Influence of Preharvest Applications of Malathion and Indole-3-Acetic Acid on Anthocyanin Development in *Vaccinium macrocarpon*, var. 'Early Black'[1]

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Abstract. The purpose of this study was to test the effect of malathion and IAA on color development in the cultivated cranberry, *Vaccinium macrocarpon* var. 'Early Black'. Dosage and time of application were evaluated. Quantitative analyses for anthocyanins of berries fresh-frozen at harvest showed that applications of 800, 1600, 2400 ppm malathion all caused a highly significant increase in color. Applications of IAA at 30 and 50 ppm did not affect color development. Treated and untreated berries were also analyzed for anthocyanin development after 7 and 14 days in common storage. No significant differences in size or yield were observed between treated and untreated berries. It was concluded that malathion applied 2 weeks before harvest at 1600 ppm would give good color enhancement and still be within the label restrictions for the use of this material on cranberries.

Introduction

RELATIVELY large amounts of anthocyanin pigments are synthesized during the ripening period of the cranberry, *Vaccinium macrocarpon*, imparting to the mature fruit an attractive red color. Thus, as in apples and many other fruits, there is a direct relationship between color development in the cranberry and its commercial value. However, in order to obtain dark, uniformly colored berries the grower must harvest at a relatively late date. A necessary consequence of late harvest is the cost and inconvenience of frost protection. Obviously, if a method could be developed that would allow early harvest of berries containing the desired amount of color, it would be of economic importance to the grower.

Preliminary tests by the senior author (3) indicated that applications of either indole-3-acetic acid (IAA) or 0,0-dimethyl dithiophosphate of diethyl mercaptosuccinate (malathion) might be used as a practical means of increasing anthocyanin development in cranberries. Exogenously applied IAA can induce the synthesis of new RNA and protein in a variety of plant tissues (2, 12, 14). The action of IAA, in this respect, may be a controlling factor in the synthesis of anthocyanins. For example, Thimann and Radner (17) demonstrated that anthocyanin synthesis in *Spirodela* is inhibited by purine and pyrimidine antagonists. Their study implies that RNA synthesis and, consequently, protein synthesis are necessary for the formation of anthocyanins. Stafford (16) found that protein synthesis is a rate-limiting factor in the maximum accumulation of the 3 anthocyanins of *Sorghum vulgare*. Finally, inhibitors of RNA and protein synthesis retard anthocyanin formation.

Malathion is a toxicant commonly used on cranberry bogs for the control of several destructive pests such as the cranberry fruit worm, girdler moths, and the black-headed fireworm. Although quite toxic to these pests as a cholinesterase inhibitor, malathion was found to be nonphytotoxic with respect to the cranberry plant when applied at the correct time. However, growers using this compound on cranberries have consistently reported that where malathion is applied a much redder berry is harvested. Recently, Eck (6) has shown that preharvest applications of malathion enhance anthocyanin development in both 'Howes' and 'Early Black' cranberries. These reports suggest that malathion when applied to cranberries enhances the synthesis of anthocyanin pigments. The purpose of this study was to analyze quantitatively the effect of IAA and malathion in color development in cranberries on the vine and in common storage. Dosage, time of application, and effect on size and yield were also evaluated.

Materials and Methods

Field design and harvest procedure. A latin square consisting of 36 10-ft square test plots was established in a section of weed-free cranberry bog containing a relatively consistent stand of vines. Each test plot was divided into 4 subplots which were lettered A–D. Thus, the latin square contained 144 subplots, designated 1A–D, 2A–D, etc. In this arrangement each individual treatment was replicated 6 times. IAA was applied at concentrations of 30 and 50 ppm while malathion (25% wettable powder) was applied at 800, 1600 and 2400 ppm. Both compounds were applied at a rate equivalent to 100 gal/acre.

All A subplots were treated 21 days before harvest, B subplots 14 days before harvest, and C subplots 7 days before harvest. Subplots designated with the letter D received no treatment. The test plots were hand-harvested and the berries immediately weighed. Berry size was determined by the number of berries required to fill a standard 8-oz. cranberry measuring cup. Subsamples were taken from each sample at harvest and again after 7 and 14 days in common storage (ambient shed temperatures ranged from 17 to 24°C). Immediately after sampling, the berries were frozen to stop further color development. Pigment concentration was determined after all subsamples had been collected.

