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Color Breakdown in Anthurium (*Anthurium andreanum* Lind.) Spathes Caused by Calcium Deficiency[1]

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Abstract. **A color breakdown disorder in the lobe (proximal) section of the spathe of *Anthurium andreanum* Lind., was related to calcium deficiency. Elemental and electron microprobe X-ray analysis of spathe and leaf tissue revealed significantly lower Ca associated with color breakdown. In nutrient cultures, color breakdown was produced in the spathes of plants grown without Ca. Addition of Ca corrected the disorder. Elemental analysis of spathe and leaf tissues indicated lower Ca uptake at pH 3 and 4, higher at pH 6, and highest at pH 7. Ca was always lower in the lobe than tip (distal part) of the spathe at all pH levels. X-ray analysis also showed lower Ca in affected than in normal spathes.**

An acute problem in the anthurium cut flower industry in Hawaii was a disorder of the spathe (Fig. 1). The disorder produced watersoaked lesions on the lobes of the flower spathe, which eventually became necrotic rendering the flowers unfit for sale. Field losses of up to 50% and losses after shipments to the overseas market of up to 20% have been reported. Frequently, the symptoms were not evident during grading and packing, but developed after transit to distant markets. The disorder was more frequently observed during the cold, rainy winter months.

This study was undertaken to ascertain the cause of the disorder and to develop a treatment to reduce the losses.

Materials and Methods

Isolation and inoculation of microorganisms from diseased tissue. Tissue sections (5 mm^2) from affected areas of 10 'Ozaki Red' anthurium spathes were surface sterilized with 0.8% sodium hypochlorite for 3 min rinsed 3 times with sterile distilled water, and plated on potato dextrose agar. A fungus, identified as *Colletochrichum gloeosporioides* was isolated from the tissues. Ten spathes each were sprayed with (1) ascospore suspension, (2) conidium suspension, or (3) distilled water. The flowers were placed in the greenhouse under a continuous fine mist for 48 hr in 75% shade at 26.7°C. Daily observations were made to determine development of incipient lesions.

Elemental analysis of affected and normal spathe tissue. Eighty ¾ expanded spathes and 80 leaves from similar nodes were collected. Maturity was determined by the degree of color change of the spadix, e.g., when the basal ¾ of the spadix had changed from reddish-orange to light pink in 'Ozaki Red.' Forty of the spathes showed symptoms of the color breakdown, while 40 appeared to be normal. The spathes and leaves were divided into 4 categories: 1) color breakdown spathes; 2) leaves of color-breakdown spathes; 3) normal spathes; and 4) normal leaves. The spathes and leaves were further divided into 4 replicates, each with 10 spathes and 10 leaves.

The samples were rinsed with distilled water and air-dried. The spadix and pedicel were discarded and only the spathe and leaves were analyzed. N, P, K, Ca, Mg, B and SiO_2 levels were determined by the methods of Horwitz (2).

Electron microprobe X-ray analysis of normal and affected tissue. Fresh samples, about 5 × 15 mm, from affected and normal spathes were used for microprobe analysis. Tissues were taken from the lobe and tip of spathe even though the symptoms of color breakdown were localized in the lobe. The cyrostat method described by Rasmussen et al. (7) was used. The samples were air-dried and placed in an electron microprobe X-ray analyzer (Applied Research Laboratory, model EMX-SM), operating at 21 kV accelerating voltage and 0.056 microamperes sample current. A conductive coating was unnecessary with sections of this thickness (16 μm). The content of K, P, Na, and Ca were determined by point counting and line profile analysis.

Solution cultures. Twenty-eight mature anthurium plants were placed in coarse perlite in 20 cm plastic pots. The pots were enclosed in a wooden box (Fig. 2) to provide high humidity and high root temperature. The peat moss was kept moist at all times, providing 100% relative humidity to the roots. A heating cable in the peat moss maintained a root temperature of 26.7°C. The plants were grown in nutrient solution (7) in a fiberglass greenhouse with 80% shade. Treatments included pH levels of 3, 4, 5, and 7 at 220 ppm Ca and Ca concentrations of 0, 100, and 200 ppm at pH 6.0. Two hundred ml of each treatment solution were added 3 times a week. No additional watering was necessary. The experiment

