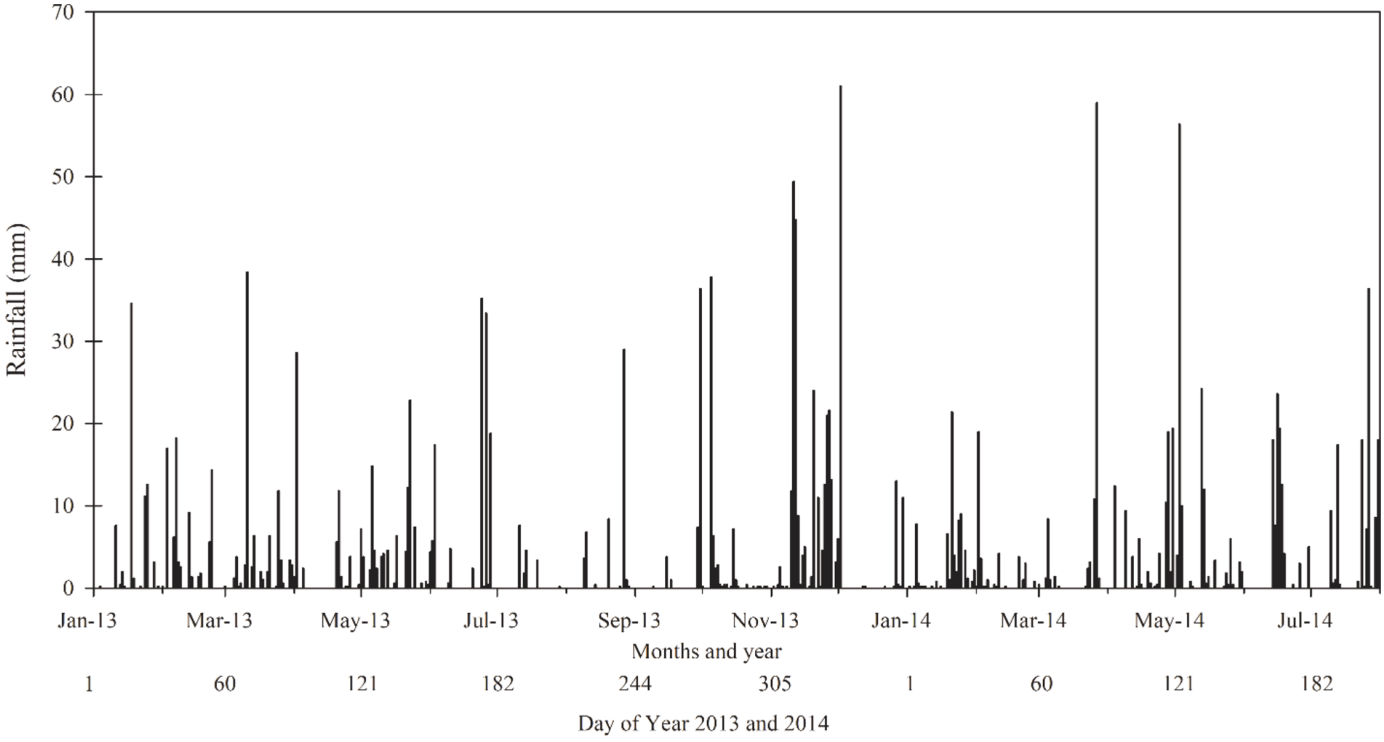

Fig. 2. Daily rainfall (mm) from January 2013 to July 2014 (Protezione Civile Marche, Regione Marche).

by presence of fruits and seeds. Furthermore, also some nutrient content analyses show an alternating trend in leaves, opposite to fruit loads (Fernandez-Escobar et al., 1999). Fernandez-Escobar et al. (2004) showed how nitrogen content in leaves was significantly lower in the on year than that recorded in the following off year. This difference could be due to N adsorption by the fruits during the on year (Fernandez-Escobar et al., 2004).

Moreover, Strippoli et al., (2013); Marra et al., (2016), and Lodolini et al., (2017) reported that a strong vegetative growth of 1-year-old shoots during spring (leading to a greater number of nodes per 1-year--old mixed and fruiting shoots) followed by a growth arrest of the shoots during summer seems to play a key role in enhancing floral bud induction and differentiation (high number of flowers per shoot).

After induction, the initiation stage, which does not show any morphological change in the bud is crucially dependent on chilling, and bud dormancy. In this stage temperatures play the most significant role; only a few hours (even less than 300) below 12.5 C being necessary to go through the initiation phase and to release dormancy (Rallo and Martin, 1991; Ramos et al., 2018).

The last stage (differentiation) begins with the formation of the inflorescence in the bud, and it is the only stage showing visible morphological modifications. It usually starts during winter, and climate conditions can anticipate or delay the completion of this stage. At this stage, inflorescences inside the buds can be damaged by frost (E.M. Lodolini et al., 2016; Valverde et al., 2020) and the morphological changes are not reversible on the shoot. Therefore, to manage the alternate bearing in short vegetative growth climatic conditions, as in central Italy, through adequate agronomic practices, it is important to control the shoot growth, and to know the best period for vegetative growth and bud induction in different cultivars, according to local climate conditions.

The objective of our study was to investigate the pattern of growth of the vegetative portion of 1-year-old mixed shoots (year n) and the return bloom in the following year (*n* 1) in six olive cultivars subjected to cultivation practices inducing strong or weak vegetative growth of the shoots.

2. Materials and methods

2.1. Plant material, experimental design, and treatments

The field trial was carried out in a commercial organic farm in Fermo, central Italy (Lat. 43 07 52.0 N, Long. 13 38 37.9 E, Alt. 200 a.s.l.). Olive trees were planted in March 2011 in a hedgerow system at 4 *m* 2 m (1250 trees per hectare) and the experiment was executed during the 3rd (2013) and 4th (2014) growth year of the trees in the field.

The soil had a clay texture, a basic pH (8.4), a high content of active limestone (139 g kg ^{1}) and a low content of organic matter (12.6 g kg ^{1}). The content of total nitrogen was low (1 g kg ^{1}) and C/N level indicated a rapid mineralization of organic matter (the ratio was 7.3). The assimilable phosphorus and potassium content were 15 mg kg ^{1} and 246 mg kg ^{1}, respectively.

Hourly mean temperatures and total daily rainfalls of the research site registered during the experimental period are reported in Fig. 1 and 2 (Protezione Civile Marche, Regione Marche, 2022).

The hourly mean temperatures during 2013 included 18 h above 35 C, and 136 h above the 30 C. In 2014, 28 h over the 30 C until the 30th of June (Fig. 1).

The weather was unseasonably rainy when compared to historic data. The average of annual rain in the period 1959 2000 in Fermo was 731 mm (Amici and Spina, 2002), whereas 1037 mm were registered in 2013 and 628 mm in the period January-July 2014 (Fig. 2).

In 2013, six olive cultivars were selected among those used in the orchard: 'Ascolana tenera , 'Maurino , 'Piantone di Falerone , 'Piantone di Mogliano , 'Rosciola , and 'Sargano di Fermo , each cultivar was represented by a row. Half of each row was subjected to a strong vegetative growth treatment. The trees of this part of the row were supplied with supplemental fertilizers and pruned using conventional methods. In order to maintain the trees in a high-vigor state and to stimulate the vegetative growth of 1-year-old mixed shoots inflorescences were thinned. The remaining half of the row was subjected to a weak vegetative growth treatment which aimed to induce a scarce resource availability and maintain a low-vigor state of the trees with limited vegetative growth of 1-year-old mixed shoots. These trees were not subjected to supplemental fertilizers, pruning and thinning of the inflorescences. Local limitations in site management did not allow for a randomized design, so the field was divided into two halves, with the weak and strong treatments located in the up-hill and low-hill portions of the orchard, respectively.

Table 1

Fruit production per tree and the total growth of the vegetative portion of 1-year-old mixed shoots per cultivar and treatments (mean standard error).

	Fruit production per tree (kg)		Vegetative growth of the shoot (cm)	
Cultivar	Strong treatment	Weak treatment	Strong treatment	Weak treatment
Ascolana tenera	0.06 0.01	0.36 0.15	28.2 3.15*	12.9 2.45
Maurino	0.06 0.01*	1.01 0.30	19.5 1.77*	11.6 1.21
Piantone di Falerone	0.02 0.01*	0.31 0.11	24.0 2.70*	11.2 1.38
Piantone di Mogliano	0.44 0.19*	1.22 0.12	21.5 2.91*	13.6 2.37
Rosciola	0.03 0.02*	0.8 0.12	24.8 2.22*	18.5 1.71
Sargano di Fermo	0.02 0.01*	0.58 0.23	21.6 1.71*	9.2 1.57

Asterisks indicate significant differences between treatments within the same cultivar according to the Tukey-Kramer HSD test (*p* 0.05).

The strong treatment consisted of:

- A pruning operation in April of 2013 which removed a mean of 0.64 0.21 kg of foliage per tree.
- A fertilization plan (93 g of N, 9 g of P_5O_2 and 93 g of KO_2 per tree). 60% of total nitrogen and potassium amount was localized on the soil along the row and close to the trees on 20th of March 2013; 15% of N was provided by fertigation twice from 15th of April to 15th of May 2013, and the remaining 25% was provided by fertigation twice from 15th of May to 15th of June 2013. The phosphorus was applied through foliar application on the canopy at the end of summer 2013 and before flowering in 2014. All applied fertilizers were allowed for organic farming.
- Hand thinning of the inflorescences to have a few fruits load during the season. To know whether this operation led to different yields per tree, the fruits were harvested per tree, and in 5 cultivars out of 6, the fruit production was different (Table 1).

Despite some inflorescences (producing very few fruits) persisting until harvest on the trees subjected to the strong treatment, a higher growth of the vegetative portion of 1-year-old mixed shoots was maintained during 2013. Average fruit production per tree and the total growth of the vegetative portion of 1-year-old mixed shoots per each studied cultivar confirmed a different vigor condition of the trees during 2013 (Table 1).

During the trial, a weekly drip irrigation was applied from April to August 2013, but the irrigation applied only a total of 160 l tree ^{1} of water in all season. If compared with the rain, the water supply with irrigation was only 5.6% of the total water (160 l versus 2860, Fig. 2), so the irrigation applied was negligible in the trial.

2.2. Measurements

In spring 2013 (year n), five homogeneous 1-year-old mixed shoots were selected in the external portion of the canopy in five of the most

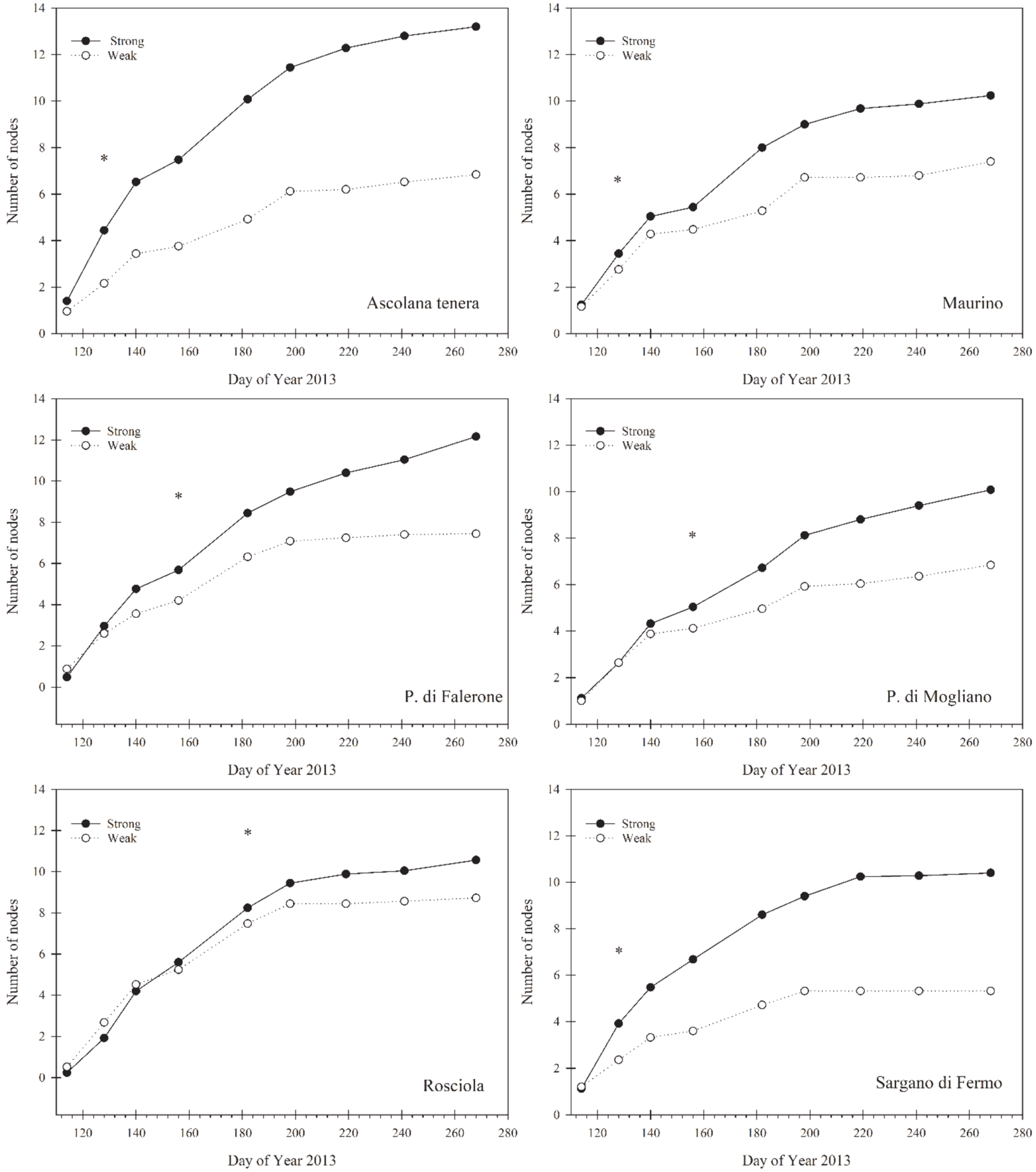

Fig. 3. Vegetative growth during the growth season 2013 expressed as nodes per shoot. Each symbol is the mean of 25 replicates (5 shoots for 5 trees). Asterisk indicates the date from when significant differences were found between the treatments (p. value< 0.05).

homogeneous trees per each treatment in each of the studied cultivars (mean trunk circumference: 31.3 ± 5.45 cm, tree height: 293±30 cm, canopy interrow cross width:129±23 cm and canopy width along the row: 146±21 cm).

During spring, summer, and autumn 2013, nine assessment sessions were executed on Day of Year (DoY) 114, 128, 140, 156, 182, 198, 219, 241, and 268 (April 24th, May 8th and 20th, June 5th, July 1st and 17th, August 7th and 29th, September 25th, respectively).

On each assessment date, the new nodes formed in the vegetative portion of the selected 1-year-old shoots were tagged in order to identify the shoot growth during each period between two consecutive assessment sessions.

Table 2

Number of nodes recorded at the end of the growing season of 2013, and number of inflorescences per node on the 2013-grown portion of the 1-year-old mixed shoot, recorded in 2014 in the different treatments of the studied cultivars.

Cultivar	Strong Number of nodes per mixed shoot (2013)	Number of inflorescences per bud (2014)	Weak Number of nodes per mixed shoot (2013)	Number of inflorescences per bud (2014)
Ascolana tenera	13.2 0.90 a	0.18 0.03 bc	6.84 1.01*	0.15 0.04 ab
Maurino	10.2 0.64 ab	0.34 0.06 a	7.4 0.63**	0.23 0.06 a
Piantone di Falerone	12.2 0.71 ab	0.05 0.02 c	7.44 0.71*	0.06 0.02 b
Piantone di Mogliano	10.1 0.96 b	0.27 0.04 ab	6.84 0.86***	0.24 0.5 a
Rosciola	10.6 0.63 ab	0.08 0.02 c	8.72 0.51***	0.21 0.03 ab**
Sargano di Fermo	10.4 0.66 ab	0.24 0.04 ab	5.32 0.77*	0.15 0.04 ab
P. value	0.0193	0.0001	No sign.	0.021

Values are mean standard error of twenty-five replicates. Different letters indicate significant differences among cultivars (Tukey-Kramer test p 0.05), while asterisks indicate significant differences between treatments for the same parameter within the cultivar:.

*** p value 0.0001;.

** p. value 0.005;.

* p. value 0.05.

At the end of the growing season (November 2013) the number of sylleptic shoots (lateral shoots growing from axillary meristems which elongated contemporary with the shoot apex growth) were counted and referred to as the node formation period of the vegetative portion of the shoot. At the same time, the fruit yield per tree was recorded in the same month.

In 2014 (year n 1), the number of inflorescences was counted for each tagged node of the vegetative portion of the shoot grown in 2013 (year n) during the return bloom period. Buds that produced a sylleptic shoot the previous year (n) were subtracted from the count of buds that could produce inflorescences in year (n 1).

2.3. Statistical analysis

The distribution of the data was assessed for normality and a one-way ANOVA was performed. The Tukey-Kramer HSD (honestly significant difference) test was used for means separation whenever the ANOVA indicated a significant influence of a variability factor. All statistical analyses were performed using JMP Software (Release 8; SAS Institute Inc., Cary, NC, USA, 2009).

3. Results

The vegetative growth of the shoots of the trees subjected to the strong treatment had a greater number of nodes at the end of the growing season than those in the weak treatment. The biggest difference between strong and weak treatments in 2013 was recorded in 'Ascolana tenera (6.36 nodes). 'Piantone di Falerone , 'Piantone di Mogliano and 'Sargano di Fermo recorded an increase of 4.72, 3.24 and 5.08 nodes, respectively. 'Maurino and 'Rosciola had the lowest increase (2.84 and 1.84 nodes, respectively) (Fig. 3, Table 2).

