

# Mechanical harvesting at dawn in a super-high-density table olive orchard: effect on the quality of fruits

Ana Morales-Sillero, María Rocío Jiménez, María Paz Suárez, Pilar Rallo and Laura Casanova 

## Abstract

**BACKGROUND:** Mechanical harvesting with over-the-row harvesters in super-high-density (SHD) table olive orchards increases the effectiveness of fruit removal, although bruising can limit the fruit quality. Additionally, an early harvest in periods less favourable to quality production is increasingly frequent as a result of global warming. The present study explores the impact on olive quality of harvesting at dawn when the environmental temperature is low. The study was carried out for 2 years on two cultivars with different tolerance to bruising ('Manzanilla de Sevilla' and 'Manzanilla Cacereña'), grown in SHD conditions and harvested at two timepoints: dawn and morning.

**RESULTS:** Fruit morphology was not modified by the moment of harvest in either of the cultivars. Fruit harvested at dawn produced less CO<sub>2</sub> and ethylene and was less damaged externally and internally compared to fruit harvested in the morning. However, environmental conditions throughout development influenced the response because the highest values of bruising (incidence, area and volume of damaged area), total internal damage and the number of tissue ruptures increased in the year with the hottest summer, and the differences between harvest treatments were less evident.

**CONCLUSION:** Mechanical harvesting at dawn contributes to reducing the damage in olive fruit.

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**Keywords:** *Olea europaea* L.; bruising; internal damage; CO<sub>2</sub> production and ethylene production

## INTRODUCTION

World table olive production has expanded by almost 197% subsequent to 1990, from 950 000 to 2 829 500 tons in 2020. Its consumption has been somehow balanced with production, and it has grown by almost 154% in the same period, from 1 065 500 to 2 709 500 tons.<sup>1</sup> Consumers demand olives with medium or large size, high pulp-to-pit ratio, and adequate colour and shape, amongst other fruit traits, and the recognition of the sensorial and nutritional quality of table olives has significantly contributed to the increase of their trade.<sup>2</sup> However, to meet this increasing demand, olive growers must face two great challenges: on the one hand, to reduce the high harvesting cost caused mainly by the large labor force required, and, on the other, to develop strategies to minimize the impact of climate change, particularly global warming, on the final product quality.

The high production costs derived from manual harvesting of table olives encouraged, beginning years ago, the search for more profitable growing techniques, such as mechanization, along with post-harvest management.<sup>3-5</sup> More recently, towards the end of the 2010s, the first super-high-density orchards (SHD) were being established for table olive production.<sup>6</sup> By now, these orchards are being designed for traditional table olive cultivars, using plant

densities of usually around 1500–2000 trees per hectare, irrigated conditions and dimensions that allow mechanical harvesting with over-the-row harvesters. This technology shortens the harvest time per hectare (2–3 h), significantly reducing the cost compared to manual harvesting, and has a high fruit removal effectiveness in the green maturation ripening stage.<sup>7</sup> Nevertheless, the most common limitation to mechanical harvesting devices is the damage caused to the olive fruit (bruising), which increases with time after harvesting, and its severity depends on the cultivar.<sup>8,9</sup> It may be considered as an abiotic stress (mechanical stress) that dramatically reduces the final quality of table olives, and the fruit appearance is critical to the acceptance by the consumer.<sup>2</sup>

Bruising is evidenced by the appearance of spots on the outside of the fruit. The determination of the level of damage in olives, both in commercial regulation<sup>10</sup> and in most published studies, is limited to a subjective evaluation of the damage extent and,

\* Correspondence to: L Casanova, Departamento de Agronomía, ETSIA, Universidad de Sevilla, Carretera de Utrera, km 1, 41013 Sevilla, Spain, E-mail: laucaler@us.es

Departamento de Agronomía, ETSIA, Universidad de Sevilla, Sevilla, Spain

at most, the measurement of the bruised area and volume of spots.<sup>8,11</sup> Bruising also causes internal damage to the mesocarp, including cell rupture and loss of cell wall thickness,<sup>12</sup> as well as a physiological response of 'over-ripening' through the increase of the respiratory rate and production of ethylene.<sup>13</sup> Complex biochemical reactions also occur in bruised fruit, mainly as a result of the action of the enzyme polyphenoloxidase (PPO), which leads to the darkening of the damaged tissue by oxidation of phenolic compounds.<sup>13,14</sup> To stop bruising in mechanically harvested fruits, immersion in NaOH diluted and cooled solutions were proposed.<sup>4,5</sup>

In addition, the current increase in temperature as a consequence of global warming affects the phenological stages and can accelerate the ripening of fruit, causing harvesting to start in periods that are less favorable to quality production.<sup>15,16</sup> In many traditionally olive producing countries, the advance of the ripening period implies an earlier harvesting for table olive, which now occurs at the middle or end of summer, when the temperatures are still high and may reach over 40 °C, as is the case in Southern Spain.<sup>16</sup> Therefore, there is a need to develop strategies for current olive orchards to maintain high-quality production and economically sustainable yields. In the case of vineyards, mainly those that produce white and sparkling wines in warmer climates, harvesting at night (manual or mechanical) is currently very popular because, under high air temperature conditions, the epidermis of the grapes could break during harvest and this would increase the risk of must alteration and oxidation. Harvesting at night contributes to maintaining the quality of the grapes and reduces this risk, therefore reducing the potential loss of aromatic qualities. Furthermore, the industry saves energy, because grapes do not have to be pre-chilled before being crushed.<sup>17,18</sup>

