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Lower Crop Load for Cv. Jonagold Apples (*Malus* × *domestica* Borkh.) Increases Polyphenol Content and Fruit Quality

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The influence of crop load on fruit quality was investigated on 7-year-old slender spindle cv. Jonagold/M.9 apple trees. In mid June five different crop loads per tree were prepared by reducing the fruit number to average 30, 59, 104, 123, and 157 fruits per crown. The fruit from low-cropping trees had more red blush, a higher percentage of soluble solids in fruit flesh, and better flesh firmness in comparison to fruit from high-cropping trees. As the crop load decreased, the concentration of all phenolic compounds in the fruit samples (cortex plus skin) increased; concentrations of the most important individual fruit phenolics were also higher. When crop load fell from 157 to 30 fruits per crown, total polyphenols increased from an average of 1300 to 1680 mg/kg of fruit fresh weight (FW) (+29%), low molecular weight polyphenols increased from 1140 to 1570 mg/kg of FW (+38%), and high molecular weight polyphenols increased from 1740 to 2070 mg/kg of FW (+19%). The average increases in single polyphenols were even greater: chlorogenic acid (+82%); 4′-*p*-coumaroylquinic acid (+22%); catechin (+178%); and epicatechin (+71%). Ascorbic acid was not significantly dependent on crop load.

KEYWORDS: ***Malus* × *domestica*; apple fruit quality; crop load; polyphenols; 4′-*p*-coumaroylquinic acid; chlorogenic acid; catechin; epicatechin**

INTRODUCTION

Several epidemiological studies have documented the protective role of fruit and vegetable consumption against the development of certain cancers, cardiovascular diseases, and stroke (*1*). Apples are one of the most frequently consumed fruits in Europe and constitute a main source of flavonoid intake in the European diet, after onions and tea (*2*, *3*). The major apple antioxidants are polyphenols and ascorbic acid. In addition to their beneficial nutritional properties, polyphenols may also contribute to plant resistance against many diseases, such as apple scab (*4*−*6*). Flavonoids and phenolic acids affect fruit firmness and may be responsible for apple quality (*7*). The major flavonoid classes in apples are flavonols (quercetin 3-glycosides), monomeric, oligomeric, and polymeric flavan 3-ols (catechin and epicatechin), dihydrochalcones (phloridzin), and anthocyanins (cyanidin 3-glycosides). Chlorogenic acid is the most important hydroxycinnamic acid in apples.

High yields from intensive apple orchards have resulted in a worldwide overproduction of apples. Interestingly, several experiments have shown improved apple quality in terms of color, size, and firmness with decreased crop loads per tree (*8*, *9*). Light cropping also advances apple fruit maturity as indicated by the background color, starch−iodine score, and soluble solids content (*10*). Some authors have found that flavonoid and chlorogenic acid levels in apple fruit vary with fruit position within the canopy, with type of bearing wood, and between orchards (*11*), whereas others report that culture and growing conditions have a limited effect on the polyphenol profiles of apple cortex and peel (*12*).

The purpose of this study was to investigate fruit quality and the variation in different classes of polyphenols under different crop loads of apple trees, cv. Jonagold.

MATERIALS AND METHODS

Plant Material. Seven-year-old slender spindle apple trees cv. Jonagold/M.9 (*Malus* × *domestica* Borkh.) with homogeneous growth vigor and bloom density were selected from an experimental orchard of the Agricultural Institute of Slovenia at Brdo during the year 2000. The field experiment was designed as a complete randomized block with six repetitions and a single tree per plot. In mid June five different crop loads of approximately 30, 60, 90, 120, and 150 fruits per crown were established by hand thinning. At that time, the average fruitlet diameter was 39 mm. The orchard received standard pest and disease management throughout the experiment.

