AHS
Advances in Horticultural Science

Quality of cold stored lemon fruit from orchards consociated to ancient olive trees

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Key words: Agroecology, agroforestry, cold storage, consociation, lemon fruit, postharvest quality.

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Citation:
ALLEGRA M., FERLITO F., TORRISI B., TROVATO S., CICCIARELLO G., STRANO M.C., 2023 - *Quality of cold stored lemon fruit from orchards consociated to ancient olive trees.* - Adv. Hort. Sci., 37(1): 41-48.

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 31 October 2022
Accepted for publication 28 December 2022

Abstract: In the hilly area of Gioia Tauro (Calabria, Southern Italy), lemon orchards are grown in consociation with centuries-old olive trees. Lemons are partially shaded by olive canopies and the microclimate at the level of their canopies is suitable for plants growth and quality productions. Under these conditions, lemon trees are grown even without irrigation, providing, despite this limitation, a quality product. This study aimed to i) investigate the qualitative characterisation of two clonal selections of the lemon cultivar Femminello, F. Siracusano (S) and F. Zagara bianca (ZB), from the described intercropping, on irrigated (I) and non-irrigated (NI) crops; ii) assess the quality preservation during cold storage, in order to evaluate the availability of lemons for marketing in a period of shortage such as the summer season. Fruits were harvested at commercial maturity, and cold stored at 10±1°C and RH 85-90%, for 60 days. Decay incidence, physiological disorders, weight loss, and the main physical-chemical parameters were assessed at harvest (T0) and every 15 days (T15, T30, T45, T60). The absence of decay and physiological disorders was observed throughout the 60-day storage period, in both clonal selections under the two management conditions. The weight loss was greater in fruits from irrigated lemon groves of both S and ZB. S_I showed significantly lower fruits weight and higher titratable acidity than S_NI. Total soluble solids and titratable acidity were statistically lower for ZB_I than for NI fruits.

1. Introduction

Several research have highlighted that some agri-food models are not sustainable from an economic, social and environmental point of view (IPES, 2016; FAO, 2017). For many local agricultural contexts, the main perspective to survive is the design and set up of agricultural systems more at the service of the right to food (food sovereignty), enhancing territoriality and reducing ecosystems degradation (Loker and Francis, 2020; Ciaccia *et al.*, 2021). In some cases these objectives are reached by the cultural consociation, adopted among crops with the aim of fostering agro-ecological services thus providing both environmental and economic benefits (Las Casas *et al.*, 2022). This represents an agroforestry model, mixed and multifunctional by definition, that should be promoted, empowering marginalized actors and farmers by creating something different (Rossi, 2020) and feasible only by a holistic approach that embrace a long-term vision, such as agroecology (Barrios *et al.*, 2020). These models are not purely productive, as the presence of trees also provides environmental services such as soil improvement, surface runoff reduction, and conservation of biodiversity. At the same time, agroforestry systems provide aesthetic services as well (Katsoulis, 2022).

In the hilly area of Gioia Tauro (Calabria, Southern Italy), lemon orchards are planted in consociation with centuries-old olive trees over 20 meters high. The general distance between olives is wide (10x10 m) according to their large and rarely pruned canopy, ensuring that the lemons are planted both in intra- and inter-rows, in a 5x5 m design. With this regular agroforestry model, olives play a key role in terms of the agroecological service, characterizing the landscape and preserving the fragile area, preventing soil erosion, and increasing biodiversity. Moreover, thanks to its long history in the area and management, compared to the other intensive woody crops, does not require high external inputs, thus contributing to reducing environmental pollution (De Graaff and Eppink, 1999). The microclimate and the adequate lighting conditions at the level of the lemon canopy is suitable for the growth of this culture and for obtaining high quality productions. Under these conditions, lemon trees can be grown rainfeed, although irrigation may influence fruit quality at harvest and during the postharvest process (Asrey *et al.*, 2018; Tadayon and Hosseini, 2020). To the best of our knowledge no studies were carried out about the effect of the consociation of olive-lemon cultivations and on postharvest performance of lemon fruit, which quality could also be prolonged by proper cold storage (Strano *et al.*, 2022, 2023). The extension of the availability to market of high quality fruit, in a period of shortage such as the summer season, would allow to obtain the maximum economic profit for the growers. This study aimed to assess i) the physical-chemical parameters of two clonal selections of lemon cv Femminello: F. Siracusano (S) and F. Zagara bianca (ZB), with high commercial value, consociated with olive cultivations, from irrigated (I) and non-irrigated (i.e., rain-fed, NI) crops; ii) the qualitative response to the cold storage of lemons coming from the two different management techniques.

