2. Lee, T. T. 1968. Effect of ozone on swelling of tobacco mitochondria. *Plant Physiol.* 43:133-139.
3. Mudd, J. B. 1965. Response of enzyme systems to air pollutants. *Arch. Environ. Health.* 10:201-206.
4. Richards, B. L., J. T. Middleton, and W. B. Hewitt. 1958. Air pollution with relation to agronomic crops: V. Oxidant stipple of grape. *Agron J.* 50:559-561.
5. Richards, B. L., O. C. Taylor, and G. F. Edmunds, Jr. 1968. Ozone needle mottle of pine in southern California. *J. Air Poll. Cont. Assn.* 18:73-77.
6. Thompson, C. R., O. C. Taylor, M. D. Thomas and J. O. Ivie. 1967. Effects of air pollutants on apparent photosynthesis and water use by citrus trees. *Environ. Sci. Technol.* 1:644-650.
7. Thompson, C. R. and O. C. Taylor. 1969. Effects of air pollutants on growth, leaf drop, fruit drop and yield of citrus trees. *Environ. Sci. Technol.* (In Press).

Table 5. Sugar and acid content of grape juice.

	Ambient Air		Carbon filtered air	
	Sugar %	Acid %	Sugar %	Acid %
	22.3	.53	25.8	.53
	21.7	.52	26.8	.46
	21.9	.48	26.8	.50
	20.9	.52	24.6	.48
	21.4	.49	26.8	.51
	22.2	.51	25.2	.44
Avg	21.7	.51	26.0	.49

Table 6. Total weights of prunings from Zinfandel grapes, dry wt, grams.

	Ambient air	Carbon filtered air	Outside checks
	374	669	367
	457	620	290
	452	606	211
	461	454	403
	339	449	489
	332	351	386
Avg	405	525	358

Effect of Preharvest Sprays of Ethrel, Alar, Malathion on Anthocyanin Content of Early Black Cranberry (*Vaccinium macrocarpon* Ait.)[1]

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Abstract. Ethrel at 600 ppm applied as a 200 gal/A preharvest spray to 'Early Black' cranberry increased anthocyanin development. Gardner Color Difference Meter readings of fresh fruit indicated that Alar-treated fruit had higher reflectance than control fruits suggesting a delay in surface coloring. Ethrel, Alar, and malathion preharvest sprays did not influence yield or size of the fruit.

The intensity of red color development in the cranberry has always been of prime importance in the marketing of cranberries as processed fruit as well as fresh fruit. The quality of cranberry fruit cocktail is largely determined by the clarity and intensity of its red color. Enhancement of the concentration of anthocyanin pigments in cranberry fruit by chemical means would be a distinct advantage to the cranberry producer. Often, because of inadequate water supplies or other cultural factors, the grower is forced to harvest before his crop has reached optimum color development. By promoting early red color development in some of his crop, the grower may harvest sooner without risk of penalty by the processor because of under-colored fruit.

Sakamura and Francis (9) have extracted the red pigments from Early Black cranberries and have determined them to be four distinct anthocyanin pigments plus a number of minor ones (5). The enhancement of the anthocyanin pigments in cranberry fruit by preharvest sprays of the insecticide, malathion, has been reported by Eck (2), Shawa and Ingalsbe (11), and by Eaton, *et al.* (1). In work with both 'Early Black' and the 'Howes' varieties, Eck has shown that 2-1/2 lbs/A of active material of a 57% emulsifiable concentrate (E.C.) of malathion significantly increased anthocyanin content in fruit when compared to unsprayed controls. The higher level of anthocyanin in the treated fruit was evident throughout the harvest season as indicated by weekly fruit samplings. In Washington, Shawa and Ingalsbe reported that 3.75 lbs/A active ingredient of 57% E. C. malathion gave the greatest ehancement of anthocyanin formation in 'McFarland' cranberries when applied three weeks before harvest as a foliage drench.

