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Postveraison shoot trimming reduces cluster compactness without compromising fruit quality attributes in organically grown sangiovese grapevines

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

Bondada, B., Covarrubias, J.I., Tessarin, P., Boliani, A.C., Marodin, G., Rombolà, A.D. (2016). Postveraison shoot trimming reduces cluster compactness without compromising fruit quality attributes in organically grown sangiovese grapevines. AMERICAN JOURNAL OF ENOLOGY AND VITICULTURE, 67(2), 206-211 [10.5344/ajev.2016.15058].

Availability:

This version is available at: <https://hdl.handle.net/11585/550341> since: 2016-07-13

Published:

DOI: <http://doi.org/10.5344/ajev.2016.15058>

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1 **Research Article**

2 **Postveraison Shoot Trimming Reduces Cluster Compactness**
3 **without Compromising Fruit Quality Attributes in**
4 **Organically-Grown Sangiovese Grapevines**

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15 Acknowledgment: Bhaskar Bondada is grateful to the University of Bologna, Bologna, Italy, for the
16 Senior Visiting Fellowship awarded through the Institute of Advanced Studies.

17 Manuscript submitted Jun 2015, revised Jul 2015, Jan 2016, accepted Jan 2016

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19
20 **Abstract:** Vine performance following preveraison shoot trimming is well document, the
21 consequences of such treatments later in the season are poorly understood. Therefore, a 4-yr
22 study was conducted in a mature vineyard of Sangiovese (clone 12T) grafted onto Kober 5BB
23 rootstock at a spacing of 1 m x 2.8 m (intra- and interrow) to analyze the influence of
24 postveraison shoot trimming on vine growth characteristics, cluster architecture (cluster
25 compactness), and yield from a physiological viewpoint. The treatments consisted of shoot
26 trimming during postveraison in a randomized block design with eight replications; each
27 replication comprised of six vines. Three shoot trimming treatments consisting of light trimming
28 (14 nodes), severe trimming (10 nodes), and an un-trimmed control were imposed when the
29 soluble solids reached 15 Brix in August (40-45 day before expected harvest). Following the
30 treatments, various vine growth characteristics, cluster morphology, and fruit quality attributes

31 were measured. Postveraison shoot trimming, especially severe trimming reduced cluster weight
32 along with cluster compactness, productivity, and total yield. In terms of fruit quality attributes,
33 these reductions were manifested as lowering of Brix and pH with minor effects on TA, yeast
34 assimilable nitrogen, the anthocyanin profile, and total anthocyanins. These results demonstrated
35 that postveraison shoot trimming can be a valuable production practice in reducing cluster
36 compactness without compromising overall fruit quality attributes in Sangiovese.

37 **Key words:** anthocyanin, Brix, growth, productivity, yield

38
39

Introduction

40 The cultivar Sangiovese is the most important Italian winegrape used for making
41 prestigious Tuscan wines such as Brunello di Montalcino, Nobile di Montepulciano and Chianti.
42 Although grown with both conventional and organic viticultural practices, organically-grown
43 Sangiovese has been increasing for the last few years as organically generated products meet the
44 standard requirements for quality as well as healthiness (Pagliarini et al. 2013). A key
45 reproductive feature of Sangiovese is that regardless of how it is grown, it produces tight
46 clusters, which are grouped as moderately compact, semi-compact, and compact (Nelson-Kluk
47 2006). Clusters of such morphology incur losses in cuticular barrier properties at the contact
48 surfaces and hence become victims of a host of fungal diseases, mostly *Botrytis* cluster rot during
49 ripening especially of grapevines grown with organic protocols. Other concerns include its
50 tendency to overcrop ensuing from its high fruitfulness of shoots regardless of origin (primary or
51 secondary buds, basal buds) and vigorous procumbent growth habit leading to dense canopies,
52 which generally result in negative effects on fruit quality, wood maturity, and vine size
53 maintenance (Poni et al. 2006).