2. Materials and Methods

Study site and fruit sample

Fruits of lemon (*Citrus limon* (L.) Osbeck) cultivar Femminello, clones Siracusano (orchard 1) and Zagara bianca (orchard 2), both grafted on sour orange (*C. aurantium* L.), planted in a 5x5 m design on a loam soil, from irrigated and non-irrigated crops of the corresponding orchard, were grown in the hills of Gioia Tauro (Calabria, Italy; lat. 38,32 N; long. 15,97 E; elevation 200 m a.s.l.).

According to the USDA (2017) texture triangle, soil of the two orchards had a loam texture (orchard 1: 462 g kg^{-1} sand, 317 g kg^{-1} silt, 222 g kg^{-1} clay; orchard 2: 602 g kg^{-1} sand, 231 g kg^{-1} silt, 167 g kg^{-1} clay), and an organic matter content of 97 (orchard 1) and 88 g kg^{-1} (orchard 2). Plants of irrigated crops were fully irrigated, corresponding to 95-98% of crop demand, while non-irrigated crops had no irrigation system, relying on rainfall for water (Ferlito *et al.*, 2014). Fruits were harvested at commercial maturity in April 2022, transported to CREA laboratories (Acireale, Sicily, Italy), selected accordingly to their uniformity in size, absence of defects and alterations (lesions and/or rot symptoms) and, washed with tap water. Three replicates of 100 fruits for each sample, were randomly placed in plastic boxes, for a total of 300 fruits, then air-dried at 20°C and stored for 60 days at 10±1°C and 85-90% relative humidity (RH).

Physical-chemical changes

Fruits were evaluated at harvest (T0), for the following parameters: weight, rind thickness, carpel axis, peel and pulp color, firmness, juice yield, total soluble solids (TSS), pH, titratable acidity (TA), maturity index (TSS/TA) and, after 15 (T15), 30 (T30), 45 (T45) and 60 (T60) days of storage, also for weight loss, decay incidence and severity of physiological disorders (chilling injury and aging).

Fruit weight loss, expressed as percentage, was evaluated on 30 fruits per sample (10 fruits/replicate) by weighting the same fruit at T0 and at each control. The results were calculated as follows: = [(m0-m1) / m0] × 100; where: m0 = the initial weight; m1 = the weight measured at each control.

The juice was extracted using an electric citrus fruit squeezer and the pooled juice of five fruits per replicate was analyzed. The juice yield was calculated as follows: juice yield (%) = (juice weight/fruit weight) x 100. TSS content was determined with a digital refractometer (ATAGO RX-5000, Atago, Japan) and results expressed as °Brix. Titratable acidity (TA), expressed as % (w/v) of citric acid equivalent, was determined by potentiometric titration (T50 Automatic Titrator, Mettler Toledo) with 0.1 N sodium hydroxide solution (AOAC, 1995; Ladaniya, 2008). Vitamin C (ascorbic acid, mg·100 mL^{-1}) concentration was determined by liquid chromatography (Rapisarda and Intelisano, 1996). The technological index 'TI' was calculated according to Kluge *et al.* (2003) by the equation:

TI = (Total soluble solids x Juice percentage) / 100.

