Fruit Varieties Journal 48(2):109-114 1994

Fruit Quality from Micropropagated Apple (*Malus domestica* Borkh.) Trees vs. Fruit from Trees on Seedling and M 7a Rootstocks

S. R. DRAKE,[1] F. E. LARSEN AND S. S. HIGGINS[2]

Abstract

'Golden Delicious' and 'Spartan' apples from micropropagated trees and scions on seedling and M.7A rootstocks were evaluated for three seasons, at harvest, and after 120 days of storage (1°C) for quality. Ethylene production indicated that apples from the three rootstocks matured at approximately the same rate and could be harvested in the same time period. Apple quality was similar among the three types of rootstocks, and no particular quality advantage was apparent for firmness, soluble solids concentration, titratable acidity, carbohydrates or mineral content. Fruit from micropropagated 'Golden Delicious' trees were more yellow at harvest than fruit from other rootstocks, but this difference did not exist after storage.

Introduction

The commercial use of micropropagated (MP) apple trees has not become a reality for several reasons. Researchers have found that MP apple trees may be poorly anchored (9, 10, 15), have delayed flowering and/or fruit production (5, 9, 16), produce trees of the same size or larger than trees on seedling roots, and may be inferior in yield efficiency than trees on clonal stocks (9, 10, 16). Few if any published reports address fruit maturity and quality as associated with MP apple trees. The influence of clonal and seedling rootstocks on fruit quality and storage, has been demonstrated (1, 2, 3, 4, 6, 8). These studies on fruit quality have indicated that rootstocks can influence size, firmness, color, soluble solids content, carbohydrate and mineral concentration, maturity and ripening, storage quality and respiration rate of the fruit before and during storage. This study was initiated to compare fruit quality of MP trees with fruit from trees on seedling and M.7A rootstocks.

Materials and Methods

Micropropagated 'Golden Delicious' and 'Spartan' apple trees and trees of the same cultivars budded on domestic seedling or M.7A rootstocks were planted in 1984 at the Washington State University, Royal Slope Research Unit, near Othello, Washington. Trees were planted in a north south orientation in a randomized complete block design with four blocks, each block containing a 3-tree replicate of each scion/rootstock combination. Trees were planted in a fine loamy sand in a triple row configuration at a spacing of 3.7 x 3.7 m within rows and a 7.3 m between sets of triple rows. Trees were trained to a central leader and were uniformly pruned. Overhead sprinkler irrigation was used, and trees were fertilized uniformly on a per hectare basis with under tree soil applications of ammonium nitrate or foliar sprays of 20-20-20 containing B (0.02%) and Zn (0.15%) as needed to maintain tree vigor. Grass sod was maintained between rows. Chemical and hand thinning practices were used annually to maintain fruit size and minimize alternate bearing.

For each 3-tree plot, two trees with a uniform crop load from each block were selected for sampling in 1989, 1991 and again in 1992. Sixty fruit of uniform size (per cultivar per rootstock per block) were harvested at estimated commercial maturity. Twenty fruit

[1]Research Horticulturist, USDA/ARS, Tree Fruit Research Laboratory, Wenatchee, WA 98801.
[2]Professor and Agriculture Research Technician, respectively, Dept of Horticulture, Washington State University, Pullman, WA 988164.

were used for each quality evaluation, at harvest (0 days) and again after 120 days in storage (1 C). At harvest, 10 fruit were evaluated immediately for quality and 10 fruit were monitored for evolved ethylene in a flow-through system (7). After storage (120 days), 10 fruit were immediately evaluated for quality and 10 fruit were evaluated after 8 days at 20 C for shelf-life quality. Quality factors evaluated were elemental analysis of fruit flesh, carbohydrates, external and internal color, firmness, soluble solids content (SSC), titratable acidity, starch index, ethylene and visual disorders.

Elemental analysis of plant tissue (exclusive of nitrogen) was determined using a Thermo Jarrell Ash, Smith-Nieftize 8000 Atomic Absorption spectophotometer. Nitrogen was determined with an LECD Model FP-228 nitrogen analyzer. Carbohydrates were determined by the high performance liquid chromatography method described by Bio-Rad (Bio-Rad, Richmond, CA). External color was determined with an Agtron Model E-5W reflectance spectrophotometer. The average values of 10 fruit were reported for external color. Firmness was determined using a Lake City Pressure Tester, Model EP1 (Kelowna, B.C.) equipped with a 1.1 cm probe. Titratable acidity (TA) was determined with a Radiometer titrator, Model TTT85 (Radiometer, Copenhagen). Acids were titrated to 8.2 with 0.1 N NaOH and expressed as percent malic acid. Soluble solids concentration (SSC) was measured with an Abbe-type refractometer, calibrated at 20 C. Starch index was determined on a scale of 1 to 6 (1 = all starch; 6 = no starch).

