exposed and shaded shoots and base, second and third clusters is necessary in order to sample them in proportion to their presence in the population. Since this is not known we opted for random selection of exposure and cluster categories.

Cluster weight, soluble solids, and acidity, respectively, can be estimated by growers by randomly selecting shoots and vines in numerical combinations indicated by the curves of Fig. 1, 2, and 3. Because of large variability among vines there is a minimum number of vines which must be sampled to gain an accurate estimate of any measurement. Sampling smaller numbers of vines would not provide an estimate of desired accuracy even if every cluster were analyzed (12). In situations where growers have not balanced-pruned, more vines must be sampled to offset higher variability among vines.

The sampling combinations for all measurements which result in the fewest total samples is that employing 1 shoot on each of the appropriate numbers of vines. This assumes that all shoots and vines are randomly selected from the vineyard of interest or that vines are selected so that areas of the vineyard which are known to vary in slope, aspect, vine vigor, soil type, etc. are repsented. It also assumes that the cost per unit samples is the same regardless of the combinations of vines and shoots (10).

Sampling for researchers is more complex. In grape research experiments, vines are balanced-pruned and harvested individually so that the earlier assumption of equal cost per unit of sampling does not apply. Here fewer vines and more shoots sampled per vine reduces the overall work in the experiment. Because vines are harvested individually (i.e., completely sampled for cluster variation) and because accurate acidity measurements require so few samples, soluble solids measurement becomes the limiting factor. Based on the data in Fig. 2, our recommendation is to sample between 24 and 36 vines per treatment combination (based on 1977 and 1978 data respectively) and 10 shoots per vine. This would provide the desired accuracy of 0.5% soluble solids while keeping the experiment small enough to be manageable. These vines would most effectively be arranged in 4-6 replicates of 6 vines each to avoid difficulties of missing data in case single vines are lost.

The data presented here provide strategies for estimating cluster weight, soluble solids, and acidity of 'Concord' grapes useful to industry and researchers. While they provide a background for sampling other grape cultivars, more research is needed to verify their applicability.

Literature Cited

1. Amerine, M. A., H. W. Berg, and W. V. Cruess. 1967. The technology of winemaking. AVI, Westport. Conn.
2. Cain, D. W. and R. L. Andersen. 1976. Sampling procedures for minimizing non-genetic wood hardiness in peach. *J. Amer. Soc. Hort. Sci.* 100:668-671.
3. Cochran, W. G. and G. M. Cox. 1957. Experimental designs. Wiley, New York.
4. Hansche, P. E. and R. M. Brooks. 1965. Temporal and spatial repeatabilities of a series of quantitative characters in sweet cherry (*Prunus avium* L.). *Proc. Amer. Soc. Hort. Sci.* 86:120-128.
5. Henderson, C. R. 1953. Estimation of variance and covariance components. *Biometrics* 9:226-252.
6. Marcuse, S. 1949. Optimum allocation and variance components in nested sampling with an application to chemical analysis. *Biometrics* 5:189-206.
7. May, P. 1965. Reducing inflorescence formation by shading individual Sultana buds. *Austral. J. Biol. Sci.* 18:463-473.
8. Partridge, N. L. 1931. The effect of fruit production and fertilizer treatments on the maturity of Concord grapes. *Proc. Amer. Soc. Hort. Sci.* 28:147-150.
9. Saltveit, M. E., Jr. 1978. Selecting an experimental design for pressure testing apples. *J. Amer. Soc. Hort. Sci.* 103:105-109.
10. Schultz, E. F. 1955. Optimum allocation of experiment material with an illustrative example in estimating fruit quality. *Proc. Amer. Soc. Hort. Sci.* 66:421-433.
11. _____. 1955. Rules of thumb for determining expectations of mean squares in analysis of variance. *Biometrics* 11:123-135.
12. _____. and G. W. Schneider. 1955. Sample size necessary to estimate size and quality of fruit, growth of trees, and percent fruit set of apples and peaches. *Proc. Amer. Soc. Hort. Sci.* 66:36-44.
13. Shaulis, N. J. 1956. The sampling of small fruits for composition and nutritional studies. *Proc. Amer. Soc. Hort. Sci.* 68:576-586.
14. Winkler, A. J., J. A. Cook, W. M. Kliewer, and L. A. Lider. 1974. General viticulture. University of California Press, Berkeley.

