Ontogenetic Changes in Calcium Concentration and Content in Pickling Cucumber Fruit as Influenced by Genotype and Environment

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Abstract. A field experiment was conducted to quantify the ontogenetic changes in Ca concentration and content of pickling cucumber fruits as influenced by environmental conditions and genotype. Pericarp tissue Ca concentrations (1.1% to 0.7% of dry weight) were higher but declined less rapidly during fruit development than endocarp concentrations (0.8% to 0.2% of dry weight). About 90% of net fruit Ca content accumulated within the pericarp of ≈ 150 g fresh weight fruit, the rest in the endocarp. The rate of Ca accumulation was highly variable during fruit ontogeny but was closely correlated with growth rate (grams fresh weight/day). Environmental conditions had the largest effect on Ca accumulation rate. Fruit tissue Ca concentrations were affected both by genotype and the cultural environment, especially at the later stages of fruit development. Calcium concentrations (1.5 to $\approx 3.0\%$ of dry weight) in fully expanded leaf tissue were higher than in rapidly growing fruit tissues on the same plants.

Calcium nutrition is considered important for high textural quality in a variety of fruit crop species. Rates of fruit ripening (Bangerth, 1979; Ferguson, 1984; Mallick et al., 1984; Sams and Conway, 1984), fruit respiration (Bangerth, 1979; Ferguson, 1984), and the loss of tissue firmness during postharvest handling or processing (Bangerth, 1979; Bramlage et al., 1979; Sams and Conway, 1984) are affected by fruit Ca status. In pickling cucumbers, loss of fruit tissue firmness during brining or following fresh-pack processing results in quality reductions in the processed product.

Altered fruit tissue Ca levels in pickling cucumbers appear to influence fruit growth and quality, including seed viability (Frost and Kretschman, 1989). Adhikari (1980) reported a correlation between misshapen fruit and fruit Ca concentration when chlorflurenol was applied to induce parthenocarpic fruit set. Water-soaked and necrotic lesions were observed in the epidermal and pericarp tissue at the distal end of fruit cultured under low Ca levels (Frost and Kretschman, 1989). Staub et al. (1988) produced the "pillowy" disorder by culturing cucumbers in a Ca-deficient nutrient solution. McFeeters noted that endogenous Ca concentrations in fresh cucumber fruit tissue were highly correlated with the rate of pericarp softening in brine following blanching (R.F. McFeeters, personal communication).

The import and accumulation of Ca into developing fleshy cucumber fruit have not been characterized nor its regulation investigated adequately. The objectives of this research were to 1) determine the ontogenetic changes in Ca concentration and content within pickling cucumber fruit and 2) evaluate the effects of environment and genotype on fruit Ca status as related to fruit growth.

Materials and Methods

Pickling cucumbers were cultured at the Horticulture Research Center in East Lansing, Mich., in 1984. The field was a Brookston loam (fine-loamy, mixed, mesic, typic Argiaquolls) soil with a pH of 5.7 and a Ca content of ≈ 1400 kg-ha⁻¹ per 17 cm depth. Seeds were directly sown on three-row flat beds

with 2.13-m centers. Plant spacing was 10 cm within and 71 cm between rows. Standard cultural practices were followed.

Four plantings were made at 2-week intervals, 7 and 20 June, 5 and 19 July, to assess environmental influence on Ca nutrition. Variability in genetic response was evaluated by planting four cultivars: 'Castlepick 2012' (A.L. Castle, Morgan Hill, Calif.), 'PSX 20580' (Petoseed Co., Saticoy, Calif.), 'Regal' (Harris Moran Seed Co., Salinas, Calif.), and 'Tamor' (Asgrow Seed Co., Kalamazoo, Mich.). A split-plot experimental design was used with planting dates as main plots, cultivars as subplots, and with four replications.

