# Yield response of mature hedgerow oil olive orchard to different level of water stress during pit hardening.

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### 11 Abstract

12 Drought sensitivity in olive trees is variable along the season, from flowering (the most) until pit hardening (the less). Yield respond is affected for the final use of the fruits 13 because oil accumulation occurs commonly in a period of low evaporative demand. The 14 aim of this work was evaluated the effect of water stress during pit hardening on the yield 15 components of oil olive trees (number of fruits, fruit size and oil percentage). The 16 17 experiment was performed during three consecutive seasons (2017-2019) in a mature hedgerow olive orchard (11 years-old, cv Arbequina). Experimental design was a 18 19 randomized completed block with 4 repetitions of four different irrigation treatments. Treatments were: Control, no water stress along the season; RDI-1, moderate water stress 20 21 during pit hardening and total recovery after the last week of August; RDI-2, same that 22 RDI-1 but with severe water stress and partial recovery; SDI, almost constant irrigation

23	rate with the same seasonal applied water than RDI-2. Irrigation scheduling in RDI-1 and
24	2 was performed with frequencies of trunk growth rate. No significant differences were
25	found in fruit and oil yield between treatments in any of the seasons. But Stress integral
26	(SI) and applied water was significantly different between treatments. When single data
27	was use, there were a significant relationship between minimum midday stem water
28	potential (SWP) and fruit yield (lineal) and between seasonal SI and oil yield (quadratic).
29	Part of the decrease in fruit yield with SWP was related with fruit moisture because no
30	significant fruit drop was found. Only conditions of water stress in 2017, before the end
31	of endocarp size, was related with a great reduction of fruit volume and then with fruit
32	and oil yield. Relationship between percentage of oil in dry weight and SWP was
33	quadratic in massive pit hardening, pit hardening and recovery. These relationships
34	supported the quadratic relationship of oil yield vs SI.
35	Keyword: Fruit development, RDI, SDI, water potential.
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## 51 INTRODUCTION

Olive (Olea europaea L.) is one of the most drought resistant fruit trees (Diaz-Espejo et 52 al., 2018) and traditionally cultivated in rainfed conditions. Irrigated orchards have 53 54 increased their surface around the world and, in some arid and semiarid countries, is one of the most important irrigated fruit species (i.e. Spain, the main world producer, with 55 more than 575.000 ha, MAPA (2021)). Tree density in commercial orchards is very 56 different from traditional, low density (less than 100 trees ha<sup>-1</sup>) until hedgerow (greater 57 than 1000 trees ha<sup>-1</sup>). In addition, fruits could harvest for different purposes: table, in 58 which fruit size is very important, and oil. Combinations of these two factors, tree density 59 60 and yield use, affect irrigation scheduling.

Yield response to drought is variable according to the moment and level of the 61 62 water stress as in other fruit trees (Hsiao, 1990). As in other drupes, drought response is very related with endocarp development (i.e. peaches, Chalmers and Wilson, 1978). In 63 64 olive trees, drought sensitivity is decreasing from shoot sprouting until pit hardening. 65 Water stress during flowering and previous days (around end of Winter to early Spring) strongly reduced fruit yield (Moriana et al., 2003; Beyá-Marshall et al., 2018; Hueso et 66 al., 2021). Final floral development, before full bloom, is the most sensitive period 67 68 because reduction in the number of fruits was maximum and compensatory fruit growth was the lowest (Rapoport et al., 2012). After flower fertilization, endocarp is growing and 69 70 hardening (Rapoport et al., 2013). Endocarp growth finishes massive endocarp hardening begins. Moderate water stress conditions in this period decreased final endocarp size and 71 72 reduced final fruit growth, even if full irrigated conditions were provided after (Gucci et 73 al., 2009; Gómez del Campo et al., 2014). Such decrease in fruit size reduced fruit yield significantly (Gucci et al., 2009; Gómez del Campo 2013) but lower than in the previous 74 stage. Severe water stress conditions in this period reduced, in addition, the amount of 75

flower in the next season (Gucci et al., 2019). When endocarp size was maximum, 76 77 hardening was increasing faster and the massive pit hardening started (Rapoport et al., 2013). In this period, the effect of water stress in yield was small, even null in moderate 78 79 conditions (Goldhamer, 1999). Most of the irrigation works defined this latter period considering central Summer months as constant dates and not evaluated the hardening 80 process. Some works used knife cutting but because pit hardening is dynamic and 81 progressive, this traditional methodology (Sanz et al., 2002) is very imprecise. Rapoport 82 et al (2013) proposed the fruit length as objective indicator to establish the beginning of 83 this period, but there are not any for identifying the end at field conditions. On the other 84 85 hand, the hardening process presented a sigmoid curve with two slow slope periods at the beginning and at the end in the middle of a fast increase (Rapoport et al., 2013). Therefore, 86 the end of the pit hardening, even with an accurate evaluation, would be difficult. Then, 87 88 regulated deficit irrigation (RDI) strategies commonly fixed a recovery period from the last month of Summer period, which changed slightly according to fruit use, earlier in 89 90 table olive (Girón et al., 2015) than in oil olive (Fernández et al., 2013). The effect of water stress during this recovery period until harvest was variable. In table olive, 91 controlled water stress with values less negative than -2 MPa of midday stem water 92 93 potential (SWP) did not reduce yield significantly (Martín-Palomo et al., 2020). The amount of oil was reduced when water stress was more negative than -2.7 MPa (Hueso et 94 al., 2019). 95

Pit hardening was the most interesting period for a RDI strategy because is the
ones with the greatest irrigation needs but the less yield-sensitive to water stress.
Moreover, in mature orchards, vegetative growth is not affected because it stopped for
the hardening process (Rallo and Suárez, 1989). RDI needs to know the response of yield
component to define an accurate irrigation scheduling that can extrapolate to different

101 conditions. In table olive, fruit size and drop have been reported as the most limiting 102 factor (Corell et al., 2020). In oil olive, the fruit oil is also very important. Oil 103 accumulation is a complex process depending on cultivar and environment (Navas et al., 104 2019). Some works reported that oil accumulation decreased in moderate water stress conditions until pit hardening (from -1.80 MPa) and final Summer/early Autumn (from 105 here so-called recovery period) (from -2.71 MPa) (Hueso et al., 2019 and 2021). During 106 107 pit hardening, some works reported a decrease in oil accumulation with severe water stress (Lavee and Wodner, 1991; Moriana et al., 2003; Fernández et al., 2013; Naor et al., 108 2013; Ahumada-Orellana et al., 2017; Ben-Gal et al., 2021). However, moderate water 109 110 stress conditions would increase fruit oil content (Moriana et al., 2003; Lavee et al., 2007; Fernández et al., 2013; Ben-Gal et al., 2021). 111

112 The aim of this work was to evaluate the yield respond of several RDI strategies that 113 consider different water stress levels during pit hardening and recovery considering fruit 114 drop, size and oil accumulation.

