Relative Humidity around Apple Fruit Influences Its Accumulation of Calcium

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Abstract. Individual fruit of ‘Delicious’ apple (Malus domestica Borkh.) were exposed to low, high, or ambient relative humidity (RH) levels during different stages of fruit development to study the importance of transpiration and the xylem system in supplying Ca to fruit. The Ca content of fruit exposed to low RH was the same or higher than that of fruit exposed to high RH. Treatments imposed early or late in the season usually affected fruit Ca levels similarly. Fruit weight was not consistently affected by RH treatments. The xylem may be a significant source of fruit Ca throughout the season.

Some disorders of apple fruit, such as bitter pit, are closely associated with low Ca concentrations in the fruit cortex (Faust et al., 1968). Calcium-related disorders result primarily from inadequate allocation of Ca to the fruit rather than limited root uptake (Himelrick and McDuffie, 1983).

The relative importance of the xylem and phloem systems in transporting Ca to fruit is not fully understood. Wiersum (1966) suggested that the xylem is the primary route of Ca supply early in the season, whereas the phloem predominates later. Early in the season Ca is distributed principally to organs such as leaves and young fruit, which transpire rapidly. Fruit accumulate Ca most rapidly early in the season (Wilkinson, 1986), likely because the young fruit have a high surface area : volume ratio and permeability to water (Blanke and Lenz, 1989). Water supply to young fruit likely is provided by the xylem, in which Ca moves comparatively freely (Wiersum, 1966).

During fruit growth, the surface area : volume ratio decreases; the fruit cuticle becomes more lipophilic (Blanke and Lenz, 1989; Ferguson and Watkins, 1989), stomata are less dense and functional (Blanke and Lenz, 1989), and the ratio of leaf : fruit number and surface area increases. These changes reduce fruit transpiration (Blanke and Lenz, 1985; Jones et al., 1983) and the movement of xylem water and Ca into the fruit. The net rate of Ca uptake decreases through the season, while the supply of phloem-mobile nutrients (K, Mg, P, N) and photosynthetic increase or remain the same (Tromp, 1975).

How much Ca might be supplied to fruit via the phloem is unclear, since Ca concentrations in the phloem of apple trees have not been reported. The phloem sap of other plant species generally contains low Ca levels (Jones et al., 1983). Although the limited remobilization of Ca from apple leaves (Himelrick and McDuffie, 1983) suggests that phloem transport is limited, indirect evidence indicates that phloem transport may occur under some conditions (Stebbins and Dewey, 1972; Faust and Shear, 1973). Since fruit typically accumulate Ca slowly later in the season (Jones et al., 1983; Tromp, 1975; Tromp and Oele, 1972; Wilkinson, 1968), the phloem could account for the limited late-season accumulation of Ca.

The importance of the xylem system in supplying Ca to tomato (Lycopersicon esculentum Mill.) fruit has been demonstrated by modifying RH levels to alter fruit transpiration rates (Ehret and Ho, 1986; Wiersum, 1966). Entire apple trees exposed to low RH environments accumulated more Ca in vegetative tissues than trees in high RH (Tromp, 1979; Tromp and Oele, 1972), but fruit Ca levels were not clearly affected (Tromp and Oele, 1972). Apple fruit covered with plastic bags on the tree had a greater incidence of bitter pit after storage (Ford, 1979), but fruit Ca concentrations were not changed (Ford, 1979; Wiersum, 1966).

If the xylem supplies the majority of Ca to apple fruit, changing fruit transpiration rates would likely alter fruit Ca levels since xylem flow is controlled partly by transpiration. If the fruit were supplied with Ca predominantly from the phloem, changing fruit transpiration rates would have little effect on fruit Ca content. The objective of this study was to determine the importance of the xylem is supplying Ca to fruit during various stages of development.

Materials and Methods

A 32-year-old planting of ‘Starking Red Delicious’/M.27 in East Lansing, Mich., was used for this study. Trees received standard pruning, fertilization, herbicide, and pest control practices, without irrigation or Ca sprays. RH treatments were imposed around individual fruit for 3 to 6 weeks during fruit development (Tables 1-3).

