Frost tolerance of 24 olive cultivars and subsequent vegetative re-sprouting as indication of recovery ability

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A R T I C L E I N F O

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A B S T R A C T

The objective of the present study was to assess the frost tolerance of 24 olive cultivars after a freezing event that occurred in February 2012 in Marche Region (Central Italy), and their recovery ability during the following growing seasons (2012 and 2013). The studied cultivars were locally, nationally and internationally spread. Trees were three-year-old and in the rest phase at the time of the freezing event. Frost tolerance was determined by two damage visual scoring: defoliation and bark split, both defined three months after the event. During the following growing seasons, the recovery ability of the cultivars was also assessed throughout a third visual index describing the vegetative re-sprouting. Results indicated differences in frost tolerance and recovery ability among the studied cultivars. In particular, 'Arbequina' recorded the highest canopy defoliation together with 'FS17', 'Raggia' and 'Sargano di San Benedetto', whereas 'FS17', showed the highest level of bark split on primary branches and trunk. This cultivar also registered a strong vegetative re-sprouting, mainly from the basal portion of the trunk. On the contrary, 'Ascolana dura' and 'Orbetana' resulted the most frost tolerant cultivars and showed the best recovery ability in 2012 and 2013, with a re-sprouting activity from the 1- and 2-year-old shoots. The results suggest low frost tolerance for the tested varieties and supply helpful information for the selection of the most suitable ones for the set of new olive orchards in cold climates.

1. Introduction

Olive is an evergreen species that lacks a true dormancy and its cultivation is greatly impeded by the inability to survive temperatures below −12 ˚C, with severe leaf damage already occurring at −7 ˚C (Larcher, 2000). Fontanazza (1986) individuated a −10 ˚C temperature threshold for frost damage induction in olive, while Barranco et al. (2005) indicated −8,6 ˚C as a lethal freezing temperature for sensitive cultivars tested in January. Ruiz et al. (2006) reported greater anatomical evidence of frost damages on 1-year-old shoots compared to bark of trunk or major branches, in olive trees exposed to cold, with freezing temperatures ranging from −6 to 0 ˚C.

Frost symptoms on the tree can range from shoot tip burns and defoliation up to bark split on branches or trunk in the case of intense injury (Gucci and Cantini, 2001). Such symptoms are frequently-used indices to evaluate the level of frost damage as indicated by Jacoboni (1985), Pezzarossa (1985), Tombesi (1986), Bini et al. (1987), Denney et al. (1993), Rotondi and Magli (1998).

Sanzani et al. (2012) reported a destructive frost on olive every 10–40 years in Italy and Gucci et al. (2003) reported that in 1907, 1929, 1956 and 1985 very low winter temperatures induced intense frost injuries to olive trees during the XX Century in Central Italy. The period of the year when freezing temperatures occur might influence plant tolerance: cold resistance in evergreen species is closely correlated with the hardening process which depends on cold acclimation in terms of exposure to a period of low but non-freezing temperatures that increases the ability to withstand the subsequent freezing temperatures (Palliotti and Bongi 1996; Travert et al., 1997; Mancuso, 2000).

Cansev et al. (2009) demonstrated that the pattern of exposure to frost could affect olive tree susceptibility since a programmed acclimation to cold produced an increase in frost tolerance in several cultivars. Nevertheless, de-acclimation process occurs in response to substantial increases in temperature, usually progresses more rapidly that acclimation, and is associated with wide-ranging structural and functional changes associated with resumption of vegetative growth. Although typically occurring in the spring, de-acclimation may occur prematurely during winter in response to transient warm spells (Kalberer et al., 2006). In woody plants of mild climates including olive, cold hardiness can be rapidly lost after a short exposure to warmer temperatures (Kozlowski and Pallardy, 1997), also depending from the increase of photosynthetic activity and total soluble sugar (TSS) accumulation (Gulen et al., 2009), thus making plants particularly vulnerable in late winter when freezing event might take place after periods of relatively warm temperatures.