54 Combatting cluster rot has always been a multifaceted endeavor to have a profitable
55 grape production. For instance, fungicides spray is one obvious option. However, it is an
56 expensive short-term operation as pathogens develop resistance to fungicides overtime and thus
57 it is unlikely that practical control of fungal diseases can be achieved by the use of fungicides
58 alone. It is indeed true in organic viticulture necessitating an alternative and a more
59 environmentally-benign approach as the chemicals that are organically certified add to more
60 environmental problems rather than remedying the situation due to their ineffectiveness
61 (Fragoulis et al. 2009). The other option is to employ various canopy management practices to
62 improve the cluster architecture and subsequently microclimate by loosening the clusters, which
63 minimizes fungal problems. For instance, defoliation decreased physical characteristics, number
64 of berries, and cluster compactness in Trebbiano and Sangiovese (Poni et al. 2006). Other
65 practices that reduced cluster compactness include cluster division (Molitor et al. 2012), type of
66 irrigation method (drip irrigation versus partial rootzone drying), pruning strategy (spur pruning
67 by hand, mechanical hedging or minimal pruning), and combination of irrigation and pruning
68 that affected the number of clusters per vine and the rate of berry maturation (Leong et al. 2006).
69 From a rootstock perspective, cluster compactness, berry skin strength, pedicel strength, and
70 weights of cane and crop are greatly influenced by choice of cultivar (Ferreira and Marais,
71 1987). For instance, clusters of Chenin blanc on Jacquez were significantly less compact than
72 those on Ramsey or 110 Richter (Ferreira and Marais, 1987).

73 Regardless of where it is practiced, the main thrust of organic farming including
74 viticulture is to exploit ecologically balanced protocols deprived of chemical usage in order to
75 avoid any environmental problem. Accordingly, organic grape growers resort to using mostly
76 eco-friendly production practices with the exception that copper may be used only when there is

77 an immediate risk of infection (Fragoulis et al. 2009). Furthermore, they treat the vineyard as an
78 interactive ecosystem known as terroir in viticulture parlance entailing the regional physical (e.g.
79 climate) and cultural domains (e.g. viticultural practices), which are put to best use towards
80 optimizing yield and fruit quality. Because of the need to adhering to the principles of organic
81 viticulture, organic grape growers seek out a wide repertoire of canopy management practices
82 such as shoot topping (Molitor et al. 2015), shoot trimming (Martínez de Toda et al. 2013),
83 pruning and leaf removal (Martin, 1990), hedging (Leong et al. 2006) etc. to maintain healthy
84 vines. Of these, an ideal practice for improving the fruit and yield of organically-grown
85 Sangiovese would be shoot trimming as it is easily mechanized and hence relatively simple to
86 perform. It involves pruning at different locations along the shoot and is generally used to
87 facilitate the movement of manpower and equipment between vineyard rows, to reduce vine
88 vigor, avoid shade from overhanging shoots, and to facilitate harvest in grapevines with upright
89 trellis system (e.g. VSP) in cool viticultural areas (Hatch et al., 2011). Since it is more of a
90 facilitator of cultural operations, little information is available with respect to its impact on vine
91 performance and fruit quality, especially when it is performed late in the season after veraison.
92 Previous studies that dealt with shoot trimming were carried out during the early part of the
93 season i.e. preveraison and examined reproductive growth, yield, ripening, and fruit and wine
94 quality (Keller et al. 1999), all of which widely varied in their responses. Furthermore, none of
95 these studies examined features such as growth characteristics of vine and cluster morphology,
96 which have a strong bearing on the incidence of fungal diseases, especially in organic viticulture.
97 This is the first study that demonstrates positive influence of postveraison shoot trimming in the
98 form of reduced cluster compactness and fruit yield in Sangiovese grown with the norms of
99 organic viticulture, which has been increasing for the last few years.

