Fruit Varieties Journal 44(3):136-141 1990

# Characterization of Apple Nectar Sugars in Selected Commercial and Crab Apple Cultivars

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## Abstract

Nectar samples from commercial and crab apple cultivars were examined by high performance thin layer chromatography to determine qualitative and quantitative differences in constituent sugars. Crab apple nectar sugars ranged from 40 to 85% sucrose, with a mean of 60%. Mean glucose and fructose proportions were 18 and 23%, respectively. Most commercial apple cultivars evaluated had similar composition to the crab apple cultivars; however, 'Redchief Delicious,' 'Golden Delicious,' and 'Royal Gala' had nearly equal amounts of sucrose and hexose. Variability in nectar sugar composition among commercial and crab apple cultivars suggests a potential influence on honeybee foraging behavior.

**Additional index words.** *Malus domestica*, *Malus* spp., high performance thin layer chromatography

Flowering crab apples are effective pollinizers for many commercial apple cultivars (4) and are often used as pollinizers in solid blocks of a single cultivar. These plantings increase the efficiency of many orchard operations including pruning, chemical fruit thinning, pest management, and harvesting. Crab apple cultivars are available with a wide range of growth habits, disease resistance, bloom colors, and bloom times. Our preliminary observations indicate differences in honeybee visitation patterns among crab apple cultivars.

Honeybee visitation was influenced by flower color (7, 8), total nectar volume (2, 3), nectar sugar concentration (10), and the ratio of constituent nectar sugars (11, 13). Differences in nectar composition among crab apple cultivars may influence their attractiveness to honeybees, and could affect their suitability as pollinizers. The objective of this study was to characterize the nectar sugar composition of many commercial and crab apple cultivars.

## Materials and Methods

Nectar samples were collected from trees growing on the Virginia Polytechnic Institute and State University Horticulture farm in Blacksburg, VA. Crab cultivars were in a single row of duplicate trees planted in 1982 at 1 m spacing. Commercial apple cultivars were in several blocks ranging in age from 7 to 20 years. All trees were maintained according to standard orchard procedures.

Nectar samples were collected for each cultivar at king or full bloom, which ranged from 10 April to 8 May in 1988 and 1989. Blossom clusters were bagged 1 or 2 days before opening with muslin material to prevent honeybee visitation before sampling. Samples were collected between 1100 and 1400 hrs during periods of warm (> 20C), sunny weather. Three samples per cultivar were collected by inserting a 1 $\mu$l microcapillary pipette into the nectary of a single (when possible) or multiple flowers, and allowing the pipette to fill by capillary action. Samples were expelled into micro-centrifuge tubes containing 100 $\mu$l of 70% (v/v) ethanol, and stored at 0C until analysis.

Received for publication _____.

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Sugars were analyzed by high performance thin layer chromatography. Samples were removed from the freezer and further diluted with 100 $\mu$l of 70% ethanol. One microliter samples were spotted on Merck pre-coated silica gel 60 plates with a Camag Nanomat II (Camag, Muttenz, Switzerland). Standard solutions containing appropriate concentrations of sucrose, glucose, and fructose were included on the plates for the development of standard curves for each sugar. Before spotting, the plates were prewashed with methanol and then pretreated with 0.1 M citrate buffer (pH 4.8) and 0.1 M sodium bisulfate solution. Plates were then placed in an oven at 110C for 1 hr. Plates were stored in a desiccator until use.

After spotting, plates were developed in covered Camag twin trough chambers using a solution of 85% (v/v) acetonitrile:water. The solution was allowed to travel 7 cm up the plate, and the plate was removed and dried for a minimum of 1 min with a hair dryer. Plates were allowed to cool, and the procedure was repeated 2 additional times. Plates were dipped into ceric sulfate/sulfuric acid solution and then heated for 10 min at 110C in a drying oven to char the sugars for visualization. The dipping reagent consisted of one part 0.1 N ceric sulfate in 2 N sulfuric acid (Ricca Chemical Corp., Arlington, Texas) and 10 parts 15% sulfuric acid. Plates were removed, allowed to cool, and sugar concentrations were determined with a Camag TLC scanner II interfaced to an SP-4270 integrator. Sugar concentration data are expressed on the basis of a 200-fold dilution of the original 1 $\mu$l nectar sample. Data were not statistically analyzed because there was no true replication; samples contained nectar from single or multiple flowers that were collected from one or two trees.