TI is an important variable linked to the quality of the juices destined for the citrus industry. Higher TI values correspond to better quality (Chitarra and Chitarra, 1990).

Decay incidence (%) was determined by a visual evaluation of fruit infected by rots. The severity of chilling injury was determined by the visual examination of the fruit pericarp, using a four-grade scoring system to estimate the damage of the rind surface: 0 = none, 1 = less than 5% (light), 2 = 5% to 25% (moderate), and 3 = over 25% (severe). The incidence of the fruits affected by aging (%) was determined by the presence of fruits with a necrotic area of rind tissue around the stem-end button.

Firmness measurement

Firmness was tested by a texture analyser (Zwick/Roell DO-FB0.5 TS model 2002, Genoa, Italy) using an 8 mm flat probe (Mitcham *et al.*, 1996). Two measurements were made on two opposite of the equatorial zones of 15 fruits per sample. The results were reported as the peak force in Newton (N) (Nasrin *et al.*, 2020).

Colour evaluation

Fifteen fruits for each sample were taken, at each time interval, for the determination of peel and pulp colour by CIELAB coordinates *L** (lightness), *a** (red-green component) and *b** (yellow-blue component) using a Minolta Spectrophotometer CM-2500d (Minolta, Milan, Italy). Three color measurements were made for each sample fruit and the results were expressed as Citrus Colour Index (CCI) = (1000 × *a**)/(*L** × *b**) (Jiménez-Cuesta *et al.*, 1981).

The effect of cold storage at 10°C on the color of lemon fruits was also evaluated, at each time interval, through the Chroma (*C**), which defines the color intensity (higher *C** values indicate brighter yellow color); the Hue angle (*h*°), with values closer to 90° for the more yellow fruits and increasing for the greener fruits; and Browning index (BI), both for peel and pulp (Kluge *et al.,* 2003). The browning index (BI) was used as an indicator of the intensity of brown discoloration. BI was calculated as follows (Palou *et al.*, 1999; Olivas *et al.*, 2007):

$$BI = [100(x-0.31)] / 0.172$$

where: $x = [(a^* + 1.75)] / [5.646 L + a^* - 3.012 b^*]$.

Statistical analysis

One-way and factorial analysis of variance (ANOVA) was performed using STATISTICA 6.0 software (Stat Soft Italia srl) according to a completely randomized experimental design. Data are means of three independent determinations. Means comparisons were performed by Tukey's HSD test at $p \leq 0.05$, 0.01, 0.001, based on the F-test significance.

3. Results

Physical-chemical changes

Observation of the fruits revealed the absence of decay and physiological disorders in both clonal selections under the two management conditions (I and NI) throughout the 60-day storage period (data not showed).

As shown in figure 1, the weight loss was significantly greater in fruits from irrigated lemon groves of both clonal selections, with average values of 23.7%

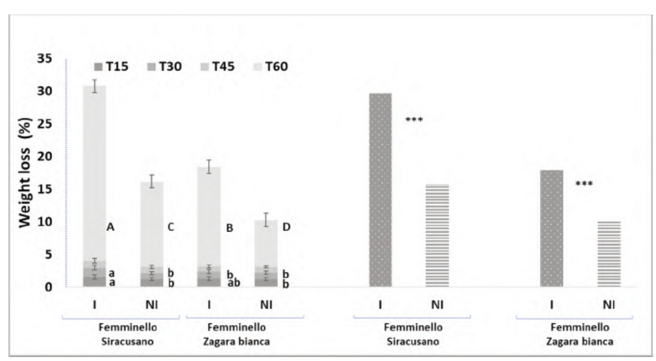

Fig. 1 - Weight loss percentage of 30 lemon fruits from each clonal selection (Femminello Siracusano and F. Zagara bianca) and irrigation management (I: Irrigated; NI: Non-irrigated) monitored during cold storage at 10±1°C and RH 85-90%. Textured bars represent total weight loss from T0 to T60 (p≤0.001 ***). Means of the same time interval indicated by different letters are significantly different (lowercase p≤0.05, uppercase p≤0.001) based on Tukey's HSD test. Error bars show the standard deviation.

