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Rootstock and seasonal variations affect anthocyanin accumulation and quality traits of 'Kyoho' grape berries in subtropical double cropping system

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Summary

The double cropping system has been commercially adopted in subtropical viticulture regions. However, very limited information about rootstock and seasonal effects on berry quality traits are available for this unique production system. Developing 'Kyoho' berries from own-rooted vines and from vines on 5C and 1202C rootstocks were periodically sampled from veraison until harvest in two consecutive cropping cycles to document the potential seasonal influence on rootstock effects. Anthocyanin concentration in berry skin, total soluble solids content (TSS), and titratable acidity (TA) were analyzed. In both cropping cycles, own-rooted vines produced berries with the highest anthocyanin concentration while vines on 1202C produced berries with the lowest anthocyanin concentration among the three scion/rootstocks. Anthocyanin concentrations were not differentiated by the differential climate pattern between the summer and the winter cropping cycles. Berries of own-rooted 'Kyoho' and 'Kyoho'/5C vines accumulated satisfactory and equal amount of TSS in both cropping cycles. 1202C rootstocks did not affect berry TSS in the summer cropping cycle but reduced TSS in the winter cropping cycle. Significant rootstock and seasonal effects on berry TA were detected. Own-rooted vines produced berries with the lowest TA while vines on 1202C produced berries with the highest TA among the three scion/rootstock combinations. TA of berries from the winter cropping cycle was significantly higher than that from the summer cropping cycle especially in 'Kyoho'/1202C. Relationships between anthocyanins and TSS of developing berries after veraison properly fitted into a sigmoidal function regardless of rootstocks and cropping cycles. However, the duration of the initial lag phase, the onset and the trend of both quality triats in the increasing phase, and the presence and degree of the final lag phase in the relationship were all modulated by rootstocks and by seasonal variations.

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

Introduction

In the hot and wet subtropical viticulture region, the double cropping system in which a grapevine completes two cropping cycles in twelve months has been commercially adopted (Araujo 1994, Lin *et al.* 1985, Shikhamany 2001, Yaacob and Subhadrabanduhu 1995). Briefly, budbreak of a vine is enforced by chemical agents between late January and mid-February (in the northern hemisphere), resulting in the first bloom in early April and the first crop, the summer crop, by mid-July. The vine is then pruned and forced again around mid-August, resulting in the second bloom in mid-September and the second crop, the winter crop, by mid-January the following year. Due to the significant difference in the climate pattern between the two cropping cycles (Fig. 1), several quality traits of the berries, e.g. color, sugar content, acidity, etc. are often distinct between the summer and the winter harvests.

'Kyoho' (*Vitis labruscana*) is a tetraploid table grape favored by East Asians and has been the major cultivar in Japan, Korea, and Taiwan (Morinaga 2001; Song 2001). Berry skin color is one of the main quality determinants for the market value of 'Kyoho' grape. Similar to other red or black cultivars, 'Kyoho' grape accumulates anthocyanins in berry skin after veraison. Adverse environmental conditions or inappropriate vineyard practices often result in poor coloration especially at high temperature (Coombe 1987, Spayd *et al.* 2002). In central Taiwan, the average summer temperature often exceeds 29 °C and the mid-day

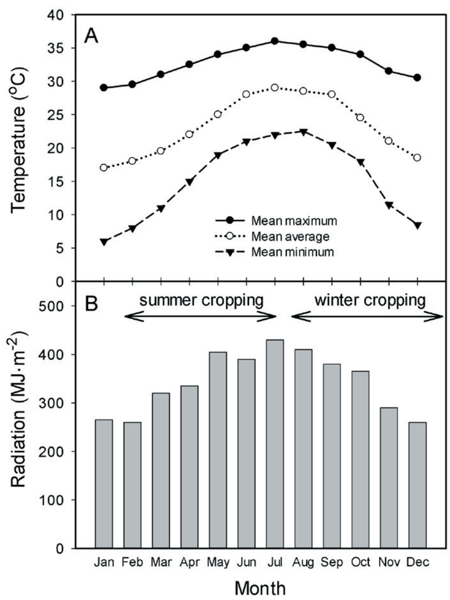

Fig. 1: **A**) Monthly mean temperature and **B**) monthly cumulative radiation in the viticulture region in central Taiwan. Data were average values of 1989-2007 records from a climatological station at Taiwan Agricultural Research Institute (24.01°N, 120.41°E, 85 m alt.). Primary source: Central Weather Bureau. The double-head arrows represent the two growing cycles in the standard double cropping system in Taiwan.