## Results

Sucrose/hexose ratios presented in Tables 1 and 2 were calculated by dividing the sucrose concentration by the combined concentration of glucose and fructose (1). These ratios determine nectar type, where $> 0.99$ is sucrose-dominant, 0.5 to 0.99 is sucrose-rich, and 0.1 to 0.49 is hexose-rich. A balanced nectar has equal concentrations of sucrose, glucose and fructose, giving a sucrose/hexose ratio of 0.5. The standard errors of the percentage data are omitted for table clarity; all standard errors were less than $\pm$ 5.

In 1988, all crab apple nectars were sucrose-dominant except 'Simpson 10-35,' which was sucrose-rich (Table 1). In 'Arrow,' 'Golden Hornet,' and 'Henry F. Dupont' nectar, sucrose constituted more than 70% of the total nectar sugar. Most crab apple cultivars had sucrose proportions between 50 and 62% of the total nectar sugar, except for 'Simpson 10-35.' For the crab apple cultivars, sucrose averaged 61%, and glucose and fructose averaged 18 and 21%, respectively. Of the commercial apple cultivars evaluated, sucrose accounted for 50 to 60% of the nectar sugar (similar to crab apples). 'Empire' had the greatest proportion of sucrose (65%). 'Redchief Delicious' (Campbell strain) nectar approached a sucrose/hexose balance, with a ratio of .7, or 43% sucrose. 'Lodi,' 'Granny Smith,' and 'Redchief Delicious' had the most total sugar; 'Empire' had the least.

Total nectar sugar concentrations were greater in 1989 (Table 2), with an average of 3.4 $\mu g \cdot \mu l^{-1}$ compared to 2.5 $\mu g \cdot \mu l^{-1}$ in 1988. However, sucrose/hexose ratios were similar for both years, with all cultivars except 'Royal Gala' being sucrose-dominant or sucrose-rich. 'Red Flesh,' 'Indian Magic,' and 'Golden Delicious' had total sugar levels of about 4 $\mu g \cdot \mu l^{-1}$, and 'Geneva,' 'Red Jacket,' 'Manchurian,' 'Indian Summer,' 'Kobenza,' 'David,' 'Henry F. Dupont,' and 'Eley' had total sugars

