Pak. J. Bot., 45(5): 1609-1615, 2013.

INFLUENCE OF GIRDLING AND FOLIAR-APPLIED UREA ON APPLE (*MALUS DOMESTICA* L.) FRUIT QUALITY

YONGGUI ZHAO[1], LIXIN ZHANG[1*], MEI GAO[1], LI TIAN[1], PENG ZHENG[1], KAI WANG[1], LINSEN ZHANG[2], BINZHI LI[2], MINGYU HAN[2] AND ASHOK K. ALVA[3]

[1]*College of Life Sciences, Northwest A&F University, 712100, Yangling, Shaanxi, P.R. China*
[2]*College of Horticulture, Northwest A&F University, 712100, Yangling, Shaanxi, P.R. China*
[3]*USDA-ARS-Vegetable and Forage Crops Research Unit, 99350, Prosser, WA, USA*
**Corresponding author's e-mail address: zhanglixin@nwsuaf.edu.cn*

Abstract

The influence of four rates of foliar-applied urea (0, 0.2, 0.5 and 0.8%; i.e., N0, N1, N2 and N3 rates, respectively) without or with girdling was evaluated on 'Fuji' apple fruit quality parameters and activities of two key enzymes in aroma metabolism. Girdling treatment at a given rate of foliar-applied urea increased fruit weight, size, contents of soluble sugars (SSC), vitamin C (VCC) and total amino acids (TAAC), while decreased soluble protein content (SPC), and titratable acidity (TAC) as well as activities of alcohol acyltransferase (AAT) and alcohol dehydrogenase (ADH). With the increasing rates of foliar-applied urea regardless of girdling treatments, increased SPC, VCC, TAAC, and TAC as well as AAT activity, while decreased SSC. The combination of girdling and urea application increased fruit size and weight, VCC and TAAC. Favorable fruit quality responses were obtained at 0.5% urea rate. This study demonstrated that combined application of girdling and foliar spray of urea improved most of fruit quality parameters.

Introduction

In recent years, the production of 'Fuji' apples has increased steadily in China (Echeverr *et al.*, 2004; Gu *et al.*, 1992; Ruiz *et al.*, 1998). However, fruit quality has continuously decreased, especially for inner quality such as soluble sugar, soluble protein, aroma and flavour (Demirsoy *et al.*, 2012; Nava & Dechen, 2009). These quality characteristics are markedly influenced by tree production practices, such as girdling and nutrient supplementation, primarily nitrogen (N) application (Ashraf *et al.*, 2012; Housley *et al.*, 1977; Nava *et al.*, 2008; Drake *et al.*, 2002). Among the above quality traits, fruit aroma is vital for consumer acceptability and market competition (Dimick & Hoskin, 1983; Taylor & Hort, 2004). It is well known that fruit aroma components such as ethyl-2-methylbutyrate, ethyl butyrate, 1-butanol, 3-methyl butyl acetate and ethyl acetate are synthesized in apple tree by amino acid metabolism, fatty acid oxidation and other secondary metabolism (Rowan *et al.*, 1996; Nie *et al.*, 2004). The amino acid metabolism is an important pathway to synthesize ester for aroma (Chen & Xiang, 2011). Most of the previous studies related to flavor precursors mainly focused on amino acid metabolism (Tress & Drawert, 1973; Drawert & Berger, 1982), involved two key enzymes i.e. alcohol acyltransferase (AAT) and alcohol dehydrogenase (ADH) (Perez *et al.*,1992). Some management practices have been adopted to improve aroma formation, such as fertilizer (Shi *et al.*, 2010), girdling (Khandaker *et al.*, 2011), and storage (Echeverria *et al.*, 2004).

Girdling could improve fruit setting and yield as well as physical and chemical properties of fruit quality (Khandaker *et al.*, 2011). Girdling involves removal of a strip of bark from the trunk or major limbs of a fruit tree, which blocks the downward translocation of photosynthates and metabolites through the phloem (Dannenmann *et al.*, 2009). The best-known effects of girdling are presumably brought about by accumulation of assimilates above the girdle (Li *et al.*, 2003). Consequently, main parameters of fruit quality such as fruit weight and size as well as soluble sugar (SSC) and vitamin C contents (VCC) were improved greatly (Arakawa *et al.*, 1998; Meintjes *et al.*, 2004; Jing, 2007). Jing (2007) reported that girdling a few weeks before flowering decreased contents of titratable acid (TAC) and total amino acids (TAAC) in red bayberry fruit. Parrott *et al.,* (2007) stated that soluble protein content (SPC) decreased by girdling in apple fruit.

Optimal nutrient management, primarily N, has a major impact on fruit quality (Batjer *et al.*, 1966; Petri *et al.*, 2002). Application of N fertilizer at the optimal rate and timing can increase fruit weight and size (Abou *et al.*, 1975; Raese *et al.*, 2007; Aujla *et al.*, 2007), SPC (Guo, 2003), TAAC (Guo, 2003) & TAC (Peng, 2001; Housley *et al.*, 1977). However, the SSC of fruits was negatively correlated with N fertilizer application as reported by Gu (1992).

