1 **Post-bud burst spur-pruning reduces yield and delays fruit sugar accumulation**

2 **in cv. Sangiovese in Central Italy**

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21 **Abstract:** The influence of pruning date on yield control and ripening rate of spur-pruned

22 Sangiovese grapevines was investigated over two years (2013 and 2014). Winter pruning was

23 applied on February 1,4 (mid-dormancy); March 1,5 (late-dormancy); April 2,7 (bud swell);

24 May 2,7 (flowers closely pressed together) and June 1,6 (40-50% of flower caps fallen). Vine

25 yield and fruit composition at harvest were not affected by shifting from the standard pruning

26   dates of mid and late dormancy to the bud swell stage. By contrast, the number of

27   inflorescences from compound buds was significantly reduced for vines pruned early May.

28   No inflorescences were retained on vines pruned at the beginning of June. Early May pruning

29   reduced fruit-set and berry weight, and fruit ripening was slower when compared to the other

30   pruning dates. At harvest, must soluble solids and titratable acidity were 1.6 Brix lower and

31   1.8 g/l higher, respectively, for the May treatment as compared to the standard pruning dates.

32   The early May pruning dates also achieved higher total anthocyanins and phenolic

33   concentrations than the standard pruning dates, indication that this technique can potentially

34   decouple the accumulation dynamics of these components. Further studies are needed to

35   better calibrate winter pruning date for managing yield and berry maturation rate.

36

37   **Key words:** berry composition, bud fertility, leaf area, yield, winter pruning, reserve storage

38

39   Climate-related changes in several major grape growing regions are leading to earlier vine

40   growth phenology and altered or atypical fruit ripening patterns (Schultze et al. 2014). In

41   many wine-production regions worldwide sugar accumulation can occur too rapidly, leading

42   to low acidity, low aromatic and phenolic concentrations and unbalanced wine profiles (Jones

43   et al. 2005). Market analyses currently show that consumers prefer wines with a moderate

44   alcohol content, good acidity and distinct aromatic profiles (Salamon 2006, Seccia and Maggi

45   2011). Accordingly, many growers are searching for innovative management practices to

46   delay fruit soluble solids accumulation (Keller 2010, Gu et al. 2012; Palliotti et al. 2013a and

47   2013b, Poni et al. 2013, Palliotti et al. 2014).

48   Winter pruning is basically designed to regulate vine vigor and yield and, in turn, achieve

49   desired must chemical composition by harvest. In Mediterranean growing areas, it is normally

50   carried out any time throughout winter after leaf fall and before bud burst. Delaying pruning

to late winter or early spring has been well studied (Anticliff et al. 1957, Barnes 1958, Coombe 1964, Bouard 1967). A primary reason for late pruning date was to delay bud burst in order to prevent spring frost damage in cool growing areas (Howell and Wolpert 1978, Trought et al. 1999). Spur-pruning at the swollen bud phenological stage is expected to delay vegetative growth, flowering, fruit-set and fruit maturation. Friend and Trought (2007) have shown that pruning performed on Merlot in New Zealand when apical shoots on the canes were about 5 cm long resulted in lower sugar and higher organic acid content in grapes.

Delaying pruning after bud break is likely to cause a sudden and severe source limitation due to two main mechanisms: storage reserves used to support initial stages of vegetative growth are removed by pruning and, if performed following bud break, pruning can remove a fraction of the foliage producing carbohydrates. According to Champagnol (1984) any primary leaf that has reached 30% of its final size becomes a source of carbohydrates, the size ratio being slightly higher for lateral leaves.

Unpruned vines normally commence vegetative growth in early spring with the burst and growth of apical buds, bud emergence proceeding based on apical dominance along the cane. When vines are spur-pruned, they are forced to regrow from the basal buds. Shifting winter pruning to post-bud burst is expected to delay vine growth and fruit ripening; it is also expected to change canopy demography (Gatti et al. 2016). The canopy may reach an active carbon balance later in the season and, especially from veraison onward, the late-pruned vines may benefit from enhanced ripening potential of a younger canopy. The aim of our trial was to evaluate the effects of delayed spur-pruning in two consecutive years (2013 and 2014) on vine growth, yield, and fruit ripening of Sangiovese grapevines grown in central Italy.

Materials and Methods

76

77   *Plant material, climatic conditions and experimental design*. The trial was carried out in

78   2013 and 2014 in a commercial vineyard near Deruta, Perugia Province, in central Italy

79   (Umbria region, 42°58'N 12°24'E, elev. 405 m a.s.l., loamy soil). The vineyard was a 15-

80   year-old planting of cv. Sangiovese (clone VCR30) grafted to 420A stocks at 2.5 m × 1 m

81   inter- and intra-row spacing, respectively. The cordon was trained 0.9 m above ground with

82   three pairs of catch wires on a canopy wall extending 1.2 m above the cordon; vines were

83   spur-pruned to about 10 nodes per vine. In both years and on different dates individually

84   encoded according to the BBCH phenological scale (Lorenz et al. 1995), five adjacent rows of

85   80 vines each were selected for completely randomised blocks, with each row as a block.