During 1988 four treatments were replicated 15 times on single, uniformly sized fruit selected randomly among two adjacent trees: 1) untreated control, 2) control, 3) high RH, and 4) low RH. High and low RH treatments were imposed by enclosing fruit in 5.1 × 10^3-mm-thick, 1-liter, low-density polyethylene bags (Zip-Lock, Dow Chemical Co., Midland, Mich.) with or without a CaCl desiccant. Bags were secured around fruit with the help of a plastic container with a 12.5-cm-diameter screw-top lid. An 8-mm hole was made in the center of each lid and a slit was cut between the hole and the lid edge. The pedicel of the fruit ran through the hole and was surrounded by polyethylene foam to cushion it from the lid. The bottom had been cut from the container, leaving a 3- to 5-cm-long threaded cylinder. A bag containing one apple was placed inside each cylinder and the bag top was folded over the lip. The cylinder was screwed to the lid to provide an enclosed chamber. The apparatus was secured to the nearest stable branch using fibrous, weather-resistant tape. A rubber septum (10.5 mm in diameter, 25 mm).

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height) was placed in the top of the lid for gas sampling (using a syringe) or for insertion of a humidity/temperature sensor into the sealed plastic chamber. Fruit not enclosed in bags but fitted with lids served as the control, while normally exposed fruit was designated as the untreated control.

The low RH treatment was imposed by placing 50 g of CaCl₂ desiccant in the bags. The desiccant was sealed in spunbound olefin (Tyvek) (Dupont Chemical Co., Flint, Mich.) packages (Shirazi and Cameron, 1990), which prevented fruit contact while allowing moisture adsorption by the desiccant. High RH was imposed by enclosing fruit in the bags without desiccant.

During 1989 two additional treatments were imposed to alter fruit transpiration. Entire fruit were dipped in either melted paraffin wax or antitranspirant [10% (v/v), Wilt-Pruf; Wilt-Pruf Products, Greenwich, Conn.). In 1989 there were 30 single-fruit replicates. In 1990 treatments were the same as applied in 1988, but with 21 replicates.

At the end of each treatment period, individual fruit, minus pedicels, were removed and weighed. Fruit weight was estimated at the initiation of periods in 1989 so that the treatment effect on weight gain could be calculated. First, we measured fruit diameter (d) and length (l), using calipers, at the initiation and termination of each treatment period to estimate the surface area of the detached fruit {fruit surface area = [d × (½d)² + (½l)²]} (Long, 1980). Then, surface area was used to estimate the weight of the fruit when attached to the tree, by means of an equation that correlated fruit weight taken at the termination of each treatment period with fruit surface area.

RH and temperature were monitored in the low- and high-RH treatment bags, and in the external environment during the last treatment period in 1988 and 1989. Analog temperature/humidity sensors (model 850-242; General Eastern, Boston) connected to a Polyorder data logger (model 516C-64; Omni Data International, Logan, Utah) provided unattended continuous recording of the conditions for 3 days. Carbon dioxide and O₂ concentrations from each of five high-RH, low-RH, and control (atmospheric levels) fruit were monitored once each treatment period by analyzing 1 cm³ of air with a standard infrared gas analyzer.

Individual fruit were homogenized in a food processor. Tissue moisture content was determined by weight loss following oven-drying at 65°C (1988) or freeze drying (1989 and 1990). Tissue samples were ashed in a muffle furnace at 550°C for 6 h, dissolved in 10% (v/v) nitric acid, and filtered through low-ash Whatman #41 paper. A portion was prepared in 1000 µg lanthanum/ml and 2% (v/v) nitric acid and analyzed for Ca by atomic absorption spectrophotometry.

Results

Fruit exposed to low RH (bagged with CaCl₂ desiccant) had higher Ca concentrations (micrograms per gram of fresh weight) than untreated fruit during period 1, 1988 (Table 1); periods 3 and 4, 1989 (Table 2); and period 1, 1990 (Table 3). Low-RH fruit also contained higher Ca concentrations than high-RH fruit (bagged without desiccant) during periods 3 and 4 in 1989 (Table 2) and period 2 in 1990 (Table 3), but lower Ca levels during period 2, 1989 (Table 2). Fruit treated with antitranspirant contained higher Ca concentrations than untreated fruit only during period 4, 1989 (Table 2). Treating fruit with wax did not change Ca concentrations relative to untreated fruit (Table 2).