The Washington workers found that no yield reductions occurred as the result of malathion sprays during the three years in which the color enhancement properties of the material were tested. Eck has reported however, that malathion is no longer recommended as an insecticide for use on cranberries in New Jersey because of observed phytotoxicity when applied to actively growing cranberry plants (2).

Alar has been reported to enhance anthocyanin development in apples (6, 12) and cherry (8). Eck (2) has reported, however, that Alar delayed anthocyanin development in the cranberry.

Russo, *et al.* (7) have associated

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Ethrel with the loss of chlorophyl in banana in a manner similar to the effect of ethylene. Preliminary reports have shown that Ethrel may enhance anthocyanin development in apples.[3]

Francis (4) used the Hunter Color and Color Difference Meter to measure color differences in whole cranberry fruit, and found that the Hunter "a" reading correlated very highly with pigment content for 'Early Black', although in later experimentation with an Agtron Model H and a Colormaster Differential Colorimeter, Francis was unable to correlate instrument reading of raw fruit with total pigment content.

In addition to measuring yield responses and total pigment content of the fresh fruit, color difference meter readings were also made to determine whether treatment differences in pigment content coincide with differences in reflectance and chromaticity. The use of a rapid objective method for evaluating red color intensity in cranberries would be highly desirable to the processor who must make a quality judgment on shipments of cranberries entering the plant.

On August 26, 1968 treatments of 2.5 lb/A active ingredient of 57% E. C. malathion, 2000 and 4000 ppm Alar[4] and 600 ppm Ethrel[5] were applied by hand sprayer at 100 psi to 'Early Black' cranberry vines at the rate of 200 gal/A. The malathion and alar plots received the same treatments in 1967 (2) but the Ethrel treatment was new in 1968. Each treatment and a check plot were replicated 5 times in a Latin square design. Fruit was harvested on September 11, 1968, counted, weighed, sized and frozen for color analysis at a later date. Duplicate samples of treated and control berries were analyzed for anthocyanin content according to the method of Servadio and Francis (10). A Gardner Color Difference Meter (GCDM) using a color standard with the following reflectance and color coordinate values: R^d, 5.7; a, + 23.7; b, + 6.1, was used to evaluate visual color differences exhibited by a ground sample of cranberries rather than by whole fruit as utilized by Francis (4). The sample for GCDM analysis was prepared by grinding an undiluted quantity of frozen and subsequently thawed fruit in a Waring blender for two minutes. The ground sample was immediately placed in a sample cup and read on the GCDM. The Duncan Multiple Range Test was used to test for significance between treatments.

Table 1. Color enhancement of 'Early Black' cranberry fruit from preharvest chemical treatment.

Treatment	Pigment content[1] mg C.R./g	Color-difference meter readings Rd	a	b	a/b
2000 ppm Alar	.140 a[2]	5.0 a	31.4 a	5.2 a	0.6 a
4000 ppm Alar	.149 a	5.0 a	3.14 a	5.3 a	0.7 a
Check	.154 a	4.2 b	30.9 ab	3.8 b	0.9 a
2-1/2 lbs Malathion	.173 ab	3.7 bc	29.9 bc	2.5 bc	1.4 a
6000 ppm Ethrel	.189 b	3.2 c	29.1 c	1.3 c	2.7 b

[1]Expressed as mg of Congo red/g fresh fruit.

[2]Any two means in a column that do not share a similar letter are significantly differently at the 5% level of confidence.

Table 2. Cranberry yield and fruit size from plots treated with various chemicals to enhance color development.[1]

Treatment	Gm of fruit/ ft²	No. of berries/ ft²	Cup count, no. berries/standard cup
Check	112	149	139
2000 ppm Alar	114	154	138
4000 ppm Alar	112	148	141
2-1/2 lbs Malathion	112	144	138
600 ppm Ethrel	102	139	142

[1]No significant difference between treatments in yield or berry size.

