1 **Research Article**

2 # Impacts of Early Leaf Removal and Cluster Thinning on
3 # Grüner Veltliner Production, Fruit Composition,
4 # and Vine Health

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24 **Abstract:** A traditional crop load regulation technique, cluster thinning (CT), was compared to a

25 more innovative technique, early leaf removal (ELR) applied either at trace bloom (TBLR) or fruit

26 set (FSLR), on high-yielding and vigorous *Vitis vinifera* Grüner Veltliner. Treatment effects on

27 key production parameters, *Botrytis* bunch rot, tolerance to winter temperatures, and production

28 costs were evaluated and compared to an untreated control over two years. Compared to CT, we

29 hypothesized that ELR would improve fruit composition, reduce *Botrytis* bunch rot, and decrease

30 grower costs. Yield regulation imposed by CT was significantly higher (39.3%) than that of

31 TBLR (12.6%) or FSLR (13.3%), but neither CT nor ELR consistently improved fruit chemistry.

32   Our results suggest that the number of leaves removed (five) at trace bloom or fruit set were

33   insufficient to induce a carbohydrate-limiting response, as ELR vines did not have lower fruit set

34   or bud fruitfulness. Concurrently, TBLR and FSLR vines did not show recovery mechanisms

35   such as greater production of lateral leaves or higher shoot efficiency. Although the overall level

36   of bunch rot severity was lower than 5%, ELR consistently decreased bunch rot intensity (TBLR,

37   FSLR) and severity (FSLR). TBLR also improved bud freezing tolerance during vine

38   acclimation in both years. CT was the most expensive treatment, and the lack of a consistent

39   improvement in fruit chemical composition or tolerance to winter temperatures indicated that

40   Grüner Veltliner can properly ripen more than one cluster per shoot.

41   **Key words:** canopy management, cold hardiness, crop level, economic sustainability, grape

42   production, viticultural practice

43   **Introduction**

44   Grüner Veltliner (*Vitis vinifera*) is a white grape cultivar widely planted in Austria,

45   representing about 47% of the total grape acreage (Austria Wine Marketing Board 2015). Grüner

46   Veltliner has historically been grown in the neighboring countries of the Czech Republic and

47   Hungary, and more recently, in areas of southern New Zealand (Robinson et al. 2012). In

48   Pennsylvania, it was first planted in 2003 (G. Troxell, personal communication, 2014) and, based

49   on anecdotal observations from Pennsylvania commercial grape growers, Grüner Veltliner

50   plantings have expanded throughout the state due to its suitability to regional growing

51   conditions. Because of its recent introduction, very little is known about the best production

52   practices for Grüner Veltliner under eastern US environmental conditions. A study conducted in

53   Virginia, which has a warm and humid climate, suggests that Grüner Veltliner is a fruitful

54   cultivar capable of ripening high crop yields (5.0 kg/m of cordon; Wolf and Warren 2000). The

55   reported levels of bunch rot were similar to Chardonnay, a cultivar with known susceptibility to

56   bunch rot, but higher than other white grape cultivars such as Petit Manseng and Viognier (Wolf

57   and Warren 2000). However, there is still uncertainty on how crop load (fruit versus vegetative

58   biomass) management can be adjusted for optimum fruit production and quality while

59   minimizing potential for bunch rot infection.

60   Cluster thinning (CT) is a crop load management practice traditionally used to reduce

61   crop level and enhance fruit ripening of wine grape cultivars that tend to overcrop (Dami et al.

62   2006 and 2013, Gatti et al. 2012). Despite growers need for high fruit yield to maximize

63   economic return, excessively high crop levels can lead to delayed fruit ripening and wood

64   maturation, decreased carbohydrates storage, and potentially increased vine susceptibility to

65   winter injury (Dami et al. 2006). While CT may improve fruit composition (Dami et al. 2006)

66   and wine quality (Prajitna et al. 2007), the improvement in wine sensory perception might be not

67   sufficient to justify the costs associated with the treatment (Preszler et al. 2013). Therefore, the

68   additional costs of labor and yield loss associated with CT might discourage some producers

69   from adopting it.

70   Alternatively, removing leaves in the fruit-zone shortly before or at beginning of bloom

71   through fruit set (early leaf removal; ELR) has shown promise as a crop regulation technique

72   mainly in European countries where grape yield is regulated by law (Poni et al. 2006, Intrieri et

73   al. 2008, Tardaguila et al. 2010, Gatti et al. 2012). Early leaf removal reduces crop yield by

74   imposing a carbohydrate limitation during bloom and early stages of fruit development (Poni et

75   al. 2006). Mechanisms behind ELR-induced yield reduction depend on the timing of application.

76   Limiting carbohydrate supply shortly before or at beginning of bloom may decrease fruit set

77   through increased flower abscission (Lebon et al. 2008), thus decreasing the total number of

78   berries per cluster. When basal leaves are removed at the onset of fruit set, lower carbohydrate

79   supply may limit cellular division during the initial stages of berry growth, thereby decreasing

80   final berry size (Jona and Botta 1988). In some cases, but not always, ELR applied at pre-bloom

81   reduced berry weight in addition to fruit set (Poni et al. 2006, Gatti et al. 2012).

82   Grapevines can exhibit mechanisms of physiological recovery from ELR, such as

83   increased leaf carbon assimilation (A), greater production of lateral leaves (i.e., younger mean

84   leaf age), and similar or greater leaf area-to-yield ratio as compared to undefoliated vines (Poni

85   et al. 2006, Palliotti et al. 2011). Those recovery mechanisms might improve post-veraison

86   canopy efficiency and in part explain why carbohydrate limitation imposed by ELR does not

87   typically have a negative impact on fruit ripening and might even improve secondary metabolite

88   composition in juice (Tardaguila et al. 2010, Bubola et al. 2017) and wine (Sivilotti et al. 2016,

89   Hickey et al. 2018).

90   Although previous work often reports data on ELR effects on fruit chemical composition,

91   to our knowledge, those data have not been related to the position of the cluster on the shoot (i.e.,

92   basal versus distal nodes). Several studies on ELR analyzed fruit composition of the basal cluster

93   only, despite wine chemistry reflects the composition of both basal and distal clusters (Intrieri et

94   al. 2008, Tardaguila et al. 2010, Gatti at al. 2012). Other work analyzed berry samples from all

95   clusters regardless of their position on the shoot (Silvestroni et al. 2016, Bubola et al. 2017).