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maximum temperature frequently exceeds 35 °C. Even during the second cropping cycle in the cool season, the maximum mid-day temperature may still exceed 30 °C (Fig. 1). High temperature has been reported to be detrimental for anthocyanin accumulation in 'Kyoho' grape skins (MORI *et al*. 2004). In addition to temperature, anthocyanin accumulation in grape skins has been reported to be affected by many other factors including water status (BUCCHETTI *et al.* 2011), soil (MORLAT and SYMONEAUX 2008), cropload (KITAMURA *et al.* 2005), and radiation (KELLER and HRAZDINA 1998, KLIEWER 1977).

Both own-rooted vines and grafted vines on rootstocks are common in Taiwan. Own-rooted 'Kyoho' vines are precocious and easy to manage in high density plantings but the longevity of the vine appears much shorter than that of vines on rootstocks, thus requiring frequent vineyard renewal. On the other hand, 'Kyoho' vines on rootstocks are usually long-lived but color and quality of berries may vary depending on rootstock varieties. Currently, own-rooted vines are predominant in Taiwan's vineyards. Nevertheless, vines on several rootstock cultivars, e.g. 1202C (*V. vinifera* 'Mourvèdre' x *V. rupestris*), 420A (*V. berlanderi* x *V. riparia*), 5BB (*V. berlanderi* x *V. riparia*), and 8B (*V. berlanderi* x *V. riparia*), etc., have been used to a limited extent.

Rootstocks may affect growth of a vine by changing its water potential (KELLER *et al.* 2012), nutrient status (DALBÓ *et al.* 2011, IBACACHE and SIERRA 2009), vigor (FOOTT *et al.* 1989, KELLER *et al.* 2012), stress tolerance (TRAMONTINI *et al*. 2013), or by the interactions between rootstocks and scion cultivars (KOCSIS and LEHOCZKY 2000, MOTOSUGI *et al*. 2002), consequently leading to the modification of berry quality (REYNOLDS and WARDLE 2001, HARBERTSON and KELLER 2012). Rootstocks often altered berry compositions (CIRAMI *et al.* 1984, HEDBERG *et al.* 1986, RÜHL *et al.* 1988, HUANG and OUGH 1989, REYNOLDS and WARDLE 2001, HEUVEL *et al*. 2004) and skin anthocyanin content (KUBOTA *et al.* 1993, OLLAT and LAFONTAINE 2003). However, there is no report so far documenting the seasonal consistency of rootstock effects on berry quality traits in the subtropical double cropping system.

In this paper, berries from own-rooted 'Kyoho' vines and from grafted vines were periodically sampled and measured prior veraison until harvest in two continuous cropping cycles of the double cropping system. Differences in anthocyanin concentrations and other major quality traits among root systems and between cropping cycles were assessed. Potential decoupling of skin anthocyanins and berry sugar content between cropping cycles and among root types were also tested. The hypothesis was that the potential rootstock effect on berry quality is modulated by seasonal variations in the subtropical double cropping system.

Material and Methods

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. The vineyard was on the alluvial plain of the Da-An River. The vineyard soil was classified as Entisol according to the US soil taxonomy system (SOIL SURVEY STAFF 1999). The soil type was well-drained sandy loam, and the depth of soil was < 50 cm with gravel formations underneath. Three adjacent blocks of grapevines on different root systems were used. Soil analysis indicated that the three experiment blocks had identical soil profile, a similar soil pH around 6.1, and organic matter content around 6 %. Vines were trained to a standard overhead horizontal trellis system and irrigated with a under canopy sprinkler system during the dry period mainly from October to March and immediately after bud forcing at the beginning of each cropping cycle. All vines from the three experiment blocks were managed with a commercial standard fertilization and pest control program.