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#### 116 MATERIAL AND METHODS

## 117 *Site and treatments descriptions*

The experiment was performed during three consecutive years (2017 to 2019) in a commercial hedgerow olive orchard in Carmona (37.49°N, -5.67°W, Seville, Spain). The orchard, cv Arbequina, 4\*1.5 m spacing (1667 tree ha<sup>-1</sup>), was 11 years-old at the beginning of the experiment. Irrigation system was a single line of drips ( $3.4 \text{ L} \text{ h}^{-1}$ ) which were spaced 0.4 m. Irrigation scheduling was daily based on trunk growth rate (TCT) measurements. Data of water relations respond, and usefulness of this approach are widely discussed in Corell et al (2019) and Martín-Palomo et al (2021). In the current work, the most important data of these latter works were summarized or presented as complementary information. The soil had a sandy-loam texture with a high pH level (8.4) and a high percentage of carbonates (greater than 25%). The amount of  $P_2O_5$  and  $K_2O$  in the soil was adequate, and so was the percentage of organic matter (1.8%).

129 Four irrigation treatments were performed in a completed randomized block design with 4 repetitions. Irrigation season was divided in three phases which were 130 131 considered for deficit treatments: a) before pit hardening, b) until the las week of August (pit hardening period) and c) until harvest (rehydration period). No irrigation was 132 provided in postharvest. The beginning of pit hardening was dated according to Rapoport 133 134 et al. (2013). Briefly, sharply change in the increase of longitudinal growth of the fruit was identified as the beginning of the pit hardening period. Irrigation was scheduled daily 135 with a remote programming device (Ciclon, C-146 v 3.53, Maher, Almeria, Spain). 136 Applied water was measured every week with a water meter in each plot. Irrigation 137 138 treatments were:

Control. The objective of this treatment was full irrigated conditions along the season. During 2017, applied water was based on Steduto et al. (2012) approach using (Kc) was 0.55 and the reduction coefficient (Kr) was 0.47. However, this approach decreased SWP at mid-season. Then to ensure an optimum water status, the amount of water applied in the 2018 season was around 150% ETc and, in 2019, it was around 175% ETc.

RDI-1. The objective of this treatment was an optimal RDI with no limitation in applied water. Before pit hardening and during rehydration periods near optimum water status was scheduling. During pit hardening, moderate water stress conditions were performed. Water stress level was controlled using trunk growth rate (TGR) frequencies (Corell et al., 2019 and Martín-Palomo et al., 2021 for

more details). Briefly, only values of TGR lower than -0.1 or greater than 0.3 mm
day<sup>-1</sup> was considered as water stress indicators. TGR weekly frequency values
were minimized during no/low water conditions while was progressively
increased during water stress.

- RDI-2. The objective of his treatment was to performed an RDI strategy but with a maximum amount of seasonal water (2017, 150 mm, 2018 170 mm, 2019, 270 mm). The increase in the seasonal maximum applied water in 2019 was due to very scarce amount of rains. Irrigation scheduling was based on the TGR approach but with more severe water stress during pit hardening and a partial recovery during rehydration.
- Sustained deficit irrigation (SDI). The objective of this treatment was applied the
   same amount of water than RDI-2 but with no control of water stress along the
   season.
- 163 *Water relations and shoot growth*

Climatic data were obtained from the Andalusian network climatic station 164 "Villanueva de Rio Minas" 165 y (http://eportal.mapa.gob.es/websiar/SeleccionParametrosMap.aspx?dst=1), 9.4 km far 166 167 from the experimental orchard. Average seasonal data were obtained of this station in the 168 period (2007-2016) to compare with the current data obtained. Plant water relations were 169 described using SWP, gas exchange measurements, trunk diameter fluctuations and soil 170 moisture determinations. These data are described and analyzed in Corell et al (2019) and 171 Martín-Palomo et al (2021), only part of SWP data are presented in the current work. SWP was measured weekly at midday with a pressure bomb. Leaves near the main trunks 172 173 were covered with aluminum bags two hours before measured. Stress integral (SI) was

174	estimated using the seasonal SWP data according to Myers (1988) using as maximum
175	value -1.2 MPa, as suggested Moriana et al (2012). The expression used was:
176	$SI =  \sum (SWP - (-1.2)) * n  $ (1)
177	Where:
178	SI: Stress Integral
179	SWP: average midday stem water potential between two consecutive measurements
180	n: number of days between two consecutive measurements
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182	Ten one-year-old shoots per plot were randomized selected at the beginning of
183	each season and marked. Expansion was measured every 15 days along the experiment.
184	The number of fruits was counted at the beginning of the pit hardening. Percentage of
185	fruit drop was estimated as the ratio of the difference between this first date and the
186	amount of fruit just before harvest vs the initial number of fruit.
187	Fruit development
188	Fruit dimensions (longitudinal and equatorial dimensions) were measured in 10 fruits per
189	plot periodically from fruit set until harvest and fruit volume estimated. Fruit longitudinal
190	pattern was used for estimating the beginning of the pit hardening (Rapoport et al., 2013).
191	From pit hardening until harvest, a 250 g of fruit sample per plot was randomized selected
192	to estimate oil content (fresh and dry weight) and fruit moisture several times along the
193	season. The last determination was always at harvest and fruit oil content was determined

194 with Soxhlet method.

Accurate description of the pit hardening process was conducted during 2019 195 196 season fruit set until harvest. A randomized sample of 40 fruits per plot were randomized collected weekly. The half of the sample was weight and dry until constant weight in an 197 oven at 70°C to determine fruit dry weight. The rest were used for determination of pit-198 breaking pressure using the experimental device described in Rapoport et al (2013). Pit-199 breaking pressure curves were adjusted to a sigmoid curve in order to estimate the 200 201 parameters: (a) range of pit-breaking pressure, (b) slope coefficient, (c) date of inflection 202 and (d) minimum pit-breaking pressure (Rapoport et al, 2013). Slope coefficient was used to estimate the beginning and the end of the massive pit hardening. Date f inflection was 203 204 considered the ones when 50% of pit hardening occurred.

