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Energy Dispersive X-ray Analysis of Ca and K in Apple Leaves

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Abstract. **Energy dispersive X-ray analysis of Ca and K was conducted in apple (*Malus domestica* Borkh.) leaves. Larger amounts of Ca and K were detected in the xylem than in the mesophyll tissue. The X-ray images of both the elements increased in intensities in samples from trees receiving gypsum and foliar sprays with $CaCl_2$. Conversion of the X-ray images into graphical data produced a better quantitative comparison of element concentrations. The range of Ca concentration in the xylem of 206 to 350 ppm compared favorably with that reported in the literature for xylem sap of assorted tree species, including apple trees.**

Elemental analysis of plant tissue usually is conducted by destruction of the plant matrix and concentrating the elements in question. With new developments in electron microbeam analyzers, a rapid method is available for nondestructive analysis of soil and plant samples (10, 12). Although costly, this method can yield useful information where other analytical techniques cannot be applied (1–4, 8, 9, 11, 13, 14, 17). Using a scanning electron microscope equipped with an electron microbeam analyzer, Tan and Nopamornbodi (15, 16) reported the X-ray imaging of elemental distribution zones in the soil rhizosphere and various parts of the root. Such a capability is very important for the analysis of Ca disorders in fruit trees, where poor Ca movement in the xylem and low Ca concentration in the cortex are assumed to contribute to physiological diseases (7). Therefore, this investigation was conducted to study a) the semiquantitative determination of nutrient elements in apple leaves by X-ray imaging, and b) the possible transformation of the latter into graphical data.

This study was conducted in an 8-year-old 'Starkrimson Delicious' apple orchard at the Univ. of Georgia Mountain Experiment Station, Blairsville, on 13 June 1985. The trees were part of an experiment to study the effect of annual soil application of gypsum and $CaCl_2$ sprays to leaves on the control of cork spot or bitter pit in apples. The treatments, replicated four times, were a) 0 kg $CaSO_4$, no spray; b) 9 kg $CaSO_4$/tree; and c) 9 kg $CaSO_4$/tree + spray. The foliar sprays were applied at 2-week intervals from petal fall to near harvest at a rate of 4.4 kg $CaCl_2$/1000 liters per ha. Leaf samples from 28 trees were taken at random from spurs located in the interior of the crown. They were washed thoroughly with distilled water, dried on tissue paper, and immediately stored in a Dewar flask filled with liquid N for transport to the laboratory. The frozen samples were freeze-fractured and prepared for analysis according to methods described previously (6, 15). A Philips 505 scanning electron microscope (Philips, The Netherlands), equipped with a solid-state energy dispersion detector (EDAX model 707A, EDAX, Prairie View, Ill) was used for SEM pictures and X-ray imaging. Graphical data were obtained by analysis with the Tracor Northern 5500 Energy Dispersive X-ray Analyzer (Tracor Northern, Middleton, Wis.) Quantitative measurements were made by using standard samples containing known concentrations of Ca, K, and other elements (16).

Scanning electron microscopy yielded micrographs of leaf cross sections with well-preserved cellular details. The epidermis was clearly separated from the mesophyll, and the vascular bundles were interspersed within the lower area of the mesophyll layer. The xylem cells were characterized by a typical spiral structure (Fig. 1A). Figure 1B shows high-density of X-ray images, localizing Ca and K in a zone following the pattern of the vascular bundles. Transformation of the EDAX images into graphical data yielded curves in which the concentrations of the elements were represented by the intensity of the peak heights. Large amounts of Ca and K were detected in the mesophyll of leaf tips from control trees (0 gypsum), and comparatively low concentrations of Cl, P, S, and Mg were noticed. Applications of gypsum resulted in an increase in peak intensities for Ca and K (Fig. 2). The increase in intensities of these two peaks was evident in tissue from trees that received both gypsum and $CaCl_2$ sprays (Fig. 2, bottom). Xylem tissue of the leaf apex showed a different element composition. The peak for Mg was absent (Fig. 3), indicating lower concentrations of Mg in xylem than in mesophyll tissue. This lack of Mg was expected, since chlorophyll, which contained Mg, was concentrated in the mesophyll. On the other hand, the peaks for Ca and K in the xylem were stronger than those in the mesophyll.

