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Girdling improved berry coloration in summer but suppressed return growth in the following spring in 'Kyoho' grapevines cultivated in the subtropical double cropping system

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Summary

Improving berry skin coloration is one of the major challenges in the tropical and subtropical viticulture. In this paper we aimed to document the effects of girdling at veraison on berry coloration and quality in grapevines on different rootstocks and we assessed the seasonal variations of girdling effects in the subtropical double cropping system. In the first experiment, girdling at veraison was tested in 'Kyoho' on 5C rootstocks. In the second experiment, own-rooted 'Kyoho', 5C or 1202C rootstocks were compared. Vines were trained to a horizontal overhead trellis with a single trunk and two short arms. One arm of each vine was girdled at veraison and the other arm served as the control. Girdling at veraison significantly improved skin color of berries from own-rooted vines or 5C rootstocks in the summer cropping cycle but was less effective in the winter cropping cycle. Girdling improved total soluble solids in own-rooted vines in the summer cropping cycle and in vines on 1202C in the winter cropping cycle. Girdling made in the winter cropping cycle did not completely heal until post-bloom in the following spring and reduced length of the fruiting shoot, number of leaves per shoot, and length of inflorescences of the next summer cropping cycle. We concluded that girdling at veraison is a good practice to improve berry color and quality for the summer cropping cycle but is not recommended for the winter cropping cycle.

K e y w o r d s : *Vitis* spp.; 5C; 1202C; own-root; tropical viticulture.

Introduction

Poor coloration and inferior berry quality have been the major limitation for tropical and subtropical viticulture (Carbonneau 2011). In the subtropical double cropping production system, the use of rootstocks and the distinct temperature and light environment between the summer and winter cropping cycles often resulted in distinct berry skin coloration, sugar and acid contents (Chou and Li 2014). Such inconsistency of the berry quality dispels market attraction for local table grape.

Girdling is an ancient horticultural practice to improve fruit production and quality (Goren *et al*. 2004). Multiple purposes can be achieved by trunk or cane girdling at different growing stages of a grapevine. Girdling after bloom improved fruit set and berry weight but did not affect skin color, total soluble solids or titratable acidity at harvest (Brown *et al*. 1988, Carreño *et al*. 1998, Jensen *et al*. 1975). On the other hand, girdling at veraison often encouraged anthocyanin and sugar accumulations (Carreño *et al*. 1998, Fujishima *et al*. 2005, Peacock *et al*. 1977, Yamane and Shibayama 2007). Girdling has been a standard practice in the table grape production in California and is recommended in Japan as a remedy for poor coloration in some cultivars due to the impact of global warming (Maff 2007). However, girdling has yet to be accepted in Taiwan where the double cropping system is the standard production model. Growers are reluctant to practice girdling in concerns of the potential *Phomopsis* cane and leaf spot (*Phomopsis viticola*) and bitter rot (*Greeneria uvicola*) invasion through the girdling wound in the humid subtropical climate. In addition, it has been reported that the temporary interruption of carbohydrate translocation towards roots after girdling impaired accumulation of carbohydrate reserves and root growth in grapevines (Goren *et al*. 2004, Yamane and Shibayama 2006). In the double cropping system a vine is enforced to complete two growing cycles in one year, resulting in a very high total yield and thus, a high carbohydrate demand for fruiting. It is assumable that the balance of carbohydrate supply and demand of a grapevine in the subtropical double cropping system is more prone to the potential negative effect of girdling than a vine in the typical temperate annual cropping system.

In this study, two field trials were carried out to assess the effects of girdling on berry quality and return growth in ‘Kyoho’ grapevines. The objectives were 1) to document girdling effects on berry coloration and quality in vines on different rootstocks, 2) to detect the differential seasonal influence on girdling effects, and 3) to investigate the potential negative effect of repeated girdling on return growth in the subtropical double cropping system.