Materials and Methods

Plant material and experimental layout. The experiment was conducted over four years from 2008-2011 in a mature vineyard of Sangiovese (clone 12T) grafted onto Kober 5BB rootstock at a spacing of 1 m x 2.8 m (intra- and interrow) for a density of 3571 vines/ha located in a hot hilly area of Emilia Romagna Region in Italy (lat. 44°17'7"N; long. 11°52'59"E, elevation 117 m asl). Since 2007, the vineyard established on diverse sub-soils of alluvial deposits has been managed with the principles of organic viticulture in accordance with Reg. EC 834/2007 (EC. 2007). Vines were trained to cordon de Royat training system consisting of a short trunk with a unilateral cordon trained to only one side of the trunk and hence extends from one vine to another. The vines were spur-pruned during winter to two count nodes equating to 12-14 nodes per vine. The noncount shoots (shoots arising from basal buds of the spur) were removed at the beginning and the cluster number was adjusted by cluster thinning. Throughout the experiment, the vines were maintained without any irrigation and fertilization.

The treatments consisted of shoot trimming during postveraison in a randomized block design with eight replications; each replication comprised of six vines (total of 144 vines). Prior to imposing the treatments, first vines were regularly (twice) trimmed to normalize a shoot length of 145 cm in each year. Thereafter, three shoot trimming treatments were imposed when the soluble solids reached 15 Brix in August (40-45 day before expected harvest). These include light trimming (LT of 14 nodes), severe trimming (ST of 10 nodes), and an un-trimmed control (CK 18 nodes). Shoot trimming was performed manually using large pruners. Following trimming, the shoot length in CK, LT and ST vines was 145, 102 and 64 cm, respectively. The amount of leaf area removed following trimming treatments and maintained on vines were

122 measured in 2009 and 2010 growing seasons using a leaf-area meter (Li-CoR Biosciences,
123 Lincoln, NE) by removing all leaves in single plants per replicate.

124 **Climatic data.** During each season, climatic data (mean, maximum and minimum daily
125 air temperatures (T), relative humidity (RH) and total rainfall) were recorded from bud burst to
126 harvest from a meteorological station located close to the vineyard.

127 **2008.** In 2008, from bud burst (March 26th) to harvest (September 24th), the growing
128 season was characterized by average T of 19.4 C with a maximum temperature of 34 C occurring
129 at the beginning of August. During the same period, the average RH varied from 45.5% to
130 89.5%. The total rainfall from bud burst to harvest (243 mm) occurred generally during April-
131 June.

132 **2009.** In 2009, the average T recorded from bud burst (April 3rd) to harvest, (September
133 23rd) was 22 C; maximum T of 38.7 C occurred on July 23rd. The average RH varied from 43.1%
134 to 100%. The total rainfall from bud burst to harvest was 190 mm and predominantly took place
135 in April and during the first week of July.

136 **2010.** In 2010, from bud burst to harvest (September 27th), the average daily T was 20 C
137 and the average RH varied from 39.3% to 92.8%; there was an abundance of rainfall (464 mm),
138 which occurred mostly in the spring and prior to harvest date.

139 **2011.** Overall, the 2011 growing season was marked by average T well above the
140 seasonal normal with a maximum T of 30 C occurring in the month of August. From bud burst to
141 harvest (September 21st), the average RH varied from 40 to 70%; highest values were observed
142 during spring (92%) and the lowest (38%) during the latter part of August. The total rainfall from

143 bud burst to harvest was 204 mm, occurred generally during spring and almost none during
144 ripening.

145 **Measurement of growth characteristics, yield, and yield components.** Leaf area
146 maintained on vines and removed by trimming, were measured through leaf-area meter (Li-CoR
147 Biosciences, Lincoln, NE), by removing all leaves from one vine per replicate and leaves
148 belonging to a representative shoot in each vine. Cane length and pruning weight were
149 determined during winter. All prunings were weighed fresh. Yield and yield components (yield,
150 number of clusters, and cluster weight) were measured when berries met optimal universal set of
151 criteria (e.g. Brix, TA) to determine the same harvest date for all treatments. This was
152 determined by periodic sampling of berries starting in July. Accordingly, all treatments were
153 harvested when berries attained same maturity levels, i.e. when soluble solid concentration
154 reached a minimal value of 20 Brix, TA of less than 7.0 g/L etc., by taking into account the
155 qualitative (e.g. berry ripening disorders) and sanitary (e.g. cluster rot) status of the clusters.
156 Cluster compactness was determined according to the 1983 OIV classification. Chlorophyll was
157 measured with a SPAD-502 chlorophyll meter (Minolta, Osaka, Japan).