Nitrogen fertilizer and girdling also influence aroma component generated by amino acid metabolism of ester. However, most of the past studies were conducted for evaluation of the effects of the above single factor, in separate experiments, on most common fruit parameters. In the present study, we investigated the effect of either girdling or foliar urea spray as a single factor or in combination on fruit weight, size and SSC, SPC, VCC, TAAC and TAC as well as activities of AAT and ADH in apple fruit on the Loss Plateau of China.

Materials and Method

Plant material and trial location: The study was conducted in 2011 in the experimental apple orchard of the Northwest A & F University in XunYi county, Shaanxi province, China. The apple cultivar 'Fuji' was planted at 2×3 m spacing (1666 trees/ha) in 2001. The orchard soil was a typical loam soil and its organic matter content was 14.00g/kg, pH 7.64, available N content 47.93 mg/kg, available P content 22.89 mg/kg and available K content 168.20 mg/kg. The organic content and available N, P, K were examined by potassium dichromate titration method, alkaline hydrolysis diffusion method, molybdenum antimony colorimetric method and flame photometer method, respectively (Lv *et al.*, 2012).

Experimental design: Twelve apple trees were selected and divided into three groups of four trees each. More than four branches with similar fruit load were selected to conduct treatments of girdling and foliar urea application from each direction (north, south, east, and west). Girdling treatment was performed on May 15, 2011 with 3 mm width of the girdle on two branches per direction of each tree at a distance of 30cm from fruits using a girdling knife. The remaining three branches in each direction were not girdled. All the selected branches were sprayed with urea solution at 0 (N0), 0.2% (N1), 0.5% (N2) and 0.8% (N3). A single tree per replication was used for each urea concentration spray. Foliar urea spray was done four times, i.e., May 15th, June 15th, July 15th, and August 15th. The total volume 2500mL of the urea solutions were sprayed on the selected branches until the leaves were completely wet and the solution ran off the leaves.

Sampling and measurement: One apple from each branch was harvested from the tested trees in October 16th, 2011 to measure fruit quality parameters including fruit weight, size, SSC, SPC, VCC, TAAC and TAC as well as activities of AAT and ADH. Eight apples were randomly sampled from each replicate of each treatment from the labeled branches. Fruit samples were taken to the lab and stored in a refrigerator at 4°C until analyses.

The fruit weight was measured by an electronic scale and fruit size (diameter) measured by a calipers. SSC was determined by anthrone-sulfonic acid method (He, 2010). TAC was determined by sodium hydroxide titration (Kader, 1992). VCC was determined by molybdenum blue colorimetric method (Gao, 2006). SPC was determined by Coomassie brilliant blue G-250 (Gao, 2006). TAAC was measured by Hitachi 835-50 amino acid analyzer (Sui *et al.*, 2007).

Assay of AAT activity was conducted according to Fellman *et al.,* (2000) and Pérez *et al.,* (1992) with some modifications. Apple cortical tissue (3 g) without skin, was frozen in liquid nitrogen and homogenized with a mortar and pestle in 2 mL g^{-1} (tissue) of 100 mM potassium phosphate buffer (pH 7.0) and 0.33 mg g^{-1} of polyvinylpyrrolidone (PVPP). After filtration and centrifugation, the supernatant was recovered and used for the enzyme assay. The standard assay mixture consisted of 0.85 mL of 0.5 M Tris-HCl, pH 8.0, buffer containing 11.6 mM $MgCl_2$, 0.3 mM acetyl-CoA, 10mM butanol, and 0.15mL of the enzyme solution. The mixture was incubated at 35°C for 15 min, and then 50 mL of 20mM 5, 5-dithiobis (nitrobenzoic acid) (DTNB) was added. Blank samples containing all of the constituents of the standard assay mixture except the alcohol moiety were carried out in parallel with each AAT assay. The increase in absorbance at 412 nm over time due to the yellow thiophenol product formed by the reaction of DTNB with the free CoA-SH liberated was measured.

ADH activity was measured following the procedure described by Longhurst *et al.,* (1990), with some modifications. Apple cortical tissue (3g) without the associated skin, was frozen in liquid nitrogen and homogenized with a mortar and pestle in 6 mL extracting solution, and it was precooled in 4°C. The extracting solution consisted of 100mM MES-Tris buffer (pH 6.5), 2mM DL-Dithiothreitol (DTT), and 1% of PVPP. The mixture was centrifuged (4°C) for 30 min at 15000 rpm and filtered. The supernatant was recovered and used for the enzyme assay. The standard assay mixture consisted of 2.4mL of 100mM MES-Tris buffer (pH 6.5), 0.15mL of 1.6mM NADH, 0.15mL of 80mM acetaldehyde and 0.3mL enzyme solution. Reaction temperature was maintained at 30°C and OD values measured at 340nm.

Statistical analysis: Analysis of variance for different parameters were evaluated by SAS software package (Anon., 1996). Least significance difference (LSD) test was used to determine the significant differences among the mean values at the 0.05 level.

Results

Fruit weight and fruit size: Across all foliar N treatments, girdling significantly increased fruit weight. Fruit weight increased with increase in application rates of urea from N0 to N2 regardless of girdling. Further increase in foliar N rate to N3 showed somewhat negative effects on fruit weight (Fig. 1). A similar response was also evident on the effects of foliar N and girdling on fruit size (Fig. 2).

