then filtered through no. 1 Whatman filter paper in a Buchner funnel into a 500 ml filtering flask, marked at the 450 ml level. After the first run filtrate of approx 250 ml is complete the pomace is broken up and mixed with 125 ml of extracting solvent. This is then filtered and the residue washed with solvent until a total of 450 ml of extract is collected. The extract is quantitatively transferred to a 500 ml volumetric flask and made to volume using solvent washings of the filtering flask. Two ml of the extract are transferred to a 100 ml volumetric flask and made to vol using extracting solvent. The extract is stored, in the dark, for 2 hr at room temp and the pH measured to ensure that it is 1.0 ± 0.1. The absorbance is then measured at 535 nm for the total anthocyanin content, using 1 cm cuvettes and distilled water as a blank. The total anthocyanin content of the samples in mg anthocyanin/100g of cranberries can then be calculated using the appropriate wt, volume, and dilution factor and the average E value for the anthocyanins, as outlined by Fuleki and Francis (6):

$$\text{Total anthocyanin (mg/100g)} = \text{Absorbance} \times \text{dilution factor} \times 10/\text{Avg } E^{1\%}_{535} = \frac{\text{Absorbance} \times \text{dilution factor}}{98.2}$$

If desired the total flavonol content, in terms of mg quercetin/100 g of cranberries, can then be determined on the same extract, as outlined by Lees and Francis (7). The total flavonol content is measured at 374 nm using distilled water as a blank and then calculated as follows:

$$\text{Total flavonol (mg quercetin/100g)} = \text{Absorbance} \times \text{dilution factor} \times 10/E^{1\%}_{374} = \frac{\text{Absorbance} \times \text{dilution factor}}{76.5}$$

A sample application of these calculations can be seen in the following example:

Sample size = 100g

Original cranberry extract = 500 ml, of which 2 ml was diluted to 100 ml for absorbance readings.

Anthocyanin reading at 535 nm = absorbance of 0.250

Flavonol reading at 374 nm = absorbance of 0.112

$$\text{Dilution factor} = \frac{500}{2} \times 100 = 25{,}000$$

$$\text{Total anthocyanin} = \frac{0.250 \times 25000}{98.2} = 63.4 \text{ mg/100g}$$

$$\text{Total flavonol} = \frac{0.112 \times 25000}{76.6} = 33.6 \text{ mg/100g}$$

It is believed that the use of these methods singularly or together, would result in a uniform method of reporting the findings of experiments on cranberry pigmentation and thus enable an easier evaluation of the experiments in this field on the same basis, i.e. in terms of actual pigment content.

Some workers may comment that they do not have a 100g sample available. In this case, they may have to use what they have and increase the final answer accordingly. It may or may not be necessary to scale down the amount of extracting liquid or the dilution. Similarly, if a 5 mm cuvette was used, the answer could be multiplied by 2.

The anthocyanin portion of this method works well for a wide variety of other materials such as apple skins, raspberries, gooseberries, red currents, viburnum berries, etc., and the results expressed in terms of cyanidin galactoside.

Literature Cited

1. Craker, L. E. 1971. Postharvest color promotion in cranberry with ethylene. *HortScience.* 6:137-139.
2. Devlin, R. M., B. M. Zuckerman, and I. E. Demoranville. 1969. Influence of preharvest applications of malathion and indole-3-acetic acid on anthocyanin development in *Vaccinium macrocarpon,* var. 'Early Black'. *J. Amer. Soc. Hort. Sci.* 94:52-55.
3. Eaton, G. W., B. M. Zuckerman, A. Y. Shawa, P. Eck, M. N. Dana, R. Garren, and H. R. Lockhart. 1969. The effect of preharvest malathion sprays upon cranberry fruit color. *J. Amer. Soc. Hort. Sci.* 94:590-592.
4. Eck, P. 1968. Chemical color enhancement of cranberry fruit. *HortScience* 3:70-72.
5. ———. 1969. Effect of preharvest sprays of ethrel, alar, malathion on anthocyanin content of Early Black cranberry (*Vaccinium Macrocarpon* Ait.) *HortScience* 4:224-226.
6. Fuleki, T. and F. J. Francis. 1968. Quantitative methods for anthocyanins. I. Extraction and determination of total anthocyanin in cranberries. *J. Food Sci.* 33:72-77.
7. Lees, D. H. and F. J. Francis. 1971. Quantitative methods for anthocyanins. VI. Flavonols and anthocyanins in cranberries. *J. Food Sci.* In press.
8. Servadio, G. J. and F. J. Francis. 1963. Relation between color of cranberries and color and stability of sauce. *Food Technol.* 17:124-128.
9. Shawa, A. Y. and D. W. Ingalsbe. 1968. Anthocyanin enhancement in 'McFarlin' cranberries at optimum maturity. *Proc. Amer. Soc. Hort Sci.* 93:289-292.