86   Groups of 16 vines within each row were randomly assigned to winter pruning treatments

87   based on date of application. For 2013 and 2014, respectively, February 1,4 (BBCH0-A) and

88   March 1,5 (BBCH0-B) were the pruning dates representative of mid and late dormancy

89   (Table 1). Delayed pruning was applied on April 2,7 (BBCH1), beginning of bud swelling,

90   and on May 2,7 (BBCH55), when the apical shoot of unpruned canes had inflorescence

91   elongating and flowers still closely packed together. The last pruning treatment was applied

92   on June 1 in 2013 and June 6 in 2014 (BBCH64), when about 40-50% of apical-shoot flower

93   caps had fallen. In 2015, all vines were pruned February 6 (mid-dormancy stage). Standard

94   pest-management practices based on scouting and local experience were applied in both years,

95   and no leaf removal was performed during the season. Shoots were mechanically trimmed as

96   needed to maintain canopy shape when most started to outgrow the last pair of catch wires.

97   Trial weather conditions were monitored by an automatic meteorological station located

98   nearby the vineyard.

99   *Leaf area development and vine vigor*. In both years, twelve fruiting shoots per treatment

100   were randomly collected from twelve vines within the trial blocks; total leaf area per shoot

101 was measured by an AAM-7 leaf area meter (Hayashi-Denko, Tokyo, Japan) and calculated

102 by multiplying mean leaf area per shoot by shoot number per vine. Canes from 20

103 representative vines per treatment were weighed yearly after spur-pruning to estimate annual

104 vine growth, and the resulting data used to calculate the Ravaz index (yield-to-pruning weight

105 ratio, kg/kg) (Ravaz 1903). Vine balance was assessed by calculating the total leaf area-to-

106 yield ratio in all the treatments.

107 *Vine yield, ripening kinetics and must composition at harvest.* In 2013 and 2014, total

108 soluble solids (°Brix), titratable acidity (TA) and must pH were periodically analysed up to

109 harvest from 70 and 54 days after full bloom in 2013 and 2014, respectively, by random

110 sampling of 100 berries in three replicates per treatment. Within each treatment x replicate

111 sample, 25 clusters were sampled to remove 2 berries from the top, and 1 berry for the middle

112 and bottom of the cluster. Total Brix and pH were measured with a temperature-compensating

113 RX 5000 refractometer (Atago-Co Ltd, Tokyo, Japan) and a digital PHM82 pHmeter

114 (Radiometer, Copenaghen, Denmark). A Titrex Universal Potentiometric Titrator (Steroglass

115 S.r.l., Perugia, Italy) was used to measure TA by titrating with 0.1N NaOH to an end point of

116 pH 8.2; the results are expressed as g/L of tartaric acid equivalent. Harvest date was 105 days

117 after full bloom (DAFB) in 2013 and 113 DAFB in 2014. In both years, vines from all

118 treatments were harvested the same day when grapes from mid-dormancy pruning reached an

119 average 20 Brix. Grapes from all trial vines were individually picked, crop weight and cluster

120 number per vine recorded, average cluster weight was calculated and berry fresh weight and

121 number of berries per cluster measured. Total anthocyanin and phenolic concentrations were

122 determined after Iland (1993) on 250 berries per treatment (five replicate samples of 50

123 berries each) and expressed as mg per kg of fresh berry weight.

124 *Carbohydrate storage in permanent vine organs.* Roots (fine brown 1.5 ± 0.2 mm diam.,

125 taken at 20- 30 cm soil depth) and canes (3rd internode) were sampled in ten replicates per

126 treatment in December to determine carbohydrate concentration. Alcohol-soluble sugars and

127 starch in both organs were determined after Loewus (1952) using anthrone reagent (Merck,

128 Darmstadt, Germany); absorbance was read at 620 nm with a Jasco V-630 spectrophotometer

129 (Tokyo, Japan).

130 *Bud fertility in the year following the treatments*. Bud fertility was assessed on twenty

131 vines per treatment in mid-June of both years by counting the number of inflorescences on all

132 shoots. The same measurements were carried out in 2015 in all treatment replicates subjected

133 to winter spur-pruning at mid-dormancy.

134 *Statistical analysis.* A two-way analysis of variance (ANOVA) was used to analyse winter

135 pruning date and year effects on leaf area development, yield components, grape composition

136 at harvest and reserve storage in canes and roots using the SigmaStat 3.5 software package

137 (Systat Software Inc., San Josè, CA, USA). Mean separation was performed by Student-

138 Newman-Keuls test ($p \leq 0.05$). Results of the seasonal evolution of total soluble solids, must

139 pH and titratable acidity are shown as means ± standard error (SE).

