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Research article

FOLIAR APPLICATIONS WITH SUNRED® BIOSTIMULANT ADVANCE AND UNIFORM FRUIT RIPENING IN ORANGE AND OLIVE

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ABSTRACT: This study evaluated the effect of foliar biostimulants on fruit quality of adult 'Valencia' orange and 'Biancolilla' olive trees. Half of the selected orange and olive trees were sprayed twice before harvest with the SUNRED® commercial mix containing phenylalanine, methionine, mono-saccharides and oxylipins from plant extracts as well as potassium salts and urea. Orange and olive yields and fruit quality were measured at harvest, and fruit peel color was determined by digital image analysis. Phenolic and sensory profiles were also determined in the olive oil. In orange, the foliar spray increased TSS:acid ratio and peel color uniformity, while in olive, it increased peel color uniformity, oleocanthal, and 3,4-DHPEA-EDA and decreased oil spiciness. The results of this study suggest that foliar sprays with the SUNRED® biostimulant in pre-harvest may improve fruit quality of 'Valencia' orange and 'Biancolilla' olive by advancing and uniforming fruit ripening.
Key words: *Citrus sinensis*, juice soluble solids, oil panel test, fruit peel color, polyphenols, yield.

INTRODUCTION

Quality at harvest is a key factor for fruit production in general. The unevenness of the fruit ripening level, particularly evident in olive, but also in orange, may determine significant cost increases, mainly in manually harvested oranges, and a substantial decline of average product quality, mainly in mechanically harvested olives. During ripening, fruits accumulate sugars (glucose, fructose and sucrose) derived from the hydrolysis of starch, from their re-synthesis or accumulated directly as such; organic acids are also transformed into sugars [1]. The increase of the primary metabolism is directly related to fruit growth (final size) [2] and indirectly related to eating and nutritional fruit quality as the biosynthesis of secondary metabolites such as pigments, vitamins and aromas arises from intermediates or monomeric products of primary metabolism [3].

Fruit ripening is also characterized by color development, due to degradation of chlorophyll and accumulation of anthocyanins (red, orange and purple) and carotenoids (yellow and orange); decrease of organic acids, mainly transformed into sugars, and further into fatty acids in olive; softening of the pulp, due to the activation of enzymes that degrade the main components of cell walls [1]. Anthocyanins are polyphenols and their synthesis starts from phenylalanine [4]. Biosynthesis of carotenoids involves several steps catalyzed by ethylene-promoted enzymes [5]. Ethylene synthesis, in turn, depends on methionine [6] and is known to regulate ripening, also by acting on sugar accumulation and carotenoid synthesis [7]. During ripening, chlorophyll is degraded by chlorophyllase, which is regulated by methyl jasmonate [8], an oxylipin. Thus, oxylipins indirectly participate in the development of fruit color. Phenylalanine, salicilic acid and jasmonic acid are also involved in lignin biosynthesis [9], which determines cell wall structure and resistance and has consequences on fruit flesh firmness.

Biostimulants are becoming more and more important as they improve the uptake and efficient use of other agricultural inputs, thus contributing to make agriculture more sustainable and food products more safe. Scientific papers that investigate biostimulant effects on yield and quality of fruit crops provide useful information for all the stakeholders involved in agriculture. In particular, the use of biostimulants, including phenylalanine, methionine, jasmonates and mono-saccharides, has been shown to improve external color, sugar content and uniformity of ripening in table grapes, apple, melon, strawberry, cherry, and loquat [10, 11, 12, 13]. It was hypothesized that similar improvements could be obtained in 'Valencia' oranges and 'Biancolilla' olives with foliar applications of SUNRED® (Biolchim, Bologna, Italy), a commercial mix that contains also the above biostimulants.

