Simulated Low-gravity Environments and Respiratory Metabolism in Avena Seedlings

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Summary. Using horizontal and vertical axis clinostats and sand-grown oat seedlings (Avena sativa), it was found that horizontal clinostat rotation at 2 rpm increased respiration and inorganic and organic phosphorus content of seedlings. Increased coleoptile geotropism and root growth are attributed to rotational nullification of the directional component of the gravitational stimulus. These growth modifications are mechanistically explicable by the relationship between plant metabolism and auxin concentration in these organs.

Both geotropic curvature of coleoptiles and root growth are enhanced by horizontal clinostat rotation and concomitant nullification of the directional component of the gravitational stimulus (3). Though coleoptile curvature and root growth are auxin dependent, the observed horizontal clinostat-induced growth differences are not explicable on bases of auxin economy (4). These auxin studies, however, infer that horizontal clinostat rotation results in higher rates of respiration.

To directly test this inference, respiration rates of plants grown with horizontal or vertical axis clinostat rotation were compared. These comparisons, based on rates of CO₂ evolution, were supplemented by data on the effect of gravity on phosphorus metabolism. This manuscript deals with the results of these respiration measurements and the further interpretation of past results on a basis of these new data.

Materials and Methods

The measurement of respiration by CO₂ evolution requires that the rate of evolution be adjusted for differences in sample size in the various treatments. As the horizontal clinostat rotation increases seedling growth, expression of data as amount of CO₂ evolved per plant hour is precluded. Growth differences attributable to gravity treatment similarly preclude the use of initial soaked weights of seed to estimate fresh sample weight during the time respiration was measured. Rendering the entire problem more challenging is the pragmatic consideration that sample weights in the CO₂ evolution experiments could be accurately assessed only after the final CO₂ evolution determination had been made. Consequently, it was decided to determine the time trend of seedling growth and the influence of horizontal and vertical axis clinostat rotation on this growth pattern. Fitted growth curves thus obtained could then be employed in back calculating the probable weights of the plants at the various times that respiration had been measured.

Oat seedlings (Avena sativa cv. Victory 1, Svalöf, Sweden) were grown in moist quartz sand (15 % H₂O w/w). Prior to planting, the hulled oat seeds were soaked for 2 hours in tap water at 25 ± 1°C, the temperature at which the seedlings were grown. Beakers were covered with transparent plastic film to decrease desiccation and were attached to either horizontal or vertical axis clinostats rotating at 2 rpm or were placed in a stationary vertical position adjacent to clinostat rotated samples. Mesocotyl elongation was inhibited by continuous red light (1,6) (G. E. Ruby Red, irradiance ca 1 mw/cm²).

Thirty hours after initial soaking and every 5 hours thereafter, the average fresh weights of plant roots and tops were determined for each of 6 samples from each of the 3 gravity treatments. Seed not showing visible extension of the root (ca 1 mm) were not included in average weight determinations. The order in which the plants were harvested and weighed was random within sets of 3 samples, one from each gravity treatment. This harvesting sequence was used to negate possible gravity-treatment bias which may have been introduced during the 40 to 60 minutes required to harvest the 18 samples employed in estimating average plant weight at each harvest time. These...
average weights were subjected to an analysis of variance. Descriptions of growth trends over time were obtained by incorporation of least squares curve fitting into the analysis of variance.

Clinostats composed of 10 interconnected chambers with gas inlet and outlet ports were constructed as the chamber component of a continuous flow respirometer. Individual chamber dimensions, seed placement, media, and light regime following planting were the same as those used in obtaining growth data.

Plant CO₂ evolution was determined colorimetrically (2) every 4 hours from the forty-eighth through sixty-eighth hour following initiation of the seed soaking and hourly thereafter through the seventy-eighth hour. Following the final CO₂ measurement, average fresh weights of plants in each gravity treatment were determined.

Plant weights at previous times of CO₂ measurement were back calculated on the basis of defined growth curve (fig 1). These back calculated fresh weights, observed flow rates (ca 100 ml/min) and plant derived CO₂ concentration were used to establish the respiration trends over time.

Using data from a series of 4 successive experiments and regression methods, the time trends of respiration of horizontally and vertically rotated samples, as well as the general trend over time disregarding gravity treatment, were determined (fig 2).

