

Powell (5) to separate a no. of indole derivatives and naturally occurring plant growth regulators. We found that Bio-Gel P (Bio-Rad Laboratories, Richmond, Calif.) was easier to handle than silica gel and was equally effective in the method. Accordingly, a Bio-Gel P column was substituted for the silica gel column used by Powell. Eight g of Bio-Gel P6 (50-100 mesh, wet) were mixed with enough distilled water to cover and allowed to swell overnight with constant stirring. A 14 cm column was packed by gravity. The column was flushed with 150 ml of the 0.2% butyl alcohol eluting solvent (petroleum ether: *n*-butyl alcohol saturated with 0.5 M formic acid, 99.8:0.2) at a flow rate of 2-3 ml per min. Next, the sample was placed on top of the column and sequentially eluted with a series of 7 solvents, containing 0.2, 3, 10, 25, 50, 75 and 100% *n*-butyl alcohol.

Residues from the ether extraction procedure and from each of the column fractions were bio-assayed by the standard method of Nitsch and Nitsch (4) using first internode sections of 'Brighton' oat seedlings. In the gradient elution series only the 75% butyl alcohol fraction responded in the bio-assay. Moreover the response in this fraction was greater than that obtained by the standard ether extraction. Powell (5) found that indoleacetic acid (IAA) separates out in the 3% butyl alcohol fraction. Control extractions in which IAA was added to the plant extract prior to gradient elution confirmed this.

Other bio-assays were conducted by the coleoptile elongation method of Sirois (6) using basal coleoptile segments of 'Brighton' oats. In this procedure Sirois was able to differentiate between an IAA effect and a GA effect based on the duration of the bio-assay. Both substances produce

responses of the same magnitude when assayed for 20 hr but only IAA is active when the assay is limited to 5 hr. Our results (Table 1) showed that extracts of short-day buds gave no response at the end of 5 hr, but did following the 20 hr exposure. Extracts from long-day buds showed no response in the bio-assays.

Table 1. Biological activity of blueberry flower bud extracts in the *Avena* coleoptile test at 5 and 20 hr. Each figure (average of 20) is the difference in elongation of treated and control coleoptile sections.

Type of extract	$E_{tr} - E_0$ in mm	
	5 hr	20 hr
Short-day flower buds	0	0.8
Long-day flower buds	0	0
IAA standard 1 mg/l	0.8	1.2
GA standard 1 mg/l	0	1.2

Additional evidence of the gibberellin-like nature of the substance was shown by thin-layer chromatography. Thin layers of silica gel (Camag Kiesel-Gel D-5, 500u) were prepared on glass plates and were spotted with extracts from short-day and long-day buds as well as with samples of gibberellins A₁, A₃, A₅, A₉ and A₁₃. Benzene:acetic acid:water (2:1:1) was employed as the solvent system and the solvent was run without equilibration approx 15 cm at room temperature. When dry, the plates were sprayed with a 1% sulfuric acid solution and were heated at 100°C for about 10 min. The gibberellins were located on the plates by fluorescence in long wave ultra-violet light. We found that a single spot was separated from the short-day flower bud extract with an R_F value similar to that demonstrated for GA₁ and GA₃ (Table 2).

Attempts to purify and more closely identify the unknown compound using

Table 2. R_F values of gibberellins and bud extracts.

Sample	R _F value
Short-day buds	0.21
Gibberellin A ₁	0.21
Gibberellin A ₃	0.22
Gibberellin A ₅	0.54
Gibberellin A ₉	0.62 and 0.68
Gibberellin A ₁₃	0.66
Long-day buds	No spot

infra-red spectrophotometry were not successful. However, the evidence obtained supports the premise that the growth regulator produced in blueberry flower buds formed under short-day conditions is similar in its behaviour to the gibberellins. The development of these buds into naturally parthenocarpic fruit if maintained under short-day conditions indicates that this substance is under short-day control and continues to be produced so long at this regime is maintained.

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Volatiles of Lowbush Blueberry Nectar¹

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Abstract. Nectar from flowers of the lowbush blueberry, *Vaccinium angustifolium* Ait. was found to evolve acetaldehyde at a rate of approx 0.047 µg/g nectar/hr and ethyl alcohol, the only other volatile detected, in smaller amounts.

Most clones of lowbush blueberry are self-sterile and require cross pollination (1). Under natural conditions pollination of *Vaccinium* species in Nova Scotia is accomplished

by bumble bees (*Bombus terricola* Kirby, and *B. ternarius* Say), solitary bees (*Andrena lata* Vier, *Andrena carlini* Ckll., and *Dialictus cressonii* (Robt.)), and closely related species (4). Although bumble bees collect pollen for food, most insects visit the blossoms for nectar.

Since the flowers of the lowbush blueberry are an inconspicuous, pale-white color and often hidden by foliage, pollinating insects may find nectar by olfactory stimuli. Here we report our findings on nectar volatiles by isothermal gas liquid chromatography analysis.

A sample of nectar was obtained by centrifuging 50 flowers at approx 5,000 rpm for 2 min. A small piece of wire screen in the base of the wide portion of the tube allowed the nectar about 0.1 ml to settle in the base of the tube. Flowers and screen were removed before the tube was closed with a 1-hole cork. Two layers of tape were placed over the cork to allow removal of a gas sample for GLC analysis as in previous studies (2,3).