(I) *vs* 12.9% (NI), in the interval from T45 to 60 days of cold storage, although it remained 3.6% (I) and 3% (NI) up to T45.

The response to irrigation management resulted clonal selection-specific. The response of each clonal selection over time is reported. The width of the carpellar axis did not reveal significant differences in the comparison between the irrigation managements of each clonal selection, except at T15 in the case of S, and at T30 in the case of ZB, in favor of NI (Fig. 2).

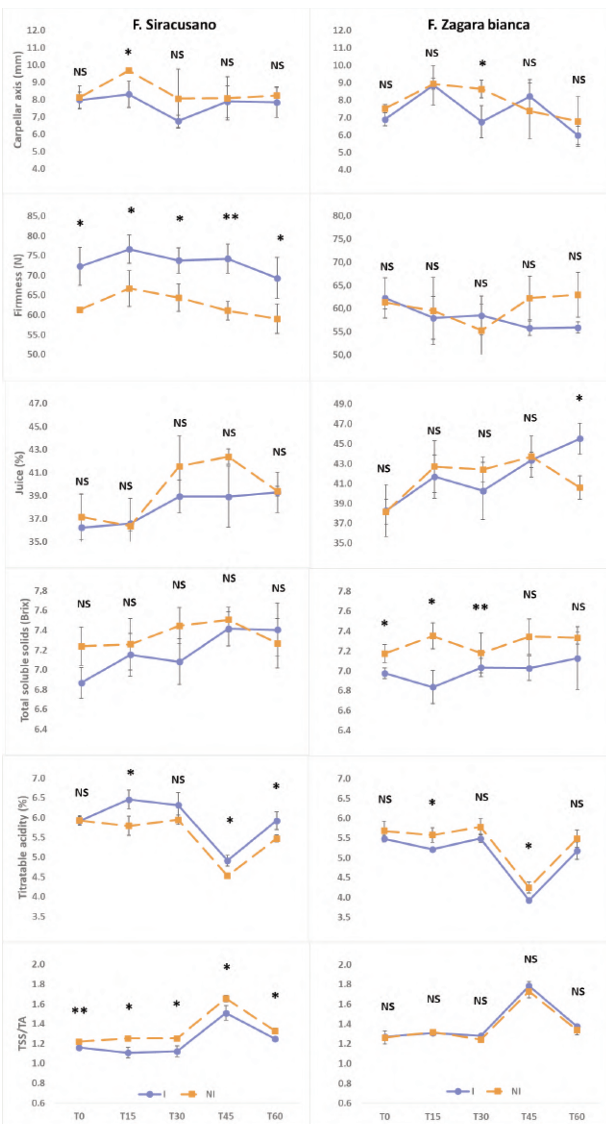

Fig. 2 - Response of the physicochemical parameters (carpellar axis, mm; firmness, N; juice, %; total soluble solids, TSS °Brix; titratable acidity, TA %; TSS/TA ratio) monitored during cold storage at 10±1°C, RH 85-90%, of lemon fruits from two clonal selections (Femminello Siracusano and F. Zagara bianca) to irrigation management (I= Irrigated; NI= Non-irrigated). Means of the same time interval indicated by different letters are significantly different (lowercase p≤0.05*, uppercase p≤0.01** and p≤0.001***; NS, no significant differences) based on Tukey's HSD test. Error bars show the standard deviation.