205 Yield response.

Harvest date changed between seasons from the earliest in 2019 (24<sup>th</sup> October, day of the 206 year (DOY) 297) to the latest in 2018 (11<sup>th</sup> December, DOY 345). These changes were 207 related with commercial decisions of the owner (climatic conditions, oil prices, fruit oil 208 209 content, machinery availability). The central line of each plot was harvested with a grape 210 straddle harvester for hedgerows and weight individually. Then a sample of each plot of 211 around 1.5 kg were obtained for oil content and other fruit features at laboratory. Size of 212 fruits were estimated with the number of fruits per kilogram in a sample of 250g. Mature index (IM) was determined in 100 fruits per plot according to Hermoso et al. (1997). Oil 213 214 yield was estimated as the results of fruit yield by the percentage of oil content in fresh 215 weight.

216 Statistical analysis

Statistical analysis were carried out with ANOVA and mean separation (Tukey's test)
using the Statistix (SX) program (8.0). Significant differences were considered for the p-

level <0.05 in both tests. The data normality was tested using a Shapiro-Wilk test. 219 220 Calculations of the p-level were performed considering the F-test of variance equality 221 (Homoscedasticity). When conditions of variance equality could not be obtained, a 222 decrease in the degree of freedom and, therefore, a more restrictive p-value was calculated. The number of samples measured is specified in the text and figures. In order 223 224 to evaluate irrigation treatments according to water stress level, lineal regressions were calculated between percentage of fruit drop, percentage of oil in dry weight, fruit yield 225 and oil yield vs SI and vs Minimum Midday SWP ( $\Psi_{min}$ ) at different periods. 226 Multivariable analysis was performed using several water stress indicators but they did 227 228 not improve the single relationship and they are not present.

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#### 230 **RESULTS**

## 231 *Water relations and shoot growth.*

232 The pattern of the rainfall and reference evapotranspiraion (ETo) along the experiment 233 was the one expected in a Mediterranean climate (Figure 1). Most of rainfall was concentrated during Autumn and Winter. Null amount of rainfall was measured in long 234 periods of Spring/Summer. The beginning of the rainfall period was delayed until second 235 236 half of September, even October in 2017 season. Seasonal amount of rain was below the 20 years-average of the station (539 mm) in 2017 (278 mm) and 2019 (328) but greater 237 in 2018 (705 mm). ETo was maximum around mid-Summer with values near 8 mm day 238 <sup>1</sup>. ETo values greater or around 6 mm day<sup>-1</sup> was measured from mid-May until last week 239 of August in the three seasons. 240

Applied water changed between seasons in order to maintain the water status defined in each treatment (see Materials and Methods). In each season, all treatments

started irrigation at the same dates but these changes between years (Figure 2). The 243 244 earliest season was 2019 because of the scarce rains during Winter though the real beginning was around the end of April. Irrigation in Control treatments was almost lineal 245 246 and increased from 2017 (around 400 mm) to 2019 (near to 1000 mm). RDI-1 presented a lineal pattern also along the season but with lower slope than Control. After deficit 247 248 period, RDI-1 slightly increase the application rate. Seasonal applied of water was lower 249 in 2017 (180 mm) than in the other two (around 400 mm), such differences were likely related with climatic conditions (Figure 1) and yield, the lowest in 2017. RDI-2 and SDI 250 presented a similar seasonal amount of water in all years but changes the application 251 252 pattern. In both treatments, seasonal irrigation was between 150 to 180 mm in 2017 and 2018 but higher in 2019, 250 mm. In RDI-2 application rate was slower, sometimes null, 253 254 during the deficit period but greater during recovery than SDI.

Midday stem water potential (SWP), soil moisture and gas exchange data were 255 256 presented in previous published work (Corell et al., 2019 and Martín-Palomo et al., 2021). 257 Table 1 summarizes the average SWP of each season in order to describe the general pattern. In all seasons, the less negative SWP were obtained before pit hardening, during 258 the active shoot growth. In this period, water status was similar between treatments, only 259 260 in 2019, Control presented a trended of lower negative SWP than the rest. The most negative values of SWP were measured during pit hardening period. Control presented 261 262 the less negative values in all seasons with the greatest differences during 2019. The most negative were obtained in RDI-2, while RDI-1 and SDI presented similar values. Finally, 263 264 rehydration improved the water status in all treatments, though this was almost null in 265 SDI in comparison of the rest. However, only RDI-1 was near to Control in 2018 and 2019, then RDI-2 presented only a partial recovery. 266

Water stress integral (SI) is presented in Figure 3. Significant differences were 267 268 found in 2018 and 2019. In all treatments, SI during phase II (pit hardening) was greater than during Phase III (rehydration). Control presented the lowest value in all seasons and 269 270 SI was decreasing from 2017 to 2019, when was almost null. RDI-1 presented similar values than the rest of RDIs treatments with not significant differences with them but 271 either with Control. SI was clearly reduced in RDI-1 during 2018 and 2019. On the 272 273 opposite, RDI-2 obtained the greatest SI values in all seasons though only significantly 274 different of Control during 2018 and 2019.

275 Shoot expansion data are presented in Figure 4 in the three seasons of the 276 experiment. In all years and treatments, the seasonal patterns were similar. The main 277 period of shot growth was until the beginning of pit hardening period when it stopped. Stopped date was equal for all treatments. Only in Control data during 2019 season, shoot 278 279 growth presented an increased until the half of the pit hardening period. The effect of irrigation treatments in shoot expansion was significant in some seasons but not clear. No 280 281 significant differences were found during 2017 in any dates. In 2018, when maximum shoot expansions were measured, significant differences were found only between SDI 282 283 and Control, with greater growth in the former than in the latter. In 2019, SDI, RDI-1 and 284 Control were not significantly different between them. Only Control was significantly greater than RDI-2 most of the season. 285

Figure 5 presented the relationship between percentage of fruit drop in each shoot and SWP and SI. In this Figure, prediction of the Corell et al (2020) is also presented. According to this latter equation, fruit drop would be maximum in the most stressed treatments. However, no significant relationships were found with any of the indicators selected and data are clearly out of the equation suggested in Corell et al (2020). In both Figures, fruit drop was almost independent of water status of the tree and almost constantaround 20%.