140

141 **Results**

142 *Environmental conditions*. Accumulated heat expressed as growing degree days (GDD,

143 calculated on a 10°C base temperature from 1 Apr to 30 Sept) was lower in 2014 than in 2013

144 (1558 vs. 1712 GDD, respectively); total rainfall over the same period was slightly lower

145 (Figure 1). Rainfall was concentrated in May (239 mm), June (78 mm) and September (151

146 mm) in 2013 but was more uniformly distributed between spring and summer in 2014 except

147 for an unseasonably rainy July (162 mm). Summer 2013 was marked by high daily maximum

148 air temperatures: 30°C in May, 35°C in June and 38°C in July and August (Figure 1). Despite

149 such trends and the absence of irrigation, no visual symptoms of water stress or significant

150 leaf yellowing were observed throughout the 2013 season.

151   *Effects of delayed winter spur-pruning on vine yield, grape composition and ripening*

152   *kinetics.* In both 2013 and 2014, delaying pruning until early April (apical buds at swelling)

153   had no effect on crop weight or yield components (Table 2). Early may pruning reduced yield

154   per vine by about 55%, evincing lower cluster number per vine (~44%), lower cluster weight

155   (~26%) and fewer berries per cluster (~17%) compared to the earlier treatments. Pruning

156   performed early June, when the apical cane buds were at the phenological stage of 40-50%

157   fallen flower caps, resulted in the total absence of inflorescences (Table 2). The May-pruned

158   vines in both years exhibited delayed soluble solids accumulation and juice organic acid

159   degradation as compared to the other treatments (Figure 2). These vines also showed a late

160   increase of juice pH, totalling about 1.6 Brix lower than the average value found in the other

161   treatments, whereas TA was higher by about 1.8 g/L. Conversely, must pH was unaffected by

162   treatments (Table 3). Anthocyanin and total phenolic concentrations in May-pruned vines

163   were significantly greater, by 19% and 11% respectively, compared to the standard pruning

164   timings (Table 3).

165   Comparing vintages indicates that the cooler 2014 led to a significant decrease of soluble

166   solids content and higher titratable acidity compared to 2013; must pH, anthocyanin and

167   phenolic contents (Table 3), and yield components (Table 2) were unaffected.

168   *Phenology, vegetative growth, vine vigour and replenishment of carbohydrate reserves.*

169   Full bloom (B) and onset of veraison (V) in standard winter pruning dates (i.e. February and

170   March) occurred 2 June and 1 August, respectively. In 2014, the same phenological stages

171   occurred with 7 and 8 day delay, respectively. There were no differences in total leaf area per

172   vine or one-year-old pruning weight across treatments at the end of vegetative growth (Table

173   4). Due to their lower yield, the May-pruned vines had a significantly higher leaf-to-fruit ratio

174   (175%) and lower yield-to-pruning weight ratio (55%) than the other treatments (Table 3). In

175   2014, a significant reduction of 32% in total leaf area and of 24% in the yield-to-pruning

176   weight ratio was found in comparison to 2013, whereas the leaf-to-fruit ratio increased by

177   about 21% (Table 4). Alcohol soluble sugars and starch content in canes and roots in

178   December did not vary across treatments, while non-structural carbohydrates (i.e. soluble

179   sugars + starch) were significantly lower in both canes and roots in 2014 (Table 5).

180   *Bud fertility*. In neither year did April pruning, which retained an average of one cluster per

181   vine, have any effect (Figure 3). Bud fertility was halved by May pruning in both years but

182   recovered to usual values after standard winter pruning in 2105. June pruning in both years

183   left no inflorescences, which increased to 0.55 inflorescences per bud after standard winter

184   pruning in 2015.

185

186   **Discussion**

187   The marked apical dominance of *Vitis vinifera* inhibits the development of the subtending

188   median and basal nodes. When basal buds of spur-pruned vines are forced to delay growth, all

189   phenological stages are postponed (Martin and Dunn 2000, Friend 2005). Floral primordia

190   differentiation, flower development, fruit-set and fertilization, berry growth, vine yield and

191   fruit composition were notably influenced by delayed spur-pruning in our trial. Indeed, the

192   February and March pruning at dormant buds elicited similar responses as per our vine and

193   fruit quality parameters, thereby indicating that winter pruning can be applied until late in this

194   season, i.e. up to the onset of bud burst, without adversely affecting vine performance.

195   In neither year did April pruning, coinciding with apical-cane bud burst, exert an effect on

196   vine growth, yield and grape composition at harvest, nor were any effects recorded in the

197   following year. Although April pruning considerably slowed post-veraison sugar

198   accumulation during the cool 2014 summer, it did not delay final fruit ripening rate in either

199   year (Figure 2, Table 3).