There is no literature about the effect of temperature at harvest on olive fruit quality. Nevertheless, it is known that the rupture of cellular tissue occurring during mechanical harvesting causes the release of phenolic compounds and oxidase enzymes,<sup>14</sup> and the activity of the PPO enzyme increases with temperature and negatively affects the degree of bruising damage to the fruit. On the other hand, the immersion of fruit in different cold solutions after mechanical harvesting contributes to reducing the loss of phenols, and this leads to lighter bruised areas.<sup>14,19</sup> In this sense, the hypothesis of the present study is that low temperatures at harvest can modulate the response of olive fruit to bruising.

The present study aimed to evaluate the effect of mechanical harvesting at dawn, that is, when the lowest temperature of the day is recorded, on the quality of olives, with a particular emphasis on bruising. The study was carried out in two consecutive years and used fresh and unprocessed fruits from two cultivars with different tolerance to bruising, growing in SHD conditions.

## MATERIALS AND METHODS

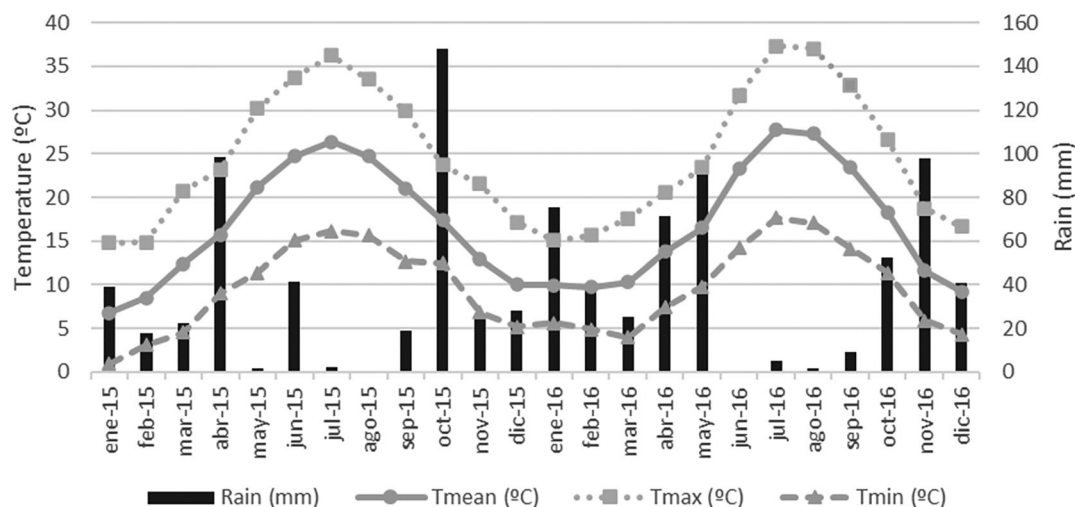
### Experimental design and harvesting

The study was carried out in 2015 and 2016 on 'Manzanilla de Sevilla' (MS) and 'Manzanilla Cacereña' (MC) hedgerows in a commercial SHD orchard located in Portugal [Campo Maior; latitude: 38° 55' 55.1' N; longitude: 7° 02' 36.8' W; altitude: 201 m (WGS84)], with trees planted at 3.75 × 1.35 m. MS is the most internationally widespread Spanish table olive cultivar. The fruit is highly appreciated for its quality, but its highly susceptible to bruising. MC is widespread mainly throughout Southwestern Spain and Portugal, and the fruit usually shows higher tolerance to bruising. Both cultivars, considered of double aptitude because of the quality of their oils, can be grown under super-high density conditions.<sup>7</sup>

The Mediterranean climate characterizes the area. Figure 1 shows the distribution of temperature and rain in the years studied. From fruit set to harvest, the average monthly temperature and the average values of the maximum and minimum temperatures were higher in 2016. Total degree days<sup>20</sup> for this period were around 1018 in 2015 and 1155 in 2016, considering 15 °C as the threshold temperature.<sup>21</sup> Trees were irrigated each year with approximately 320 mm ha<sup>-1</sup> between March and September.

Treatments were Dawn = Harvesting initiated at around 06.30 h, and Morning = Harvesting initiated around 3 h later, at 10.30 h. For each cultivar, three random rows of trees (approximately 90) were selected each year as replicates.