No significant difference between managements in the percentage of juice and TSS for S. Only at T60, the juice yield of ZB_I was significantly higher than ZB_NI, while TSS already differed at T0 up to T30 in favor of ZB_NI (Fig. 2). The titratable acidity had a different behavior for the two clonal selections, with I statistically higher for S at T15, T45 and T60, and NI for ZB at T15 and T45 (Fig. 2). TSS/TA was statistically higher for S_NI, compared to S_I, while no difference was recorded for ZB (Fig. 2).

Peel percentage and peel to pulp ratio did not show significant differences. Only at the end of the storage (T60) ZB_NI showed significantly higher values (Fig. 3).

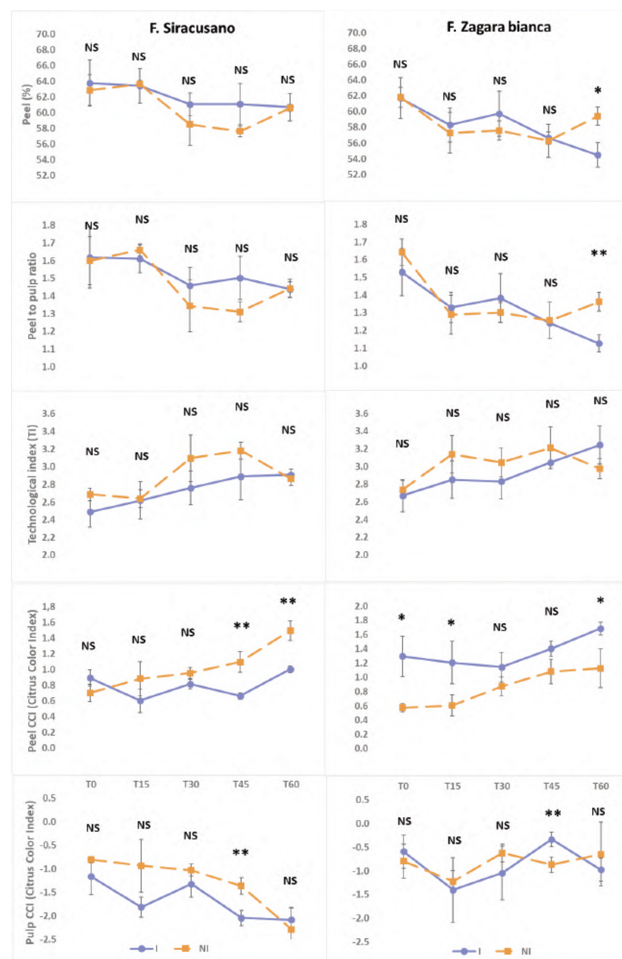

Fig. 3 - Response of the physicochemical parameters (peel, % w/w; peel to pulp ratio; Technological index, TI; peel and pulp Citrus Color Index, CCI) monitored during cold storage at 10±1°C, RH 85-90%, of lemon fruits from two clonal selections (Femminello Siracusano and F. Zagara bianca) to irrigation management (I= Irrigated; NI= Non-irrigated). Means of the same time interval indicated by different letters are significantly different (lowercase p≤0.05*, uppercase p≤0.01** and p≤0.001***; NS, no significant differences) based on Tukey's HSD test. Error bars show the standard deviation.

Vitamin C values were significantly higher for S, while no significant differences emerged between I and NI management (Fig. 4).

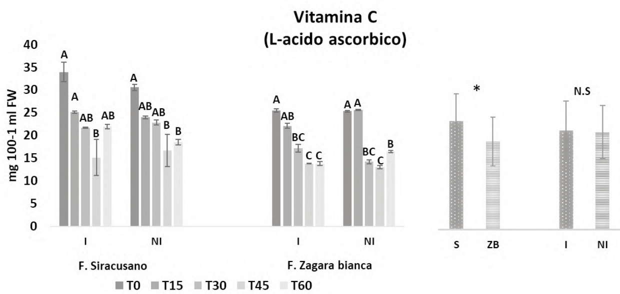

Fig. 4 - Variation of the vitamin C content during cold storage at 10±1°C, RH 85-90%, of lemon fruits from two clonal selections (Femminello Siracusano 'S' and F. Zagara bianca 'ZB') and two irrigation management (I= Irrigated; NI= Non-irrigated). Means observed at each time interval relative to each irrigation management of the clonal selections indicated by different letters are significantly different (p≤0.001) based on Tukey's HSD test. Textured bars represent the results of the factorial ANOVA (p≤0.05*; NS, no significant differences). Error bars show the standard deviation.