The seasonal pattern of growth of the shoots in terms of number of nodes was not changed by the strong treatment following a sigmoid trend similar to that of weak treatment (Fig. 3). 'Ascolana tenera , 'Maurino , and 'Sargano di Fermo cultivars showed a significant difference in the cumulative number of nodes between the treatments from DoY 128, while 'Piantone di Mogliano and Piantone di Falerone in the strong treatment significantly increased the cumulative number of nodes from DOY 156. 'Rosciola significantly differed after DoY 182 (Fig. 3).

Differences between weak and strong treatments were recorded also for the growth rate of the vegetative shoots in 2013; all the studied cultivars, except 'Rosciola and 'Piantone di Mogliano , had significantly different daily growth rates starting from the second assessment date (Fig. 4). The peak of the trend was registered on DoY 128 (5th of May) and at DoY 140 (20th of May), i.e. the growth trend from DoY 114 (24th of April) to DoY 140 (20th of May), corresponding to the month before blooming, was the fastest of the whole growing season. Other phases of the season showed significant differences between weak and strong treatments, within each cultivar.

Another peak of growth, but smaller than the first one, was recorded between DoY 182 and 200 (about one month after bloom, in July).

Both peaks showed a slight delay for the trees subjected to weak treatment compared to the strong one in 'Ascolana tenera and 'Maurino (Fig. 4).

In the period between DoY 140 and DoY 156 (close to bloom time), the formation of new nodes was strongly reduced, near zero per day, especially in the weak treatment of 'Ascolana tenera , 'Maurino , 'Piantone di Mogliano , and 'Sargano di fermo cultivars.

'Maurino , 'Piantone di Falerone , and 'Rosciola had a small increase in the growth rate at the end of the growing season, for both treatments in the first cultivar and only in the strong treatment for the other two.

In 'Ascolana tenera , 'Maurino and 'Sargano di Fermo the strong treatment showed more sylleptic shoots along the 1-year-old mixed shoots at the end of the growing season 2013 (Fig. 5).

The emission of sylleptic shoots in the nodes formed during the second growing peak between DoY 156 and DoY 182 was remarkable only in 'Maurino in comparison with that of the other cultivars, in particular, showed a significant increase in the strong treatment (Fig. 6).

In 2014, the number of inflorescences per bud in the 2013-grown portion of the shoot (both on 1-year-old shoot and on the sylleptic shoots) registered significant differences between treatments only in 'Rosciola (Table 2, and 3). In particular, the trees in the weak treatment had higher values compared to those in the strong treatment (0.21 0.16 and 0.08 0.09, respectively). The number of inflorescences per bud in the other cultivars were not significantly different between weak and strong treatments (Table 2). 'Maurino had higher number of inflorescences per bud compared to the other studied cultivars (0.29 0.04, mean of both treatments), differences were not significantly different from 'Piantone di Mogliano , and 'Sargano di Fermo , (0.26 0.03, and 0.21 0.03, means of both treatments respectively). These two cultivars did not have a significant difference from 'Ascolana tenera (0.17 0.03, mean of both treatments), that, in turn was not significantly differentiate more flower buds than 'Rosciola (0.15 0.02, mean of both treatments) . The 1-year-old shoots of 'Piantone di Falerone produced fewer inflorescences per bud (0.06 0.01, mean of both treatments) and significantly differed from all the other cultivars except 'Rosciola .

Generally, the newly formed nodes from DoY 128 to 182 (8th of May to 1st of July) in 'Maurino , 'Piantone di Mogliano and 'Sargano di Fermo had at least one inflorescence for every four buds. The nodes in 'Piantone di Falerone , 'Rosciola and 'Ascolana tenera always

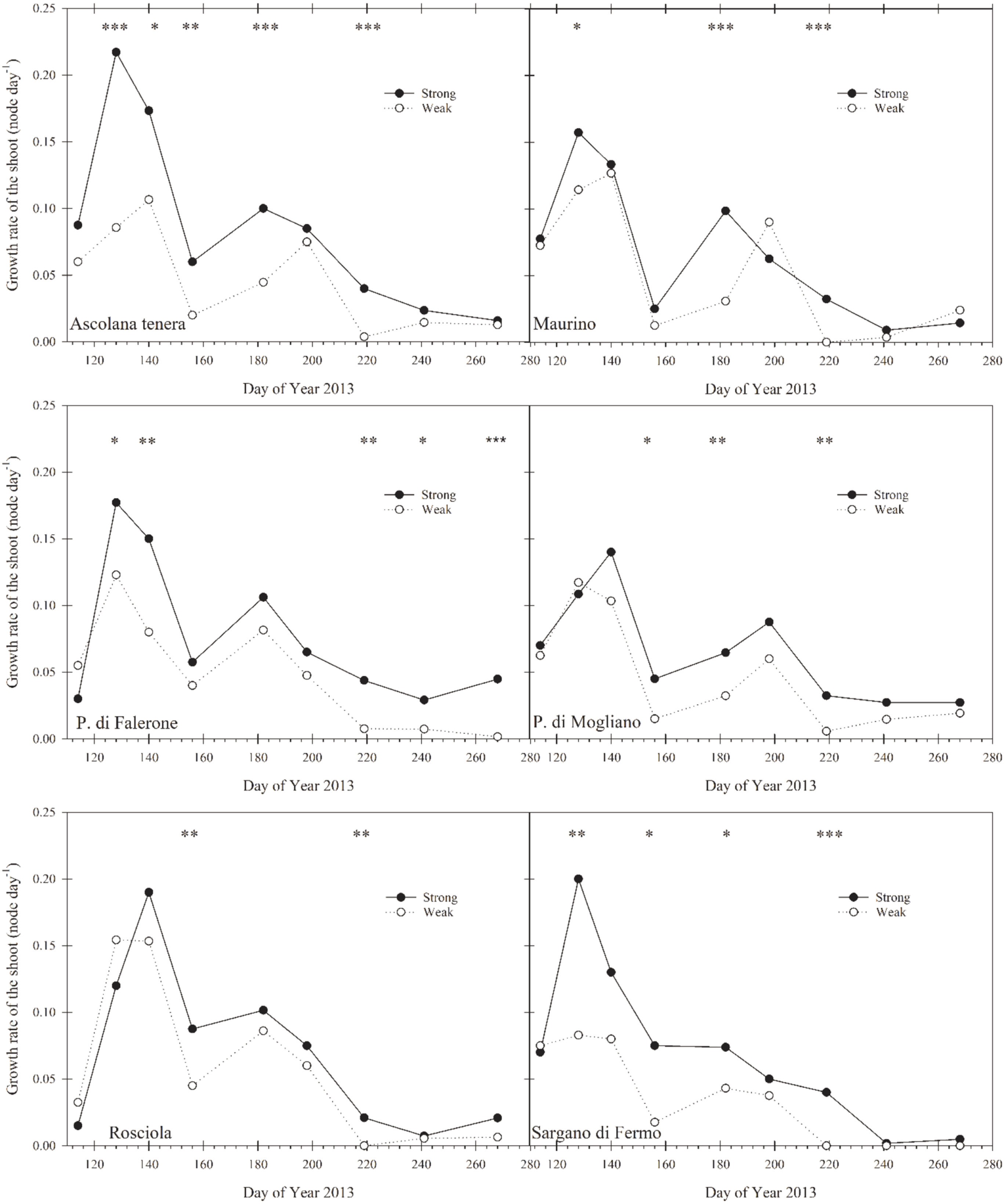

Fig. 4. Vegetative growth rate (number of nodes per day) in the vegetative portion of the shoot per day (symbols are mean of 25 replicates). Asterisks indicate significant differences between treatments within the cultivar: ***p value <0.0001; **p. value <0.005; *p. value <0.05.

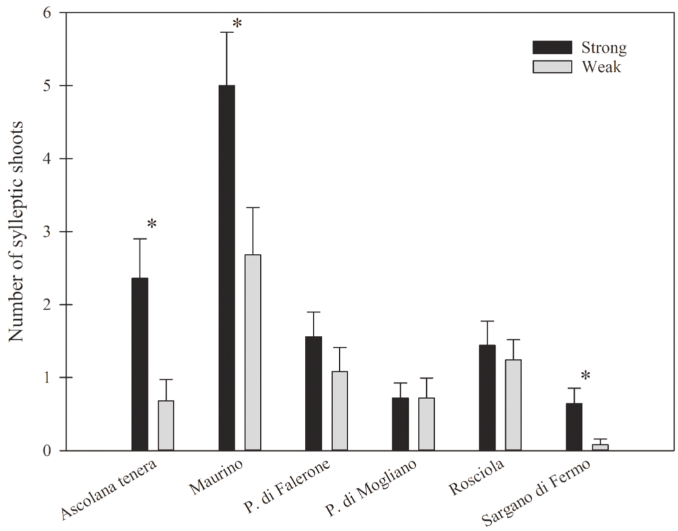

Fig. 5. Number of sylleptic shoots along the 1-year-old mixed shoots recorded at the end of the growing season 2013. Data shown as mean + standard error of 25 replicates. Asterisks indicate significant differences between treatments within the same cultivar according to the Tukey-Kramer HSD test ($p < 0.05$).

remained below that level, recording almost no inflorescences in the buds that were formed after DoY 182 (1st of July 2013) (Fig. 7).

4. Discussion

As mentioned by several authors (Iniesta et al., 2009; Gómez-del-Campo et al., 2014; Zipori et al., 2020; Tadayon and Hosseini, 2022) cultivation practices can strongly affect the level of crop load and the vegetative growth of 1-year-old mixed shoots and therefore the return bloom in the following year. In our study, the vegetative portion of 1-year-mixed shoots of the trees subjected to the "weak" treatment grew less than those of the trees in "strong" treatment conditions (Table 2). Crop load, fertilization, and pruning in 2013 could have affected the vegetative growth of 1-year-old mixed shoots in the same year and thus the amount of the total inflorescences in the following year (2014).

Previous studies reported that irrigation increases the vegetative growth and the fruit production, both in young and adult trees (Tognetti et al., 2006; Gucci et al., 2007; Pérez-López et al., 2007; Martín-Vertedor et al., 2011; Erel et al., 2013; E.M. Lodolini et al., 2016; Freihat et al., 2021). The amount of water can be accompanied by other factors to increase the growth of the shoots. Nutrients and fruit load are responsible for the vegetative growth, both individually (Rodrigues et al., 2011;Rosati et al., 2018b; Leskovar and Othman, 2019; Mazeh et al., 2021; Famiani et al., 2022) and in combination (Martín-Vertedor et al., 2011).

The effect of reproductive buds (i.e. presence of flowers and fruits) on the vegetative growth in the same growing season and on the return bloom has already been reported in several studies (Monselise and Goldschmidt, 1982; Rallo and Suarez, 1989; Krueger et al., 2005; Dag et al., 2010; Castillo-Llanque and Rapoport, 2011; Fernandez et al., 2015; Famiani et al., 2022). Our results confirmed that in young trees, non-limiting resource conditions can enhance a higher formation of new nodes in the vegetative portion of 1-year-old shoots with some cultivars doubling the final number of nodes in trees where vigor state has been induced by the "strong" treatment compared to the "weak" one, leading to more potential inflorescences in the next year (return bloom).

Significant differences between the treatments ("strong" and "weak") were observed for the growth rate of the vegetative portion of 1-year-old shoots in the studied cultivars. Strippoli et al., (2013), showed a strong deceleration of the vegetative growth already at DoY 150 in long vegetative growth climatic conditions as in Southern Italy, while the results of our trial referred to a short vegetative growth climatic condition (central Italy) resulted in a deceleration (almost an arrest) at between DoY 156 and 180. In both study areas, the heavy deceleration of the vegetative growth of 1-year-old shoots coincided more or less with blooming time (Orlandi et al., 2010), perhaps due to the nutritional need of the inflorescences in this period (Famiani et al., 2019). Moreover, as reported by Lodolini and Neri (2012), during this stage and later, during fruit development, the fruit becomes the main sink attracting the assimilates so that the growth of the vegetative portion of 1-year-old mixed shoot progressively decreases. Such a trend was seen also in our study showing differences between resources availability (higher resources less decrease) and cultivars. In particular, 'Sargano di Fermo' did not show a second peak of growth rate acceleration between DoY 187 and 200 as registered in the other studied cultivars.

Observing the vegetative growth rate and the climatic conditions of our trial, the period with the highest rate of node formation (steeper slope) appeared to be late spring before blooming, and early summer

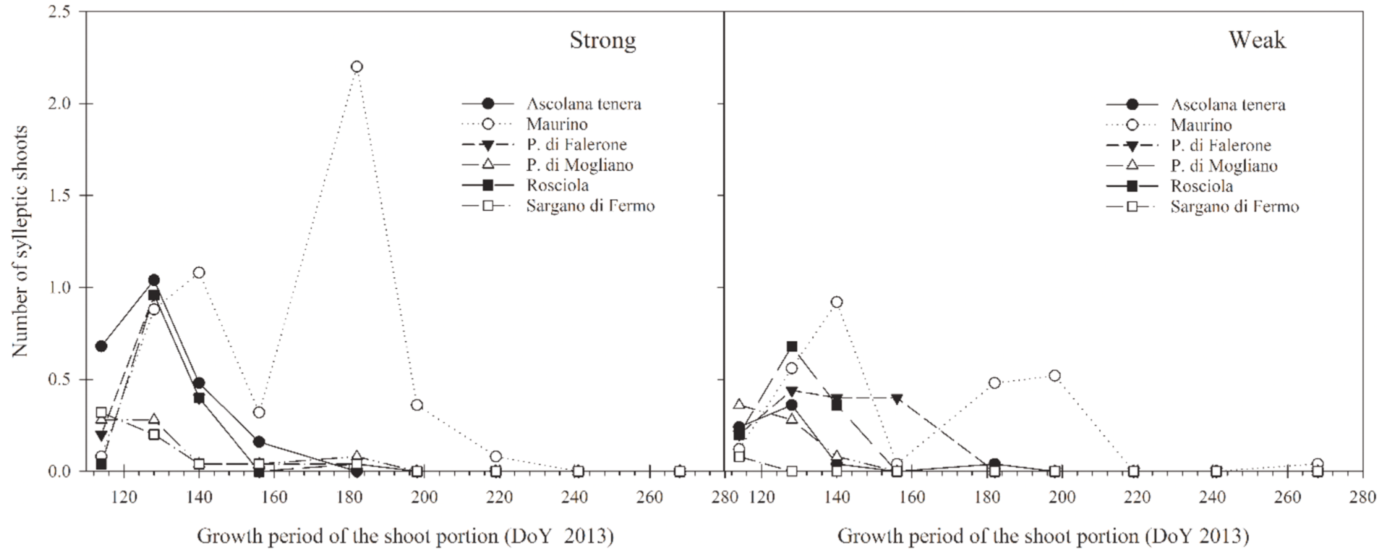

Fig. 6. Number of sylleptic shoots emitted along the 1-year-old mixed shoots. Symbols are mean of 25 replicates. No significant differences were measured between the treatments, or among the cultivars, or among the days (according to the Tukey-Kramer HSD test ($p < 0.05$)).

Table 3
Number of nodes recorded at the end of the growing season of 2013, and number of inflorescences per node on the 2013-grown portion of the sylleptic shoot, recorded in 2014 in the studies cultivars and for the different treatments.

Cultivar	“Strong”		“Weak”	
	Number of nodes per sylleptic shoot (2013)	Number of inflorescences per bud (2014)	Number of nodes per sylleptic shoot (2013)	Number of inflorescences per bud (2014)
Ascolana tenera	6.21±0.39 b	0.40±0.03 ab	5.00±0.69 bc	0.47±0.06 a
Maurino	6.40±1.21 ab	0.43±0.07 abc	6.75±1.80 abc	0.25±0.09 ab
Piantone di Falerone	8.19±0.49 a	0.23±0.03 c	7.33±0.43 ab	0.21±0.02 b
Piantone di Mogliano	7.33±0.71 ab	0.54±0.07 a	7.89±0.86 a	0.41±0.04 a
Rosciola	5.86±0.42 b	0.30±0.04 bc	4.03±0.36 c[***]	0.46±0.04 a[***]
Sargano di Fermo	6.93±0.94 ab	0.51±0.08 ab	6.60±1.47 abc	0.62±0.16 a
P. value	0.0243	<0.0001	<0.0001	<0.0001

Values are mean ± standard error of different number of samples (for the strong treatment: ‘Ascolana tenera’: 56; ‘Maurino’: 15; ‘Piantone di Falerone’: 36; ‘Piantone di Mogliano’: 18; ‘Rosciola’: 35; ‘Sargano di Fermo’: 15; for the weak treatment: ‘Ascolana tenera’: 15; ‘Maurino’: 4; ‘Piantone di Falerone’: 30; ‘Piantone di Mogliano’: 18; ‘Rosciola’: 31; ‘Sargano di Fermo’: 5). Different letters indicate significant differences among cultivars (Tukey-Kramer test p <0.05). Asterisks indicate significant differences between treatments for the same parameter within the cultivar:.

[***] p value <0.0001; **p. value <0.005; *p. value <0.05.