Phosphorus transport and metabolism were studied by adding carrier-free disodium phosphate (³²P at 3.5 mc/50 ml) to the tap water used during the 2-hour preplanting seed soaking. Seed were planted and grown as in the growth study but only the vertically and horizontally rotated gravity treatments were employed. About 72 hours after soaking, paired 6-plant samples were harvested, one sample from each gravity treatment. Harvested plants were separated as to roots and tops, the parts independently weighed, and immediately frozen. Paired frozen samples were subsequently removed from the freezer in the order harvested and homogenized in a 2-phase extraction system. The aqueous phase, a solution containing 5% trichloroacetic acid, 10% silicotungstic acid and 1% ammonium molybdate, was added so that the volume of tissue and solution equaled 5 ml. The organic phase was 5 ml of isobutanol. The entire extraction procedure was performed at about 0°. Following centrifugation, 0.5 ml aliquots were withdrawn, one from the aqueous phase containing organic phosphates and one from the organic phase containing P, and the lipophosphates (5,9). These aliquots were plated and dried. Radioactivity of the samples was assayed with an end-window Geiger counter in the same order as the samples were harvested. This harvesting and counting sequence eliminated need for half-life corrections in these comparative data.

**Results and Discussion**

Gravity treatment did not alter the time at which seedling growth was initiated. Growth of seedlings, regardless of gravity treatment, was adequately described by a polynomial equation of the form \( y = a + bx + cx^2; \) where \( y \) is the weight of the seedling and \( x \) is the time after commencing soaking. As the time of growth initiation is uniform regardless of gravity treatment and growth over time is describable by the above polynomial, the minimum values of the curves depicting the time trend of growth in any of the gravity treatments occur at a common time, \( x \) minimum. The value of these polynomial equations at their common minimum estimates the weight of the imbibed embryo, a relative constant varying only within the limits of pretreatment, random selection of seeds, not with gravity treatment. Consequently, it can be shown as follows that previously reported (3) gravity treatment-induced differences in weights of plants after 72 to 80 hours of growth must have been accrued through constant differences in growth rate.

Let \( a, b, \) and \( c_1 \) and \( a_2, b_2, c_2 \) be the coefficients of the equations describing changes in seedling weights \( y_1 \) and \( y_2, \) respectively, over time \( x. \)

\[
\frac{dy_1}{dx} = b_1 + 2c_1x \quad \text{and} \quad \frac{dy_2}{dx} = b_2 + 2c_2x.
\]

At \( x \) minimum \( \frac{dy_1}{dx} = \frac{dy_2}{dx} = 0. \)

\[
\begin{align*}
\frac{dy_1}{dx} &= \frac{dy_2}{dx} = 0. \\
b_1 &= b_2 \\
2c_1 &= 2c_2
\end{align*}
\]

minimum, and \( \frac{dy_1}{dx} = \frac{dy_2}{dx} = 0. \)

There exists a number \( k \) such that \( b_1 = kb_2. \)

By substitution \( \frac{dy_1}{dx} = \frac{dy_2}{dx}; \) \( b_2c_1 = kb_2c_2 \) and \( c_1 = kc_2. \)

Therefore, \( b_1 + 2c_1x = k(b_2 + 2c_2x) \)

and \( \frac{dy_1}{dx} = \frac{dy_2}{dx} = k \)

at \( x \) minimum. The value of the polynomial equations at this time (the estimated wt of imbibed embryos) was essentially equal regardless of gravity treatment. Therefore, it has been proven that gravity treatment-induced differences in plant weights are accrued through constant differences in growth rates.

When these growth equations are translated so that their common minimum corresponds with the ordinate, the increase in weight with time can be described by equations of the form \( y = i + b't + c't^2; \) where \( y \) is the weight of the seedling \( b' \) and \( c' \) the translation adjusted values of the linear and quadratic coefficients, respectively, \( t \) the number

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of hours beyond the time at which the minimum weight in the nontranslated growth equation occurred, and $i$ the value of the growth equation at its minimum, i.e., the equation estimated weight of the imbibed embryo.

The 2 previously used growth equations when translated so that their common minimum value corresponds with the origin are $y_1' = i + b_1't + c_1't^2$ and $y_2' = i + b_2't + c_2't^2$. By arguments paralleling those previously advanced: $b_1' = k_b y_1'$ and $c_1' = k_c y_1'$. $y_1' - i = k(b_2't + c_2't^2)$ by substitution and $y_2' - i = b_2't + c_2't^2$. Therefore: $y_1' - i = k(y_2' - i)$ and $y_1' - i = k$.

This derivation provides the nondestructive method of obtaining calculated seedling fresh weights during the times respiration was measured. These were calculated for each respiration experiment, gravity treatment, and time of measurement using values from the experimentally generated growth curve and its associated equation (fig 1) and the following formula: $Rt = n \frac{W_0 - i}{W_f - i} (Ft - i) + i$. Where: $Rt$ = the calculated fresh weight at time $t$ of a sample on which respiration was measured; $n$ = the number of seedlings in the sample on which respiration is being measured; $W_0$ = the average seedling weight at the termination of the respiration experiment; $W_f = \text{the fitted average weight of the seedling at the time of termination of the respiration experiment (fig 3)}$; $i$ = the fitted average minimal weight in the equation describing the time trend of growth (fig 3); $Ft$ = the fitted average weight of a seedling at time $t$ corresponding with the time of $Rt$.