Firmness measurement

No significant difference during storage regarding firmness for ZB, while S showed higher values for I as early as T0 (Fig. 2).

Colour evaluation

While the color parameters and indices relating to the pulp very rarely showed significant differences between irrigation managements I and NI (Fig. 5), those relating to the peel show in some cases significant, and often antithetical, differences between the two clonal selections. This is the case of CCI, Chroma and BI, for which S_NI had statistically higher values than S_I, while the opposite occurred for ZB. The peel *h*° had a specular behavior with respect to what was described for the other indices (Fig. 5).

Differences at harvest (T0) occurred in the case of S, for firmness (Fig. 2), peel CCI (Fig. 3) and peel BI (Fig. 5) with higher values for I, which was probably later in ripening, as the lower TSS/TA value seems to demonstrate (Fig. 2).

Zagara bianca showed differences already at harvest (T0) in TSS (Fig. 2) and peel *h*° (Fig. 5), higher in NI, while peel CCI (Fig. 3), C* and BI (Fig. 5) were higher in I.

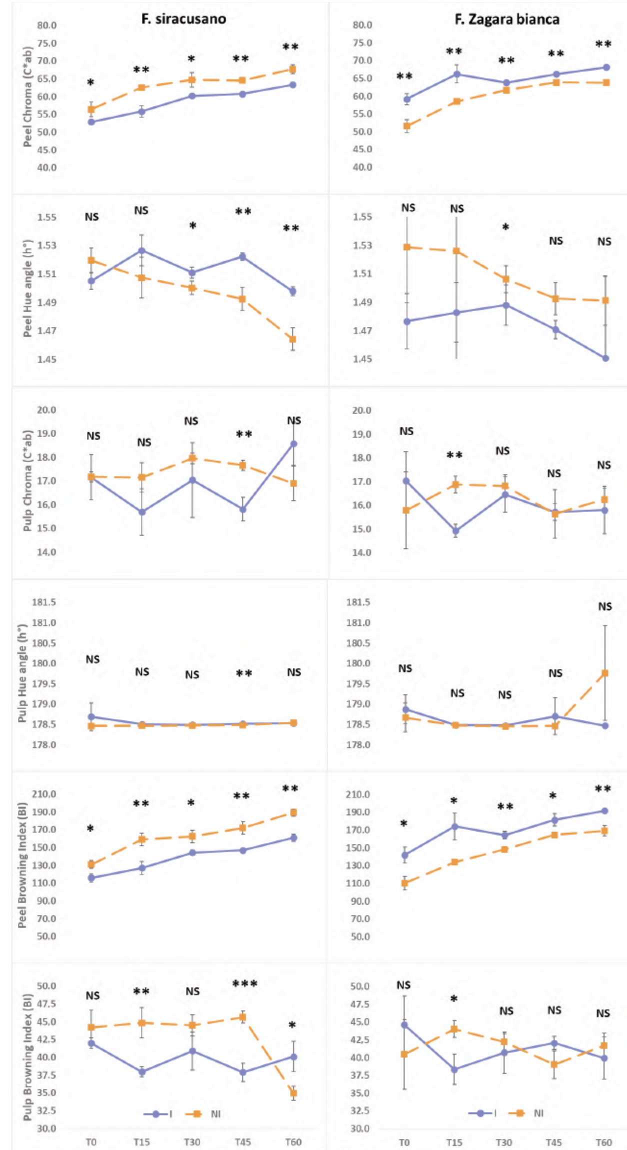

Fig. 5 - Response of the colour parameters of fruit peel and pulp (Chroma, C*ab; Hue angle, h°; Browning index, BI) monitored during cold storage at 10±1°C, RH 85-90%, of lemon fruits from two clonal selections (Femminello Siracusano and F. Zagara bianca) to irrigation management (I= Irrigated; NI= Non-irrigated). Means of the same time interval indicated by different letters are significantly different (lowercase p≤0.05*, uppercase p≤0.01** and p≤0.001***; NS, no significant differences) based on Tukey's HSD test. Error bars show the standard deviation.

4. Discussion and Conclusions

The modern woody crops systems are generally characterized for a strong modification on agroecosystem structure and functioning. In a climate change scenario also a modification of the related agronomical processes such as the water and mineral availability, the solar radiation and the spontaneous flora growth could be affected (Ciaccia *et al.*, 2019). In the present work the response of the particular consociation between the lemon and the old-century olives" in Calabria was investigated, assuming that it could allow to lengthen the lemon quality traits and shelf life throughout the response to long periods of cold storage (Fung *et al.*, 2019). Furthermore, the response of irrigated and non-irrigated lemon groves was compared.

Understanding these linkages, the obtained data can predict how the effect of a single agronomic practice at the micro and macro-area scales can reduce the water input for irrigation. Moreover, the lemon storage could drastically reduce the lengthen of the irrigation season. Moreover, from the economical point of view, the proposed study can be an evaluable way for the marketing of fresh lemons. In fact, as observed by Ciriminna *et al.* (2020), the fresh lemons have a constant demand from consumers which in some seasons cannot be entirely satisfied by Italian production. Finally, the consociated model could be diversified also adopting other species that are well adapted to shade such as berries (Cicala *et al.*, 2002).