## 293 Fruit development

Fruit volume was significantly affected for water stress in all seasons (Figure 6). The 294 295 seasonal pattern of fruit development was similar between seasons with a period of low fruit growth during pit hardening and great increased from the end of August. Fruit 296 297 volume was lower in all treatments during 2017 season in comparison to 2018 or 2019. 298 RDI-1 was significantly lower than Control only at the end of 2017. In 2018 and 2019, 299 fruit volume of this treatment was slightly lower but not significantly of Control at the 300 beginning of the rehydration period and almost equal in the last 2-4 weeks before harvest. 301 Significant and persistent differences were found in all seasons in the most severe water 302 stress treatments from the second part of pit hardening. From this date, in all seasons, 303 Control treatments were significantly greater than RDI-2 and only in 2018, the recovery 304 of this treatment reached values similar to the maximum. The pattern of SDI was similar 305 to the RDI-2 but with less significant differences with Control. SDI treatments commonly 306 oscillated between RDI-1 and RDI-2 with not significant differences between them in most of the dates. Only at the end of all seasons, RDI-2 and SDI were almost equal. 307

308 The pattern of oil accumulation was almost lineal in all treatments and all seasons (Figure 7). The last measurements of 2018 season (the ones with the latest harvest date) 309 310 suggests, that in fact, there was a saturation curve. There were no significant differences in any of the season in percentage of oil (dry weight) in fruit and in the slope of these 311 accumulation lines. At the beginning of the recovery period around the half of the total 312 313 oil content was reached. In all seasons, slightly trends of greater values in SDI and RDI-1 than Control and RDI-2 were found. The difference at harvest date between the greatest 314 315 values (in all season SDI) and the lowest (Control in 2017 and RDI-2 in 2018 and 2019) varied from 6 in 2017 to 2 in 2018, which suppose a relative increase of 18 and 5%,respectively.

318 The relationship between fruit moisture and SWP was significant in the three 319 seasons (Figure 8). These fits showed that fruit moisture strongly decreased with SWP. 320 Data obtained in 2017 and 2018 presented an almost equal fit with a range in fruit 321 moisture between 45 to 65%. On the contrary, data of 2019 season presented lower fruit 322 moisture than the two previous years. The fit obtained in this latter season was significantly different for the other two. The period of sampling in the three seasons were 323 similar from the end of July to mid-September. There were more fruit sample later than 324 325 the ones of Figure 8 but they are not included because SWP measured occurred in a 326 different date.

327 The relationship between minimum SWP during pit hardening and percentage of 328 fruit oil in a dry weight at harvest is presented in Figure 9a. The percentage of oil in all 329 the plots during 2019 were greater than the ones obtained in 2017 and 2018. The best fit 330 in 2017 data were quadratic but with a pattern of increase when SWP was less negative 331 in all the range considered (maximum value greater than 0). Two data of 2017 were not included because they corresponded with two Control plots in which problem with 332 333 irrigation affected strongly trees water status. No significant relationships were found with the 2018 data. But, when both dataset (2017 and 2018) were included a significant 334 regression was fitted. This common regression using both seasons showed that though oil 335 content increase with less negative values of SWP, there were a maximum around -1.8 336 337 MPa. The best fit was obtained with 2019 data, error was the lowest and the range of 338 SWP was the widest. In this latter season, the best fit was also quadratic with a maximum value around -3.6 MPa. Fits did not improve using Stress integral values. 339

The relationship between oil data of Figure 9a and minimum SWP during recovery 340 341 was analyzed in two ways (Figure 9b). First, only data below -2.1 MPa was considered 342 according to Hueso et al (2019). In this case, only 2017 data presented a significant lineal 343 relationship between oil content and SWP. In this equation, oil percentage in the fruit decreased with the decrease of SWP. Second way considered all data available in each 344 345 season. No significant relationship was found with 2017 and 2018 data. But when both 346 seasons were considered a poor quadratic fit was adjusted. In this latter equation, greater fruit oil would reach with the increase of SWP (maximum at positive value, greater than 347 0). Data of 2019 season presented the best fit, and also quadratic equation. In this case, 348 349 maximum oil content would be around -3.4 MPa. No better relationships were found with Stress Integral in any of the seasons. The equations calculated in this Figure were poorer 350 than the ones of Figure 9a. Adjusted was not improved when both SWP used. 351

Additional fruit features (fruit dry weight and pit breaking pressure) were 352 characterized only in 2019 (Figure 10). Fruit dry weight presented a lineal increase along 353 354 the experiment in all treatments (Figure 10a). Only at the end of the stress period significant differences were found between RDI-2 and Control. From this date, RDI-2 355 356 trended to lower values that the rest of treatments but differences were not significant. 357 The seasonal pattern of the pit-breaking pressure was a sigmoidal curve with a short period of great increase from DOY 164 to 199 (Figure 10b). Small significant differences 358 359 were found between Control and SDI in the last period of hardening when maximum 360 values were reached. There was a significant relationship between average SWP of this 361 period of greatest hardening increase and fruit oil at harvest (Figure 10c). This equation 362 was similar in accurate and shape to the ones obtained in other periods (Figure 9). However, maximum values of percentage of oil would be obtained with SWP around -363 364 1.6 MPa.

Table 2 contains the average parameters that defined the individual pit-hardening 365 366 pressure curves. These parameters showed a significant delay in the pit hardening curve of Control in comparison to the rest of treatments. Date when 50% of maximum values 367 368 were reached were significantly later in Control (around DOY 181) than the rest of treatments (around DOY 179). The slope of increase trended also to lower values in 369 370 Control than in RDI-2 and SDI though no significant differences were found. Maximum 371 values of pressure trended also to greater values in Control than in RDI-2 and SDI. RDI-1 data presented intermediate pattern with a significant advance to Control curve but 372 similar slope and maximum pressure values. 373