Flower Opening, Pollen Shedding, Stigma Receptivity and Pollen Tube Growth in the Cranberry[1]

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Abstract. **Cranberry flower development was studied in the greenhouse on uprights thinned to a single flower. Flowers started opening each hour of the day. The interval from petal separation to fully open flowers varied from 2 to 12 hr with 80% of the flowers fully open within 6 hr. Elongation of the style and emergence of the stigma through the anther ring occurred on 94% of the flowers during the 24 - 48 hr period after the petals were fully reflexed. The stigma was pollen receptive at the time of petal separation. The pollen tube had traversed the style 48 hr after pollination in 37% of the flowers examined. Removal of the style 72 hr after pollination no longer prevented fruit development.**

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The cranberry, *Vaccinium macrocarpon* Ait., flower bud opens by separation of the petals which curl back (reflex) exposing the ring of stamens surrounding the pistil. When the petals are first reflexed the stigma is not visible unless the stamens are mechanically separated. The pollen is mature at the time of petal separation but the stigma is not exposed for natural pollination until sometime later. The cv. Stevens was used to determine the time sequence of these developmental stages under greenhouse conditions.

Uprights with dormant (flower) buds were rooted in sphagnum moss before transfer to nutrient solution in the greenhouse. Natural daylight was supplemented with a combination of incandescent and fluorescent lamps to provide a 16 hr photoperiod with temp of 18°C night and 24-32° day. After the shoots developed to the "hook" stage each upright was thinned to one flower.

Flower opening and pollen shedding. More flowers opened during 2 hr intervals during daylight than at night. Differences were attributed to temp effects. Approx 80% of the flowers were fully open in 6 hr after petal separation. Flowers starting to open late in the day developed more slowly than those in the middle of the day, a response to high mid-day temp. In 24-48 hr, 94% of the flowers moved from fully reflexed petals to first appearance of the stigma.

A microscope slide painted black was placed 6 mm below and perpendicular to the anther tubes of each flower to intercept pollen tetrads shed by gravity or by the flower opening processes. After full flower development, microscopic examination of 100 slides showed that 97 had no pollen tetrads and 3 had a negligible amount. Apparently, the pendulous flowers shed pollen only when agitated.

Receptivity of the stigma. At the petal separation stage 200 flowers were emasculated and immediately hand pollinated with pollen from different plants. At this stage the stigma was about 1 mm short of reaching the distal end of the anther tubes. The test flowers were shielded against any further pollination. Another 50 flowers were emasculated at this early development stage but were pollinated 6 days later when the styles had fully elongated and the stigma was known to be pollen receptive. Of the flowers pollinated at petal separation, 90% showed ovary swelling indicative of ovule fertilization and 70% developed mature fruit. Of the flowers pollinated 6 days after petal separation, 92% developed mature fruit. The stigma was therefore receptive at the petal separation stage of flower development. Normally the stigma is not exposed this early and the pollen may be shed before the stigma approaches the same position as the terminal pores in the anther tubes.

Bain (1) reported that *"on the second or third day after flower opening the style elongates . . . and the flower now for the first time becomes receptive to pollen."* Cross (2) reported that Bergman found that the stigma did not become receptive until 24–36 hr after the flower has begun to shed pollen. Roberts and Struckmeyer (3) observed that the stigma was receptive when the style was equal in length to the anther tubes. Apparently receptivity of 'Stevens' is earlier than reported for other cultivars.