The xylem and mesophyll in the basal part of the leaves from control trees appeared to contain more Ca than that of the leaf tip (Fig. 3). The peaks for Ca also increased substantially after gypsum and $CaCl_2$ spray treatment. As a result of foliar sprays, the peak height for Cl^- in the leaf base was twice that of Cl^- in the leaf tip.

The quantitative measurements of Ca and K by integration of peak heights with those of the standard samples confirmed the observation for higher concentrations of Ca and K in xylem than in mesophyll tissue, but

Table 1. Calcium and K concentrations in xylem and mesophyll of tip and basal sections of apple leaf.

	Concn (ppm)			
	Xylem		Mesophyll	
Treatment	Ca	K	Ca	K
	Leaftip			
0 kg gypsum	206.2[z]	940.9	196.9	886.4
9 kg gypsum	309.4	940.9	203.0	654.5
9 kg gypsum + spray	309.4	1159.1	243.7	854.5
LSD_{05}	21.5	16.4	3.9	12.9
	Leafbase			
0 kg gypsum	253.1	940.9	206.2	927.3
9 kg gypsum	303.1	895.4	206.0	572.7
9 kg gypsum + spray	351.5	1009.1	351.5	940.9
LSD_{05}	25.4	64.6	18.5	18.4

[z]Mean separation within leaf tissue, location, and element by LSD ($p = 0.05$).

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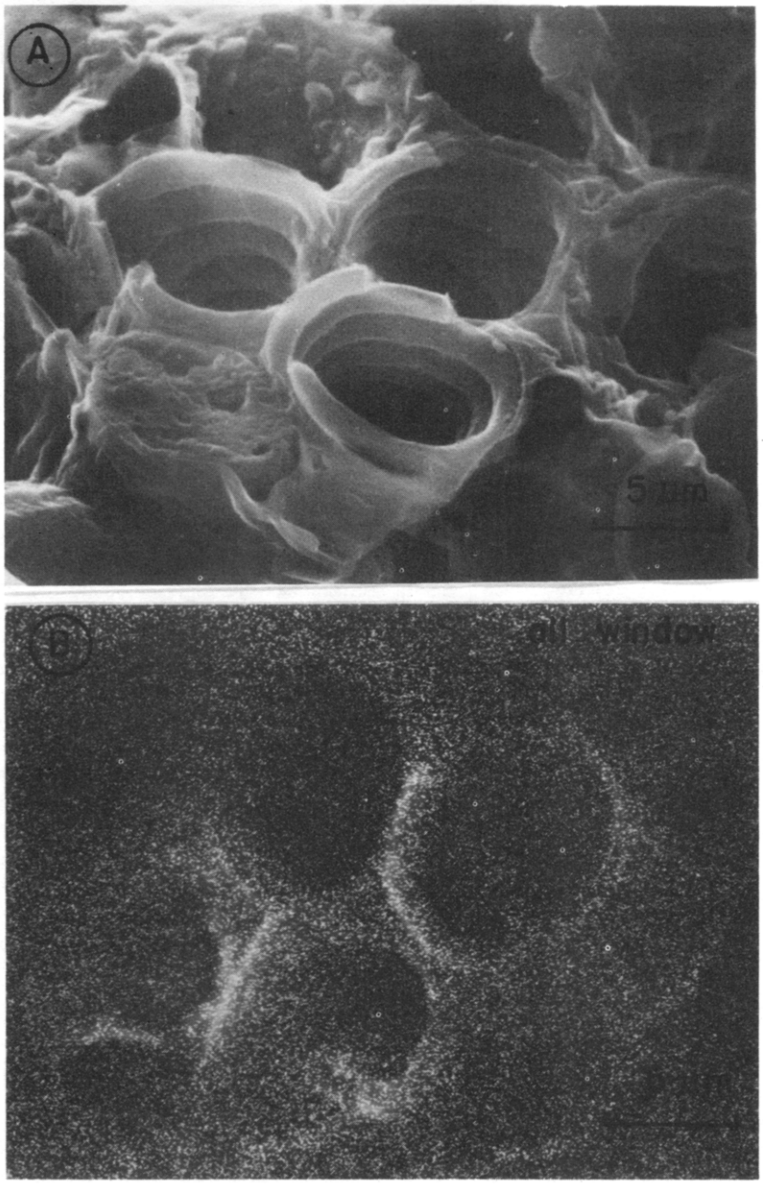

Fig. 1. Scanning electron and electron microbeam micrographs of a cross-section of the vascular bundles in an apple leaf (0 gypsum): (**A**) SEM micrograph of xylem tissue, (**B**) all window X-ray distribution image of the elemental composition.