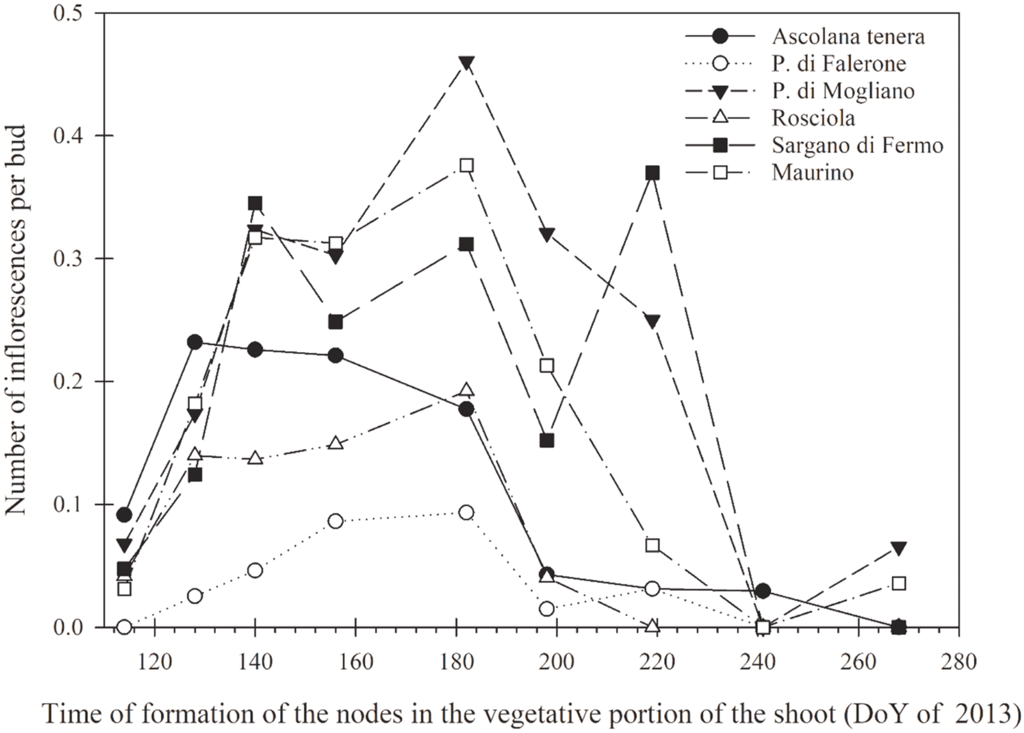

Fig. 7. Number of inflorescences per node recorded in 2014 on the nodes formed in the vegetative portion of 1-year-old shoots grown in 2013. Symbol are mean of 25 replicates.

after fruit set in 2013. In fact, in June 2013 the shoots reached 50–70% of their whole yearly growth in almost all the cultivars and treatments, and in early July 2013 it reached 70–90% of yearly growth depending on the variety.

The development of sylleptic shoots can contribute to the formation of new fertile nodes in the vegetative portion of 1-year-old shoots. Fertilization, low crop load, and pruning induced a higher number of sylleptic shoots in three of the six studied cultivars. This kind of shoots was prevalently emitted on the axillary meristems of the nodes formed during the pre-bloom period, which is the fastest growth period of 1-year-old shoots. In fact, all the cultivars had this behavior, except for ‘Maurino’ which had a high number of sylleptic shoots also in the portions grown during summer (DoY 180–200 JD).

The new vegetative growth, which occurred in early summer after bloom in the 1-year-old mixed shoots, was less than the vegetative growth that occurred in late spring. The average growth per single shoot in the two phases were different, which can be explained, in addition to the higher presence of fruits on the trees of “weak” treatment, by the larger number of growing points in the canopy. Early growth relied on a limited number of spring buds while in the summer there were numerous newly formed axillary meristems, which developed into axillary shoots. The axillary shoots (sylleptic shoots) were, for this study, very important because of the high number of inflorescences per bud which were higher than the number of inflorescences per bud of the 1-year-old mixed shoots.

For some cultivars, the formation of the sylleptic shoots could be important for increasing the number of the potential inflorescences per tree (Moutier et al., 2004). In our study the sylleptic shoots produced more inflorescences per bud than the 1-year-old shoots, suggesting the importance of these shoots in controlling the fruit load and likely alternate bearing. For this reason, the study of the architecture, the period of formation, and how production of this kind of shoots is pivotal (Strippoli et al., 2013), especially in increased planting densities. As found by Castillo-Lanque and Rapoport (2011), the trees with more nutrients available (the non-bearing in the case of the cited paper, and in the weak treatment in our paper) presented more sylleptic shoots.

Although irrigation, fertilization, and low crop load improved the formation of nodes in the vegetative portion of 1-year-old shoots and in the sylleptic shoots, the number of inflorescences per node in the same portion in the following year was similar in both treatments in five of the six studied cultivars (Table 2 and 3). In previous studies, the high crop load of the previous year (n) caused a reduction in number of inflorescences of the following one ($n + 1$) (Dag et al., 2010), and, therefore, also of crop load (Dag et al., 2009). Perhaps the fruit load of the previous year (n) in our research was not as high and did not affect the return bloom as much ($n + 1$).

All studied cultivars showed the highest number of inflorescences per node in 2014 (year $n + 1$) when the nodes were formed between DoY 120 and 219 in 2013 (year n). In ‘Ascolana tenera’, ‘Rosciola’ and ‘Piantone di Falerone’ the nodes formed from July (DoY 182) to the end of the season in 2013, had very low or null return bloom in 2014. On the other hand, on the $n + 1$ shoots, ‘Maurino’, ‘Piantone di Mogliano’, and ‘Sargano di Fermo’, registered the presence of inflorescences at the nodes that were formed in the vegetative portion of the 1-year-old shoots (n year) up to August (DoY 220). Possibly a separate regulation of summer bud induction may exist among the olive cultivars confirming diversified adaptation to climate conditions and presenting different reproductive patterns of 1-year-old mixed shoots (Lodolini et al., 2017). Moreover, in the specific climatic conditions of our study (central Italy), the formation of new nodes at the end of the summer, as occurred with ‘Piantone di Mogliano’ and ‘Maurino’ could be very concerning given the newly-formed meristems may not have enough time to harden and resist winter cold (Lodolini et al., 2022).

The higher inflorescences bud capacity of sylleptic shoots and the portion of 1-year-old shoots with highest inflorescences bud formation suggests, as reported by Piedra et al., (2012), that the best period to create new nodes (*i.e.*, buds) for forming inflorescences fate is summer, while in spring it is possible to produce many meristems that create the sylleptic shoots.

5. Conclusion

This study contributes to understanding the reproductive and vegetative growth behavior of several olive cultivars, and in particular climatic conditions (central Italy) with a short vegetative growth season. Good irrigation (or rainfall in this case) is not enough to stimulate vegetative growth and good pruning and fertilization were necessary for an adequate fruit (or flower) load. Furthermore, this study found that while nutrition and fruit load can affect the number of new buds, in young trees it does not affect the bud initiation to flower.

Considering the climatic conditions of central Italy, we conclude it is possible to concentrate the vegetative growth in spring and summer. In spring, the meristems which do not sprout and do not originate sylleptic shoots likely will originate inflorescences the following year. In summer, the meristems (at the base and at the apical portion of the shoot) rarely produce new sylleptic shoots but new nodes promote the formation of buds, which will induce and finally form large amounts of flowers in the following year.

This information can be useful for applying precision agronomic management to increase or decrease the formation of new nodes in the vegetative portion of the 1-year-old mixed shoots and consequently to balance the vegetative-reproductive behavior during consecutive years in order to contain the alternate bearing.

We suggest management of the orchard should focus on spring with amendments and fertilisers to the soil and in summer with fertigation (fertilisers and irrigation) to stabilize production and reduce alternate bearing. To add new tools to manage the alternate bearing, other studies are necessary to understand the behavior of the induction of flower buds and the impact of thinning.

CRediT authorship contribution statement

Matteo Zucchini: Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Enrico Maria Lodolini:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Supervision. **Alessandro Tarragoni:** Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – review & editing. **Davide Neri:** Conceptualization, Methodology, Validation, Investigation, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Response of different olive cultivars to late frosts in the Marche region (Italy)

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Abstract

In Europe, the intensity and frequency of late-winter frost, as well as spring frost, is increasing, because of climate change. In olive (*Olea europaea* L.), frost damage can affect different tissues, from the leaves to the trunk, including inflorescences. A partial loss of inflorescences may not change the fruit set number, but a too high loss of flowers would lead to a high loss of production.

In central Italy, on the Adriatic coast, during the night of the 11th of April 2022, a few hours below zero were recorded. The low temperatures damaged the inflorescences of olives with different intensities based on cultivar and area.

To evaluate the intensity of the damages, in several orchards, the number of alive and dead inflorescences were collected in several cultivars.

From the data it emerges that the entity of the damage is not correlated to the cultivar; each cultivar showed a different behaviour in different orchards. Arbequina showed 6% of dead inflorescences in the orchard of Maiolati Spontini, and 95% in the orchard of Agugliano. In Maiolati Spontini, only FS-17 showed a great loss of inflorescences. In the orchard of Fermo, Rosciola showed the higher damages. This different behaviour could be explained by small differences in temperature and humidity that are site specific, and to the exact phenological stage of the flowers in that particular moment. These observations could be helpful in determining the most adaptable cultivars to the future climate of central Italy. This objective seems possible only by considering a mixture of cultivars, due to the different responses of the cultivars to the different and unpredictable types of frosts each year (winter, late winter, spring).

Keywords: Frost, late-frost, inflorescences, flowers.

INTRODUCTION

In the early future, the extreme environmental phenomena can increase (IPCC, 2022). The global warming could also increase the power of winter frosts, especially the late ones (Hamouda et al., 2021; Zohner et al., 2020).

In this scenario, also the olive productivity and the agronomical techniques will change (Fraga et al., 2021). For example, the behavior of agriculture techniques about the frost.

In central Italy, five strong frost phenomena have been dated in the last 100 years (1929, 1956, 1985, 2012, and 2018, (Lodolini et al., 2016; Valverde et al., 2020)).

In olive tree, as in evergreen species, the frost tolerance could be correlated to hardening processes (Cansev et al., 2009), so the same intensity of frost event could cause different damages in the tree according to the event period, the weather conditions before the event, the genotype and all the mentioned factors combined all together. Previous studies reported that some olive organs can tolerate up to -17° C in some periods (Antognozzi et al., 1994).

Depending on the involved organs, the damage observed can range from light to severe when leaves/shoots and trunk/primary branches are affected, respectively (Gucci and Cantini, 2001; Lodolini et al., 2016). The main symptom that can be observed in the trunk and main branches is the bark split (Valverde et al., 2020) while the main symptoms observed in leaves are brown coloration and appearance of chlorosis (Lodolini et al., 2016; Petruccelli et al., 2022). Another organ that is affected in these late frost episodes are the inflorescences (Rapoport, 2014). The inflorescences can be damaged in whole flower clusters, as well as single flowers and their pistil (Sanzani et al., 2012). Furthermore, frost damage can initiate the infectious activities of olive knot (Valverde et al., 2020, Zucchini et al., 2023a)

Shoots and trunk frost tolerance is reported up to -17/-18° C in full acclimation period (winter), while a decrease in resistance is registered during de-acclimation: in early spring, the shoot and trunk of the olive tree cannot tolerate temperatures below -7/-10° C (Antognozzi et al., 1994).

Buds and leaves can show damages at -2/-3° C in some periods of the year, but in the wintertime, leaves can tolerate temperatures around -12/-13° C (Antognozzi et al., 1994).

Inflorescences and flowers do not tolerate temperatures under -3/-4° C (Petrucelli et al., 2022).

Although late frost is difficult to manage in olive, the olive tree presents a good strategy to recover from frost damage, so that it can sprout from latent and adventitious meristems along the trunk and branches, or from accessory buds at the axilla of the inflorescences on the productive shoots (Rapoport, 2014). The re-sprouting process depends on the cultivar and on the level of damage (Lodolini et al., 2016). Preventively, control the nitrogen winter status can result important to reduce the frost susceptibility, in fact Fernandez-Escobar et al. (2010) showed a decrease of tolerance in increasing nitrogen concentration in April. Therefore, the potassium concentration in the leaf can affect the frost tolerance (Saadati et al., 2020). Also, it could be important not to create new leaves and buds in the late season (Zucchini et al., 2023b).

Different studies and observations were done in the last years to know the frost tolerance of the olive cultivars. Mancuso et al. (2000) in a laboratory evaluation determined 'Ascolana Tenera' and 'Coratina' as the most and less frost tolerant cultivars, respectively, when compared with 'Frantoio' and 'Leccino'. Other authors observed the cultivar behavior in field conditions, that is, after natural frost event. Alfei et al. (1999) reported 'Leccino' and 'Piantone di Mogliano' as the most frost tolerant cultivars (presented low level of damage) and 'Canino' and 'Rosciola' highly susceptible. On the contrary, Lodolini et al. (2016) didn't confirm the higher frost tolerance of 'Piantone di Mogliano', that, on the contrary, resulted among the low-frost tolerant cultivars together with 'Piantone di Falerone' and 'Arbequina'. Similar results were found when another frost event occurred in 2018 in the same studied area (Lodolini et al., 2022).

The aim of this study was to evaluate the effect of a late frost event (April frost) in olive inflorescences in several cultivars in the Marche region, central Italy.

2. MATERIAL AND METHODS

2.1 Experimental fields and cultivars

To evaluate the frost damages in the olive inflorescenses 7 groves in the Marche region (central Italy) were selected. The fields were: Augliano HD, Maiolati Spontini HD, Maiolati Spontini LD, Fermo HD, Fermo LDS, Fermo LDN, and Pesaro MD (Table 1), (where HD: High Density, LD: Low Density, LDS: Low Density South-west (exposure), LDN: Low Density North-west (exposure), and MD: Medium Density). The selected cultivars were 'Arbequina', 'Piantone di Mogliano', 'Piantone di Falerone', 'FS-17', 'Ascolana tenera', 'Koroneiki', 'Rosciola colli Esini', 'Carboncella', 'Rosciola', and 'Maurino'. For each location, different number of groves and cultivars were evaluated (Table 1) with a not-balanced factorial design.

Figure 1: Map of the Marche region (central Italy) and the points of the studied locations.

Table 1: Not-balanced factorial design used for the evaluation of the frost tolerance of the cultivars in each observed grove, HD: High Density, LD: Low Density, LDS: Low Density South-west (exposure), LDN: Low Density North-west (exposure), and MD: Medium Density.

	Agugliano HD	Maiolati Spontini HD	Maiolati Spontini LD	Fermo HD	Fermo LDS	Fermo LDN	Pesaro MD
Planting density (tree/ha)	1250	1250	260	1250	260	260	550
Sun exposure	S-E	E-S	E-S	E	S-W	W-N	E-S
Coordinate	43°32'55"N 13°21'51"E	43°28'38"N 13°07'35"E	43°28'34"N 13°07'35"E	43°07'52"N 13°38'37"E	43°08'52"N 13°40'53"E	43°08'12"N 13°38'15"E	43°48'50"N 12°48'56"E
A.S.L. (m)	145	377	366	95	174	141	276
Cultivar							
Arbequina	x	x		x			
Piantone di Mogliano	x	x	x	x			
Piantone di Falerone	x	x	x				

FS-17		x		x			
Ascolana Tenera			x	x	x	x	x
Koroneiki				x			
Rosciola colli Esini	x						
Carboncella					x		
Rosciola				x			
Maurino	x						

2.3 Frost episode occurence

On the 11th of April 2022 in some locations of the Marche region (central Italy), the temperatures reached 0 °C during the night (Figure 1). In Maiolati Spontini the minimal temperatures reached 2 °C, the weather station close to Pesaro recorded 0.7°C, in Fermo -1.2 °C, while in Augliano -0.7 °C. It is notable that two days before this frost episode, the maximum temperatures reached 18.5 °C, 19.9 °C, 23.9 °C and 19.4 °C in Maiolati Spontini, Pesaro, Fermo, and Agugliano, respectively (Figure 1).

In this period the stage of the phenology was around the BBCH growth stage 54 (Sanz-Cortés et al., 2002).

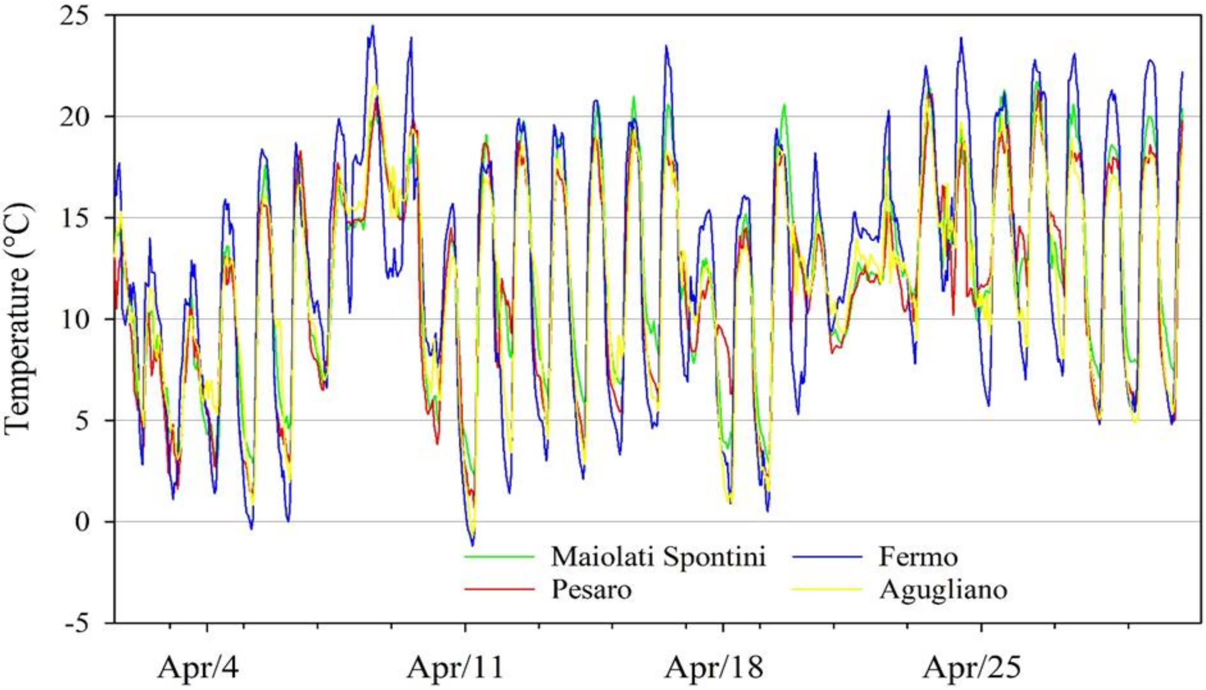

Figure 2: Hourly temperatures of the April 2022 in the four locations of the observed groves.