Fruit was harvested at commercial maturity in mid September and graded into two classes of sizes: >80 and <80 mm diameter, respectively. After the yield of each tree had been weighed and counted, 20 >80 mm fruits per crown were randomly sampled to estimate fruit color, starch−iodine score, firmness, and percent of soluble solids of each fruit sample at harvesting time. Fruit skin color was estimated visually from 1 (0−10% blush) to 10 (90−100% blush), flesh firmness by hand penetrometer with an 11 mm probe (1 cm^{-2}), and percent of soluble solids by hand refractometer (using juice obtained during measurement of flesh firmness). The starch−iodine score was estimated from 10 apple halves immersed in 0.1 M iodine solution (1−10, 1 =

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Table 1. Effect of Crop Load of Cv. Jonagold/M.9 Apple Trees on Size, Weight, Color (Blush Intensity), Soluble Solids, Firmness, and Starch–Iodine Score of Apple Fruit

crop load (no. of fruit/tree)	no. of fruit <80 mm/tree	av fruit wt (g)	color (1–10)[a]	soluble solids (%)	firmness (N)	starch–iodine score (1–10)[b]	sunfleck[c] (%)
30	10	228	8.0	15.8	97	5.1	37
59	24	220	7.7	14.4	89	6.4	24
104	58	197	5.7	13.4	82	7.7	14
123	98	175	4.6	13.4	79	6.9	23
157	121	171	4.4	13.1	78	7.5	18
S_{yx}[d]	*18.6*	*15.5*	*0.96*	*0.57*	*5.2*	*0.80*	*13.0*
significance[e]							
L	***	***	***	***	***	***	*
Q	NS	NS	NS	***	**	*	NS

[a] 1 = 0–10% red blush of apple skin; 10 = 90–100% red blush. [b] 1 = all flesh stain black; 10 = no staining, no starch present. [c] Sunfleck represents the percent of direct sunlight penetration to the bottom part of the canopy. [d] S_{yx} is standard error of estimate of the linear regression line (n=30). [e] NS, *, **, ***: nonsignificant or significant regression for linear (L) or quadratic (Q) trend at $P < 0.05$, $P < 0.01$, or $P < 0.001$, respectively.

highest starch content). The remaining fruit samples from each tree were stored for 3 months at 4 °C until they were fit to eat, when the analysis of polyphenols was carried out. The percentage of direct sunlight penetrating through the canopy (sunfleck) to the lower part of the crown (1 m from the ground) was measured by a sunfleck ceptometer (Decagon) 2 weeks before harvesting. Six readings per crown were used to determine the average sunfleck data per tree.

Extracts. To limit enzymatic and chemical reactions (especially oxidation), both apples and the extraction solution were cooled to 4 °C. The pith of six fruits per sample (tree) was removed with a corer, and each apple was cut into 10 equal slices. Two slices (cortex plus skin) from the opposite sides of each fruit were used to prepare aqueous acetone extracts. For 120 g of representative samples, two slices from at least five various fruits were taken per sample. Apples were extracted by homogenizing 120 g of the apple fraction with 250 mL of cool 70% aqueous acetone in a blender at maximum speed for 90 s. Extraction took 15 min, and the extract was transferred to a centrifuge tube. Residue from the blender was rinsed with 20 mL of 70% aqueous acetone and added to the centrifuge tube. The extract was centrifuged for 5 min at 4100 rpm to partially remove solid parts. Sediments from all centrifuge tubes were combined, and 200 mL of extraction solution was added. The second extraction, centrifuged as the first, took 15 min. Both supernatants were put in a 500 mL volumetric flask and brought to exact volume with the extraction solution. The two extracts were put into two 250 mL glass bottles. Nitrogen was insufflated in the headspace, and the bottles were closed and stored at −18 °C until the analysis.

Sample Preparation. To remove acetone from the extract, 20 mL of extract was evaporated, to about one-third of the volume in a 100 mL pear-shape flask by rotary evaporation under reduced pressure at 35 °C. This sample was brought to 20 mL with deionized water. For spectrophotometric methods, the sample was then applied to a C18 SPE column (0.5 g, Sep-Pak, Waters). For HPLC analysis the final sample was filtered through 0.45 μm, 13 mm PTFE syringe-tip filters (Millipore, Bedford, MA) into LC vials.

Standards and Reagents. All chromatographic solvents (methanol and formic acid) were of HPLC grade and were purchased from Merck (Darmstadt, Germany). (−)-Epicatechin, (+)-catechin, and chlorogenic acid were purchased from Roth, and Folin−Ciocalteu reagent and vanillin were purchased from Merck.