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Calcium Deficiency of *Anthurium andreanum* Lind. Spathes[1]

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Abstract. **Color breakdown of spathe tissue of anthurium, *Anthurium andreanum* Lind., exhibited typical calcium deficiency symptoms. Anatomical studies revealed cell separation and collapse in affected tissue. Cell separation may have resulted from instability of the middle lamella due to calcium deficiency. Calcium application in the field, either as nitrate or silicate, significantly reduced the incidence of the disorder.**

Anthurium is one of Hawaii's principle ornamental exports. The long vase life and ease of handling and packaging make it a durable product for shipping throughout the world. The flower consists of a large colorful spathe and a spadix protruding from the base of the spathe.

One of the serious problems facing growers is the appearance of water soaked lesions which eventually discolor the lobes of the spathe. These lesions turn dark brown and eventually dry out, rendering the flowers unsuitable for sale. Preharvest losses of 50% have been reported, while losses after shipments to the overseas markets have been as high as 20%. Visual symptoms may not be evident during grading and packing, but appear after

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transit to distant markets. Higaki et al. (2) reported that this color breakdown disorder was caused by calcium deficiency in the lobe tissue of the spathe.

The purpose of this study was to investigate the internal anatomy of the anthurium spathe and to test 2 types of calcium fertilizers for correcting the disorder.

Materials and Methods

To study the effect of the color breakdown disorder on internal anatomy, spathe (lobe and tip), spadix and pedicel tissue, with and without color breakdown symptoms were killed and fixed in FAA, stained with safranin and fast green as outlined by Jensen (3) and examined with a light microscope.

Application of Ca was made in the field to determine whether Ca deficiency caused the color breakdown disorder. Seventy-two mature 'Ozaki Red' anthurium plants were planted in 1.8 x 3.7 m plots in black cinder medium under 75% Saran shade in a completely randomized block designed with 3 treatments and 3 replications. The Ca treatments were: 1) $Ca(NO_3)^2$ at 2242 kg/ha·yr; 2) $CaSiO_3$ at 1569 kg/ha·yr; and 3) No Ca. The Ca was applied in 3 equal parts as a soil additive every 4 months. A standard fertilizer program of Osmocote (14N-6P-11.6K) 3 times a year (336 kg/ha·yr) and standard pesticide practices were followed. The number of normal flowers and flowers with color breakdown symptoms were determined at harvest throughout the year. Production data were collected starting one month after the first application of Ca.

Results and Discussion

Cells of spathes with color breakdown were collapsed; more so in the mesophyll than in the epidermis (Fig. 1). All tissue was torn and damaged. Unaffected flowers showed no evidence of cellular distortion. Tearing occurred between cells (e.g. separation of the middle lamella) as reported by Rasmussen (5) in "tender" tobacco leaves and rarely through the cells (Fig. 2). Rasmussen indicated a lack of Ca resulted in the solubilization of the pectin of the middle lamella and separation of cells. Studies of the spadix and pedicel showed no sign of pectin dissolution or cell separation. This indicated sufficient Ca for minimum pectin cross linking but under stress resulted in separation between cells. No differences in the general anatomical structure of lobe and tip sections of a color breakdown spathe were found.

Cell outlines and total protoplasmic content were readily visible. Cells showing color breakdown stained less intensely than cells of the normal tissue in the spathe, spadix, and pedicel. This phenomenon suggested loss of membrane integrity (e.g. plasmalemma, tonoplast and nuclear membrane) and with the resulting internal leakage, a lack of cytoplasmic clarity.

There was no sign of cell hypertrophy or formation of intumescence as found in "oedema" (a physiological disorder) nor was there a difference in Ca crystal content between affected and normal tissue.