Harvesting was carried out at the end of September, when the ripening index was 1, that is, green-yellowish coloured epidermis,<sup>2</sup> and the pulp was separated easily from the stone. The temperature was lower both at dawn (approximately 12 °C) and in the morning (22 °C) in 2015 than in 2016 (18 °C and over 30 °C) (Table 1). The harvester was a New Holland Braud 9090X Olive (CNH Global, Zedelgem, Belgium). The nominal speed was



**Figure 1.** Distribution of rain and temperature ( $T_{\text{mean}}$ , average temperature;  $T_{\text{max}}$ , maximum temperature;  $T_{\text{min}}$ , minimum temperature) in 2015–2016.

2 km h<sup>-1</sup> and the beating frequency was around 450 Hz. After harvesting, samples of fruit (3 kg) were randomly selected by the experimental unit from the harvested fruit.

### Fruit CO<sub>2</sub> production and ethylene production

Immediately after harvest, 500 g of fruit per replicate were placed in a 2-L glass jar and hermetically sealed for 3 h. Later, the fruit CO<sub>2</sub> production (nmol CO<sub>2</sub> kg<sup>-1</sup> s<sup>-1</sup>) and the ethylene content (nmol ethylene kg<sup>-1</sup> s<sup>-1</sup>) were measured with a G100 portable gas analyser (Geotechnical Instrument Ltd, Leamington Spa, UK) and an ICA portable ethylene analyser (International Controlled Atmosphere Ltd, Paddock Wood, UK), respectively.<sup>22</sup>

### Fruit weight, volume, shape, pulp-to-pit ratio and colour

Mean fruit weight (g) was measured using a sample of 1 kg of fruit per experimental unit. Volume and pulp-to-pit ratio were estimated using samples of 0.5 kg. Fruit volume (mL) was determined from the volume displaced after immersion of each sample in a graduated measuring cylinder filled with water. Pulp-to-pit ratio was estimated in fresh weight calculating the difference between the weights of fruit and pits. Fruit shape was determined as the ratio between the maximum longitudinal and equatorial diameters (mm) in 50 fruit samples.<sup>2</sup>

### Skin colour

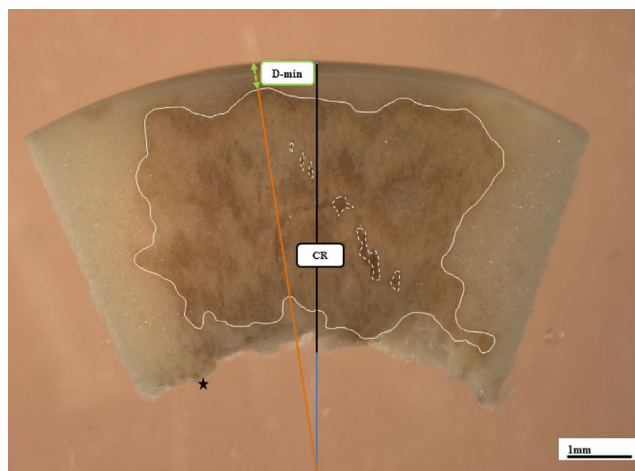
The skin colour was measured on the equatorial zone of 30 fruits with a Minolta CM-700d (Konica Minolta Inc., Tokyo, Japan) spectrophotometer. The International Commission on Illumination colour notation system was applied to determine the parameters *L\** (lightness), *a\** (colour axis from green to red) and *b\** (colour axis from blue to yellow) and the colour index was estimated.<sup>23</sup>

### Fruit cuts and bruising incidence

The proportion of fruit with cuts and the incidence of bruising were estimated 2 h after harvesting using samples of 100 fruits. For the latter parameter, fruits were classified into three categories<sup>7</sup>: non-bruised, low damage (< 25% of the skin surface affected) and severe damage (25%–100% of the skin affected).

### Olive fruit fixation and bruising measurements

The fruits were fixed in a FAE solution [formalin, acetic acid, 95% ethanol and distilled water (10:5:50:35, v/v/v/v)] 2 h after harvest.<sup>24</sup> Later, in the laboratory and for the anatomical study, 30 fruits per cultivar, harvesting treatment and replicate were randomly selected each year to measure the external bruising area (*BA*, mm<sup>2</sup>) and bruising volume (*BV*, mm<sup>3</sup>).<sup>25</sup> These traits were determined by measuring the length (*W*<sub>1</sub>, mm), width (*W*<sub>2</sub>, mm) and depth (*d*, mm) of the fruit damaged surface using a digital caliper (COMECTA-ICT, Barcelona, Spain).