Material and Methods

E x p e r i m e n t o n e

P l a n t m a t e r i a l a n d e x p e r i m e n t s i t e : Five-year-old ‘Kyoho’ grapevines (*Vitis labruscana* x *V. vinifera*) on 5C (*V. berlandieri* × *V. riparia*) rootstocks in a commercial vineyard in Miaoli, central Taiwan (24°18’

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N, 120°51' E, 420 m alt.) were used. The soil was well drained loamy soil with pH around 6.5 and organic matter content > 5 %. A standard overhead horizontal trellis was adopted. The plant spacing was 2.8 x 2.8 m and each vine was trained to a single trunk with two short arms below the trellis. A flat panel of canopy completely covered the vineyard land area was formed from two to four cordons on each arm. Vines were managed with a fertilization and pest control program commonly used for this region. Vines were cane pruned to eight compound buds per cane and forced with 50 % (v/v) 2,2-dichloroethanol on February 17, 2008. Cluster and berry thinning was carried out on April 20 (full bloom) and May 17, respectively, to achieve a final target of forty clusters per vine or five clusters·m^{-1} land area and 20 berries per cluster. Clusters were individually enclosed in single layer paper bags on May 25 to protect berries from oriental fruit flies (Diptera: Tephritidae) and bird attack. The paper bag was not removed until harvest. Six uniform and representative vines in the experiment block were randomly selected for the trial. Veraison was on June 12 and fruit were harvest on July 20. The final yield for this harvest cycle was 10 tons·ha^{-1}.

Girdling treatments: Girdling was applied to the vine on 16 June. One arm of each vine was randomly chosen and subjected to girdling while the other arm was not girdled and served as the control. The chosen arm was girdled at the base 10 cm above the conjunction to the trunk. A 5-mm wide girdle was made with a sharp utility knife. The girdle was examined 1 hr after girdling to ensure complete removal of the phloem tissue.

Measurements of berry skin color and quality: Ten berries were randomly sampled from individual arms every week from 15 June until harvest. Skin color was visually evaluated by comparing with a color index chart specifically designed for dark-colored grape cultivars (Nichienren, Tokyo, Japan) that numerically categorizes purple color into 13 grades with 0 to be the lightest and 12 to be the darkest. Total soluble solids (TSS) and titratable acidity (TA) of the juice collected from the berry sample were determined with a hand refractometer (N-1, Atago, Tokyo, Japan) and an automatic potentiometric titrator (702 SM Titrino, Metrohm, Herisau, Switzerland).

At harvest, ten clusters were sampled from each arm. All berries on each cluster sample were individually evaluated with the color index chart and the percentage of berries in each color category was calculated.

Measurements of total soluble sugar and starch in fruiting shoots after harvest: After harvest, five pre-bearing shoots were randomly sampled from each arm. The green and lignified internodes of the shoot were separated. Total soluble sugars and starch concentration of the sample were determined according to DUBOIS *et al*. (1956).

Measuring return growth: For the following winter cropping cycle of 2008, vines were cane pruned to 12 buds per cane and the terminal compound bud on each cane was enforced on 29 August. Date of budburst and length of young fruiting shoots were measured from 13 September until full bloom.

Experiment two

Plant material and experiment site: A commercial vineyard located in Miaoli, central Taiwan (24.18°N, 120.51°E, 320 m alt.) was chosen and served as the experiment site. Three adjacent blocks of grapevines on three different rootstocks were used. Own-rooted 'Kyoho' vines were planted in 2006 with a spacing of 3.6 x 1.2 m; vines on 5C (*V. berlandieri* x *V. riparia*) and 1202C (*V. vinifera* 'Mourvèdre' x *V. rupestris*) rootstocks were planted in 2000 with a spacing of 3.6 x 3.6 m. The soil was well-drained sandy loam, and the depth of soil was < 50 cm with gravel formations underneath. Soil analysis indicated that the three experiment blocks had similar soil profiles, a similar soil pH of ca. 6.1, and organic matter content of ca. 6 %. The training, pruning, and other vineyard managements were similar to those of the vineyard used for experiment one. A standard cane pruning and forcing as described in experiment one was practiced. To produce the first (summer) crop, the own-rooted vines were pruned and forced with 50 % (v/v) 2,2-dichloroethanol on January 29, 2009. The vines on 5C and 1202C rootstocks were cane pruned and forced a week later. The own-rooted vines were in full bloom on April 10 and the grafted vines were in full bloom three d later. Veraison was on May 23 in own-rooted vines and on May 30 in vines on 5C and 1202C rootstocks. The own-rooted vines were harvested on July 16; Vines on 5C and 1202C rootstock were harvested on July 28 and August 3, respectively. To produce the second (winter) crop, the own-rooted vines were pruned and forced on August 12, 2009 and the grafted vines were pruned and forced a week later. The own-rooted vines bloomed on September 7 and the grafted vines on September 15. Veraison was on October 23 in own-rooted vines and on October 30 in 5C and 1202C rootstocks. Berries on own-rooted vines were harvested on December 18, 2009 and berries on vines grafted on both rootstocks were harvested on January 1, 2010. All vines were forced again in late January, 2010.