Isothermal GLC analysis for volatiles was carried out on 1.0 ml aliquots of gas with a Varian aerograph Model 204B equipped with a hydrogen flame ionization detector. The following liquid phases in stainless steel columns (1/8 in O.D.), their lengths, and the oven temperatures were:

- 20% Halcomid M18, 12 ft at 84°C.
- 10% Carbowax M20, 10 ft at 70°C.
- 20% Diethylene Glycol Succinate (DEGS), 10 ft at 70°C.

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The stationary phase in each of the columns was Chromosorb P AW DMCS. For all analyses helium carrier gas was supplied at 30 ml/min, and hydrogen flow was 20 ml/min with Hallcomid and 25 ml/min for Carbowax and DEGS.

The retention times in min of acetaldehyde, ethyl alcohol and 2 volatiles from lowbush blueberry nectar are given in Table 1. Since the retention times of the 2 volatiles corresponded with those of acetaldehyde and ethyl alcohol on the 3 columns, it is assumed that the latter 2 chemicals are the volatiles of lowbush blueberry nectar. From 3 separate samples, acetaldehyde was given off by the nectar at a rate of approx 0.0473 $\mu\text{g/g}$ nectar/hr. Ethyl alcohol production was somewhat less than this amount. In our work with

Table 1. Retention time in min of acetaldehyde, ethyl alcohol, and volatiles from 3 samples of lowbush blueberry nectar using 3 columns for GLC separation.

Volatiles	Retention time (min)		
	20% Hallcomid	10% Carbowax	20% DEGS
Acetaldehyde	1.25 \pm 0.00	1.75 \pm 0.00	2.38 \pm 0.00
Ethyl alcohol	5.25 \pm 0.13	5.75 \pm 0.00	7.13 \pm 0.00
Nectar volatile #1	1.25 \pm 0.00	1.75 \pm 0.00	2.38 \pm 0.00
Nectar volatile #2	5.25 \pm 0.13	5.75 \pm 0.00	7.13 \pm 0.00

blueberry flowers, we have not been able to smell these volatiles.

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Highbush Blueberry Fruit Set in Relation to Flower Morphology^{1,2}

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Abstract. Various flower parts were measured from 35 different cultivars and selections of highbush blueberry and correlated to their fruit setting capability. High fruit set was associated with a short distance between stigma and anther tip. The ideal flower from the standpoint of max fruit set appears to be one with a short corolla that widened at the middle to more than 8 mm and then narrowed perceptibly at the base. It is suggested that flower structure might serve the plant breeder as an additional criterion for evaluating the potential fruit setting capability of a blueberry selection.

The failure to obtain the high fruit set for commercial crops of highbush blueberries, particularly for certain cultivars such as 'Earliblue', 'Coville', and 'Stanley', may be related to inadequate pollination (2). There is probably more than one factor involved in determining the pollination efficiency of the blueberry flower of any particular cultivar. If these factors can be determined and evaluated with respect to their relative importance in the expedition of pollination and hence fruit set, the plant breeder may be able to eliminate plant selections that have characteristics predisposing them to poor pollination. The morphology of the flower represents one factor that might be related to insect pollination efficiency.

Although the blueberry flower is self-fruitful, its structure is such that insect pollination must occur in order to achieve fruit set. Individual flowers hang

in a pendant position from the raceme. The pistil is surrounded by 10 stamens, and extends beyond them—in some instances protruding beyond the rim of the corolla. The receptive surface of the stigma is never exposed to the pollen from the anther, the angled sides of the stigma serving to deflect the pollen after its release from the anther. The size and shape, and the relative position to one another of the individual flower parts may affect the foraging of the pollinating insect and thereby indirectly influence fruit set.

To test this hypothesis, individual flowers of the major highbush blueberry cultivars and of a number of selections were measured (Table 1). The measurements that were considered as possibly influencing the efficiency of a pollinator were (A) corolla length, (B) filament length, (C) anther length, (D) the distance from stigma to anther tip, (E) the distance from stigma to flower rim, (F) corolla opening, (G) corolla diam at base, (H) corolla width at widest diam, (I) length from base to widest diam of corolla.

diam at the base, (H) corolla width at its widest diam, and (I) the length from the base to the widest diam of the corolla (Fig. 1).

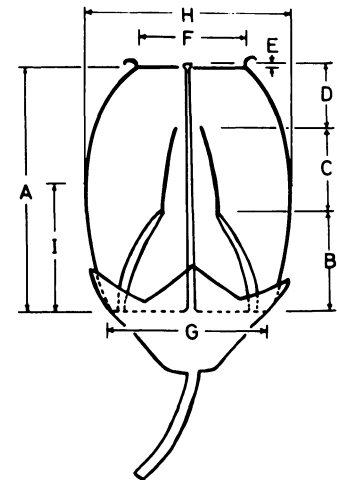


Fig. 1. Highbush blueberry flower parts measured: (A) corolla length, (B) filament length, (C) anther length, (D) stigma to anther tip, (E) stigma to flower rim, (F) corolla opening, (G) corolla diam at base, (H) corolla width at widest diam, (I) length from base to widest diam of corolla.

Table 1. Highbush blueberry cultivars used in determining the relationship between flower morphology and fruit set.

Relative fruit set	Range in % fruit set	Cultivars
Excellent	86-100	June, Rubel, Bluecrop, Rancocas, E-30, Murphy, Angola
Good	71- 85	Cabot, Wolcott, Croatan, Darrow, Ivanhoe, Burlington, Pemberton, Dixi, Concord, Weymouth, Blueray, E-6, M-23, M-88
Fair	56- 70	G-72, G-94, G-101, G-103, Atlantic, Berkeley, Collins, Jersey, Herbert
Poor	< 56	G-105, Stanley, Coville, Earliblue, G-80

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