2.4 Frost damage evaluations

The evaluation of the symptoms of the frost damages was performed in 3 1-year-old mixed shoots for 9 trees randomly spread in the field per each cultivar. For each shoot, the inflorescences still alive and the dead inflorescences (i.e., fallen or burned) were counted (figure 3).

Figure 3: inflorescences (1) and flower (2) burned by the frost, and inflorescence alive (3).

2.5 Data Analysis

One-way ANOVA was performed for each location, and in the presence of significant differences, the Tukey-Kramer (HSD) test at $P<0.05$ was used for mean separation.

3. RESULTS

In Augliano, where the temperatures reached -0.7°C, the loss of inflorescences was higher than 80% for 'Arbequina', 'Maurino' and 'Piantone di Mogliano' cultivars. 'Piantone di Falerone', with less of 80% of inflorescences lost, did not show significant differences with the other studied cultivars, while 'Rosciola colli Esini' resulted the best cultivar with more than 40% of inflorescences alive (Figure 4).

In Maiolati Spontini, the cultivars in the HD grove did not show a high number of inflorescences damaged except for 'FS-17' that registered more than 72% of inflorescences burned by the frost. In the cultivars in the LD grove the inflorescences damaged were lesser except for 'Ascolana tenera'. The data recorded in 'Piantone di Falerone' HD were not significantly different compared to the same cultivar in the LD grove, while in 'Piantone di Mogliano' the difference was significant.

HD and LDS groves in Fermo resulted the most affected by the frost event, with all the inflorescences fallen in 'Rosciola' as the major affected and 'Arbequina' as the less affected cultivar with only the 26.5% of inflorescences lost in HD grove, while Fermo LDN showed very few early falls of inflorescences (Figure 4).

Considering only 'Ascolana tenera', Fermo LDN was the best grove in term of not damaged inflorescences: the percentage of the lost ones was 1.5±0.82%. In Fermo HD, 'Ascolana tenera' was the worst (73.9±6.9%), while in the other three groves, this cultivar showed values slightly higher than 40% in terms of inflorescences burned by the frost event (Figure 4).

DISCUSSION

In some groves the temperatures were not 0°C or less, but in almost all the groves was possible to see inflorescences burned because of cold temperatures. Where the temperatures were very low the damages were high (Agugliano HD, Fermo HD and LDS). The exception of

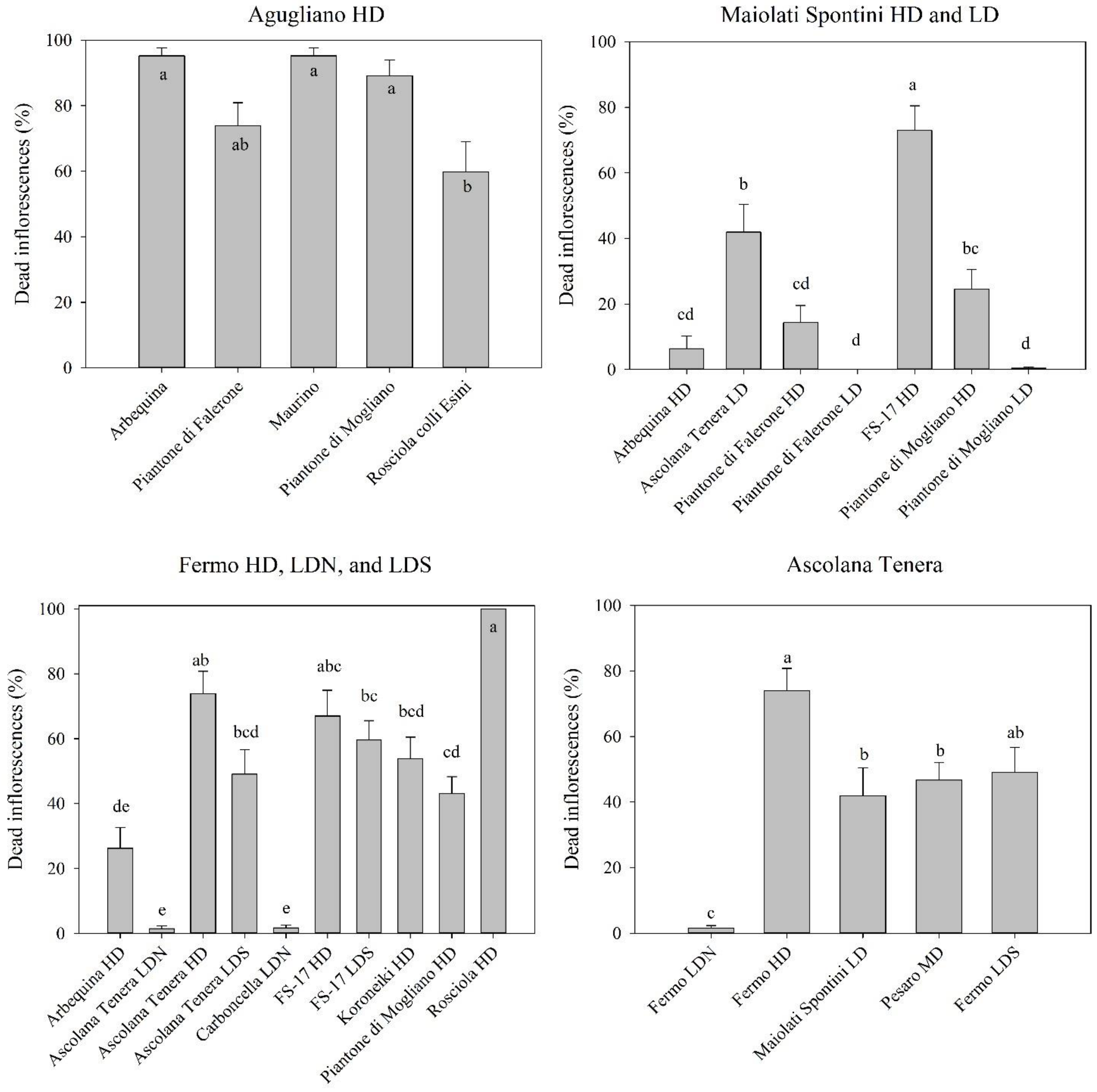

Figure 4: Percentage of the dead inflorescences per analysed shoot (mean ± SE). Letters indicate significant differences of Tukey-Kramer HSD test (P value<0.05).

Fermo LDN could be traced back to the exposition of the field that might entail a delay of the phenological stage and the less thermic daily excursion. Despite in some groves the inflorescences lost were few (for example in 'Arbequina' in Fermo HD), the total absence of fruit after fruit set stage was communicated by the farmer with a personal information, maybe because of the damages on pistil or other tissues not visible during the survey.

Contrarily to other study on frost damages on the wood and leaves (Lodolini et al., 2016; Lodolini et al., 2022, Valverde et al., 2020), analysing the cultivars for different groves was not possible to say which cultivar could present more tolerant to inflorescence damage at these temperatures. The maximal temperatures found during the episode were 2°C (in Maiolati

Spontini), although it is possible that the temperatures on the canopy were lesser, we can consider 2°C the dangerous threshold for the inflorescences in this phenological stage and is possible to update the temperature for inflorescences in Petrucelli et al. (2022). Frost that can affect inflorescences is an interesting problem due to climate change, so further studies indicating the real threshold will be needed.

CONCLUSIONS

The cultivation of olive is spreading toward the north in Italy, but the climate change is jeopardizing the production every year (Hamouda et al., 2021; Zohner et al., 2020; Tanasijevic et al., 2014; Fraga et al., 2019; Cabezas et al., 2020; Gabaldón-Leal et al., 2017). Knowing the tolerated temperature for the olive trees tissues can be strategic for the next years. With this study, we can put a starting point for this objective, and we can say that for inflorescences and flowers is difficult to discriminate the more resistant cultivar. In any case the loss of shoots, leaves, or inflorescences determine an important loss of production for the year, and stimulate the alternate bearing of olives (Lavee, 2007) differently in each cultivar. We can suggest to study more the impact of the olive grove exposure and water and nutritional management to control phenological phase and finally the possibile use of tempestive biostimulant treatment in case of decreasing temperature during flower development in olive.

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PLOS ONE

RESEARCH ARTICLE

Knot formation and spread along the shoot stem in 13 olive cultivars inoculated with an indigenous pathobiome of 7 species of *Pseudomonas* including *Pseudomonas savastanoi*

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Data Availability Statement: The data set necessary to replicate our study findings has been uploaded to stable, public repositories. In more detail, the sequences of the 30 newly identified isolates are available at the GenBank DNA database of NCBI (accession numbers OQ990727-OQ990756); the data related to the observations

Abstract

Olive knot is a widely spread disease among olive (*Olea europaea* L.) trees. *Pseudomonas savastanoi* pv. *savastanoi* is recognized as the primary causative agent of the disease however, recent evidence indicated that consortia of bacteria (pathobiome), may favor its development. Several factors are involved in the host-plant relationship and affect the intensity of the symptoms. Among these the presence of wounds, or damages to the plants' tissues may affect the intensity and propagation of the disease. It remains unknown whether or not bacteria move from an infected wound to another not infected one via shoot tissues. The present investigation focused on the susceptibility to olive knot of several cultivars after inoculating artificial wounds with selected *Pseudomonas* species, while spreading the disease from these to wounds on the same stem, that had not been purposefully inoculated. The pathobiome for the inoculum was prepared with 7 species of *Pseudomonas* (including *Pseudomonas savastanoi* pv. *savastanoi*), isolated from knot samples collected from two different, heavily infected olive orchards. The inoculation was done after the manual execution of 10 horizontal wounds on the stem of potted plants of 13 olive cultivars grown in the greenhouse. Only the lowest 5 wounds were inoculated. The inoculated wounds showed a maximum percentage of knots after 187 days. All 13 cultivars showed knots yet, the cultivar with the most severe disease level to *Pseudomonas savastanoi* pv. *savastanoi* was 'Rosciola colli Esini'. The metataxonomic analysis performed on the olive knots removed after 225 days confirmed the dominance of the inoculated species *Pseudomonas savastanoi* in all the assayed cultivars. The not inoculated wounds did not show the knot disease likely because the bacterium's inability to transmigrate from the inoculated wounds to the non-inoculated ones.

and measurements are available in the BioStudies database (https://www.ebi.ac.uk/biostudies/) under accession number S-BSST1089; the 16S rRNA gene sequences related to the metataxonomic analyses are available at the Sequence Read Archive of NCBI (accession number PRJNA972624).

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Competing interests: The authors have declared that no competing interests exist.

Introduction

Olive knot is a well-known pathology of olive (*Olea europaea* L.) trees and its symptoms can be easily recognized by tumors (or galls), growing on the woody tissues or, sometimes on roots, leaves and/or fruits [1–3]. Although the Gram-negative bacterium *Pseudomonas savastanoi* pv. *savastanoi* (herein referred to as Pss) has been recognized as the primary agent of olive knot disease [4], in the recent years evidence has emerged to suggest that a consortium of bacteria (pathobiome) may favor the development of the disease [5–9]. In addition, Pss can affect several other species [10–13] including oleander (*Nerium oleander* L.), pomegranate (*Punica granatum* L.) and myrtle (*Myrtus communis* L.).

Several factors are involved in the host-pathogen relationship thus, affecting the intensity of disease manifestations. The interaction among the bacteria of the pathobiome with Pss can affect the development of the knots [14], whereas the cultivar of the host species can influence the diversity of the pathobiome population [15].

Another key factor is the intensity of damages and injuries in the tissues of the host plant. Pss enters the host through wounds, stimulating the infection process through a series of reactions that lead to knots developments through an activation of hrp genes and secretion of phytohormones via the hypersensitive response reaction [16].

The wounds triggering the infection in the host's tissues can be caused by natural and mechanical-physical events, some of which are attributable to harvest, pruning, hailstorms and, as studied by Valverde et al. [17], by late winter frost damages. Although it is not clear the importance of tolerating late frost damages compared to those caused by the bacterium, it has been demonstrated that frost events in late winter can affect the intensity and severity of the disease in different cultivars [17, 18].

Environmental conditions remain important to determine the success of an infection outbreak, or the time of expression of specific symptoms, since the penetration of Pss in the host plant tissues. In fact, the knots can appear in a period ranging from two weeks to several months after inoculation, according to temperature and humidity conditions [19]. Yet, the literature is still unclear about reporting Pss mobility through the trees' stem.

Various researchers have analyzed the susceptibility of olive cultivars to Pss under controlled environmental conditions. For example, Varvaro and Surico [18] found different responses in the time of disease appearance during 90 days since an artificial inoculation of Pss in 6 cultivars, where 'Nocellara del Belice' and a 'wild olive' trees showed 100% of the inoculated wounds with knots as early as 8 days since inoculation. Cultivars 'Leccino' and 'Frantoio' showed knots on 85% of the inoculated wounds, whereas knots appeared only on the 18% of the inoculated wounds in trees of the 'Coratina' cultivar. Benjama [20] found different behaviors among different cultivars and different bacterial strains with 'Frantoio' being slightly more susceptible than 'Ascolana Dura'. Penyalver et al. [1] reported that 'Arbequina' was a highly susceptible cultivar to two different Pss strains whereas, 'Ascolana Tenera' presented a variable susceptibility depending on the strain, and 'FS-17'® showed low susceptibility to both Pss strains. In a field evaluation, Salaman et al. [21] found that susceptibility to olive knot varied significantly among cultivars and environments.

The purpose of this study was to assess the olive knot formation in 13 olive cultivars (international, Italian, including autochthonous varieties from the Marche region), after controlled inoculations with an olive knot (indigenous pathobiome of 7 Pseudomonas species, including Pss), of intentionally-made wounds and the knot propagation along the stem, from inoculated to not inoculated wounds.

Materials and methods

Isolation of *Pseudomonas* spp.

In September 2018, knot samples were taken from different olive trees in two orchards where several local and international cultivars were grown. The first orchard is at the Polytechnic University of Marche experimental farm "Pasquale Rosati" (AN, Italy), which is located at: 43˚ 35'21.1 "N; 13˚17'22.8"E. The second orchard is located at Monte San Vito (AN, Italy), at: 43˚ 32'29.6"N; 13˚22'38.3"E. Trees in both the orchards were heavily affected by olive knot as a consequence of the late frost, which occurred in February 2018 [17]. To isolate *Pseudomonas* spp. for the preparation of an inoculum containing *Pseudomonas savastanoi*, the knot samples were randomly taken from 'Frantoio', 'FS-17®', 'Piantone di Mogliano', 'Leccino', 'Maurino', 'Piantone di Falerone', 'Rosciola Colli Esini', 'Ascolana Tenera', and 'Carboncella' cultivars.

The sampled olive knots were transported to the laboratory under refrigerated conditions and stored at 4˚C until use. Olive knots were homogenized using a sterile mortar in a sterile 0.85% (w v^{-1}) NaCl saline solution. Two different samples were made according to the orchard of origin. Ten-fold serial dilutions were prepared in sterile physiological solution (NaCl 0.85%, w v^{-1}) and aliquots (100 μL) of each dilution were spread onto *Pseudomonas* agar base (VWR, International, Radnor, PA, USA), supplemented with *Pseudomonas* CFC selective supplement (VWR) and incubated at 30˚C for 48 h.

Thirty well separated colonies were randomly picked from the plates and streaked to purity for three times onto the same selective growing medium. Pure cultures were stored in Luria Bertani broth (LB) (tryptone 10 g L^{-1}, NaCl 10 g L^{-1}, yeast extract 5 g L^{-1}) added with 25% (v v^{-1}) glycerol.

Identification of the pure cultures

The 30 presumptive *Pseudomonas* isolates underwent a preliminary Gram staining followed by DNA extraction using the method described by Hynes et al. [22] with slight modifications as reported by Osimani et al. [23]. DNA quantity and purity of the extracts were assessed spectrophotometrically, as previously described [23]. Molecular identification of the isolates was accomplished by sequencing the 16S rRNA gene with the primers 27f (`5'-GAG AGT TTG ATC CTG GCT CAG-3'`) and 1495r (`5'-CTA CGG CTA CCT TGT TAC GA-3'`) [24]. In detail, 3 μl (corresponding to ~ 100 ng of bacterial DNA) of each extract was amplified in a reaction volume of 50 μL, containing 1 U of Taq polymerase (SibEnzyme Ltd, Novosibirsk, Russia), 1 × reaction buffer (60 mM Tris-HCl, 1.5 mM MgCl2, 25 mM KCl, 10 mM 2-mercaptoethanol, 0.1% Triton X-100), 0.2 mM of dNTPs, and 0.2 μM of each primer. Polymerase Chain Reaction (PCR) was performed in a thermal cycler (My Cycler, Bio-Rad Laboratories, Hercules, USA), using the following cycling program: initial denaturation at 95˚C for 5 min, followed by 35 cycles of denaturation at 94˚C for 1 min, annealing at 55˚C for 1 min and extension at 72˚C for 2 min, and final extension at 72˚C for 15 min. The PCR products were sent to Genewiz Europe (Leipzig, Germany) for sequencing. The isolates were then identified by aligning the obtained sequences to the sequences deposited at the GenBank database (http://www.ncbi.nlm.nih.gov/) using the BLAST algorithm [25]. The sequences of the 30 newly identified isolates are available at the GenBank DNA database of NCBI (accession numbers OQ990727-OQ990756) as detailed in Table 3.