After bloom of each cropping cycle, clusters were hand thinned to average five clusters m^{-2} land area with 40 berries per cluster. Fruit clusters were individually enclosed in single layer white paper bags when berries were ca. 1.5 cm in diameter to protect the berry from pests and birds. The paper bags were not removed until harvest. The yield of the vineyard was 19 tones ha^{-1} for each cropping cycle and was similar among the three experiment blocks.

Girdling treatments: In each scion/rootstock combination, ten uniform vines were selected before veraison of the 2009 summer cropping cycle. One arm of each vine was randomly chosen and subjected to girdling while the other arm was not girdled and served as the control. In the summer cropping cycle of 2009, the chosen arm was girdled at the base ca. 10 cm from the conjunction to the trunk as described in experiment one. The same arm of each vine was girdled again at veraison of the 2009 winter cropping cycle. The second girdle was made at 5 cm above the previous girdling position.

Measuring berry skin color, total soluble solids, and titratable acidity: Ten fruit clusters per arm were labeled after girdling. Two berries with normal size (ca. 9.5 g F.W.) were randomly sampled from each cluster at harvest. The skin color of the berry sample was visually evaluated by comparing with the color index chart and TSS and TA of the berry sample were determined as described in experiment one.

Measuring return growth after repeated girdling: Return growth before bloom was measured when shoots were fully extended. Length of shoots, number of leaves, and length of inflorescences of five representative fruiting shoots per arm were measured.

Statistical analyses: In this study each vine was an experiment unit or replicate, *i.e.* n = 6 in experiment one and n = 10 in experiment two. Repeated measures, e.g. berry color, TSS, length of shoots, etc. on each arm were averaged before subjected to statistical analyses. Percentage data, *i.e.* color distribution within a cluster, were arcsine transformed before analyses but original data were presented. Differences between girdling and the un-girdled control were tested with paired t-test using SigmaStat software (version 3.1.1.0, Systat Software, Inc., San Jose, California, USA). Significances were determined at $P \leq 0.05$.

Results and Discussion

Girdling effect on berry skin coloration: In experiment one, skin coloration of berries was slightly but consistently improved by girdling after veraison (data not shown). At harvest, the girdled arm produced a significant higher percentage of dark-colored berries (color index > 10), and a significant lower percentage of moderate-colored berries (color index = 8), than did the un-girdled arm (Figure).

A similar positive effect was observed in the summer cropping trial of experiment two. Regardless of the rootstocks, the berry skin of the un-girdled control in the summer cropping trial was poorly colored at harvest. Girdling significantly improved skin color in own-rooted vines and vines on 5C rootstock (Tab. 1). In the winter cropping trail, berries from own-rooted vines and vines on 5C rootstock were naturally well-colored in comparison to those in the summer trail. The effect of girdling on skin color was only significant in vines on 5C rootstock.

Coloration of dark grapes is the result of anthocyanin accumulation and is regulated by numerous physiological and environmental factors, e.g. cropload (Kitamura *et al.* 2005), water status (Bucchetti *et al.* 2011), soil (Morlat and Symoneaux 2008), radiation (Keller and Hrazdina 1998), temperature (Mori *et al.* 2004), etc. In a previous study, anthocyanin accumulation in 'Kyoho' berry skins was remarkably affected by rootstocks but less affected by cropping cycles (Chou and Li 2014). Similarly in this study, vines on 1202C produced berries with inferior skin color and girdling had little effect on coloration regardless of cropping cycles. On the other hand, although berry skin coloration in own-rooted vine and vines on 5C was also poor in the summer cropping cycle, it was significantly improved by girdling at veraison, especially in own-rooted vines. Koshita *et al.* (2011) reported that girdling effect on skin color of 'Aki Queen' grape was only significant in low