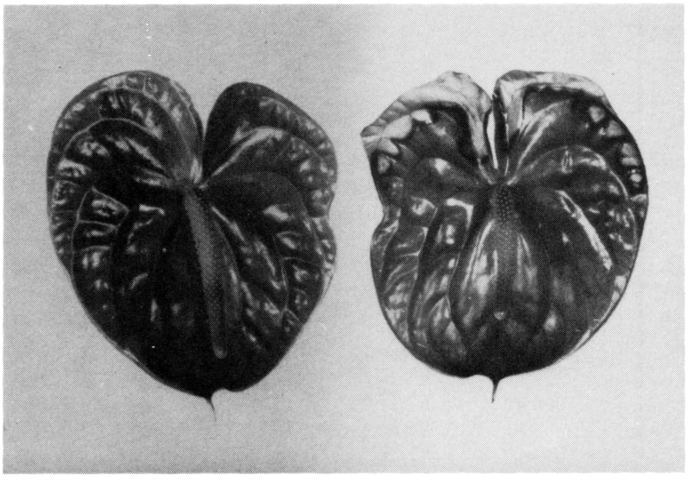

Fig. 1. 'Ozaki Red' anthurium spathes showing the color breakdown disorder. Right = flower with the lobe section turned brown and necrotic.

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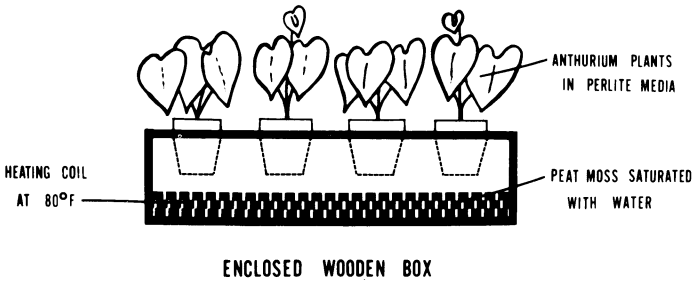

Fig. 2. Planting system used to grow anthuriums in nutrient culture.

was designed to study Ca uptake at various pH levels and Ca concentrations and its effect on the color break symptoms. The solutions were pre-mixed in 20 liter plastic carboys and pH readings taken weekly. When necessary, the pH was adjusted with either H_2SO_4 or NaOH. Weekly production data and any observable nutritional deficiency symptoms were recorded. Calcium content of the spathe and the leaf associated with the flower was determined spectrophometrically (2).

Radioisotopic studies. Twelve mature anthurium plants were grown in 11 liter (3 gallon) plastic containers in aerated solution culture in a fiberglass greenhouse under 80% shade with supplementary Cool White florescent lighting of 5.2 klx from 5 PM to 5 AM daily.

Plants were grown in deionized water for 5 days and transferred to nutrient solutions adjusted to pH 3, 5, 7, and 9. Three plants per treatment were grown for 7 days, the solution was replaced, the pH adjusted, and 0.05 microcuries per ml of ^{45}Ca added to each container. The plants took up ^{45}Ca for another 14 days. The pH was checked twice daily and adjusted as necessary.

After 14 days, the plants were prepared for microautoradiography and SEM studies. Fresh tissue 5 x 15 mm was taken from the spathe and leaf blade arising at the same node. Tissues were sampled from the lobe and the tip. Sections were fixed in a 3:1, 95% alcohol-acetic acid solution (by volume) and dehydrated through tertiary butyl alcohol and embedeed in tissuemat (3). Sections (8 μm) were mounted on gelatin-chrome-alum coated glass slides. The paraffin was removed with xylene and the sections were coated with Kodak Nuclear Track NTB2 emulsion and stored in the dark (1). After the microautoradiographs were developed, fixed, air-dried, and a cover glass mounted permanently with Pro Tex mounting media, the sections were viewed through phase contrast or bright field microscopy to determine silver localization in the tissue.

Table 1. Elemental analysis of anthurium spathe and leaf from color breakdown and normal plants.[z]

Organ	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	SiO_2 (%)	B (ppm)	Moisture (%)
Spathe								
Normal	1.55	.180	2.13	.830	.176	.10	16.0	13.17
Color break	1.76	.201	2.44	.372**	.150	.12	14.0	13.78
Leaves								
Normal	2.12	.178	2.01	.805	.266	.10	11.8	21.68
Color break	2.20	.173	1.98	.363**	.196	.10	13.6	24.18

[z]Each figure represents an average of 10 plants per sample with 4 replications.