Several methods have been proposed for selecting frost tolerant genotypes in olive species, such as stomatal density (Roselli et al., 1989), photosynthetic activity (Antognozzi et al., 1990), stomatal size (Roselli and Venora, 1990), release of phenolic compounds (Roselli et al., 1992), differential thermal analysis (Fiorino and Mancuso, 2000), ionic leakage (La Porta et al., 1994) and electrochemical techniques combined with leaf fractal analysis (Azzarello et al., 2009).

The conclusions of the numerous analytic methods where generally confirmed by visual observations (Bartolozzi and Fontanazza, 1999). Furthermore, small portions of the plants do not always reflect the frost tolerance of the whole plant and the response of isolated tissues and cells to low temperature may differ in some respects with that known for the whole plant system, thus supporting the power of a whole plant approach to the analysis.

Fiorino and Mancuso (2000) studied the freezing temperatures of different organs in four Italian cultivars (‘Ascolana tenera’, ‘Frantoio’, ‘Leccino’ and ‘Coratina’) acclimated to cold and reported ‘Ascolana tenera’ and ‘Coratina’ to be the most and the least frost tolerant, respectively.

Alfei et al. (1999) studied the frost tolerance of 3-year-old trees of Italian local and nationally spread olive cultivars after a natural freezing event, and reported that ‘Piantone di Mogliano’ and ‘Leccino’ showed a high frost tolerance (low level of damage), while ‘Canino’ and ‘Rosciola’ resulted highly susceptible. Lodolini et al. (2014) did not confirm the high frost tolerance of ‘Piantone di Mogliano’ showing strong damages and no significant differences when compared to ‘Oliva Grossa’, ‘Sargano di San Benedetto’, ‘Sarganella’, ‘Piantone di Falerone’, ‘Ascolana tenera’ and ‘Carboncella’.

Several studies successfully discriminated between frost-tolerant and frost-susceptible olive cultivars, though they have focused on a limited number of genotypes. In particular, local varieties of Center-North Italy, potentially greatly adapted to cold condition, had rarely been tested.

The use of the more tolerant olive cultivars together with the understanding of the mechanism of frost hardiness could represent the most effective methods to avoid frost damages. The objective of the present study was to evaluate 24 olive cultivars (20 of them locally spread) for the level of frost damage and recovery ability in terms of vegetative re-sprouting after a freezing event occurred in February 2012 in Marche Region (Central Italy).

2. Materials and methods

2.1. Study site

The study was carried out in a three-year-old experimental olive orchard (1.5 ha) located in Maiolati Spontini (latitude, 43 °28′37″N; longitude, 13 °07′09″E; altitude 405 m a.s.l.) in Central Italy with a planting density of 416 trees ha^{-1} (i.e. 6.0 m × 4.0 m arrays). Trees were trained as the central leader free canopy system and were regularly fertilized during spring and rain-fed from planting. The trees were coetaneous and homogeneous at October 2011 in terms

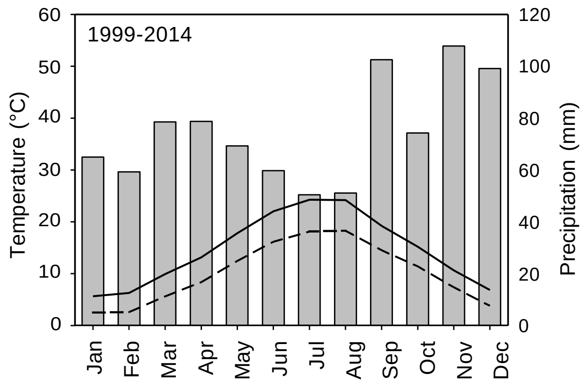

Fig. 1. Monthly average (solid line) and minimum (dotted line) air temperature and rainfall (columns) over a period of fifteen years (1999–2014) climatic records for the studied area (source: Centro Agrometeo ASSAM).