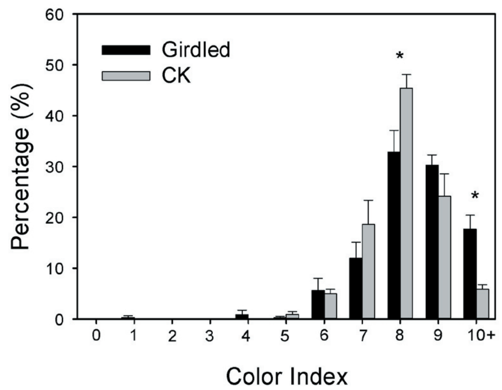

Figure: Girdling effect on berry skin color distribution in 'Kyoho'/5C vines. One arm on each of the six tested vines was girdled at veraison of the summer cropping cycle of 2008 and the other arm was un-girdled as the control (CK). The skin color of berries from ten clusters on each arm was visually evaluated using a 13-grade color chart with index 0 to be the lightest and index 12 to be the darkest. Vertical bars represent standard errors and * indicates significant differences between girdling and the control at $P \leq 0.05$ by paired t-test.

Table 1

Girdling effects on berry skin color and total soluble solids in 'Kyoho' grapevines on own-root, 5C or 1202C rootstocks

	Summer cropping cycle			Winter cropping cycle		
Rootstock	Girdled	CK	*P*	Girdled	CK	*P*
			Skin color index			
Own-root	10.2 ± 0.58	6.6 ± 1.04	0.008	10.0 ± 0.47	10.0 ± 0.44	0.982
5C	7.5 ± 0.33	6.9 ± 0.47	0.017	10.6 ± 0.21	9.8 ± 0.34	0.017
1202C	7.0 ± 0.25	6.2 ± 0.39	0.081	7.8 ± 0.52	6.5 ± 0.35	0.079
			Total soluble solids (°Brix)			
Own-root	17.5 ± 0.70	15.6 ± 0.77	0.01	14.9 ± 0.76	15.1 ± 0.59	0.756
5C	17.8 ± 0.32	17.8 ± 0.43	1.00	17.6 ± 0.34	17.3 ± 0.50	0.505
1202C	16.6 ± 0.36	16.3 ± 0.37	0.21	14.9 ± 0.58	13.3 ± 0.35	0.017

Data were means ± standard errors of ten replicates (vines). Significant differences between the girdled and the un-girdled control (CK) within each scion/rootstock combination were detected by paired t-test.

temperature (21 °C) but not in high temperature (31 °C). Contrary to their findings, our result showed that although berries of own-rooted 'Kyoho' and 'Kyoho' on 5C rootstock were also naturally well-colored in the cooler winter cropping season, girdling effect was less remarkable than in the much warmer summer cropping season (Tab. 1)

Effect of girdling on total soluble solids and titratable acidity: TSS of berries from own-rooted vines was significantly improved by girdling in the summer cropping cycle but not in the winter cropping cycle while an opposite result was found in vines on 1202C (Tab. 1).

Other than a moderate reduction in TA from 2.8 to 2.0 g·L^{-1} F.W. in berries on own-rooted vines in the summer cropping cycle of the experiment two ($P = 0.03$), TA was not affected by girdling in either experiment (data not shown). Similar results were reported in 'Pione' (Fujishima *et al*. 2005) and 'Aki Queen' (Yamane and Shibayama 2006) grape.

The positive effects of girdling on berry coloration and quality were largely the result of temporary interruption of carbohydrate outflow from the canopy (Goren *et al*. 2004). Before the reconnection of phloem tissues, girdling increased the concentration of non-structure carbohydrate in the leaf while reduced the concentration of carbohydrate in the root (Roper and Williams 1989). Previous studies often showed that girdling at veraison resulted in increased berry TSS at harvest (Carreño *et al*. 1998, Yamane and Shibayama 2006). However, it has also been reported that girdling at veraison did not improve berry quality or maturity due to heavy cropload (Peacock *et al*. 1977) or vigorous vegetative growth after girdling (Novello *et al*. 1999). Reduced TSS in berries was reported in 'Himrod' grapevines after cane girdling and was attributed to off-balanced carbohydrate supply due to inadequate leaf to fruit ratio (Zabadal 1992).