96   Bloom typically begins in the basal inflorescence of a shoot (Vasconcelos et al. 2009); therefore,

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97   when ELR is applied, distal inflorescences might be at an earlier phenological stage compared to

98   those developed from basal nodes. Currently, it is unclear how ELR influences cluster

99   morphology and chemical composition of distal clusters and the resulting impact on total fruit

100   biomass used for winemaking.

101   When compared with CT, ELR has several potential advantages and drawbacks. Early

102   leaf removal might reduce cluster compactness (Poni et al. 2006, Tardaguila et al. 2010) and

103   therefore decrease fruit susceptibility to bunch rot (*Botrytis cinerea*), a major fungal disease in

104   humid regions (Hed et al. 2015). In contrast, CT tends to increase berry growth and consequently

105   cluster compactness, a compensatory result from removal of carbohydrate sinks (Ferree et al.

106   2003). Additionally, ELR improves canopy microclimate by increasing fruit-zone sunlight

107   exposure, air flow, and pesticide spray penetration (Hed and Centinari 2018, Hickey and Wolf

108   2018). This might contribute to reducing bunch rot and improving primary and secondary

109   metabolites to desirable levels in the harvested fruit (Bubola et al. 2017).

110   To its detriment, repeated use of ELR over multiple years has unintentionally reduced

111   bud fruitfulness (i.e., number of clusters per shoot) in some cultivars despite increased sunlight

112   exposure during bud formation (Sabbatini and Howell 2010, Hickey and Wolf 2018). Moreover,

113   it is still uncertain how carbohydrate source-sink manipulation from ELR might impact storage

114   of soluble carbohydrates (e.g., sucrose, fructose, glucose, and raffinose oligosaccharides) that

115   play a critical role in vine freeze tolerance. Potential effects of ELR on vine susceptibility to

116   winter injury is an important consideration for grape growing regions that experience low winter

117   temperatures capable of damaging dormant tissues of *Vitis vinifera* cultivars.

118   The overall goal of this study was to provide targeted crop load management

119   recommendations for Grüner Veltliner production under cool and humid growing conditions. We

120   compared CT, a traditional crop load regulation practice, to a more innovative and still

121   experimental practice, ELR, imposed at two different phenological stages. We hypothesized that,

122   regardless of timing of application, ELR would be effective at reducing yield and improving fruit

123   composition, while reducing cluster compactness and bunch rot compared with CT and an un-

124   thinned, non-early defoliated control. Due to the expense and time required to perform crop load

125   management practices, an economic assessment was developed to determine whether ELR would

126   be a less expensive practice for yield reduction as compared to CT. We expected CT to have

127   higher economic costs due to a longer application time required relative to ELR. To investigate

128   effects of ELR on vine susceptibility to winter low-temperature, we estimated bud freeze

129   tolerance using differential thermal analysis (DTA) during vine acclimation, maximum winter

130   hardiness, and de-acclimation.

Materials and Methods

132   **Vineyard site and experimental design.** The study was conducted in 2015 and 2016 on

133   Grüner Veltliner cl. 01 (*Vitis vinifera*) at a commercial vineyard in Lewisburg (40°59′N; 76°5′W;

134   elevation: 171 m above sea level), Pennsylvania, US. The vineyard soil was classified as Elliber

135   silt-loam (https://websoilsurvey.sc.egov.usda.gov/). The vines were grafted on 101-14 Mgt (*V.*

136   *riparia × V. rupestris*) rootstock and planted in 2010. The vine spacing was 1.5 m within rows

137   and 2.4 m between rows for a density of 2778 vines per hectare with rows oriented north-south.

138   Vines were trained to a bilateral cordon at 0.8 m height and vertically shoot positioned. Winter

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139 pruning was performed to retain eight two-bud spurs (16 nodes) per meter. Shoot density was

140 adjusted to an average of 15 shoots per meter of cordon on 25 May 2015 and 26 May 2016 when

141 shoots reached an average growth stage E-L 14 according to the modified Eichhorn-Lorenz

142 system (E-L; Coombe 1995). Shoot hedging was performed two to three times per season

143 between mid-July and late-August. Standard disease and insect control practices for *V. vinifera*

144 cultivars in the eastern US were used (Wolf 2008).

145   Four adjacent rows were selected for the study. The experimental design was a complete

146 randomized block design with four blocks per treatment that were assigned across the four rows.

147 Each experimental unit consisted of 12 contiguous vines, with 10 inner vines used for data

148 collection and the outer two serving as guard vines. The treatments consisted of a control (un-

149 thinned and non-early defoliated vines); ELR performed at E-L 19, when flowers began to open

150 (trace bloom leaf removal; TBLR); ELR performed at E-L 27 representing the onset of fruit set

151 with clusters at 90° angle from the shoot (fruit set leaf removal; FSLR); and CT performed at E-

152 L 32, bunch closure. Phenological assessment was not conducted seperately for basal and distal

153 inflorescences. Three shoots from each one of the four central vines of each experimental unit

154 (12 shoots per experimental unit) were flagged on the same day of shoot thinning for data

155 collection.

156   Early leaf removal treatments were implemented on both sides of the canopy by hand-

157 removing the first five well-developed basal main leaves on each shoot and any associated lateral

158 shoots that developed from the same nodes. Treatments were applied on 3 Jun 2015 and 9 Jun

159 2016 (TBLR) and 18 Jun 2015 and 21 Jun 2016 (FSLR). Cluster thinning was performed by

160 removing distal clusters and retaining only one cluster per shoot on 1 Jul 2015 and 13 Jul 2016.

161　The day CT was performed, three basal leaves were removed on both side of the fruit-zone of

162　control and CT vines in accordance with standard grower practice. Treatments were applied on

163　the same vines in 2015 and 2016. No additional passes of leaf removal were applied to any of the

164　treatments during the season to mimic the grower's practice.