At anthesis of test fruits, previously set fruit were detached. During the following 2 weeks, fruits were harvested at ≈ 2 -day intervals from the center row of each subplot. The sample size per subplot was 15 fruits in the first harvest and eight fruits in subsequent harvests. Fruits were similar in maturity at each harvest from each subplot.

Fruit growth was evaluated by measuring fruit length, diameter, and weight at each harvest. Fruit ≥ 25 g fresh weight were cut longitudinally and partitioned into pericarp (fruit wall) and endocarp (seed cavity) tissues and weighed individually. Whole fruit tissue from harvests 1 and 2 and pericarp and endocarp tissues from the later harvests were dried at 60°C for 72 hr in a forced-air oven, weighed, and ground in a mortar and pestle.

Leaf tissues were collected at anthesis for Ca analysis. The first fully expanded leaf (fourth or fifth leaf from the apex) was sampled, separated into lamina and petiole tissues, dried at 60°C for 72 hr, and ground in a Wiley mill to pass a 40-mesh screen.

Calcium concentrations in fruit and leaf tissues were determined by atomic absorption spectrophotometry. Tissue samples of 0.1 g were digested in 10 ml of analytical-grade nitric acid and the oxidation completed with H₂O₂. Samples were diluted with a LaCl₃ solution to achieve a final La concentration of 1000 ppm in order to minimize background interference during atomic absorption spectrophotometric analysis.

Rates of Ca accumulation and growth were estimated by regressing a nonlinear function through the time course data of fruit Ca content and weight using piecewise cubic spline interpolation (Tektronix, 1980). The first derivative of the function for the best-fitting curve provided rate estimates for various stages of fruit development, 5 to 150 g fresh weight. Both direct measurements and estimated rates were statistically tested using

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analysis of variance for each harvest and at specific stages of fruit growth. When the F value was significant, mean separation was determined by least significant difference.

Results

Environmental conditions during the vegetative and reproductive periods of plant development differed among the four cucumber plantings. For both the 7 June and 5 July plantings, ample rainfall (75 mm) was received before flowering but only 7 mm or no rainfall, respectively, was received following fruit set. In contrast, periodic rains (131 mm total) occurred during the entire developmental period following the 20 June planting. The 19 July planting experienced drought stress due to limited rainfall (9 mm) from 10 days before flowering throughout fruit development.

Calcium concentrations in pickling cucumber fruit tissues declined during fruit development (Fig. 1). The highest concentrations were consistently observed in pericarp tissue, where they ranged from $\approx 1.0\%$ to 0.7% of dry weight, as compared to 0.65% to $<0.2\%$ dry weight in endocarp tissue. In most situations, the endocarp tissue exhibited the largest ontogenetic decline in Ca concentration.

Both cultivar and planting time significantly affected Ca concentrations in pericarp tissue from fruit of 50 to 150 g fresh

weight and in endocarp tissue from fruit of >100 g fresh weight (Fig. 1). Of the cultivars tested, 'Tamor' fruit consistently contained the lowest Ca concentrations in both pericarp and endocarp tissues. The highest endocarp Ca concentrations were measured in fruit from 'Regal'. Fruits harvested from the 20 June planting had higher Ca concentrations than fruit from either July planting. The early season planting (7 June) produced fruit relatively high in endocarp Ca concentration but moderately low in Ca within the pericarp.

The net Ca content of pickling cucumber fruits increased continuously (0.5 to 48 mg Ca/fruit) during fruit growth and development (Fig. 2). However, imported Ca was partitioned unequally between pericarp and endocarp tissues within the fruit. In 100 to 150 g fresh weight fruit, $<10\%$ of the total fruit Ca content had accumulated within the endocarp. In these same fruit, however, the endocarp constituted $\approx 25\%$ to 30% of the total fruit fresh weight.