Fruit quality response: The SSC under girdling treatment at a given rate of foliar urea was substantially increased, while it decreased by urea spray at N0 to N3 rates. It was significantly lower in N3 urea treatment as compared to that with all other rates of foliar-applied urea, regardless of girdling treatments (Table 1). The TAC was lower with girdling as compared to that without girdling at each foliar urea rate. The foliar urea spray at N2 and N3 rates in un-girdled treatment resulted in maximum TAC. There was a significant positive correlation between the TAC and foliar urea rates in both girdled and un-girdled treatments (Table 1). Fruit VCC was greater in girdled than that in the un-girdled treatment. VCC decreased with increasing rate of foliar-applied urea, regardless of girdling treatment (Table 1). Fruit SPC decreased by girdling treatment at each rate of urea spray. The SPC increased with increasing rate of urea only within the range of N0 to N2, in both girdled and un-girdled treatments. The SPC then decreased at N3 of urea spray (Table 1). Girdling significantly increased TAAC only in N2 and N3 rates of urea spray. In girdled treatment, TAAC significantly increased at each increment in foliar-applied urea. In un-girdled treatment, the TAAC increased with an increase in foliar-applied urea in the range of N0 to N2, and then TAAC decreased at N3 rate of urea (Table 1).

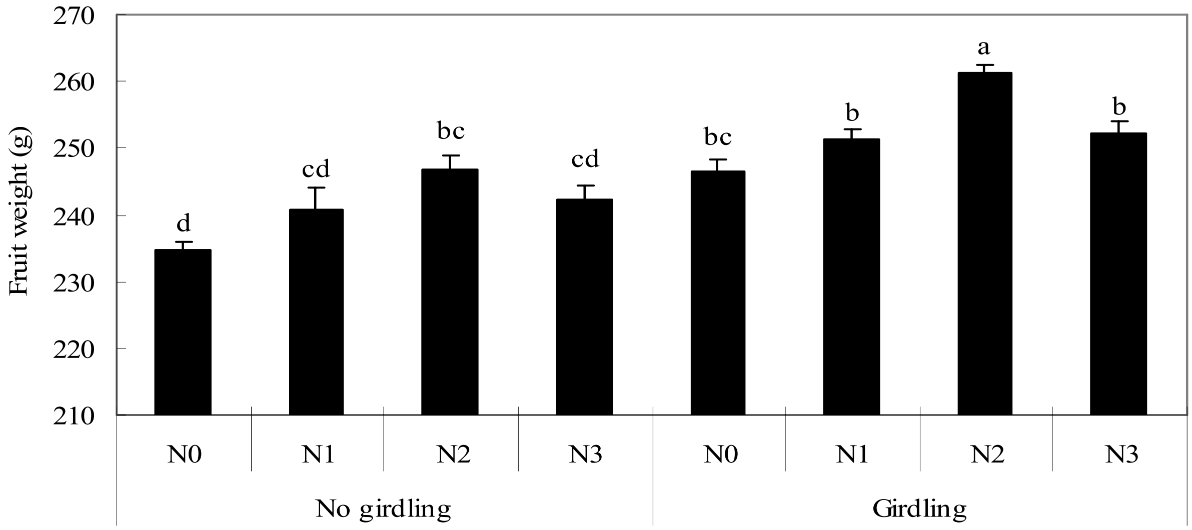

Fig. 1. Effects of foliar-applied urea (0, 0.2, 0.5 and 0.8%; i.e. N0, N1, N2 and N3 rates, respectively) with no or girdling on fruit weight of 'Fuji' apple. Means followed by a common letter are significantly different by Duncan's Multiple Range Test ($p < 0.05$).

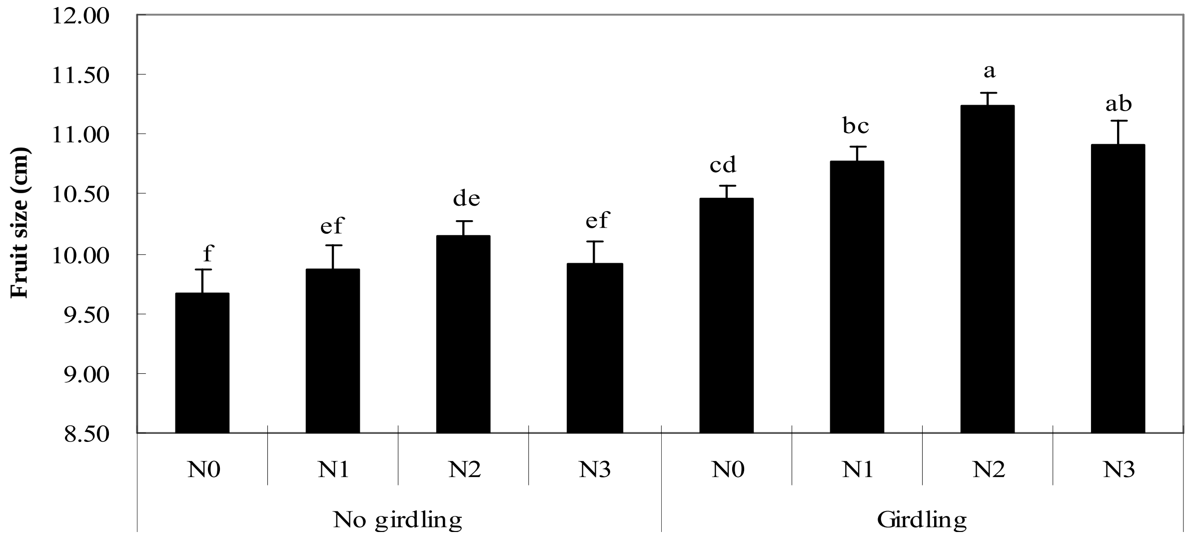

Fig. 2. Effects of foliar-applied urea (0, 0.2, 0.5 and 0.8%; i.e. N0, N1, N2 and N3 rates, respectively) with no or girdling on fruit size of 'Fuji' apple. Means followed by a common letter are significantly different by Duncan's Multiple Range Test ($p < 0.05$).