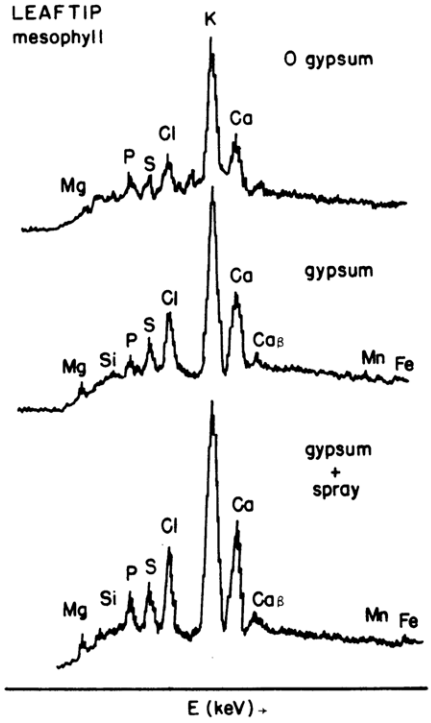

Fig. 2. Graphical presentation of the nutrient element X-ray images in the mesophyll of leaf tips. Intensity of peak heights represents relative concentration.

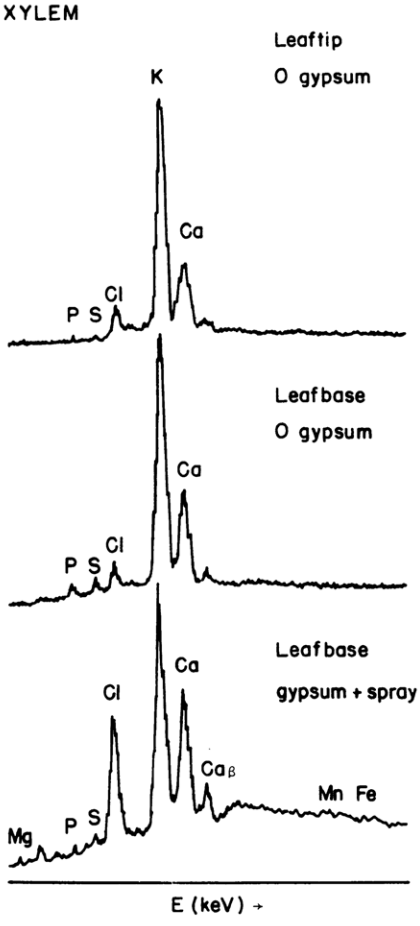

Fig. 3. Graphical presentation of the nutrient element X-ray images in the xylem of tip and basal sections of an apple leaf. Intensity of peak heights represents relative concentrations.

differences in composition between leaftip and leafbase were less obvious (Table 1). Only at 0 gypsum was a tendency noticed for higher Ca and K contents in the basal sections of leaves than in leaf tips. This higher Ca concentration was attributed perhaps to the fact that the basal sections of leaves were the main ports of entry for Ca. The Ca concentrations in the xylem (206.2–351.5 ppm) were considerably higher than those mentioned in the literature. Jones et al. (7) reported a Ca concentration of 32 to 165 $\mu g \cdot ml^{-1}$ in xylem sap of assorted tree species. In apple sap, they detected a Ca concentration of 4.1 to 8.2 $\mu g \cdot ml^{-1}$. The evidence discussed above indicated that energy-dispersive analysis with X-rays provides a method for determining nutrient elements in various parts of the plant tissue. Since Ca-disorders in apple trees are caused by low Ca content in the cortex (7) and poor redistribution of Ca from leaf to fruit tissue (5), methods that analyze a localized section of the tissue will provide better information concerning deficiency problems. It will not be surprising if other types of physiological diseases in agronomic crops are also attributed to some disorder in a specific part of the plant tissue. In the determination of such a disorder, the use of energy dispersive analysis by X-rays may increase in importance and application.