of trunk cross sectional area at 0.25 m height from the ground (2.5 ± 0.05 cm^2) and total tree height (1.8 ±0.01 m). The orchard was arranged with one cultivar per row and each row was about 100 m long. Twenty-four cultivars (‘Ascolana dura’, ‘Ascolana tenera’, ‘Capolga’, ‘Carboncella’, ‘Cornetta’, ‘Coroncina’, ‘Lea’, ‘Mignola’, ‘Mignolone’, ‘Nebbia del Menocchia’, ‘Oliva Grossa’, ‘Orbetana’, ‘Piantone di Falerone’, ‘Piantone di Mogliano’, ‘Raggia’, ‘Raggiola’, ‘Rosciola Colli Esini’, ‘Sarganella’, ‘Sargano di Fermo’ and ‘Sargano di San Benedetto’ locally spread in Marche Region; ‘FS17’, ‘Don Carlo’ and ‘Giulia’, nationally distributed; and ‘Arbequina’ internationally spread) were assessed for their frost tolerance and recovery ability after the freezing event occurred in February 2012. At the time of the event, all the trees were in the rest phase, without any noticeable apical growth. Chilling units during the winter reached the 950 h, which in olive is barely enough to induce acclimation against cold (Bartolozzi and Fontanazza, 1999). The monthly precipitations and the average air temperatures of the studied area are reported in Fig. 1 according to climatic records referring to a period of fifteen years (1999–2014) (source: Centro Agrometeo ASSAM). The winter 2011–2012 showed ten days (from January 7th to 17th) with mild minimum temperatures (mean value of the decade of 6.5 °C), thus significantly higher than the historical monthly average minimum temperature of 2.6 °C in January. The relatively mild period was followed by a 12-days freezing event with a mean minimum temperature of 4.7 °C in the period included between February the 3rd and the 15th and an absolute minimum temperature of - 6.4 °C on the 15th. Abundant snow events (cumulated precipitation of 200 mm) and strong and cold wind (wind gust speed up to 43 km/h) from northeast affected the considered area for the whole mentioned period.

2.2. Visual indicators

Three months after the freezing event, 20 trees per cultivar were selected in the central portion of each row and assessed throughout damage visual scoring.

Two visual indexes (Fig. 2) were used to evaluate the level of frost damage in terms of canopy defoliation ranging from 0 to 3 (0: no leaf drop, 1: <50%, 2: >50% and 3: totally defoliated) and bark split ranging from 0 to 4 (0: none, 1: only on 1-year-old shoots, 2: extended to 2- and 3-years-old branches, 3: extended to primary branches and 4: extended to the trunk) adapting the methodology reported by Ruiz Baena et al. (2007) and Alfei et al. (1999). After five months from the freezing event, the recovery ability of the studied cultivars in terms of vegetative re-sprouting was described on the same trees using a visual index ranging from 0 to 3 (0: only in the apical portion of the canopy, 1: in the 1- and 2-year-old shoots in

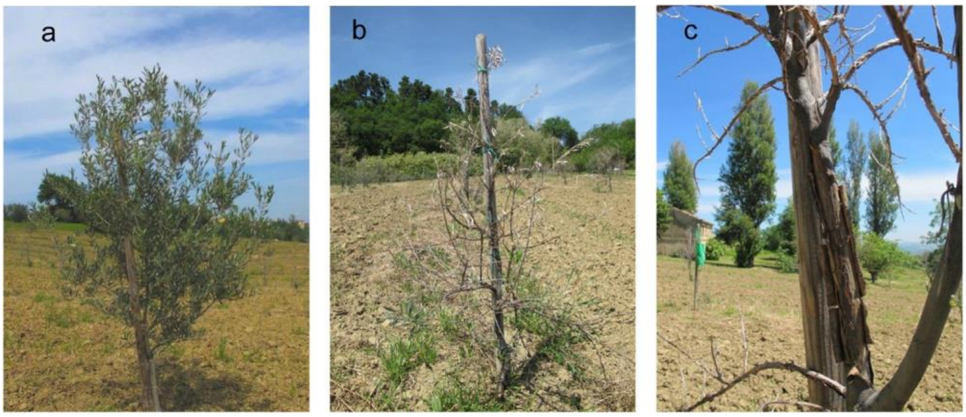

Fig. 2. Visual indexes used to evaluate the level of frost damage. In particular a) no leaf drop and no bark split (score 0 for both parameters), b) totally defoliated (score 3) and c) bark split extended to the trunk (score 4).

the internal part of the canopy, 2: in the trunk and in the primary branches and 3: only in the basal portion of the trunk). The same evaluation of re-sprouting pattern was performed on the same trees in the following growing season, on April the 30th, 2013.