Growth of the pollen tube. At anthesis, 200 flowers were emasculated and hand pollinated. At intervals of 24, 48, 72, and 96 hr after pollination the styles were cut adjacent to the ovaries on 40 flowers for each time interval. In the controls, the styles were left intact. Flowers with styles cut 24 hr after pollination showed no growth of the ovaries. Flowers with styles cut at 48 hr showed fruit development in 37% of the ovaries. Removal of the style at 72 or 96 hr or not at all resulted in 66% of the flowers developing into fruit. At the greenhouse temp used (18-32°C) the time between pollination and ovule fertilization was more than 24 and less than 72 hr. Cooler temp probably would extend the time nearer to or beyond the 72 hr period.

The rate of flower development was related to diurnal temp differences with comparatively rapid growth during the day and slow changes at night. These differences would be intensified in the field with temp commonly reaching 10°C at night and 30° during the day. We anticipate that flower opening in the field would be concentrated during daylight but not limited to any portion of that period. In the field, the period for pollen tube growth and ovule fertilization would be longer than that found under our conditions because of temp differences. A cold period following pollination of a flower and a long delay before ovule fertilization may result in a failure to obtain fruit set.

The solitary flower in the axil of a bract appears to develop to fruit set independently of its neighbors on the main stem axis. Thinning flowers to one per upright should not influence the results found for early flower stages.

Literature Cited

1. Bain, H. F. 1933. Cross pollinating the cranberry. *Proc. Wisc. State Cranb. Growers' Assoc.* 47:7-11.
2. Cross, C. E. 1953. Cranberry flowers and the set of fruit. *Cranberries.* 17(12):7-9.
3. Roberts, R. H. and B. Esther Struckmeyer. 1942. Growth and fruiting of the cranberry. *Proc. Amer. Soc. Hort. Sci.* 40:373-379.

Nitrate Reductase in Leaves of Ericaceae[1]

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Abstract. **Nitrate reductase was induced in detached leaves of a number of species of Ericaceae by infiltration with KNO_3 solution after which enzymatic activity was determined. Considerable activity was found in the leaves of some species and cultivars indicating an inherent capability to synthesize the enzyme in the presence of nitrate. For many members of the ericaceous family, however, the very low level of activity found in the leaves suggests this organ may play little or no part in the reduction of nitrate.**

The utilization of nitrate by plants requires the presence of nitrate reductase, an adaptive enzyme occurring in most plant cells and tissues (2, 7). In most crop plants studied, the bulk of the activity appears to be in the leaves (6). However, appreciable activity occurs in the roots of some species (8), and the distribution of activity may depend on stage of maturity and on NO_3^- levels (14).

Most higher plants grow well with NO_3^- as a source of nitrogen, but members of the family Ericaceae have a well known preference for ammonium N (4, 5). Although blueberries (*Vaccinium* sp.) apparently can utilize both forms (10), there is little doubt that superior growth is achieved with NH_4 as the sole or partial source (12).

Townsend and Blatt (11) initially reported the absence of a NO_3^- reducing system in lowbush blueberry, but later discovered appreciable activity in the roots only (13). Since nitrate reductase is an adaptive enzyme, found only in the presence of NO_3^-, the question arises whether the lack of activity in blueberry leaves is due to an inability to synthesize the enzyme or because no NO_3^- reaches the leaves from the roots.

The objective of this study was to establish if leaves of blueberries and other ericaceous plants do in fact have the capability of synthesizing nitrate reductase. All plants were grown in the greenhouse in a peat:perlite mixture and fed with a complete commercial fertilizer containing only NH_4-N.

For enzyme induction, freshly harvested leaves were infiltrated for 24 hr with 0.05 M KNO_3 solution in enameled pans under cool-white fluorescent lamps. Humidity was maintained at approx 85% and light intensity at 1800μw/cm^2.

The enzyme assay was based on that described by Mulder et al. (9) as modified by Bar-Akiva et al. (3) and Townsend (11, 13). In this procedure, leaf discs are incubated in a solution of KNO_3; the NO_2^- which is produced by the enzyme diffuses into the solution and is measured colorimetrically. Because there is a min of tissue disruption, the enzyme is not affected

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