**Table 1. Constituent sugar composition[z] of selected commercial and crab apple cultivar nectars in 1988.**

| Cultivar | Sucrose | | Glucose | | Fructose | | Total | Ratio[y] | Type[x] |
|---|---|---|---|---|---|---|---|---|---|
| | $\mu g \cdot \mu l^{-1}$ | % | $\mu g \cdot \mu l^{-1}$ | % | $\mu g \cdot \mu l^{-1}$ | % | $\mu g \cdot \mu l^{-1}$ | | |
| **Crab apple cultivars** | | | | | | | | | |
| Arrow ($R^w$) | 1.82 ± .04[v] | 85 | 0.19 ± .03 | 9 | 0.13 ± .02 | 6 | 2.13 ± .07 | 5.7 | SD |
| Donald Wyman (WP) | 1.22 ± .07 | 50 | 0.57 ± .13 | 23 | 0.65 ± .06 | 27 | 2.44 ± .13 | 1.0 | SD |
| Golden Hornet (WP) | 1.87 ± .17 | 74 | 0.33 ± .05 | 13 | 0.32 ± .03 | 13 | 2.52 ± .09 | 2.9 | SD |
| Henry F. Dupont (R) | 1.48 ± .22 | 75 | 0.24 ± .09 | 12 | 0.26 ± .08 | 13 | 1.98 ± .39 | 3.0 | SD |
| Indian Magic (R) | 1.36 ± .18 | 52 | 0.61 ± .19 | 23 | 0.63 ± .13 | 25 | 2.60 ± .36 | 1.1 | SD |
| Manchurian (W) | 1.35 ± .30 | 50 | 0.52 ± .09 | 20 | 0.81 ± .12 | 30 | 2.68 ± .04 | 1.0 | SD |
| Simpson 10-35 (WP) | 1.03 ± .10 | 40 | 0.71 ± .02 | 28 | 0.82 ± .07 | 32 | 2.56 ± .17 | 0.7 | SR |
| Snowdrift (W) | 1.75 ± .18 | 62 | 0.45 ± .01 | 16 | 0.61 ± .11 | 22 | 2.81 ± .07 | 1.7 | SD |
| Mean | 1.48 | 61 | 0.45 | 18 | 0.53 | 21 | 2.41 | 2.1 | — |
| **Commercial apple cultivars** | | | | | | | | | |
| Empire (W) | 1.06 ± .13 | 65 | 0.28 ± .01 | 17 | 0.30 ± .03 | 18 | 1.64 ± .16 | 1.8 | SD |
| Granny Smith (W) | 1.68 ± .01 | 55 | 0.67 ± .10 | 22 | 0.68 ± .10 | 23 | 3.03 ± .19 | 1.3 | SD |
| Jerseymac (W) | 1.40 ± .23 | 56 | 0.61 ± .07 | 24 | 0.50 ± .07 | 20 | 2.51 ± .37 | 1.2 | SD |
| Lodi (W) | 1.79 ± .20 | 50 | 0.83 ± .19 | 23 | 0.94 ± .06 | 27 | 3.56 ± .46 | 1.0 | SD |
| Redchief Delicious (W) | 1.19 ± .01 | 43 | 0.62 ± .20 | 25 | 1.11 ± .18 | 38 | 2.92 ± .42 | 0.7 | SR |
| Winesap (W) | 1.40 ± .15 | 59 | 0.46 ± .07 | 20 | 0.49 ± .14 | 21 | 2.35 ± .35 | 1.4 | SD |
| Winter Banana (W) | 1.35 ± .28 | 58 | 0.43 ± .07 | 19 | 0.52 ± .10 | 23 | 2.31 ± .45 | 1.4 | SD |
| York (W) | 1.44 ± .41 | 57 | 0.58 ± .15 | 23 | 0.49 ± .08 | 20 | 2.52 ± .48 | 1.3 | SD |
| Mean | 1.41 | 55 | 0.56 | 22 | 0.63 | 23 | 2.61 | 1.3 | — |

[z]Expressed on the basis of a 200-fold dilution of the 1 µl sample.
[y]Sucrose/hexose ratio calculated by dividing the total amount of sucrose by the combined total amount of glucose and fructose.
[x]Nectar type: > 1.0, sucrose dominant (SD); 0.50 to 0.99, sucrose rich (SR).
[w]Flower color: W = white, WP = white/pink, R = red.
[v]Mean ± SE for three observations per cultivar.

less than 3 $\mu g \cdot \mu l^{-1}$. In 'Red Flesh,' 'Arrow,' 'Golden Hornet,' and M. coronaria 'Charlottae,' sucrose constituted more than 70% of the total nectar sugar. 'Snowdrift' and 'Donald Wyman' differed from the previous year with more balanced nectars in 1989. Average sucrose proportion for the crab apple cultivars was 58%, and glucose and fructose proportions were 18, and 25%, respectively. Among the commercial apple cultivars evaluated, all except 'Red Prince Delicious,' and 'Winter Banana' approached a balanced nectar. 'Royal Gala' was the only cultivar in the entire study with a hexose rich nectar.

## Discussion

The crab and commercial apple cultivars evaluated in this study bloomed over a 4-week period in both years, necessitating collection of nectar samples on multiple dates. To minimize variation, samples were collected in the afternoon on days with similar weather conditions. In California, Vansell (10) found a 3-fold increase in plum nectar sugar concentration from 0930 to 1400 hrs associated with the concomitant decrease in atmospheric relative humidity. We did not collect samples on days following periods of cool, cloudy weather because nectar production was minimal. Wykes (14) found that carbohydrate availability influenced both total nectar production and nectar sugar concentration.