MATERIALS AND METHODS

Two similar trials were carried out using adult orange (*Citrus sinensis*, cv Valencia) and olive (*Olea europea*, cv Biancolilla) trees. Orange trees were located in one of the experimental plots of the Department of Agricultural and Forest Sciences, University of Palermo (30.06N, 13.21E, and 31 m a.s.l.). In March 2012, 48 adult orange trees grown on sour orange (*Citrus aurantium* L.) were selected and divided into two treatments (24 control trees and 24 sprayed trees) each assigned to four randomized blocks (6 trees per block). About 30 and 20 days before harvest (7 May 2012), one half of the trees were sprayed with a 4 ml l^{-1} solution (manufacturer's recommended rate) of the commercial product SUNRED® (Biolchim, Bologna, Italy) using a shoulder sprayer and 3 l/tree of solution. SUNRED® is a proprietary mixture containing 26.6 g l^{-1} of organic N, 13.3 g l^{-1} of mineral N, 93.1 g l^{-1} of K_2O, and 186.2 g l^{-1} of organic C. These components derive from inorganic fertilizers (potassium salts, urea) and liquid plant extracts rich in oxylipins, phenylalanine, methionine, and mono-saccharides. Trees of the two treatments were separated by an appropriate number of buffer trees (minimum two) to avoid drifts and contamination of control. Trees were sprayed at sundown of dry days and in absence of wind.

At harvest, all oranges were collected, counted and weighed to obtain the production per tree. A sub-sample of fruits from each tree (at least 10%) was randomly taken and brought to the laboratory for qualitative determinations, such as weight, peel color, juice yield (% of fruit weight), juice color, total soluble solids (TSS, with a digital refractometer) and titratable acidity (TA, with an automated titrator). Intensity of peel and juice color of each fruit was determined by digital image analysis (see below for details).

A similar trial was conducted using 'Biancolilla' olive trees from a grove located near Caccamo (Palermo, Italy, 37.42N, 13.33E, and 216 m a.s.l.) and for three consecutive years (2011-2013). In this case, 40 adult trees were selected and divided in two treatments as above. In each year, about 25 and 15 days before harvest, one half of the trees trees were sprayed with a 4 ml l^{-1} of SUNRED®, using 3 l/tree of solution, again at sundown and in absence of wind.

Olives were harvested on 10 November 2011, 22 November 2012, and 18 November 2013. At harvest, all olive fruits were collected and weighed to obtain the production per tree. A sub-sample of 90-120 drupes from each tree was randomly taken to the laboratory to determine average drupe weight and degree of veraison by digital image analysis. In 2013, olive oil was micro-extracted from 10 samples of drupes (five for each treatment) and used for sensory analysis by panel test and to determine polyphenol content according to the method approved by the International Olive Council.

Specifically, polyphenols were extracted from a 2-g aliquot of olive oil by adding 1 ml of syringic acid (1.5 g l^{-1} solution) as an internal standard and 5 ml of 80% (v:v) methanol. The mix was ultrasonicated for 15 min at room temperature, centrifuged at 3000 × *g* for 25 min and the supernatant filtered with a 0.45-µm PVDF filter. Polyphenols were quantified by HPLC analysis using an Agilent 1200 Series system (Agilent Technologies, Santa Clara, CA, USA) equipped with a photodiode detector (200-400 nm) and a Spherisorb ODS-2 column (250 mm × 4.6 mm i.d., 5 µm particle size). The column was eluted with 0.2% H_3PO_4 in millipore water (v:v), methanol and acetonitrile (96:2:2 v:v:v) at a flow rate of 1 ml min^{-1} and re-equilibration time between two individual runs was 2 min. Syringic acid and tyrosol were used as external standards.

Digital images of the fruits were acquired with a Nikon Coolpix L10 digital camera (Nikon Corporation, Tokyo, Japan) under standard light conditions and used to determine proportion and intensity of peel color according to the method described in Policarpo et al. [14]. Specifically, we used an algorithm that converts images from RGB (red-green-blue) to CIE (Commission internationale de l'éclairage) 1976 (L*, a*, b*) color space system, extracts the fruit from the image (removing the image background), separates the total fruit area into two sub-regions (only in olive), cover color (closer to purple-red) and ground color (closer to green) according to an adjustable green-red threshold, and quantifies color characteristics of each region as the weighed distance of each pixel in the image from an interactively chosen reference (best colored area from olive or orange fruit). The output is an index for the entire fruit area and one for each of the two regions ranging from 0 (maximum distance from reference) to 1 (identical to reference). Proportion of cover color (degree of veraison) was calculated in olive dividing the number of pixels of the purple-red region by the number of pixels of the entire fruit area.