Color analysis. The techniques used in the analysis for anthocyanin content were primarily those of Francis and Atwood (7) and Servadio and Francis (15). Fifty g aliquots of berries from each subsample were blended for 4 min with a 400 ml mixture of 0.1*N* HCl and 95% ethanol (15:85). The homogenate was decanted and allowed to stand for 30 min, during which time most of the solid materials settled. A 2 ml aliquot of the clear supernatant was removed and diluted 10-fold with the aforementioned acid-ethanol solvent. After standing for 30 min, a small sample of the diluted juice was read at 535 mμ in a Beckman DU spectrophotometer. Beckman DU readings were converted to mg of anthocyanin per g of fresh fruit. The factors involved in the conversion of DU readings to mg of anthocyanin were determined by Fuleki and Francis (9) who have isolated, identified, and established the absorption coefficients for the anthocyanins present in the cranberry. The data were analyzed by analysis of variance (*f* value test).

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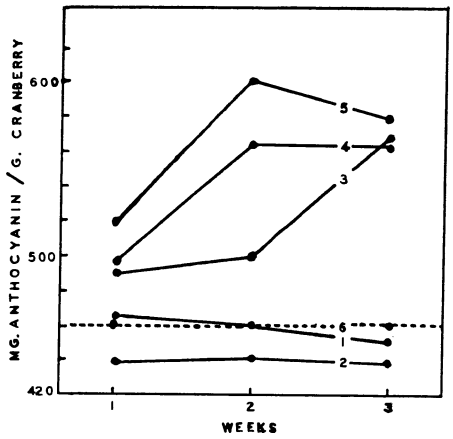

Fig. 1. Effect of preharvest applications of IAA and malathion on anthocyanin development in 'Early Black' cranberries. Each point on the graph represents an average of 6 replicates except for the control which represents an average of 9 replicates. Curves 1–5 describe the effect of IAA and malathion applied 1, 2, and 3 weeks before harvest. Curve 1 = 30 ppm IAA; 2 = 50 ppm IAA; 3 = 800 ppm malathion; 4= 1600 ppm malathion and 5 = 2400 ppm malathion. Curve 6 = control.

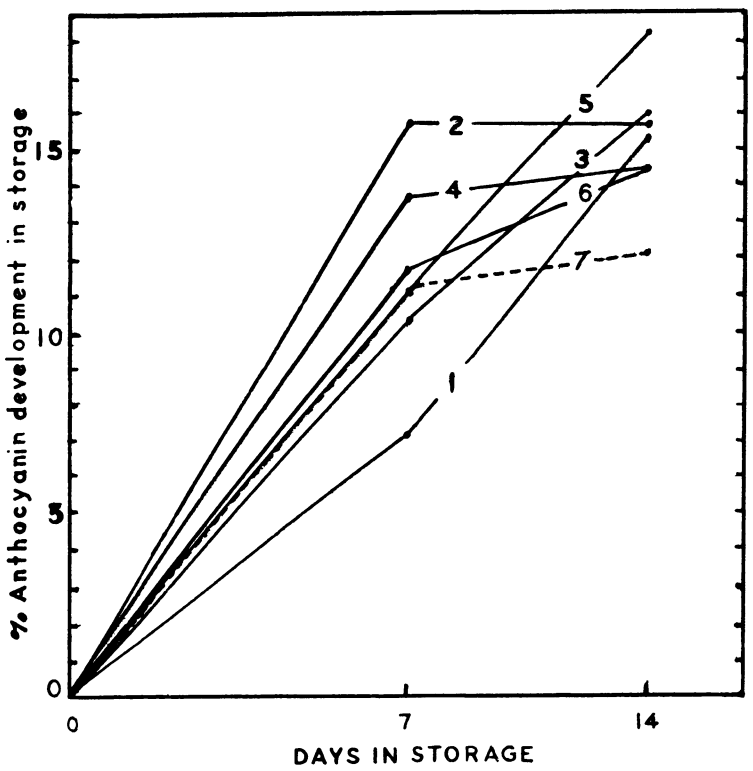

Fig. 2. Effect of preharvest applications of IAA on anthocyanin development in storage. Anthocyanin development is expressed as the per cent increase over the amount of anthocyanin found at harvest. Each point on the graph represents an average of 6 replicates except for the control which represents an average of 9 replicates. Curves 1–3 describe anthocyanin development in storage by berries treated with 30 ppm IAA 1, 2, and 3 weeks before harvest. Curves 4–6 describe anthocyanin development in storage by berries treated with 50 ppm IAA 1, 2, and 3 weeks before harvest. Curve 7 represents the control.