Preparation of the inoculum

With reference to recent research works [8, 9, 14], which showed how the pathobiome interaction in the olive knot contributes to form bigger knots, a consortium of 7 different *Pseudomonas* species was formulated to inoculate the wounds (Table 1).

Table 1. *Pseudomonas* isolates selected for the formulation of the inoculum.

Field of isolation	Isolate cod.	Species
Experimental farm "Pasquale Rosati"	AGU2	*Pseudomonas coleopterorum*
	AGU9	*Pseudomonas caspiana*
	AGU10	*Pseudomonas caspiana*
	AGU15	*Pseudomonas graminis*
	AGU18	*Pseudomonas lutea*
Orchard located at Monte San Vito	SAN1	*Pseudomonas savastanoi*
	SAN13	*Pseudomonas harudinis*
	SAN16	*Pseudomonas bohemica*

https://doi.org/10.1371/journal.pone.0289875.t001

More in detail, 8 isolates (one for each identified species and one more for the most represented species *Pseudomonas caspiana*) were selected and used, in equivalent proportions, for the preparation of the inoculum. Each isolate was sub-cultured twice, with an initial 3% (v v-1) inoculation in LB broth incubated at 30°C for 48 h. At the end of the incubation period, the bacterial load of each microbial suspension was determined spectrophotometrically, with readings at 600 nm (OD600 = 0.9 = 1 * 10^9 CFU mL^{-1}) [2]. Then, aliquots containing 8 Log CFU mL^{-1} were collected from each microbial suspension, mixed, and centrifuged at 4.000 rpm for 10 min; the obtained cell pellets were re-suspended in sterile physiological solution to reach a final load for each isolate, of 8 Log CFU mL^{-1}.

Experimental design and plant material

Before starting some plantlets were removed because they were not conforming to the standard, hence the experiment was represented by a complete randomized design formed by different numbers of trees basing on the cultivar.

Self-rooted, two-year-old plotted olive plants were used for the cultivars 'Rosciola Colli Esini', 'Ascolana Tenera', 'Ascolana Dura', 'Carboncella', 'Piantone di Mogliano (A)' and 'Maurino (A)'. The average of the Stem Cross Sectional Area (TCSA) of these olives was 46.0 ±12.9 mm^2 on first internode.

Self-rooted, one-year-old potted olive plants were used for the cultivars 'Frantoio', 'FS-17Ⓡ', 'Piantone di Mogliano (B)', 'Leccino', 'Leccio del Corno', 'Pendolino', 'Maurino (B)', 'Arbequina' and 'Piantone di Falerone'. The average of the TCSA of these olives was 20.4±9.6 mm^2 on first internode.

'Maurino A' and 'Piantone di Mogliano A' differ from 'Maurino B' and 'Piantone di Mogliano B' not only for the age of the plants, but also for the nursery provenience.

The experimental layout was a complete randomized design formed by: 10 plants of 'Ascolana Tenera', 'Leccino', 'Leccio del Corno', 'Pendolino' and 'Piantone di Mogliano B' with 6 inoculated plants and 4 control (not inoculated plants); and 15 plants of 'Arbequina', 'Ascolana Dura', 'Carboncella', 'Frantoio', 'Fs-17Ⓡ', 'Maurino A', 'Maurino B', 'Piantone di Falerone', 'Piantone di Mogliano A', 'Rosciola colli Esini' with 10 inoculated plants and 5 control (not inoculated) plants.

In all plants, the wounds consisted in 10 horizontal 5 mm long cuts (about one per internode), that were manually executed on the bark of the stem using a sterile surgical scalpel. On the inoculated plants the first 5 wounds made in the lower section of the stem were inoculated with aliquots (10 μL) of the *Pseudomonas* spp. suspension (~8 * 10^8 CFU mL^{-1}), prepared with the isolates listed in Table 1. The 5 wounds in the upper section of the stem of the inoculated trees were treated with distilled water as it was done in all the 10 wounds made on control plants (Fig 1).

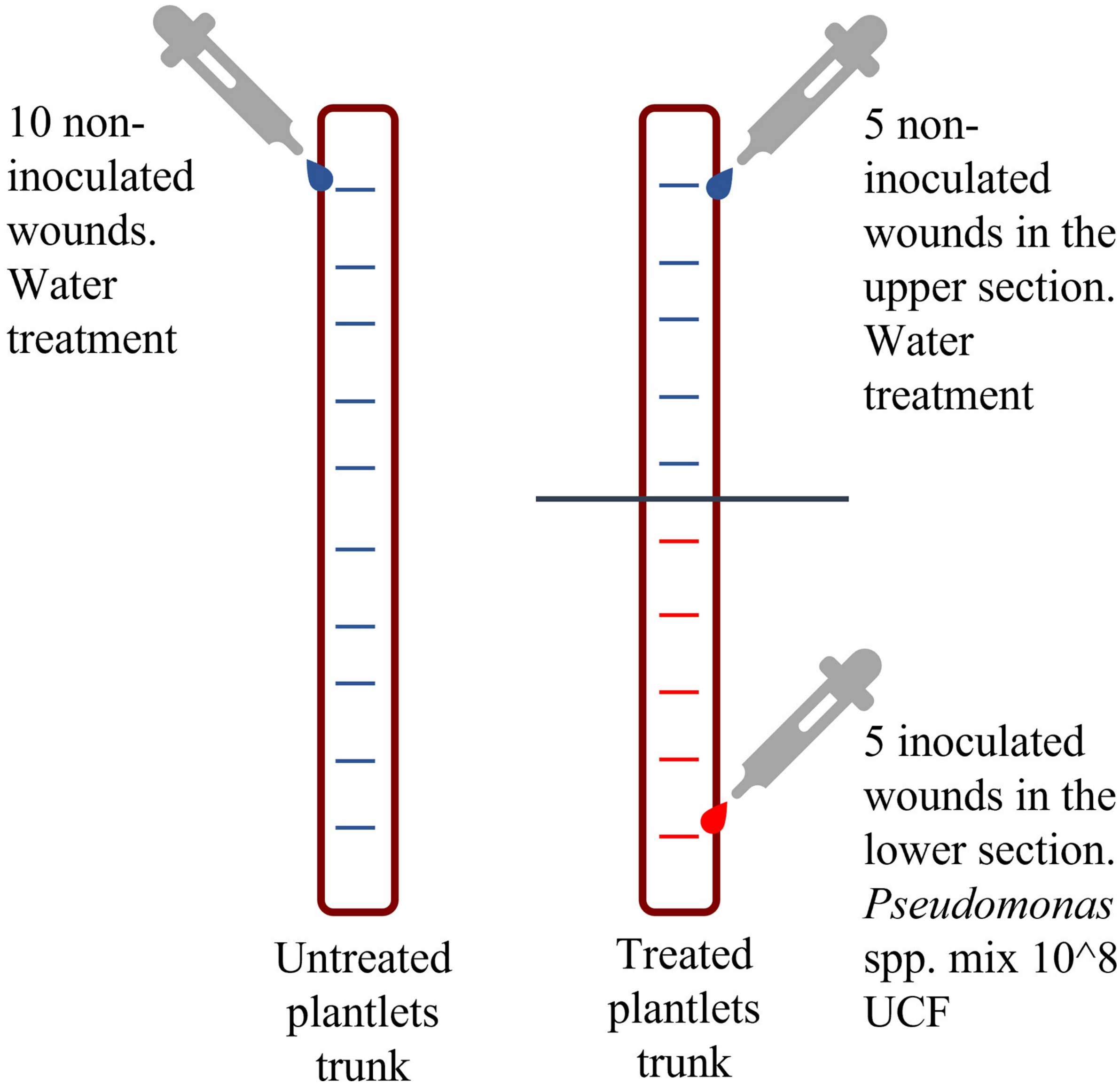

Fig 1. Design of experiment on plants.

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The experimental trial was executed in a heated greenhouse (fixed minimum 18˚C) and began on the 3rd of March 2019.

Observations and measurements

From DAI (day after inoculum), 64 (6th of May 2019) to DAI 225 (14th of October 2019), the evaluation of the disease was carried out for 6 times. A visual index for the formed knot was attributed to each wound in each assessment date (value 0 = absence of knot, value 1 = the presence of knot was doubtful, value 2 = visible knot, value 3 = big and well-defined knot), (Fig 2).

For each assessment, the percentage of the sum of visual index 3 and 4 of each plant was used to calculate the Area Under Incidence Progress Curve (AUIPC) index with the following

Fig 2. Visual index for the formed knot attributed to each wound: Value 0 = absence of knot, value 1 = the presence of knot was doubtful, value 2 = visible knot, value 3 = big and well-defined knot.

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formula:

$$AUIPC = \sum_{i=1}^{n} \left(\frac{y_i + y_{i+1}}{2}\right)(t_{i+1} - t_i)$$

Where: y is the % of the intensity 3 and 4 of the visual index of knot; t is the day of the assessment (i). In the same assessment dates, transversal and longitudinal diameters and height of each knot were measured to calculate the volume, according to an ovoid-based cylinder. On DAI 225 (14th of October, the last assessment date), all knots were removed from the stem using a sterile surgical scalpel and these were individually weighed.

For those knots whose dimensions did not allow a measurement of their diameter, the volume was obtained by the interpolation of volume/weight (volume = 66.58+1352.55*weight, R square 0.84, P value <0.0001).

The transversal diameter of the stem was measured at each height where the knots were formed, and the TCSA calculated to calculate the volume of knot/TCSA index.

Data are available in the BioStudies database (https://www.ebi.ac.uk/biostudies/) under accession number S-BSST1089.

Metataxonomic analyses

A metataxonomic approach was applied to analyze the total DNA of fourteen samples collected from eleven cultivars of olive plants (R1-R11, Table 2) to study the microbiota composition of the olive knots removed from the stem on DAI 225. Samples R11, R11A, R11B and R11C were collected from the same cultivar (Cultivar Rosciola colli Esini), but from different plants.

Table 2. Samples identification (ID) and the relative cultivars of the sampling collection.

Sample-ID	Cultivar
R1	cultivar 'Arbequina'
R2	cultivar 'Ascolana dura'
R3	cultivar 'Ascolana tenera'
R4	cultivar 'Carboncella'
R5	cultivar 'Frantoio'
R6	cultivar 'Fs17®'
R7	cultivar 'Maurino A'
R8	cultivar 'Piantone di Mogliano A'
R9	cultivar 'Piantone di Mogliano B'
R10	cultivar 'Piantone di Falerone'
R11	cultivar 'Rosciola Colli Esini'
R11A	cultivar 'Rosciola Colli Esini' plant 1
R11B	cultivar 'Rosciola Colli Esini' plant 2
R11C	cultivar 'Rosciola Colli Esini 'plant 3

https://doi.org/10.1371/journal.pone.0289875.t002

The V3-V4 regions of the 16S rRNA gene were amplified following the parameters defined by Maoloni et al. [26]. Illumina guidelines were used to purify, tag, pool and sequence PCR products. 250-bp paired-end reads were generated by a MiSeq platform (Illumina). Raw files (.fastq) were elaborated by QIIME 2 software [27]. Cutapter was used to remove primer sequences and DADA2 algorithms were employed to denoise the reads [28] through the q2-dada2 plugin in QIIME 2 in order to obtain the ASVs. Taxonomic assignment was performed by the QIIME2 feature-classifier against the Greengenes 16S rRNA gene database v. 13.8. To increase the confidence of sequence reads we excluded the ASVs with less than five read counts in at least two samples. Sequence reads were normalized by verification with an equal representation of 10,000 sequences per sample at the lowest sequence/sample reads. Alpha and beta diversity analyses were performed by QIIME2 diversity plugin. The 16S rRNA gene sequences are available at the Sequence Read Archive of NCBI (accession number PRJNA972624).

Statistical analysis

One-way ANOVA was performed and, in presence of significant differences, the Tukey-Kramer (HSD) test at $P<0.05$ was used to detect statistically significant differences among the mean scores. All statistical analyses were performed with JMP 8.0 software (SAS Institute Inc., Cary, NC, USA, 2009).

Differences between alpha or beta diversities and ASVs abundance were analyzed by R software or QIIME2 as appropriate.

Results

For the identification of the 30 Gram negative isolates collected from olive knots a threshold of 98.65% sequence similarity [29] was set; based on the alignment of the 16S rRNA gene sequences the 30 isolates were ascribed to the following species: *Pseudomonas caspiana*, *Pseudomonas coleopterorum*, *Pseudomonas graminis*, *Pseudomonas lutea*, *Pseudomonas savastanoi*, *Pseudomonas harudinis*, and *Pseudomonas bohemica* (Table 3).

The wounds inoculated in the lower section of stems showed a growing trend for the values 2 and 3 in the visual index for the formed knot, opposite to values 0 and 1. The trend is best shown if value 0 is added to value 1, and value 2 is added to value 3 (Fig 3D). In fact, the first

Table 3. Identification of the Gram-negative bacteria isolated from olive knots in the University farm "Pasquale Rosati" and a private orchard located at Monte San Vito (AN, Italy).

Field of isolation	Isolate code	Species	Sequence similarity %[a]	E value	Accession N.[b]	Accession N.[c]
Experimental Farm "Pasquale Rosati"	AGU1	*Pseudomonas caspiana*	98.93	0.0	OQ990727	NR_152639.1
	AGU2	*Pseudomonas coleopterorum*	98.89	0.0	OQ990728	NR_137215.1
	AGU3	*Pseudomonas caspiana*	99.11	0.0	OQ990729	NR_152639.1
	AGU4	*Pseudomonas graminis*	99.41	0.0	OQ990730	NR_026395.1
	AGU6	*Pseudomonas lutea*	99.22	0.0	OQ990731	NR_029103.1
	AGU8	*Pseudomonas graminis*	99.63	0.0	OQ990732	NR_026395.1
	AGU9	*Pseudomonas caspiana*	99.72	0.0	OQ990733	NR_152639.1
	AGU10	*Pseudomonas caspiana*	99.53	0.0	OQ990734	NR_152639.1
	AGU11	*Pseudomonas caspiana*	99.47	0.0	OQ990735	NR_152639.1
	AGU13	*Pseudomonas graminis*	99.51	0.0	OQ990736	NR_026395.1
	AGU14	*Pseudomonas caspiana*	99.33	0.0	OQ990737	NR_152639.1
	AGU15	*Pseudomonas graminis*	99.80	0.0	OQ990738	NR_026395.1
	AGU17	*Pseudomonas graminis*	99.30	0.0	OQ990739	NR_026395.1
	AGU18	*Pseudomonas lutea*	99.12	0.0	OQ990740	NR_029103.1
	AGU19	*Pseudomonas caspiana*	99.35	0.0	OQ990741	NR_152639.1
	AGU20	*Pseudomonas caspiana*	99.22	0.0	OQ990742	NR_152639.1
	AGU21	*Pseudomonas graminis*	99.49	0.0	OQ990743	NR_026395.1
Orchard located at Monte San Vito	SAN1	*Pseudomonas savastanoi*	99.81	0.0	OQ990744	NR_117822.1
	SAN6	*Pseudomonas caspiana*	99.43	0.0	OQ990745	NR_152639.1
	SAN8	*Pseudomonas caspiana*	99.48	0.0	OQ990746	NR_152639.1
	SAN11	*Pseudomonas caspiana*	99.61	0.0	OQ990747	NR_152639.1
	SAN12	*Pseudomonas bohemica*	99.15	0.0	OQ990748	NR_159101.1
	SAN13	*Pseudomonas harudinis*	98.81	0.0	OQ990749	NR_181730.1
	SAN15	*Pseudomonas harudinis*	98.73	0.0	OQ990750	NR_181730.1
	SAN16	*Pseudomonas bohemica*	98.71	0.0	OQ990751	NR_159101.1
	SAN18	*Pseudomonas caspiana*	99.66	0.0	OQ990752	NR_152639.1
	SAN19	*Pseudomonas harudinis*	98.80	0.0	OQ990753	NR_181730.1
	SAN20	*Pseudomonas harudinis*	98.73	0.0	OQ990754	NR_181730.1
	SAN21	*Pseudomonas caspiana*	99.17	0.0	OQ990755	NR_152639.1
	SAN22	*Pseudomonas caspiana*	99.55	0.0	OQ990756	NR_152639.1

[a] Similarity % between the analyzed sequence and the GenBank sequence.

[b] Access number of the analyzed sequence deposited and available at the GenBank database.

[c] Access number of the GenBank sequence with the highest identity % with the analyzed sequence.

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couple of values decreased until 18%, whereas the second couple reached the 82% of the total of wounds.

Contrary to the inoculated wounds (on lower section), the non-inoculated wounds (on upper section) limited rates of value 2 and 3 in the visual index for the formed knots. The maximum value as the sum of values 2 and 3 corresponded to about 0.5% of the total non-inoculated wounds, recorded on DAI 187 (September 6th, Fig 3B).

In the non-inoculated plants (control), the value 2 of the visual index for the formed knots reached the maximum intensity on DAI 137 (July 18th), corresponding to 0.55% of the total of the non-inoculated wounds (Fig 3A). On DAI 187 (September 6th) the maximum degree reached for values 2 plus 3, that corresponded to 0.56% of the total of non-inoculated wounds. The value 1 increased from 64 DAI (May 6th, 17%) to 73 DAI (May 15th, 36%) before it fell down. On 225 DAI (October 14th) value 1 was not used.