200   Friend and Trought (2007) found that in cv. Merlot late winter spur-pruning, i.e. pruning

201   performed when apical cane shoots were ~5 cm long, resulted in delayed grape ripening,

202   lower Brix and TA at harvest, but significantly greater yields per vine. The latter result was

203   explained by higher average berry weight leading to heavier clusters, an increased proportion

204   of large seeded berries, and fewer shot berries. The authors attributed the findings to enhanced

205   flower fertilization and seed development due to postponement of bloom to a period (October,

206   Southern Hemisphere) when climatic conditions were more favourable than those in July

207   (standard winter pruning date in New Zealand).

208   May and June spur pruning in our environment caused a significant reduction of yield per

209   vine (more than 50%) due to lower berry per-cluster and cluster per-vine number. Yield

210   decreases were mostly due to the reduction of basal-shoot cluster number following delayed

211   pruning. These results are likely linked to the sudden source limitation on the developing

212   cluster primordia. This source limitation likely resulted from two main components. Apical

213   shoots have some mature leaves as sources of carbohydrates for the sinks that are eliminated

214   by pruning. Sources of carbohydrates and nitrogen compounds derived from storage reserves

215   that are invested in new growth are also eliminated by late pruning and cannot contribute to

216   post-pruning development of basal buds (Gatti et al. 2016). The reproductive and vegetative

217   organs of woody species compete for carbohydrates provided by current photosynthesis

218   and/or reserve remobilization (Wardlaw 1990, Smithyman et al. 1998, Lebon et al. 2008,

219   Tombesi et al. 2015). Candolfi-Vasconcelos and Koblet (1990) reported that the number of

220   clusters developed in the subsequent season is greatly dependent on carbon budget and

221   competitive relationships among vine organs during the onset of inflorescence. Our May 2013

222   pruning caused a significant decrease of clusters per vine, precisely 8.9 vs. 13.3 counted in the

223   earlier pruning treatments, whereas in 2014 this parameter dropped to 5.6 clusters per vine,

224   suggesting a possible additive, negative effect on bud fruitfulness. Indeed, the main factor

225   influencing bud induction in a much delayed bud burst is the notably limited vine source at

226   the usual time induction takes place. A non-limiting source-to-sink ratio by the time of bud

227   induction is historically acknowledged as a primary regulator of bud fruitfulness (Coombe

228   1962). The significant difference in berry number per cluster suggests that delayed pruning

229   can also affect current season fruit-set. A likely explanation is that delayed bud break causes

230   shoot growth to occur at higher daily rates under higher air temperatures, thereby exerting

231   stronger competition among the differentiating bud meristems (May 2004).

232   Early June pruning, when apical shoots bear flowers with ~40% of fallen flower caps,

233   caused the complete loss of yield. Basal-bud shoots failed to develop flowers and remained

234   vegetative after pruning. Interestingly, the leaf area produced by the June pruning did not

235   differ from that of the other treatments despite the fruitless shoots. The higher vine capacity

236   expected to occur due to the absence of competing clusters was fully offset by a shorter

237   season for canopy development.

238   The increase of total anthocyanins and phenolics in the May-pruned vines seems in

239   contrast with final Brix and TA, indicating a ripening delay. Solute concentration due to

240   reduced berry surface area would help to explain the anthocyanin and phenolics count but not

241   the decrease in sugar concentration. Higher total anthocyanins and phenolics recorded in May

242   pruning could likely be a consequence of smaller berry size. Although we did not quantify

243   relative skin mass and flesh components, several papers have shown that inferring higher

244   relative skin mass in smaller berries simply based on the geometrical features of a spherical

245   berry shape can be quite misleading (Roby et al. 2004, Walker et al. 2005, Poni and

246   Bernizzoni 2010). Work by Kliewer and Dokoozlian (2005) has shown that, under an array

247   of conditions and genotypes, both sugar and color accumulation in grapes correlate with the

248   leaf area-to-fruit ratio according to a negative exponential curve featuring a plateaux at

249   approximately 1.5 m^2/kg of fresh fruit mass. This relationship would explain the improved

berry color while sharply contrasting with the low final sugar level recorded by the May pruning. However, hints to account for this rather anomalous sugar-storage response are provided by the variation in the Ravaz index (i.e. yield-to-pruning weight ratio), which progressively decreases the more pruning is delayed (Smart and Robinson 1991). We submit that the sugar accumulation we found was primarily delayed due to excessive and/or too prolonged vegetative competition. It remains to be explained, however, why fruit phenolic concentrations were not affected by the same phenomenon. Recent studies have clearly shown that sugar and color accumulation can become quite decoupled depending on environmental conditions and specific management practices. For instance, Sadras and Moran (2012) provide evidence of a temperature-driven decoupling of sugars and anthocyanins in berries of Shiraz and Cabernet Franc. Other researchers have examined whether the onset and rate of sugar and anthocyanin accumulation can be selectively modified via canopy management practices such as the application of plant hormones (Böttcher et al. 2011), apical-to-cluster late leaf removal (Palliotti et al. 2013b, Poni et al. 2013) and post-veraison shoot trimming (Filippetti et al. 2015). Gatti et al. (2016) report that a pre-veraison, anti-transpirant application alone or in combination with a pre-flowering spray proved effective in slowing sugar accumulation in cv. Barbera while avoiding concurrent delay of color development. While a similar effect was seen in the present study, more in-depth research is needed to assess how the color : sugar ratio changes during ripening, thereby offering the chance to determine differences in onset and rates of ripening to verify repeatability of such a decoupling under a wider range of conditions. Since all our treatments were harvested on the same day, it was not possible to assess how prolonged hang time could have improved the already remarkable fruit ripening pattern shown by the May pruning (i.e. lower °Brix and higher color than the earlier prunings). Given the long growing season the trial site allows for and the well-known relationship between berry-color accumulation and temperature, which