Results and Discussion

Color analysis at harvest (IAA). Berries treated with 30 ppm IAA 1 and 2 weeks before harvest were not significantly different from the control (Fig. 1). However, berries treated 3 weeks before harvest with 30 ppm IAA contained approximately 2.6% less anthocyanin than control berries. No significant difference in pigment development due to timing of treatment was observed. If all treatments are considered, IAA at 30 ppm retards color development (significant at the 5% level).

The retarding influence of IAA on pigment synthesis increased with increase in concentration from 30 to 50 ppm. Plots that received foliar sprays of 50 ppm IAA 3, 2, and 1 week before harvest contained berries with 5.0, 4.3, and 5.2% less anthocyanin than control berries (Fig. 1). Again, as in berries treated with 30 ppm IAA, no significant difference in pigment development was observed due to timing of treatments. Data in Fig. 1, demonstrating the inhibition of pigment development in berries treated with 50 ppm IAA, are significant at the 1% level.

Numerous studies have shown that anthocyanin formation is accompanied by increased oxygen consumption. Zanoni (18, 19) for example, observed that the rates of respiration in anthocyanin-containing tissues of many leaves were higher than those of green leaves. Increased respiration in the fruits of *Sambucus* and in the leaves of *Parthenocissus* during anthocyanin formation was noted by Eberhardt (5). Although IAA is known to increase respiration when applied to certain plant growth systems (10), it retards rather than accelerates anthocyanin development in cranberries.

As previously mentioned in the Introduction, IAA has been shown to induce new RNA and protein synthesis in several different plant growth systems (2, 12, 14). Conceivably, this could cause a shortage in protein metabolites some of which would include the aromatic amino acids phenylalanine, tyrosine, and tryptophan. Numerous studies have shown that these amino acids are involved in the synthesis of anthocyanins as noted in the review by Neish (13) and if their supply is depleted the synthesis of anthocyanins could be retarded.

Color analysis at harvest (malathion). Malathion applied at concentrations of 800, 1600, and 2400 ppm significantly increased pigmentation (Fig. 1). Berries from plots treated with 800 ppm malathion 3 weeks before harvest contained 24% more anthocyanin than control berries. Berries treated with the same concentration 2 and 1 week before harvest contained 9 and 6% more anthocyanin than untreated berries. Pigmentation increased with increase in malathion applied. Increases in anthocyanin production of 23, 22, and 7% were observed in berries treated 1600 ppm malathion 3, 2, and 1 week prior to harvest. The highest concentrations of anthocyanin were found in berries treated with 2400 ppm malathion. The per cent increases in pigmentation induced by 2400 ppm malathion applied 3, 2, and 1 week before harvest were 25, 30 and 8%, respectively. Analysis of the data show that malathion treatments caused a highly significant (1%) increase in pigmentation.

In almost all malathion treatments, pigment development was greatest in those berries exposed to malathion for the longest period of time (3 weeks). Only in the 2400 ppm malathion application, 3 weeks before harvest, was there a slight decline in anthocyanin production as compared to berries exposed to the same application for 2 weeks. However, the slight difference found in this case was not significant. These data confirm Eck's study (6) in which he found that preharvest appli-

