

by V_g in these 2 populations (Tables 1 and 2) is attributable to genes that have additive statistical effects on phenotype. In fact, they indicate that 90% of the total variability associated with full bloom, ripening date, and fruit size in these seedling populations is attributable to genes with additive statistical effects.

The heritability of yield, -0.17 ± 0.21 , was a conspicuous exception on 2 counts: 1) its estimated heritability was not significantly different from zero, whereas the heritabilities of the other traits were near one; and 2) subjective measurement of this trait is known to be open to far greater error than the objective measures used for the other traits (3 and 4). It thus remains unknown whether or not any real differences in yields of seedling trees during their first year of bearing were due to genes with additive effects. All that these results indicated was that the subjective measure of yield we used was not precise enough to discriminate whatever genetic differences in yield there may be among these seedlings.

The potential rates of genetic improvement of these breeding stocks can be estimated from the relationship $R = h^2S$, where S is the selection differential and R is the response per generation (2). Assume that only 20% of the seedlings are selected on the basis of superior performance and are allowed to mate randomly, *inter se*. Then the expected response of their progeny is as given in Table 3. These estimates indicate that mass selection could cause large per-generation responses in fruit size and ripening date and a moderate response in percent soluble solids.

When correlations among these traits are large, responses to selection are also expected to be correlated, enhancing or impeding genetic improvement when selection is practiced on 2 or more traits. We found the phenotypic correlation between ripe date and fruit wt to be .22, between ripe date and % soluble solids to be .22 and between fruit wt and percent soluble solids to be -.13. (The 95% confidence intervals for those estimates, computed via Fisher's Z statistic are all $\pm .11$.) All of these correlations are significantly different from zero

with 95% confidence. They are small, however, suggesting that selection would not be affected greatly by basing selection on multiple traits.

Conclusions

This analysis gives results comparable to those obtained with *P. avium* L. (sweet cherry) and *P. persica* L. (peach) (3, 4). In each of those species, expression of date of bloom, date of fruit ripening, and fruit size are largely controlled by genes, whereas percent soluble solids is under slightly less genetic control. Further, heritability estimates indicate that the genes that control full-bloom date, ripening date, and fruit size are almost entirely additive in effects. The statistical properties of genes controlling percent soluble solids have yet to be determined. Finally, no large adverse correlations appear to exist among traits of ripening date, fruit size, and percent soluble solids in these populations. Consequently, we concluded that inter-mating of parents selected on the basis of their own performance could be very effective and efficient in improving the genetic potential of such breeding stocks in bloom date, ripening date, fruit size, and probably (though not certainly) soluble solids.

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Stress Ethylene Production in Apple Shoots¹

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Abstract. Increases in internal ethylene concentrations occurred after attached and detached apple (*Malus domestica*, Borkh. cv. Winesap) shoots were placed in a horizontal position. Ethylene was higher in the underside of the shoot, and decreased linearly in a basipetal direction along the axis. Bent and horizontally-oriented detached shoots did not increase in ethylene at 20°C, but increased to identical levels at 30°, suggesting that responses to the 2 types of stress were identical. Although gravitational stress alone increased ethylene levels, an experiment designed to separate bending stress from gravitational stress was inconclusive because rotation decreased shoot ethylene levels.

Tying stems into arcs increased internal ethylene levels as much as 10-fold (11, 23), but it was not determined if the ethylene increase resulted from physical bending stress or from gravitational stress caused by stem reorientation. Horizontal positioning of apple stems (7, 25, 26) has long been known to decrease growth and increase flower production. Gravitational stress has also been reported to influence flowering of such unrelated plants as pineapple (21) and soybean (5). Flowering is regulated in pineapple by ethylene (4, 24). Coleus plants placed in a horizontal position produced more ethylene with a subsequent enhancement of leaf abscission (2). Pretreatment of erect sunflower plants with ethylene or horizontal orientation inhib-

ited polar transport of ¹⁴C-IAA and induced similar characteristic symptoms, suggesting that effects of horizontal orientation might result from a stimulation of ethylene production (22). In this study we examined ethylene levels and distribution in horizontally positioned apple shoots.