Table 1. Effects of foliar urea application (0, 0.2, 0.5 and 0.8%; i.e. N0, N1, N2 and N3 rates, respectively) with no or girdling on fruit internal quality parameters measured of 'Fuji' apple in this study.

Treatments	Soluble sugar content (%)	Titratable acidity (%)	Vitamin C content (mg $100g^{-1}$)	Soluble protein content (mg g^{-1})	Total amino acid content (%)
N0	11.582c	0.253cd	7.311cd	2.780cd	64.410e
N1	11.373c	0.258c	7.383c	2.980 ab	66.070de
N2	11.137d	0.279ab	7.117de	3.080 a	68.963bc
N3	10.727e	0.297a	6.940e	2.897 bc	66.210de
N0+Girdling	12.137a	0.227e	8.241ab	2.603 e	66.296de
N1+Girdling	11.840b	0.236e	8.360a	2.757 cde	67.831cd
N2+Girdling	11.557c	0.253cd	8.140 ab	2.996 ab	72.272a
N3+Girdling	11.123d	0.263bc	8.021b	2.620 de	70.109b

Means in each N rate followed by a common letter are significantly different by Duncan's Multiple Range Test ($p < 0.05$)

Fruit AAT and ADH activities in aroma metabolism: Girdling decreased the AAT activity only in N0 and N1 of foliar-applied urea. At the higher urea rates, i.e., N2 and N3, girdling had no effects on AAT activity. In un-girdled treatment, AAT activity was not influenced by foliar urea rates. In contrast, in girdled treatment the AAT activity was significantly greater with N2 and N3 rates of urea as compared to that in N0 and N1 (Fig. 3). Somewhat similar response was also evident with respect to ADH activity (Fig. 4).

Correlations between either of the parameters measured and urea application rate under without or with girdling conditions: Correlation analysis results showed that application concentration of urea and TAC/AATA showed a significant positive correlation, while those of urea and VCC/SSC exhibited a negative correlation under both conditions. There were also positive correlations between either of Si and TAAC/ADHA and urea concentration only under girdling condition (Table 2).

Interaction of foliar urea treatment and girdling for all parameters measured in this study: Girdling and foliar urea treatment had significant effects on all parameters. The magnitudes of *F* values across the above parameters were in the order: girdling >foliar urea. The interaction effects among the above treatments were not mostly significant for all variables except FU × Gi for weight (Table 3).

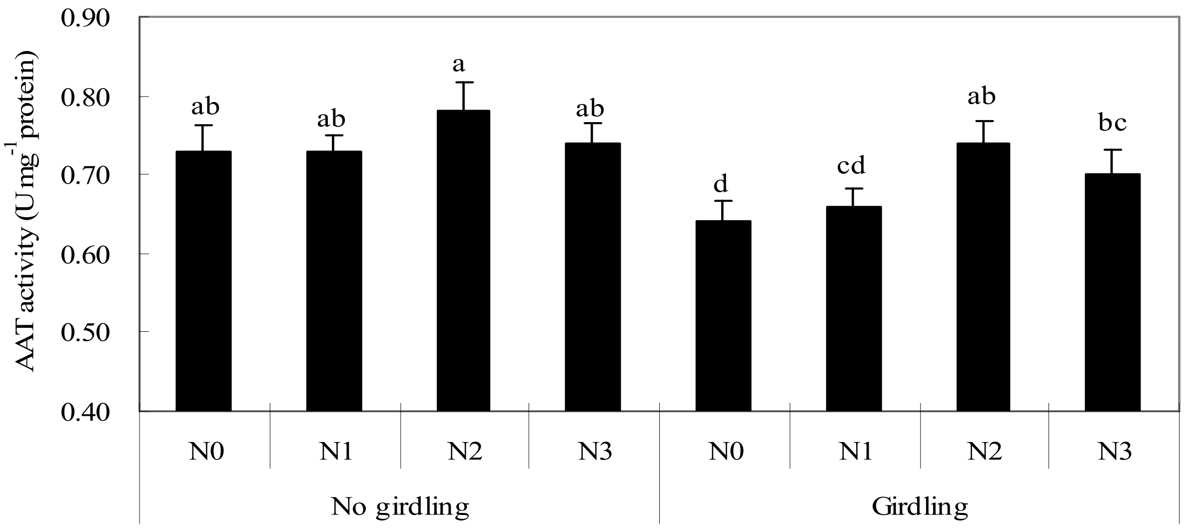

Fig. 3. Effects of foliar-applied urea (0, 0.2, 0.5 and 0.8%; i.e. N0, N1, N2 and N3 rates, respectively) with no or girdling on fruit alcohol acyltransferase (AAT) activity of 'Fuji' apple. Means followed by a common letter are significantly different by Duncan's Multiple Range Test ($p< 0.05$).

