Interpretation of One Dimensional Chromatograms: Inhibitor or Promoter?[1]

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Abstract. **Limitations in interpretation of data from 1 dimensional chromatograms is illustrated using growth substances extracted from developing seeds of sour cherry (*Prunus cerasus* L. cv. Montmorency).**

Fractionation of plant growth substances in crude alcoholic extracts by means of a single chromatographic step is often the only purification attempted prior to biological assay. Such fractionation rarely results in an adequate spatial separation of all promotive and inhibitory substances in the extract. Consequently, the observed bioassay response from any one zone on the chromatogram represents the net effect of both promoters and inhibitors moving to that zone. Such data are of limited value in understanding the role of endogenous growth substances, and may lead to mis-interpretation of the chromatographic data. We illustrate such a limitation in this report using growth substances extracted from developing 'Montmorency' sour cherry seeds.

Seeds from lyophilized whole fruits (42 days after anthesis) were ground to pass a 20 mesh screen and extracted with 3 successive (8 hr) portions of 90% methanol at -25°C. The filtrates were combined and evaporated to the aqueous phase (flash evaporator, 30°), which was then adjusted to pH 8.5 and partitioned against peroxide-free ethyl ether (2). The aqueous phase was adjusted to pH 3.0 and further partitioned against ethyl ether to yield an acidic ether fraction. The ethyl ether phase from the initial partitioning step was evaporated to near dryness and the residue was resuspended in acetonitrile. After partitioning against hexane (3), the acetonitrile was evaporated to near dryness. The residue was resuspended in distilled water at pH 6.5 and partitioned against ethyl ether to yield a neutral ether fraction. All solvents were redistilled before use.

The acidic and neutral ether fractions, either alone or in combination, were subjected to ascending paper chromatography (Whatman 3 MM) using the following solvent systems: A, isopropanol:water (4:1); B, isopropanol:water (9:1); C, hexane:water (upper phase). All papers were prewashed in the developing solvent to remove potential inhibitory substances (1). Chromatograms were equilibrated over the solvent for 10 hr, developed to a distance of 20 cm, dried in a cool air stream, and cut into 10 equal sections. Each section was bioassayed directly by the *Avena* first internode test (4). Control strips, equal in size to strips from the developed chromatogram, were taken from the area below the origin.

The bioassay response (final section length minus initial section length) was expressed as a percentage of the initial section length and plotted against chromatogram R_f in the manner of a histogram. Upper and lower 5% fiducial limits were calculated (5), outside of which a treatment must fall in order to be significant.

Bioassay of eluates from a 1 dimensional chromatogram of the combined acidic and neutral ether fraction developed in solvent A revealed 2 zones of growth promotion (R_f 0.0-0.1 and R_f 0.5-0.7) and 1 zone of growth inhibition at R_f 0.9 (Fig. 1A). Re-chromatography of the acidic ether fraction in solvent A (after prior chromatography in solvent system B) resulted in a single zone of growth promotion at R_f 0.5-0.6 (Fig. 1B), which corresponded, in part, with the zone of growth promotion at R_f 0.5-0.7 (Fig. 1A). The zone of growth inhibition found at R_f 0.4 (Fig. 1B) was considered to be due to a supraoptimal concn of the acidic promoter, since equal gram equivalents chromatographed in acetonitrile:water (4:1) showed only a broader distribution of a promoter and no

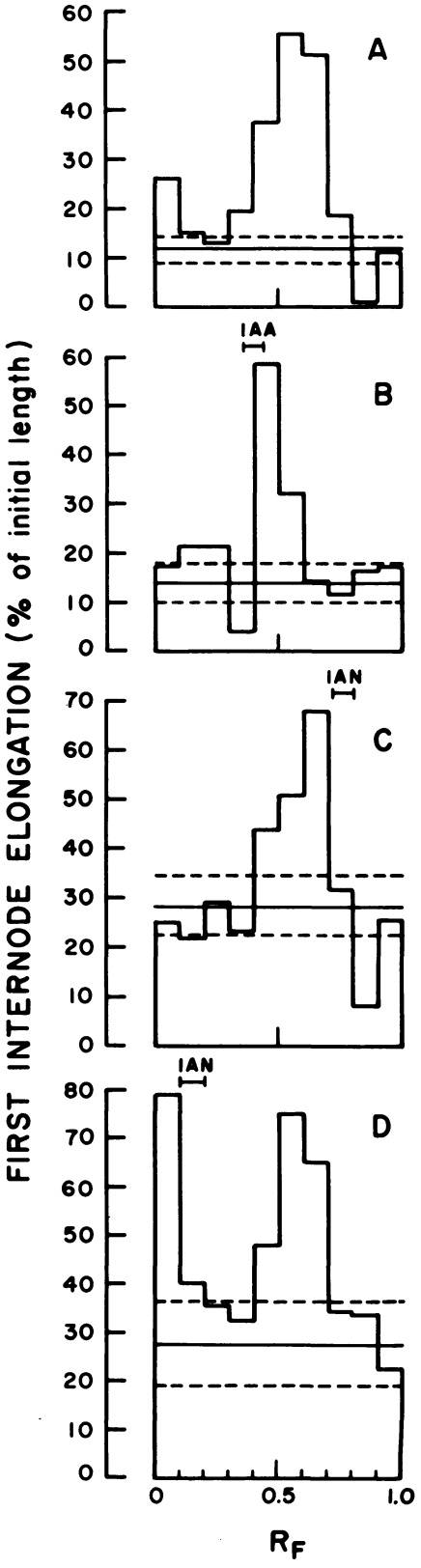

Fig. 1. Histograms depicting the *Avena* first internode bioassay response to growth substances in sour cherry seed tissue. Control value (———), 5% fiducial limits (- - - - - - -); IAA = Indoleacetic acid, IAN = indoleacetonitrile.
A. 1.9 g equivalent (GE) of the combined acidic and neutral ether fractions chromatographed in isopropanol:water (4:1).
B. 1.5 GE of the acidic ether fraction chromatographed in isopropanol:water (4:1).
C. 1.0 GE of the neutral ether fraction chromatographed in isopropanol:water (9:1).
D. 1.0 GE of the nuetral ether fraction chromatographed in hexane:water (upper phase).