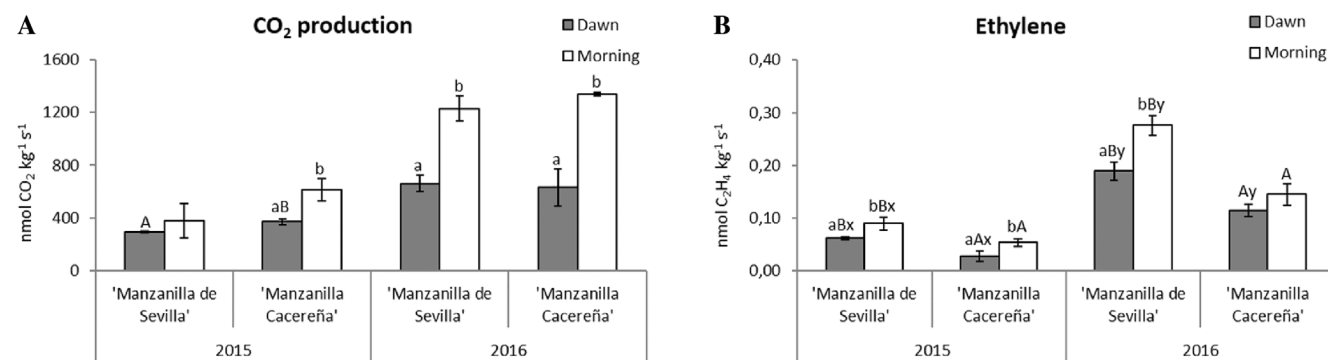
**Figure 2.** Mesocarp portion where the total internal damage (*TDA*) is delimited (area outlined by the continuous white line) and distances measured to locate damage are indicated: the central radius (*CR*) extends centripetally from the center of the bruised area and *D-min* is radial distance from the fruit exterior to the damaged area closest to the epidermis (mm). Tissue ruptures are indicated by discontinuous lines. ★ internal limit of the fruit mesocarp.

To describe and quantify the internal damage, 10 fruits were randomly selected. The fruit were subjected to a rehydration and cutting process to obtain the damaged tissue portions of the mesocarp.<sup>12</sup> Later, photographic images of the fixed portions were taken with a digital camera (Sight DS Ri 1; Nikon, Tokyo, Japan) connected to a binocular loupe (SMZ 1270; Nikon). All samples were measured under the same conditions of light and binocular magnification, and several parameters related to the internal damage were quantified<sup>12</sup>: total damaged area (*TDA*, mm<sup>2</sup>), damaged area location (distance from the epidermis) and browning intensity, all of which were determined using these images with NIS ELEMENTS AR, version 3.2 (Nikon).

Different measurements were made to locate the damaged area in the fixed mesocarp portions (Fig. 2): central radius (*CR*, mm) and radial distance from the fruit exterior to the damaged area closest to the epidermis (*D-min*, mm). With all the obtained data, the percentage of *D-min* over the central radius (% *D-min*) was calculated. The numbers of tissue ruptures inside the damaged area were also quantified in the fixed tissues of the mesocarp. The browning intensity of the damaged area was estimated through the *Mean Intensity*, which was measured using the RGB component (red, green, and blue channel intensities). Pixel values for each component ranged from 0 (black) to 65 535 (white) in 16-bit depth. The larger the values of *Mean Intensity*, the lower the browning in the internal damaged area.

**Table 1.** Agroclimatic conditions: temperature (°C) and humidity (%) at the beginning of the harvest

Year	Climatic parameters	'Manzanilla de Sevilla'		'Manzanilla Cacereña'	
		Dawn	Morning	Dawn	Morning
2015	Temperature	13	23	11	21
	Humidity	50	40	71	65
2016	Temperature	18	32	18	27
	Humidity	60	42	60	30



**Figure 3.** Fruit CO<sub>2</sub> production and ethylene production 3 h after harvest. Lowercase letters (a, b) indicate significant differences in harvesting treatment for each cultivar. Uppercase letters (A, B) indicate significant differences in cultivars for each harvesting treatment. 'x' and 'y' indicate significant differences in years for each cultivar and harvesting. An absence of letters indicates a non-significant effect. Vertical bars represent the SD.

### Statistical analysis

Analysis of variance for factors such as cultivar, harvesting treatment and year was performed using the StatGraphics Centurion XVIII (Statgraphics Technologies, Inc., The Plains, VA, USA). Data were previously transformed for some values to achieve variance normality and homogeneity, using the Box–Cox power transformations.<sup>26</sup> Significant differences between the mean values were discriminated using the Tukey's test ( $P < 0.05$ ).

## RESULTS

### Fruit CO<sub>2</sub> production and ethylene production

CO<sub>2</sub> production was lower in the fruit harvested at dawn than in the fruit harvested in the morning (Fig. 3) and the differences between harvesting treatments were significant. For MS, CO<sub>2</sub> production in 2015 was 379 nmol kg<sup>-1</sup> s<sup>-1</sup> when fruit was harvested in the morning and 296 nmol kg<sup>-1</sup> s<sup>-1</sup> when fruit was harvested at dawn. The values obtained in 2016 were 1230 and 660 nmol kg<sup>-1</sup> s<sup>-1</sup>, respectively. For MC, CO<sub>2</sub> production in 2015 was 613 nmol kg<sup>-1</sup> s<sup>-1</sup> when fruit was harvested in the morning and 370 nmol kg<sup>-1</sup> s<sup>-1</sup> when fruit was harvested at dawn. The values obtained in 2016 were 1337 and 632 nmol kg<sup>-1</sup> s<sup>-1</sup>, respectively. The differences in the fruit harvested at dawn in 2015 were also important between the different cultivars.