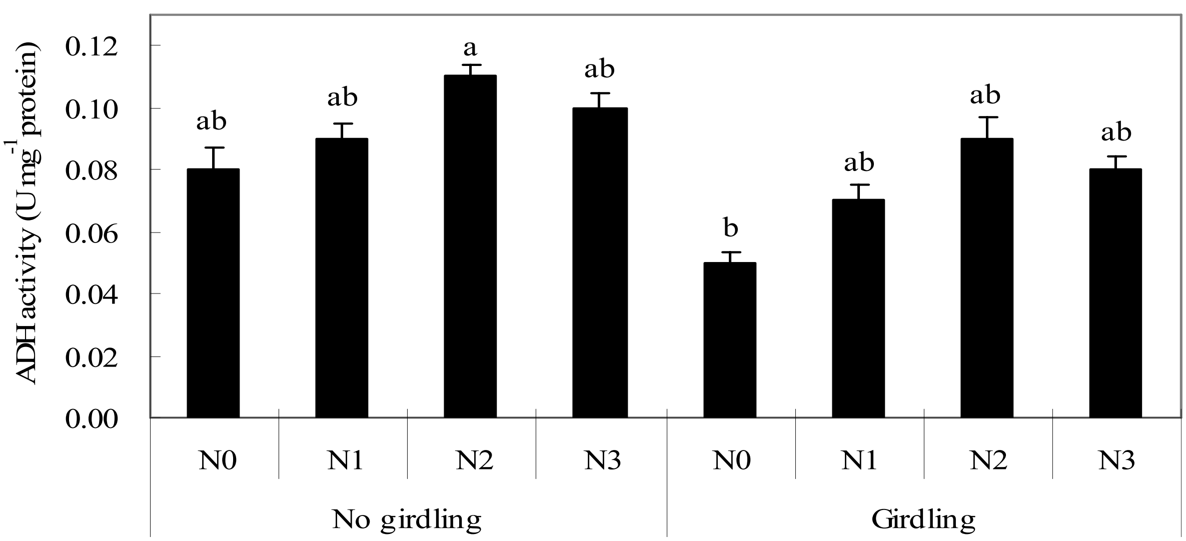

Fig. 4. Effects of foliar-applied urea (0, 0.2, 0.5 and 0.8%; i.e., N0, N1, N2 and N3 rates, respectively) with no or girdling on fruit alcohol dehydrogenase (ADH) activity of 'Fuji' apple. Means followed by a common letter are significantly different by Duncan's Multiple Range Test ($p< 0.05$).

Table 2. Correlation coefficients for the relationship between urea concentration and fruit quality parameters measured in this study under no (N) or (Gl) conditions.

Treatment	Wg	Si	SSC	VCC	SPC	TAC	TAAC	AATA	ADHA
N	0.008	0.453	-0.916**	-0.934**	0.385	0.807**	0.503	0.609*	0.425
Gl	0.490	0.609*	-0.969**	-0.787**	0.187	0.786**	0.722**	0.824**	0.688**

*, **, *** significance at 5%, 1% and 0.1 % level of significance, respectively. AATA, alcohol acyltransferase activity; ADHA, alcohol dehydrogenase activity; TAC, titratable acidity content; TAAC, total amino acid content; Si, fruit size; SPC, soluble protein content; SSC, soluble sugar content; VCC, vitamin C content; Wg, fruit weight

Table 3. *F* values of the effects of foliar urea treatment (FU), girdling (Gl), and their interactions on all parameters measured in this study

Source of variation	Girdling (Gl)	foliar urea (FU)	FU×Gl
Wg	22.729***	140.103***	3.891*
Si	7.970*	107.279***	0.487
SSC	50.312***	66.575***	0.378
TAC	11.502***	37.642***	1.317
VCC	18.632***	596.386***	0.777
SPC	13.469***	20.939***	0.955
TAAC	5.402*	0.319	1.423
AATA	23.023***	34.396***	1.312
ADHA	0.380*	0.799	0.089

*, **, *** significance at 5%, 1% and 0.1 % level of significance, respectively. AATA, alcohol acyltransferase activity; ADHA, alcohol dehydrogenase activity; TAC, titratable acidity content; TAAC, total amino acid content; Si, fruit size; SPC, soluble protein content; SSC, soluble sugar content; VCC, vitamin C content; Wg, fruit weight

Discussion

Optimal nutrient management and horticultural practice such as girdling, are two effective measures to affect fruit quality of fruit trees such as apple (Raese *et al.*, 1997; Quaggio *et al.*, 2002; Zhang *et al.*, 2013; Figs 1&2; Figs. 3 & 4; Table 1). The immediate effect of a girdle is to interrupt the transport of photosynthates through the phloem. This increases foliar carbohydrates (sugars) and other nutrient in parts of a branch above the girdle, which helps flowering (Roper & Williams, 1989). Moreover, a positive correlation between most parameters measured and concentration of urea were evident in apple plants under both girdling and no girdling treatments, especially for girdling condition (Table 2). Additionally, the effects of girdling or foliar-applied urea treatment on all parameters measured were much significant (Table 3). It is, therefore, suggested that optimal application of foliar urea or girdling treatment dose benefit for increasing apple fruit quality.