374 Yield response.

375 Figure 11 and Table 3 present the main results related with yield and its components along the experiment. The lowest fruit yield was measured in 2017 and the greatest in 2018 in 376 377 all treatments (Figure 11). Fruit and oil yield were not significantly different in any of the 378 seasons (Figure 11). Control trended to the greatest fruit yield value in the three seasons 379 and RDI-2 or SDI to the lowest (Figure 11). This result was in agreement with fruit size 380 at harvest. The number of fruits per kg were significantly lower or trended to lower values in Control and RDI-1 (depending of the season) than RDI-2 which was always the greatest 381 382 (Table 3). However, differences in fruit yield between treatments were reduced or even 383 changed in oil yield (Figure 11) when the oil percentage basic on fresh weight (Table 3) 384 was considered. Although this latter measurement was not either significant, all deficit treatments trended to greater oil percentage in fresh weight than Control (Table 3). 385 386 Differences between Control and the rest of treatments in percentage of oil varied from 387 0.3 (2018 Control vs RDI-2) to 3 (2017 Control vs RDI-2 and SDI) (Table 3). Such 388 differences in oil percentage were not related with the ripening process because the 389 mature index data were not significantly different between treatments in any of the

seasons (Table 3). Harvest occurred at the beginning of the verasion (IM around 2, Table 3) in all seasons. Applied water increased along the experiment with significant differences between Control (maximum), RDI-1 and the other two treatments. RDI-2 and SDI were very similar and not significantly different between them (Table 3). On the other hand, water productivity was significantly greater in RDI-2 and SDI than Control in most of the seasons, with maximum values during 2018 and minimum during 2019 season (Table 3).

Figure 12 presents the analysis of fruit and oil yield with single plot data and their 397 water status. Fruit yield presented significant regressions in 2017 and 2019 seasons but 398 399 not in 2018, the season with the latest harvest date (Figure 12a). In both seasons, 2017 400 and 2019, the best relationships were obtained with minimum SWP during pit hardening. Fruit yield decreased progressively with more negative values of SWP. No common 401 regressions were found between 2018 and the other two set of data. Oil yield presented 402 significant regressions also in 2017 and 2019 seasons with the total SI data (Figure 12 b). 403 404 Both of them were significantly different. In 2017, the best fit was a lineal regression with a continuous decrease of oil yield with water stress. There was a significant quadratic 405 406 adjust with 2018 and 2019 data, but this fit improved if only 2019 season was considered. 407 The relationship of 2018 and 219 data showed a maximum value at 109.3 MPa\*day, but in the interval until this value the equation looked almost constant. The decrease in oil 408 409 yield was strong from around 200 MPa\*day. The range of SI data in 2018-2019 equation 410 were wider than in 2017 adjust. In fact, there were two groups of data in 2017 season: lower than 100 MPa\*day (average oil yield 1049±105 kg ha<sup>-1</sup>) and greater (average oil 411 yield  $780 \pm 66 \text{ kg ha}^{-1}$ ). 412

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#### 414 **DISCUSSION**

Drought resistance of oil and fruit yield was extremely great with deficit irrigation from 415 416 pit hardening. Extremely low SWP from this period (Figure 9) with a great duration of the level of water stress (Figure 3) and even partial rehydration (Table 1) resulted in a no 417 418 significant fruit and oil yield reduction (Figure 11). However, when water status of each plot was considered, there was a significant affection in oil and, some seasons, fruit yield 419 420 (Figure 12). Several works suggest a great response of olive to irrigation (Moriana et al., 421 2003; Ben-Gal et al., 2011). But when water stress was controlled (Ben-Gal et a., 2021) 422 and not coincident with sensitive period such as olive flowering (Moriana et al., 2003) the decrease in oil yield was low (Moriana et al., 2003; Ben-Gal et al, 2021). The decrease 423 424 presented in the current work (Figure 12) was similar to the ones reported in other previous work with moderate water stress (Fernández et al., 2013; Naor et al., 2013; 425 426 Ahumada-Orellana et al., 2017). The lack of results when average treatment is considered 427 (Figure 11) suggested that variability of water status between plot compensated the effect 428 of irrigation strategies. These support the conclusion of Ben-Gal et al (2021) about the 429 limitations of SDI in comparison to RDI strategies and the utility of water status measurements in olive irrigation scheduling (Moriana et al., 2012; Girón et al., 2015; 430 Ahumada-Orellana et al., 2017; Hueso et al., 2019; Corell et al., 2020; Ben-Gal et al, 431 432 2021; Hueso et al., 2021).

Crown volume and fruit load are two factors which could affect yield response to irrigation (Naor et al., 2013; Corell et al., 2020; Pierantozzi et al., 2020). However, the former is associated with young irrigated olive orchard and the latter with cultivars with alternate bearing pattern. None of these factors are commonly presented in mature hedgerow olive orchards. In these orchards, cultivar (the most common is Arbequina) has not a strong alternate bearing pattern (Barranco, 1997) and crown volume is similar at the beginning of each season to facilitate mechanic harvest. Therefore, current seasons

factors are more important than in intensive or traditional orchards. Oil production has 440 441 three main yield components: number of fruits, fruit size and oil content. Fruit drop was almost null in the current work (Figure 5) with some levels and duration of water stress 442 443 very severe. Fruit drop is not commonly evaluated in irrigation works, but previous work in Manzanilla (table olive) reported that yield reduction would be significant with water 444 445 stress level more negative than -2 MPa of SWP (Girón et al., 2015; Corell et al., 2020). 446 Such yield reduction would be lower than the ones predicted in the shoots (Corell et al., 2020) but it could be important in the greatest level of water stress. The lack of results in 447 this component was likely related with the cultivar (Arbequina, oil olive vs Manzanilla, 448 table olive). 449

Fruit size was significantly affected along the experiment (Figure 6) but partially 450 recovered at harvest even in the most stressed treatments (Figure 6 and Table 3). Most of 451 the differences in fruit yield (Figures 11 and 12) were likely related with this component. 452 453 In fact, part of the differences in fruit yield are only differences in fruit moisture. The 454 absence of relationship between fruit yield and SWP during 2018 suggests that the fruit moisture could be not limiting in that season. 2018 season was the latest harvest date with 455 456 a lot of rains previously (Figure 1) and fruit volume was almost equal (Figure 6). On the 457 contrary, 2017 and 2019 seasons, in which harvest date was before most of the rains, presented reductions fruit volume at harvest (Figure 6) some of them likely related with 458 459 fruit moisture (Figure 12). Other data that support that part of fruit yield differences are 460 related with fruit moisture is the comparison between the two graphs of Figure 12. 2018 461 and 2019 oil yield data are in the same adjust while they are clearly different in fruit yield 462 (Figure 12). In 2019 season, the year with the greatest level of water stress (Figure 3), no significant differences were found in fruit dry weight (Figure 10). Therefore, according 463 464 to the strong relationship between fruit moisture and SWP (Figure 8) the lineal decrease