The ethylene production followed similar pattern. In 2015, for MS, it was 0.06 versus 0.09 mg kg<sup>-1</sup> s<sup>-1</sup> for fruit harvested in the morning; for MC, these values were 0.03 versus 0.05 mg kg<sup>-1</sup> s<sup>-1</sup>; in 2016, from the results were 0.19 versus 0.28 mg kg<sup>-1</sup> s<sup>-1</sup> and 0.11 versus 0.15 mg kg<sup>-1</sup> s<sup>-1</sup>, respectively. Differences between harvesting treatments were always significant and the MS fruit always released the highest values.

### Fruit traits

Mechanical harvesting at dawn did not modify fruit morphology between harvesting treatments in any cultivar, regardless of the year.

Mean values of fruit weight in 2016 were 4.9 g in MS and 4.3 g in MC and were higher than in 2015 (3.5 and 3.7 g, respectively). Similar results were found in the fruit volume. Mean values in 2016 were 4.9 mL in MS and 4.3 mL in MC and were higher than in 2015 (3.6 and 3.8 mL, respectively). Fruit shape ratio was 1.2 and pulp-to-pit ratio was between 6.5 and 7.3 without a significant difference in any case.

### External damage of the fruit

In 2015, the colour index mean values were around 30, with no differences between harvesting treatments or between cultivars (Table 2). The percentage of fruit cuts did not depend on the harvesting treatment, although it was significantly higher in MS fruit (mean values 13%) than in MC (3.0%). Nevertheless, for both cultivars, the bruising incidence was significantly lower in the fruit harvested at dawn than in those harvested in the morning. MS fruit showed the highest values (1.5 in the Dawn treatment and 1.6 in the Morning treatment). For this cultivar, all of the fruits were damaged in both harvesting treatments, although the percentage of severely damaged fruit was lower (by 9%) when harvested at dawn. MC fruit showed the lowest values of bruising incidence. For this cultivar, bruising incidence was 1.1 in the fruit harvested at dawn and 1.2 in the fruit harvested in the morning. The lower value of bruising incidence in the fruit harvested at dawn was indicated by the decrease of fruit with severe damage (by 20%) and the increase in a similar proportion (22%) of the fruit with low damage.

In 2016, the colour index was significantly lower for MS fruit harvested at dawn (22.9) than for the fruit harvested in the morning (24.6), whereas no significant differences were found in the MC fruit, for which the mean value was 24.5. The percentage of cut fruit was lower than in 2015. In MS, it was significantly lower in the fruit harvested at dawn (6%) than in the fruit harvested in the morning (9.7%), whereas, in MC, no differences between harvesting treatments were found, and cut fruit represented approximately 2%. That year, the bruising incidence was similar, regardless of harvesting treatment. Mean values were 1.6 in MS fruit and 1.3 in MC fruit. As in the previous year, most fruit were damaged, and the MC cultivar showed the highest percentages of fruit with low damage (approximately 68%) compared to MS (approximately 40%).

### Bruising area and bruising volume

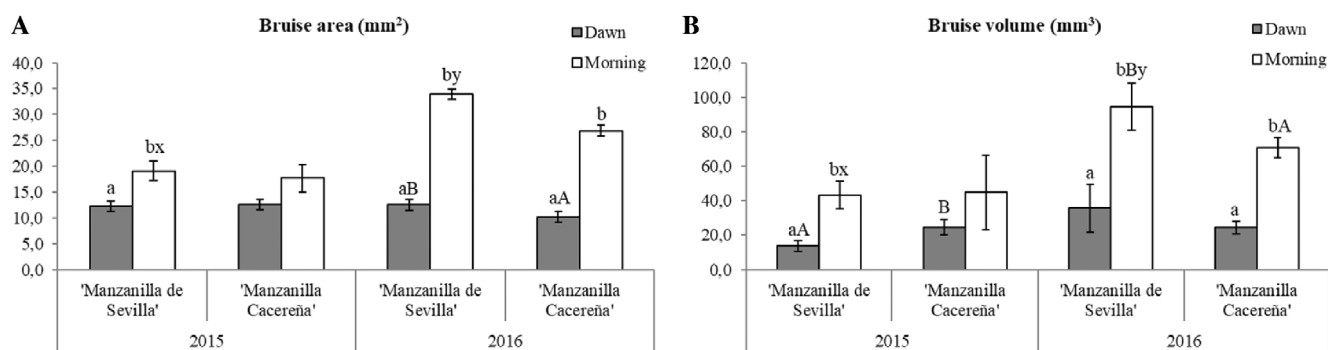
The bruising area (BA, mm<sup>2</sup>) and bruising volume (BV, mm<sup>3</sup>) values were significantly lower in the fruit harvested at dawn, regardless of the cultivar and year (Fig. 4). In 2015, the BA increased from 12.2 mm<sup>2</sup> in the MS fruit harvested at dawn to 19.1 mm<sup>2</sup> in the fruit harvested in the morning, and from 12.6 to 17.7 mm<sup>2</sup> in the MC fruit, whereas BV increased from 13.7 to 43.2 mm<sup>3</sup> in MS fruit, and from 24.5 to 44.8 mm<sup>3</sup> in MC.