The microclimatic effects induced by the presence of the olive trees are reflected on the preservation of the fruit quality, on the protection from natural adversities such as excessive insolation or hail, and seems to result in a postponement of ripening, particularly in non-irrigated lemon groves (Brunori *et al.*, 2019).

The results of this study seem to support the hypothesis of the effectiveness of the shading effect and of the microclimate induced by the presence (cover, protection) of the olive trees, on the quality of the production of both clonal selections studied, and on the sustainability of non-irrigated crops, given the comparability of many of the qualitative parameters with those of irrigated crops. Therefore, despite the considered period is not too long to draw general conclusions the preliminary results of the research show that the Agroecological system and the use of the non-economical valuable olive as an Agro-ecological service crop could realize a mutual relationship into the agroecosystem.

Storage at 10°C avoided the development of decay and physiological disorders regardless of clonal selection and irrigation management.

Management strategies that increase the productivity of existing agricultural land are increasingly needed (Salmon *et al.*, 2015). The diffusion of the consociation here studied could allow the exploitation of large tracts of land that host centuries-old olive groves, which must be preserved for their environmental and cultural importance, but no longer usable at a production level, given the very high management and harvest costs (Brunori *et al.*, 2019). Additionally, the possibility of eliminating or reducing the water supply makes this intercropping particularly interesting.

Increased agricultural productivity is generally achieved by increasing inputs. Irrigation, which currently accounts for about 70% of global freshwater withdrawals, is one of the most important and widespread means of achieving this goal (Lobell *et al.*, 2009). Rain-fed croplands, also called dryland farming, include all cropland where no water from any storage or delivery mechanism is utilized, but crops are not flooded, and where harvesting occurs at least once per year (Salmon *et al.*, 2015).

As a result of the present work, the comparable quality of the fruits at harvest, both with and without irrigation, and their qualitative maintenance with cold storage would allow to have a good product on the market even in periods of shortage.

The comparison with lemon groves without olive trees will be investigated in order to estimate any qualitative improvement induced by the two crops consociation.

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