Anthocyanin accumulation in berry skins has been closely associated with berry TSS. However, temperature has a strong influence on this relationship (Koshita *et al*. 2011, Sadras and Moran 2012, Zheng *et al*. 2009). Distinct patterns of the relationship between anthocyanin and TSS accumulation in developing berries were detected in 'Kyoho' vines on different rootstocks and between the two cropping cycles of the subtropical double cropping system (Chou and Li 2014). In addition to temperature, the physiological status and carbohydrate dynamics of a grapevine are also responsive to photoperiod (Fennell and Hoover 1991). The short day length in the winter cropping cycle appeared to be another environmental restraint in anthocyanin-TSS relationship and may have substantially influenced the degree of girdling effects on berry coloration and quality among rootstocks used.

Repeated girdling suppressed return growth in spring: In both experiment trials, the wound of girdling at veraison in the summer cropping cycle healed within three weeks. The bark and phloem tissues above and below the girdling wound were visually re-connected before harvest. In experiment one where TSS and starch concentrations in shoots were measured after harvest, no difference between girdling treatment and the control was detected. The average TSS concentrations of the lignified and green internodes were 0.02 and 0.03 g·g^{-1} D.W., respectively, and the average starch concentrations of the lignified and green internodes were 0.03 and 0.02 g·g^{-1} D.W., respectively. After forcing, there was also no detectable difference in the length of shoots, the number of leaves per fruiting shoot, or the length of inflorescences between the girdled and the un-girdled arms in the following winter cropping cycle (data not shown). However, in experiment two, negative effects of repeated girdling on the return growth were recorded (Tab. 2). In any rootstock tested, few callus formed on girdling wound but the healing process discontinued. The girdling wound remained open after budbreak of the next summer cropping cycle and was not completely healed until post-bloom. Before bloom, the length of fruiting shoots and the number of leaves per shoot on the girdled arm were both significantly suppressed by the previous girdling treatment. The length of inflorescences of own-rooted vines was also severely reduced.

Cane girdling before budbreak restricted carbohydrate availability and reduced shoot growth rate and number of internodes in 'Sauvignon Blanc' grapevines (Eltom *et al*. 2013). Likewise, the prolonged disconnection of phloem after girdling in our winter trial might have prevented carbohydrate reserves to be accumulated in the trunk and root after winter harvest and to be transported toward growing shoots upon budbreak in the following spring.

Table 2

Effects of repeated girdling on return growth in 'Kyoho' grapevines on own-root, 5C or 1202C rootstocks

Rootstock	Girdled	CK	P
Length of fruiting shoots (cm)			
Own-root	36.8 ± 2.85	69.1 ± 5.07	< 0.001
5C	42.8 ± 2.89	79.0 ± 3.02	< 0.001
1202C	47.0 ± 1.93	64.0 ± 3.99	0.008
Number of leaves per shoot (cm)			
Own-root	7.7 ± 0.26	11.9 ± 0.53	< 0.001
5C	9.8 ± 0.38	15.0 ± 0.36	< 0.001
1202C	8.9 ± 0.44	12.0 ± 0.54	< 0.001
Length of inflorescences (cm)			
Own-root	11.2 ± 0.29	16.1 ± 0.45	< 0.001
5C	17.2 ± 1.16	19.2 ± 0.64	0.189
1202C	15.0 ± 0.75	16.4 ± 1.22	0.281

Data were means ± standard errors of ten replicates (vines). Significant differences between the girdled and the un-girdled control (CK) within each scion/rootstock combination were detected by paired t-test.

Conclusion

For the first time the seasonal and rootstock influences on girdling effects were evaluated in the subtropical double cropping viticulture. From the result we suggested that girdling at veraison is a feasible and convenient practice to improve berry coloration of 'Kyoho' grape in the summer cropping cycle, especially in own-rooted vines. However, in the cool winter cropping cycle, girdling effects on berry color and quality were less effective. Concerning the slow healing of girdling in winter and the consequent reduced return growth in the following spring, girdling is unnecessary and not recommended for the winter cropping cycle of the subtropical double cropping production.

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