Materials and Methods

Experiments were conducted in July and August, 1972, using vigorous new vertical shoots, both attached and detached, from the tops of mature apple trees at the Purdue University Farm. Unless otherwise designated, similar shoots 100 cm long and 1 cm thick at the base were used, and ethylene was obtained from a 10 cm section taken 45 cm away from the shoot tip. Each experimental replicate contained 4 shoots.

As illustrated in Fig. 1, the term "horizontal shoot" denotes shoots

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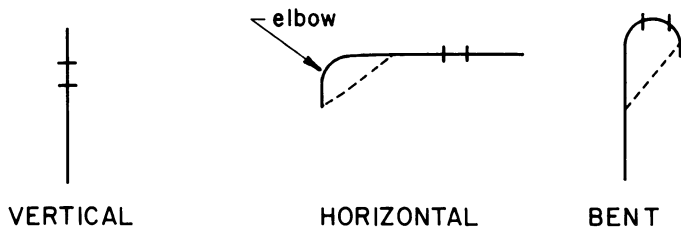


Fig. 1. Two tying positions were used to obtain gravitational stress alone or gravitational stress plus bending stress. The 10 cm sections indicated were sampled for measurement of internal ethylene concentration.

Table 1. Effect of bending and orientation on ethylene levels in attached and detached apple shoots.

Shoot status	Ethylene ($\mu\text{l/liter}$)		
	Vertical	Shoot treatment Horizontal	Bent
Attached	0.05a ²	0.44b	0.41b
Detached	0.79a	1.23b	1.23b

² Mean separation in rows by Duncan's multiple range test, 1% level.

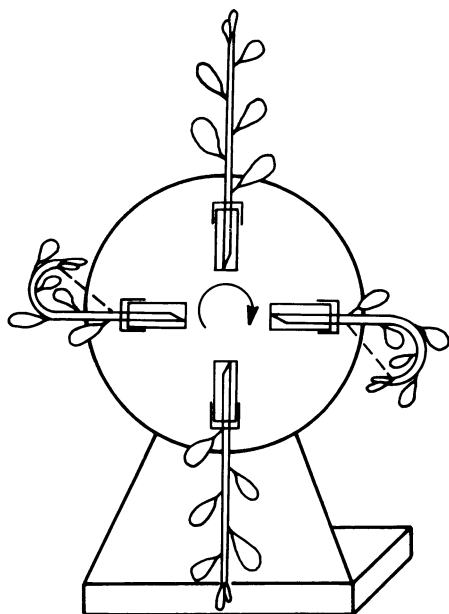


Fig. 2. Shoots, perpendicularly oriented to a horizontal rotation axis, were turned at 1 rpm for 24 hr to remove gravitational stress which results from horizontal orientation.

placed in a horizontal plane by tying close to the shoot base, while the term "bent shoot" describes shoots tightly curved by tying close to the shoot tip. In both types of treatments the sampled sections were under gravitational stress from horizontal orientation. In bent shoots the sampled portion was also under physical stress. Whether attached or detached, however, bent and horizontal shoots had identical ethylene levels (Table 1). However, detached shoots showed less response to treatment due to higher ethylene background levels, perhaps as a result of cutting injury.

Since previous results indicated that maximum ethylene production occurred between 1 and 4 days after stress application (23), the time of treatment in these experiments, with the exception of clinostat studies, was 48 hr. Light intensity and temperature were not controlled except in growth chamber studies designed to study effects of temperature on ethylene production. Growth chambers were maintained at either 20 or 30°C, with a 16 hr photoperiod and a light intensity of 15 klx at horizontal plant height and at 1/2 vertical plant height. To obtain

bending stress in the absence of gravitational stress, shoots were bent by tying and rotated at 1 rpm for 24 hr with their axes perpendicular to the horizontal axis of rotation (Fig. 2). Bent and vertical shoots, 30 cm long, were pushed to the bottoms of 25 ml vials through water-tight slits in serum caps. Although the bases of rotating shoots were out of water a few seconds each min while the shoots were in the upside-down position, shoots remained fully turgid. Vials were refilled with distilled water every 4 hr. Additional unbent and bent shoots were held as controls in vertical and horizontal positions adjacent to the clinostat.