Fruit weight and size are external indices of fruit quality. In our study, fruit weight and size increased with increase in urea application rate and girdling treatment (Figs. 1&2). The urea concentration 0.5% was best for improving the weight and size of ‘Fuji’ apple, which was parallel to what has been earlier observed in ‘Granny Smith’ apple (Fallahi *et al.*, 1997 and 2001; Raese *et al.*, 1997). Girdling can improve carbohydrate availability to fruits, which in turn can lead to an increase in fruit-set and yield as well as number of fruits (Rivas *et al.*, 2004). The increase in size due to girdling may indicate its ability to stimulate carbohydrate translocation to the fruit in combination with their effect on increasing cell wall elasticity. Our results are in agreement with the findings of Mostafa & Saleh (2006), who reported that girdling alone or with N spray increased the fruit size and fruit weight in Balady mandarin orange.

TAC and SSC are important indicators of measuring fruit quality and evaluating the fruit taste. Feng *et al.*, (2008) stated that TAC was promoted by N fertilizer, while SSC decreased. However, the opposite response was found with girdling (Jing, 2007). Our results revealed that in 10-year-old apple (Fuji), TAC increased by N application, while decreased by girdling (Table 1). Raese *et al.*, (1997) reported similar results in apple tree, which stated that TAC was the highest from trees fertilized with highest N rate (0.8%). Our results are supported by the findings of Wu *et al.*, (2009).

Our results revealed that apple fruit SPC was promoted by different rates of urea application, while decreased by girdling (Table 1). Seung (2000) reported that VCC in fruits decreased at high rates of N. VCC increased with girdling, while SPC decreased. Guo *et al.*, (2003) indicated that N could improve SPC and improve fruit quality. With the increasing of N addition, ratio of SPC and TAAC of fruit increased (Peng *et al.*, 2001). Krapp (1995) stated that SPC was decreased by girdling.

TAA is one of main metabolites in aroma metabolic pathway. It has been demonstrated that only sufficient N and carbon are important for amino acid synthesis. Furthermore, it is illustrated that TAAC was positively affected by N application. Thus, N is essential to the synthesis process of amino acids (Gu *et al.*, 1981). In our study, TAAC increased after spraying N with 0.5% concentration, which presented the best results, whereas girdling decreased fruit TAAC (Table 1).

AAT and ADH are key enzymes in aroma metabolic pathway. Fellman and Fallahi found that the fruit N status of 'Redspur Delicious' apple determines the amount and nature of flavor, which volatiles present in fruit flesh, but has little effects on enzyme activity. Our results showed that increased N rate enhanced AAT activity, but not the ADH activity. Girdling decreased both enzymes (Figs. 3 & 4).

Conclusion

Girdling and foliar urea application are two effective measures to improve apple quality. Girdling, at each rate of foliar urea increased fruit weight, size, SSC, VCC and TAAC, but decreased SPC, TAC as well as activity of AAT and ADH. The increasing rate of foliar N, regardless of girdling, increased SPC, VCC, TAAC, TAC and activity of AAT, while decreased SSC. The combination of girdling and foliar-applied urea exhibited the greater significant effects as compared to application of foliar N only. Further research is required to elucidate molecular mechanisms and signaling pathways involved in girdling and/or foliar urea application effects on apple fruit quality.

Acknowledgments

This research was supported by the Special Fund for Agro-scientific Research in the Public Interest (201303104) and West Light Foundation of CAS (2060299-14), Program for Agricultural Sci-Tech Innovation of Shaanxi Province (2011NXC01-18), Environment Protection Program (2012-47) and Sci-tech Coordinating Innovative Engineering Projects of Shaanxi Province (2011KTZB02-02-05).

Reference

Abou Aziz, A.B., I. Desouki and M.M. El-Tanahy. 1975. Effect of nitrogen fertilization on yield and fruit oil content of avocado trees. *Sci. Hortic.*, 1(3): 89-94.

Anonymous. 1996. *Getting started with PROC ANOVA. SAS.* Institute Inc., Cary, NC.

Arakawa, O., A. Kanetsuka, K. Kanno and Y. Shiozaki. 1998. Effects of five methods of bark inversion and girdling on the tree growth and fruit quality of 'Megumi' apple. *J. Japan. Soc. Hort. Sci.*, 67(5): 721-727.

Ashraf, M.Y., M. Yaqub, J. Akhtar, M.A. Khan, M. Alikhan and G. Ebert. 2012. Control of excessive fruit drop and improvement in yield and juice quality of kinnow (*citrus deliciosa* x *citrus nobilis*) through nutrient management. *Pak. J. Bot.*, 44: 259-265.

Aujla, M.S., H.S. Thind and G.S. Buttar. 2007. Fruit yield and water use efficiency of eggplant (*Solanum melongena* L.) as influenced by different quantities of nitrogen and water applied through drip and furrow irrigation. *Sci. Hortic.*, 112: 142-148.

Batjer, L.P., J.M. Bloxom, G. Auvil and N.R. Benson. 1966. Tree fruit nutrition in central Washington. *Proceedings Washington State Horticulture Association*, 62: 201-207.

Chen M. and M.L. Xiang. 2011. The research progress in the Synthesis of fruit aroma. *Modern Horticulture*, 6: 17-19.