The rate of Ca accumulation within fruits was highly variable and did not exhibit a consistent ontogenetic trend (Fig. 3). Environmental conditions during fruit development, as influenced by planting time, had a large effect on the developmental trends. In general, Ca accumulation rates in fruit increased rapidly during the early stages of ontogeny, up to 25 g fresh weight, with the exception of fruit from the 19 July-planting. As the fruit continued to develop, the rate of Ca accumulation either continued to accelerate (20 June and 19 July plantings), stabilized and remained relatively constant (7 June planting), or even declined after the fruit reached ≈ 100 g fresh weight (5 July planting), depending on the environmental conditions of the respective plantings. The pickling cucumber cultivars did not differ in Ca accumulation rate throughout fruit ontogeny nor in their re-

Fig. 1. Influence of planting date and cultivar on Ca concentration within pericarp and endocarp tissues of pickling cucumber fruits at specific stages of fruit weight. For analysis of planting date effects, each point for 25 to 125 g fresh weight fruit is a mean of four replications $\times$ four cultivars, but for 150-g fruit, it is a mean of three replications $\times$ four cultivars. Vertical bars represent LSD at $P = 0.05$.

Fig. 2. Ontogenetic changes in Ca content and fresh weight of pericarp and endocarp tissues within pickling cucumber fruit. Each point is an overall mean of four replications $\times$ four planting dates and four cultivars.

Fig. 3. Influence of planting date on the rates of Ca accumulation and of increase in fresh weight of pickling cucumber fruit at specific stages of fruit weight. For 25 to 125 g fresh weight fruit, each point is a mean of four replications $\times$ four cultivars, but for 150-g fruit, it is a mean of three replications $\times$ four cultivars.

sponse to the various planting times. The only exception was 'Tamor', for which lower Ca accumulation rates were observed in small fruit, i.e., those <25 g in fresh weight (data not presented).

Ontogenetic trends in expansive fruit growth rate, expressed on a fresh-weight basis, were affected by the cultural environment in a manner similar to Ca accumulation rate (Fig. 3). A relatively high correlation was found between fruit growth rate and the net rate of Ca accumulation in 150-g fresh weight cucumber fruit (Fig. 4).

Calcium concentrations in fully expanded leaf tissue were 50% to 300% higher than those of rapidly growing fruit tissues (<1.0% dry weight) (Table 1). Within an individual leaf, Ca concentrations in the lamina mostly were higher than in the petiole. Environmental conditions during plant development, as influenced by planting date, affected lamina but not petiole Ca concentrations and contents. The Ca levels in the leaf tissue as determined at anthesis were not correlated with fruit Ca concentrations (data not presented).

Discussion

Calcium import and accumulation in pickling cucumber fruit during fruit ontogeny were related to expansive fruit growth and the net accumulation of water within the fruit. Conditions that were conducive to rapid fruit growth enhanced movement of Ca into the developing fruit (Fig. 3, 4). This relationship is consistent with the view that the xylem, which is the primary vascular tissue supplying water to fruit, is also involved in the transport of Ca and other ions into the fruit (Clarkson, 1984; Hanger, 1979). Xylem sap has been reported to contain up to

Fig. 4. Correlation between the rate of fruit growth expressed on a fresh-weight basis and the net rate of accumulation of Ca within pickling cucumber fruit weighing 150 g fresh weight. Each point is comprised of estimates from the rate functions from each of the treatment subplots.

Table 1. The effect of planting date on the Ca concentration and content of pickling cucumber leaf tissues².

Planting date (1984) ¹	Ca concn (% dry wt)		Ca content (mg/tissue)	
	Lamina	Petiole	Lamina	Petiole
7 June	3.10	1.84	14.2	1.09
20 June	2.36	1.45	7.96	0.54
5 July	1.85	1.54	4.41	0.33
19 July	2.42	1.76	9.05	0.62
LSD 0.05 ^x	0.31	NS	2.30	0.19

²The most recent fully expanded leaf at time of anthesis.

¹Each entry is an average of four replications $\times$ four cultivars.