165　　　**Weather conditions.** Air temperature was recorded every 30 min throughout the study

166　period using wireless temperature data loggers (iButton Fob, Model DS9093Fl; Embedded Data

167　Systems, Lawrenceburg, KY). One temperature sensor was placed on the first set of catch wires

168　at the height of the fruit-zone in each experimental unit of the control treatment. Growing degree

169　days (GDD, base 10 °C) were calculated for each sensor from 1 Apr to harvest as GDD =

170　[(maximum daily temperature + minimum daily temperature)]/2] – 10. Daily precipitation from 1

171　Apr to harvest for the town of Lewisburg was acquired from the National Center for

172　Environmental Information (https://www.ncdc.noaa.gov/cdo-web/datasets#GHCND).

173　　　**Vegetative growth measurements.** To determine treatment impacts on canopy density

174　and light availability in the fruit-zone, enhanced point quadrat analysis (EPQA, Meyers and

175　Vanden Heuvel 2008) was assessed twice per season. EPQA measurements were performed

176　preveraison on 16 Jul 2015 and 28 Jul 2016 and post-veraison on 25 Aug 2015 and 7 Sep 2016.

177　Point quadrat analysis was conducted using a thin metal rod and an insertion guide marked with

178　20 cm intervals to make 36 insertion points per experimental unit. Photosynthetically active

179　radiation (PAR) was measured with a LI-250A ceptometer (LI-COR Bioscience, Lincoln, NE)

180　within 2 hrs of solar noon on the same day under full-sun conditions. Ambient PAR was

181　measured for each experimental unit by averaging one measurement per second over 15 sec with

182　the ceptometer positioned above the canopy in the middle of the row and the sensors oriented

183 parallel to the sky. Ten intracanopy PAR measurements per experimental unit were taken with

184 the ceptometer placed parallel to the vine row in the interior of the canopy at fruit-zone height

185 with the sensor directly facing upward. In-canopy photon flux was calculated as the ratio of

186 intracanopy canopy PAR and ambient PAR. Canopy density and in-canopy flux measurements

187 were analyzed with Canopy Exposure Mapping Tools (v. 1.7, freeware from J.M. Meyers,

188 Cornell University, Ithaca, NY), which was developed to calculate occlusion layer number, leaf

189 and cluster exposure flux availability (Meyers and Vanden Heuvel, 2008).

190 All flagged shoots were collected one day prior to harvest to assess end-of-season shoot

191 leaf area. Main and lateral leaves were measured separately using a scanning leaf area meter (LI-

192 3100c, LI-COR Bioscience, Lincoln, NE). Pruning weights were taken on the 10 inner vines of

193 each experimental unit during the dormant season (9 Mar 2016 and 9 Mar 2017) with a 0.01 kg

194 accuracy hanging scale (Pelouze 7710, Rubbermaid, Inc., Huntersville, NC).

195 **Fruit set, yield parameters, and fruit composition.** Fruit set was estimated in 2016 for

196 each inflorescence of the control and ELR flagged shoots at stage E-L 18, as reported by Poni et

197 al. (2006). The basal and distal inflorescence of each flagged shoot, as well as 50 inflorescences

198 from non-experimental Grüner Veltilner vines, were photographed on a flat, white background

199 on the same day. The number of visible flowers in each photo was counted using MS Paint

200 (Microsoft, Bellevue, WA). After taking the photos, the 50 inflorescences from the non-

201 experimental vines were collected, transported to the laboratory, and the actual number of

202 flowers per inflorescence were counted. The regression equation ($y = 1.404x + 11.347$; $r^2 =$

203 0.831) established between the actual number of flowers per inflorescence and that counted on

204 the 50 photos was used to estimate the number of flowers on the inflorescences of the flagged

205 shoots. The percentage of fruit set was then determined at harvest as ratio between the total

206 number of berries per cluster and the number of estimated flowers at stage E-L 18. Because of

207 lack of personnel resources, we were unable to take pictures of the inflorescences at stage E-L 18

208 in 2015; therefore, fruit set was not estimated in the first year of the study.

209   Vines were harvested by hand on 24 Sep 2015 and 28 Sep 2016 the day before

210 commercial harvest. Immediately prior to harvest, 30 randomly selected basal and distal clusters

211 per experimental unit were visually assessed for bunch rot incidence (percentage of clusters

212 infected) and severity (percentage of infected cluster area; Horsfall and Barratt 1945). Clusters

213 from the 10 inner vines of each experimental unit were counted and weighted using a hanging

214 scale accurate to 0.01 kg (Pelouze 7710). The average cluster weight was calculated by dividing

215 yield by the number of clusters per experimental unit. Ravaz index was calculated as the ratio of

216 yield to pruning weight collected in the following dormant season.

217 In both years, basal and distal clusters of flagged shoots were collected separately two days prior

218 to harvest. Cluster compactness was rated for each cluster using a scale from 1 to 5, where 1

219 described a cluster as "very loose; no berry contact; bending of the stem to 90° possible" and 5 as

220 "very compact; berries not flexible; bending of the stem not possible" (Ipach 2005). Harvested

221 clusters were then frozen at – 20 °C until they were deconstructed to count the number of berries

222 per cluster and measure average berry weight. Frozen berries were divided in the following

223 categories: full size and healthy berries, berries infected by rot, small seedless berries (< 5 mm

224 diameter, 'chicken' berries), and live green ovaries (small, firm, green, seedless berries; Iland

225 2011). Berries were counted and weighted by category, thawed in a water bath at 60 °C, crushed,

226 and strained to remove skin and seeds for juice chemistry analysis. Total soluble solids (TSS)

227   were measured using a hand-held refractometer (Master, Atago USA, Inc., Bellevue, WA), pH

228   using a pH-meter (Orion Star A111, Thermo Fisher Scientific, Waltham, MA), and titratable

229   acidity (TA) was measured using an autotitrator (G20, Mettler Toledo, Columbus, OH).

230   Titrations were made using a 10 mL juice sample size titrated with 0.1 N NaOH to an endpoint

231   pH of 8.2. Juice chemistry analysis was conducted for basal and distal clusters separately but

232   also for both clusters combined.

233   **Bud freeze tolerance and fruitfulness.** Primary bud freeze tolerance was estimated monthly

234   throughout the dormant season from November to March. The differential thermal analysis

235   (DTA) method was used in accordance to Mills et al. (2006). Eight canes were collected

236   randomly across the 10 inner vines of each experimental unit and stored at 4 °C until analysis.