Spectrophotometric Methods. The following assays were tested: total polyphenols (FC), high molecular weight polyphenols (PROC), and index of vanillin (VAN) (*13*).

Total polyphenols were assessed by the reduction of phosphotungstic−phosphomolybdic acids (Folin−Ciocalteu reagent) to blue pigments by phenols in alkaline solution. Concentrations were determined by means of a calibration curve as (+)-catechin.

High molecular weight polyphenols were evaluated by transformation into cyanidin and expressed by means of a calibration curve with cyanidin chloride. PROC are more responsive for high molecular weight polyphenols, especially high molecular weight proanthocyanidins.

Table 2. Effect of Crop Load on the Contents of Total Polyphenols (FC), Low Molecular Weight Polyphenols (VAN), High Molecular Weight Polyphenols (PROC), and Ascorbic Acid in Fresh Weight Samples of Cv. Jonagold/M.9 Fruit (Cortex plus Skin)

crop load (no. of fruit/tree)	FC[a] (mg/kg)	VAN[a] (mg/kg)	PROC[b] (mg/kg)	ascorbic acid (mg/kg)
30	1680	1570	2070	94.4
59	1510	1340	1970	78.2
104	1360	1180	1800	92.2
123	1400	1190	1820	94.7
157	1300	1140	1740	100.4
S_{yx}[c]	*136*	*139*	*224*	*16.5*
significance[d]				
L	***	***	*	NS
Q	NS	**	NS	NS

[a] Equivalent of (+)-catechin. [b] Equivalent of cyanidin. [c] S_{yx} is standard error of estimate of the linear regression line (n = 30). [d] NS, *, **, ***: nonsignificant or significant regression for linear (L) or quadratic (Q) trend at $P < 0.05$, $P < 0.01$, or $P < 0.001$ respectively.

Index of Vanillin. Concentrations were calculated as (+)-catechin by means of a calibration curve. VAN is more responsive for low molecular weight polyphenols, especially catechins and low molecular weight proanthocyanidins.

HPLC Analysis. The HPLC analyses of flavan-3-ols and hydroxycinnamates were performed on a Hewlett-Packard series 1090 instrument, equipped with an HP ChemStation using a reversed-phase column Hypersil ODS RP-18 (250 × 2.1 mm, 5 μm particle size) and precolumn Hypersil ODS (20 × 2.1 mm, 5 μm particle size). The solvents used were A (0.5% acetic acid in water) and B (2% acetic acid in methanol). Gradients were as follows: from 8 to 22% B in 14.5 min, from 22 to 27.8% B in 5.5 min, from 27.8 to 100% B in 1 min, 100% B for 2 min, from 100 to 8% B in 2 min, and 8% B for 5 min. Flow rate was 0.4 mL/min, injection volume 10 μL, and oven temperature 40 °C. The detection of flavan-3-ols was at 280 nm and that of hydroxycinamates at 320 nm. For this analysis, 4′-*p*-coumaroylquinic acid was expressed as chlorogenic acid.

Ascorbic Acid Analysis. Ascorbic acid was analyzed by using the 2,4-dinitrophenylhydrazine method (*14*).

Statistical Analysis. Data were subjected to statistical analyses using the statistical program Statgraphics 5.0 (STSC, Rockville, MD). The main effect of crop load was analyzed with regression analysis to evaluate linear and quadratic trends. Standard errors of the estimate show the standard deviation of the residuals on the regression line.

RESULTS AND DISCUSSION

Crop load significantly affected size, color, and internal quality of cv. Jonagold fruit. Increasing the crop load increased the number of small fruit on the tree (of diameter <80 mm)