2.3. Statistical analysis

Data referring to frost damage and consequent vegetative re-sprouting resulted as non-normally distributed and were subjected to the non-parametric test of Kruskal-Wallis for the comparison of the medians (Rank sums). Scores frequencies for each variety were compared throughout a contingency analysis according to the Likelihood ratio test. Differences were considered significant with a $p < 0.002$. The p value for significance was set by applying a Bonferroni correction to the initial 0.05 due to the large number of cultivars tested in the trial (Bonferroni, 1936; Shaffer, 1995). A principal component analysis (PCA) using a correlation matrix was carried out to visually discriminate cultivars response to frost. Finally, a K-mean iterative clustering test was performed using all data referring to frost damage and vegetative re-sprouting, and used to overlay confidence ellipses on the PCA score plots. The correlations between frost damage and vegetative re-sprouting were determined according to the Pearson Product Moment Correlation analysis. A stepwise regression analysis with forward direction and probability to enter = 0.100 was proposed for vegetative re-sprouting prevision based on the two damage indicators and the degree of cultivar frost tolerance as dummy variable. The factors selected according to the stepwise screening were tested by a standard least square analysis ($p = 0.05$). All statistical analyses were performed using JMP 10 (SAS Institute Inc., Cary, NC).

3. Results

Olive cultivar medians significantly differed either in terms of defoliation ($y^2 = 297.3$ and $p < 0.0001$ according to Kruskal-Wallis test) and bark split ($y^2 = 425.3$ and $p < 0.0001$) (Fig. 3a,b). 'Ascolana dura' and 'Orbetana' showed the lowest canopy defoliation (95% and 70% of the scores≤1.0, respectively) and bark split (94% and 80% of the scores≤1.0, respectively) and resulted the most tolerant among the tested cultivars. Defoliation resulted intermediate for 'Mignolone' (65% of the scores 1 ≤ and ≤ 2) and intense for the remaining 21 tested cultivars (over 80% of the scores ≥ 2.0). Frost induced the highest degree of defoliation in 'Arbequina', 'FS17′', 'Raggia' and 'Sargano di San Benedetto' (100% = 3.0). When frost damages were described in terms of bark split, 'Mignolone', 'Coroncina', 'Mignola' and 'Rosciola Colli Esini' recorded the lower values (over 85% ≤ 2), while intermediate values (over 80% ≥2 and ≤3)