The Ca content (milligrams per fruit) of low-RH fruit was 44%, 37%, and 24% higher than that of high-RH fruit during periods 1, 3, and 4, respectively, in 1989 (Table 2), and 27% and 15% higher in periods 2 and 3, respectively, in 1990 (Table 3). The Ca content of low-RH fruit was higher than that of untreated fruit during period 4, 1990 (Table 3), but similar during other periods. The Ca content of waxed fruit was significantly less than that of untreated fruit during periods 2 and 3, and lower than control fruit in periods 1 and 2 in 1989 (Table 2). Antitranspirants had little influence on fruit Ca content (Table 2).

Treatments had inconsistent effects on fruit weight and estimated fruit growth (measured in 1989 only). Final weight of control fruit (lid apparatus only) was different from that of untreated fruit only in period 3 of 1989 (Table 2). The final weight of low-RH fruit was less than that of high-RH fruit in period 1, 1988 (Table 1), and period 1, 1990 (Table 3). The estimated growth of low-RH fruit was less than that of high-RH fruit during period 3, 1989, and less than control fruit during periods 3 and 4 (Table 2). Paraffin wax often reduced fruit weight (periods 1, 2, and 3, 1989) and estimated fruit growth (periods 3 and 4, 1989) relative to untreated fruit (Table 2). Antitranspirant did not affect final fruit weight relative to untreated fruit, but reduced fruit growth relative to untreated fruit during period 4 in 1989 (Table 2). Low RH reduced the fruit moisture content relative to high RH only during periods 3 and 4, 1989 (Table 2), and period 4, 1990 (Table 3).

Temperature and RH levels for high-RH, low-RH, and control fruit over 3 days in 1988 (Fig. 1) are representative of measurements taken on several other dates. High-RH treatments ranged from 75% RH at midday to 100% at night; low RH ranged from 20% to 40%, while the RH surrounding untreated fruit (ambient levels in the tree canopy) remained between the high- and low-RH treatments. Air temperatures surrounding the bagged fruit (Fig. 1) were similar to ambient temperatures (data not given) during each measurement period. Carbon dioxide and O₂ levels within the bagged treatments were not significantly different from ambient levels (data not presented).

Discussion

Fruit Ca concentration and content were often reduced by high RH, indicating that Ca accumulation in apples is influenced
by fruit transpiration throughout development. High RH had similar effects on Ca in tomato (Banuelos et al., 1987; Ehret and Ho, 1986), paprika (Capsicum annuum L.), and bean (Phaseolus vulgaris L.) fruit (Mix and Marschner, 1976a, 1976b).

The RH treatments likely affected fruit Ca levels by altering the xylem supply rather than phloem transport, since consistent reductions in fruit weight would be expected to accompany reduced phloem transport. The high- and low-RH treatments resulted in similar fruit weights in all but three instances (period 1, 1988; period 3, 1989; period 1, 1990). High-RH fruit during these periods accumulated more weight than low-RH fruit (Tables 1-3).

The plastic bags and lids used to impose high- and low-RH treatments around fruit were adequately sealed to maintain different RH levels. However, CO₂ and O₂ levels in the bags were similar to ambient conditions, suggesting that some gas exchange occurred through small openings around the pedicels. The plastic lid did not appear to constrict or abrade pedicels, since the weight and Ca content of control fruit (treated with lid apparatus only) were generally similar to untreated fruit.

Some responses were inconsistent. It is unclear why treatments had little effect on fruit Ca levels in 1988. The 1988 season was characterized by higher temperatures and less precipitation than 1989 or 1990. These trees were not irrigated and may have been under moisture stress in 1988. How this stress might have influenced treatment effects specifically is not clear. Although fewer replications were used in 1988 (15) than 1989 (30) or 1990 (21), the variability of fruit Ca levels did not appear to limit our ability to detect treatment differences in 1988 since coefficients of variation in 1988 were similar to those in 1989.