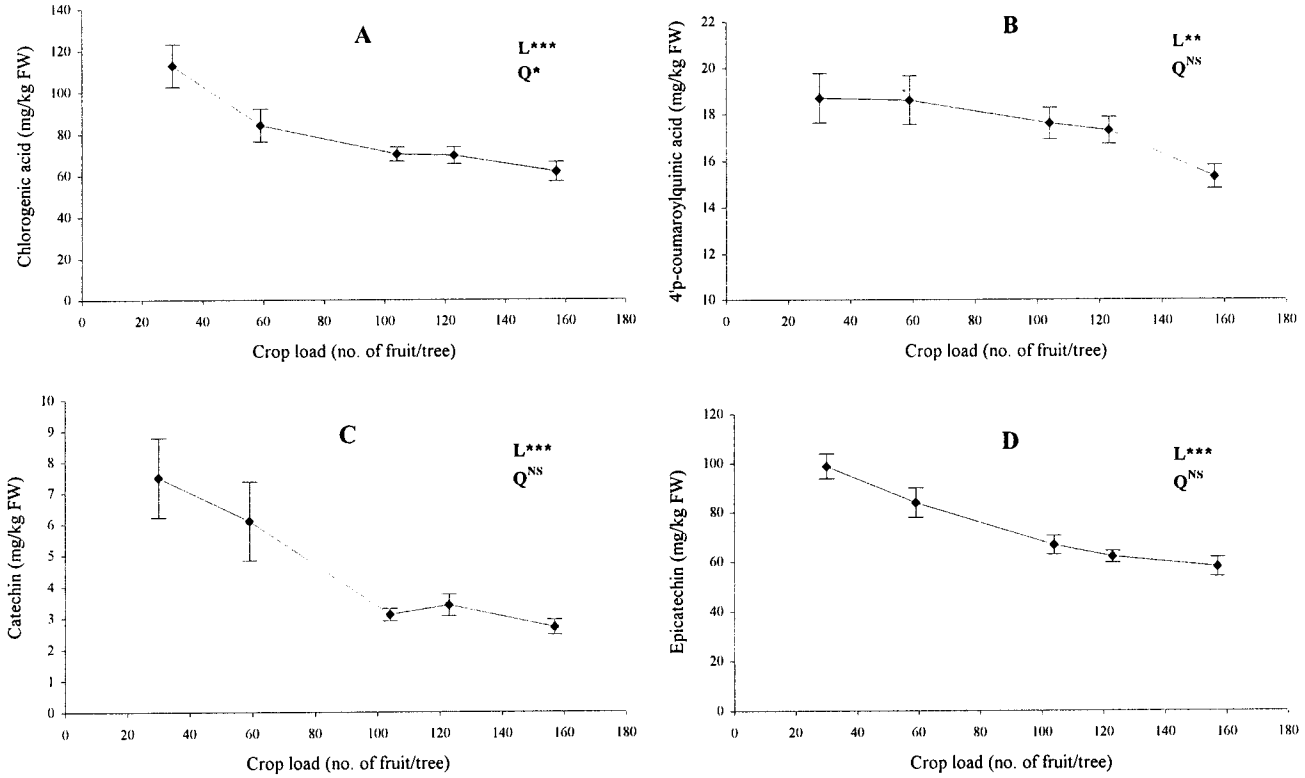

Figure 1. Effect of crop load of cv. Jonagold/M.9 apple trees on chlorogenic acid (A), 4′-*p*-coumaroylquinic acid (B), catechin (C), and epicatechin (D) content in fresh weight (FW) samples containing fruit cortex plus skin. NS, *, **, ***: nonsignificant or significant polyphenol/crop load regression analysis ($n = 30$) with linear (L) or quadratic (Q) trend at $P < 0.05$, $P < 0.01$, or $P < 0.001$, respectively. Vertical bars indicate standard errors of each mean.

and decreased the average fruit weight, both with a linear trend (**Table 1**). This inverse relationship between fruit size and fruit number on crown has been confirmed by many researchers and is one of the main reasons for using fruit thinning in apple orchards (*15*, *16*). Fruit from the low-cropping trees had significantly more red blush color, a higher percentage of soluble solids, and better flesh firmness compared with the high-cropping trees (**Table 1**). Data are comparable with previous research; total sugar content and blush color increased with decreased crop load (*17*, *18*). A higher percentage of sunfleck on low-cropping trees (**Table 1**) may have contributed to the better red coloration of apples because there is a strong correlation between direct sunlight reaching the fruit and anthocyanin content in the exposed part of fruit skin (*19*, *20*). However, the percentage of direct sunlight penetrating through the canopy decreased only slightly as the crop load increased, whereas the range of blush intensity of skin color decreased strongly with a significant linear trend (**Table 1**). In a study on cv. Mutsu, no correlation was found between ratings for tree vigor and tree openness with apple fruit color, whereas crop load was highly correlated to the fruit color (*9*). Therefore, we expect that crop load in our experiment affected blush intensity more strongly, and this was independent of sunlight reaching the fruit. Our starch−iodine tests indicated a delayed maturity (more starch) in apples from low-cropping trees (**Table 1**) contrary to a report of a cv. Braeburn apple experiment in which the authors found less starch present on the fruit cortex of low-bearing apple trees (*21*). The results of a cv. Braeburn bearing experiment (*21*) confirmed that apple fruit firmness and soluble solids content increase linearly with decreasing crop loads.