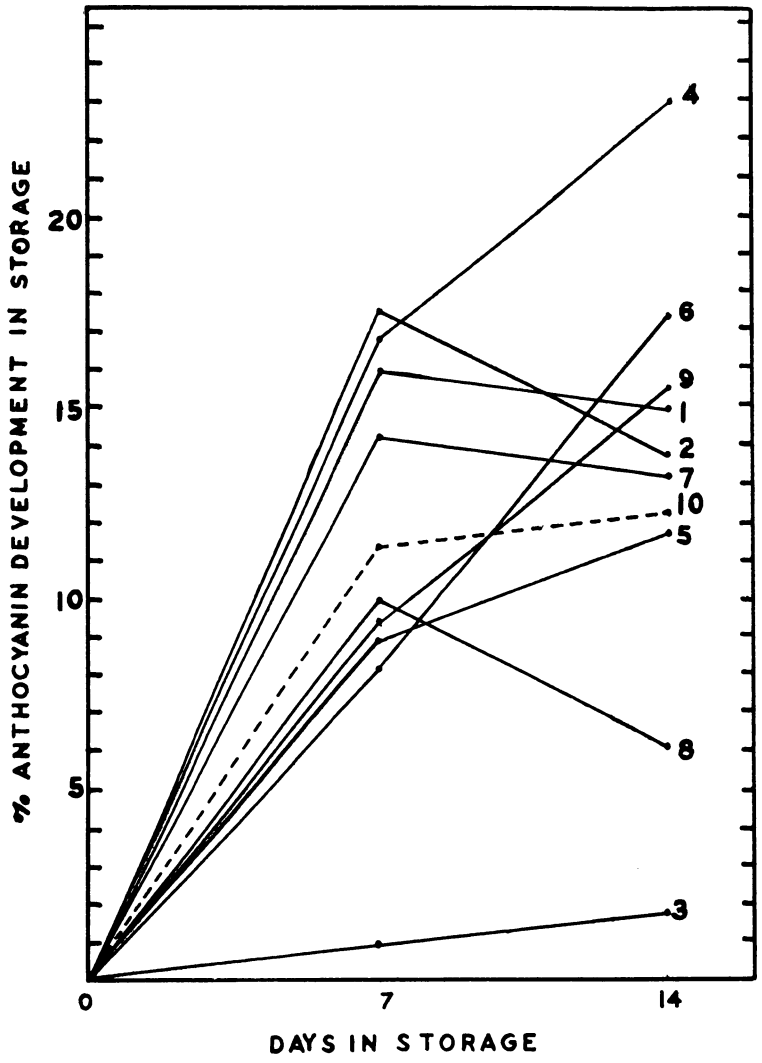

Fig. 3. Effect of preharvest applications of malathion on anthocyanin development in storage. Anthocyanin development is expressed as the per cent increase over the amount of anthocyanin found at harvest. Each point on the graph represents an average of 6 replicates except for the control which represents an average of 9 replicates. Curves 1–3 describe anthocyanin development in storage by berries treated with 800 ppm malathion 1, 2, and 3 weeks before harvest. Curves 4–6 describe anthocyanin development in storage by berries treated with 1600 ppm malathion 1, 2, and 3 weeks before harvest. Curves 7–9 describe anthocyanin development by berries treated with 2400 ppm malathion 1, 2, and 3 weeks before harvest. Curve 10 represents the control.

cations of malathion enhance color development in cranberries.

The simplest explanation for the stimulatory influence of malathion on pigmentation is that the compound is phytotoxic when applied to cranberries. Cranberries which have not completely ripened typically respond to injury with a rapid synthesis of anthocyanins. This occurs when the berries are frost-nipped, punctured, bruised, infected, or otherwise damaged. However, it should be pointed out that, aside from a few reports of plant injury (1, 11), malathion when applied as recommended is considered nonphytotoxic.

Color development in storage (IAA). It is a well established fact that pigment development by cranberries continues while in common storage (4, 8). Therefore, it is of interest to determine what effect, if any, preharvest treatments with IAA and malathion have on color development in storage. Pigment development by IAA-treated and control berries after 7 and 14 days in common storage is illustrated in Fig. 2. Each point on the graph represents the per cent increase in anthocyanin production while in storage over the amount of anthocyanins at harvest.

Color analyses after 7 days in common storage revealed no particular correlation between color development in storage and preharvest treatments with IAA. That is, no pattern of acceleration or inhibition of anthocyanin synthesis due to the concentration of IAA used could be established. However, after 14 days in common storage it was apparent that color development in IAA-treated berries had proceeded at a more rapid pace than in the untreated berries (Fig. 2). Anthocyanin development in storage by berries receiving 30 ppm IAA 1, 2, and 3 weeks prior to harvest exceeded the control by 3.4, 3.5, and 3.7%, respectively. Anthocyanin development in storage by berries receiving 50 ppm IAA 1, 2, and 3 weeks prior to harvest exceeded the control by 2.5, 5.5, and 2.5%, respectively. It appears that berries at harvest having less anthocyanins due to IAA treatments, regain this loss after 14 days in common storage.

Color development in storage (malathion). Again as in the IAA treatments, no particular relationship between the concentration of malathion applied prior to harvest and anthocyanin development in storage could be established (Fig. 3). Quite obviously, the greatest amount of pigment development takes place the first 7 days in storage no matter what treatment is applied. Color analyses after 14 days storage of treated and untreated berries show that the synthesis of anthocyanin pigments slows and in some cases completely stops during the period between 7 and 14 days of common storage (Fig. 3). This trend is also quite noticeable in Fig. 2. It can be concluded from this study that preharvest treatment of cranberries with either IAA or malathion does not impede color development in storage.