In 2016, the mean values of BA and BV were significantly higher than in 2015. The BA increased from 12.5 to 33.9 mm<sup>2</sup> in the MS

**Table 2.** Colour index and external fruit damage by harvesting treatment and variety in 2015 and 2016

Year	Fruit traits	'Manzanilla de Sevilla'		'Manzanilla Cacerena'	
		Dawn	Morning	Dawn	Morning
2015	Colour index	30.3	30.0	29.2	30.6
	Cut fruit (%)	14.7 By	12.1 B	2.0 A	4.0 A
	Bruising incidence	1.5 aB	1.6 bB	1.1 aA	1.2 bA
	Bruised fruit (%)				
	Total	100.0 B	99.3	97.4 A	95.7
2016	Low damage	50.2 bA	40.4 aA	87.5 bBy	66.1 aB
	Severe damage	49.8 aB	58.9 bB	9.8 aA	29.6 bA
	Colour index	22.9 aA	24.6 b	24.2 B	24.8
	Cut fruit (%)	6.0 aBx	9.7 b	2.7 A	1.7
	Bruising incidence	1.6 B	1.6 B	1.2 A	1.3 A
	Bruised fruit (%)				
	Total	99.7	100.0	96.0	98.0
	Low damage	42.0 A	40.0 A	71.3 Bx	65.3 B
	Severe damage	57.7 B	60.0 B	24.7 A	32.7 A

Note: Lowercase letters (a, b) indicate significant differences in harvesting treatment for each cultivar. Uppercase letters (A, B) indicate significant differences in cultivars for each harvesting treatment. 'x' and 'y' indicate significant differences in years for each cultivar and harvesting. An absence of letters indicates a non-significant effect.



**Figure 4.** Bruise area (BA, mm<sup>2</sup>) and bruise volume (BV, mm<sup>3</sup>) by harvesting treatment cultivar and year in 2015 and 2016. Lowercase letters (a, b) indicate significant differences in harvesting treatment for each cultivar. Uppercase letters (A, B) indicate significant differences in cultivars for each harvesting treatment. 'x' and 'y' indicate significant differences in years for each cultivar and harvesting. An absence of letters indicates a non-significant effect. Vertical bars represent the SD.

fruit, and from 10.2 to 26.9 mm<sup>3</sup> in MC; and the BV increased from 35.6 to 94.7 mm<sup>3</sup> in MS and from 24.4 to 70.9 mm<sup>3</sup> in MC. Differences between cultivars were not always significant.

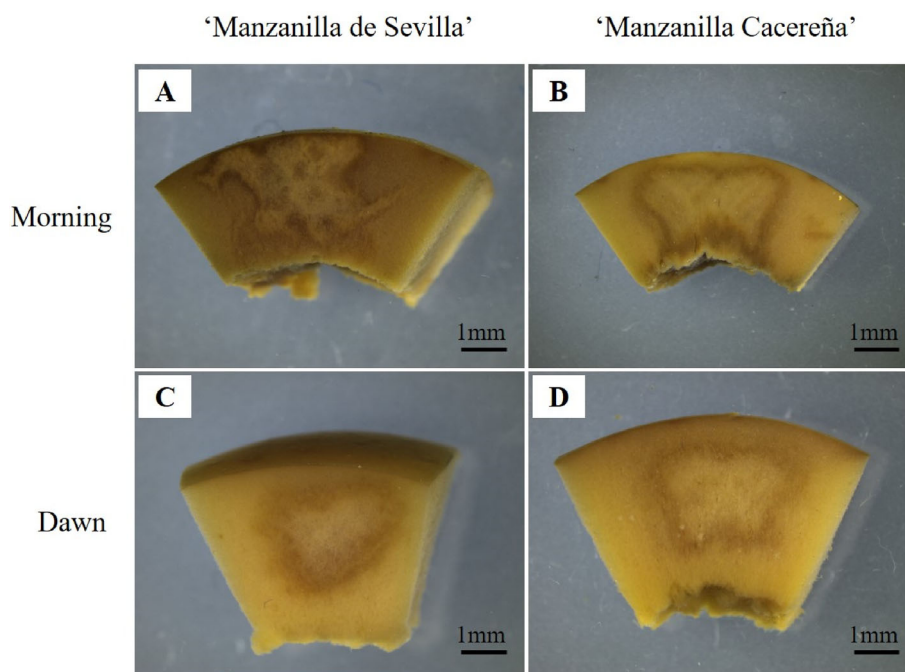
### Fruit internal damage

Darkened spots with some gaps were found, regardless of harvesting treatment, cultivar and year. The apparent size of these spots was similar in both harvesting treatments, although the darkening of the damaged area was lower in the fruit harvested at dawn, particularly in MC (Fig. 5). In 2015, differences between harvesting treatments were also observed in the location of the spots in MS because they were deeper in the fruits harvested at dawn than in those harvested in the morning, in which the spots were closer to the epidermis. In MC, no differences between harvesting treatments were observed, and the darkened spots were always located in a deep position. In 2016, the internal damaged area was greater than in 2015 for both cultivars and no clear differences between harvesting treatments were observed, nor in the size of the spots or in their location. In the MS, the spots were nearer the epidermis and were darker than those of MC.