As the crop load decreased, the concentration of all phenolic compounds, as well as concentrations of the most important individual phenolics in fruit (cortex plus skin), increased (**Table 2**; **Figure 1**). The total polyphenols (FC) content of cv. Jonagold fruit increased from an average of 1300 mg/kg of FW (157 fruits per crown) to an average of 1680 mg/kg of FW (30 fruits per crown) (+29%). Low molecular weight polyphenols (VAN) increased from an average of 1140 mg/kg of FW for the highest crop load to 1570 mg/kg of FW for the lowest crop load (+38%). The concentration of high molecular weight polyphenols (PROC) increased from an average of 1740 to 2070 mg/kg of FW (+19%) (**Table 2**). All three groups of polyphenols increased significantly with a linear trend in the regression analysis ($n = 30$), whereas VAN increased with a quadratic trend as well. Concentrations of single polyphenols (**Figure 1**) show similar trends, but the average increases were much higher compared with the average increases of total polyphenols and low and high molecular weight polyphenols. Except for of 4′-*p*-coumaroylquinic acid, which increased by 22%, other single polyphenols exhibited a rapid increase: chlorogenic acid by 83%, catechin by 178%, and epicatechin by 71% when the crop load fell from 157 to 30 fruits per crown (**Figure 1**). Regression analysis demonstrates that all investigated individual phenols in cv. Jonagold apples increased significantly with linear trend as the crop load decreased. However, another apple bearing experiment (*11*) showed that crop load had no significant effect on the level of flavonoids and chlorogenic acid in cv. Elstar apple skin. This apparent contradiction could be explained by the different profiles and absolute amounts of polyphenols in the cortex and skin and by the variation between cultivars (*22*). Ascorbic acid, an important antioxidant in apple fruit cortex and skin, did not change significantly when crop load decreased (**Table 2**).

In conclusion, our results demonstrate that for cv. Jonagold apple fruit the content of all polyphenol classes investigated, as well as some single polyphenols, strongly depends on the crop load per tree. A lower crop load results in a higher content of polyphenols, known to be effective antioxidants in apple fruit.

At the same time, lightly cropped apple trees bear fruit with a higher marketable value; they have a more attractive appearance with bigger fruit and more blush and better internal properties such as higher cortex firmness and more soluble solids content in fruit juice. Lower crop loads per tree (or per hectare) would help to prevent overproduction of apples while producing a high-quality fruit of improved nutritional value.

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LITERATURE CITED

(1) Hertog, M. G. L.; Hollman, P. C. H.; Katan, M. B.; Kromhout, D. Intake of potentially anticarcinogenic flavonoids and their determinants in adults in The Netherlands. *Nutr. Cancer* **1993**, *20*, 21−29.

(2) Hertog, M. G. L.; Feskens, E. J. M.; Hollman, P. C. H.; Katan, M. B.; Kromhout, D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study. *Lancet* **1993**, *342*, 1007−1011.

(3) Scalbert, A.; Williamson, G. Dietary intake and bioavailability of polyphenols. *J. Nutr*. **2000**, *130*, 2073−2085.

(4) Treutter, D.; Feucht, W. The pattern of flavan-3-ols in relation to scab resistance apple cultivars. *J. Hortic. Sci*. **1990**, *65*, 511−517.

(5) Picinelli, A.; Dapena, E.; Mangas, J. J. Polyphenolic pattern in apple tree leaves in relation to scab resistance. A preliminary study. *J. Agric. Food Chem*. **1995**, *43*, 2273−2278.