158 **Analysis of fruit composition.** At harvest, berry weight, soluble solids (refractometer
159 PAL-1, ATAGO, Tokyo, Japan), titratable acidity (TA) and pH (Crison Compact Titrator, Crison
160 Instruments, Barcelona, Spain) were determined by randomly collecting 100 berries per
161 replication in each treatment. On the same samples, must YAN was determined by the method
162 described by Aerny (1996) and berry skin anthocyanins using HPLC methodology described by
163 Venencie et al. (1997). Briefly, for each sample, skins were peeled from 30 berries, weighed and
164 placed in 20 mL hydroalcoholic solution (EtOH:H₂O, 10:90 v/v) containing 15 mL distilled
165 water and 2.5 g tartaric acid. The samples were homogenized with an Ultraturrax (IKA

166 Labortechnik, Staufen, Germany) and centrifuged for 5 min at 3000 x g at 20 C. The final
167 solution weight was adjusted to 100 g and pH to 3.6. The samples were then macerated for 24 hr
168 at 4 C in dark and centrifuged (ALC International, PK121R, Italy) for 20 min at 1400 g.
169 Thereafter, the samples were filtered under vacuum with Whatman Filter Paper 40, porosity 8
170 μm . Skin anthocyanins were determined using a Waters 1525 instrument equipped with a diode
171 array detector (Waters Corporation, Milford, MA) and a reversed-phase column RP18 (Supelco
172 Inc., Bellefonte, PA); 5 μm pore size; 250 mm \times 4 mm. Signals were detected at 520 nm. The
173 elution gradient consisted of the following solvents. Solvent A: water/formic acid (10%, v/v)
174 (Romil Ltd., Cambridge, UK); solvent B: acetonitrile (Sigma-Aldrich, Milan, Italy). The binary
175 gradient applied was as follows: 0-7.9 min of 96% A-4% B; 7.9-23.0 min of 85% A-15% B;
176 23.0-27.0 min of 80% A-20% B; 27.0-43.0 min of 70% A-30 % B; 43.1-45.0 96%A-4% B.
177 Anthocyanins were quantified by measuring peak area at 520 nm. A calibration curve was
178 prepared from standard solutions of purified Oenin Chloride (Sigma-Aldrich, Milan, Italy) with
179 the concentrations between 50 mg/L and 500 mg/L. The anthocyanins were expressed as mg/g of
180 berry skin. For each treatment, eight field replicates (each one deriving from a 6-plant
181 experimental plot) were measured. All field replicates were maintained separately and measured
182 in the laboratory. Therefore the number of field replicates equaled to analytical replicates.

183 **Statistical analysis.** Analysis of variance and comparison of means between treatments
184 were done by using SAS 6.04 software (SAS Institute, Cary, NC). Means were compared by the
185 Student-Newman-Keuls test ($P \leq 0.05$). Cluster weight and cluster compactness were subjected
186 to Kruskall Wallis non-parametric test.

187

Results

188
189 **Growth, yield and compositional characteristics.** The postveraison shoot trimming
190 treatments were imposed on the same vines each year with the consequence that the treatment
191 effects accumulated throughout the experiments resulting alterations in growth and fruit quality
192 attributes. For instance, the crop load (Ravaz index) increased with an increase in shoot trimming
193 (Table 1), which needs to be interpreted with caution. This is because, the trimming treatments,
194 while they altered the yield to pruning weight ratio, it was rather an imposed response, not a
195 developing response. On the other hand, berry weight declined with no differences among the
196 treatments during the first two years of experiment (2008-2009), remained the same in 2010, but
197 differed during the final year i.e. 2011 following the adoption of production practices of organic
198 viticulture in 2007 (Table 1). This indicated that reduction in berry weight following shoot
199 trimming was a cumulative process, nevertheless the possible effects of smaller vine size and
200 relatively warmer season cannot be ignored. Cluster weight and yield per vine declined starting
201 in 2008 (Table 1). The amount of leaf area removed and that remained on the vine following
202 shoot trimming showed differences among the three treatments (Table 1). Once again, such
203 differences primarily stemmed from severity of pruning treatments *per se* and were not the
204 consequence of treatments. Analogous to cluster weight and yield per vine, cluster compactness
205 decreased in response to shoot trimming, especially against the severe one (Table 2). Regarding
206 fruit composition, the Brix values were reduced during the first two seasons (2008-2009),
207 whereas no difference was observed during the latter two years (2010-2011) (Table 3). No
208 difference was observed in pH in 2008 and 2010 whereas the 2009 and 2011 seasons displayed
209 differences (Table 3). In contrast, the TA and YAN except for one year were immune to shoot