Own-rooted 'Kyoho' vines were planted in 2006 with a spacing of 3.6 x 1.2 m; vines on 5C (*V. riparia* x *V. berlianderi*) and 1202C (*V. vinifera* 'Mourvèdre' x *V. rupestris*) rootstocks were planted in 2000 with a spacing of 3.6 x 6 m. Regardless of the difference in planting design between own-rooted and grafted vines, the three experiment blocks were routinely cane pruned at the beginning of each cropping cycle and a uniform overhead canopy completely covering the land area was established. Timing of bud forcing in different rootstocks were slightly adjusted by the dormant status of the vine. 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 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 days later. Veraison was observed 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, 2009; Vines on 5C rootstock were harvested on July 28 and vines on 1202C were harvested on August 3. 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 observed 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.

After bloom of each cropping cycle, fruit clusters were hand thinned to 4.5 clusters·m^{-2}, with about 40 berries per cluster. Fruit clusters were individually enclosed in single layer white paper bags when berries were approximately 1.5 cm in diameter to protect the berry from oriental fruit flies (Diptera: Tephritidae) and bird attack. The paper bags were not removed until harvest. The yield of the vineyard was 16.2 t·ha^{-1} for each cropping cycle and was similar among the three experiment blocks.

Berry sampling: On May 23 and October 9, 2009, ten uniform vines in each scion/rootstock combination were selected from each experiment block and two normal developing berries on each vine were randomly sampled every week until harvest. Berry samples were sealed in plastic zip lock bags, placed in a portable ice chest, and then stored at -20 °C before anthocyanin extraction within 24 hrs after sampling.

Anthocyanin extraction and determination: In the lab, each berry sample was individually peeled. A quarter of the berry skin was weighted and immediately soaked in 20 mL methanol solution containing 2 % HCl (v/v). Anthocyanin in the berry skin was extracted by shaking the sample in the methanol-HCL solution for 20 min. The extraction process for each sample was repeated three times to reach > 90 % extractability. All extraction process was operated in an ice bath and in the dark or dimmed red light to avoid degradation of anthocyanins. For each berry sample a final extraction solution of 60 mL was obtained.

Absorbance of the extraction at 520 and 700 nm was measured with a spectrophotometer (U-2001; Hitachi, Tokyo, Japan). Total anthocyanins, expressed as malvidin 3-*O*-glucoside equivalents ($mg \cdot g^{-1}$ skin F.W.), were determined using the procedure proposed by Niketic-Aleksic and Hrazdina (1972).

Measuring total soluble solids content and acidity of the berry: After skin removal, the flesh of berry samples was pressed and the juice was collected. Total soluble solids content (TSS) of the juice was measured with an electronic refractometer (PAL-1; ATAGO, Tokyo, Japan) and titratable acidity (TA) was determined at an endpoint pH of 8.2 using an automatic titrator (720 SM Titrino; Metrohm AG., Herisau, Switzerland).

Statistical analyses: In this experiment each vine was an experiment unit. Berry samples for each vine were individually analyzed and measurements averaged before statistical analysis. Rootstocks, *i.e.* own-root, 5C, and 1202C, and cropping cycles, *i.e.* summer cropping and winter cropping, were the two experimental variables. Data were subjected to two-way analysis of variance (ANOVA), using SigmaStat software (version 3.1.1.0, Systat Software Inc., San Jose, California, USA), with Duncan's new multiple range test. Significances were determined at $P \leq 0.05$. The relationship between anthocyanin concentrations and TSS in ripening berries was tested with a 3-parameter sigmoidal nonlinear regression using the dynamic fitting protocol of SigmaPlot (version 12.0, Systat Software Inc., San Jose, California, USA).

Result and Discussion

Anthocyanin concentration was affected by rootstocks but not by seasonal variations between summer and winter cropping cycles: The anthocyanin concentration in berry skins from own-rooted 'Kyoho' vines increased in a relative consistent linear rate after veraison (Fig 2A). A maximal anthocyanin concentration was recorded on July 10, 5 d before harvest, and no further increase occurred by harvest. Anthocyanins in berry skins from 'Kyoho'/5C vines also quickly accumulated during the first three weeks after veraison. However, the accumulation rate remarkably declined after late June and maintained at a consistently slower rate in comparison to that in own-rooted vines until harvest. The accumulation of anthocyanins in 'Kyoho'/1202C berry skins after veraison was much slower than that in own-rooted vines and vines on 5C rootstocks, remained consistent between early and mid-July then slightly increased until harvest. The final anthocyanin concentration at harvest in 'Kyoho'/5C and 'Kyoho'/1202C berry skins was 22 % and 56 %, respectively, less than that in own-rooted vines (Fig 2A).