$W_0 - i / W_f - i$ in the above equation is the previously derived uniform rate adjustment factor. This renders gravity treatment adjusted curves even though the fitted values were derived from an equation describing the time trend of oat seedling growth with the gravity treatments averaged.

Weights so calculated were used to estimate the fresh weight of tissues arising from the embryo.

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**Fig. 1.** Growth curve of *Arvna* seedlings 30 to 80 hours. $W = 24.759 - 1.493t + 0.026t^2$; where $W =$ weight in milligrams; and $t =$ time in hours after initial wetting of seeds.

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**Fig. 2.** Rates of CO$_2$ evolution of horizontally and vertically rotated *Arvna* seedlings: Horizontally rotated mg CO$_2$ kg - hr $\overline{\text{mg CO}_2 \over \text{kg - hr}} = 3459.4473 - 87.6217t + 0.5903t^2$; Vertically rotated mg CO$_2$ kg - hr $\overline{\text{mg CO}_2 \over \text{kg - hr}} = 729.4098 - 7.5513t$; where $t =$ time in hours after initial wetting of seeds.
and disregard the respiratory contribution of endosperm tissues. This procedure is supported by findings in related cereal grains which indicate that endosperm tissue of germinating seeds contributes little, if at all, to CO₂ evolution (7).

Time trends of CO₂ evolution from horizontally and vertically rotated samples show that horizontal clinostat rotation nullifying the directional component of gravity force enhances respiration (fig 2).

The respiration data presented for each of the gravity treatments could not be adequately described by a single regression line incorporating data from both treatments (odds greater than 99:1). Equations and curves provided are adequate descriptions of the data from each gravity treatment (odds greater than 19:1). No higher degree polynomial equation through the fourth degree describes the time trends of respiration in these gravity treatments better than the second and first degree polynomial equation used for horizontally and vertically rotated samples, respectively.

The amounts of phosphorus incorporated into organic forms and inorganic phosphorus content per unit fresh weight are greater in the horizontally rotated than in the vertically rotated seedlings (table I). Though seedlings grown with horizontal rotation had higher total ³²P contents per unit fresh weight, the fraction of the total ³²P in organic plant constituents was uniform (61%) regardless of gravity treatment. This indicates that the functional activity of the metabolic system within the tissues was enhanced rather than modified. There were no material differences in organic or inorganic ³²P contents per unit weight attributable to plant part. Nor were differences attributable to plant part apparent when this variable was considered with gravity treatment and phosphorus fraction, jointly and singly. Both roots and tops translocate absorbed ³²P from the endosperm and incorporate it into organic forms at similar rates. These rates are uniformly and similarly effected by gravity treatment regardless of organ. It may, therefore, be deduced that mechanistically both roots and tops respond similarly to the gravity treatments imposed.

Collectively, the data on CO₂ evolution and phosphorus metabolism demonstrate that a major effect of nullification of the directional component of the gravity-force vector is the enhancement of respiratory metabolism. This increase in metabolic activity affords the bases for a mechanistic explanation of increased root growth and coleoptile curvature responses of seedlings grown under conditions nullifying the directional component of the gravity stimulus.

The fact that oat coleoptiles curve in response to geotropically induced lateral asymmetry of auxin demonstrates that auxin is limiting the growth of the coleoptiles. When this auxin-imposed growth limitation is partially ameliorated by geotropic stimulation, the coleoptiles with the higher respiration rate show the greatest increase in growth in response to higher auxin levels in the lower side of the coleoptile (i.e., horizontally rotated coleoptiles curve more than vertically rotated). Increased metabolism without the localized auxin increases introduced by geostimulation will little modify coleoptile growth; for in this case, auxin supply, not metabolism, remains limiting throughout the organ.

Roots, except when very young or senescent, have auxin concentrations at or near superoptimal levels (8). Increased metabolism in these organs results in greater growth; for in roots, respiratory metabolism, not auxin supply, limits growth. The growth favoring increase in metabolism imparted by the horizontal clinostat rotation is evinced by increased root growth. Thus, higher rates of metabolism attributable to horizontal clinostat rotation when contrasted to lower metabolic rates attributable to vertical clinostat rotation explain and render consonant the empirical data on growth, curvature, and auxin economy. Remaining undefined is the mechanism by which these simulated low-gravity environments enhance respiratory metabolism.

**Literature Cited**