^xSignificant LSD at $P = 0.05$ or the analysis of variance F value was nonsignificant (NS).

4 mM Ca (Hocking et al., 1978; Wilcox et al., 1977) in herbaceous annual species. In the present study, the increase in Ca content relative to the increase in fresh weight of the fruit per unit time, as determined for 50 to 100 g fresh weight fruit, ranged from 200 to 240 μg Ca/g fresh weight. Assuming that the fresh weight gain in fruit is attributable largely to the accumulation of water, and assuming that transpirative water loss from cucumber fruit due to a low surface to volume ratio is minimal, the estimated increase in Ca relative to that of water within developing fruit (4 to 5 $\mu\text{mol}\cdot\text{ml}^{-1}$) approximated the typical Ca concentrations within xylem solution.

The rate of accumulation of Ca within cucumber fruit was not correlated with tissue Ca concentration. Since Ca concentration is both a function of the rate of change in net Ca content and the rate of biomass (dry matter) accumulation within a particular organ, the ontogenetic decline in Ca concentration within the pericarp and endocarp tissue (Fig. 1) might be interpreted as being due to a relatively high rate of increase in tissue biomass. Conversely, one cannot discount the effects of fluctuations in Ca import rate relative to growth. In fruit from the 7 July planting, for example, the rate of Ca accumulation remained virtually constant while the growth rate declined substantially as the fruit grew beyond 100 g fresh weight (Fig. 3).

The low endocarp vs. pericarp tissue Ca concentrations (Fig. 1) could be due in part to a slow rate of import of Ca via the xylem into the endocarp, the seed cavity region of the cucumber fruit (Fig. 2). Anatomical examination (data not presented) revealed extensive vascularization throughout the pericarp. The vascular bundles extend primarily to the point of attachment of the ovules on the outer placental wall, resulting in limited distribution within the seed cavity. Since volume flow of water via the xylem into the endocarp is driven by osmotically generated water potential gradients, the rate of Ca ion transport within the xylem would be expected to be slow. Moreover, if the imported Ca passes first through the pericarp, cells along the vascular pathway would have first opportunity to absorb the Ca and thus deplete the Ca from the apoplastic solution entering the seed cavity. The observed gradient in Ca concentration from the proximal to the distal end of cucumber fruit by Frost and Kretchman (1989) also supports the hypothesis for Ca depletion from the xylem solution during transport.

We have shown that genetic selection or environmental modification through cultural manipulation might provide two viable strategies for enhancing fruit Ca. Environment and, to a lesser extent, genotype were found to significantly influence the concentration and cumulative content of Ca within cucumber fruit tissues. Soil moisture appears to be an important factor influencing the Ca status of pickling cucumber fruit. Both July plantings experienced drought stress following fruit set, and this was associated with depressed expansive growth and lower Ca levels in the fruit (Figs. 1 and 3). Soil moisture deficits have been reported to limit Ca uptake by roots (Haber et al., 1983; Wiersum, 1979) and accumulation within developing tomato fruit (Geraldson, 1957; Mullins and Wolt, 1983). However, the involvement of other environmental factors on Ca uptake and transport cannot be discounted, since the lowest fruit tissue Ca concentrations were both observed from late-season plantings.

The ability to predict and anticipate potentially low Ca levels within pickling cucumber fruit from leaf tissue analysis appears to be limited. Calcium concentrations in leaf tissue sampled at anthesis were not correlated with subsequent fruit Ca levels. In addition, Ca accumulation rates were found to be highly variable during the development of the fruit and modified rapidly by changes in cultural environment. However, leaf tissue analysis might serve as a diagnostic tool for confirming the occurrence of Ca-limiting conditions if recently expanded leaves are sampled during the fruiting period. The Ca content within these leaves may reflect the uptake and transport of Ca within the xylem during the fruiting period, since remobilization of stored Ca via the phloem from mature leaves is thought to be minimal (Iwahashi et al., 1982).

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