**Significantly different from normal at 1% level.

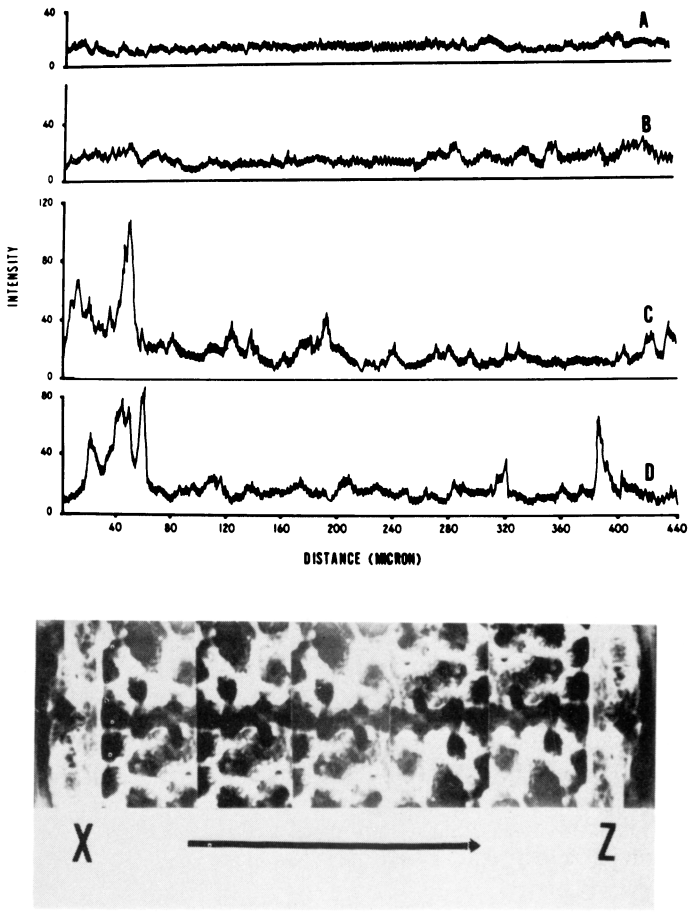

Fig. 3. Microprobe line profile X-ray analysis for Ca in the spathe of anthurium plants. Intensity gives relative calcium concentration. A = Spathe with color breakdown, lobe section; B = spathe with color breakdown, tip section; C = normal flower, tip section; D = normal flower, lobe section. The line scan proceeded from X, upper epidermis, to Z, lower epidermis.

The paraffin embedded tissue was cut (10 μm), mounted on gelatin chrome-alum coated polished aluminum disc (17 mm in diameter), and the paraffin removed with xylene prior to energy disperive X-ray analysis. The sections were air-dried, coated with 20 nm of carbon by evaporation, and analyzed in a SEM (Cambridge Steroscan S4 with an EDAX detector) (8).

Results and Discussion

Anthurium spathes sprayed with ascospore and conidium suspensions of *Colletochrichum gloeosporioides* showed no sign of the color breakdown disorder. The normal senescence symptoms took place within 15 days after treatment. Since lesions could not be reproduced with applications of conidium and ascospore suspensions, it was concluded that the fungus, *Colletochrichum gloeospoioides,* was not the cause of the disorder in anthuriums.

Elemental analysis of affected and normal spathe and leaf tissues showed significant differences in Ca content between normal and color breakdown spathes and with their associated leaves (Table 1). No other element varied significantly between color breakdown and normal tissues, suggesting that low Ca may be the cause of the disorder.

Electron microprobe X-ray analysis showed a higher concentration of Ca in normal spathes compared to color breakdown

Table 2. Ca X-ray analysis of a cross-section of the spathe of anthurium in normal and color breakdown tissue.[z]

Spathe tissue	Ca distribution (counts/sec.)	
	Normal spathe	Color break spathe
Upper epidermis	922a[y]	76a
Middle	699b	67b
Lower epidermis	679c	124b
Avg	767	89**

[z]Each figure is an average of 9 counts taken from 3 spathes.

[y]Mean separation within columns by Duncan's multiple range test, 1% level.

**Significant difference from normal 1% level.

spathes (Fig. 3 and Table 2). Ca was higher in the upper epidermis of normal tissues than in the mesophyll or lower epidermis. Ca in color breakdown tissue was uniformly low. In the normal spathe, Ca increased significantly from the lower to upper epidermis. However, in the color breakdown spathes, the lower epidermis had significantly higher Ca than the middle and upper epidermis areas. Lanning (5) found variations in Ca content of the strawberry in the same plant part and the same section. It also varied between diseased and healthy plant parts.