(6) Piretti, M. V.; Gallerani, G.; Brodnik, U. Polyphenol polymerisation involvement in apple superficial scald. *Postharvest Biol. Technol.* **1996**, *8*, 11−18.

(7) Lidster, P. D.; Dick, A. J.; DeMarco, A.; McRae, K. B. Application of flavonoid glycosides and phenolic acids to suppress firmness loss in apples. *J. Am. Soc. Hortic. Sci.* **1986**, *111*, 892−896.

(8) Link, H. Significance of flower and fruit thinning on fruit quality. *Plant Growth Regul*. **2000**, *31*, 17−26.

(9) Daugaard, H.; Grausland, J. Fruit colour and correlations with orchard factors and post-harvest characteristics in apple cv. Mutsu. *J. Hortic. Sci. Biotechnol.* **1999**, *74* (3), 283−287.

(10) Palmer, J. W.; Giuliani, R.; Adams, H. M. Effect of crop load and leaf photosynthesis of 'Braeburn'/M26 apple trees. *Tree Phys.* **1997**, *17*, 741−746.

(11) Awad, M. A.; de Jager, A.; Dekker, M.; Jongen, W.; van der Krol, A.; van der Plas, L. Flavonoids and chlorogenic acid levels in apple skin as affected by mineral nutrition and crop load. *XXth International Conference on Polyphenols, Freising-Weihenstephan*; 2000; Vol. 2, pp 657−658.

(12) McRae, K. B.; Lidster, P. D.; De Marco, A. C.; Dick, A. J. Comparison of the polyphenol profiles of the apple fruit cultivar by correspondence analysis. *J. Sci. Food Agric.* **1990**, *50*, 329−342.

(13) Rigo, A.; Vianello, F.; Clementi, G.; Rosetto, M.; Scarpa, M.; Vrhovsek, U.; Mattivi, F. Contribution of the proanthocyanidins to the peroxy-radical scavenging capacity of some Italian red wines. *J. Agric. Food Chem.* **2000**, *48*, 1996−2002.

(14) *Methods of Vitamin Assay*, 2nd ed.; Interscience Publishers: New York, 1951.

(15) Wertheim, S. J. Chemical thinning of deciduous fruit trees. Proceedings of the 8th Symposium on Plant Bioregulators. *Acta Hortic*. **1997**, *463*, 445.

(16) Neilsen, D.; Millard, P.; Neilsen, G. H.; Hogue, E. J. Nitrogen uptake, efficiency of use, and partitioning for growth in young apple trees. *J. Am. Soc. Hortic. Sci.* **2001**, *126*, 144−150.

(17) Magness, J. R.; Overley, L. Relation of leaf area to size quality of apples and pears. *Proc. Am. Soc. Hortic. Sci.* **1929**, *26*, 160−162. In *Temperate-Zone Pomology: Physiology and Culture*; Westwood, M. N., Ed.; Timber Press: Portland, OR, 1993; p 314.

(18) Schechter, I.; Proctor, J. T. A.; Elfving, D. C. Apple fruit removal and limb girdling affect fruit and leaf characteristics. *J. Am. Soc. Hortic. Sci.* **1994**, *119*, 157−162.

(19) Jackson, J. E. Light interception and utilization by orchard systems. In *Horticultural Reviews*; Janick, J., Ed.; AVI Publishing: Westport, CT, 1980; pp 2−6.

(20) Zhiguo, J.; Chenlian, L.; Yongbing, Y.; Yongzhang, W.; Gongschi, L. Color potential, anthocyanin accumulation and enzyme activity in fruit of commercial apple cultivars and their F1 progeny. *Sci. Hortic.* **1999**, *79*, 39−50.

(21) Wünsche, J. N.; Palmer, J. W.; Greer, D. H. Effects of crop load on fruiting and gas-exchange characteristics of 'Braeburn'/M.26 apple trees at full canopy. *J. Am. Soc. Hortic. Sci.* **2000**, *125*, 93−99.

(22) Awad, M. A.; de Jager, A.; van Westing, L. M. Flavonoid and chlorogenic acid levels in apple fruit: characterization of variation. *Sci. Hortic.* **2000**, *83*, 249−263.

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