210 trimming treatments (Tables 3). In a similar pattern, the anthocyanin profile (cyanidin, peonidin,
211 petunidin, delphinidin, malvidin) and total anthocyanins remained unaffected except for peonidin
212 and total anthocyanins in 2010, which tended to decline in response to shoot trimming
213 (Supplemental Table 1). Between the two shoot trimming treatments, the severe one in general
214 mostly induced differences (lowered the values) on the measured variables.

215

Discussion

216 Shoot trimming, the mainstay of this study, is one of the cultural repertoire of production
217 practices to which grapevines adjust by forming tylose in the xylem vessels below the cut ends as
218 a sealing mechanism to avoid water loss and pathogen entry into the vine (Sun et al. 2006). In the
219 aftermath of shoot trimming following the sealed cut ends, the vine attains an altered state of
220 shoot physiology and growth characteristics. This is particularly true when shoots are trimmed
221 after veraison, the phenological event marking the inception of ripening (Poni and Intrieri, 1996).
222 As a result, the effects of postveraison shoot trimming would be manifested in altered cluster and
223 berry morphologies and eventually fruit composition. These are evident in our study wherein the
224 postveraison shoot trimming reduced cluster compactness by loosening the clusters in
225 Sangiovese, a cultivar well-known for its vigorous growth and large sized compact clusters of
226 varying degree (moderately compact, semi-compact, and compact) (Nelson-Kluk 2006). A
227 similar effect was observed when shoot topping (shoot tips tipped off ~5 cm below the apex) was
228 delayed until four weeks after the end of flowering in Riesling and Pinot gris (Molitor et al.
229 2015). Other canopy management practices that yielded similar effects include techniques of
230 pruning and leaf removal (Martin 1990), but it was not clear as to what might have contributed to
231 reducing cluster compactness and the consequent effects on fruit composition. As per this study,

232 it was reduced berry size (weight) and cluster weight that loosened the clusters and the
233 ramification of this physiological activity in terms of productivity was reduced yield per vine,
234 which was expected as berry and cluster weights are a function of productivity. This trade-off is
235 a highly sought after feature by the practitioners of organic viticulture as loosened clusters by
236 virtue of increased epicuticular wax load and cuticle thickness become less susceptible to cluster
237 rots (Martin 1990). Accompanying the loosened clusters were low Brix, pH, and yield. This is a
238 reflection of constrained ripening associated with reduced leaf area following shoot trimming
239 (Stoll et al. 2009). Contrary to our study, these attributes reacted far more negatively (i.e. drastic
240 reductions) when a similar form of severe shoot trimming was performed after fruit set (berry
241 diameter 3-4 mm) by excising the internode just above the distal clusters of Grenache and
242 Tempranillo (Martínez de Toda et al. 2013). Then again, analogous treatments of our study
243 (trimming to the 10th node) performed early in the season (pre-veraison) not only elongated
244 clusters but also increased yield and Brix (Cartechini et al. 2000) except for its combination with
245 nitrogen application, wherein shoot trimming decreased fruit quality (Keller et al. 1999). While
246 the Brix, pH, and yield were reduced, the anthocyanin profile and total anthocyanins remained
247 unaffected by shoot trimmings. Herrera et al. (2015) observed the same phenomenon when
248 Merlot vines were subjected to severe canopy reduction during early stages of ripening. These
249 studies confirm that many of the precursors for anthocyanin development accumulate pre-
250 veraison (Dokoozlian and Kliewer 1996) whereas the postveraison anthocyanin accumulation is
251 mediated by sugars inducing genes for encoding enzymes in the anthocyanin biosynthesis
252 pathway (Conde et al. 2007). If this is the case, then what was the source of sugars for
253 accumulating anthocyanins in trimmed shoots? The obvious one is the reserves, which are
254 stimulated to hydrolyze by pruning activities into sugars so as to remobilize them into the sink