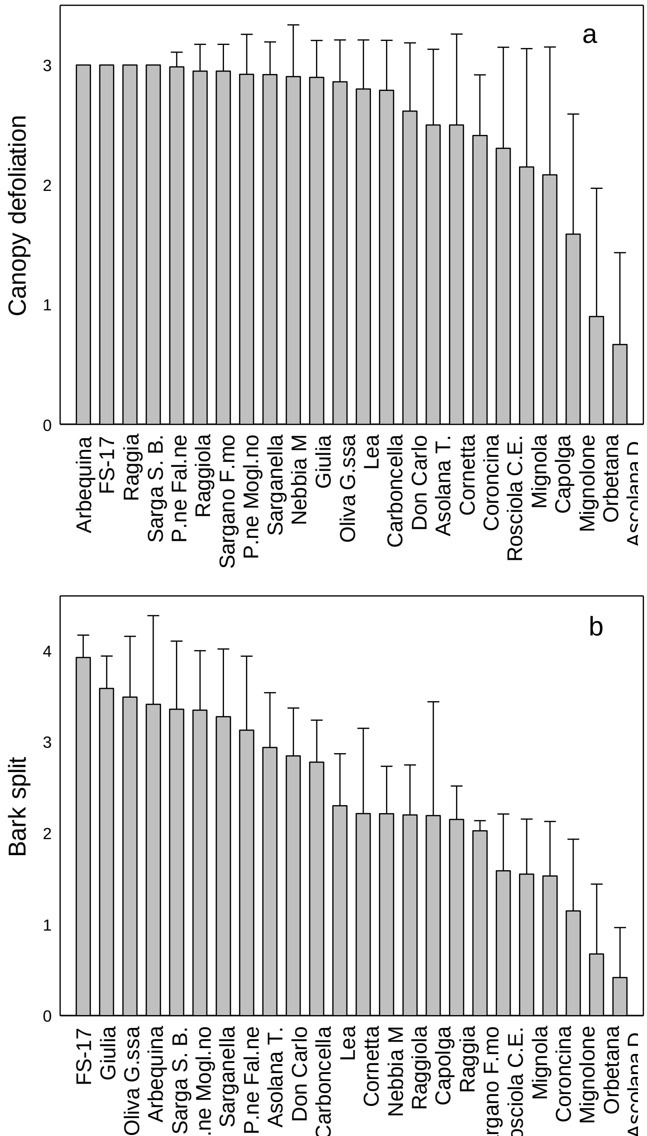

Fig. 3. Level of frost damage expressed in terms of canopy defoliation (a) and bark split (b) visual scores. Data are reported as mean ± standard deviation of 20 replicates (Kruskal-Wallis test $p < 0.0001$).

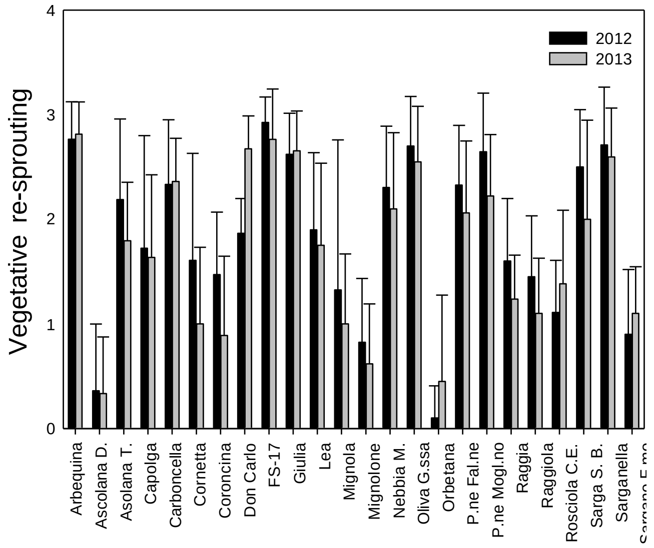

Fig. 4. Level of vegetative re-sprouting recorded after five months from the freezing event in 2012 (black column) and in the following growing season in 2013 (grey column). Data are reported as mean ± standard deviation of 20 replicates (Kruskal-Wallis test p < 0.0001).

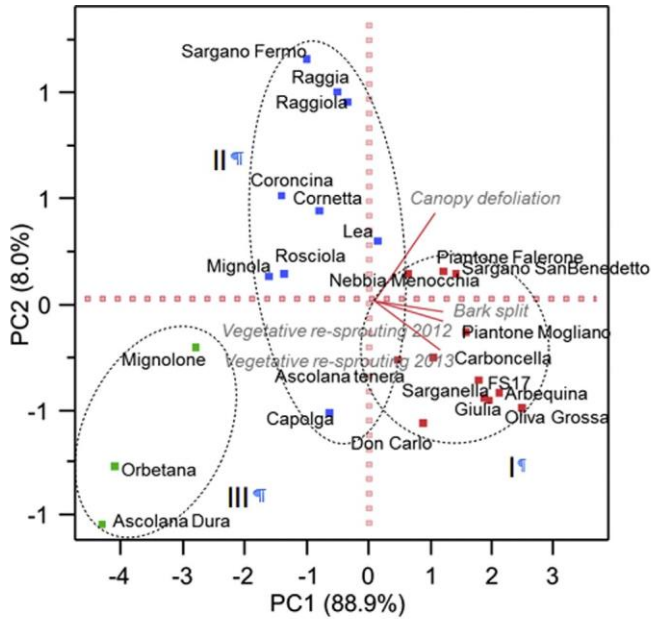

Fig. 5. Loading (a) and score (b) plots of principal components analysis (PCA) based on frost damage and vegetative re-sprouting of the tested olive cultivars (for interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).
Clusters I (red), II (blue) and III (green) indicate less, moderately and more tolerant cultivars, respectively and are showed by dotted ellipses according to the iterative K-mean clustering.