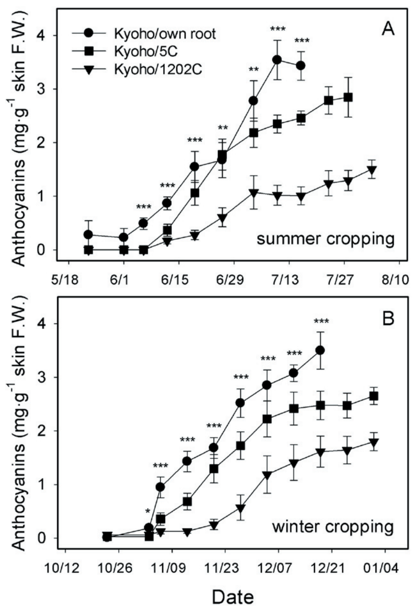

Fig. 2: Anthocyanins of developing berry skin in 'Kyoho' vines on own-root, 5C, and 1202C rootstocks in **A**) the summer and **B**) the winter cropping cycles of 2009. Vertical bars represent standard errors. *, **, and *** indicate significant differences at $P \leq 0.05$, 0.01, and 0.001, respectively (n = 10).

Similar trends were recorded in the winter cropping cycle (Fig 2B). In own-rooted 'Kyoho' vines, the anthocyanin concentration in berry skins quickly and continuously increased until harvest. Again, berries in 'Kyoho'/5C had a rapid initial anthocyanin accumulation rate similar to that in own-rooted vines. However, the quick accumulation stopped by Dec 11, 20 d before harvest and no further significant increase by harvest. The anthocyanin concentration in berry skins from 'Kyoho'/1202C remained low for the first three weeks after the onset of veraison and then quickly but briefly increased by early December. Overall, berry skin anthocyanin concentrations were significantly different among 'Kyoho' vines on different rootstocks in both cropping cycles, while as in any tested scion/rootstock combination, no detectable difference between the two cropping cycles occurred at harvest (Fig. 3).

Berry skin coloration in grapes can be altered by rootstocks. 'Cabernet Sauvignon' (*V. vinifera*) grafted on 101-14MGT (*V. riparia* x *V. rupestris*), SO-4 (*V. berlanderi* x *V. riparia*), and RG (*V. riparia*) rootstocks considerably differed from each other in their berry skin anthocyanin content (Ollat and Lafontaine 2003). 'Fujimori' (*V. vinifera* x *V. labruscana*) table grape on 3306 (*V. riparia* x *V. rupestris*) rootstocks produced berries with higher level of anthocyanins than berries from vines on six other dif-

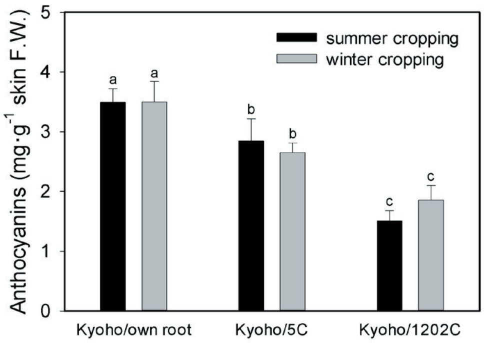

Fig. 3: Anthocyanins of mature berry skin in 'Kyoho' vines on own-root, 5C, and 1202C rootstocks in the summer and winter cropping cycles of 2009. Vertical bars represent standard errors (n = 10). Mean separation by Duncan's multiple comparison ($P \leq 0.05$).