255 organs-in-need (Clair-Maczulajtys et al. 1996). Conversely, shoot-trimming during pre-veraison
256 (Cartechini et al. 2000) or when shoots were trimmed to two leaves per cluster reduced
257 polyphenols including anthocyanins due to inhibition of anthocyanin biosynthetic enzymes (Wu
258 et al. 2013). The same phenomenon is responsible for anthocyanin losses under conditions of
259 high temperature (Mori et al. 2007), which explains the lower anthocyanin levels in 2011, a
260 relatively much warmer season than the preceding wet seasons receiving high rainfall (190-455
261 mm) from budbreak to harvest. Such results are a testimony to climatic differences playing a
262 major role in inducing seasonal variations in growth and fruit quality attributes (Herrera et al.
263 2015). The TA analogous to anthocyanins was not affected either. Such a response was expected
264 of as grape berries complete acid accumulation by synthesizing most of it *in situ* not later than
265 veraison (Conde et al. 2007). In contrast, the accumulation of nitrogen in the form of α -amino
266 acids and ammonia contributing to the pool of YAN increases at the onset of veraison (Bell and
267 Henschke 2008) and requires continuous supply of photoassimilates (Perez and Kliewer 1982).
268 Hence, not surprisingly, the YAN levels declined with the removal of leaves due to shoot
269 trimming.

270 Since the physiological status of vines at a given phenological event determines
271 morphological features and shapes fruit quality attributes, the next logical proceeding is to
272 analyze cluster architecture and fruit composition of trimmed shoots in the context of vine
273 physiology and growth characteristics to understand the mechanics behind observed changes. In
274 view of that, what ensued from postveraison shoot trimming was an unexpected and accelerated
275 senescence of the shoot system. When this happens, the source:sink ratio declines and
276 subsequently the overall photosynthetic capacity is compromised (Poni et al. 2003). This is
277 simply due to the fact that the most photosynthetically active leaves during ripening are located

278 at the top of the canopy and those arising from lateral shoots (Candolfi-Vasconcelos et al. 1994).
279 These sources provide abundant supply of assimilates for increasing both yield and Brix (Poni
280 and Intrieri 1996), which did not occur in our study due to severe shoot trimming after veraison.
281 On the other hand, if shoots are trimmed lightly (e.g. shoot topping) early in the season (before
282 veraison), Brix and yield increase due to elimination of competing organs of shoot tip
283 (Cartechini et al. 2000). Based on these observations, it is logical to infer that source limitation
284 reduces Brix and yield. But then again, one may argue that shoot trimming should enhance
285 ripening as it induces lateral growth with more photosynthetically efficient leaves for
286 transporting assimilates into the berry (Koblet et al. 1996). The compensatory lateral growth for
287 supporting berries did not surface in our study as shoots trimmed after veraison induce little
288 regrowth (Poni and Intrieri 1996).

289 Other consequences of postveraison shoot trimming relates to an imbalanced (decreased)
290 source:sink ratio resulting in a shift in sink strength, the ability to attract photosynthates towards
291 stems and roots limiting partitioning into berries (Kriedemann and Lenz 1972). In this study,
292 roots whose sink strength is generally weakened in the presence of fruits, the organs with the
293 highest sink potential (Minchin and Lacoïnte 2005), became the next strongest sink as these are
294 the site of cytokinin synthesis and any tissue or organ enriched with cytokinins is known to
295 attract assimilates (Ronzhina 2004). Further support comes from Ravaz index (yield:pruning
296 weight ratio), a measure of crop load and source-sink relationship (Kliewer and Dokoozlian
297 2005) increased with trimming treatments. However, such evidence needs to be interpreted with
298 caution as the increase in Ravaz index primarily ensued from losses of leaves and stems caused
299 by shoot trimming *per se*. Given the severity of the trimming, the loss in shoot dry matter in our
300 study is estimated to be more than 25%. As a result, most of the assimilates freed by fruits were

301 mobilized into roots, a cogent course of action not only to avoid death of fine roots but also to
302 replenish the sugars lost to bud break and early summer growth.