Further studies on the effect of low Ca on cellular detail should be conducted to determine the role of Ca in the color breakdown disorder. The observed tissue damage and cell separation from pectin solubilization do not appear to be the cause of this disorder, since the color breakdown disorder is reversible in its early stages with changes in environmental conditions (e.g. moisture and temperature). Ca is essential to the formation and maintenance of the cell-membrane system which control cell metabolism. The lack of membrane integrity would more logically have an effect on cellular chemistry and color change. The color change may be dependent on environmental

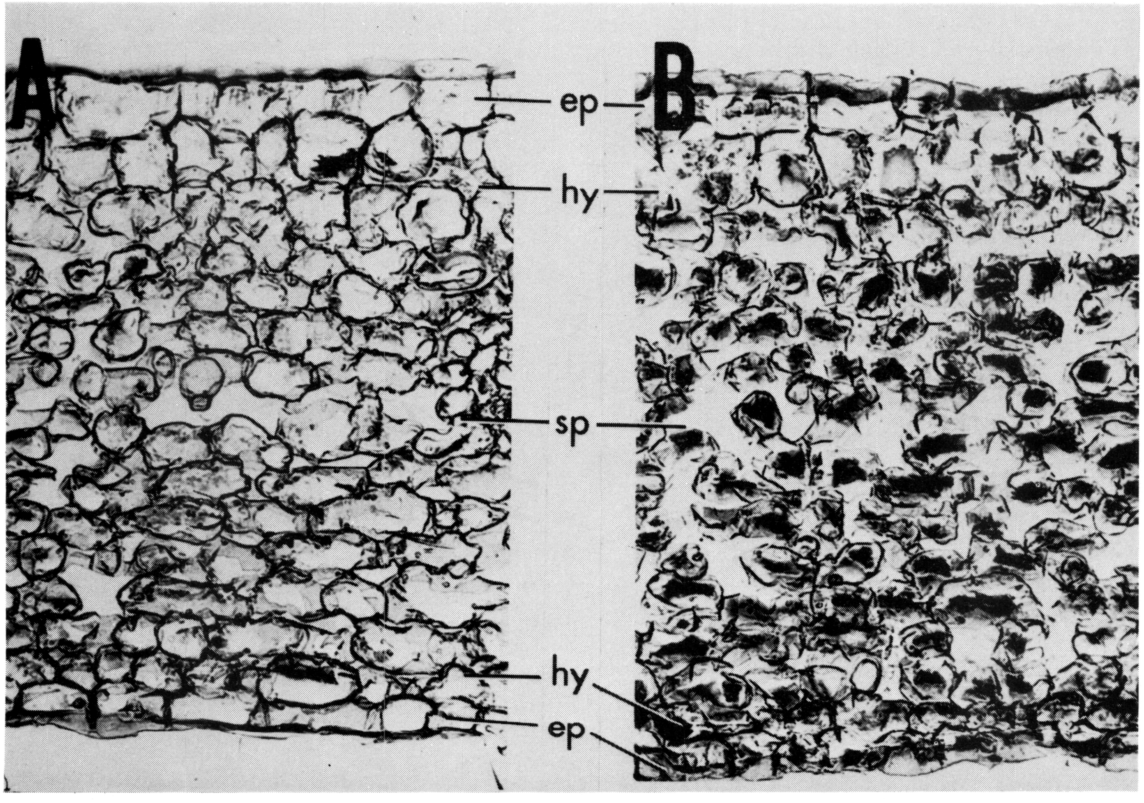

Fig. 1. Photomicrographs of cross-sections of anthurium spathes. A = cross-section of normal spathe tissue, ×100; B = cross-section of color-breakdown spathe tissue showing collapsed mesophyll tissue, ×100; ep = epidermis, hy = hypodermis, sp = mesophyll.