During period 2 in 1989, it is unclear why fruit Ca content was higher in the high-RH treatments than controls since this contradicted the effect observed during other treatment periods. Waxing fruit generally reduced fruit Ca levels and increased

| Table 2. Effect of RH, antitranspirant, and wax treatments on ‘Delicious’ apple fruit weight, fruit growth, moisture content, and Ca levels, 1989.² |
|---------------------------------------------------------------|
| **Treatment** | **μg·g⁻¹** | **Final fruit wt (g)** | **Estimated fruit growth (g)** | **Moisture (%)** |
|----------------|-------------|------------------------|-------------------------------|-----------------|
| **Period 1: 21–48 DAFB (19.5C EPAN 0.47 cm)** | | | | |
| Untreated* | 202 | 4.1 ab | 20.4 ab | 15.0 | 86.4 |
| Control° | 233 | 4.8 a | 21.6 a | 16.2 | 87.6 |
| Low RH | 229 | 4.9 a | 21.7 a | 16.4 | 86.3 |
| High RH | 156 | 3.4 b | 22.1 a | 16.5 | 87.0 |
| Antitranspirant* | 169 | 3.2 b | 18.8 bc | 13.6 | 87.0 |
| Paraffin wax | 200 | 3.3 b | 17.0 c | 11.9 | 87.0 |
| **Period 2: 50–84 DAFB (22.2C EPAN 0.54 cm)** | | | | |
| Untreated | 110 b | 8.7 ab | 78.7 a | 47.4 | 85.9 ab |
| Control | 106 b | 7.8 b | 73.7 a | 44.3 | 85.4 b |
| Low RH | 108 b | 8.3 ab | 75.9 a | 46.0 | 85.5 b |
| High RH | 132 a | 9.7 a | 73.5 a | 43.0 | 85.2 b |
| Antitranspirant | 110 b | 8.1 b | 75.0 a | 45.8 | 85.5 b |
| Paraffin wax | 101 b | 6.0 c | 58.3 b | 28.0 | 86.6 a |
| **Period 3: 86–115 DAFB (19.8C EPAN 0.47 cm)** | | | | |
| Untreated | 82 b | 10.7 ab | 129 bc | 50.1 ab | 83.9 b |
| Control | 67 c | 9.4 bc | 140 a | 51.1 a | 86.5 a |
| Low RH | 96 a | 12.3 a | 128 bc | 43.7 bc | 83.0 c |
| High RH | 66 c | 9.0 bc | 136 ab | 51.8 a | 86.6 a |
| Antitranspirant | 69 bc | 8.7 c | 125 cd | 47.8 ab | 86.2 a |
| Paraffin wax | 73 bc | 8.6 c | 117 d | 38.1 c | 86.6 a |
| **Period 4: 115–145 DAFB (10.9C EPAN 0.29 cm)** | | | | |
| Untreated | 61 bc | 9.7 ab | 157 | 28.2 a | 85.3 cd |
| Control | 70 a | 10.8 a | 152 | 24.2 ab | 85.8 bc |
| Low RH | 73 a | 10.7 a | 148 | 16.0 c | 85.2 d |
| High RH | 56 c | 8.6 b | 152 | 22.0 abc | 85.8 ab |
| Antitranspirant | 70 a | 10.9 a | 154 | 19.4 bc | 85.5 bcd |
| Paraffin wax | 66 ab | 9.5 ab | 143 | 17.3 bc | 86.3 a |

*Mean separation within columns by LSD; numbers followed by similar letters not significantly different at P = 0.05.
°DAFB = days after full bloom. Full bloom 18 May. Average daily temperature and pan evaporation (EPAN).
*Untreated fruit.
**Fruit treated with lid apparatus only.
¹°%, %w/w) ‘Wilt-Pruf’ antitranspirant.
levels (Table 2), although antitranspirants applied to whole trees have reduced the incidence of bitter pit (Schumacher et al., 1976). Repeated applications might have been effective since a single application may not have persisted on the fruit to the end of the period of observation.