Analysis of variance (ANOVA) was determined by MSTAT (12) as a factorial experiment with rootstock (three levels), and storage (two levels). Each cultivar was analyzed separately. Years were combined for data analysis. No consistent interactions were evident and thus not addressed. Based on a significant F test, means were separated by Tukey's HSDT.

Results

Maturity at harvest was not affected by rootstock. Starch values for both cultivars were approximately 3.0 to 3.6 on a scale of 1 to 6. Regardless of rootstock, the initiation of ethylene production for 'Golden Delicious' apples was in the same time period, and the fruit from the 3 rootstocks would be considered of like maturity (data not shown). 'Spartan' apples from MP trees initiated ethylene production 5 days earlier than fruit from M.7A rootstocks, but all other maturity indicators suggested a similar maturity for 'Spartan' apples regardless of the rootstock.

Firmness is a major consideration in the evaluation of apple fruit quality. In this study there was little difference in fruit firmness that would be considered of commercial interest. 'Golden Delicious' fruit grown on M.7A rootstocks were firmer (1.8 N) than fruit grown on seedling (Table 1). A firmness difference of 2.2 N or more is generally required for the difference in apple fruit firmness to be of commercial significance (13). There was no firmness difference between 'Golden Delicious' fruit from M.7A and MP, or MP and seedling rootstocks. Firmness of 'Spartan' apple fruit was similar regardless of rootstock (Table 2).

During 120 days of refrigerated storage, apple firmness declined 27% for 'Golden Delicious' fruit (Table 1) and 32% for 'Spartan' apple fruit (Table 2). This decline in firmness during storage would limit the sale of 'Spartan' apples if a minimum firmness of 53.4 N was required as in the case of 'Golden Delicious' apples (14). There was no consistent interaction between rootstock and storage and the fruit from the three rootstocks lost firmness at a similar rate.

Table 1. Fruit quality at harvest and after storage for 'Golden Delicious' apples on three rootstocks.

Treatment	Firmness (N)	Color (E-5W)	Soluble solids (%)	Titratable acidity (% MAE)	Sucrose g/100 ml	Fructose g/100 ml	Glucose g/100 ml	Sorbitol g/100 ml	Total carbo-hydrates g/100 ml	CA ppm	Mg ppm	K %	Mn ppm	Fe ppm	Cu %	N
Rootstock																
MP	66.2 ab[z]	52.2 a	12.8 NS	0.45 b	3.87 NS	6.54 NS	1.93 a	0.33 NS	12.7 NS	192 b	257 a	0.82 a	1.74 NS	7.79 NS	6.83 a	0.30 a
Sdlg	65.1 b	49.8 b	12.7	0.47 a	3.91	6.58	1.73 b	0.33	12.6	220 a	240 b	0.77 b	1.70	8.36	3.94 b	0.27 b
M.7A	66.9 a	49.6 b	12.8	0.48 a	3.97	6.48	1.72	0.32	12.5	214 ab	236 b	0.72 c	1.75	8.43	3.73 b	0.25 b
Storage (days)																
0	76.2 a	45.7 b	12.1 b	0.57 b	3.72 b	6.33 b	1.80 NS	0.30 b	12.2 b	170 b	260 a	0.78 a	1.48 b	7.32 b	3.95 b	0.31 a
120	55.9 b	55.4 a	13.4 a	0.36 a	4.11 a	6.73 a	1.78	0.36 a	12.9 a	248 a	229 b	0.76 b	1.98 a	9.05 a	5.70 a	0.23 b

[z]Means separation within cultivar and among rootstocks or between storage times by Tukey's Honestly Significant Difference Test (P = 0.05).

Table 2. Fruit quality at harvest and after storage for 'Spartan' apples on three rootstocks.

Treatment	Firmness (N)	Color (E-5W)	Soluble solids (%)	Titratable acidity (% MAE)	Sucrose g/100 ml	Fructose g/100 ml	Glucose g/100 ml	Sorbitol g/100 ml	Total carbo-hydrates g/100 ml	CA ppm	Mg ppm	K %	Mn ppm	Fe ppm	Cu %	N
Rootstock																
MP	63.9 NS	82.3 NS	12.5 NS	0.54 a	3.73 NS	6.52 NS	1.46 NS	0.43 NS	12.1 NS	193 a	269 a	0.92 NS	1.44 a	7.51 a	6.36 a	0.28 NS
Sdlg	65.0	83.9	12.5	0.52 ab	3.59	6.48	1.42	0.42	11.9	170 b	261 ab	0.87	1.20 b	6.50 b	4.73 b	0.26
M.7A	63.9	84.2	12.5	0.49 b	3.65	6.55	1.43	0.43	12.0	181 ab	249 b	0.88	1.20 b	7.01 ab	4.85 b	0.28
Storage (days)																
0	76.6 a	82.0 b	11.4 b	0.59 a	3.49 b	6.40 b	1.19 b	0.36 b	11.4 b	159 b	263 NS	0.83 b	1.24 NS	6.83 NS	4.01 b	0.33 a
120	52.0 b	85.0 a	13.6 a	0.45 b	3.82 a	6.63 a	1.68 a	0.49 a	12.6 a	204 a	256	0.95 a	1.32 a	7.18 a	6.61 a	0.21 b