237   DTA analysis was performed twice over the following 48 hrs; four canes for each experimental

238   unit were used for each DTA run: five buds per cane were excised from nodes two through six

239   with approximately 2 mm of intact surrounding tissue and placed on a thermoelectric module

240   (Melcor Corporation, Trenton, NJ). Six trays, each containing nine cell modules each, were

241   placed in a programmable temperature-controlled freezer (Tenney, Thermal Products Solutions,

242   New Columbia, PA). The temperature was lowered from 4 °C to – 40 °C at a rate of 4 °C hr^{-1},

243   held at – 40 °C for 1 hr, and then increased to 4 °C at the rate of 4 °C hr^{-1}. Lethal bud

244   temperature was expressed as median low temperature exotherm (LT_{50}) or the temperature at

245   which 50% of primary buds died. Bud fruitfulness was evaluated as number of inflorescences per

246   shoot the spring following treatments application. Measurements were taken prior to shoot

247   thinning on 25 May 2016 and 9 May 2017.

248   **Economic analysis.** An economic analysis was conducted to estimate the additional price per

249   tonne of Grüner Veltilner grapes and retail price per 750 mL bottle of wine if CT, TBLR, and

250   FSLR were adopted compared no additional crop load management practices except shoot

251   thinning (Preszler et al. 2013). The time necessary to apply each treatment was recorded for each

252   experimental unit in 2015 and 2016 and averaged across the two years. The additional labor costs

253   per hectare were estimated according to Yeh et al. (2014). Expected revenue per hectare was

254   calculated by multiplying yield by the average industry price per tonne for Grüner Veltliner for

255   2015 and 2016 (https://flgp.cce.cornell.edu). The additional production cost per tonne, the

256   additional price per tonne (i.e., grower preferred price), and retailed price per 750 mL bottle of

257   wine required to provide the same grower revenue were estimated.

258   **Statistical analyses.** Data analyses were performed using JMP statistical software (v.

259   12.1, SAS Institute, Cary, NC). All data were analyzed using a mixed-effects analysis of

260   variance (ANOVA) model with year and treatment as fixed-effects and block as a random-effect.

261   Treatment differences were assessed using Tukey's honestly significant difference (HSD) pair-

262   wise comparison test. In the instance of a treatment by year interaction effect, parameters were

263   analyzed separately within each year. We chose to use a more liberal critical value to test our

264   hypotheses (probability value of 10% rather than 5%). Data collected under field condition could

265   be quite variable and, therefore, it would require a very large sample size to detect difference at

266   the 5% level (Marini 1999). We also elected to report exact P-values to help the reader with data

267   interpretation.

Results

269   **Weather conditions.** Seasonal GDD calculated from 1 Apr to harvest was similar

270   between the two growing seasons. Cumulative GDD was 53 GDD higher in 2015 than 2016

271 (1884 *vs.* 1831). However, 2015 was a wetter year with 161 mm more precipitation than 2016

272 (555 *vs.* 394 mm). Cumulative precipitation from Apr 1 to harvest was close to the past 10-year

273 average (589 mm) in 2015, but much lower in 2016.

274   **Vine vegetative growth.** Overall, ELR had modest effects on canopy density and no

275 impact on the amount of sunlight reaching the clusters and the leaves in the fruit-zone (Cluster

276 and leaf exposure flux availability; Table 1). Results were consistent between the two years;

277 differences were mainly observed preveraison, while tended to disappear later in the season, after

278 veraison. As expected, ELR reduced the number of leaf layers (TBLR, FSLR), the number of

279 shade-producing leaves and clusters (occlusion layers; TBLR), and the percentage of interior

280 leaves and clusters (FSLR) preveraison when compared to the control (Table 1). Main, lateral,

281 and total leaf area did not differ amongst treatments in 2015; however, in 2016, FSLR vines had

282 lower main and total leaf area than CT vines (Table 2). Pruning weight was lowest in the TBLR

283 and highest in the CT treatment (Table 3).

284   **Yield parameters, fruit set and composition.** The crop load management treatments

285 had lower yield compared to control vines in both years (Table 3). Cluster thinned vines had the

286 lowest yield and Ravaz index; on average, CT vines had 39.3% lower yield than control vines.

287 Yield reductions were less severe for TBLR and FSLR vines; they averaged to 13.3% and

288 12.6%, respectively, compared with the control. As expected, the lower yield of CT vines was

289 due to a lower number of clusters per vine compared to the control and ELR (Table 3). Cluster

290 thinned vines had the greatest average cluster weight when compared to basal and distal clusters

291 combined of C and ELR vines (Table 3). However, when comparing basal clusters only, berry

292 weight of CT was not higher than that of the control or TBLR vines (Table 4). Although TBLR

293 and FSLR vines exhibited lower yield than control vines, they did not have lower cluster weight,

294 as we predicted, or fewer clusters (Table 3). Regardless of the timing, ELR did not affect fruit set

295 of either the basal ($P = 0.477$) or distal cluster ($P = 0.378$) when compared to the control; fruit

296 set averaged to 49.2% for the control, 45.1% for TBLR, and 48.6% for FSLR. Similarly, total

297 number of berries per cluster, percentage of 'chicken' berries, and percentage of live green

298 ovaries were similar between ELR and control for both basal or distal clusters (Table 4).

299   Early leaf removal, either applied at trace bloom or fruit set, consistently reduced the

300 percentage of clusters infected by bunch rot (Table 3). When compared to the control, FSLR

301 vines also had lower bunch rot severity and cluster compactness. The percentage of berries

302 infected with bunch rot in basal and distal clusters confirmed the visual assessment of bunch rot

303 conducted at harvest. The lowest percentage of infected berries was in the basal cluster of the

304 FSLR vines and distal cluster of the TBLR vines (Table 4).

305   Juice chemistry was affected differently by treatments in the two vintages (Table 5).

306 When combining juice from basal and distal clusters, the only consistent effect across the two

307 years was that FSLR had lower TSS than CT. Concurrently, FSLR had the lowest shoot

308 efficiency, defined as total sugar per shoot or unit of leaf area, and lower leaf area-to-yield ratio

309 compared to CT (Table 6). When analyzing juice chemistry of the basal cluster alone, CT fruit

310 had higher TSS than control in both years, but differences were not consistent across vintages

311 when analyzing the total fruit produced by the shoot (i.e., combined basal and distal clusters for

312 the control treatment).