Ethrel-treated cranberries contained more anthocyanin than any other berries (Table 1). The increase in pigment content over the control and Alar levels was significant, but the difference in pigment content between malathion-treated and Ethrel-treated fruit was not significant. Alar treatment resulted in the poorest colored fruit. Color differences as indicated by data from the GCDM coincided with the pigment differences noted between treatments. Reflectance values from Alar-treated fruit were significantly greater than from the check, malathion, and Ethrel-treated fruit. Both malathion and Ethrel-treated fruit had higher 'a' readings but lower 'b' readings than did the Alar-treated fruit. The a/b ratio for the ethrel–treated fruit was significantly greater than for any of the other treatments.

Yield as measured by the weight or the number of berries produced per square foot was not affected by the treatments (Table 2). The size of the fruit, as measured by the number of fruit required to fill a standard cup, was not influenced by the treatments.

Ethrel appears to be a relatively strong red–color enhancement agent for cranberry fruit. The loss in greeness noted in the Ethrel-treated fruit may be an indication that some of this color enhancement may come about as the result of chlorophyll breakdown as noted by Russo, *et al.* (7). The increase in anthocyanin pigment concentration, however, would suggest that some biochemical mechanism is involved that has accelerated the production of anthocyanin in the fruit. Although not significant, malathion treated fruit was higher in anthocyanin than the control fruit. In the previous years data, these same treatment plots yielded fruit that had significantly more anthocyanin than the control plots. The failure again to produce a significant increase in anthocyanin content may suggest that environment may influence the malathion response. As in 1967, Alar-treated berries were again the poorest colored fruit.

The fact that the reflectance and chromaticity values obtained by the GCDM for ground cranberry fruit coincided with differences in pigment content occurring between treatments suggests that the analysis of a ground sample of cranberries by the GCDM may be useful to evaluate pigment content of fruit. Under the conditions of this experiment, color quality differences that were not discernible in the pigment extractions of Alar-treated fruit were evident when R_d and "b"

[3]Personal communication. Mr. Armin Furrer, Amchem Products, Ambler, Pa.

[4]Trade mark of the U.S. Rubber Company for succinic acid 2, 2-dimethyl hydrazide.

[5]Trade mark of Amchem Products, Inc. for 2-chloroethane phosphonic acid.

values were compared to the controls.

Yields from the plots which received malathion were not different from the controls. These plots had received a similar malathion treatment the previous season; therefore, any effect the malathion may have had on the following seasons potential fruit production would have become evident. It appears that malathion does not have any deleterious effect on cranberry growth when applied late in the growing season, unlike the noticeable phytotoxicity when this insecticide is applied in the early part of the growing season for insect control. These results support Shawa's findings that malathion does not adversely influence the fruiting potential of the cranberry when applied late in the growing season as a preharvest spray. Whether or not Ethrel has influenced fruit production cannot be determined until next year's harvest, when Ethrel's influence on flowering and fruit set can be evaluated.

Literature Cited

1. Eaton, G. W., B. M. Zuckerman, A. Y. Shawa, P. Eck, M. N. Dana, R. Garren, and C. L. Lockhart. 1969. The effect of preharvest malathion sprays upon cranberry fruit color. *Proc. Amer. Soc. Hort. Sci.* (In Press).
2. Eck, P. 1968. Chemical color enhancement of cranberry fruit. *HortScience* 3(2):70-72.
3. Francis, F. J. 1957. Color and pigment measurements in fresh cranberries. *Proc. Amer. Soc. Hort. Sci.* 69:296-301.
4. ————. 1964. Cranberry color measurement. *Proc. Amer. Soc. Hort. Sci.* 85:312-317.
5. Fuleki, T. and Francis, F. J. 1967. Co-occurrence of monoglucosides and monogalactosides of cyanidin and peonidin in the American cranberry. *Phytochem.* 6:1705-8.
6. Mattus, G. E. 1967. Alar sprays in 1967. *Horticulture News* 48:8-11.
7. Russo, L., Jr., H. C. Dostal, and A. C. Leopold. 1968. Chemical regulation of fruit ripening. *BioScience* 18:109.
8. Ryugo, K. 1966. Persistence and mobility of Alar and its effect on anthocyanin metabolism in sweet cherries. *Proc. Amer. Soc. Hort. Sci.* 88:160-166.
9. Sakamura, S., and F. J. Francis. 1961. The anthocyanins of the American cranberry. *J. Food Sci.* 26:318-321.
10. Servadio, G. J. and F. J. Francis. 1963. Relation between color of cranberries and color and stability of sauce. *Food Technology* 170:124-218.
11. Shawa, A. Y., and D. W. Ingalsbe. 1968. Anthocyanin enhancement in 'McFarlin' cranberries at optimum maturity. *Proc. Amer. Soc. Hort. Sci.* 93:289-292.
12. Shutak, V. G., J. T. Kitchin, and M. M. Dayawon. 1966. Effects of N-dimethyl amino succinamic acid on quality of Cortland apples. *HortScience* 1(1):27-28.