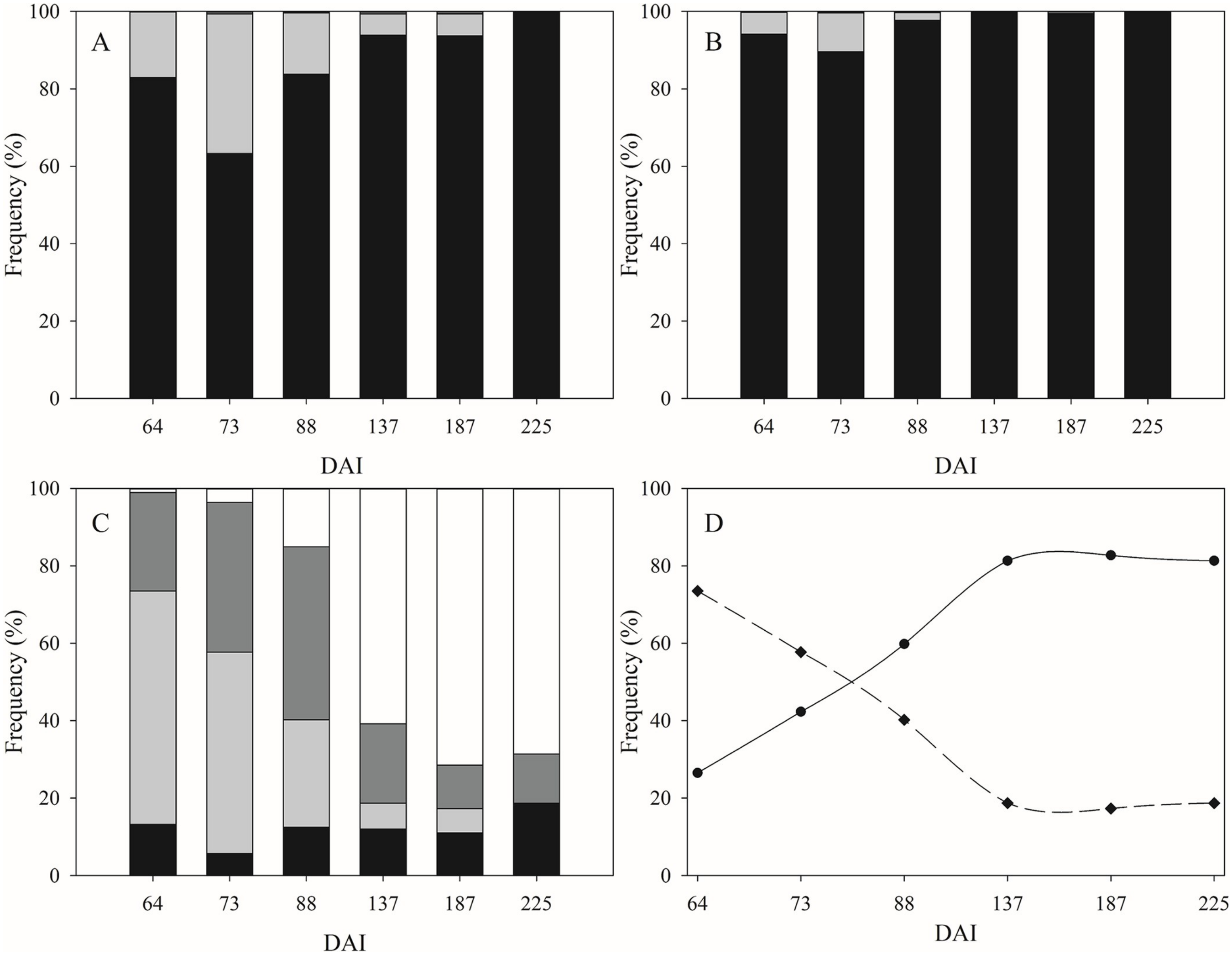

Fig 3. Evolution of the frequency of the visual indexes for the formed knot attributed to each wound in time (DAI: Day after inoculum) on the whole set of plants and cultivar tested. (A) Non-inoculated wounds in the non-inoculated plants (control); (B) Non-inoculated wounds in the upper section in the inoculated plants; (C) and (D) Inoculated wounds in the lower section in the inoculated plants. Graph A, B, and C: value 0 = absence of knot (■), value 1 = the presence of knot was doubtful (▨), value 2 = visible knot (■), value 3 = big and well-defined knot (□). Graph D: value 0 plus 1 (slashed line), value 2 plus 3 (continuous line).

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On DAI 64 (May 6th) all cultivars except 'Pendolino' formed knots and showed values 2 and 3 of the visual index. Instead, the 'Pendolino' cultivar showed the value 2 starting from DAI 73 (May 15th, Fig 4).

The severity of the disease followed an increasing trend starting from DAI 64 (May 5th). On this day only 'Carboncella', 'Maurino A' and 'Rosciola Colli Esini' cultivar already showed the presence of big tumors (value 3, Fig 4).

On 18th July (137 DAI) 100% of the inoculated wounds formed knots in 'Leccio del corno', followed by 'Maurino A' and 'Ascolana Tenera' with 90.0%, 'Rosciola colli Esini' with 76.0%, 'Frantoio' with 75.6%, 'Arbequina' with 68.9%, 'Leccino' with 63.3%, 'Piantone di Mogliano A' and 'Piantone di Mogliano B' with 60.0%, 'Ascolana Dura' with 57.8%, 'Pendolino' with 53.3%, 'Carboncella' with 52.0%, 'Piantone di Falerone' with 44.4%, 'FS-17Ⓡ' with 35.0%, and 'Maurino B' with 6.67%.

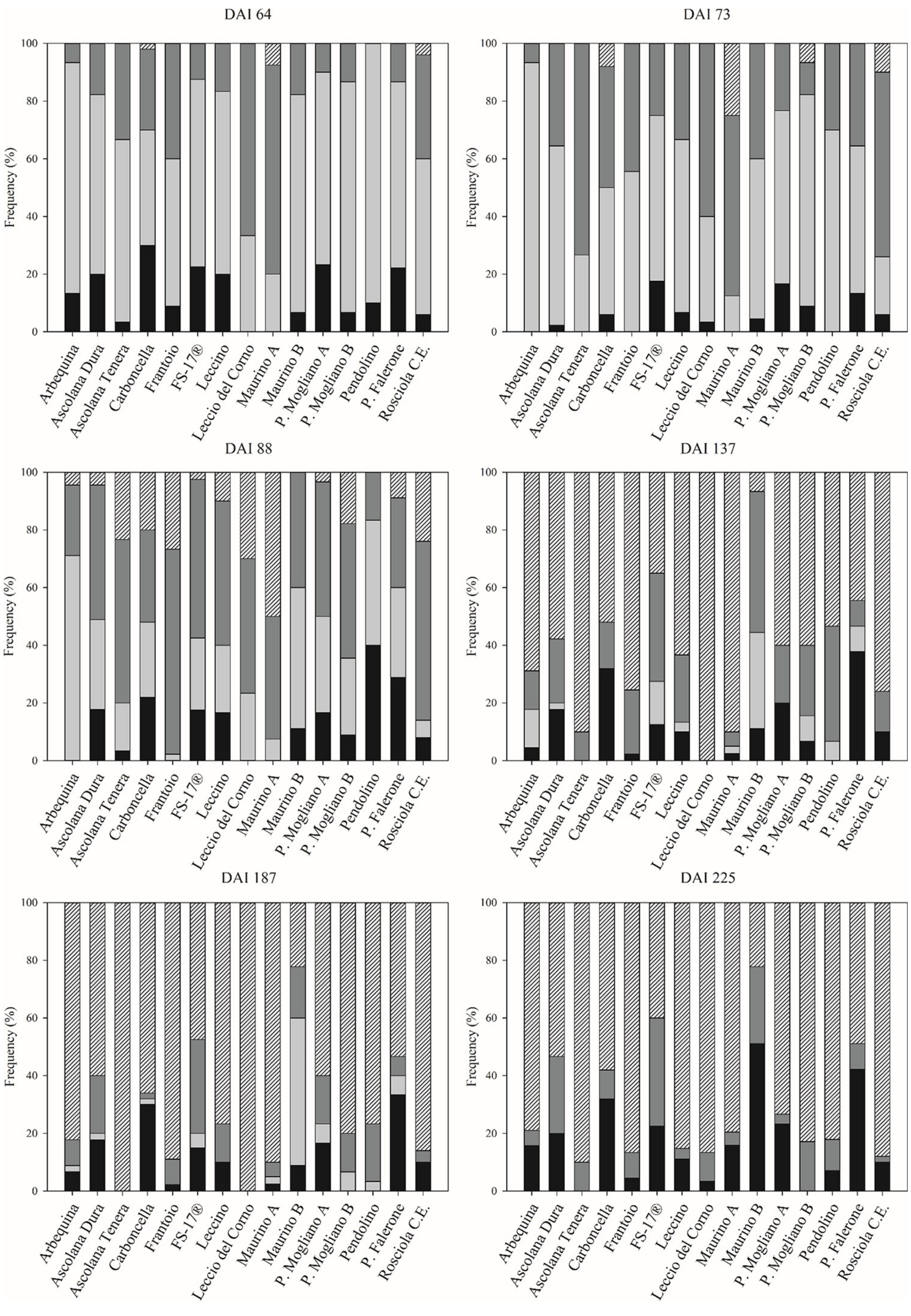

Fig 4. Evolution of the frequency per cultivar of the visual indexes for the formed knot attributed to each inoculated wound (the wounds in the lower section) of each treated plant in time (DAY: Day after inoculum). Value 0 = absence of knot (■), value 1 = the presence of knot was doubtful (░), value 2 = visible knot (▓), value 3 = big and well-defined knot (▨).

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On 137 DAI all the cultivars reached percentages higher than 50% of knots-forming wounds. In DAI 225 "Maurino B" and 'Piantone di Falerone' cultivars presented the highest percentage of absence of knot (value 0 of the visual index), showing 51% and 42% of the inoculated wounds, respectively. In the same DAI 225 'Piantone di Mogliano A' showed 23% of the inoculated wounds without knots, while 'Piantone di Mogliano B' cultivar showed the presence of knot in all of the inoculated wounds. 'Maurino B' showed approximately the 51% of the inoculated wounds without knots, which corresponds to an increase of 35% compared to 'Maurino A' (16%). 'Maurino A' had also the highest AUIPC value, while 'Maurino B' showed the lower (149 and 73 respectively).

Considering the values 2 and 3 of the visual index of the presence of the knot, 'Rosciola Colli Esini' showed the highest mean value of knot volume (727±511 mm^3, Fig 5). Only 'Maurino A' presented a significantly comparable knot (494±335 mm^3) to 'Rosciola Colli Esini', and a slight, but not significant, difference with 'Maurino B' (322±104 mm^3). Also 'Piantone di Mogliano A' and 'Piantone di Mogliano B' did not present significantly difference on knot volumes (369±188 mm^3 and 254±190 mm^3).

Although 'Leccio del Corno' showed the greater intensity of the disease than other cultivars (Fig 4), the severity of the disease was low (164 ± 72 mm^3), although it showed significant differences only with 'Rosciola Colli Esini', 'Maurino A', and 'Carboncella' (Fig 5).

Regarding the index 'volume of knot / TCSA', 'Maurino A' and 'Maurino B', 'Piantone di Mogliano A' and 'Piantone di Mogliano B' showed similar values without significant differences (Fig 6).

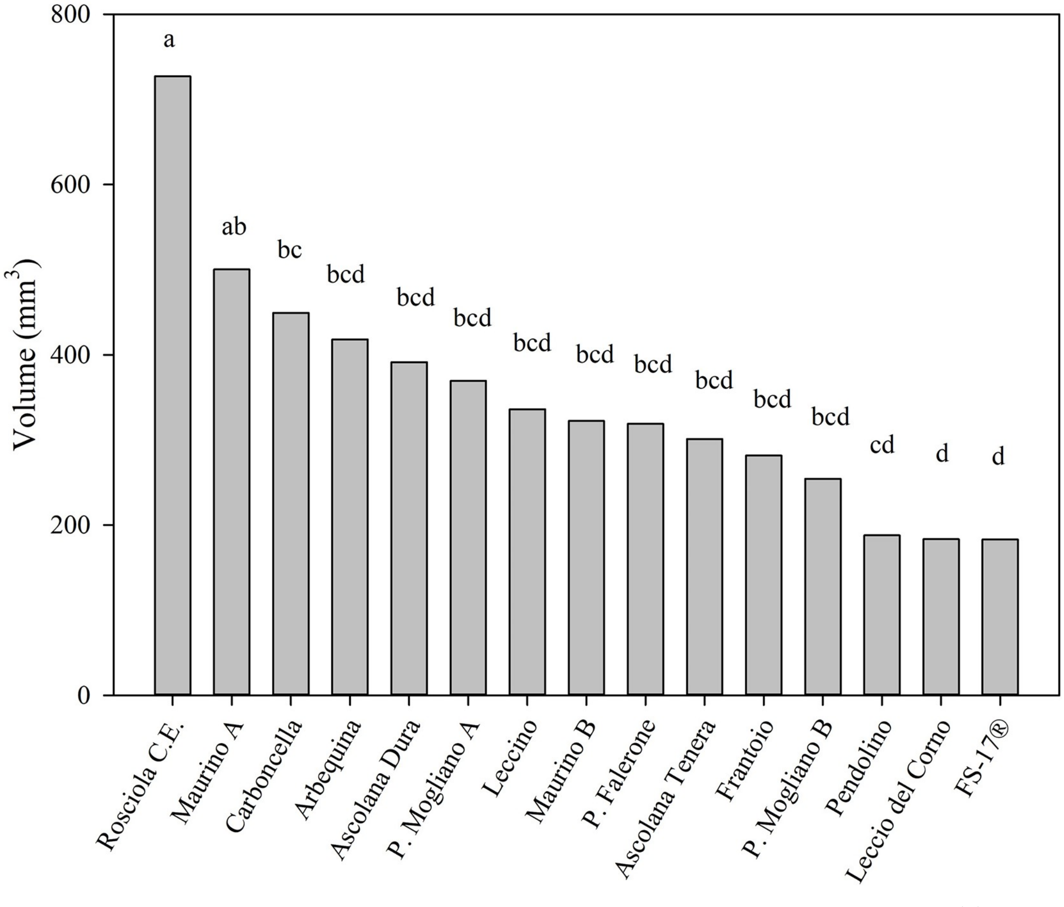

Fig 5. Mean in each cultivar of the volume of the knots at the value 2 and 3 of the visual index at 225 DAI. Each bar represents the mean value of a different number of replicates: 'Rosciola C.E.', 44; 'Maurino A', 33; 'Carboncella', 34; 'Arbequina', 31; 'Ascolana Dura', 29; 'P. Mogliano A', 23; 'Leccino', 19; 'Maurino B', 9; 'P. Falerone', 22; 'Ascolana Tenera', 28; 'Frantoio', 41; 'P. Mogliano B', 20; 'Pendolino', 22; 'Leccio del Corno', 29; 'FS-17®', 26. Different letters indicate significant differences among cultivars according to the Tukey test (α = 0.05), P.value: <0.0001.

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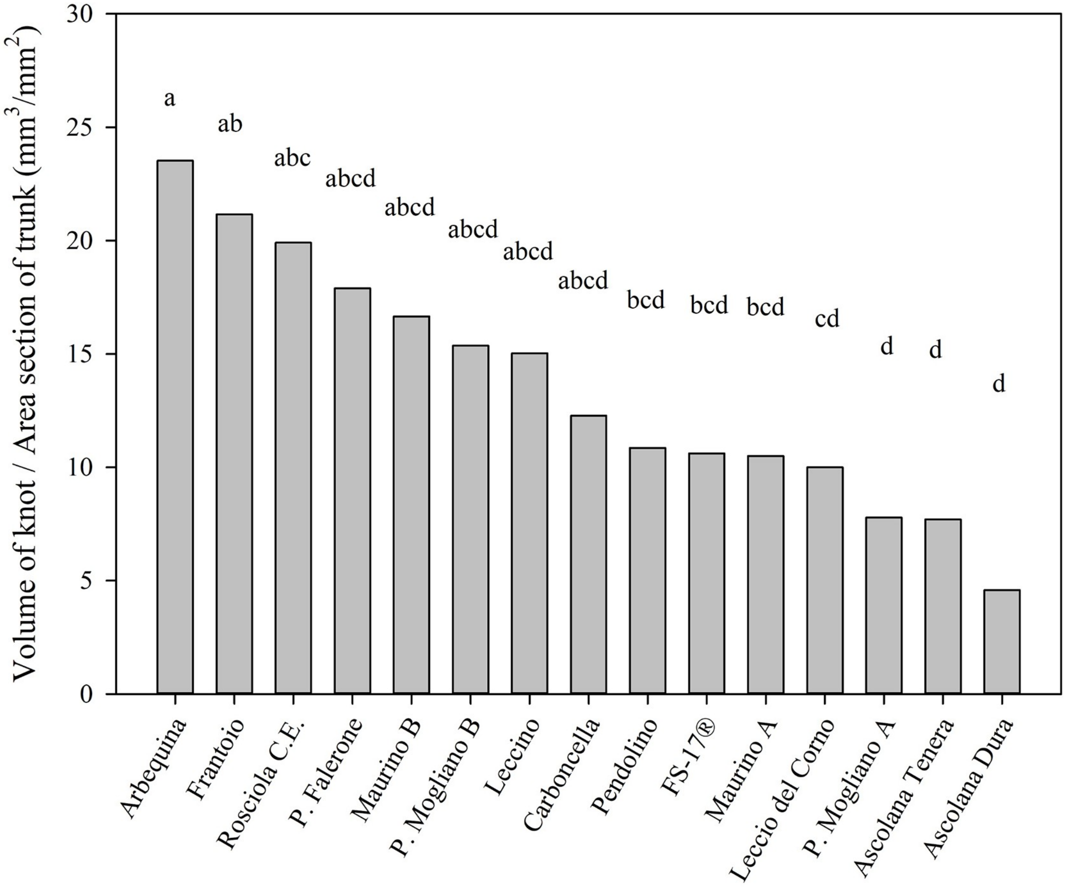

Fig 6. Relation between tumor volume and the area of the stem section where the knot was positioned at DAI 225. Each bar represents the mean value of a different number of replicates: 'Arbequina', 23; 'Frantoio', 32; 'Rosciola C.E.', 40; 'P. Falerone', 20; 'Maurino B', 9; 'P. Mogliano B', 20; 'Leccino', 9; 'Carboncella', 24; 'Pendolino', 21; 'FS-17®', 21; 'Maurino A', 25; 'Leccio del Corno', 29; 'P. Mogliano A', 23; 'Ascolana Tenera', 18; 'Ascolana Dura', 17. Different letters indicate significant differences among cultivars according to the Tukey test (α = 0.05), P.value: <0.0001.