Literature Cited

1. Cecas, M.P., E.H. Tyner, and L.J. Gray. 1968. The electron microprobe x-ray analyzer in its use in soil investigations. Adv. Agron. 20:153–198.
2. Cecas, M.P., E.H. Tyner, and J.K. Syers. 1970. Distribution of apatite and other mineral inclusions in a rhyolitic pumice ash and beach sands from New Zealand: an electron microprobe study. J. Soil Sci. 21:78–84.
3. Gallaher, R.N., H.F. Perkins, and K.H. Tan. 1974. Classification, composition, and mineralogy of iron glaebules in a southern coastal plain soil. Soil Sci. 117:155–164.
4. Hill, D.E. and B.L. Sawhney. 1971. Electron microprobe analysis of soils. Soil Sci. 112:32–38.

5. Hill, J. 1980. The remobilization of nutrients from leaves. J. Plant Nutr. 2:407–444.
6. Humphreys, W.J. 1975. Drying soft biological tissue for scanning electron microscopy, p. 707–714. In: O. Johari and I. Corvan (eds.). Scanning Electron Microscopy. Illinois Inst. of Technol. Res. Inst. Chicago.
7. Jones, H.G., K.H. Higgs, and T.J. Samuelson. 1983. Calcium uptake by developing apple fruits: I. Seasonal changes in calcium content of fruits. J. Hort. Sci. 58:173–182.
8. Lee, F.Y., and J.A. Kittrick. 1984. Electron microprobe analysis of elements associated with zinc and copper in an oxidizing and an anaerobic soil environment. Soil Sci. Soc. Amer. J. 48:548–554.
9. Qureshi, R.H., D.A. Jenkins, R.I. Davies, and J.A. Rees. 1969. Application of microprobe analysis to the study of phosphorus in soils. Nature (London) 221:1142–1143.
10. Rasmussen, H.P. and B.D. Knezek. 1971. Electron microprobe techniques and uses in soil and plant analysis, p. 209–222. In: L.M. Walsh (ed.). Instrumental methods for analysis of soils and plant tissue. Soil Sci. Soc. Amer., Madison, Wis.
11. Sawhney, B.L. 1973. Electron microprobe analysis of phosphates in soils and sediments. Soil Sci. Soc. Amer. Proc. 37:658–660.
12. Sawhney, B.L. 1986. Electron microprobe analysis, p. 271–290. In: A. Klute (ed.). Methods of soil analysis. Part I, 2nd ed. Amer. Soc. Agon. and Soil Sci. Soc. Amer., Madison, Wis.
13. Smith, J.V. and R.C. Stenstrom. 1965. Chemical analysis of olivines by the electron microprobe. Mineral Mag. 34:436–459.
14. Syers, J.K., J.D. Williams, A.S. Campbell, and T.W. Walker. 1967. The significance of apatite inclusions in soil phosphorus studies. Soil Sci. Soc. Amer. Proc. 31:752–756.
15. Tan, K.H. and O. Nopamornbodi. 1979. Electron microbeam scanning of element distribution zones in soil rhizosphere and plant tissue. Soil Sci. 127:235–241.
16. Tan, K.H. and O. Nopamornbodi. 1981. Electron microbeam analysis and scanning electron microscopy of soil-root interfaces. Soil Sci. 131:100–106.
17. Tousimis, A.J. 1964. Electron probe x-ray microanalysis of medical biological specimens. ASTM Spec. Tech. Publ. 349:193–206.

