ular sieve column and 0.25-ml samples were used for O_2. At the start of the experiment the chamber air contained 0.4 percent CO_2 and 21 percent O_2. Before the chamber was opened on the last day, it contained 0.13 percent CO_2 and 63 percent O_2. Oxygen had slowly accumulated to 21.5 percent after 1 day, 23.5 percent after 3 days, 26 percent after 8 days, 34 percent after 13 days, 40 percent after 17 days, 43 percent after 30 days, 53 percent after 40 days, 55 percent after 48 days, and 58 percent after 61 days. Carbon dioxide was well controlled, usually being analyzed at 0.2 percent or less. Only one analysis exceeded 0.8 percent (31st day). A value of 5 percent was obtained on the 28th day, after a weekend when algal growth had attained 1.7 percent packed cell volume. The mouse did not show respiratory signs or lack of activity consistent with this high value. Analysis had shown 0.2 percent CO_2 on the 24th day, so the analysis was considered to be in error for unknown reasons.

At no time were gas chromatograph peaks for methane or ethane seen. The chamber contents were checked for CO with the Mine Safety Appliances analyzer at the end of the experiment, and no accumulation of CO was found. Both room air and chamber gas were alike, containing less than 0.001 percent CO. The CO reached toxic levels in blue-green algal gas exchangers (*5*).

The mouse was normally active, though confined, throughout the run. It weighed 43.5 g when removed, a weight gain of 4.6 g. It remained healthy for more than 9 months after the experiment. The increased O_2 in the atmosphere (30 percent rising to 60 percent over a period of 50 days) and restoration to 21 percent had no adverse effect.

Odor in the mouse chamber containing the accumulated excreta of 66 days was considerably less than that of an uncleaned cage after 1 week. This is explained by the fact that relatively dry air entered the chamber and by the deodorant action of the algal culture. No activated charcoal or other absorbent was used. Ammonia, volatile fatty acids, hydrogen sulfide, and mercaptans would be metabolized and removed by the algae. Chlorophyll has also been claimed to be a deodorant.

No precautions were taken to begin with or to maintain a pure culture of *Chlorella*. Bacteria were present, but created no problem. Fecal bacteria in a previous experiment (*3*) had demonstrated that their aerobic metabolism can disrupt the functioning of the system. Close to 400 ml of tap water were used by the mouse, except for a slight loss by wastage and evaporation. Exhaustion of this supply was the reason for terminating the experiment.

The variation in chamber N_2 is unexplained. With the rise in O_2 and the maintenance of CO_2, the N_2 decreased progressively. Nitrogen storage in the mouse's weight gain could explain a very small part of the decrease. It is estimated that about 2 liters of N_2 disappeared from the chamber air. Removed culture should have contained less than the added medium because the temperature change reduced the nitrogen's solubility. Undetected leaks should also have the effect of increasing low values of N_2. *Chlorella* does not fix N_2. Bacteria could, but this seems to be a large amount of nitrogen fixation, and in other experiments no evidence for nitrogen fixation has been found. The oxygen buildup can be explained by imbalance in the respiration quotient of the mouse and the assimilation quotient of the algae. This could be prevented by use of ammonium salts as the nitrogen supply in the medium, and such an experiment might supply an answer to the N_2 picture.

One minor accident occurred on the 22nd day. While the medium was being admitted, the separatory funnel was inadvertently emptied, and room air estimated to be 100 ml or less entered the chamber. This would be 2 percent or less of room air added to chamber air of 0.2 percent CO_2, 40 percent O_2, and 59.8 percent N_2.

An average man's weight is three orders of magnitude greater than that of this rather large mouse. The respiratory exchange is two orders of magnitude higher. The present experiment indicates 100 gallons of 1 percent packed cell volume of algae could support life for one man. The lighting was designed for the standard strain of *Chlorella*. Optimum lighting for the thermophilic strain is much higher (*6*). Improved performance can be developed even in the absence of gravity where the separation of gas from liquid is a problem. Relatively long-term safety and reliability have been shown for this system.

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24 January 1961

Diageotropism in Vanilla Roots

Abstract. Diageotropic growth in the dark and geotropic growth in the light occurred in the roots of cuttings of three *Vanilla* species. The diageotropic response also occurred in far-red, red, orange, and green light, while positive geotropism resulted only if blue light was present.

Diageotropism, or the orientation of plant parts at right angles to the direction of gravity, was mentioned by Darwin (*1*) as occurring in certain rhizomes and probably in some secondary roots. Bennet-Clark and Ball (*2*) reported that the diageotropic response of the rhizome of *Aegopodium* occurred only in the absence of light and that positive geotropism occurred in the presence of light of any color. They suggested that growth curvatures in response to light and darkness served as a depth-regulating mechanism. A search of the available literature did not reveal reports of a similar light-dependent mechanism in roots, or of a plant with a diageotropic terrestrial roots system.

The pendent roots of *Vanilla* vines may grow downward as far as 30 ft before reaching the ground. After they reach the ground, the roots turn at right angles and ramify through the accumulated organic matter and seldom penetrate the mineral soil. Although the physical resistance of the soil may be a factor in controlling root growth, recent work at this station indicates that light may play a major role in determining the direction of growth of *Vanilla* roots.

To determine the effect of light on root growth, single-node cuttings of *Vanilla planifolia* Andrews with attached leaves were rooted under intermittent mist spray. After they were selected for uniformity, cuttings with adventitious roots were placed in 1-gallon glass chambers with a relative humidity of 100 percent. The direction of the root tips was recorded at that time. Replicate chambers were exposed to fluorescent light (500 ft-ca) for 9 hr daily, and additional chambers were maintained in continuous darkness. All chambers were opened daily to allow a brief circulation of air.