Concerning internal damage, no effect of harvesting treatment was found on the TDA, and the number of tissue ruptures (Table 3). In 2015, the mean values for both harvesting treatments were approximately 9.5 mm<sup>2</sup> in MS fruit and 15.1 mm<sup>2</sup> in the MC, and the number of tissue ruptures located inside was approximately two and three, respectively. The *D-min* and % *D-min* were significantly higher in the fruit harvested at dawn (0.24 mm and 4.6%, respectively) in MS; therefore, the damage was deeper than in the fruit harvested in the morning (0.04 mm; 0.8%). For MC no differences were found between harvesting treatments.

The highest values of the *Mean Intensity* (Table 3) and, in consequence, the lowest intensities of browning, were found in the fruit harvested at dawn, although differences from the fruit harvested in the morning were significant only in MS. For all parameters related to internal damage, no differences were found between cultivars in the fruit harvested at dawn, whereas, in the fruit harvested in the morning, higher and more significant values were found in MC than in MS.

In 2016, no differences between the harvesting treatments were found for the TDA with respect to either the parameters related to



**Figure 5.** Fixed mesocarp sections of MS (A, C) and MC (B, D) for both harvesting treatments in 2015.

Year	Internal damage traits	'Manzanilla de Sevilla'		'Manzanilla Cacereña'	
		Dawn	Morning	Dawn	Morning
2015	TDA (mm <sup>2</sup> )	10.4 x	8.5 Ax	14.7 x	15.4 B
	D-min (mm)	0.24 b	0.04 aA	0.16	0.25 By
	% D-min	4.6 b	0.8 aA	3.1	4.8 B
	Number of tissue ruptures	2.5	1.5 Ax	3.8	2.4 Bx
	Mean Intensity	22 205.7 b	17 558.2 aA	23 653	22 066.7 B
2016	TDA (mm <sup>2</sup> )	17.5 Ay	21.3 y	20.4 By	20.8
	D-min (mm)	0.04 A	0.03 A	0.18 B	0.14 Bx
	% D-min	0.9 A	0.6 A	3.7 B	2.9 B
	Number of tissue ruptures	4.4	4.7 y	3.1	4.5 y
	Mean Intensity	24 098.5 A	19 420.3 A	27 929.6 bB	24 423.2 aB

*Note:* Lowercase letters (a, b) indicate significant differences in harvesting treatment for each cultivar. Uppercase letters (A, B) indicate significant differences in cultivars for each harvesting treatment. 'x' and 'y' indicate significant differences in years for each cultivar and harvesting. An absence of letters indicates a non-significant effect.

its location or the tissue ruptures. For both cultivars, the mean values of TDA were approximately 20 mm<sup>2</sup> and the number of tissue ruptures was approximately 4; both were significantly higher than in 2015. The internal damage location was always closer to the epidermis in the fruit of MS, as can be concluded from the lowest values of D-min and % D-min (approximately 0.04 mm and 0.8% in MS, and 0.16 mm and 3.3% in MC). Furthermore, the fruit of MS harvested at dawn showed the lowest values of TDA compared to those of MC.

The Mean Intensity was significantly higher in the fruit of MC, which implies that they showed lower browning than MS fruit. Differences between harvesting treatments were found to be such that the fruit harvested at dawn showed the lowest browning. However, these differences were significant only in MC.

## DISCUSSION

For two consecutive years, we have explored to what extent the quality and particularly the damage by bruising in olive may be reduced by carrying out the mechanical harvesting in a SHD orchard at the time of day when the ambient temperature is at its lowest, which usually occurs at dawn.

As expected, CO<sub>2</sub> production (Fig. 3) was significantly lower with the lowest temperature. Lower values at 8 °C than at 25 °C have been reported in the literature.<sup>13</sup> On the other hand, fruit produced ethylene after mechanical harvesting, which has been described previously for the same cultivars as a reaction to stress caused by an over-the-row harvester.<sup>22</sup> However, the lowest amounts of ethylene were produced by the fruit

harvested at dawn, which was probably related to a better quality of the olive because, in general, less damaged fruits produce less ethylene.<sup>22</sup>

Fruit morphology did not change, regardless of the time and type of harvesting. Diurnal variations of fruit size have been described, however, for different fruit species including olives.<sup>27–29</sup> Harvesting at dawn contributed to decreasing the level of damage in the fruit of the studied cultivars compared to harvesting later in the morning. Nevertheless, the decrease of damage was not always visible as the results of bruising incidence reveal in 2016 (Table 2). It is particularly in the bruising area and volume (Fig. 4) where the effect of temperature at harvesting was different, at a significant level and for both years, in the fruit of MS, and in 2016 in MC fruit. Furthermore, the internal browning was lower in the fruit harvested at dawn, as indicated by the higher *Mean Intensity* data (Table 3), which is in agreement with the description of the damage found in the mesocarp sections (Fig. 5).