Polyploid Mutants in Grapes

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The value of tetraploid plants of *Vitis* for breeding has been pointed out on a number of occasions (9, 2, 13). Such mutants occur spontaneously in vineyards and are noticeable mainly by enlarged berries, thicker bunch stems, and darker green leaves. Collections have been accumulated either from vines or parts of vines found in the field (10) or from colchicine-induced mutants (2). Such plants are often cytochimaeras (4, 12) and a 2–4 constitution has been most frequently reported. If the inner layer determines behavior in breeding (3) either 2–4 or 4–4 plants could be used for this purpose.

As well as direct chromosome estimates or counts, estimation of nuclear size (12) or quantitative DNA estimation (11) has been used to determine the ploidy of nuclei. Chromosome counts are especially laborious in grapes as the chromosomes are small and only few cell divisions are present in an apex. Measurements of the size of nuclei may be misleading, since Leuchtenberger and Schrader (7) have shown for animal tissues that the size of a nucleus depends on its protein content and not on the amount of DNA present. The determination of the amount or DNA by Feulgen spectrophotometry of fluorometry as reviewed by Leuchtenberger (6) and Ruch (11) is simple and accurate, and represents a true indication of ploidy.

Mutants which appeared to be tetraploid were collected as potential breeding material from some of the varieties most commonly grown in the Mildura district (Victoria, Australia) as follows:

GORDO (syn. Muscat of Alexandria): –Sweeney, Jones
WALTHAM (syn. Rosaki, Dattier): –Centennial, Metcalf
LISTAN (syn. Palomino): –Baumann
SULTANA (syn. Thompson seedless, Sultanina): –Case

The mutants are named after the growers on whose properties they were found, with the exception of the Centennial, which had been propagated from a Waltham vine near Bendigo over a century ago.

These mutants were examined for polyploidy together with material from an apparently diploid Pinot Noir. The apices of rapidly growing shoots were dissected and fixed in 4% neutral formalin for 24 hours. After washing in water they were dehydrated, embedded in wax and sectioned on a rotary microtome at 7μ thickness. Alternate slides were stained with red Feulgen and with fluorescent Feulgen respectively (6, 5). Nuclear size was determined in the sections stained with red Feulgen, the fluorescence was used for quantitative DNA determination. Forty nuclei were examined in each case.

Fig. 1 shows the distribution of nuclei according to diameter and to DNA content expressed as relative fluorescence. With respect to DNA content, there were two clearly defined classes of nuclei emitting fluorescence of 5 and 10 arbitrary units respectively. No such clearly defined classes could be distinguished for nuclear size.

The DNA values indicate that all the mutants except Jones Gordo have the constitution 4–4 and Jones Gordo is 2–4 while Pinot Noir is fully diploid (2–2). The values for nuclear size on the other hand might suggest that Sweeney Gordo, Centennial, Metcalf Waltham and Case Sultana are 2–4, that Baumann Listan is 4–2 and that Pinot Noir could not be classified at all. The nuclear sizes of the two apical layers differed highly significantly in all except Pinot Noir.

The two tetraploid mutants of Muscat of Alexandria are similar in appearance. While their fruit is indistinguishable, the leaves of Jones Gordo (2–4) are slightly but distinctly