in fruit yield with SWP (Figure 12) was strongly related with differences in fruit water 465 466 content. Reduction in fruit size was reported as very sensitive to water stress (Girón et al., 2015; Ahumada-Orellana et al., 2017; Martín-Palomo et al., 2020) and related with less 467 468 absorption of water during the recovery period in table olive (Girón et al., 2015; Corell et al., 2020). But, though fruit size is an important yield component, it is not a quality feature 469 470 in oil production. Moreover, if the increase in fruit yield is related with an increase in fruit 471 moisture, this will affect oil industrial extractability (García et al., 2013) and, in fact, reduced commercial oil yield. Then, irrigation scheduling should be adapted to minimize 472 this effect (Ben-Gal et al, 2021), which autumn rains would enhance. Slightly lower fruit 473 474 yield than Control (such as RDI-1) but with similar oil yield than full irrigated would secure greater industrial oil yield and then an optimization of the grower's profit. On the 475 476 other hand, the reduction in fruit size in 2017 season in comparison with the rest of the 477 years (Figure 6 and Table 3) was the main factor that limiting fruit and oil yield in this 478 season. This decrease was not related with alternate bearing pattern because low fruit 479 yield was not associated with greater fruit size (i.e. Girón et al., 2015; Corell et al., 2020). 480 Such lower fruit size was likely produced because of the water stress around the beginning of pit hardening (Table 1 and supplementary material S1) that likely reduce endocarp size 481 482 and fruit volume. Affection of endocarp growth reduced fruit yield even though full irrigation recovery was applied (Gómez Del Campo et al., 2014). Water stress level of 483 this latter work (Gómez del Campo, 2013) was similar to the supplementary material S1 484 485 but did not affected next season flowering (Figure 11) as described in more severe 486 conditions (Gucci et al., 2019).

487 Oil accumulation curves (Figure 7) and oil yield (Figure 11) was not significantly
488 affected. But significant relationships between oil yield and IS in the plot data were found
489 (Figure 12b). Those support the trends of Figures 7 and 11 of lower values in percentage

490 of oil in Control than some deficit irrigation strategies (RDI-1 and SDI). Such differences 491 compensated in oil yield the differences in fruit yield (Figure 12). Therefore, moderate water stress would enhance oil accumulations while severe water stress or full irrigated 492 493 conditions reduced. Oil accumulation are commonly reported as sensitive to water stress (Lavee and Wodner, 1991; Gómez del Campo, 2013; Ahumada-Orellana et al., 2017, 494 495 Hueso et al., 2019; Hueso et al., 2021). However, other works suggested that moderate 496 water stress promoted oil accumulation (Moriana et al., 2003; Grattan et al., 2006; Gucci et al., 2007; Lavee et al, 2007; Fernández et al., 2013; Ben-Gal et al., 2021). Such 497 differences in the literature could be related with the level of water stress but also with 498 499 the moment. Similar relationships between oil content and SWP were found in three 500 different part of the season in 2019 (massive pit hardening (Figure 10c), pit hardening (Figure 9a) and rehydration (Figure 9b)) and in two of them in 2017-2018 (Figure 9). 501 502 Data of the current work cannot conclude which is the most limiting or if there was a 503 synergy between periods. In fact, all of them could be related because the best agreement 504 of oil yield was with seasonal IS (Figure 12b). Even in 2017 season, when oil yield was 505 limited for fruit growth, the relationship suggested a similar respond to the 2018-2019 (Figure 12b). Rehydration period, the nearest to harvest, presented the worse agreement 506 507 (Figure 9b). Hueso et al. (2019) concluded that in this period oil content is almost constant until SWP around -2 MPa and presented a lineal decrease from this threshold. This latter 508 509 relationship was not found in most of the data of Figure 9b and suggest that could be the 510 less important. Better agreements were found with the water stress in pit hardening 511 (Figure 9a) with maximum oil values reached at -1.7 MPa (2017-2018) and -3.6 MPa 512 (2019). Such range is the common in irrigation works that reported increase of oil accumulation (Moriana et al 2003; Grattan et al., 2006; Fernández et al., 2013; Ben-Gal 513 et al., 2021). Finally, water stress during the fast-hardening period (massive pit hardening) 514

around -1.6 MPa (Figure 10) maximize oil accumulation. Hueso et al. (2021) reported a 515 516 lineal decrease of fruit oil with more negative values than -1.8 MPa, but not described any improvement. Massive hardening process needs a great amount of assimilates (Rallo 517 518 and Suárez, 1989), then if it was ended before in deficit than in Control irrigation (RDI-1, Table 2) part of these assimilates could be use in oil production. Hammani et al (2013) 519 520 concluded that water status affected the timing of sclerification, faster in rainfed than in 521 full irrigated. Recently, López-Bernal et al (2021) concluded oil content could be 522 modelling with two parameters: slope of accumulation ( $\beta$ ), independent of fruit carbon 523 assimilation, and fruit dry weight at onset of oil accumulation (w<sub>fo</sub>), depending between 524 others, the water relation in the earlier stage of fruit growth. This latter parameter ( $w_{fo}$ ) 525 could be associated with changes in massive pit hardening process related with water stress (Figure 10). 526

#### 527 CONCLUSIONS

Regulated deficit irrigation optimized yield responds in olive trees. Regulation of the 528 moment and the water stress level was decisive in identify the best respond. Oil yield was 529 related with two main components: fruit size and oil content because the effect of water 530 531 stress conditions in fruit drop was null. This latter result was cultivar dependent and could 532 change significantly the respond in other orchards. Fruit size variations was mainly 533 produced for fruit moisture and its decrease would be strongly reduce with irrigation or rain. Similar fruit size with low fruit yield reduction were obtained in RDI-1 strategy 534 which were associated with low fruit moisture. Oil content was increased with moderate 535 536 water stress in different periods and RDI-1 and SDI trended to the greatest values at harvest. Maximum oil yield was obtained in plots which seasonal SI was lower than 100 537 MPa\*day. These levels of water stress included plots with moderate water stress during 538 539 pit hardening and moderate or null in rehydration which maximize the two factors 540 considered, fruit size and oil content. The period between the end of maximum endocarp 541 growth and the end of massive pit hardening was critical. Water stress in this period 542 decreased fruit size irreversibly and then reduced oil yield. But also, oil content was 543 maximized with less severe water stress during massive pit hardening. Endocarp 544 development, growth and hardening, are the most critical moment in oil yield.

