Nitrate Reductase Activity in the Leaves of the Highbush Blueberry and Other Plants[1]

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Abstract. A tissue infusion method was used to demonstrate nitrate reductase activity in the leaves of *Vaccinium corymbosum* L. grown with 3 N sources and in 2 pH regimes. Activity was not detectable by the extraction and assay of homogenized leaves of *Kalmia latifolia* L., *Leucothoe catesbaei* Gray, *Malus sylvestris* Mill., *Pieris japonica* D. Don., and *Rhododendron catawbiense* Michx. or in the roots of *Leucothoe* and *Zea mays* L., but it was detectable in both leaves and roots by the tissue infusion method.

There has been considerable controversy concerning the source of N for the growth and development of both the lowbush (*Vaccinium angustifolium* Ait.) and highbush (*Vaccinium corymbosum*) blueberries and other ericaceous plants. Several investigators (2, 3, 5, 14, 15, 16) have advocated the superiority of NH_4-N over NO_3-N while others (7, 10, 12) have stated that N source made little difference as long as other limiting factors were present in optimum amounts. Oertli (10) suggested that the absence of a biochemical system to utilize NO_3-N would be an interesting ecological explanation for the natural restriction of blueberries to acid soils. Recently, Townsend (17) presented evidence for a nitrate reducing system in the roots of the lowbush blueberry grown on NO_3-N at pH 4 and 6 and NH_4-N at pH 6. He was, however, unable to detect activity in the leaves, usually the site of highest nitrate reductase activity in plants (11).

Materials and Methods

Cultural. Thirty 3-year-old highbush blueberry, *Vaccinium corymbosum* cv. Jersey, plants were grown in a 2:2:1, soil:peat:perlite medium adjusted to pH 4.2 or 6.2. Three N sources, $(NH_4)_2SO_4$, KNO_3 plus $(NH_4)_2SO_4$, or KNO_3 were supplied daily at a concn of 50 ppm N in 250 ml volume. The other essential elements were supplied daily at one-half Hoagland's (6) concn. The initial pH of the medium was 4.2. Adjustment to approx 6.2 was made by the addition of CaO at the rate of 18 g per 1,000 g of growing medium. The treatments were initiated on June 21, 1971 and leaves were sampled for nitrate reductase activity 30 and 50 days later.

Kalmia latifolia, Leucothoe catesbaei, Malus sylvestris cv. McIntosh seedlings. *Pieris japonica, Rhododendron catawbiense* cv. Roseum Elegans and *Zea mays* cv. Harris Gold Cup were grown in the same potting medium and were supplied with Hoagland's (6) solution at least 2 days before sampling for enzyme assays.

Analytical. A modification of Mulder's (1, 9, 17) procedure for nitrate reductase extraction was employed. One-g tissue samples were used in all assays. The leaves were washed with distilled water and blotted dry with paper towels. Sterilization of the leaves with 2% hypochlorite before sectioning did not affect nitrate reductase activity and was not routinely used. The tissue was then sectioned into millimeter-sized sections and placed in a side-arm test tube. To the test tube were added 5 ml 0.06*M* phosphate buffer, pH 7.5, 1 ml 0.1 *M* succinate neutralized to pH 7.0 with NaOH, 1 ml of 0.1*M* KNO_3 and 2 ml distilled water. The preparation was incubated for 30 min. No advantage was gained through vacuum evacuation of the tubes to hasten the diffusion of the incubation medium into the tissue sections.

Townsend (17) reported nitrite loss upon cessation of the reaction and addition of the color developing reagents. This problem was circumvented by filtering the incubation solution directly into a 250 ml Erlenmeyer filtering flask containing the color developing reagents: 2 ml of 1% w/v sulfanilamide in 1.5*N* HCl plus 2 ml of 0.02% w/v N-(1-naphthyl)-ethylenediamine dihydrochloride in 0.2*N* HCl. The color which developed with these reagents was read at 540 nm with a Beckman DU-2 spectrophotometer.