The roots of plants exposed to light responded rapidly to the stimulus of gravity. Figure 1 shows the positive geotropism that occurred in illuminated roots regardless of the original direction of the tips. The root tips responded faster when oriented directly upward than when oriented horizontally. The curvature could be detected 3 hr after positioning; in 20 hr the root tips completed a curvature of 180°.

Cuttings, which had been kept in darkness for a 2-day conditioning period and which were then oriented with tips up and retained in darkness, made initial growth responses in approximately the same time as those grown in light. However, after the root tips had turned 90° from the vertical, this response invariably halted. Growth in a horizontal plane continued for 10 days if the plants remained in darkness. Roots placed in a horizontal position continued to grow in that plane, while roots originally down required 12 hr to turn toward the horizontal (Fig. 2).

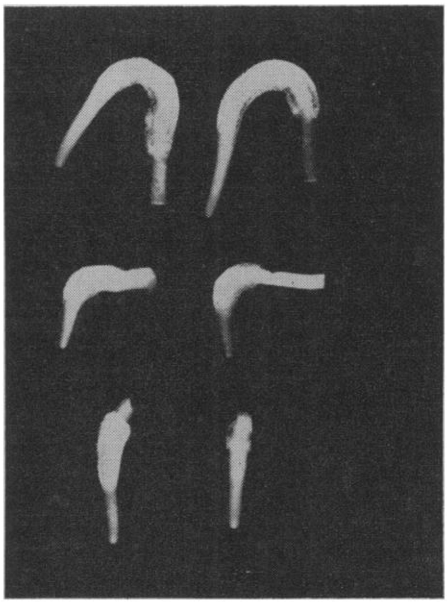

Fig. 1. Light-induced geotropic response in roots of *Vanilla planifolia*. The cut portion of the roots indicates the original basipetal position.

In other tests the chambers were wrapped with colored and clear cellophane to provide illumination by far-red, red, orange, green, blue, and white light. Cuttings were placed in the chambers, and the lighting was arranged to provide a similar light intensity (15 ft-ca) in each container. A consistent diageotropic response occurred after the root tips remained for 48 hr in far-red, red, orange, and green light. Positive geotropism resulted only in those roots maintained in blue and in white light.

Some photic responses may be initiated by the absorption of light in the blue region of the spectrum by carotenoids (*3*) or caroteno-protein (*4*). We extracted 50 root tips with acetone and obtained a mixture of two yellow pigments. The mixture was separated on filter paper with hexane as the solvent. Spectrophotometric curves obtained from the eluted pigments indicated that they were beta-carotene ($A_{\max}$ at 425, 451, and 480 mμ) and lutein ($A_{\max}$ at 419, 447, and 477 mμ). These data are compatible with the concept that the absorption of blue light by carotenoids mediates the positive geotropic response of *Vanilla* roots.

Responses to applications of indoleacetic acid by roots grown in darkness suggest that the diageotropic growth was not due to a complete loss of sensitivity to auxin. Applications of indoleacetic acid (1, 10, and 100 parts per million in lanolin) to a point 1 mm from the root apex resulted in pronounced growth curvatures toward the site of application. Applications of indoleacetic acid to roots grown in light resulted in similar curvatures. Lanolin alone was ineffectual in roots grown in both darkness and light. The growth rate of roots in both treatments was approximately 5 mm a day for the first 6 days.

Rooted cuttings of *V. dilloniana* Correl, *V. phaeantha* Reichb. f, and a hybrid between *V. planifolia* and *V. phaeantha* responded to light and dark treatments in a manner similar to *V. planifolia*. The aerial roots of two other semi-epiphytic, climbing orchids, *Vanda* sp. and *Aerides* sp., failed to respond to the gravitational stimulus in this experiment.

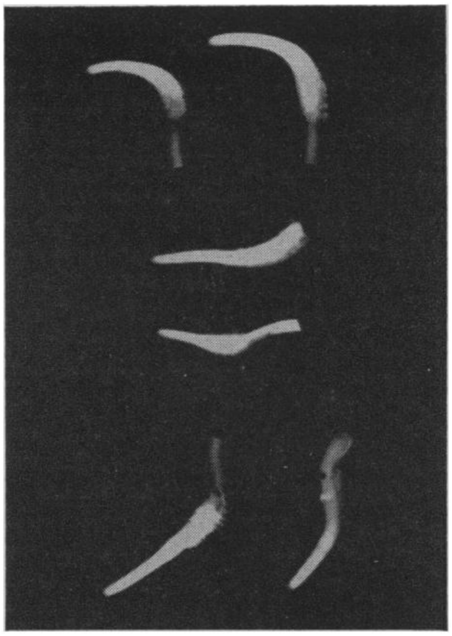

Fig. 2. Dark-induced diageotropic response in roots of *Vanilla planifolia*. The cut portion of the roots indicates the original basipetal position.

The geotropic response of *Vanilla* roots may be separated into two phases. The first consists of the curvature of the roots from the vertical (tip up) to the horizontal, the response to gravitational stimulus occurring both in light and in the dark. The second, occurring in the presence of blue light, is the curvature of the root from the horizontal to the vertical, but with the tip down. The effects of the light-induced curvature in the second phase may be reversed in the absence of blue light, resulting in an upwards curvature of the root and the resumption of the diageotropic position.

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