ferent rootstocks (KUBOTA *et al.* 1993). The effect of rootstocks on berry quality was generally considered to be an indirect result of altered scion vigor. In wine grape production, 1202C was in the category of very vigorous rootstocks (FOOTT *et al.* 1989) while 5C did not significantly increase scion vigor in comparison to own-rooted vines (BOSELLI *et al.* 1992). On the other hand, although coloration developments were different among 'Shiraz' on different rootstocks, final coloration or wine color density were not affected by rootstocks or related to canopy size (WALKER *et al.* 2000). Temperature strongly affects coloration in grapes (SPAYD *et al.* 2002). 'Kyoho' grapevines in low temperature produced much better colored berries than those in high temperature (TOMANA *et al.* 1979). On the other hand, high temperature especially high night temperature dramatically suppressed anthocyanin accumulation and skin coloration (KOSHITA *et al.* 2007, MORI *et al.* 2004, 2005). In the double cropping system, although temperature of the post veraison period was higher in the summer cropping cycle than in the winter cropping cycle (Fig. 1), we did not observe any significant difference in final anthocyanin concentrations between the two cropping cycles in any tested scion/rootstock combination, indicating that other environmental or physiological factors may have overwhelmed the influence of temperature on berry coloration.

Rootstock and seasonal variations affected berry TSS and TA: In the summer cropping cycle, the initial TSS was around 8 °Brix at the beginning of veraison, continued to increase towards harvest. TSS at harvest was >18 °Brix in all three scion/stock combinations (Fig. 4A). Regardless of the apparent lower TSS in 'Kyoho'/1202C than in own-rooted 'Kyoho' and 'Kyoho'/5C, there was no significant difference in TSS during the final ripening phase among three scion/rootstock combinations.

In the winter cropping cycle, the initial TSS at the beginning of veraison was slightly lower than that in the summer. The accumulation rates of TSS in own-rooted 'Kyoho' and 'Kyoho'/5C berries were similar through the ripening phase and the final TSS at harvest was also >18 °Brix (Fig. 4B). Although the initial TSS at veraison in 'Kyoho'/1202C was similar to that in 'Kyoho'/5C, the accumulation of TSS in 'Kyoho'/1202C was significantly lower than that in the other two scion/rootstock combinations. After mid-December, 20 d prior to harvest, no obvious change in TSS was measured in 'Kyoho'/1202C berries. TSS at harvest in 'Kyoho'/1202C was only slightly >15 °Brix (Fig. 5A).

In the summer cropping cycle, TA was around 20 g L^{-1} at the beginning of veraison in all three scion/rootstock combinations, reduced quickly during the early ripening phase, and continued gradually towards harvest (Fig. 4C). Final TA in mature berries at harvest was < 2 g·L^{-1} in own-rooted 'Kyoho', < 2.4 g·L^{-1} in 'Kyoho'/5C, and slightly > 2.5 g·L^{-1} in 'Kyoho'/1202C (Fig. 5B).

In the winter cropping cycle, TA in berries was > 30 g·L^{-1} at veraison in all three scion/rootstock combina-

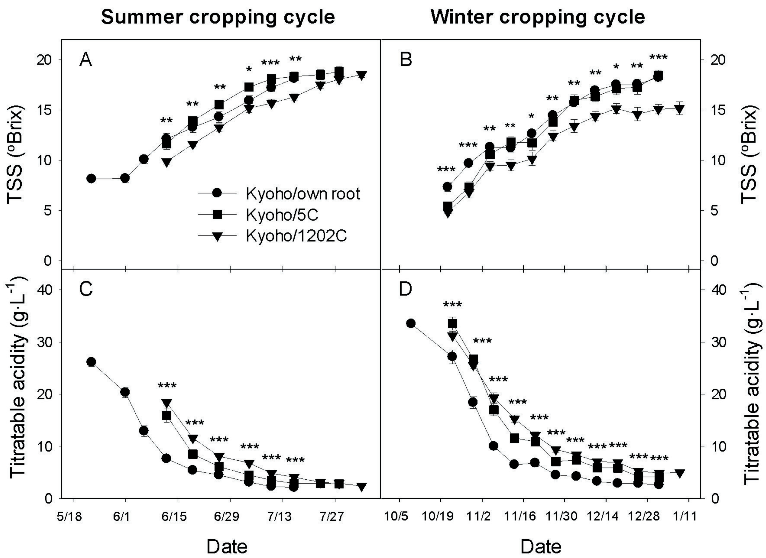

Fig. 4: **A-B**) Total soluble solids content (TSS) and **C-D**) titratable acidity of developing berries in 'Kyoho' vines on own-root, 5C, and 1202C rootstocks in the summer and winter cropping cycles of 2009. Vertical bars represent standard errors. *, **, and *** represent significant differences at $P \leq 0.05$, 0.01, and 0.001, respectively (n = 10).

tions (Fig. 4D). The reduction trend was similar to that in summer with a quick decrease in the early ripening phase followed by a gradual decline. However, final TA at harvest was still much higher than that in the summer crop in all three scion/rootstock combinations (Fig. 5B). TA in berries at harvest was 2.6 g·L^{-1} in own-rooted vines, 4.1 g·L^{-1} in 'Kyoho'/5C, and 5 g·L^{-1} in 'Kyoho'/1202C. The differences among scion/rootstocks and between cropping cycles were both significant.