were registered for 'Sargano di Fermo', 'Raggia', 'Raggiola', 'Nebbia del Menocchia', 'Lea', 'Carboncella' and 'Don Carlo'. Seven cultivars registered high values for this parameter, with 'FS17′ resulting as the less tolerant with a level of bark split 100% ≥ 3.

When the vegetative re-sprouting during the same season following the frost damage was evaluated in 2012 (Fig. 4a,b), results indicated differences among cultivars (y^2 = 393.8 and p < 0.0001 according to Kruskal-Wallis test). Namely the highest values were recorded for 'FS17′, 'Giulia', 'Sargano di San Benedetto' and 'Arbequina' (100% ≥ 2). Strong vegetative re-sprouting (over 90% of the scores ≥ 2) starting from the basal portion of the trunk was registered also for, 'Sarganella', Oliva Grossa', Piantone di Mogliano' and 'Piantone di Falerone'. Intermediate values (95% ≤ 1 and ≤ 2) were recorded for 'Lea', 'Don Carlo', 'Carboncella', 'Raggia', 'Coroncina', and 'Raggiola', 'Nebbia del Menocchia' 'Ascolana tenera'. 'Sargano di Fermo', 'Mignolone', 'Asconala dura' and 'Orbetana' showed the lowest vegetative re-sprouting concentrated mainly in the 1- and 2-year-old shoots (85% ≤ 1.0).

During the following season (2013), differences in vegetative re-sprouting were still significant among cultivars (y^2 = 381.5 and p < 0.0001 according to Kruskal-Wallis test). Results indicated the highest visual index values for 'Arbequina' and 'FS17′ (68% = 3) and 90% of the values ≥ 2 for 'Don Carlo', 'Giulia', 'Sarganella', 'Oliva Grossa', 'Carboncella' and 'Nebbia del Menocchia'. Vegetative re-sprouting values were at 75% ≥ 1 and ≤ 2 for 'Ascolana tenera', 'Lea', 'Rosciola Colli Esini', 'Raggia', 'Raggiola' and 'Sargano di Fermo' and were 85% ≤ 1.0 for 'Coroncina', 'Mignolone', 'Orbetana' and 'Ascolana dura' (Fig. 4b). Differences highlighted above for all the parameters resulted significant with a p < 0.001 in all cases.

When a K-mean iterative clustering test was carried out, it was possible to individuate three different clusters for cultivars tolerance to frost, visualized throughout a principal component analysis (PCA) in Fig. 5. Three cultivars, namely 'Ascolana dura', 'Orbetana' and 'Mignolone' (cluster III) resulted more tolerant to frost. Nine cultivars ('Sargano di Fermo', 'Raggia', 'Raggiola', 'Cornetta', 'Coroncina', 'Rosciola Colli Esini', 'Mignola' and 'Capolga') were included in cluster II showing an intermediate frost tolerance. The other 12 cultivars resulted included in cluster I characterized by lower tolerance to frost. In this last group, international ('Arbequina')

Table 1
Factors predicting vegetative re-sprouting in 2012 and 2013, p value and estimate calculated by nominal logistic regression on factors selected according to the stepwise analysis.

	Re-sprouting 2012		Re-sprouting 2013	
	Est.	Prob>|t|	Est.	Prob>|t|
Sensitivity[Very–Less sensitive]	−0.5	<0.0001	−0.7	<0.0001
Defoliation	0.1	0.0015	0.1	0.0092
Bark split	0.6	<0.0001	0.4	<0.0001

and national spread ('FS17′, 'Don Carlo' and 'Giulia') cultivars were included.