The lower total sugar concentrations measured in 1988 may have been due to the cool, cloudy weather prevalent during bloom. However, we feel that warmer temperatures during sample collection in 1989 resulted in alcohol evaporation, and inflated sugar concentrations. Nectar sugar concentra-

**Table 2. Constituent sugar composition[z] of selected commercial and crab apple cultivar nectars in 1989.**

| Cultivar | Sucrose | | Glucose | | Fructose | | Total | Ratio[y] | Type[x] |
|---|---|---|---|---|---|---|---|---|---|
| | $\mu g \cdot \mu l^{-1}$ | % | $\mu g \cdot \mu l^{-1}$ | % | $\mu g \cdot \mu l^{-1}$ | % | $\mu g \cdot \mu l^{-1}$ | | |
| **Crab apple cultivars** | | | | | | | | | |
| Adams (P[w]) | 1.89 ± .03[v] | 52 | 0.74 ± .02 | 21 | 0.97 ± .03 | 27 | 3.61 ± .10 | 1.1 | SD |
| Almey (R) | 2.12 ± .33 | 58 | 0.59 ± .14 | 16 | 0.95 ± .09 | 26 | 3.67 ± .55 | 1.4 | SD |
| Arrow (R) | 2.82 ± .01 | 76 | 0.43 ± .08 | 12 | 0.45 ± .10 | 12 | 3.70 ± .17 | 3.2 | SD |
| M. coronaria Chatlottae (W) | 2.53 ± .04 | 76 | 0.37 ± .07 | 11 | 0.41 ± .07 | 13 | 3.31 ± .11 | 3.2 | SD |
| David (WP) | 1.45 ± .15 | 52 | 0.51 ± .05 | 19 | 0.81 ± .06 | 29 | 2.77 ± .02 | 1.1 | SD |
| Donald Wyman (WP) | 1.56 ± .05 | 43 | 0.88 ± .01 | 24 | 1.19 ± .07 | 33 | 3.63 ± .13 | 0.8 | SR |
| Eley (P) | 1.42 ± .14 | 50 | 0.59 ± .19 | 21 | 0.83 ± .13 | 29 | 2.84 ± .44 | 1.0 | SD |
| Evelyn (R) | 1.86 ± .03 | 59 | 0.49 ± .05 | 15 | 0.82 ± .11 | 26 | 3.17 ± .15 | 1.4 | SD |
| Floribunda Rossa (WP) | 2.17 ± .07 | 60 | 0.61 ± .07 | 18 | 0.80 ± .17 | 22 | 3.58 ± .32 | 1.6 | SD |
| Geneva (R) | 1.20 ± .22 | 58 | 0.39 ± .06 | 19 | 0.48 ± .10 | 23 | 2.06 ± .37 | 1.4 | SD |
| Gibbs Golden Gage (WP) | 1.74 ± .11 | 53 | 0.57 ± .11 | 18 | 0.97 ± .07 | 29 | 3.28 ± .08 | 1.1 | SD |
| Golden Hornet (WP) | 2.51 ± .37 | 75 | 0.35 ± .06 | 10 | 0.50 ± .01 | 15 | 3.36 ± .43 | 3.0 | SD |
| Henry F. Dupont (R) | 1.93 ± .40 | 66 | 0.33 ± .11 | 11 | 0.64 ± .10 | 23 | 2.90 ± .62 | 2.0 | SD |