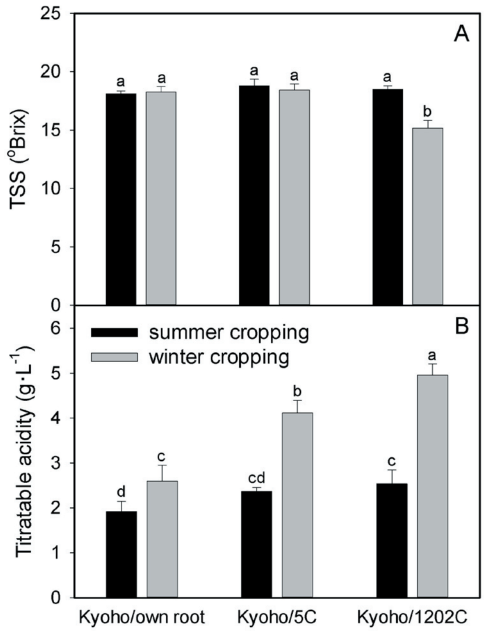

Fig. 5: **A**) Total soluble solids content (TSS) and **B**) titratable acidity of mature berries in 'Kyoho' vines on own-root, 5C, and 1202C rootstocks in the summer and winter cropping cycles of 2009. Vertical bars represent standard errors (n = 10). Mean separation by Duncan's multiple comparison ($P \leq 0.05$).

Rootstocks often modified berry sugar and acid content in grapes (Jackson and Lombard 1993, Keller *et al.* 2001, Reynolds and Wardle 2001) but great variations have also been reported among scion cultivars or clones (Rühl *et al.* 1988, Benz *et al.* 2007). Rootstock effect on berry TSS largely appeared to be the consequence of altered cropload or carbohydrate sink: source ratio (Coban and Kara 2002, Kliewer and Weaver 1971, Kliewer and Dokoozlian 2005). With the same canopy management and bunch density in our trials, berries were capable of accumulating satisfactory amount of sugars by harvest in the summer cropping cycle in all three scion/rootstock combinations regardless of the high temperature during ripening. Seasonal variation of rootstock effect on TSS was not significant in own-rooted 'Kyoho' or 'Kyoho'/5C vines. Berries from either vine accumulated the same amount of sugars in either cropping cycle. However, TSS in 'Kyoho'/1202C was strongly affected by the seasonal difference between the summer and the winter cropping cycle. Temperature is one of the major environmental variables that may alter berry TSS and TA. High temperature especially high night temperature during maturation phase often reduced berry TSS and TA (Coombe 1987, Mori *et al.* 2005). In the double cropping production system, post veraison night temperature in the summer cropping cycle was much higher than that in the winter cropping cycle (Fig. 1). The lack of significance in TSS between the two cropping cycles in own-rooted 'Kyoho' and 'Kyoho'/5C vines and the contradictory result in 'Kyoho'/1202C from our trial indicate other environmental factors, e.g. differential day lengths and radiations, may compensate for the differential temperature effect between the summer and the winter cropping cycles. Nevertheless, the difference in berry TA between the two cropping cycles in all three scion/rootstocks was in agreement with previous research that low temperature during post veraison period increased berry acidity due to low respiration (Coombe 1987, Mori *et al.* 2005). Although berry TA in the winter cropping cycle was significantly higher than in the summer cropping cycle in all three scion/rootstock combinations, the difference was relatively small in own-rooted vines, whereas berry TA in the winter cropping cycle almost doubled the amount in the summer cropping cycle in 'Kyoho'/1202C. In addition to the poor skin color, the low sugar content but high acidity in the winter berries in 'Kyoho'/1202C often resulted in an unbalanced sugar to acid ratio, and thus less attractive to consumers.