303 The reduced sink strength of berries in the shoot-trimmed clusters is also a reflection of
304 resistance to flow in the phloem pathway (Minchin and Lacoïnte 2005) due to plugging of sieve
305 plates of the phloem sieve tubes with callose, a polymer of carbohydrate, which is synthesized
306 naturally when vines go into dormancy (Bondada, 2014). Its synthesis can also be induced by
307 abiotic stress (Bondada 2014), hence, it is highly possible that the stress caused by trimming
308 (Candolfi-Vasconcelos et al. 1994) might have accelerated an early synthesis of callose thereby
309 reducing sugar export into the berries and the fact that postveraison berry expansion relies on
310 phloem influx (Bondada et al. 2005), a resistant phloem will reduce berry size by reducing its
311 sink strength. Although these premises lend credible physiological explanations for the lower
312 Brix of shoot-trimmed clusters, they need to be evaluated in future studies.

313

Conclusions

314 Grapevines are subjected to many different cultural practices that may or may not favor
315 fruit quality. The results of this study on shoot trimming are in favor of promoting positive
316 effects on cluster morphology and fruit quality i.e. a reduction in cluster compactness and sugar
317 levels, which ensued from source-sink imbalance associated with postveraison shoot trimming.
318 Despite reductions in yield and sugar levels, the overall fruit quality was not lost to shoot
319 trimming. This is a reflection of adaptive nature of grapevine conferring them with adjustments
320 to maintain homeostasis. Grapes with reduced sugar levels is highly attractive to wineries as they
321 can be fermented into balanced wines of reduced alcohol content with relative ease; such
322 qualities are highly desired in today's market and by consumers. From an environmental

323 perspective, the cultural practice of shoot trimming is ideal to organic viticulture, which aims at
324 promoting and enhancing biodiversity and soil biological activity. Hence, this practice is worthy
325 of deserving recommendation to organic grape growers as an effective cultural practice for
326 reducing sugar levels in tandem with cluster compactness in Sangiovese.

327

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Table 1 Growth characteristics, crop load, and yield and yield components in control, lightly and severely trimmed Sangiovese vines from 2008-2011.

Year	Treatment	Pruning			Yield (kg/plant)	Ravaz index	Cluster	Berry
		wt (kg/vine)	LA1 (m ²)	LA2 (m ²)			wt (g)	wt (g)
2008	CK	0.47 a	4.5 a	-	5.6 a	11.9 b	332.0 a	2.2
	ST	0.39 b	2.9 b	1.3	5.2 ab	13.3 b	318.4 ab	2.1
	LT	0.25 c	2.4 c	2.5	4.7 b	18.8 a	282.6 b	2.0
	Significance	***	**	***	*	***	*	ns
2009	CK	0.55 a	3.6 a	-	3.4 a	6.0 b	268.1 a	2.6
	ST	0.42 b	2.8 ab	1.1	3.2 ab	7.6 ab	245.9 ab	2.5
	LT	0.29 c	2.1 b	1.6	2.6 b	9.0 b	210.2 b	2.4
	Significance	***	***	ns	*	**	*	ns
2010	CK	0.66 a	2.9 a	-	5.4 a	8.2 b	294.5 a	2.5
	ST	0.55 a	2.4 b	0.9	4.7 ab	8.5 b	260.5 b	2.5
	LT	0.40 b	1.3 c	0.6	4.0 b	10.0 a	246.8 b	2.5
	Significance	**	***	***	*	*	*	ns
2011	CK	0.42 a	4.9 a	-	2.5 a	6.0 b	216.1 a	2.1 a
	ST	0.32 b	2.7 b	0.8	2.2 a	6.9 ab	191.6 b	1.9 b
	LT	0.21 c	1.9 c	1.2	1.8 b	8.6 a	158.9 b	1.8 b
	Significance	***	***	*	**	*	**	***

*Significant at $p < 0.05$, ** significant at $p < 0.01$, *** significant at $p < 0.001$, ns, not significant. Means within a column followed by different letters are significantly different according to the Student-Newman-Keuls test (pruning weight, Ravaz index, LA, berry weight, yield) and Kruskal-Wallis test (cluster weight). CK, control vines; LT, lightly trimmed vines; ST severely trimmed vines; LA1, leaf area before imposing the treatments; LA2, leaf area after imposing the treatments.