313   **Bud freeze tolerance and fruitfulness.** Differences in bud freeze tolerance (i.e., LT_{50})

314 amongst treatments were mainly found in November, during the acclimation period (Table 7). In

315 both years, TBLR vines had higher bud freeze tolerance than the control in November; the LT_{50}

316 of TBLR buds was 0.34 °C (2015) and 0.91 °C (2016) lower than that of the control. However,

317 LT_{50} was lower in FSLR (0.35 °C) and CT (0.37 °C) compared with the control only in

318 November 2015. There were no differences in LT_{50} amongst treatments during mid-winter, when

319 buds reached maximum winter freeze tolerance, except for January 2016 when FSLR vines had

320 higher bud freeze tolerance as compared to CT and TBLR vines. Bud freeze tolerance was not

321 affected by CT or ELR in March, as the vines start to de-acclimate. The minimum winter

322 temperatures during the two dormant seasons were –19.5 °C on 14 Feb 2016 and –11.7 °C on 9

323 Jan 2017, which may have not reached critical values likely to have caused extensive damage on

324 Grüner Veltliner (Shellie et al. 2014). Although there were no differences in bud fruitfulness

325 between the crop load management treatments and the control in either year, TBLR vines had

326 fewer clusters per shoot (1.44) than CT (2.04) after two years of treatments application. Overall,

327 bud fruitfulness ranged from 1.60 (control) to 1.88 (TBLR) in 2015 and from 1.44 (TBLR) to

328 2.04 (CT) in 2016.

329   **Economic analysis.** Unsurprisingly, crop load management practices increased grower

330 costs and reduced expected revenue in both years (Table 8). Cluster thinning was the most

331 expensive treatment; if CT was applied, the expected revenue ($/ha) for Grüner Veltliner

332 growers would have been reduced by 44% in 2015 and 46% in 2016. Applying TBLR would

333 have generated 26% (2015) and 16% (2016) less revenue, while applying FSLR would have

334 resulted in 23% (2015) and 19% (2016) less revenue. As a result, Grüner Veltliner producers

335 would have had to increase the retail price for a 750 mL bottle of wine by approximately $0.33

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336 (FSLR) to $1.18 (CT) in 2015 and $0.17 (TBLR) to $1.34 (CT) in 2016 to maintain revenue

337 similar to that of the control (Table 8).

Discussion

339   The primary objective of this study was to understand how CT and ELR at trace bloom

340 and fruit set impact key production parameters and tolerance to winter temperatures of Grüner

341 Veltliner under humid, cool-climate conditions. Early leaf removal, regardless of timing, and CT

342 reduced yield in both years of the study. However, the extent of yield reduction was much higher

343 in CT vines than in those defoliated either at trace bloom or fruit set.

344   Yield reduction induced by CT was predictable and has been well-documented (Dami et

345 al. 2006 and 2013, Gatti et al. 2012, Preszler et al. 2013). When comparing basal clusters, CT

346 vines had a slight but not significant increase in berry weight (50 mg), which was not enough to

347 counter balance yield-reduction effects from CT, as it occurs when clusters are removed at

348 earlier phenological stages (Ferree et al. 2003). Low yield and crop load of the CT vines

349 indicated that Grüner Veltliner can support more than one cluster per shoot. Crop load of CT

350 vines was below the Ravaz index range, 4 to 10, and above the leaf area-to-yield ratio, 8 to 12

351 cm^2/g, suggested for optimal *V. vinifera* wine quality and vine balance (Kliewer and Dokoozlian

352 2005). Furthermore, CT had inconsistent effects on fruit ripeness at harvest, increasing TSS and

353 pH only in one of the two years.

354   Yield reduction induced by ELR is much less predictable than CT and, in our study, was

355 less severe than expected. Defoliation of the first six nodes of the shoot just prior to bloom

356 reduced yields anywhere from 20% to 50% on several *V. vinifera* cultivars (Gatti et al. 2012,

16

357   Silvestroni et al. 2016, Hed and Centinari 2018, Hickey and Wolf 2018). In our study, yield

358   reduction averaged to 12.6 % (TBLR) and 13.3 % (FSLR). More than five leaves would likely

359   need to be removed from vigorous Grüner Veltliner vines under our environmental conditions to

360   significantly decrease fruit set and reduce yield to levels comparable to previous work.

361   Although crop regulation was less severe than what anticipated, yield of ELR vines was

362   significantly lower than that of control vines. The mechanism behind yield reduction remains

363   unclear as ELR vines did not have lower fruit set, number of berries per cluster, or cluster

364   weight. However, it should be noted that yield data was collected for all the experimental vines,

365   which included a greater and more diverse population of shoots compared to the 12 shoots

366   selected per experimental unit for fruit set and berry data collection. We cannot exclude that ELR

367   might have significantly reduced fruit set and berry development when applied on shorter shoots

368   with lower total leaf area than those selected. It also possible that, although not significant at the

369    $P = 0.1$ level, a numerical reduction of average cluster weight for both ELR treatments (10.5%),

370   berry weight for FSLR (basal cluster: 5.5%; distal cluster: 4.5%), and number of berries per

371   cluster for TBLR (basal cluster: 12.5%; distal cluster: 14.7%) might have contributed to the

372   lower overall yield of the ELR vines.

373   Removing five leaves likely did not limit the availability of carbohydrates in a way that

374   could induce recovery mechanics that improve fruit chemical composition. Contrary to our

375   hypothesis, all fruit reached similar technological maturity in both years regardless of crop load

376   management. Previous studies indicated that improved fruit maturity of ELR vines was related to

377   source-sink recovery mechanisms, which included higher late-season canopy efficiency due to

378   greater production of lateral leaves, higher or similar leaf area-to-yield ratio, and greater leaf A

379   rate as compared to undefoliated vines (Poni et al. 2006, Palliotti et al. 2011). Grüner Veltliner

380   vines exhibited vegetative recovery from ELR as main and lateral leaf area and leaf area-to-yield

381   ratio were similar to that of the control vines. However, ELR vines did not have a younger and

382   more efficient canopy (i.e., greater lateral leaf area and/or shoot efficiency) as reported in

383   previous studies (Poni et al. 2008, Palliotti et al. 2011). Under our growing conditions, vines

384   were hedged multiple times until late-August, which might explain why the lateral leaf area was

385   similar amongst treatments. We don't exclude, however, that the effects of CT and ELR on fruit

386   maturity, would be different in cooler and rainy years, since weather conditions during ripening

387   influence the efficacy of CT and leaf removal (Frioni et al. 2007).