Since fruit transpiration rates decrease as the season progresses (Blanke and Lenz, 1985), and fruit often accumulate little additional Ca late in the season, it is interesting that RH still influenced CA accumulation toward the end of the season. RH treatments affected the water and CA content of the fruit during period 4 of 1989 just before harvest even though fruit Ca contents were similar at the end of periods 3 and 4 and fruit appeared to accumulate little additional Ca during this time.

A possible explanation for the late-season influence of RH on fruit Ca involves Ca export from fruit. The decline in Ca content of apple fruit observed late in the season (Tromp and Oele, 1972; Wilkinson, 1968) may result from a reversal of the xylem flow. Apple fruit contract and expand diurnally during periods of high evaporative demand (Tukey, 1964), indicating a net loss of water from fruit during part of the day. Although Jones and Higgs (1982) argued that transpiration from the fruit surface could account for these losses, studies by Lang (1990) indicate that water flows out of apple fruit into the tree in response to water potential gradients, and that similar flows occur in grape (Vitis vinifera L.) vines (Lang and Thorpe, 1989). This reverse flow could result in a net loss of fruit Ca if concentrations in the export stream are high. During several periods, the moisture content of fruit exposed to high RH was higher than that of low-RH fruit (Tables 1–3); this difference may have promoted the movement of water and Ca out of the fruit during periods when evaporative demand on the rest of the tree was high.

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Table 3. Effect of RH treatments on ‘Delicious’ apple fruit weight, moisture content, and Ca levels, 1990.*

| Treatment       | μg/g-fresh wt | mg/fruit | Final fruit wt (g) | Moisture (%) |
|-----------------|---------------|----------|--------------------|--------------|
| Period 1: 31–55 DAFB (20.4°C EPAN 0.66 cm)* |               |          |                    |              |
| Untreated       | 154 b         | 3.2      | 21.0 ± 0.0         | 88.9         |
| Control         | 183 ab        | 3.7      | 20.0 ± 0.0         | 88.4         |
| Low RH          | 210 a         | 3.6      | 17.9 ± 0.0         | 87.9         |
| High RH         | 181 ab        | 3.8      | 20.8 ± 0.0         | 86.9         |
| Period 2: 58–85 DAFB (20.4°C EPAN 0.49 cm) |               |          |                    |              |
| Untreated       | 76 ab         | 5.2 ab   | 68.1 ± 0.0         | 88.9         |
| Control         | 77 ab         | 5.5 ab   | 70.9 ± 0.0         | 88.2         |
| Low RH          | 88 a          | 6.1 a    | 69.3 ± 0.0         | 87.9         |
| High RH         | 69 b          | 4.8 b    | 70.1 ± 0.0         | 88.4         |
| Period 3: 88–111 DAFB (18.7°C EPAN 0.37 cm) |               |          |                    |              |
| Untreated       | 65            | 6.8 ab   | 104 ± 0.0          | 88.9         |
| Control         | 58            | 6.2 b    | 107 ± 0.0          | 89.2         |
| Low RH          | 65            | 7.0 a    | 109 ± 0.0          | 89.2         |
| High RH         | 56            | 6.1 b    | 108 ± 0.0          | 88.8         |
| Period 4: 114–144 DAFB (17.2°C EPAN: 0.39 cm) |               |          |                    |              |
| Untreated       | 52            | 7.6 a    | 144 ± 0.0          | 88.1 ab      |
| Control         | 61            | 8.8 ab   | 145 ± 0.0          | 87.3 a       |
| Low RH          | 63            | 9.3 b    | 146 ± 0.0          | 87.7 ab      |
| High RH         | 60            | 8.7 ab   | 144 ± 0.0          | 88.7 b       |

* Mean separation within columns by LSD, P = 0.05.

**DABF = days after full bloom. Full bloom 10 May. Average daily temperature and pan evaporation (EPAN).

* Untreated fruit.

* Fruit treated with lid apparatus only.

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Fig. 1. Temperature and RH surrounding low-RH-treated fruit (bagged with CaCl₂ desiccant), high-RH-treated fruit (bagged without desiccant), and untreated control (ambient RH) in 1989. Temperature is the mean of all three treatment temperatures. RH levels are a mean of two replications.
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