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evidence of an inhibitor[3]. Thus based on these data it could be hypothesized that the seed tissue contained 1 acidic promoter and 1 neutral inhibitor.

To verify this hypothesis, the neutral ether fraction was chromatographed in solvent B. The results (Fig. 1C) resembled those obtained when both fractions were co-chromatographed in solvent A (Fig. 1A), except for the absence of the promoter at R_f 0.0-0.1. It could now be argued that the seed tissue contained 1 neutral inhibitor and 2 promoters, 1 acidic and 1 neutral. However, on further chromatography in a solvent system of markedly different polarity (solvent C) 2 well-separated zones of growth promotion (R_f 0.0-0.1 and at R_f 0.5-0.7) were found in the neutral ether fraction (Fig. 1D).

Subsequently, we found that each of the 2 neutral promoters was chromatographically homogeneous, and that the zone of promotion at R_f 0.0-0.1 (Fig. 1D) was not an artifact caused by overloading[3]. Furthermore, the 2 neutral promoters overlapped on chromatograms developed in solvents A and B such that the promoter concn at R_f 0.9 (Fig. 1A, 1C) was supraoptimal in the *Avena* first internode bioassay, resulting in inhibition of internode elongation.

The minor zone of growth promotion located at R_f 0.0-0.1 (Fig. 1A) was not found when either the acidic or neutral fractions were chromatographed alone (Fig. 1B, 1C). In view of the large amount of material chromatographed (1.9 g equivalents) this zone of promotion may be attributed to overloading.

Our data illustrate the need to critically insure that complete chromatographic separation is achieved before attempting bioassay and interpretation. We have illustrated that with varying degrees of separation considerably different conclusions can be drawn. The overloading problem can be reduced by first bioassaying serial dilutions of the crude extract and determining the optimum concentration for chromatography. The problem of promoters and inhibitors moving to the same zone, however, can only be resolved by repeated chromatography of the active zone in solvents of markedly different polarity (Fig. 1C, 1D) to ensure that the growth substance under investigation is satisfactorily separated from other promoters and inhibitors that may be present in the original extract.

[3]Hopping, M. E. 1972. Isolation, characterization and role of endogenous auxins and cytokinins in sour cherry (*Prunus cerasus* L. cv. Montmorency) fruit development. Ph.D Dissertation, Michigan State Univ., East Lansing.

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New Portable Colorimeter to Evaluate External Fruit Color of Tomato and Peach[1]

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Abstract. **A new portable colorimeter appears promising as a tool for evaluating color of horticultural products. G(green) reflectance values (546 nm) for external fruit color of tomato (*Lycopersicon esculentum* Mill.) correlated highly with visual scores and Hunter a/b ratios. For ground color of peach [*Prunus persica* (L.) Batsch], G values decreased as fruits matured and softened.**

External color is an accepted indicator of maturity and/or ripeness of fruits of tomato and peach. In tomato-ripening studies, some researchers visually evaluate external color, assigning numerical scores on a predetermined scale from green to red (1, 3). The most frequently reported instrumental indicator of external tomato color is the a/b ratio calculated from readings on the Hunter or Gardner Color Difference Meter (7). A combination of redness and yellowness, a typical a/b ratio may change from -0.5 to +2.0 as color changes from green to red.

Ground color of peaches is reported to be a reliable maturity index, especially when combined with Magness-Taylor firmness measurements (4, 5). Printed paper color charts and the a/b ratio are used most often for visual and instrumental ground color evaluation.

Current methods have several disadvantages for many horticulturists. Visual scores may be affected by the skill of the observer, scoring system used, lighting conditions, and physical fatigue after many samples. A color-difference meter (2) is a non-portable laboratory instrument that costs several thousand dollars.

A new colorimeter (Tri-Colorphot)[3,4] is portable, simple to use, and relatively low in cost (about $750). Its readings are expressed as percentage reflectance of the 3 basic or tristimulus colors, blue (B, 436 nm), green (G, 546 nm), and red (R, 640 nm).

The colorimeter consists of 2 units joined by a cable (Fig. 1). The probe unit, which touches the sample, contains the light source, 3 colored glass filters, and 3 photocells, 1 for each filter. Light reflected from the sample is detected by the photocells and signals are transmitted to the second unit, containing the power supply and meter. Any of the 3 values (B, G, and R) may be read on the meter by moving the selector switch. White and gray cards are provided by the manufacturer for standardization.

This report describes the evaluation of the new colorimeter's usefulness in measuring external color of tomatoes and peaches.

Tomato. 'Homestead' tomatoes were sorted by a light-transmittance technique (6) into 3 similar groups, each containing a known range of maturities, from immature green to breaker. External color was evaluated after 2 weeks storage at 10°, 13°, or 16°C ± 1° (50°, 55°, or 60°F).

B, G, and R values were measured with the colorimeter at the blossom end of each tomato. Preliminary tests showed that blossom-end measurements were more indicative of ripeness and easier to duplicate than measurements

Fig. 1. New colorimeter, consisting of probe and power supply with meter.

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[3]Manufactured by Phototronic, Inc., Jenkintown, Pa., 19046.

[4]The mention of specific instruments, trade names, or manufacturers is made for the purpose of identification and does not imply any endorsement by the U. S. Government.