275   indicates that that the 15-25°C range optimizes accumulation of anthocyanins and phenolic

276   (Spayd et al. 2002) and temperatures >35 °C begin to degrade them (Mori et al. 2007), further

277   postponement of harvest via this treatment is likely.

278   Non-structural cane and root carbohydrates at the end of December were unaffected by

279   pruning timing. This finding suggests that the replenishment of carbohydrate reserves

280   followed the same pattern regardless of pruning date.

281

282   **Conclusions**

283   Delaying spur-pruning to early spring may be used to reduce vine yield, slow sugar

284   accumulation and increase fruit anthocyanin and phenolic concentrations. Winter spur-

285   pruning carried out after bud burst caused a ripening modulation, still evident at harvest,

286   whose significant magnitude may be related to the time elapsed from bud burst to pruning.

287   Late pruning caused a yield decline close to 50% due to reduced flower differentiation and

288   development on shoots grown from basal buds. Further delaying pruning elicited vine

289   unproductivity and negative carry-over effects the following year. This study represents the

290   first attempt to understand and calibrate winter pruning date as a management tool in pursuit

291   of the right compromise between mild yield limitation and a delay in fruit ripening.

292

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Figure captions

Figure 1. Maximum, minimum and average air temperature and daily rainfall during the 2013

and 2014 growing seasons. B, V, and H indicate full bloom, onset of veraison and harvest

dates, respectively.

Figure 2. Seasonal trend of total soluble solids, must pH and titratable acidity in 2013 (solid

symbols) and 2014 (empty symbols) for Sangiovese vines pruned at different dates: February

1,4 (BBCH0- A), March 1,5 (BBCH0- B), April 2,7 (BBCH1) and May 2,7 (BBCH55) for

2013 and 2014, respectively. Data points are means of three replicates of 50-berry samples.

Vertical bars represent standard error (SE) around means. Top panels report daily maximum

air temperature. V = onset of veraison; H = harvest.

Figure 3. Bud fertility of Sangiovese vines pruned at different dates in 2013, 2014 and 2015:

February 1,4 (BBCH0 - A), March 1,5 (BBCH0 - B), April 2,7 (BBCH1), May 2,7 (BBCH55)

and June 1,6 (BBCH64) for 2013 and 2014, respectively. In 2015, winter pruning date was