388   Our results also indicated that leaf removal would need to be applied multiple times

389   throughout the season to maintain an open fruit-zone in vigorous Grüner Veltliner vines. Lateral

390   shoot growth was observed in the fruit zone after applying ELR treatments but was not

391   quantified. Removing five leaves at bloom or fruit set only temporarily improved canopy density

392   and did not increase sunlight availability to leaves and clusters a few weeks after treatment

393   application. This result contrasted with what was found in Riesling (*V. vinifera*) in a similar

394   grape growing region, where lower canopy density and increased cluster and leaf sunlight

395   exposure effects from mechanical and hand-applied ELR were still present after veraison (Hed

396   and Centinari 2018).

397   Although ELR did not affect fruit set or berry weight, FSLR vines had lower cluster

398   compactness and bunch rot severity. It might be possible that a numerical decrease in average

399   berry weight for the basal and distal cluster (average to 5%), which was not significant at the $P =$

400   0.1 level, was enough to cause a visible reduction in cluster compactness. Cluster loosening is

401 typically a desirable effect from ELR, as it can reduce susceptibility to late-season bunch rot

402 especially in humid climates and for cultivars with compact clusters. The fewer leaf layers in

403 TBLR (preveraison) and FSLR (pre- and post-veraison) compared to the control could have also

404 improved fungicide penetration resulting in lower bunch rot infections. However, overall levels

405 of bunch rot severity were below 5% in both years. Rainfall in September 2015 (41 mm) and

406 2016 (23 mm) was lower than the 10-year average (116 mm) and likely resulted in low late-

407 season disease pressure. Under those weather conditions, implementation of standard canopy

408 management practices (shoot thinning, shoot positioning, hedging, and basal leaf removal at

409 bunch closure) were sufficient to adequately control bunch rot.

410 Overall, ELR effects on cluster morphology or chemical composition were not influenced

411 by the cluster position on the shoot. However, comparing basal clusters of CT shoots with both

412 basal and distal clusters produced by control and ELR shoots instead of the basal cluster only,

413 provided more accurate information on the effect of CT on juice chemistry. When comparing

414 basal clusters, CT fruit had higher TSS than the control in both years, but the difference

415 disappeared in 2015 when combining basal and distal clusters for the control.

416 One of the objectives of this study was to investigate the effects of source-sink

417 manipulation on vine susceptibility to winter injury and bud fruitfulness. The most relevant result

418 was a higher bud freeze tolerance during fall acclimation for the TBLR vines as compared to the

419 control in both year; however, by December, buds amongst all treatments had similar ability to

420 withstand low temperatures. Decreasing crop load resulted in increased bud freeze tolerance in

421 overcropped vines (Dami et al. 2006), but it had also no effect on freeze tolerance when vines

422 were already in balance before crop load adjustments (Dami et al. 2013, Preszler et al. 2013).

423   The higher freeze tolerance during the acclimation period of TBLR vines may have not been

424   caused by a lower crop load but, instead, related to a temporary increase in bud sunlight exposure

425   early in the season during bud development. Bud sunlight exposure can improve bud maturation

426   (Fennell and Hoover 1991) and freeze tolerance during cold temperature acclimation (*V.*

427   *labrusca*; Howell and Shaulis 1980). Crop load management treatments did not impact bud

428   fruitfulness compared to the control in either 2015 or 2016. While the carbohydrate-limiting

429   effect from ELR may negatively impact the development of the inflorescence primordia within

430   the bud, which occurs between bloom and veraison (Sabbatini and Howell 2010, Hickey and

431   Wolf 2018), in our study the amount of leaves removed, as previously stated, were likely

432   insufficient to induce a stress (lower fruit set or bud fruitfulness) or recovery response.

433   When crop load management is implemented, a grape grower might be able to recover

434   economic losses from reduced yield and increased labor costs by harvesting healthier fruit and

435   improving wine quality. Under our experimental conditions, CT generated the greatest loss in

436   revenue among the crop load management treatments because of the lowest yield and highest

437   labor costs but did not improve fruit health or chemistry. Although applying ELR might cause

438   economic losses under relatively dry late-summer conditions, we recognized that outcomes can

439   change under different weather conditions. Costs associated with ELR may be recovered in wet

440   season with higher disease pressure through the reduction of bunch rot severity (FSLR) and

441   intensity (TBLR and FSLR). In our study, secondary metabolites in juice or wine were not

442   analyzed; hence, we cannot determine if an improvement in wine quality due to either ELR or

443   CT might have justified an increase in the retail price per bottle of wine. Estimating costs of crop

444   load management practices can be challenging since many variables can influence the outcomes.

445   Our estimates should be used as an example, and we suggest growers to develop their own cost

446   analysis based on their specific situation.

Conclusion

448   For two consecutive seasons, ELR applied either at trace bloom or fruit set on vigorous Grüner

449   Veltliner vines did not reduce carbohydrate availability in a way that could decrease fruit set or

450   improve fruit quality as reported in previous studies on other *V. vinifera* cultivars. Although

451   significant, yield reduction from ELR was overall less than 15% and much lower than that

452   imposed CT. The most relevant and consistent effects of ELR included higher bud freeze

453   tolerance during fall acclimation (TBLR) and lower bunch rot incidence (TBLR and FSLR) and

454   severity (FSLR) as compared to the control. Therefore, in humid climates, costs associated with

455   ELR may be justified by a greater amount of healthy fruit at harvest. The lower yield of CT vines

456   did not translate to a consistent improvement in fruit chemical composition, suggesting that

457   Grüner Veltliner can be cropped to more than one cluster per shoot to minimize or avoid loss in

458   revenue.

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Table 1 Enhanced point quadrat analysis (EPQA) characteristics of Grüner Veltilner vines with different crop load management treatments. Measurements were taken at preveraison (16 Jul 2015 and 28 Jul 2016) and post-veraison (25 Aug 2015 and 7 Sep 2016).