Sampled sections were evacuated within 15 min after cutting. Internal gases were collected and ethylene levels determined using the method of Leopold et al. (11). Identical internal ethylene concn were obtained despite variations in amounts of tissue extracted or length of evacuation time. Collected gases were sampled in 1 ml syringes for injection into a gas chromatograph fitted with an activated alumina column. Five peaks were usually obtained; the ethylene peak was identified by retention time, by co-chromatography with pure ethylene, and by removal from gas samples by a saturated solution of mercuric perchlorate.

Results

Internal ethylene concn in shoots increased in response to horizontal positioning. In vertical shoots ethylene levels were uniform throughout the shoot regardless of the wood maturity (Fig. 3). In all but the basal sections, which were vertical, ethylene levels were higher in horizontal shoots than in corresponding sections of vertical shoots. In horizontal shoots levels were highest 10 to 20 cm behind the shoot tip and declined linearly toward the basal end of the shoot. Section 5, the only section under bending stress, showed a smaller ethylene increase than did the first 4 sections which were under gravitational stress only. Degree of developmental maturity apparently influenced responsiveness.

When attached shoots were bisected longitudinally while in the horizontal plane, the ethylene concn of 1.58 $\mu\text{l/liter}$ present in the

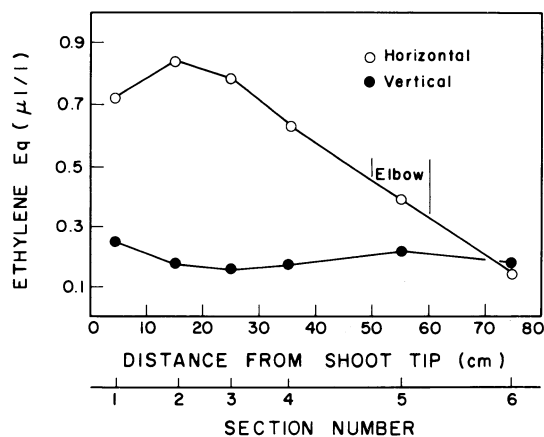


Fig. 3. Longitudinal localization of ethylene in attached apple shoots. Vertical shoots and shoots which had been tied in a horizontal position for 48 hr were divided into 10 cm segments, and ethylene concn was determined in the internal atmosphere of each segment.

Table 2. Effect of temperature on ethylene production in vertical, horizontal and bent apple shoots. Detached shoots were held for 48 hr under 16 hr photoperiods at a light intensity of 15 klx.

Shoot treatment	Ethylene ($\mu\text{l/liter}$)	
	20°C	30°C
Vertical	0.19a ²	0.26a
Horizontal	0.18a	0.53b
Bent	0.18a	0.51b

² Mean separation by Duncan's multiple range test, 1% level.

Table 3. Effect of orientation on ethylene levels in potted trees. Trees had approx 50 cm of new growth. Ethylene was determined 48 hr after positioning.

Tree orientation	Ethylene ($\mu\text{l/liter}$)	
	Shoot tip	Remaining shoot
Vertical	0.19a ^z	0.08a
Horizontal	0.40b	0.40b

^z Mean separation by Duncan's multiple range test, 1% level.

Table 4. Effect of rotation on ethylene production in detached shoots. Vertical and bent shoots were rotated at 1 rpm for 24 hr prior to ethylene determination.

Shoot treatment	Ethylene ($\mu\text{l/liter}$)
Rotated control	0.38a ^z
Rotated bent	0.36a
Stationary control	0.64b
Stationary horizontal	0.97c
Stationary bent	1.13c

^z Mean separation by Duncan's multiple range test, 1% level.

lower half was significantly higher at the 1% level than the concn of 0.54 $\mu\text{l/liter}$ present in the upper half. Following horizontal placement, therefore, internal ethylene concn in the shoot increased primarily in the lower half of the shoot, and decreased basipetally and linearly along the shoot axis.

At 20°C there was no influence of bending or gravitational stress on ethylene production. At 30°, however, ethylene levels in vertical (control) shoots were slightly increased (Table 2). Levels in bent and horizontal shoots were identical, and significantly higher than those in control shoots. Besides illustrating a temperature dependence for ethylene production, the data suggested that bending and gravitational stress are either identical or equally effective in triggering ethylene synthesis.