February 6 (mid-dormancy). Each bar is the mean of twenty vines ± SE.
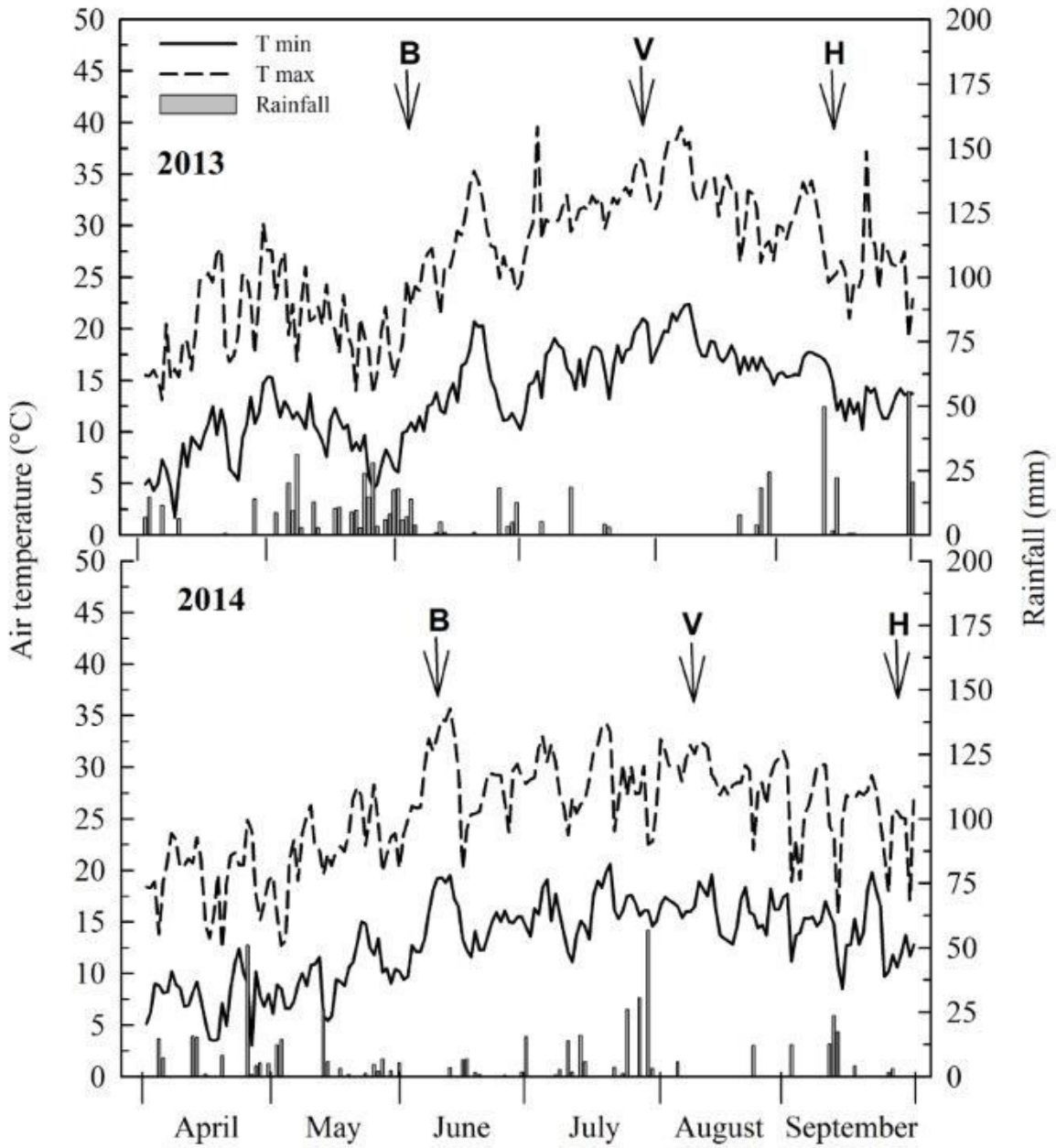