In nutrient culture studies, color breakdown symptoms were consistently produced on spathes after 6 months of culture in the "No Ca" treatment at pH 6. The symptoms initially occurred at the lobe of the spathe and later spread throughout the spathe. The leaves developed necrotic spots with eventual dieback of the growing tip of the plant. Other treatments (100 and 200 ppm Ca) at pH 6 and other pH levels showed no symptoms of color breakdown after 12 months. The "No Ca" treatment plants which developed color breakdown symptoms again produced normal flowers when Ca was added.

The Ca uptake increased in both spathe and leaf with increasing Ca concentration (Table 3). In the spathe tissues, except for "No Ca" treatment, there was less Ca in the lobe than in the tip, with no significant difference between lobe and tip in leaf tissues. This explains why the disorder is confined to the lobe section of the spathe. If symptoms occurred on the leaves, they were distributed throughout the leaf. When Ca was added, uptake was greater in the tip than the lobe of the spathe (Fig. 4). This may be due to the fact that all major vascular bundles extend out from the base of the spadix toward the margin of the spathe, converging again at the tip of the spathe. In the leaf, all the major vascular bundles are uniformly scattered throughout the leaf blade. This anatomical difference

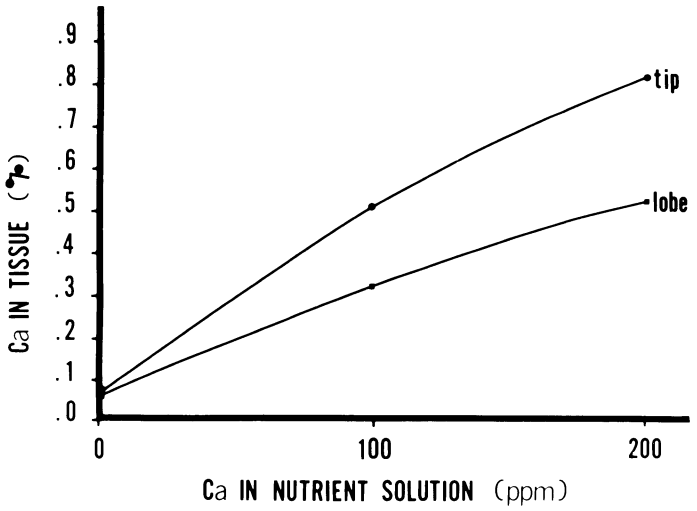

Fig. 4. Ca concentration in the spathe tissue of plants grown in nutrient solutions of different Ca levels.

may explain why Ca is lower in the lobe than in the tip and why the symptoms always occur only in the lobe. The leaf blade has a more uniform Ca distribution and symptoms, and when they occur, are uniformly distributed.

In general, Ca uptake increased with increasing pH from 3 to 7 in both spathe and leaf (Table 4). Although there were significant differences between pH levels on uptake of Ca, none of the treatments showed color breakdown symptoms. Significant differences in Ca content are shown between lobe and tip of the spathe, but not in the leaf. The interaction between Ca treatment levels and pH levels were not significant in either spathe or leaf.

Tissue samples of the lobe and tip of the spathe and of leaves of plants grown at various pH levels with 200 ppm Ca were analyzed by energy dispersive X-ray analysis. Ca uptake was lowest at pH 3, increased through pH 5, reached a maximum at pH 7, and decreased again at pH 9. These results support previous work on pH and Ca uptake (4, 6).

Color breakdown disorder occurs most frequently on plants in nurseries using volcanic black cinder. This inert material is light and porous, providing excellent drainage and aeration. It is low in cost and readily available as a product of Hawaiian volcanoes. Its wide spread utilization occurred after the development of slow-release fertilizers. Ordinary fertilizers were leached

Table 3. Ca content of anthurium spathe and leaf tissues grown at various Ca concentrations.[z]

Ca (ppm)	Ca concentration in tissue (%)			
	Spathe[y]		Leaf[y]	
	Lobe	Tip	Lobe	Tip
0	0.06d	0.07d	0.13c	0.13c
100	0.32c	0.50b	0.83b	0.88b
200	0.52b	0.83a	1.16a	1.15a
Avg	0.30**	0.47	0.71	0.72

[z]Each figure is the average of 4 plants.

[y]Mean separation within groups within organ by Duncan's multiple range test, 1% level.