To test this it was necessary to induce each stress type independently. Potted greenhouse trees could be subjected to gravitational stress without injury or any additional stresses, and, when these trees were placed on their sides, the ethylene concn in the new growth was significantly higher than in identical trees maintained in a vertical position (Table 3). These trees had approx 50 cm of new growth when stressed. Although trees showed strong geotropic responses, the ethylene level in the shoot tip was identical to that in the rest of the shoot.

To isolate bending stress, slow rotation was used to nullify the gravity force vector. After 24 hr of rotation ethylene levels were identical in bent and non-bent detached shoots (Table 4), suggesting that bending increases ethylene levels not because of physical stress but rather because it changes the shoot's orientation to gravity. This conclusion is equivocal, however, because rotation significantly reduced non-bent shoot ethylene levels and therefore might have also interfered with any ethylene produced in response to physical bending. Non-rotated bent and horizontal shoots had similar ethylene levels, and this level was significantly higher than that in vertical shoots.

Discussion

The induction of gravitational stress by horizontal positioning results in higher internal ethylene levels in apple shoots. The pattern of ethylene localization strongly suggests that ethylene participates in gravimorphic events. If apple stems are tied down horizontally there is a reduction in terminal growth and a change in the pattern of lateral shoot growth (17). Buds on the upper side of the stem produce shoots while those on the lower side remain dormant or produce spurs. Lateral buds exhibit a gradient in growth vigor, with the longest developing from buds close to the first vertical stem deviation. The longitudinal ethylene gradient also correlates well with total growth

and individual shoot growth as indicated by analysis of bud groups isolated at distal, proximal, and basal regions on 1-yr-old layer shoots (18). However, older shoots of uniform maturity may not show the same ethylene gradient found in young shoots although ethylene does increase in response to gravity in 1-yr-old wood (23), and mature and young shoots do display similar gravimorphic patterns. Most important is the fact that ethylene increases only in the horizontal section of a shoot, and to a significantly greater extent in the underside of a horizontally placed shoot.

Time course experiments have shown that ethylene levels in shoots pulled down and tied in a horizontal position returned to normal in about 20 days (11, 23). Thus, ethylene differentials could initiate observed gravimorphic patterns which would later continue according to a previously described "fast augmentation" concept (15). The new, vigorously growing laterals intercept acropetally moving growth substances at the expense of the terminal shoot and other lateral buds. However, the theory that an inhibitory substance is transported along perpendicular vectors basipetally and downward from the apex in horizontal shoots (18) is not entirely contradicted. The proposed inhibitor which may be ethylene (or auxin which results in increased ethylene production) and its distribution may only initiate observed responses.

That ethylene often parallels auxin concn in plant tissues (3, 11) suggests that ethylene production by plant tissue may give an estimate of internal auxin levels (1). More ethylene was found on the lower side of geotropically stimulated bean seedlings (3), and this probably reflects the asymmetrical distribution of auxin which occurs during tropistic responses (6). If apple shoot data are interpreted likewise, they provide further evidence for asymmetrical auxin distribution in horizontal woody shoots. Supporting data include the preferential accumulation of radioactive auxin on the lower side of horizontal poplar stems (9) and the extraction of larger quantities of growth substances from the lower side of horizontal pine and poplar stems (9, 19).

Horizontal placement alone was sufficient to increase ethylene levels but we did not show conclusively that bending alone was effective. That both types of stress resulted in nearly identical increases in ethylene levels, and that ethylene levels in both control and bent rotated shoots were identical, suggests that bending might act simply by causing horizontal reorientation.

Lyon showed that the rotation of a horizontally placed plant around its long axis (12) and ethylene application (13) both induced epinastic curvature in tomato leaves. Although he suggested that the curvature was due to auxin imbalance, Leather et al. (10) showed that this ethylene might be directly responsible for the observed epinasty. In my experiments shoots were oriented so that the long tissue axis/gravity force vector angle varied continuously, and was not a constant 90° (Fig. 2) as was the case in other horizontal rotation studies. Additional studies on the effects of rotation and movement on ethylene production are needed to determine why ethylene production decreased in my rotation studies. Although movement, as by shaking, is known to affect plant growth (16, 20), shaking and tactile stimulation increase plant ethylene levels (C. A. Mitchell, personal communication).