| Indian Magic (R) | 2.20 ± .10 | 54 | 0.76 ± .01 | 19 | 1.10 ± .03 | 27 | 4.02 ± .01 | 1.9 | SD |
| Indian Summer (P) | 1.43 ± .09 | 52 | 0.52 ± .14 | 19 | 0.78 ± .03 | 29 | 2.73 ± .25 | 1.1 | SD |
| John Downing (W) | 1.71 ± .40 | 47 | 0.89 ± .21 | 24 | 1.07 ± .12 | 29 | 3.66 ± .61 | 0.9 | SR |
| Kelsey (P) | 1.56 ± .08 | 46 | 0.73 ± .05 | 22 | 1.06 ± .04 | 32 | 3.34 ± .17 | 0.9 | SR |
| Kobenza (P) | 1.51 ± .29 | 54 | 0.49 ± .09 | 17 | 0.82 ± .16 | 29 | 2.82 ± .54 | 1.2 | SD |
| Manchurian (W) | 1.55 ± .28 | 52 | 0.64 ± .01 | 22 | 0.78 ± .11 | 26 | 2.97 ± .38 | 1.1 | SD |
| Pioneer Scarlet (R) | 2.59 ± .08 | 66 | 0.58 ± .06 | 15 | 0.75 ± .14 | 19 | 3.92 ± .13 | 2.0 | SD |
| Red Jacket (R) | 1.74 ± .05 | 62 | 0.37 ± .12 | 14 | 0.67 ± .08 | 24 | 2.78 ± .15 | 1.7 | SD |
| Red Flesh (WP) | 3.19 ± .19 | 78 | 0.42 ± .07 | 10 | 0.46 ± .06 | 12 | 4.06 ± .06 | 3.7 | SD |
| Rosedale (P) | 1.55 ± .02 | 47 | 0.77 ± .08 | 23 | 1.01 ± .18 | 30 | 3.32 ± .23 | 0.9 | SR |
| Snowdrift (W) | 1.65 ± .25 | 45 | 0.77 ± .16 | 21 | 1.18 ± .08 | 34 | 3.60 ± .48 | 0.9 | SR |
| Turesi (WP) | 2.00 ± .11 | 59 | 0.55 ± .09 | 17 | 0.83 ± .10 | 24 | 3.38 ± .28 | 1.5 | SD |
| Whitney (W) | 1.62 ± .24 | 53 | 0.62 ± .18 | 21 | 0.79 ± .18 | 26 | 3.03 ± .60 | 1.2 | SD |
| Mean | 1.90 | 58 | 0.58 | 18 | 0.81 | 25 | 3.29 | 1.6 | — |
| **Commercial apple cultivars** | | | | | | | | | |
| Golden Delicious (W) | 1.53 ± .23 | 38 | 0.93 ± .01 | 24 | 1.50 ± .16 | 38 | 4.00 ± .40 | 0.6 | SR |
| Redchief Delicious (W) | 1.45 ± .05 | 38 | 0.88 ± .07 | 23 | 1.48 ± .15 | 39 | 3.82 ± .04 | 0.6 | SR |
| Red Prince Delicious (W) | 1.86 ± .03 | 50 | 0.67 ± .01 | 18 | 1.20 ± .03 | 32 | 3.73 ± .07 | 1.0 | SD |
| Royal Gala (W) | 0.96 ± .18 | 30 | 0.83 ± .08 | 26 | 1.42 ± .02 | 44 | 3.22 ± .20 | 0.4 | HR |
| Winter Banana (W) | 1.86 ± .53 | 53 | 0.68 ± .26 | 19 | 0.95 ± .29 | 28 | 3.48 ± .90 | 1.2 | SD |
| Mean | 1.53 | 42 | 0.80 | 22 | 1.31 | 36 | 3.65 | 0.8 | — |