The stepwise analysis individuated the level of defoliation and even further the level of bark split as good predictors of vegetative re-sprouting the following year (Table 1)

Correlations between damage score and vegetative re-sprouting were in all cases significant according to Pearson Product Moment analysis (p < 0.0001) (Fig. 6 a, b, c and d) but linear regression was stronger for vegetative re-sprouting with bark split (r^2 = 0.88 in 2012 and r^2 = 0.82 in 2013) than with canopy defoliation (r^2 = 0.63 in 2012 and r^2 = 0.350 in 2013). Thus indicating that the position of vegetative re-sprouting in the canopy was determined by the damage intensity and the age of the injured portion. Correlation with vegetative re-sprouting was consistently stronger in 2012 than in 2013, either for bark split and defoliation.

4. Discussion

The freezing event in our study reached the temperature of −6.4 °C. Although Barranco et al. (2005) reported − 8.6 °C threshold as a dangerous temperature for injuries only for less tolerant olive cultivars, damages at cell level had been recorded by Ruiz et al. (2006) after the exposure to temperatures ranging from 0 to-6 °C. Furthermore, the freezing event took place in mid-February after

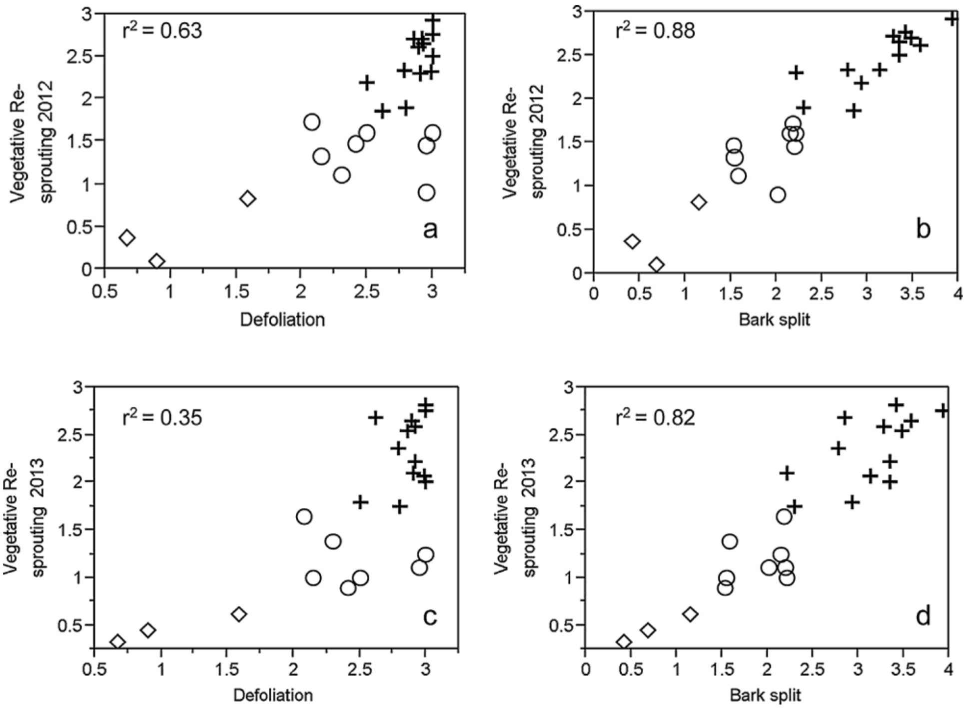

Fig. 6. Correlation between canopy defoliation and vegetative re-sprouting in 2012 (a) and 2013 (c), and bark split and vegetative re-sprouting in 2012 (b) and 2013 (d). Different symbols indicate cultivars classified as less (+), moderately (◯) and more tolerant (◆). R^2 values were calculated according to the Pearson Product Moment analysis.

experiencing a relatively warm decade (average minimum temperature of 6.5 °C). Such temperatures might have induced a quick loss of cold hardiness in olive trees (Kozlowski and Pallardy, 1997) and might explain the generally elevated degree of damage recorded for several tested cultivars.