[z]Means separation within cultivar and among rootstocks or between storage times by Tukey's Honestly Significant Difference Test (P = 0.05).

'Golden Delicious' apples from MP trees were more yellow (higher E-5W values) at harvest than apple fruit from either seedling or M.7A rootstock trees (Table 1). This difference in color was very evident visually and apples from micropropagated trees would be considered earlier ripening if color were the only factor used to assess maturity. Color of 'Spartan' apples was not influenced by the rootstock (Table 2). Based on subjective observations, 'Spartan' apples appeared to have more red color when grown on either seedling or M.7A than fruit from

MP trees, but objective, E-5W color measurements detected too much variation to allow ANOVA to discriminate among rootstocks.

Both 'Golden Delicious' and 'Spartan' apples developed additional color during storage. This increase in color for 'Golden Delicious' apples was almost 10 E-5W units and was very distinct visually. Color increase, during storage, for 'Spartan' apples was only 3 E-5W units, but this increase was also apparent visually. There was no difference in color development during storage due to rootstock for either 'Golden Delicious' or 'Spartan' apples.

Rootstocks had no influence on the SSC of either 'Golden Delicious' (Table 1) or 'Spartan' (Table 2) apples. There were differences in titratable acidity of apples due to rootstocks, but these differences were not consistent between the 2 cultivars. 'Golden Delicious' apples from MP trees had the lowest acid content (Table 1), but 'Spartan' apples from MP trees had the highest acid content (Table 2). The reverse was true for acid content of apples grown on M.7A. Acidity of apples grown on seedling rootstocks was intermediate between apples from MP trees and trees on M.7A rootstocks.

Regardless of cultivar, rootstocks had no significant influence on individual carbohydrates except for glucose in 'Golden Delicious' apples (Table 1). MP 'Golden Delicious' apples contained 11% more glucose than apples from either seedling or M.7A rootstocks. MP 'Golden Delicious' apples contained more glucose than fruit from trees on other rootstocks, but they contained similar amounts of sucrose, fructose, sorbitol and total carbohydrates as apples from seedling and M.7A rootstocks. Similar total carbohydrates between the 3 rootstocks agrees with the SSC data. No difference in carbohydrates for 'Spartan' apples due to rootstock was evident (Table 2).

'Golden Delicious' and 'Spartan' displayed increased carbohydrates during storage (Tables 1 and 2). During 120 days of storage sucrose increased 10.5% in 'Golden Delicious' and 9.5% in 'Spartan' apples. The increase in fructose was smaller during storage (6.3 and 3.6%, respectively) but was significant. Glucose did not change during storage for 'Golden Delicious', but increased 41% for 'Spartan.' Sorbitol increased during storage was also significant, with a 20% increase in 'Golden Delicious' and a 36% increase for 'Spartan.' Increases in total carbohydrates were not as large (5.7 and 10.5%), but were significant. Increases in soluble solids concentration for 'Golden Delicious' and 'Spartan' apples were larger but were consistent with the increases in total carbohydrates.

MP 'Golden Delicious' apples contained generally less Ca and more Mg, K, Cu and N than apples from either seedling or M.7A rootstock (Table 1). Mn and Fe content of 'Golden Delicious' apples was not influenced by rootstock. The mineral content of 'Golden Delicious' apples grown on seedling or M.7A was similar, except in the case of K. Apples grown on M.7A rootstock had a lower K content. The N:Ca ratio was highest in MP 'Golden Delicious, but was similar between apples from seedling and M.7A rootstocks.

MP 'Spartan' apples contained more Ca, Mn and Cu than apples from either seedling or M.7A rootstocks and more Ca and Fe than seedling rootstock (Table 2). Although high in Mg and Fe, MP apples had Mg values similar to apples from seedling trees and Fe values similar to apples from trees on M.7A rootstocks. K and N values for 'Spartan' apples were not affected by rootstock. There were no differences in the N:Ca ratio among rootstocks for 'Spartan' apples.