[z]Expressed on the basis of a 200-fold dilution of the 1 µl sample.
[y]Sucrose/hexose ratio calculated by dividing the total amount of sucrose by the combined total amount of glucose and fructose.
[x]Nectar type: > 1.0, sucrose dominant (SD); 0.50 to 0.99, sucrose rich (SR).
[w]Flower color: W = white, WP = white/pink, R = red.
[v]Mean ± SE of the mean for three observations per cultivar.

tions of 4 $\mu g \cdot \mu l^{-1}$ would indicate an 80% nectar sugar solution (correcting for the 200-fold dilution), which is above the saturation level of sucrose in water. Previous research has shown 55% nectar sugar to be a reasonable upper limit for apple nectar (10). However, sample concentrations due to alcohol evaporation should not influence the relative proportions of the constituent sugars.

Sucrose, glucose, and fructose were identified in all cultivars evaluated, supporting previous findings for apple nectar (9, 12). There was no evidence of maltose or other "rare" sugars which have been reported in nectars from other plant species (5, 12). In general, the sucrose/hexose ratios (nectar types) were consistent between years for each cultivar. Baker and Baker (1) found the sucrose/hexose ratio to be consistent over a range of environmental conditions.

Honeybees have shown a strong fidelity for foraging on white or non-white flowers (8), and the interchange between commercial and crab apple cultivars was greatest when flower color was similar (6). Flower color ranged from pure white to pink or red among the cultivars evaluated in our study, and flower color was independent of nectar composition. Also, there was no relationship between bloom time and nectar composition, with a wide range of sucrose/hexose ratios occurring in both early- and late-blooming cultivars.

Honeybee preferences differed among apple cultivars with similar flower color (2), suggesting an influence of nectar composition on honeybee foraging behavior. Generally, honeybees prefer greater nectar volume, and high sugar content, but Wykes (13) found honeybee preference was also influenced by the specific constituent sugars of artificial nectar solutions, and their concentrations. In single sugar solutions, sucrose was more attractive to foraging honeybees than glucose or fructose, but a solution of equal parts sucrose, glucose, and fructose was most attractive. In contrast, Waller (11) found that a single sugar solution of 30-50% sucrose was most attractive to foraging honeybees, and differential preferences for nectar sugar constituents were most prevalent with fewer numbers of foraging honeybees. Mayer et al. (8) suggested that constituent nectar sugars of crab apples had no effect on honeybee foraging behavior, but their data analysis did not allow for comparisons between honeybee foraging and constituent sugar concentrations.

Most of the commercial cultivars evaluated had sucrose-dominant nectars similar to the crab apple cultivars, but 'Golden Delicious,' 'Redchief Delicious,' and particularly 'Royal Gala' had nearly balanced nectars. These findings suggest that the attractiveness of these cultivars to honeybees may be quite different from the other commercial and crab apple cultivars in this study. The difference in sucrose/hexose ratio found between 'Redchief Delicious' and 'Red Prince Delicious' suggests that significant variation may occur in the nectar constituents between strains of apple cultivars. Further research is needed to determine the influence of constituent sugar concentrations on honeybee foraging behavior.

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**Fruit Varieties Journal 44(3):141-148 1990**

# Multiplication of *Rubus* Germplasm *In Vitro*: A Screen of 256 Accessions

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## Abstract

*Rubus* germplasm at the National Clonal Germplasm Repository, Corvallis, Oregon, was screened to determine tissue culture growth media suitable to the diverse collection being maintained. Explants were taken from mature pot-grown plants. Murashige and Skoog (MS) medium with 1 mg/l benzyladenine (BA) and 0.1 mg/l indole butyric acid (IBA) was used for initiation (explant establishment) of 256 different accessions of *Rubus*. Evaluation was based on a three fold (3X) increase in the number of plantlets following three weeks on the medium. Following two initial transfers, those which did not multiply 3X in three weeks were divided into two groups based on the vigor of the clone. Group 1 (low multiplication but growing well) were placed on MS with 1 mg/l BA, 0.1 mg/l IBA and 0.1 mg/l $GA_3$ while Group 2 (low multiplication and poor growth) were grown on Anderson's medium with 1 mg/l BA and 0.1 mg/l IBA. Accessions which did not respond with 3X growth after two transfers of three weeks each were placed on media with additional modifications. The majority (62%) will multiply 3X in three weeks on MS medium with 1 mg/l BA, 0.1 mg/l IBA and 0.1 mg/l $GA_3$ while another 18% respond on Anderson's medium at the same hormone levels. The remaining 20% require BA at 2 mg/l, 0.1/mg/l IBA and $GA_3$ 0.1 mg/l to produce 3X growth in three weeks. This is the first report of the *in vitro* culture of many of these *Rubus* species and cultivars. Chemical names used: N(-phenylmethyl)-1H-purin-6-amine (BA); Indole-3-butyic acid (IBA); Gibberellic Acid $A_3$ ($GA_3$).

## Introduction

*In vitro* propagation in individual cultivars of raspberry and blackberry and optimal basal media and hormone concentrations are well documented (1, 2, 3, 4, 7). Most studies have developed media formulations for one to five cultivars. The development of

[1]USDA/ARS National Clonal Germplasm Repository, 33447 Peoria Road, Corvallis, OR 97333. The author acknowledges the assistance of Trudy McFarlane in collecting and Margaret Norton in culturing and storing the explants.