All assessed cultivars showed low tolerance to frost (over 80% of the scores ≥ 2.0) in terms of canopy defoliation with the only exception of 'Ascolana dura', 'Orbetana' and 'Mignolone', whereas a wider distribution was seen in terms of bark split: seven cultivars ≥ 3.0, 8 with values largely included between 2.0 and 3.0, four between 1.0 and 2.0 and two cultivars, 'Ascolana dura' and 'Orbetana' with over 80% of the scores ≤ 1.0.

Considering the damage level, 'Ascolana dura' resulted the most tolerant cultivar to frost, whereas 'FS17′ among the most susceptible.

Results showed in this study did not confirm the low frost susceptibility indicated for 'Ascolana' by Fiorino and Mancuso (2000) and confirmed a moderate cold-hardiness as reported by Cansev et al. (2009), even though for both experiments the full name of the tested variety ('dura' or 'tenera') was not indicated, thus making uncertain the comparison, due to the extremely different frost tolerance of the two cultivars reported by our study.

'Ascolana dura' and even greatly 'Orbetana' showed the lower tendency to vegetative re-sprouting after the freezing event (2012) and in the following year (2013) with the maintenance of the canopy and the re-sprouting concentrated exclusively in the 1- and 2-year-old shoots.

Among the previously studied cultivars with local diffusion, 'Piantone di Mogliano' and 'Mignola' showed a relatively low and high frost tolerance, respectively. Such results are in contrast for 'Piantone di Mogliano' and in line for 'Mignola' with those reported by Alfei et al. (1999), where visual indices for defoliation and bark split on shoot, branches and trunk were used on ten cultivars to define the level of frost tolerance in three-year-old olive trees in two experimental field.

The cultivars that were most affected by frost were not able to recover the damages from the shoots and the branches and showed a vigorous vegetative re-sprouting directly from the base of the trunk, replacing the whole damaged canopy (i.e. 'FS17' and 'Arbequina'). Thus confirmed the age of structures presenting bark damages as a good predictor of the vegetative re-sprouting position (Table 1), because of the increasing damage effect on the tissues from old to young organs (from bark of older trunks or branches to young shoots and leaves, Ruiz et al., 2006). Consistently also the damages to the different bud typologies affect the vegetative re-sprouting position: buds in young shoots may result completely destroyed by frost because of their high water content and the absence of protecting structures (Ruiz Baena et al., 2007), whereas latent and adventitious buds confined mainly under the bark of mature organs (primary branches and trunk) might better resist to frost and thus originate a strong vegetative re-sprouting from those positions.

The international cultivar 'Arbequina' resulted very susceptible to frost in this trial. This latest result is not consistent with Ruiz et al. (2006) that reported a high frost tolerance for 'Arbequina' (no fissures in the xylem or medulla were found in freeze exposed 1-year-old shoots), nor with Gomez-del-Campo and Barranco (2005) that indicated a low percentage of frost-damaged shoots in 1-year-old 'Arbequina' olive trees subjected to −6.5 and −10.5 °C on early November and December, respectively and nor with Barranco et al. (2005) that registered a low leaf lethal freezing temperature (−11.8 °C) for 'Arbequina' by measuring the electric conductivity of electrolyte leakage from leaf disks. Nevertheless, the high vegetative re-sprouting capacity after frost for this cultivar was confirmed also in this trial.

5. Conclusions

This study allowed the characterization of twenty locally spread olive cultivars (Central Italy), three national ones and one widely spread international cultivar with regards to frost tolerance. Only three of the cultivars tested were found with a very high frost tolerance (namely 'Ascolana dura', 'Orbetana' and 'Mignolone') showing limited canopy damages and a balanced vegetative growth on 1-year-old shoots in spring.

Among the national cultivars, 'Don Carlo' showed an intermediate frost damage level and comparable with the most of the locally spread cultivars. On the contrary, 'FS17' and 'Giulia' resulted particularly susceptible to frost so that they can be considered not suitable for plantation in cold areas.

'Arbequina' resulted particularly susceptible to frost reporting heavy damages in the whole scaffold of the tree and showing a vigorous vegetative re-sprouting from the base of the trunk.

Presented results can be helpful to select the less susceptible olive varieties to frost when a new olive orchard has to be planted in cold areas.

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