Table 2 Cluster compactness (OIV rating) in control, lightly and severely trimmed Sangiovese vines from 2010 and 2011 experiments.

Treatment	Cluster compactness	
	2010	2011
CK	6.5 a	7.3 a
LT	6.6 a	5.5 b
ST	5.6 b	5.6 b
Significance	*	*

*Significant at $p < 0.05$. Means within a column followed by different letters are significantly different according to the Student-Newman-Keuls test. CK, control vines; LT, lightly trimmed vines; ST, severely trimmed vines.

Table 3 Berry compositional attributes in control, lightly and severely trimmed Sangiovese vines from 2008-2011.

Year	Treatment	TSS (Brix)	pH	TA (g/L tartaric acid)	YAN (mg/L)
2008	CK	22.7 a	3.30	6.8	
	ST	21.5 ab	3.30	7.0	
	LT	20.7 b	3.20	7.1	
	Significance	*	ns	ns	
2009	CK	24.2 a	3.40 a	4.6	165 a
	ST	23.4 b	3.30 ab	4.4	142 b
	LT	23.5 b	3.4 b	4.9	140 b
	Significance	*	***	ns	*
2010	CK	20.9	3.11	7.0	106
	ST	20.5	3.11	7.1	105
	LT	20.6	3.13	6.7	116
	Significance	ns	ns	ns	ns
2011	CK	25.6	3.49 a	7.0	99
	ST	25.4	3.45 b	6.9	91
	LT	25.3	3.41c	6.7	81
	Significance	ns	***	ns	ns

*Significant at $p < 0.05$, ** significant at $p < 0.01$, significant at $p < 0.001$. Means within a column followed by different letters are significantly different according to the Student- Newman-Keuls test. CK, control vines; LT, lightly trimmed vines; ST, severely trimmed vines; TA, titratable acidity; TSS, total soluble solids; YAN, yeast assimilable nitrogen.

Supplemental Table 1 Concentration (mg/g of skin) of individual anthocyanins at harvest in berries of control, lightly, and severely trimmed Sangiovese vines from 2008-2011. Cyanidin-3-glucoside (cn-3G); peonidin-3-glucoside (pn-3G); delphinidin-3-glucoside (dp-3G); petunidin-3-glucoside (pt-3G); malvidin-3-glucoside (mv-3G); and total glycosylated anthocyanin (tot-G).

Year	Treatment	cn-3G	pn-3G	dp-3G	pt-3G	mv-3G	tot-G
2008	CK	0.28	0.21	0.21	0.24	0.71	1.64
	LT	0.26	0.19	0.21	0.24	0.71	1.61
	ST	0.28	0.19	0.20	0.23	0.65	1.55
	<i>Significance</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
2009	CK	0.34	0.21	0.14	0.16	0.38	1.22
	LT	0.37	0.21	0.16	0.17	0.40	1.30
	ST	0.36	0.20	0.15	0.17	0.41	1.29
	<i>Significance</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
2010	CK	0.51	0.26 a	0.28	0.29	0.71	2.04 a
	LT	0.43	0.22 b	0.23	0.25	0.59	1.71 b
	ST	0.51	0.25 ab	0.28	0.30	0.63	1.95 ab
	<i>Significance</i>	<i>n.s.</i>	*	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	*
2011	CK	0.17	0.11	0.07	0.08	0.17	0.59
	LT	0.16	0.10	0.07	0.08	0.16	0.56
	ST	0.16	0.10	0.07	0.08	0.16	0.57
	<i>Significance</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>

*Significant at $p < 0.05$; ns, not significant. Means within a column followed by different letters are significantly different according to the Student- Newman-Keuls test. CK, control vines; LT, lightly trimmed vines; ST, severely trimmed vines.