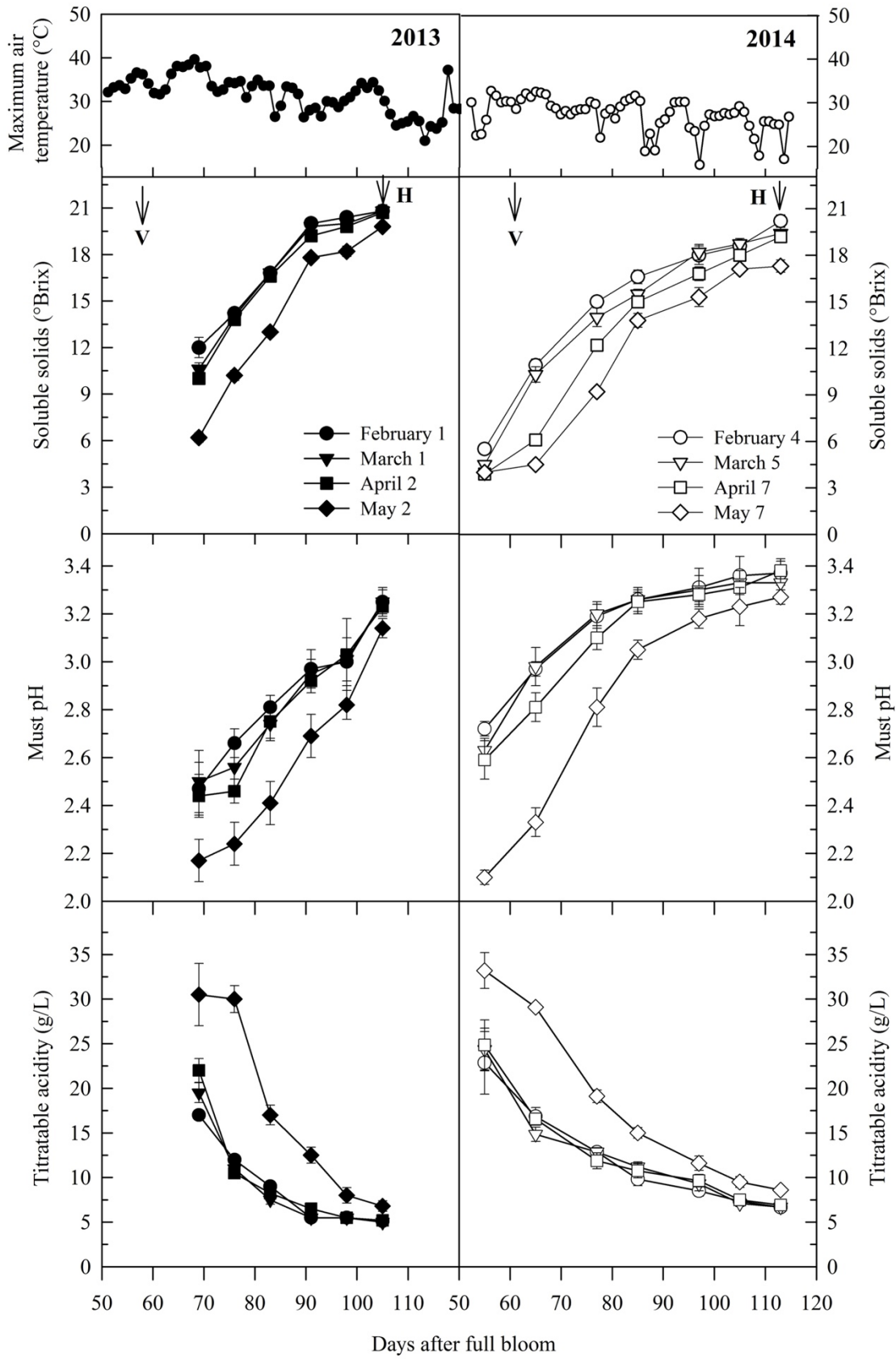
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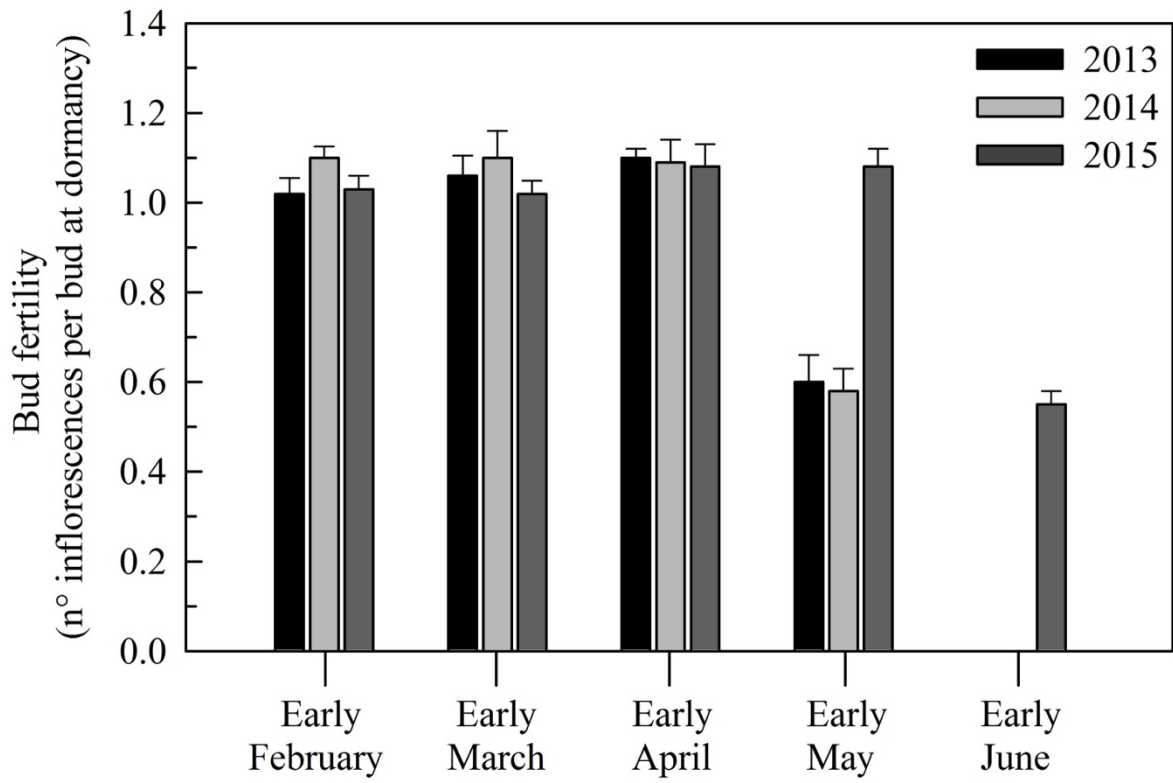427   Figure 1