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AUIPC of each cultivar: 'Arbequina' 107, 'Ascolana Dura' 111, 'Ascolana tenera' 148, 'Carboncella' 100, 'Frantoio' 148, 'FS-17' 108, 'Leccino' 123, 'Leccio del Corno' 147, 'Maurino A' 149, 'Maurino B' 73, 'Piantone di Mogliano A' 107, 'Piantone di Mogliano B' 125, 'Pendolino 115', 'Piantone di Falerone' 81, 'Rosciola' 139.

Regarding the metataxonomic composition of olive knots removed from the stem of different cultivars on DAI 225, no significant differences in alpha or beta-diversity indexes were found (S1 Table, S1 Fig). Comparison of ASVs relative abundances between the cultivars confirmed the dominance of Pss. Its relative frequency was never less than 80%. Pss represented the only identified taxon in the knots collected from cultivar 'Carboncella' (R4), 'Frantoio' (R5), Fs17 (R6), 'Piantone di Mogliano A' (R8) and 'Rosciola Colli Esini' (R11) (Fig 7).

Additional species were also detected at low relative abundances in knots of some cultivars. More specifically, 'Arbequina' (R1) and 'Ascolana dura' (R2) showed the presence of *Kocuria* in percentage less than 0.5% of the relative frequency (Fig 7), whereas *Streptomyces* was found in knots sampled from cultivar 'Ascolana tenera' (R3–3% of the relative frequency), 'Maurino A' (R7–9.3% of the relative frequency) and 'Piantone di Mogliano B' (R9–0.5% of the relative frequency) (Fig 7). The cultivar 'Maurino A' (R7) showed also the presence of *Curtobacterium citreum* (6.8% of the relative frequency). This taxon was found also in knots collected from

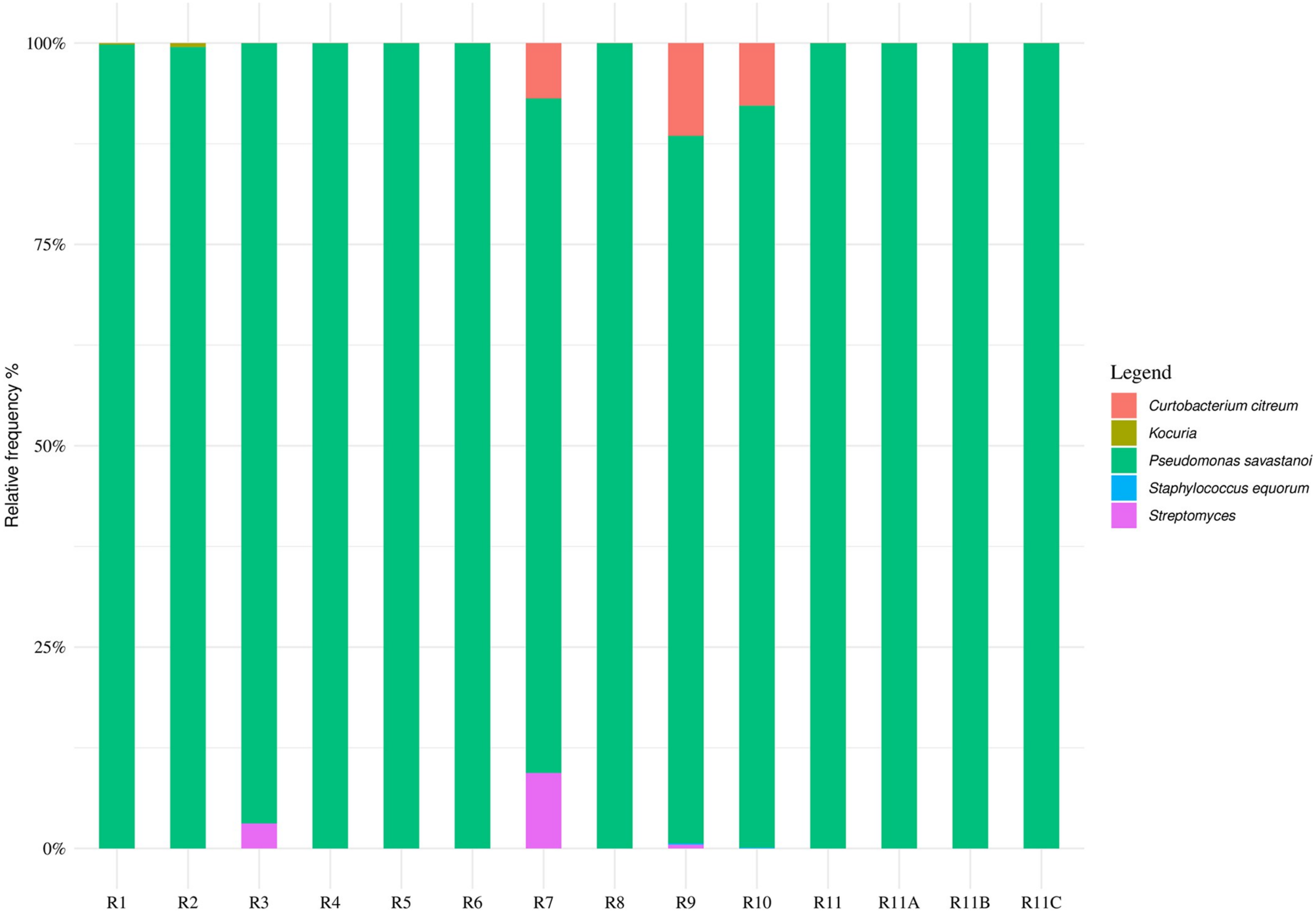

Fig 7. Relative frequency of bacterial Amplicon Sequencing Variants (ASVs) detected in the olive knots removed from the stem of different cultivars on DAI (day after inoculation) 225. For the coding of the samples see Table 2.

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cultivar 'Piantone di Mogliano B' (R9) and 'Piantone di Falerone' (R10) together with *Staphylococcus equorum* (~ 0.11% of the relative frequency) (Fig 7).

By comparing the results collected from different plants of the cultivar 'Rosciola Colli Esini', this latter was characterized by the exclusive presence of *Pseudomonas savastanoi* (100% of the relative frequency) (Fig 7).

Discussion

All the wounds inoculated with the pathobiome consortium of *Pseudomonas* species showed a high percentage of disease. On the contrary, the wounds in the not inoculated control plants did not show the disease. This evidence clearly suggests that the bacterial inoculum was responsible for the infection of the treated olive trees. This evidence was confirmed also by the metataxonomic approach analysis, which evidenced that *Pseudomonas savastanoi* pv *savastanoi* was more present than other species, in all the cultivars. Contrary to what was reported by Penyalver et al. [1], the absence of knots in non-inoculated wounds of treated plants indicates that bacteria of the consortium were unable to transmigrate within the tested time. This latter finding suggests that the spread of the disease can be confined to the inoculated wounds if the environmental condition are suitable and compartmentalization and/or a prompt disinfection of the trees is executed.

Furthermore, the cultivars herein treated differed in the time of appearance of knot even though after about 187 days since the inoculation, wounds reached the maximum percentage of knots (2 and 3). In the field the time of appearance of the disease is important to plan for appropriate disinfection treatments.

Moreover, the fact that all cultivars with the only exception of 'Maurino B' showed a high percentage of wounds with knots (more than 50%), indicates that all assayed varieties are susceptible to *Pseudomonas savastanoi* pv. *savastanoi*. As already reported by Penyalver et al. [1], plants' age might affect the incidence of this disease, with younger plants showing lower levels of olive knot. This is in alignment with the results we found for 'Maurino' where younger plants showed higher levels of intensity of disease and the AUIPC but not valid for 'Piantone di Mogliano', where higher levels of disease were found in older plants. Such different behavior legitimizes the need for conducting further research with emphasis about the susceptibility to *Pseudomonas* according to the age or the physiological status of olive shoots and plants.

Valverde et al. [17] showed how after a late frost 'Rosciola Colli Esini' was into the "low susceptibility" cluster, with low level of disease and low level of injury due to frost (confirmed also by Lodolini e al. [30, 31]). In the present investigation, opposite results to those reported by these authors were obtained in protected environment with young pot trees. In fact, after inoculation with the bacterial consortium, 'Rosciola Colli Esini' was the most susceptible cultivar among those assayed.

Hence, we can hypothesize that the formation of wounds on olive tree tissues might be the first step to trigger the disease; the higher tolerance of this cultivar to late frost can be determined by the lower presence of damages due to frost. Consequently, a modest presence of the disease is not due to a lower sensibility of the plant to the inoculum.

Moreover, the dominance of *Pseudomonas savastanoi* in the knots sampled on DAI 225 is congruent with the available literature, describing this microorganism as the primary causative agent of olive knot disease [2, 17, 32]. By contrast, the genera *Kocuria* and *Streptomyces*, and the species *Curtobacterium citreum* and *Staphylococcus equorum* detected to a lesser extent in some cultivars might likely be part of the resident epiphytic and endophytic microflora of olive trees, as previously suggested [33, 34]. The absence of the other *Pseudomonas* species besides *Pseudomonas savastanoi* indicates that they didn't develop in the knot after inoculation.

Conclusion

The present study confirms that all cultivars herein assayed are susceptible to olive knot, while the reaction time, intensity, and severity of the disease depend on the olive cultivar. This information is supportive of design decisions for new olive groves in less endemic areas with well adapted varieties to limit the risk of frost damages in the shoots and stems and then the severity of the knot disease. Furthermore, all the information herein collected and the observed inability of the inoculated bacteria to transmigrate in the tested time might help the olive grower to reduce the spread of the disease in the field by pruning the infected portion of the trees. Maintaining only the healthy parts of the branches is a priority for orchard sanitation, but it is not mandatory because from the knots the Pss does not migrate from a wound to other wounds along the same stem. There is the need for studies about other bacterial populations to better understand the interaction of the Pss with other bacterial species, after natural and artificial inoculation and possibly to improve the use of biological treatments in the olive groves.

Supporting information

S1 Table. Alpha diversity measurement for 16S rRNA amplicons analyzed.
(PDF)

S1 Fig. Principal coordinates analysis of Bray Curtis distances for 16S rRNA gene sequence data. Samples are color-coded by variety.
(PDF)

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Writing – review & editing: Matteo Zucchini, Antonietta Maoloni, Enrico Maria Lodolini, Lucia Aquilanti, Davide Neri.

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Vegetative and productive response of olive trees under anti-insect nets.

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Abstract

Anti-insect net can help to control pests in many fruit tree crops reducing the application of pesticides. It can also be useful in olive in controlling the fly (*Bractrocera oleae* Gmel.), especially for table cultivars. In addition to prevent the direct damages to the fruits by avoiding the access of the insects to the canopy, the net can be useful against other abiotic stress. For example, it can provide shade that protects them from excessive insolation leading to greater final growth.

In this study we evaluated the shading effect of anti-insect nets on the vegetative and reproductive growth of the olive tree.

In July 2021 and 2022, the nets (shading factor 15%) were installed before and after the pit hardening (15 days difference). Uncovered trees were used as control. In 2021, the trees didn't show differences regarding the fruit yield, but the trees covered with the nets after the pit hardening showed bigger fruits than the control without nets. The installation of nets after pit hardening induced also a higher number of nodes in the vegetative portion of 1-year-old mixed shoots during the period of installation of the nets (from July to October 2021). In 2022 no differences were recorded among the applied treatments and the control, probably because of a low crop load of the trees.

The use of anti-insect nets in table olive cultivars, in addition to prevent damages to the fruits from *Bactrocera oleae*, can positively affect the physiological parameters of the canopy, inducing higher growth of the shoots and of the fruits when crop load is high. Nevertheless, the installation time needs to be studied to not negatively affect vegetative-reproductive growth and flower induction.

Keywords: *Bactrocera Oleae,* olive fly, vegetative growth, fruit, olive.

INTRODUCTION

During the last years the use of multifunctional nets in agriculture is increasing (Kotilainen et al., 2018). The nets are not only used against abiotic factors such as wind, hail, or excess sunlight (Crescenzi et la. 2022; Neri et al. 2021) but also for biotic stress like insects (Grassi et al., 2007; Grassi and Pallaoro, 2012). On olive (*Olea europaea* L.) the use of nets is a recently used method against the *Bactrocera oleae* fly (Lodolini et al., 2018) with interesting results, but the scientific literature lacks scientific works. As studied in other crops, Lodolini et al. (2018) reported that in addition, the use of anti-insect nets, can modify the microclimate of the canopy, acting on PAR (Photosynthetic Active Radiation), temperature, and humidity (Kotilainen et al., 2018; Manja & Aoun, 2019).

The conditions of the microclimate of the canopy can influence the performance of the tree. For example, high light intensity decreases the efficiency of photosynthesis in olive, (Jifon and Syvertsen, 2003; Sofo et al., 2009), with a saturation point around 500-800 $\mu molm^{-2}s^{-1}$ (Bongi and Long, 1987; Proietti et al., 2012; Sofo et al., 2009). Given the marked tendency toward alternate bearing (Lavee, 2007), if the use of the net increases the performances, above all during summer, then it could increase the production of the fruits of the same year and of the following one. In olive, the inflorescences develop from one-year-old buds bare on one-year-old portion of the shoots. The first step to develop new inflorescences is, although is not enough, to create new nodes (i.e., a couple of buds) during the previous year of the blooming (Moutier et al., 2004), and adequate agronomic practices can increase the shoot growth (Zucchini et al., 2023). In olive, as in other fruit trees, the vegetative growth is in contrast with the fruit growth, the presence of a high crop load negatively affects the growth of new nodes, as if fruits dominated nutrient intake (Dag et al., 2009 and 2010).

Our study continues the study of Lodolini et al. (2018), answering the question, does increasing photosynthesis performance increase tree yield under the netting system? And moreover, what is the best time of the season to install the net?

MATERIAL AND METHODS

2.1 Experimental field

The experiment was performed during 2021 and 2022 in an olive orchard planted in 2001, with a density of 277 tree ha^{-1}, in Mogliano (Marche Region, Central Italy, latitude 43°11’09”N; longitude 13°28’50”E; altitude 313 m a.s.l.). All the trees were ‘Piantone di Mogliano’ cultivar and were trained according to a polyconic vase system. The temperatures during the two years of the trial are represented in figure 1 as the temperatures recorded in a weather station far 9 km from the experimental field.

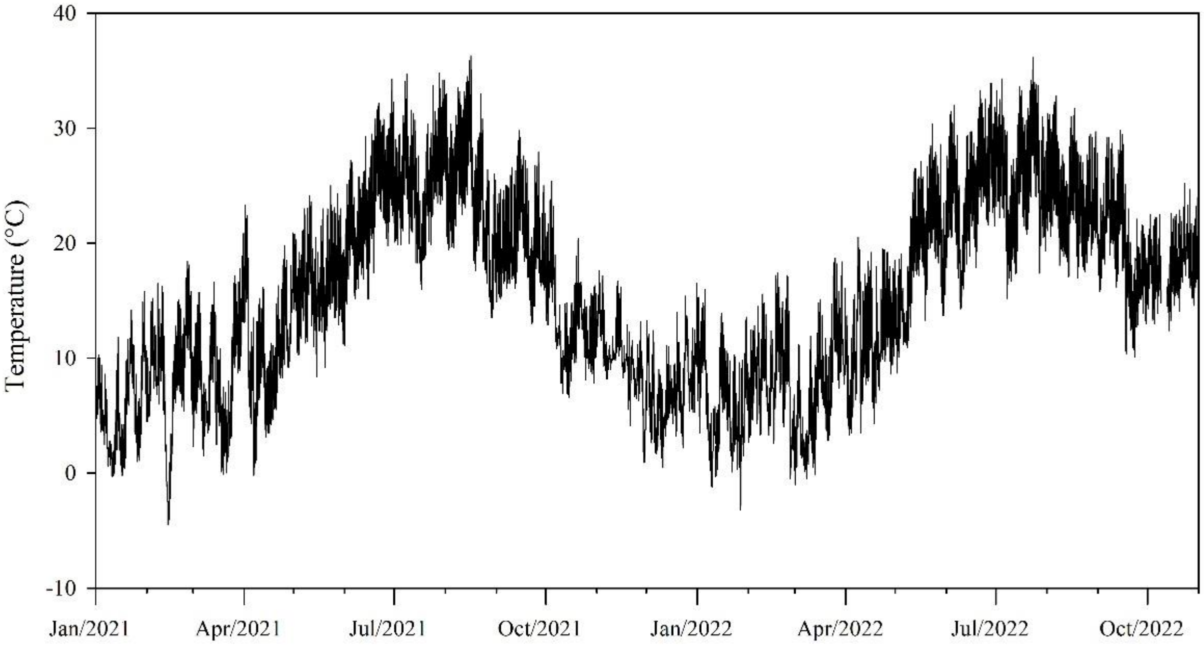

Figure 1: Hourly temperatures recorded in a weather station 9 km far from the experimental field.

2.2 Treatment and measurements

An anti-insect net in HDPE “Monotex 50” (Agrintech, Italy) of neutral color, with 15% shading, mesh size of 0.97 mm x 1.54 mm and weight of 95 g/m2 was used for the field trial. The plants were covered with rectangles of nets measuring 6x10 m.

The treatments were:

- ‘Pre’, installation of the net at the begging of pit hardening (early July).
- ‘Post’, installation of the net after the pit hardening (late July).
- Uncovered trees as ‘Control’.