Nitrate reductase was also extracted and assayed for activity utilizing the method of Hageman and Flesher (4). In this case 4 g of tissue were homogenized in 16 ml of 0.2*M* tris, 0.01*M* L-(+)-cysteine, and 3 x 10^{-4} *M* EDTA adjusted to pH 7.5 with 2*N* HCl and incubated for 30 minutes.

Results and Discussion

Nitrate reductase activity was detected in leaves of blueberry plants grown under various pH and N regimes (Table 1) when the modified Mulder assay method, henceforth designated as the tissue infusion method, was employed. The tissue homogenate method of Hageman and Flesher (4) did not permit detection of enzyme activity. With the infusion method, no significant differences among treatments occurred on the 1st sampling date. On the 2nd sampling date, 20 days later, nitrate reductase activity was greatest in the leaves from plants grown on NO_3-N. Soil pH had a significant effect on activity when both NO_3 and NH_4 were provided.

Although measurements were not made of blueberry growth, no visual differences were noted during this experiment. All plants appeared healthy and vigorous with no chlorosis or toxicity symptoms. Several weeks after treatments were ceased and only water was provided, all the plants receiving NO_3-N plus NH_4-N died. No plants in the other treatments died. In previous experiments (unpublished data) with *Leucothoe* and *Rhododendron*, NO_3-N proved to be as good or superior to urea and NH_4-N in many parameters employed in determining the growth of these 2 species.

Table 1. Nitrate reductase activity of leaves of blueberry.

N source	pH	Assay and days on treatment: Homogenate 30	Homogenate 50	Infusion 30	Infusion 50
		mμmoles NO_2^- produced/g fr wt/hr[z]			
NO_3	4	0	0	15.0a	15.0c
NO_3 plus NH_4	4	0	0	15.0a	12.0b
NH_4	4	0	0	15.0a	11.0b
NO_3	6	0	0	12.5a	14.0c
NO_3 plus NH_4	6	0	0	12.7a	8.3a
NH_4	6	0	0	13.0a	11.5b

[z]All values are the mean of 5 determinations. Means followed by different letters are significantly different ($P \leq 0.05$) within columns.

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[4]Polyclar AT. Available from GAF Corporation, Dyestuff and Chemical Division, 140 West 51 Street, New York, N. Y. 10020.

Table 2. Nitrate reductase activity in leaves and roots of some species as determined by 2 different extraction procedures.

Species	Plant part	Tissue homogenate −PVP	Tissue homogenate +PVP	Tissue infusion
		mμmoles NO_2^- produced/g fr wt/hr[z]		
Kalmia latifolia	Leaves	0	0	120 ± 18
Leucothoe catesbaei	Leaves	0	0	35 ± 15
Leucothoe catesbaei	Roots	0	0	80 ± 16
Malus sylvestris McIntosh	Leaves	0	0	333 ± 56
Pieris japonica	Leaves	0	0	16 ± 4
Rhododendron catawbiense Roseum elegans	Leaves	0	0	20 ± 6
Zea mays	Leaves	2240 ± 70	2240 ± 70	750 ± 13
Zea mays	Roots	0	0	140 ± 36

[z]All values are the mean of 10 determinations.

Although the tissue homogenate assay is used by many investigators, we believe that this method has certain disadvantages which have thus far precluded the detection of nitrate reductase in leaves of the Ericaceae. When leaves are homogenized, cells are broken, and phenols and other components of the vacuoles are mixed with the cytoplasmic matrix. Since phenols will inactivate nitrate reductase, Klepper and Hageman (8) modified Hageman and Flesher's (4) assay procedure by adding insoluble polyvinylpyrrolidone (PVP)[4] to apple leaves to complex the phenolic compounds. No nitrate reductase activity was detected in apple leaves (Table 2) by this procedure in our study, but activity was detected by the tissue infusion method. Blueberry and other ericaceous plants are also high in phenolic compounds. Comparisons of optical densities of leaf extracts show that blueberry and *Leucothoe* contain from 6 to 8 times the phenolic content of corn. PVP reduced this ratio to a 1- to 2-fold difference between the 2 ericaceous species and corn (Fig. 1) but did not permit detection of nitrate reductase activity in homogenized leaves. Although the phenolic content of *Leucothoe* in the presence of PVP was similar to that of corn with PVP, no activity could be detected in *Leucothoe*. This suggests that other compounds may act as inhibitors of nitrate reductase in this species.