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Table 1. Actual pruning dates, description and classification (BBCH scale) of phenological stages for the 2013 and 2104 trial years.

Phenological growth stage	BBCH scale (Lorenz et al. 1995)	Pruning dates 2013	Pruning dates 2014
Mid-dormancy	BBCH 0-A	February 1	February 4
Late-dormancy	BBCH 0-B	March 1	March 5
Beginning of bud swelling	BBCH 1	April 2	April 7
Inflorescence elongating with flowers closely pressed together	BBCH 55	May 2	May 7
40-50 % of flower caps fallen	BBCH 64	June 1	June 6

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Table 2. Crop weight and yield components recorded at harvest on Sangiovese vines pruned in 2013 and 2014 at different timings: February 1, 4 (BBCH0-A), March 1, 5 (BBCH0-B), April 2, 7 (BBCH1), May 2, 7 (BBCH55) and June 1, 6 (BBCH64).

	Yield/vine (kg)	Clusters/vine (n)	Cluster weight (g)	Berry weight (g)	Berries/cluster (n)
Treatment (T)					
February 1, 4	3.55^a	13.4^a	266^a	2.63^a	103^a
March 1, 5	3.40^a	13.0^a	261^a	2.69^a	100^a
April 2, 7	3.45^a	12.6^a	270^a	2.66^a	102^a
May 2, 7	1.55^b	7.3^b	197^b	2.34^b	85^b
June 1, 6	0	0	---	---	---
Significance	**	**	**	*	*
Year (Y)					
2013	3.30	12.1	275	2.59	107
2014	3.05	10.9	269	2.70	98
Significance	ns	ns	ns	ns	ns
T × Y	*	ns	ns	ns	ns

489 Means within columns noted by different superscript are different by Newman-Student-Keuls test.
490 *, **, ns indicate significant differences between treatments and years at $p \leq 0.05$ and 0.01 or not
491 significant, respectively.
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Table 3. Grape composition recorded at harvest on Sangiovese vines pruned in 2013 and 2014 at different timings: February 1, 4 (BBCH0-A), March 1, 5 (BBCH0-B), April 2, 7 (BBCH1) and May 2, 7 (BBCH55).

	Soluble solids (Brix)	Titratable acidity (g/L)	Must pH	Total anthocyanins (mg/kg)	Total phenolics (mg/kg)
Treatment (T)					
February 1, 4	20.5[a]	5.85[a]	3.31	220[a]	1990[a]
March 1, 5	20.1[a]	5.80[a]	3.29	206[a]	1988[a]
April 2, 7	19.9[a]	6.05[a]	3.31	214[a]	1959[a]
May 2, 7	18.5[b]	7.70[b]	3.21	254[b]	2206[b]
	*	**	ns	*	*
Year (Y)					
2013	20.5[a]	5.52[b]	3.33	210	1983
2014	19.0[b]	7.21[a]	3.22	248	2091
Significance	*	**	ns	ns	ns
T × Y	ns	*	ns	ns	ns

495 Means within columns noted by different superscript are different by Newman-Student-Keuls test.
496 *, **, ns indicate significant differences between treatments and years at $p \leq 0.05$ and 0.01 or not
497 significant, respectively.
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Table 4. Total leaf area, winter pruning weight and balance indicesrecorded on Sangiovese vines pruned in 2013 and 2014 at different timings: February 1, 4 (BBCH0-A), March 1, 5 (BBCH0-B), April 2, 7 (BBCH1), May 2, 7 (BBCH55) and June 1, 6 (BBCH64)

	Total leaf area (m²/vine)	Leaf-to-fruit ratio (m²/kg)	Pruning weight (kg/vine)	Yield/pruning weight (kg/kg)
Treatment (T)				
February 1, 4	3.63	1.02^b	0.68	5.22^b
March 1, 5	3.54	1.05^b	0.78	4.36^b
April 2, 7	3.42	1.04^b	0.80	4.31^b
May 2, 7	3.47	2.87^a	0.74	2.09^a
June 1, 6	3.45	---	---	---
Significance	ns	**	Ns	*
Year (Y)				
2013	4.17^a	1.35^b	0.78	5.38^b
2014	2.83^b	1.63^a	0.71	4.08^a
Significance	**	**	ns	*
T × Y	ns	*	ns	ns

503 Means within columns noted by different superscript are different by Newman-Student-Keuls test.
504 *, **, ns indicate significant differences between treatments and years at $p \leq 0.05$ and 0.01 or not
505 significant, respectively.
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Table 5. Cane wood and root reserves recorded at the end of December 2013 and 2014 in Sangiovese vines pruned at different timings: February 1, 4 (BBCH0-A), March 1, 5 (BBCH0-B), April 2, 7 (BBCH1), May 2, 7 (BBCH55) and June 1, 6 (BBCH64).

	Cane wood		Roots	
	Soluble sugars (mg/g dw)	Starch (mg/g dw)	Soluble sugars (mg/g dw)	Starch (mg/g dw)
Treatment (T)				
February 1, 4	103.2	104.7	116.0	162.8
March 1, 5	102.9	97.6	110.9	176.0
April 2, 7	98.8	92.1	97.8	157.4
May 2, 7	115.2	101.1	96.0	166.5
June 1, 6	112.6	93.9	99.6	154.7
Significance	ns	ns	Ns	ns
Year (Y)				
2013	123.8^a	84.5^b	111.0^a	170.8^a
2014	88.9^b	107.3^a	97.7^b	156.2^b
Significance	*	*	*	*
T × Y	ns	ns	Ns	ns

513 Means within columns noted by different superscript are different by Newman-Student-Keuls test.
514 *, ns indicate significant differences between treatments and years at $p \leq 0.05$ or not significant,
515 respectively.
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