	Percent gaps (%)	Leaf layer number (n)	Occlusion layers (n)	Interior clusters (%)	Interior leaves (%)	Cluster exposure flux availability (%)	Leaf exposure flux availability (%)
Preveraison							
Treatment (T)[a]							
Control	11.46	1.01 a[b]	1.86 a	18.2 a	20.4 a	57.1	53.1
CT	12.50	0.84 ab	1.59 ab	11.8 ab	10.2 b	59.5	56.9
TBLR	14.93	0.42 c	1.39 b	14.3 ab	12.2 ab	64.0	54.4
FSLR	17.24	0.58 bc	1.50 ab	13.7 b	5.9 b	64.1	57.5
P-value (T)	0.318	< 0.001	0.009	0.079	0.008	0.208	0.627
Year (Y)							
2015	12.09	0.78	1.62	10.7	13.1	62.4	56.6
2016	15.97	0.65	1.55	14.8	11.3	59.9	54.3
P-value (Y)	0.109	0.777	0.475	0.064	0.496	0.363	0.627
P-value (TxY)	0.781	0.647	0.720	0.680	0.100	0.674	0.966
Post-veraison							
Treatment (T)							
Control	13.20	0.98 ab	1.78	22.0	13.9	55.3	57.1
CT	12.85	1.15 a	1.73	19.5	13.6	53.0	53.6
TBLR	9.38	1.12 a	1.88	23.4	14.3	60.5	53.4
FSLR	12.85	0.78 b	1.62	16.0	13.7	53.9	59.9
P-value (T)	0.669	0.019	0.128	0.253	0.995	0.133	0.075
Year (Y)							
2015	11.98	1.08	1.80	21.9	17.0	56.4	56.2
2016	12.15	0.94	1.71	18.5	10.8	54.9	55.8
P-value (Y)	0.945	0.105	0.224	0.253	0.007	0.544	0.846
P-value (TxY)	0.677	0.581	0.541	0.665	0.822	0.241	0.790

[a]CT = cluster thinning; TBLR = trace bloom leaf removal; FSLR = fruit set leaf removal.
[b]Treatment means followed by different letters within a column are significantly different (Tukey's HSD test, *P* < 0.10).

Table 2 Main, lateral, and total leaf area at harvest for Grüner Veltilner vines with different crop load management treatments. Data are separated by year due to significant treatment by year interaction.

	Main leaf area (cm^2)	Lateral leaf area (cm^2)	Total leaf area (cm^2)
2015			
Treatment (T)[a]			
Control	1836.6	1691.2	3527.8
CT	1884.5	1582.7	3467.2
TBLR	1724.8	1758.6	3483.4
FSLR	1805.8	1878.6	3684.3
P-value (T)	0.951	0.666	0.955
2016			
Treatment (T)			
Control	1823.5 ab[b]	2026.6	3850.1 ab
CT	2009.8 a	2427.2	4437.0 a
TBLR	1894.7 ab	2217.6	4112.3 ab
FSLR	1556.4 b	1840.9	3397.3 b
P-value (T)	0.072	0.128	0.015

[a]CT = cluster thinning; TBLR = trace bloom leaf removal; FSLR = fruit set leaf removal.
[b]Treatment means followed by different letters within a column are significantly different (Tukey's HSD test, *P* < 0.10).

Table 3 Effects of treatment and year on yield components, bunch rot, pruning weight, and crop load for Grüner Veltliner vines.

	Yield/ vine (kg)	Clusters/ vine (n)	Cluster wt (g)	Pruning wt/ vine (kg)	Ravaz index (yield/prun. wt [kg/kg])	LA/yield (cm^2/g)	Cluster compactness (1-5)[b]	Rot severity (% cluster area)	Rot intensity (% clusters)
Treatment (T)[a]									
Control	8.89 a[c]	49 a	190 b	1.18 ab	7.91 a	8.11 b	3.50 ab	4.42 a	71.7 a
CT	5.40 c	36 b	220 a	1.35 a	3.78 b	14.65 a	3.75 a	4.10 ab	69.2 a
TBLR	7.77 b	46 a	170 b	1.07 b	6.99 a	8.82 b	3.13 bc	2.81 ab	55.8 b
FSLR	7.71 b	45 a	170 b	1.19 ab	6.56 a	9.85 b	2.88 c	2.69 b	57.1 b
P-value (T)	< 0.001	< 0.001	< 0.001	0.031	< 0.001	< 0.001	0.001	0.012	0.005
Year (Y)									
2015	7.54	47 a	210 a	1.22	6.44	11.45 a	3.19	4.81 a	76.7
2016	7.34	35 b	160 b	1.18	6.17	9.27 b	3.44	2.19 b	50.2
P-value (Y)	0.418	< 0.001	< 0.001	0.469	0.588	0.005	0.048	< 0.001	< 0.001
P-value (TxY)	0.477	0.110	0.149	0.635	0.534	0.870	0.542	0.436	0.467

[a]CT = cluster thinning; TBLR = trace-bloom leaf removal; FSLR = fruit set leaf removal.

[b]Scale range is from 1= very loose clusters, no berry contact, bending of the stem to 90° possible to 5 = very compact, berries not flexible, bending of the stem not possible (Ipach et al. 2005).

[c]Treatment means followed by different letters within a column are significantly different (Tukey's HSD test, *P* < 0.10).

Table 4 Effects of treatment and year on berry weight, total number of berries per cluster, and percentage of normal size, 'chicken' and infected berries, and live green ovaries for basal and distal clusters of Grüner Veltliner vines.

	Berry wt (g)	Total berries /clusters (n)	Normal size berries (%)	'Chicken' berries (%)	Rot-infected berries (%)	Live green ovaries (%)
			Basal cluster			
Treatment (T)[a]						
Control	1.65 ab[b]	160	89.02	3.91	4.58 a	1.81
CT	1.70 a	157	90.66	3.78	3.06 ab	2.17
TBLR	1.63 ab	140	89.36	3.46	4.16 ab	1.90
FSLR	1.56 b	157	89.41	4.91	2.67 b	2.40
P-value (T)	0.024	0.161	0.529	0.255	0.070	0.635
Year (Y)						
2015	1.57	138	87.19	6.14	5.29	0.02
2016	1.70	170	92.03	1.89	1.95	4.12
P-value (Y)	< 0.001	0.161	< 0.001	< 0.001	< 0.001	< 0.001
P-value (T x Y)	0.270	0.933	0.600	0.102	0.465	0.629
			Distal cluster			
Treatment (T)						
Control	1.68	109	88.02	4.71 ab	4.44 a	2.32
CT	NA[c]	NA	NA	NA	NA	NA
TBLR	1.65	93	91.17	3.56 b	2.41 b	2.12
FSLR	1.60	105	88.52	5.83 a	4.07 ab	1.46
P-value (T)	0.215	0.097	0.145	0.073	0.073	0.167
Year (Y)						
2015	1.56	86	86.69	7.30	5.09	0.02
2016	1.71	118	91.79	2.10	2.19	3.92
P-value (Y)	0.001	< 0.001	0.002	< 0.001	0.001	< 0.001
P-value (T x Y)	0.913	0.097	0.696	0.344	0.338	0.162

[a]CT = cluster thinning; TBLR = trace bloom leaf removal; FSLR = fruit set leaf removal.
[b]Treatment means followed by different letters within a column are significantly different (Tukey's HSD test, *P* = 0.10).
[c]Distal cluster removed from CT vines.