Dannenmann, M., J. Simon, R. Gasche, J. Holst and P.S. Naumann. 2009. Tree girdling provides insight on the role of labile carbon in nitrogen partitioning between soil microorganisms and adult European beech. *Soil Biol. Biochem.*, 41: 1622-1631.

Demirsoy, L., H. Demirsoy and G. Balci. 2012. Different growing conditions affect nutrient content fruit yield and growth in strawberry. *Pak. J. Bot.*, 44(1): 125-129.

Dimick, P.S. and J.C. Hoskin. 1983. Review of apple flavor-state of the art. *CRC Crit. Rev. Food Sci. Nutr.*, 18: 387-409.

Drake, S.R., J.T. Raese and T.J. Smith. 2002. Time of nitrogen application and its influence on 'Golden delicious' apple yield and fruit quality. *J. Plant Nutr.*, 25(1): 143-157.

Drawert, F. and R.G. Berger. 1982. Biogenesis of aroma compounds in plants and fruits. XVII. Anabolic Properties of strawberry fruit tissue for the biosynthesis of aroma compounds. *Z. Naturforsch., C: Biosci.*, 37: 849-856.

Echeverr, G., J. Graell, M.L. López and I. Lara. 2004. Volatile production, quality and aroma-related enzyme activities during maturation of 'Fuji' apples postharvest. *Postharvest Bio. Tech.*, 31: 217-227.

Echeverr´ıa, G., T. Fuentes, J. Graell, I. Lara and M.L. López. 2004. Aroma volatile compounds of 'Fuji' apples in relation to harvest date and cold storage technology: A comparison of two seasons. *Postharvest Biol. Technol.*, 32: 29-44.

Fallahi, E., W.M. Colt, C.R. Baird, B. Fallahi and I. Chun. 2001. Influence of nitrogen and bagging on fruit quality and mineral concentrations of 'BC-2 Fuji' apple. *Hort. Technol.*, 11(3): 462-465.

Fallahi, E., W.M. Colt and M.M. Seyedbagheri. 1997. Influence of foliar application of nitrogen on tree growth, precocity, fruit quality, and leaf mineral nutrients in young 'Fuji' apple trees on three rootstocks. *J. Tree Fruit Prod.*, 2: 1-12.

Fellman, J.K., T.W. Miller and D.S. Matttnson. 2000. Factors that influence biosynthesis of volatile flavor compounds in apple fruits. *HortSci.*, 35: 1026-1033.

Feng, H.D., B.Z. Li and L.S. Zhang. 2008. The effect of different nitrogen levels on quality, photosynthesis and leaf element content of 'Fuji' apple. *Acta Agriculturae Boreali-occidentalis Sinica*, 17(1): 229-232.

Gao, J.F. 2006. Experimental Guidebook of modern plant physiology. *Higher Education Press*, 243-247.

Gu, M.R, R.Z. Zhang and H.R. Shu. 1981. The preliminary study of nitrogen of apple. *Acta Hortic. Sin.*, 8(4): 21-28.

Gu, M.R, H.R. Shu, G.M. Qu, Y.M. Jiang and L. Miao. 1992. The relationship between fruit quality and mineral element contents in fruit of 'starking delicious' apples. *Acta Hortic. Sin.*, 19(4): 301-306.

Guo, Y.Y. 2003. The Characteristics of ^{15}N utilization and influence of nitrogen on physiological process of fruit ripening of Strawberry. *Thesis for Master Degree of Shandong Agricultural University*.

He, X.T., J.Y. Niu and J.H. Liu. 2010. The influence of different levels of fertilization on yield and quality of apple. *Journal of Gansu Agricultural University*, 45(2): 83-86.

Housley, T.L., M.P. David and L.E. Schrader. 1977. Long distance translocation of sucrose, serine, leucine, lysine, and CO_2 assimilates. *Plant Physiol.*, 59: 217-220.

Jing, L. 2007. Effects of ringing and girdling on growth and nutrition, photosynthetic characteristics in Myrica ruba and major qualities of Bayberry fruits. *Thesis for Master Degree of Fujian Agriculture and Forestry University*.

Kader, A.A. 1992. Quality and safety factors: Definition and evaluation for fresh horticultural crops. *Postharvest Tech. Hortic. crops*, 9: 185-189.

Khandaker, M.M., S.H. Abm, O. Normaniza and N.B. Amru. 2011. Application of girdling for improved fruit retention, yield and fruit quality in syzygium samarangense under field conditions. *Int. J. Agric. Biol.*, 13: 18-24.

Krapp, A. and M. Stitt. 1995. An evaluation of direct and indirect mechanisms for the 'sink regulation' of photosynthesis in spinach: changes in gas exchange, carbohydrates, metabolites, enzyme activities and steady-state transcript events after cold-girdling source leaves. *Planta*, 195: 313-323.

Li, C.Y., D. Weiss and E.E. Goldschmidt. 2003. Girdling affects carbohydrate-related gene expression in leaves, bark and roots of alternate-bearing citrus trees. *Ann. Bot.*, 92: 137-143.

Longhurst, T.J., J.F. Tung and C.J. Brady. 1990. Developmental regulation of the expression of alcohol dehydorgenase in ripening tomato fruit. *J. Food Biochem.*, 14: 421-433.

Lv, L.X., L.X. Zhang, M. Gao, Y.F. Li and C.Y. Liao. 2012. The influence of rhizosphere fertilizer injection on soil properties, soil enzymes, fruit yield and quality on the Loss Plateau. *J. Fruit Sci.*, 29(5): 782-788.