**Significantly different from tip of spathe at 1% level.

Table 4. Ca content of anthruium spathe and leaf tissues grown at various pH levels.[z]

pH	Ca concentration in tissue (%)			
	Spathe[y]		Leaf[y]	
	Lobe	Tip	Lobe	Tip
3	0.37d	0.57c	0.73a	0.79a
4	0.43d	0.71b	0.85ab	0.89abc
5	0.42d	0.70b	0.88abc	0.88abc
6	0.52c	0.83a	1.16bc	1.15bc
7	0.54c	0.85a	1.20bc	1.22c
Avg	0.46**	0.73	0.96	0.98

[z]Each figure is the average of 4 plants.

[y]Mean separation within groups within organ by Duncan's multiple range test, 1% level.

**Significantly different from tip of spathe at 1% level.

by heavy rains before the plants could utilize them. Unfertilized, fresh volcanic, black cinders have a pH of 6.0 to 7.0. When used as a medium with slow-released fertilizers, the pH drops to 4.0. The acid pH, coupled with no Ca in the cinder, may account for the color breakdown of spathe in Hawaii.

Microautoradiography with ^{45}Ca showed that Ca was concentrated primarily in the cell wall of both spathe and leaf. There was no indication of crystallization of Ca, which would render it unavailable to the plants. Instead, it appears that color breakdown symptoms may be caused by cell membrane leakage causing the "water soaked" appearance of incipient injury.

The results show that Ca deficiency in anthurium can cause color breakdown disorder of the spathe. The lower Ca content in the lobe compared to the tip explains the localization of the color breakdown disorder to the lobe of spathes.

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Relationships between Water Translocation and Zinc Accumulation in Citrus Trees with and without Blight[1]

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Abstract. **Orange trees (*Citrus sinensis* (L.) Osb.) with early-stage (sectored) and moderate blight were evaluated for zinc accumulation and water translocation characteristics. Zinc accumulated at above-normal levels in the outer 2 cm of trunk wood, but water uptake was at below-normal levels in the inner 2 to 6 cm of trunk wood of moderately blighted trees. Water-flux density of roots was not correlated with zinc accumulation. In trees with early stages of blight, zinc accumulated at above-normal levels in the healthy-appearing sides of the trunks, as well as in the blighted sides, but the water uptake in the healthy-appearing sides was similar to that in the trunks of healthy trees. Evidence suggested that the blight effect on abnormal zinc metabolism developed prior to the dysfunction of water translocation.**

Citrus blight is a wilt disease found in several citrus-growing areas of the world (1, 3, 13, 20). The disease can be characterized by restricted water movement in roots, trunks, and large limbs (3, 4, 5, 17, 18, 20), xylem-vessel obstructions in roots and trunks (1, 2, 6, 8, 9, 12, 13), and above-normal zinc accumulation in the wood of roots, trunks, and large limbs (10, 15, 16, 20). Zinc accumulates at above-normal levels in the outer wood next to the cambium (10), whereas xylem-vessel obstructions occur in larger numbers in inner wood (8, 9). The restriction in water movement through roots, trunks, and limbs occurs in the inner, older xylem tissues (4, 17).

Since the cause of blight is unknown, characterization of zinc accumulation and water transport dysfunctions induced by the disease should provide new insight into its etiology. The purpose of this paper is to report new information on the relationships between the water-translocation dysfunction and the above-normal zinc accumulation that are associated with blight.

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Materials and Methods

Plant materials. Citrus used in these studies were 10-, 14- and 16-year-old 'Valencia' orange trees, *Citrus sinensis,* on rough lemon rootstock, *C. limon* (L.) Burm. f., and 14-year-old 'Hamlin' orange trees, *C. sinensis,* on Carrizo citrange, *C. sinensis* × *Poncirus trifoliata* (L.) Raf. These trees were located in 3 groves in central Florida. Trees selected included apparently healthy ones, those exhibiting an early stage of blight where the trees were sectored, and those exhibiting a moderate stage (16). On sectored trees, only one fourth to one third of the canopy exhibited the earliest typical visible blight symptoms. The sectored portion of the trees was confined to 1 or 2 adjacent major scaffold limbs. Moderately blighted trees had typical symptoms throughout the canopy. All sectored trees selected had exhibited blight symptoms less than 1 year as determined from our annual surveys of these groves.

Water uptake, dye distribution, and water-flux density measurements. In a study to compare water uptake by healthy and early-stage (sectored) blight trees, trunk water uptake was measured by the infusion method of Cohen (3). To measure water uptake at different depths in moderately blighted tree trunks, modified infusion methods were used. Uptake by xylem up to 2 cm in depth was accomplished through a specially