Table 5 Effects of treatment and year on Grüner Veltliner juice composition for basal cluster, distal cluster, and combined basal and distal cluster at harvest 2015 and 2016.

	TSS (Brix)	pH	TA (g/ L)	TSS (Brix)	pH	TA (g/L)	TSS (Brix)	pH	TA (g/L)
				2015					
	Basal cluster			*Distal cluster*			*Basal + distal cluster*		
Treatment (T)[a]									
Control	21.4 b[b]	3.63	4.16	21.8	3.61	4.26	21.6 ab	3.61	4.20
CT	22.1 a	3.61	4.27	NA[c]	NA	NA	22.1 a	3.62	4.27
TBLR	21.3 b	3.64	4.29	21.9	3.61	4.43	21.7 ab	3.61	4.35
FSLR	22.1 a	3.62	4.25	21.4	3.61	4.44	21.3 b	3.62	4.33
P-value (T)	0.001	0.719	0.850	0.143	0.919	0.636	0.033	0.959	0.744
				2016					
	Basal cluster			*Distal cluster*			*Basal + distal cluster*		
Treatment (T)									
Control	21.1 bc	3.62 b	4.12	21.5	3.61	4.11	21.0 b	3.60 b	4.12
CT	23.0 a	3.77 a	4.07	NA	NA	NA	23.0 a	3.77 a	4.07
TBLR	21.9 ab	3.72 ab	4.24	22.2	3.72	4.10	22.1 ab	3.74 ab	4.10
FSLR	20.5 c	3.63 ab	4.21	21.2	3.64	4.10	20.8 b	3.63 ab	4.09
P-value (T)	0.005	0.025	0.959	0.261	0.110	0.989	0.009	0.016	0.999

[a]CT = cluster thinning; TBLR = trace bloom leaf removal; FSLR = fruit set leaf removal.
[b]Treatment means followed by different letters within a column are significantly different (Tukey's HSD *P* < 0.10).
[c]Distal cluster removed from CT treatment.

Table 6 Effects of treatment and year on Grüner Veltliner shoot efficiency, or source-sink balance.

| | Total sugar (g) | | | Leaf area-to-yield ratio |
	per shoot	per berry	per cm^2 leaf area	cm^2/g
Treatment (T)[a]				
Control	95.9 a[b]	0.355	0.026 a	8.11 b
CT	93.6 a	0.357	0.025 ab	14.65 a
TBLR	83.2 a	0.357	0.024 ab	8.82 b
FSLR	56.5 b	0.357	0.016 b	9.85 b
P-value (T)	< 0.001	0.998	0.089	< 0.001
Year (Y)				
2015	95. 5	0.371	0.028	11.45 a
2016	69.1	0.341	0.018	9.27 b
P- value (Y)	< 0.001	< 0.001	< 0.001	0.005
P- value (T x Y)	0.412	0.073	0.473	0.870

[a]CT = cluster thinning; TB = trace-bloom leaf removal; FSLR = fruit set leaf removal.

[b]Treatment means followed by different letters within a column are significantly different (Tukey's HSD test, *P* < 0.10).

Table 7 Bud median low-temperature exotherm (LT_{50}; °C) of Grüner Veltliner vines with different crop load management treatments from November 2015 through March 2016 and from November 2016 through March 2017.

	November 2015	December 2015	January 2016	February 2016	March 2016
Treatment (T)[a]					
Control	-12.72 a[b]	-21.13	-22.91	-22.58	-19.82
CT	-13.09 b	-21.48	-22.78	-22.56	-19.68
TBLR	-13.06 b	-20.97	-22.94	-22.61	-19.95
FSLR	-13.07 b	-21.32	-22.87	-23.03	-19.70
P-value (T)	0.039	0.181	0.913	0.360	0.659
Treatment (T)					
Control	-13.20 a	-20.50	-22.53 ab	-21.92	-19.95
CT	-13.82 ab	-20.36	-22.17 a	-21.90	-19.79
TBLR	-14.11 b	-20.36	-22.24 a	-22.50	-20.01
FSLR	-13.46 ab	-20.82	-22.91 b	-22.52	-20.21
P-value (T)	0.030	0.543	0.035	0.210	0.556

[a]CT = cluster thinning; TBLR = trace bloom leaf removal; FSLR = fruit set leaf removal.

[b]Treatment means followed by different letters within a column are different based are significantly different (Tukey's HSD test, $P < 0.10$).

Table 8 Production cost associated with early leaf removal and cluster thinning and price analysis of Grüner Veltliner.

	Additional cost of crop load management ($/ha)[a]	Additional production cost ($/t)	Yield (t/ha)	Expected revenue[a] ($/ha)	Preferred price to maintain revenue ($/t)	Additional cost[b] ($/bottle)
Treatment (T)[e]				**2015**		
Control	0	0	25	36,969	1,361	0.00
CT	133	9	15	20,760	2,207	1.18
TBLR	102	5	20	27,381	1,672	0.43
FSLR	96	5	21	28,641	1,598	0.33
				2016		
Control	0	0	23	35,764	1,406	0.00
CT	133	9	14	19,309	2,372	1.34
TBLR	102	5	21	29,923	1,533	0.17
FSLR	96	5	21	29,098	1,579	0.23

[a]The average industry price per tonne for Grüner Veltliner was $1,361 in 2015 and $1,406 in 2016.

[b]The additional retail price for a 750 mL bottle of wine under the assumptions of 491.4 L wine (655.2 bottles) per tonne of grapes, and that the producer uses the grapes for winemaking instead of selling the grapes at market price.

[c]CT = cluster thinning; TBLR = trace bloom leaf removal; FSLR = fruit set leaf removal.