The lower temperature when harvesting at dawn probably caused a partial inhibition of the PPO activity in the fruit, and this led to less browning, compared to harvesting in the morning. Phenolic compounds are related to the brown dark spots typical of damage by bruising. These compounds are oxidized in response to biotic or abiotic stresses.<sup>30</sup> Thus, in a simulation of mechanical harvesting of olives, the concentration of these compounds, particularly oleuropein, decreased to a greater extent in bruised fruit compared to unbruised fruit.<sup>13,14</sup> As in other fruit,<sup>17</sup> the activity of oxidases, mostly the PPO enzyme, can contribute to explaining the appearance of bruising. The PPO enzyme is in the chloroplasts, separated from the phenols that accumulate primarily in the vacuoles and cell walls.<sup>31</sup> When cells age or a physical stress takes place in the fruit, they come into contact and the reactions lead to browning.<sup>14</sup> The PPO activity depends on the olive pH and temperature because it is considered a temperature-dependent enzyme; this occurs in such way that it decreases at low temperatures.<sup>32</sup>

The MC cultivar showed the greatest tolerance to damage caused by mechanical harvesting, as in previous studies.<sup>7,9</sup> On average, this cultivar showed the lowest percentages of cut fruit and fruit with severe-damaged and the lowest mean values of bruised area and volume (Fig. 4 and Tables 2 and 3). In terms of internal damage, the area affected was located usually deeper and its extension was equal or even greater than in MS fruit (depending on the year and harvesting treatment), which does not match the first description of the internal damage of fruit from both cultivars.<sup>9</sup> In that previous study, *TDA* was always lower in MC fruit, and it was also located deeper than in MS. Furthermore, no tissue ruptures were found in MC fruit, and browning was higher (lower *Mean Intensity*) than in the fruit of the present study, where the browning intensity was always lower in the internal damaged area of MC fruit, regardless of the harvesting treatment and year (Fig. 5 and Table 3). We do not have a clear explanation for this. It could be related to the lower concentration of phenols in the fruit of MC in comparison to MS fruit, as we have found in our investigations (unpublished data, A. Morales-Sillero) and/or the lower activity of the PPO enzyme for this cultivar at the studied temperatures. So far, the tolerance or susceptibility of olive cultivars to bruising has been linked mostly to both parameters,<sup>32,33</sup> although the thickness of the cuticle also appears to have a relevant role.<sup>9,33</sup>

Additionally, the results show clear differences between years for most of the studied parameters. There was a significant higher

damage at an external and internal level in the fruit of 2016 (Tables 2 and 3). In that year, the fruit of both cultivars showed the highest bruising incidence, *BA*, *BV*, *TDA* values, and number of tissue ruptures. Environmental conditions in 2016 were different not only at harvesting (Table 1), but also for fruit set to harvesting (Fig. 1). The average monthly temperature and the average values of the maximum and minimum temperatures for this period were 24, 33 and 15 °C, respectively in 2015, and 26, 36 and 16 °C in 2016. In addition, the number of days with  $T_{\max} > 35$  °C and even 40 °C was also higher in 2016 (66 and 12 days, respectively) compared to 2015 (58 and 4 days, respectively), and total degree days were also higher in 2016. This means that the summer of 2016 was warmer than that of 2015. High temperatures during fruit development can negatively affect fruit weight, oil content and oil quality, including the total amount of phenols, although this depends on cultivar.<sup>34,35</sup> Furthermore, the activity of the PPO enzyme increases with temperature and negatively affects the degree of bruising damage in the fruit. In the case of other endogenous enzymes, such as peroxidase,  $\beta$ -glucosidase and esterase, the activity also appears to increase with temperature, up to around 30–40 °C,<sup>32</sup> although its relationship with this damage has been scarcely investigated.

Taking into account the aforementioned results, to achieve the maximum product quality, postharvest management procedures appear to be essential for cultivars where production is mainly intended for green dressing and where the cultivars are highly susceptible to bruising, such as MS, as well as in years and growing areas that are particularly hot. Finally, an important aspect to consider when deciding the time to harvest (dawn or morning) is the regulatory framework that may exist in this regard. In the South of Spain, for example, night harvesting (from sunset to sunrise) is temporarily prohibited in high-density olive groves because of the mortality of birds. In this case, we propose that harvesting can be carried out immediately after sunrise or in the hours before sunset, depending on the ambient temperature.

## CONCLUSIONS

Low temperatures at harvest contribute to reducing the damage in olive fruit harvested mechanically in SHD orchards. The traits that have been most influenced by the time of harvesting are the area and volume of the damage and the internal browning, and, to a lesser extent, bruising incidence and the depth of internal damage. Damage decreases as a result of harvesting at dawn have been particularly evident in the most susceptible cultivar (MS) and in the year with the less warm summer (2015).

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## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in [idUS, Depósito de Investigación Universidad de Sevilla] at [<https://idus.us.es/handle/11441/141069>], reference number [36].

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