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Capacity of Citrus Flowers to Supercool

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Additional index words. freeze avoidance, ice nucleation, exotherms, pistil, style, ovary, *Citrus sinensis*

Abstract. **'Hamlin' orange [*Citrus sinensis* (L.) Osbeck] flowers readily supercooled on young trees tested in a controlled-temperature room. Differential thermal analysis (DTA) determinations with thermocouples inserted into different flower parts indicated ice nucleation occurred from −3.8° to −6.1°C when flowers were attached to trees and from −8.1° to −11.9°C when flowers were detached. Similar supercooling levels also were noted in ovaries and young leaves. Ice-nucleation-active (INA) bacteria apparently were not involved based on total bacteria counts and flower wash extracts sprayed on flowers. Supercooling of citrus flowers was comparable to flowers of deciduous fruit trees in temperate climate zones. Data indicate a degree of freeze avoidance not previously recognized in citrus reproduction organs.**

Supercooling in citrus by some freeze avoidance standards does not appear to be of practical consequence because of limitations in relative degree of supercooling. For example, the apparent role of deep supercooling in woody plants (8) and horticultural crops other than citrus (4) is not apparent in −7°C lethal temperatures for citrus trees (16). However, the potential that does exist in citrus (17) is adequate to account for occasional uninjured trees (escapes) that are seen after injurious freezes in Florida. As yet, supercooling potential cannot be used for practical advantage during natural freezes in citriculture, largely because the event is uncontrollable, with no assurance that it will occur in trees at different freeze temperatures or persist for ≥ 1 hr. The importance of ice-nucleating agents, such as bacteria (15) or intrinsic internal factors (3), have not been resolved. It apparently is possible to modify partially the role of nucleation sites, since both cold-hardening temperatures (13) and water stress (14) increase supercooling in citrus.

The detachment of plant parts or a break in vascular continuity also suggests that nucleating sites can be modified successfully for practical advantage (2, 5). The objective of this reported work was to determine the supercooling potential in citrus flowers. Flowers are especially vulnerable to injury during natural freezes, suggesting a very low level of supercooling, probably not much below −2°C, a level of efficient ice nucleation based on activity of various INA agents (15). Such information would help to identify supercooling limits in different citrus tissues and provide useful data for modeling whole-tree survival without injury during freezes at different stages of growth and development.

Citrus trees. Thirty uniform and healthy 2.5-year-old 'Hamilin' orange trees on *C. macrophylla* rootstock from a registered Florida citrus nursery were the source trees of all flower tests. Trees in a high organic soil mix in 20-liter pots kept outdoors were watered daily with monthly applications of 3 liters of a 60:1 (v/v) dilution of 15N–3P–6K complete fertilizer (Sunniland Corp. Sanford, Fla.). Tests were done during flowering months of Feb. through Apr. 1985. Trees were 1 to 1.5 m tall above soil level, trunk diameters ranged from 2.5 to 3.5 cm 10 cm above the bud union, and 5- to 10-cm long flower-bearing stems were <2 mm at mid diameter.

INA agents. Of primary concern were INA bacteria implicated in freezing of citrus (12). A random sample of 100 flowers, open for at least 1 day, were excised from the 30 trees immediately before the first and last tests. Subsamples of 20 flowers were immersed in 20 ml of sterile glass-distilled H_2O and shaken for 30 sec on a vortex shaker, and 10 ml was serial-diluted on King's B agar containing 40 $\mu g \cdot ml^{-1}$ cycloheximide (11). Plates were incubated for 3 days at 20° ± 1°C and total bacteria counts expressed as colony-forming units (cfu) per flower. The remaining 10 ml were misted with a hand sprayer on isolated flower clusters and checked for early freez-

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Table 1. Capacity of 'Hamlin' orange flowers, isolated ovaries, and single leaves to supercool.

Item	Temperature (°C)			
	Attached to trees		Detached from trees	
	Nucleation	Range	Nucleation	Range
Open flowers	−4.5 ± 0.3[z] a[y]	−3.6 to −6.1	9.9 ± 0.9 b	−8.1 to −11.9
Isolated ovaries	−5.2 ± 0.2 a	−4.3 to −5.4	8.9 ± 0.7 b	−6.7 to −11.1
Leaves	−4.2 ± 0.5 a	−3.6 to −5	−10 ± 0.5 b	−9.5 to 10.6

[z]Mean ± SD, n = 19–21.
[y]Significant at 1% level, comparison of means between attached and detached tissues.