Effect of preharvest treatment with IAA or malathion on size and yield of cranberries. Size and yield were not significantly affected by IAA and malathion treatments (Table 1). Also, no significant difference in size or yield as a result of application timing could be discerned. However, there

Table 1. Effect of preharvest applications of IAA and malathion on yield (barrels/acre) and size of 'Early Black' cranberries at harvest. Each figure in the table represents an average of 6 replicates except for the control which represents an average of 9 replicates. CC = cup count.[a]

Treatment	1 week		2 weeks		3 weeks	
ppm IAA	CC	Yield	CC	Yield	CC	Yield
0	108.7	141.7	108.7	141.7	108.7	141.7
30	109.0	115.8	106.8	88.2	106.7	119.7
50	110.5	150.1	103.1	124.8	113.7	139.8
ppm malathion	CC	Yield	CC	Yield	CC	Yield
800	107.2	169.2	112.0	148.3	107.4	139.8
1600	108.8	142.3	109.5	115.8	114.2	126.1
2400	112.5	131.9	105.3	158.0	111.9	139.5

[a]No significant difference.

was a tendency toward larger yields (not significant) from plots treated 1 week before harvest.

It was concluded that malathion applied 2 weeks before harvest at 1600 ppm and at a rate equivalent to 100 gal/acre, would give good color enhancement and still be within the label restrictions for the use of this material on cranberries.

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Some Biochemical Effects in Apple Leaf Tissue Associated with the Use of Simazine and Amitrole[1]

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Abstract. Simazine at both 4 and 8 lb./A and amitrole at 2 lb./A, alone and in combination, altered the protein and RNA content of apple leaf tissue. Initially, simazine increased the protein content, but this effect progressively diminished until late June when it became nonexistent. Similarly, the use of amitrole caused an early season increase in protein content, later changing to a marked decrease. Greatest effects occurred in the total and globulin fractions while the soluble fraction was affected only slightly and this was limited to the first 6 weeks of growth.

Total RNA also was affected. This effect occurred in mid-May when simazine was stimulatory and amitrole acted as a depressant. These same effects persisted until late June when simazine at 4 lb./A and amitrole strongly decreased the RNA content. Enhanced action due to the simultaneous use of both chemicals did not occur, but there was evidence that the depressing effect due to amitrole was moderated when used with simazine, particularly in the period when shoot expansion ceased.

INTRODUCTION

INCREASING use of herbicide chemicals has created some apprehension concerning the possible residual effects, particularly with such chemicals as the triazines which possess marked persisting properties. Some experimental evidence indicated that their use promoted beneficial effects in the case of young apple and peach trees (4) wherein increases in leaf proteins have been observed. It also has been demonstrated that simazine, diuron and amitrole can be used at 3 to 4 times the usual rates on young apple trees with no apparent injury (6).

In 1965, a local nursery noted several unusual effects, apparently associated with the use of simazine and amitrole. One planting of spur type 'Red Delicious' developed a nice branching habit, contrasting with the usual single stem whips. Another group of trees exhibited symptoms resembling those of the rubbery wood virus (3). Such observations indicated a need for examining the use of these chemicals under more clearly defined conditions. We were unable to reproduce any of these observed abnormalties, but certain physiological changes were evaluated by measuring the effects upon the RNA and protein fractions.

MATERIALS AND METHODS

Spur type 'Red Delicious' trees were obtained from a nursery and set out in early March in 3 groups of 6 trees, each group of trees containing 6 treatments. Simazine at 4 and 8 lb./A and amitrole at 2 lb./A, alone and in combination, were applied on April 1, 1966. These treatments followed a modificated Latin square type of design, but the trees developed such poor growth during the unfavorable 1966 growing season, that no leaves were collected. On April 1, 1967, the chemical treatments were repeated and subsequent collections of leaf tissue were made at appropriate intervals. The first collection was made on April 11 when 4 to 5 fully expanded leaves appeared on the terminals. Similarly aged tip leaves were harvested periodically until growth ceased in late June. The harvested leaves were lyophilized and stored as dry leaf powder until used. Total protein was estimated on the leaf powder by a micro-Kjeldahl method. Soluble proteins were extracted with borate buffer, pH 8.3 (9) and measured ac-

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