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Carbohydrates in Two *Rhododendron* Cultivars¹

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Abstract. Determinations of carbohydrates in the plant organs of *Rhododendron* spp. cv. 'Sweetheart Supreme' and 'Hexe', were made by chemical analysis, thin-layer chromatography (TLC) and gas-liquid chromatography (GLC). Reducing sugar content was 1.5 times higher in buds than in leaves for 'Hexe' with no significant differences for 'Sweetheart Supreme'. Reducing sugars were also higher in the roots than stems with both cultivars. Sucrose content was 1.4 times greater in leaves than in buds of 'Hexe' and 1.6 times greater in 'Sweetheart Supreme'. Starch was significantly higher in leaves and buds than in stems and roots. The predominant soluble sugars identified by TLC and GLC were sucrose, glucose, and fructose. Small but detectable amounts of raffinose and maltose and an unidentified compound were also found in the plant organs.

Studies of the identification and distribution of carbohydrates in woody plant material have dealt primarily with forest trees. Wargo (10) reported the presence of starch, sucrose, glucose, fructose, stachyose, raffinose and maltose in the roots of sugar maple. In white pine stems, Parkerson and Whitmore (8) showed the occurrence of starch, sucrose, glucose and fructose but no stachyose or raffinose. However, Zimmerman (11) found traces of sucrose, raffinose and stachyose in the sieve-tube exudates of forest trees. Similar information on woody ornamental shrubs is limited, although Jeremias (3) reported the presence of sucrose, raffinose, glucose, stachyose and sedoheptulose in the bark of *Picea abies* and wood of *Euonymus europaeus*. The purpose of this study was to determine the amount and identity of the major sugars and starch in the different organs of 2 *Rhododendron* cultivars.

Materials and Methods

Leaves, buds, roots and stems of 2 fully budded field grown *Rhododendron* cultivars were purchased from a nursery on Feb. 15, 1975, and used for carbohydrate determinations. Tissues from 4 replicates with 3 plants per replicate of each cultivar were lyophilized for 36 hrs and ground in a Wiley mill to pass a 40-mesh sieve. A 200 mg composite sample from 3 plants of each replicate and for each of the organs was extracted with 80% ethanol for 4 hours in a micro-Soxhlet apparatus. The alcoholic extracts were evaporated to a cloudy aqueous phase *in vacuo* at 40°C, diluted to 15 ml with distilled water and centrifuged. The supernatants were then used for quantita-

tive and qualitative analysis of the soluble carbohydrates in the plant organs. Starch extractions were made on the residue remaining after ethanol extraction according to Aung et al. (1).

Quantitative determination of sugars in the ethanolic extracts was made by Nelson's method (7) with glucose as a standard. Sucrose was hydrolyzed to reducing sugars by adding an equal volume of 0.05 M sodium acetate buffer, pH 4.7, containing 0.2 mg/ml of yeast invertase to each sample and incubating for 30 min at 25°C. The amount of sucrose was calculated from the difference between total reducing sugar after invertase treatment and reducing sugar before enzyme hydrolysis.

Qualitative examination of the sugars in the ethanolic extracts from the roots of 'Hexe' was made by thin-layer chromatography (TLC) and gas-liquid chromatography (GLC). For TLC, 20 x 20 cm glass plates were coated with a 250 μ layer of either Kieselguhr G or silica gel G. The plates were developed to 15 cm at 26°C using solvent A consisting of n-butanol, acetone and 0.1 M phosphate buffer at pH 5.0 (4:5:1 v/v), and solvent B consisting of 60 ml ethylacetate, and 35 ml mixture of isopropanol and water (2:1 v/v). For detection of the known and unknown sugar spots, the developed Kieselguhr G plates were sprayed with a reagent mixture of 9 ml 95% ethanol, 0.5 ml concentrated sulfuric acid and 0.5 ml of anisaldehyde and the silica gel G plates a reagent mixture of equal volumes of 20% sulfuric acid and an alcoholic 0.2% naphthoresorcinol. Plates were dried at 105°C for 5-10 min. Sugars for analysis by GLC were obtained from an alcoholic extract from 25 mg of dry root tissue. This extract was chromatographed on Kieselguhr G plates developed with solvent A. Areas corresponding in R_f to authentic raffinose and maltose were

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