Before the installation the number of nodes for 10 vegetative shoots was counted for each tree (5 in the north side and 5 in the south side of the canopy), and where selected 10 tree for each treatment. A totally randomized block design and 10 trees per treatments were used for the experiment.

At harvest (November), the total fruit production, the fresh weight of 30 fruits and the number of nodes in the vegetative shoots were taken for every tree.

2.5 Data Analysis

One-way ANOVA was performed for each variable per each year, and in the presence of significant differences, the Tukey-Kramer (HSD) test at P<0.05 was used for mean separation. All statistical analyses were performed using JMP 14.0 software (SAS Institute, Cary, NC).

RESULTS

In both years the average percentage of fruits affected by the *Bactrocera oleae* in the Control trees (without the net) was very high, 26±2 and 38±4 for 2021 and 2022 respectively. The fruits under nets resulted less affected by the insect, in fact for the installation pre pit hardening were the 0.6±0.4 and 0±0 for 2021 and 2022 respectively, while in the post pit hardening the infestation was 2±1 and 4±2 for 2021 and 2022 respectively (figure 2).

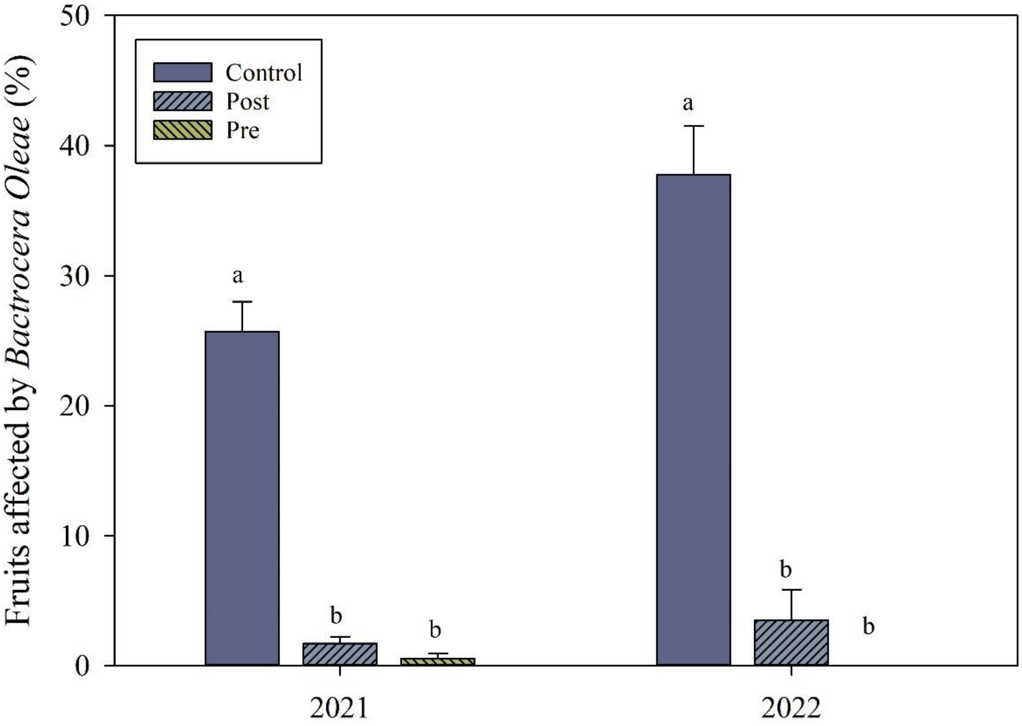

Figure 2: Percentage of fruit affected by Bactrocera oleae (mean±SE). Different letters indicate significant differences for Tukey-Kramer HSD test (P value<0.05).

The fruit productions of the two years were very different, in 2021 was about 9.61±0.94 kg, while in 2022 was about 0.7±0.25 kg. In both years, the treatments didn't show significant differences regarding the fruit production per tree. Nevertheless, in 2021 (on-year) the 'Post' treatment (after pit hardening) showed a higher fruits production than the control, while the 'Pre' treatment (beginning of pit hardening) didn't show significant differences with the control (figure 3). When the production was very low (off-year, 2022), the fruit size was similar for all the tested treatments.

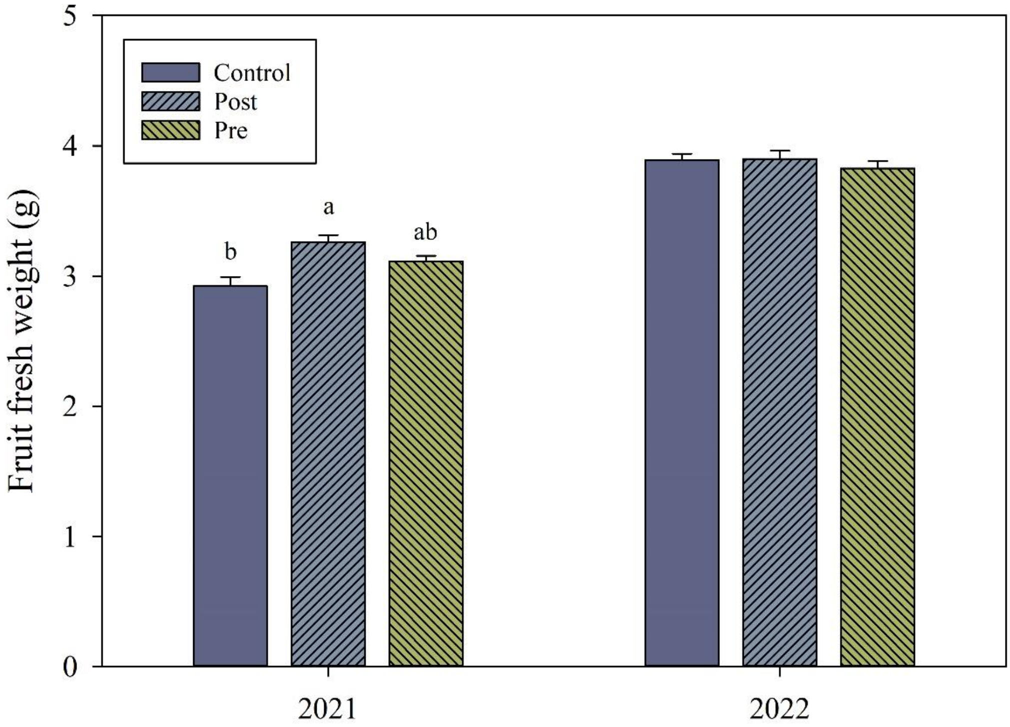

Figure 3. Mean fruit fresh weight for each tested treatment (mean±SE). Different letters indicate significant differences for Tukey-Kramer HSD test (P value<0.05).

The same results were found for the number of new nodes created in the vegetative portion of the shoot during the season: in 2021 the 'Post' treatment showed a higher value, while the 'Pre' didn't show significant differences with the 'Post' treatment and the control. In 2022 the 'Post' treatment showed the highest number of new nodes, but without significant differences with the 'Pre' treatment and the control (figure 4).

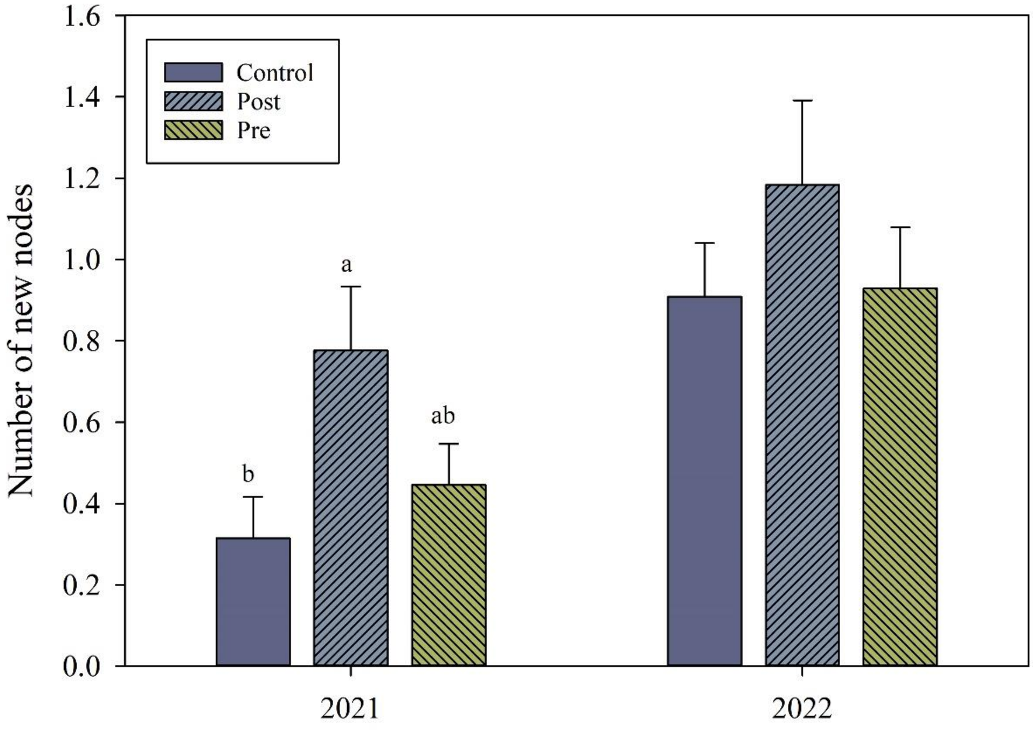

Figure 4: Number of new nodes formed during the season (mean±SE). Different letters indicate significant differences for Tukey-Kramer HSD test (P value<0.05).

DISCUSSION

The installation of anti-insect nets showed effective protection against the *Bactrocera* fly, as previously reported by Lodolini et al. (2018). Furthermore, the installation of the anti-insect net confirmed the improvement of the performances of the trees, especially whether the coverage was carried out after the complete pit hardening time. Pit hardening is an important phase in olive cultivation, in this period water reduction did not affect the production of the year (Ben-Gal et al., 2021; Corell et al., 2022; Dell'Amico et al., 2012; Marra et al., 2016). Thus, netting during beginning of pit-hardening decreased the performance of the trees. In fact, both the shoot growth and the fruit size in 2021 showed no significant differences in the trees where installation of the net was performed at early pit hardening, while there was a significant difference between trees covered after complete pit hardening and the control. After the complete pit-hardening, in the olive tree, the oil accumulation of the fruit begins (López-Bernal et al., 2021; Navas-Lopez et al., 2019; Trentacoste et al., 2010), which is a important phase (Naor et al., 2013). For this reason, installing the net after the complete pit hardening could help against stressors such as drought, high temperatures, and light. Crop load also affected the shoot and fruit growth, in off-years the nets do not increase the performance of the trees compared to the control without net (Dag et al., 2009, 2010).

Nevertheless, the installation time needs to be studied to not negatively affect vegetative-reproductive growth and flower induction.

CONCLUSION

In modern olive cultivation and in conditions of climate change, new tools need to be implemented in order to increase crop yield and quality and to decreasing the input. The use of anti-insect nets in olive can represent an effective system to decrease the use of pesticides against *Bactrocera olea*e. In addition, the netting system can improve the performance of the canopy creating a better microclimate conditions, increasing the vegetative growth of the shoots and enhancing the fruit weight. If the result to have bigger fruit size for table olive varieties is highly appreciable, further studies are required to study the oil accumulation in the fruits of trees covered with anti-insect and possibly photo-selective nets.

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7. Perspectives and conclusions

From the information in the scientific literature, the cost of fruit production from the 5th to the 50th year of life of the orchard (for conventional agriculture) was estimated at 2,033 €/ha per year in Puglia (Mohamad et al., 2014), and at 2,578 €/ha per year in the Sicily region (Sgroi et al., 2015) (without transport and milling costs for both estimates).

In central Italy (Marche, Tuscany, Lazio, and Abruzzo Regions) the production estimated by AgriIstat (2024) was 1.4 T/ha of fruits. Evaluating the cost of production of fruits as the literature, around 2,250 €/ha, the prize of fruits should be about 1,600 €/T excluding an income for the farmer. Instead, personal information from several olive stakeholders told me a price of olive fruits that can reach 1300-1400 euros per tons, with an average of 700-800. Reduce costs and increase production should be essential to maintain the olive cultivation sustainable in this area. It is possible to do a quick calculation, in the case in which the production costs were 2,500 €/ha and the selling price of the fruit was700 €/T, therefore, to break even the costs the production should be around 3.5-4 T/ha per year. So, in general, there is a lack of around 2 tons of production per hectare.

The problem is that, as mentioned earlier, the climate is changing, and the olive cultivation should change, above all in the area where olive is a secondary crop.

Starting from nutritional techniques, irrigation can first be considered.

In Italy, in 2001, the irrigated olive orchards were the 14%, with the percentage that reached the 25% in the south of Italy (Gucci, 2012). Although in central Italy the rains are usually more frequent than in south Italy during the summer, an irrigation plan can also increase production for this area (Servili et al., 2007; Caruso et al., 2022). In most of the olive orchards of this area irrigation treatment can incentivize vegetative growth and production. Caruso et al. (2002), showed, in a field of 556 trees/ha, in central Italy (Tyrrhenian coast) an increase of fruit production between the 2-3 t/ha for the 'Frantoio' cultivar, and between the 4.5-6.5 t/ha in the 'Leccino' cultivar. Irrigation could therefore already hit the goal of increasing by 2 t/ha.

However, in a context like that of central Italy where rainfall events during the summer are unpredictable, it is very important to control irrigation so as not to exaggerate vegetative growth and not to reduce the quality of the oil. To achieve this objective, it is important to follow the water status of the plants, and monitoring the water potential is the only one that returns a direct water status.

In case of drought stress, the irrigation should be managed following the water potential of the tree, and the use of microtensiometer could support the farmer to take the decision, considering the objectives to be achieved, the phenological phase, production, and other parameters.

Until now, the tree's water potential is measured with the pressure chamber (Scholander pressure bomb). The use of this machine has a cost which includes the weekly payment of the specialized operator, and the data is punctual at the seasonal and daily moment in which the measurement is carried out. Therefore, the use of the microtensiometer can help the farmer to follow the water status of the trees of the orchard during the season and throughout the day, and can also observe the response to applied irrigation. The microtensiometer records the water potential of the point where it is installed, so the data may differ from the water potential of the stem. Furthermore, the microtensiometer can eliminate operator error.

The increase in water supply in the olive grove must be accompanied with a greater availability of nutritional elements. This is also true in the event of greater rainfall, which can increase vegetative growth. Taking in account the vegetative growth, olives should need the fertilisation during spring when the apical bud of the mix-shoots is growing together with the inflorescences and flowers. Although lesser than in spring, a growth of vegetation was observed also during summer, the season when the fruit growth happens, for this reason also in this period a little fertilisation could help the production of the same year and the following year. Vegetative growth needs more nitrogen than fruit growth, while fruit growth needs more potassium, hence, the fertilisation management during the year will change according to the necessities.

In our study the "strong" treatment increased the vegetative growth, increasing the potentially productive buds, in fact in the following year the number of productive buds was doubled compared to the "weak" treatment. Furthermore, seems that on the sylleptic shoots the inflorescences frequency per bud is higher than the proleptic shoots. In the future, whether this observation will confirm, to increase these shoots (with fertilisation, irrigation, pruning) could be a technique to improve the production.

However, the grower will have to pay attention to the vegetative growth in the late season, i.e. at the end of summer. The new leaves that sprout in this period do not mature and are more susceptible to the winter and spring frost. Furthermore, an overdose of nitrogen can reduce the susceptibility to winter and spring frost of leaves and tissues (Fernández-Escobar et al., 2010) on the contrary a treatment of potassium at the end of the season increases the tolerance (Saadati et al., 2021).

In the future, for the central Italy area, the late frost events could hit the olives, not only with very low temperature that can damage the vegetative part of the tree, but also with little low temperatures (around 2°C) that can burn the inflorescences, flowers, or ovary. By protecting the olive grove from late frosts, production could thus be kept constant over the years.

Another problematic that frost can accentuate is the olive knot spreading, in fact the injuries produced on the bark can trigger the *Pseudomonas savastanoi* pv. *savastanoi*. In the work insert in the thesis was shown a lack of transmigration of the bacteria along the tree. If the bacteria cannot transmigrate, removing the diseased branch can lower the bacterial population of the phylloplane. Moreover, the choice of the cultivar plays an important role in the design of the new olive orchards, in fact, despite no-resistance was observed in no one cultivar, some cultivars like ‘Leccino’ and ‘Leccio del Corno’ are more tolerant than others like ‘Rosciola colli Esini’.

Although the olive tree is a species suitable for hot, semi-arid and sunny areas, the olive tree prefers milder temperatures to produce satisfactory quantities of fruit (in the midsummer in Mediterranean areas the plant undergoes a phase of stasis), application of water, and light shade in midsummer. In our trial, the olive covered by an anti-insect net (shadow effect by 15%) after the pit-hardening showed larger fruits, and more nodes, only when the fruit load was suitable.

Finally, we can hypothesize that in the central Italy area, the cultivation of olive needs a possibility of irrigation, that can follow the water potential of the tree to increase the efficiently water use. Stem water potential measured with the Sholander chamber can be replaced by the microtensiometer, to adjust the irrigation in all the season follow the rules of precision agriculture. Precision agriculture must also improve the administration of fertilizers, the doses of which must be rationed according with the phenological phases and fruit load. The doses of nitrogen should by decrease along the season, while the potassium doses should increase along the season to reduce the frost susceptibility. For the central Italy frost will be a problematic phenomenon to consider in the management of the orchard.

To reduce solar stress during midsummer, in addition to irrigation we can use multifunctional nets, which shade the canopy, protect from hail and harmful insects.

So, the cultivation of the olive tree does not escape the rules also used for other fruit crops, which provide for targeted and precise management, almost paradoxical for the agricultural culture created around the olive tree in central Italy.

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