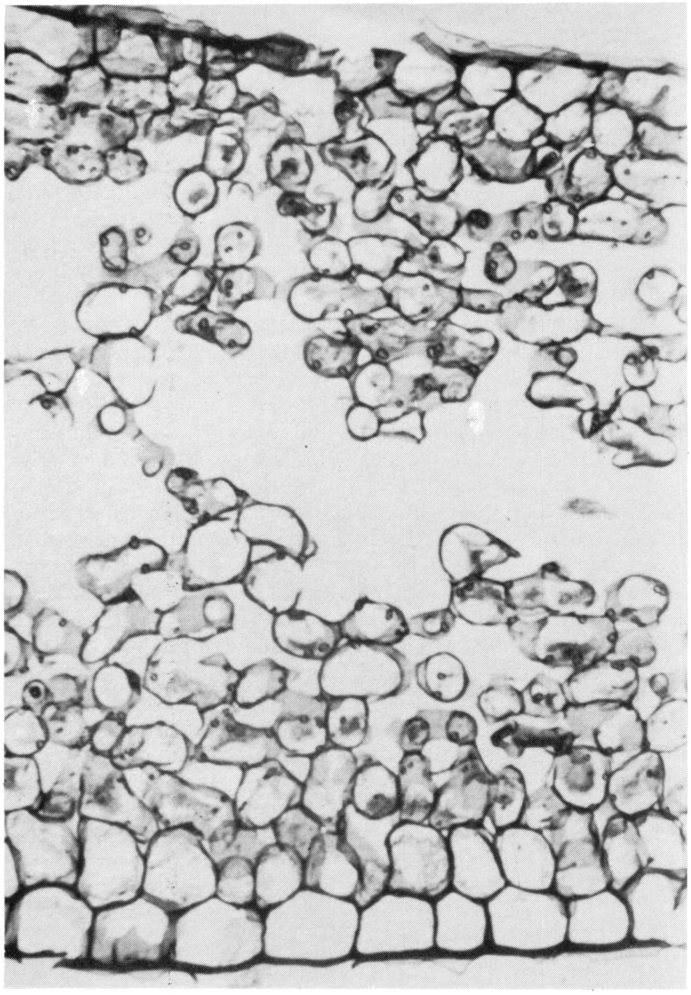

Fig. 2. Section through spathe with color-breakdown showing cellular separation. ×250.

factors which influence moisture in the cell and/or pH and, therefore, would manifest itself in a color change. Extreme cell damage would lead to irreversible breakdown and necrosis of the tissue. Ca effects on the middle lamella were probably secondary to the membrane changes.

Table 1. The incidence of color breakdown disorder of anthurium spathes with Ca applications.

Ca treatment	Flowers with color breakdown[z] (%)
None	8.8a
Ca nitrate (2242 kg/ha·yr)	1.2b
Ca silicate (1569 kg/ha·yr)	0.0b

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

The field test results indicate that (Table 1) the addition of Ca as calcium nitrate or calcium silicate significantly reduced the occurrence of color breakdown (No Ca = 8.8%; Ca nitrate = 1.2%; and Ca silicate = 0.0%, respectively). No significant difference was found between calcium nitrate (1.2%) and calcium silicate (0.0%). The application of Ca either as nitrate or silicate fertilizer significantly reduced the incidence of the color breakdown disorder of the anthurium spathe to the point of elimination of this disorder as a serious commercial problem.

Literature Cited

1. Higaki, T. 1976. Anatomical study of the anthurium plant, *Anthurium andreanum* Lind., and a color breakdown of its flower. PhD Thesis, Michigan State Univ., East Lansing.
2. _____, H. P. Rasmussen, and W. J. Carpenter. 1980. Color-breakdown in anthurium flowers, *Anthurium andreanum* Lind. caused by Ca deficiency. *J. Amer. Soc. Hort. Sci.* 105:000.
3. Jensen, W. A. 1962. Botanical histochemistry. W. H. Freeman, San Francisco.
4. Nakasone, H. Y. and H. Kamemoto. 1962. Anthurium culture with emphasis on the effects of some environments on growth and flowering. *Haw. Agr. Expt. Sta. Tech. Bul.* 50.
5. Rasmussen, H. P. 1967. Calcium and strength of leaves. I. Anatomy and histochemistry. *Bot. Gaz.* 128:219-223.