Yield and fruit quality data were compared by analysis of variance using R procedures (The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS AND DISCUSSION

The application of SUNRED® somewhat influenced fruit quality, whereas yield parameters were not affected (Table 1). Specifically, the foliar spray significantly increased TSS/TA in the juice of 'Valencia' oranges mostly by reducing TA, while no TSS increase was recorded.

On the contrary, foliar applications with the same commercial mix increased TSS in grapes and other fruit crops [12, 13]. One explanation for the lack of TSS increase in the present study is that 'Valencia' trees had already reached their limit in size and sugar content for the crop load and cultural conditions of this trial. An inverse relationship between yield or crop load and fruit size and SS has indeed been shown in many fruit trees [15, 16, 17, 18].

The average intensity of peel or juice color was not improved by SUNRED® foliar sprays. Yet, a test for differences between variances (Levene's test on medians) as well as the coefficient of variation indicated greater peel color uniformity in sprayed fruits as compared to control fruits (Table 2). A higher degree of peel color uniformity after SUNRED® applications has also been reported in loquat [14] and may be related to a greater and more even allocation of assimilates to fruits induced by the biostimulant. In turn, assimilates (i.e. intermediate or final products of primary metabolism) represent substrates needed for pigment formation [3, 4]. In addition, the greater peel color uniformity in sprayed trees may be associated to faster chlorophyll degradation in response to the oxylipins present in the commercial mix.

Moreover, both higher juice TSS/TA and more uniform peel color suggest more advanced ripening in sprayed fruits as compared to control fruits, although a confirmation of these responses is needed in further trials. The effect of SUNRED®, in this case, was expressed in terms of earlier and more uniform fruit ripening.

Olive

Olive yield and production parameters changed significantly during the three years of trial, and this is expected as olive is known to be an alternate bearing species (Table 3).

Table 1: Yield and fruit quality of adult 'Valencia' orange trees sprayed or non-sprayed (Control) with SUNRED® biostimulant in pre-harvest

	Control	SUNRED®	*P* value
Yield per tree (kg)	43.6	40.6	0.614
Number of fruits per tree	383	331	0.329
Fruit weight (g)	0.344	0.345	0.630
Juice yield (%)	57.5	58.5	0.570
Specific weight (g/cm^3)	1.056	1.059	0.299
TSS[z] (°Brix)	12.8	12.8	0.959
TA[z] ($g\ l^{-1}$)	12.2	11.2	0.049
TSS/TA[z]	1.06	1.16	0.046
Peel color index	0.963	0.960	0.376
Juice color index	0.799	0.798	0.660

[z] TSS: total soluble solids; TA: titratable acidity.

On the other hand, SUNRED® applications did not induce significant increase of yield, yield efficiency, drupe size or degree of veraison in the three years of study (Table 3). Yet, there was a repeated tendency for the sprayed trees to produce slightly more and more mature fruit than control trees. Also in olive like in orange, SUNRED® induced more uniform fruit coloration (Table 3). Drupe color changes are due to chlorophyll degradation [19] and anthocyanin accumulation [20], and peel color, or degree of veraison, is a good indicator of olive fruit maturation stage [21]. Our results indicate greater advancement of maturation and ripening uniformity in sprayed than in control fruits. Olive is known to be a strong alternate bearing species and crop load tends to be inversely related to maturation timing (i.e. the higher the crop load, the later the fruit maturation) [22]. In our study, the application of SUNRED® tended to offset this inverse relationship, anticipating fruit maturation of higher yielding trees.

Table 2: Comparison of coefficients of variation (P value from Levene's test) for production and fruit quality parameters measured in adult 'Valencia' orange trees sprayed or non-sprayed (Control) with SUNRED® biostimulant in pre-harvest.

	Control	SUNRED®	*P* value
Yield	0.378	0.293	0.396
Number of fruits per tree	0.399	0.259	0.039
Fruit weight	0.095	0.119	0.307
Juice yield	0.039	0.083	0.133
Specific weight	0.021	0.019	0.701
TSS[z]	0.046	0.060	0.603
TA[z]	0.107	0.073	0.201
TSS/TA[z]	0.102	0.071	0.813
Peel color index	0.008	0.002	0.021
Juice color index	0.009	0.007	0.851

[z] TSS: total soluble solids; TA: titratable acidity.