During storage, increases in Ca, Mn, Fe and Cu occurred in both 'Golden Delicious' and 'Spartan' apples. The K and Mg content of 'Golden Delicious' apples decreased slightly. N content of both 'Golden Delicious' and 'Spartan' apples decreased significantly (> 25%). Differences in mineral content during storage were probably related to the natural shift in mineral content from one region of the fruit to another. In this study the mineral content of only the flesh was determined.

Discussion

All of the fruit from the different rootstocks produced detectable ethylene in approximately the same time period. In addition, starch content and soluble solids were similar. There is no single apple maturity indicator, but the onset of detectable ethylene production can be used along with decreased starch, firmness and acids and increased soluble solids (13). The 3 rootstocks used in this study had little influence on ethylene, starch and soluble solids and produced only slight differences in firmness and acids. These results confirm that apples from trees grown on these 3 rootstocks can be harvested at the same time.

Regardless of cultivar, apple quality was comparable among the 3 rootstocks. When all the quality parameters were considered, no particular advantage was present for any rootstock. Quality advantages for apples due to different rootstocks have been reported (1, 2, 3, 6, 8), but in this study little difference was evident except for color. If color were a market consideration, the more yellow MP 'Golden Delicious' apples might be preferred for markets favoring yellow fruit, while the green fruit from seedling or M.7A would be more suitable for markets favoring such fruit. This difference in green color was not due to the N content of the fruit. It has been reported (11) that high N levels enhance greenness of apples. In our study, apples with higher N (MP) had less green color. Considering the reported cultural problems associated with MP trees (9, 10), coupled with no quality advantages, there is little reason to use MP trees for apple production.

References

1. Autio, W. R., Barden, J. A. and Brown, G. R., 1991. Rootstock affects ripening, size, mineral composition and storability of 'Starkspur Supreme Delicious' in the 1980-81 NC-140 Co-operative Planting. Fruit Var. J., 45:247-251.
2. Autio, W. R., 1991. Rootstocks affect ripening and other qualities of 'Delicious' apples. J. Amer. Soc. Hortic. Sci., 116:378-382.
3. Barden, J. A. and Marini, M. E., 1992. Maturity and quality of 'Delicious' apples as influenced by rootstock. J. Amer. Soc. Hortic. Sci., 117:547-550.
4. Brown, G. R. and Wolfe, D., 1992. Rootstock affects maturity of 'Starkspur Supreme Delicious' apples. HortScience 27:76.
5. Cobianchi, D., De Salvador, F. R., Faedi, W., Insero, O., Liverani, A., Rivalta, L., Minguzzi, A., and Marani, M., Preliminary field observations on *in vitro* propagated trees. Acta Hortic., 227:514-516.
6. Drake, S. R., Larsen, F. E. and Higgins, S. S., 1991. Quality and storage of 'Granny Smith' and 'Greenspur' apples on seedling, M.26 and MM.111 rootstocks. J. Amer. Soc. Hortic. Sci., 116:261-264.
7. Drake, S. R., Fellman, J. K. and Nelson, J. W., 1987. Postharvest use of sucrose polyesters for extending the shelf-life of stored 'Golden Delicious' apples. J. Food Sci., 52:1283-1285.
8. Drake, S. R., Larsen, F. E., Fellman, J. K. and Higgins, S. S., 1988. Maturity, storage quality, carbohydrate, and mineral content of 'Goldspur' apples as influenced by rootstock. J. Amer. Soc. Hortic. Sci., 113:949-952.
9. Larsen, F. E. and Higgins, S. S., 1990. Early field performance of several self-rooted, micropropagated apple cultivars vs trees on seedling or M.7A rootstocks. Fruit Var J., 44:185 192.
10. Larsen, F. E. and Higgins, S. S., 1993. Growth and fruit production of young micropropagated apple (*Malus domestica* Borkh.) trees. Scientia Hortic., 53:205-211.
11. Meheriuk, M., 1989. Skin color in 'Newton' apples treated with calcium nitrate, urea, diphenylamine and polymeric film coating. HortScience, 25:775-778.
12. MSTAT. 1988. Version 1.0. Michigan State University, East Lansing, Michigan.
13. Washington Apple Maturity Program Handbook, 1986. Washington Apple Maturity Program, Wenatchee, WA.
14. Washington State Department of Agriculture, 1988. Procedure Related to WAC 16-403-142 Red Delicious, Delicious, Golden Delicious minimum firmness, Olympia, WA.
15. Webster, A. D., Oehl, V. H., Jackson, J. E. and Jones, O. P., 1985. The orchard establishment, growth and precocity of four micropropagated apple scion cultivars. J. Hortic. Sci., 60:169 180.
16. Zimmerman, R. H. and Miller, S. S., 1991. Orchard growth and fruiting of micropropagated apple trees. J. Amer. Soc. Hortic. Sci., 116:780-785.