Cellular rupture is minimized with the tissue infusion method. Since fewer cells are destroyed, there is less mixing of cellular components in comparison to the homogenization procedure. Therefore, nitrate reductase activity was easily detected in the leaves of ericaceous plants with the tissue infusion method whereas detection was impossible in previously applied procedures.

The in vitro rates of nitrate reduction in ericaceous plants appear low when compared to corn. Corn, in comparison to ericaceous plants, is a rapidly growing plant; thus, substantially greater rates of activity are required to meet its metabolic requirements. The observation of lower rates of enzyme activity with the infusion method with corn in relation to the

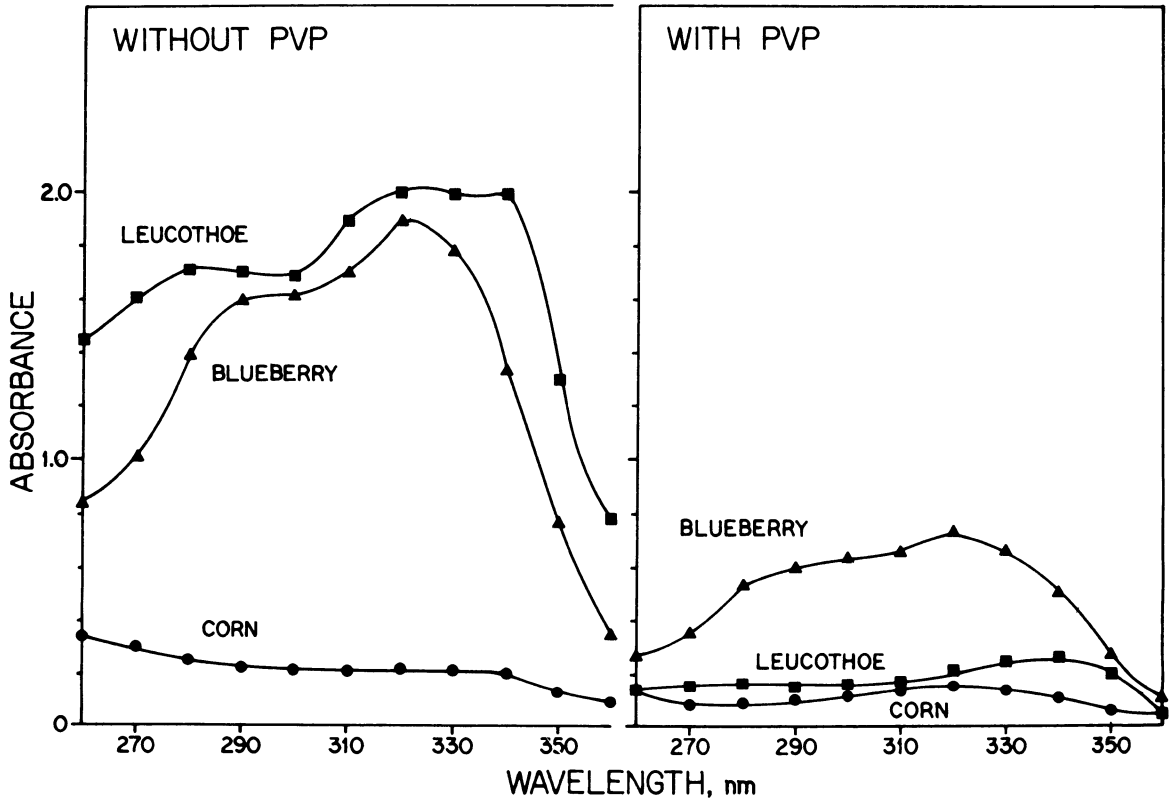

Fig. 1. Absorbance of extracts of homogenized corn, blueberry, and *Leucothoe* leaves. Leaves were ground in 0.2*M* tris buffer, pH 7.5, and diluted 100:1 with 0.1*M* phosphate buffer, pH 7.0. The absorbencies from 260 nm to 340 nm indicate the relative amounts of various phenolic compounds in the extracts (13). Left: Without polyvinylpyrrolidone (PVP) added during the homogenization and extraction. Right: 3 g of hydrated (Klepper and Hageman, 8) PVP per g fr wt of tissue added during homogenization and extraction.