Meintjes, J.J., P. Stassen and K.I. Theron. 2004. The effect of different rates of prohexadionecalcium and girdling on shoot growth and fruit quality when applied to different pear cultivars. *Int. Soc. Hortic. Sci.*, 671: 539-546.

Mostafa, E.A.M. and M.M.S. Saleh. 2006. Response of balady Mandarin trees to girdling and potassium spraysunder sandy soil conditions. *Res. J. Agric. Biol. Sci.*, 2: 137-141.

Nava, G., A.R. Dechen and G.R. Nachtigall. 2008. Nitrogen and potassium fertilization affect apple fruit quality in southern Brazil. *Commun. Soil Sci. Plant Anal.*, 39: 96-107.

Nava, G. and A.R. Dechen. 2009. Long-team annual fertilization with nitrogen and potassium affect yield and mineral composition of 'fuji' apple. *Sci. Agric. (Piracicaba, Braz.)*, 66(3): 377-385.

Nie, C.L., J.S. Sun and R.H. Huang. 2004. The formation of fruit aroma and its influencing factors. *Chinese Bull. Bot.*, 21(5): 631-637.

Parrott, D.L., K. McInnerney, U. Feller and A.M. Fischer. 2007. Steam-girdling of barley (*Hordeum vulgare*) leaves leads to carbohydrate accumulation and accelerated leaf senescence, facilitating transcriptomic analysis of senescence-associated genes. *New Phytol.*, 176: 56-69.

Peng, F.T. 2001. Regulation of nitrogen to apple fruit development, yield and quality. *The Ph.D. Dissertation of Shan Dong Agricultural University*.

Petri, J.L., L.A. Palladini and A.C. Pola. 2002. In: EPAGRI. Manual da cultura da macieira. *Florianópolis: Epagri*, 229-259.

Perez, A.G. C. Sanz and J.J. Rios. 1992. Aroma components and free amino acids in strawberry variety Chandler during ripening. *J. Agric. Food Chem.*, 40: 2232-2239.

Quaggio, J.A., D. Mattos, J.H. Cantarella, E.L.E. Almeida and S.A.B. Cardoso. 2002. Lemon yield and fruit quality affected by NPK fertilization. *Sci. Hortic.*, 96: 151-162.

Raese, J.T. and S.R. Drake. 1997. Nitrogen fertilization andelemental composition affects fruit quality of 'Fuji' apples. *J. Plant Nutr.*, 20(12): 1797-1809.

Raese, J.T., S.R. Drake and E.A. Curry. 2007. Nitrogen fertilizer influences fruit quality, soil nutrients and cover crops, leaf color and nitrogen content, biennial bearing and cold hardiness of 'Golden Delicious'. *J. Plant Nutr.*, 30: 1585-1604.

Rivas, F., H. Arbiza and A. Gravina. 2004. Caracterización del comportamiento reproductive de la mandarin 'Nova' en el sur del Uruguay. *Agrociencia*, 8: 79-88.

Roper, T. and L. Williams. 1989. Net CO_2 assimilation and carbohydrate partitioning of grapevine leaves in response to trunk girdling and gibberellic acid application. *Plant Physiol.*, 89: 1136-1140.

Rowan, D.D., H.P. Lane, J.M. Allen, S. Fielder and M.B. Hunt. 1996. Biosynthesis of 2-methylbutyl, 2-methyl-2-butenyl and 2-methyl butanoate esters in 'Red Delicious' and 'Granny Smith' apples using deuterium-labeled substrate. *J. Agric. Food Chem.*, 44(10): 3276-3285.

Ruiz, J.M. and L. Romero. 1998. Commercial yield and quality of fruits of cucumber plants cultivated under greenhouse conditions: response to increases in nitrogen fertilization. *J. Agric. Food Chem.*, 46: 4171-4173.

Seung Lee, K. and A. Kader Adel. 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biol. Technol.*, 20: 207-220.

Shi, C.Y., X.M. Li, X.D. Li and R. Zhang. 2010. The effects of two fertilizer treatments on aroma components of Red Fuji apple. *Northern Hortic.*, 3: 1-3.

Sui, J., Y.M. Jiang, F.T. Peng and Y.Y. Guo. 2007. The effect of nitrogen levels on fruit quality of strawberry. *Deciduous Fruits*, 1: 1-3.

Taylor, A.J. and J. Hort. 2004. Measuring proximal stimuli involved in flavour perception. *Oxford: Blackwell Publishing*, 1-38.

Tress, R. and F. Drawert. 1973. Biogenesis of banana volatiles. *J. Agric Food Chem.*, 21: 560-565

Wu, L.M., Y.C. Jiang and M.S. Zhou. 2009. The effect of girdling on growth, nutrition and fruit quality of the Goldwater Kom. *Hubei Agric. Sci.*, 48(11): 2762-2766.

Zhang, L.X., J. Zhou, Y.G. Zhao, Y.Y. Zhai, K. Wang, A.K. Alva and S. Paramasivam. 2013. Optimal combination acid to improve soil and leaf properties, yield and quality of apple (*Malus domestica*) in the Loess Plateau of China. *Pak. J. Bot.*, 45(4): 1315-1320.

(Received for publication 10 June 2012)