Total phenol content was similar in the olive oil from sprayed and control trees (Table 4). This seems to be in contrast with observations in grape must where applications of the same biostimulants increased polyphenols [23]. Yet, phenols accumulate gradually in the olive fruit, similar to oil, reaching a maximum level just as the fruit skin begins to change color (veraison) [24, 25]. As the fruit ripens and reaches full color, the content of polyphenols and some other flavor components decline rapidly [21]. Hence, total phenol content in the oil is strictly dependent on fruit ripening stage at harvest, and the lack of phenol increase in sprayed fruit may be due to more advanced fruit ripening.

On the contrary, SUNRED® affected the phenolic profile and specifically, it significantly increased oleocanthal and 3,4-DHPEA-EDA, while it decreased 3,4-DHPEA-EA (Table 4). The sensory profile was generally unaffected, only spiciness was slightly reduced in the olive oil from sprayed trees (Table 5). The latter is also expected in olive oils made from riper drupes [26], and confirming an advanced maturation and uniform ripening after SUNRED® applications.

Table 3: Yield and fruit quality of adult 'Biancolilla' olive trees sprayed or non-sprayed (Control) with SUNRED® biostimulant in pre-harvest.

	Yield (kg/tree)	Yield efficiency (kg cm^{-2})	Drupe weight (g)	Veraison (%)
2011	11.7	0.053	2.21	53.6 (21)[z]
2012	19.7	0.056	3.48	45.7 (40)
2013	14.7	0.048	2.16	47.0 (26)
P	<0.001	0.010	<0.001	<0.001
Control	15.2	0.050	2.61	48.2 (43)
SUNRED®	16.8	0.051	2.62	49.3 (41)
P	0.431	0.797	0.992	0.488

[z]Coefficient of variation reported within brackets

Table 4: Polyphenol content (mg kg^{-1}) in the olive oil from adult 'Biancolilla' olive trees sprayed or non-sprayed (Control) with SUNRED® biostimulant in pre-harvest.

Polyphenols	Control	SUNRED®
Hydroxytyrosol	3.81 ± 0.38^z	4.53 ± 0.58
Tyrosol	2.56 ± 0.24	2.41 ± 0.11
3,4-DHPEA-EDA	75.9 ± 2.87	90.5 ± 6.18
Oleocanthal	17.8 ± 0.89	19.4 ± 0.40
Acetoxy-pinoresinol	4.58 ± 0.97	3.85 ± 0.63
Pinoresinol	10.8 ± 2.29	9.92 ± 3.14
3,4-DHPEA-EA	343.3 ± 80.0	303.8 ± 22.4
Total	459.3 ± 76.2	434.4 ± 30.7

^{z} Means ± SE.

Table 5: Scores for sensory parameters in the olive oil from adult 'Biancolilla' olive trees sprayed or non-sprayed (Control) with SUNRED® biostimulant in pre-harvest.

	Control	SUNRED®
Fruity	1.9 ± 0.19^z	2.0 ± 0.10
Bitter	1.8 ± 0.12	1.8 ± 0.20
Spicy	2.1 ± 0.19	1.8 ± 0.12
Overall	7.4 ± 0.10	7.4 ± 0.24

^{z} Means ± SE.

CONCLUSION

Overall, our results show that SUNRED® foliar applications in pre-harvest may improve fruit quality of 'Valencia' orange and 'Biancolilla' olive, especially by advancing and uniforming fruit ripening. This effect may be specifically due to the action of oxylipins and phenylalanine (activating biosynthesis of anthocyanins and flavonols) and methionine (stimulating ethylene biosynthesis and in turn fruit ripening) contained in the commercial mix. Having most of the fruit on a tree at the same ripening stage (i.e. uniforming fruit ripening) is of great interest and value to nearly all fruit growers, for both large- and small-fruited species. Uniform maturation and ripening may in fact reduce harvesting time and costs, and concurrently increase average fruit quality (less waste/undersized fruit). This is particularly important in olive, which is often harvested by machines that rely on maturity-related fruit attachment force.

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