Seasonal decoupling of anthocyanin and TSS accumulations in the double cropping system: The relationship between anthocyanins and TSS was properly described by a sigmoidal function with moderate to high degrees of coefficient of determination (r^2) on any root system in both cropping cycles (Fig. 6). During the post veraison period, an initial

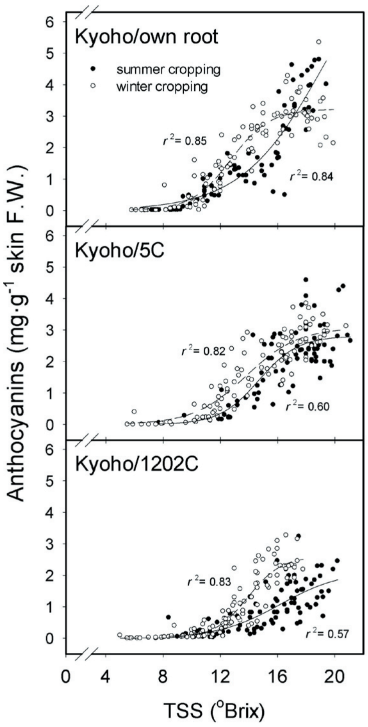

Fig. 6: Relationships between accumulation of anthocyanin and accumulation of total soluble solids (TSS) in developing berries of 'Kyoho' on own-root, 5C, and 1202C rootstocks in the summer and winter cropping cycles of 2009. Solid lines and dash lines represent the regressions for the summer and the winter cropping cycles, respectively. n = 90 in self rooted vines, 110 in vines on 5C rootstocks, and 120 in vines on 1202C.

lag phase with an increase in TSS but little change in anthocyanins was followed by concurrent increases in both quality traits. Except in own-rooted 'Kyoho' in the summer cropping cycle, the sigmoidal relationship also highlighted a late decoupling phase where TSS accumulation rate exceeded anthocyanin accumulation rate towards harvest. The values of r^2 in all three scion/rootstock combinations were greater in the winter cropping cycle than in the summer cropping cycle, indicating a closer relation between anthocyanin and sugar in the cool season than in the warm season.

Sugar content in grape berries is a major determinant for anthocyanin accumulation (ZHENG *et al*. 2009, KOSHITA *et al*. 2011). PIRIE and MULLINS (1977) reported that anthocyanin content was more correlated with sugar content of berry skin than with sugar content of whole berries during ripening. Recently, SADRAS and MORAN (2012) related concentrations of anthocyanins and TSS in developing berries with a two-phase model where in the early lag phase TSS increased with no detectable change in anthocyanins whereas in the late linear phase both quality traits increased in parallel. They concluded that elevated temperatures decoupled the relationship between anthocyanins and sugars by shifting the onset of the linear phase rather than changing the rate of accumulation. Our trials also showed clear shifts of the relationship between the two cropping cycles in all three scion/rootstocks. However, the shift in detail, *i.e.* the length of the initial lag phase, the onset and the rate of the increasing phase, and the presence and degree of the final phase, all appeared to be modulated by rootstocks in the subtropical double cropping system. Likewise, differential climatic characters other than temperature, e.g. day lengths and cumulative radiations between the two cropping cycles, may have further complicated the relationship.

Conclusion

With the benefit of high total yield and cash return, the double cropping system has great potential in subtropical and tropical viticulture regions. However, due to the distinct climate pattern and the consequent distinct phenology, the main challenge has been the limited understanding of the adaptation of grapevines to the unique climate and culture system (CARBONNEAU 2011). For the first time, the seasonal consistency of rootstock effects on berry quality traits in the subtropical double cropping system was documented. Berry quality of own-rooted 'Kyoho' vines was more consistent between the two cropping cycles while 1202C rootstock resulted in inferior berry quality, especially in the winter cropping cycle. Our results, demonstrating rootstock effects and differential seasonal relationships of anthocyanin and TSS in the double cropping system, created a foundation for future exploring the potential physiological effects and commercial necessity of canopy manipulations, e.g. girdling or cluster thinning to improve carbohydrate source: sink ratio, or environment controls, e.g. vineyard supplemental lighting or heating in winter to improve sugar: acid ratio, in subtropical viticulture.

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