#### 545 Acknowledgement

546 This research was supported by the Agencia Española de Investigación (AEI) and the

547 Fondo Europeo de Desarrollo (FEDER) project AGL2016-75794-C4-4-R. Authors

thank to Finca El Morillo for their helpful assistant during the experiment, specially to

549 Mr Javier León.

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Table 1. Average and standard error of midday stem water potential (SWP) in the three

680 periods considered in the irrigation scheduling (Growth, vegetative growth period

Period	Control	RDI-1	RDI-2	SDI
2017. Growth	$-1.07\pm0.10$	$-1.04 \pm 0.08$	$-1.06 \pm 0.07$	$-0.98\pm0.06$
2017. Pit	$-2.01 \pm 0.13$	$-2.81 \pm 0.13$	$-3.10\pm0.18$	$-2.34 \pm 0.13$
2017. Reh	$-1.07\pm0.04$	$-2.07\pm0.17$	$-2.24\pm0.20$	$-2.41 \pm 0.25$
2018. Growth	$-0.76\pm0.04$	$-0.75 \pm 0.03$	$-0.79\pm0.04$	$-0.76 \pm 0.03$
2018. Pit	$-1.57\pm0.07$	$-1.91\pm0.10$	$-2.77 \pm 0.19$	$-2.00 \pm 0.14$
2018. Reh	$-1.06\pm0.04$	$-1.11 \pm 0.06$	$-1.62 \pm 0.17$	$-1.84 \pm 0.21$
2019. Growth	$\textbf{-0.82} \pm 0.02$	$-1.01 \pm 0.04$	$-1.19\pm0.05$	$-1.14 \pm 0.05$
2019. Pit	$-1.07\pm0.04$	$-2.06\pm0.12$	$-3.40\pm0.25$	$-2.52 \pm 0.17$
2019. Reh	$-1.14 \pm 0.04$	$-1.33\pm0.07$	$-2.54\pm0.28$	$-2.34 \pm 0.21$

681 before pit hardening; Pit, pit hardening period; Reh, rehydration period).

682

Table 2. Parameters and variability of the fit adjusted in the pit-breaking pressure curves (Figure 9b). Max (Maximum pit breaking pressure, MPa), Slope (velocity of increase of pit-breaking pressure, MPa days<sup>-1</sup>), 50% hard (date when 50% of maximum value was reached, DOY), Min (Minimum pit breaking pressure, MPa). Curves were estimated in each plot with 14 data. Parameters of this table are the average of 4 data. Different letters in the same parameter indicates significant differences between treatments (p<0.05, Tukey test).

Parameter	Control	RDI-1	RDI-2	SDI
Max	168.81±5.6 a	164.42±4.5a	153.78±2.1 a	155.76±7.8 a
Slope	0.15±0.02 a	0.14±0.01 a	0.21±0.01 a	0.19±0.03 a
50% hard	180.5±0.4 a	178.8±0.5 b	178.6±0.2 b	178.7±0.3 b
Min	3.13±2.17 a	2.19±1.66 a	8.39±0.99 a	5.90±3.00 a
<b>R</b> <sup>2</sup>	0.71	0.85	0.79	0.74

691

Table 3.- Summary of water efficiency and fruit quality during the 3 years of the experiment. (average  $\pm$  standard error). Different letters indicate significant differences in the same year (p < 0.05, Tukey Test). Applied water (n=4, mm, AW); Irrigation Water Productivity (kg.m<sup>-3</sup>,WP<sub>1</sub>); Size (n=4, Fruits kg<sup>-1</sup>); % Oil content in Fresh weight (n=4, % fresh weight, %OCF); Maturity Index (n=4, MI).

		Control	RDI-1	RDI-2	SDI
AW	2017	431.53 ± 18.3 a	$188.19 \pm 33.1 \text{ b}$	$150.4 \pm 17.8 \text{ b}$	$148.6 \pm 5.1 \text{ b}$
	2018	532.7 ± 19.0 a	$333.5 \pm 14.8 \text{ b}$	$173.4 \pm 21.9 \text{ c}$	$175.2 \pm 5.1 \text{ c}$
	2019	972,3 ± 14,3 a	$446,3\pm8,6~b$	269,4 ± 33,4 c	$248,1 \pm 21,4$ c
WPI	2017	$1.72\pm0.15$	$5.23 \pm 1.53$	$5.28 \pm 1.63$	$4.58\pm0.57$
	2018	$3.3 \pm 0.4 \text{ b}$	$4.6 \pm 0.4 \text{ b}$	$10.4 \pm 2.2$ a	$8.2 \pm 0.9$ ab
	2019	$1.25\pm0.02~b$	$2.78\pm0.18\;ab$	$4.30\pm0.84~ab$	$4.83 \pm 0.55$ a
Size	2017	$1021.1 \pm 65.5$	1139.7 ± 111.6	$1179.1 \pm 85.5$	$949.5\pm65.5$
	2018	$654.1 \pm 18.3 \text{ b}$	$702.3 \pm 6.3$ ab	751.15 ± 31.7 a	$675.8 \pm 24.4 \text{ ab}$
	2019	$668.8 \pm 29.3 \text{ ab}$	$601.3 \pm 22.9 \text{ b}$	$728.9 \pm 37.6$ a	$627.5 \pm 45.1$ ab
% OCF	2017	$11,6 \pm 0,6$	$13,6 \pm 0,3$	$14,6 \pm 0,6$	$14,6 \pm 0,4$
	2018	$13,3 \pm 0,3$	$14,2 \pm 0,7$	$13,6 \pm 0,9$	$14.4 \pm 1.0$
	2019	$16,8\pm0,6$	$17,5 \pm 0,5$	$18,2 \pm 0,6$	$18,4 \pm 0,6$
MI	2017	$1.91 \pm 0.44$	$2.08 \pm 0.07$	$1.89 \pm 0.34$	$1.72 \pm 0.19$
	2018	$2.13\pm0.07$	$1.96\pm0.03$	$2.24\pm0.25$	$2.56\pm0.16$
	2019	$2.32\pm0.03$	$1.95\pm0.05$	$2.39\pm0.15$	$2.48\pm0.04$

## 694 **Figure Captions**

Figure 1.- Reference evapotranspiration (ETo) and effective rainfall (Rain) during the
2017, 2018 and 2019 growing seasons (Carmona, Spain). Source: Spanish Agrocimatic
network, "Villanueva de rio y minas" station
(http://eportal.mapa.gob.es/websiar/SeleccionParametrosMap.aspx?dst=1).