homogenization procedure is probably related to reduced intimacy between the enzyme, cofactors, and substrate, the requirement for diffusion of reaction products out of the intact cells and the possibility of further metabolism of nitrate.

With blueberry roots, we observed by the infusion method nitrate reductase activities of 48 ± 18 mμ moles NO_2^- formed per hour. This observation was made with plants grown at pH 4 with NO_3 nutrition. This value is of the same order of magnitude as that found by Townsend (17) under similar conditions.

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High Intensity Supplementary Lighting Increases Yields of Greenhouse Roses[1]

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Abstract. Flower yields from October to May increased with the duration of supplementary lighting. Lighting improved flower yields by increasing bottom breaks, stimulating axillary shoot development after flower removal, and slightly reducing the days from cut-to-cut. Development of additional axillary buds was the principal factor in the improved branching of lighted plants. Excessive plant branching from supplementary lighting during a 7-month flowering period reduced significantly flower stem length, node number, and fresh wt of cvs. Shocking Pink and Red American Beauty. The 6.2 w/ft^2 of incandescent light after the high intensity lighting period improved the flower quality of 'Forever Yours' but not of 'Red American Beauty'. Plants lighted 9, 12, or 21 hr daily had fewer blind stems than unlighted plants.

Low winter light reduces greenhouse rose plant growth and flower yield. The capacity to regulate light, as achieved for other environmental factors, is necessary for the full control of plant growth in greenhouses. Supplementary high intensity lighting, above 10 watts/ft^2, has caused improved seed germination and faster seedling growth of gloxinia and cineraria during winter (10). African violets (11), begonia (2), cyclamen (9), gloxinia (10), and stock (4) flowered much earlier when they received supplementary high intensity lighting. Lighting during dark seasons increased the numbers of cuttings from stock plants of chrysanthemum (3) and foliage plants (6). Winter rose yields have been increased 24% by lighting nightly overhead for 5 hr with W.S. Gro-Lux lamps at 21 lamp watts/ft^2 (1). 'Better Times' roses lighted continuously from Feb. 1st to May 30th with W.S. Gro-Lux lamps at 32 lamp watts/ft^2 had yield increases over unlighted plants of 96% from lamps placed between the plants and 46% when overhead. Reduction of stem lengths and fresh wt of cut roses by supplementary lighting was attributed to flower injury from contact with hot lamp surfaces (8).

Our study was conducted to determine the extent and nature of the effect of supplementary lighting on winter growth and flowering of greenhouse roses.

Materials and Methods

One hundred ninety-two dormant plants each of rose cvs. Forever Yours and Shocking Pink were planted in adjacent 54-ft, 42-inch width, V-bottom ground benches April 15, 1969. The benches were sub-divided into 6 plots of 32 plants immediately after planting, and 6-ft opaque partitions were placed between plots and at bench ends. Four plots were randomly selected to receive supplemental lighting nightly and the remaining 2 were unlighted. Two, 4 by 8 ft, metal frame fixtures, each mounting six, 96-inch, 105 w, W.S. Gro-Lux lamps, were placed vertically in each lighted replication between interior rows 1-2 and 3-4. A black sateen cloth hung vertically was pulled in the aisle between the benches nightly. Plant spacing and the cultural practices followed were those recommended for roses (8).

Lighting treatments began Dec. 15, 1969, and discontinued May 1, 1970. Plots were randomly selected to receive: (a) no supplementary lighting, (b) 8 hr of supplemantary lighting

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