Figure 2.- Applied water (mm) of each irrigation treatments during 2017, 2018 y 2019seasons. Vertical lines limited the deficit period.

Figure 3.- Stress integral (SI) during pit hardening (Phase II), recovery (Phase III) and total season in the 3 years of the experiment (2017, 2018 and 2019). Each bar is the average of 4 data. Vertical lines represented standard error. Different letters at the same season indicate significant differences (p < 0.05, Tukey test).

Figure 4.- Shoot expansion (cm) of each treatment during 2017, 2018 and 2019 growing
seasons. Each point is the average of 40 data. Vertical bars represented standard error.
Vertical lines show beginning of pit hardening period, and recovery. Stars indicates dates
where statistical differences were significant (\*p < 0,05, Tukey Test).</li>

Figure 5. Relationship between Stress Integral (SI, MPa.day<sup>-1</sup>) (a) and Minimum Midday
Stem Water Potential (SWP, MPa) (b) vs Fruit Drop per shoot (%). Each point is the

average of 4 data for each treatment and season. Only shoots with fruits (see Material and
 Methods section). No significant relationships were found. Solid lines represented the

- 713 expected fruit drop according to Corell et al (2020).
- Figure 6.- Seasonal pattern of fruit volume (cm<sup>3</sup>) during 2017, 2018 and 2019 growing seasons. Each point is the average of 40 data. Vertical bars represented standard error. Vertical lines show beginning of pit hardening period, and recovery. Stars indicates dates where statistical differences were significant (\*p < 0,05, Tukey Test).

Figure 7.- Seasonal pattern of oil content (% of fruit dry weight) along the three seasons

of the experiment. Each symbol is the average of 4 data. Vertical bars are standard error.
 Vertical lines limited the beginning of the rehydration periods. No significant differences

721 were found between treatments (p < 0.05, Tukey Test).

722 Figure 8.- Relationship between midday stem water potential (SWP) and fruit moisture 723 during the three seasons of the experiment. Each symbol is a single measurement. Data 724 were included only when SWP measured and fruit sample occurred in the same date. Square and solid line, 2017 season (Fruit moisture=3.08\*SWP+63.44; R<sup>2</sup>=0.68\*\*\*; 725 Standard deviation=3.0%, N=32); Triangle and long dash line, 2018 season (Fruit 726 moisture=3.07\*SWP+63.66, R<sup>2</sup>=0.58\*\*\*, Standard deviation =3.3%, N=32); Circle and 727 short dash line, 2019 season (Fruit moisture=2.61SWP+56.73, R<sup>2</sup>=0.56\*\*\*; Standard 728 deviation =3.4%; N=48). 729

Figure 9. Relationship between minimum midday stem water potential during pit 730 731 hardening (SWP, MPa) (a) and during rehydration (SWP, MPa) (b) vs fruit oil content at harvest (% dry weight). Each point is a single measured. The two points in a circle are 732 not included in any regressions. Square, 2017 season; Triangles, 2018 season; Circles, 733 734 2019 season. No significant regressions were found with 2018 data alone. (a) 2017 adjust no presented in the figure, Oil=0.30\*SWP2+4.42\*SWP+43.81, R2=0.52\*, Standard 735 deviation=2.7%, N=14; Solid line 2017 and 2018 season, Oil=-0.47\*SWP2-736 737 1.66\*SWP+35.46, R2=0.39\*\*, Standard deviation=3.6%, N=30; Dash line, 2019 season, Oil=-0.52\*SWP2-3.70\*SWP+36.06, R2=0.49\*, Standard deviation=1.6%, N=16. (b) 738

- Solid line, 2017 season, Oil=1.82\*SWP+38.73, SWP lower than -2.1 MPa, R2=0.43\*,
  Standard deviation=3.0%, N=12; Short dash curve 2017 and 2018 data, Oil=0.11\*SWP2+0.71\*SWP+37.79, R2=0.29\*\*, Standard deviation=3.9%, N=30; Long dash
  line 2019 season, Oil=-0.81\*SWP2-5.44\*SWP+34.15, R2=0.42\*, Standard
  deviation=1.7%, N=16.
- Figure 10.- Fruit dry weight (a), pit breaking pressure (b) and relationship between
  average SWP in the period of massive pit hardening and fruit oil content (% dry weight)
  (c) during 2019 season. Symbols are the average of 20 measurements at graphs "a" and
  "b". In graph "c", single measurements of each plot are presented. Solid line, Oil=-3.72
  SWP2-12.14 SWP+32.49, R2=0.46\*, Standard deviation=1.68%, N=16. Stars indicates
  dates where statistical differences were significant (\*p < 0,05, Tukey Test).</li>
- Figure 11.- Annual fruit yield (kg ha<sup>-1</sup>), oil yield (kg ha<sup>-1</sup>) and oil content (% dry weight) as a function of irrigation treatment. Each bar is the average of 4 data. Vertical lines represent standard error. No significant differences were found between treatments in any years of the experiment in these measurements (p<0.05, Tukey Test).
- 754 Figure 12. Relationship between fruit yield vs minimum midday stem water potential 755 (SWP) (a) and Oil Yield vs Total stress integral (SI) (b). Each symbol is a single data. Square, 2017; Triangle, 2018; Circles, 2019. (a) Solid line, regression 2017 data: 756 Fruit=12225.6+1322\*SWP, R<sup>2</sup>=0.73\*\*\*; Standard deviation=1032.1 kg ha<sup>-1</sup>, N=16; 757 758 Dash line, regression 2019 data: Fruit=13278.9+553.4\*SWP, R<sup>2</sup>=0.51\*\*, Standard deviation=1001.9 kg ha<sup>-1</sup>, N=16. No significant relationship were found with 2018 data. 759 (b) Solid line, regression 2017 data: Oil=1213.5-1.9\*SI, R<sup>2</sup>=0.36\*, Standard 760 deviation=229.8 kg ha<sup>-1</sup>, N=16; Dash line, regression 2018-2019 data: Oil=-761 0.014\*SI<sup>2</sup>+3.06\*SI+2076.34, R<sup>2</sup>=0.33\*\*, Standard Deviation=276.4 kg ha<sup>-1</sup>, N=32; 762 Regression equation of 2019 data (not presented in Figure): 763 Oil=- $0.010*SI^2+1.91*SI+2063.72$ , R<sup>2</sup>=0.52\*\*, Standard deviation=204.1 kg ha<sup>-1</sup>, N=16. 764
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