PHOTOSYNTHETIC RESPONSE OF BALSAM FIR SEEDLINGS FROM AN ALTITUDINAL GRADIENT[1]

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INTRODUCTION

Elevational gradients within plant populations are convenient for the study of the genetics, physiology, and ecology of adaptation to extreme environments by natural selection. The proximity of populations along altitudinal transects largely excludes genetic isolation and random drift as causes of population differentiation. Among altitudinal populations, variation is likely the result of natural selection exclusively.

Most wide-ranging species show divergence into ecological races or ecoclines, each adapted by natural selection to different environments (Turesson, 1930; Clausen, Keck, and Hiesey, 1948). Adaptation to the environmental complex associated with elevational zones should be no exception. Some important environmental parameters which vary considerably with elevation above sea level are air and soil temperature, length of growing season, quantity and quality of light, summer cloudiness, available soil moisture, partial pressure of oxygen and carbon dioxide, and soil fertility (Scott and Billings, 1964).

Investigations of physiological parameters are of particular importance in studies of plant adaptation. The physiology of plants at high altitudes has been the subject of numerous studies since the pioneering research of Bonniers (1895). A number of recent reviews (Pisek, 1960; Larcher, 1963; and Tranquillini, 1964) deal with the subject. Photosynthesis and respiration, which are integrated metabolic processes and basic to the effective capture, storage, and utilization of energy within the plant are examples of important physiological characteristics.

Photosynthetic CO_2-uptake of high altitude plants compared to low altitude plants has been summarized by Tranquillini (1964) in the following generalities: high altitude plants (1) require more light for both photosynthetic compensation and saturation; (2) have lower temperature optima; and (3) have greater ability to assimilate carbon dioxide at low partial pressures. However, these generalizations appear to be based on data from plants of different species, or on widely separated sub-populations of the same species, or on field measurements in which genetic differences are confounded with environmental differences. The value of these studies for the interpretation of genetic adaptation to altitude is limited because observed differences may be the result of either population divergence due to factors unrelated to elevational influences, or to the direct influence of environmental factors.

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The present study reports some characteristics of photosynthetic CO_2-uptake in one species, Abies balsamea (L.) Mill., collected over a single altitudinal transect, and grown under uniform environmental conditions. The photosynthetic responses to light and temperature were investigated. The altitudinal transect was established on Mt. Moosilauke in the White Mountains of New Hampshire, where balsam fir is an important continuous component of the vegetation from the spruce-hardwood zone below 2,500 feet to the alpine tundra above 4,700 feet. The occurrence of balsam fir varies from stands of well-formed dominants of up to 60 feet in height at the lower elevations to a stunted krummholz at the highest elevations. In a study of phenotypic variation, Myers and Bormann (1963) sampled this transect for cone morphology and concluded that balsam fir in New England changed from purely var. phanerolepis at the higher elevations to an intermediate between var. balsamea and var. phanerolepis at the lower elevations.

MATERIAL AND METHODS

Seed was collected along the southeast slope of Mt. Moosilauke in October 1969, at 2400 feet, 2800 feet, 3800 feet, 4300 feet, and 4800 feet msl. Elevation was measured with an altimeter. The length of the transect was only 2 miles. At each elevation, cones were collected from 5 to 8 widely spaced trees. The seed was bulked by elevation and stratified at 2°C for 6 weeks.

After germination of the seed, seedlings were grown in plastic pots in the greenhouse, 3 seedlings per pot, in a 1:1:1 coarse sand:loam:peat mixture. After setting a dormant bud, the seedlings were chilled at 2°C for 14 days. Two weeks of chilling was sufficient to allow the seedlings to flush and elongate normally when returned to greenhouse conditions. After 6 months of growth, the seedlings were again given a chilling period followed by a return to the greenhouse. Greenhouse conditions averaged 27°C day temperature, and 23°C night temperature, and seedlings flushed within 2 to 3 weeks. In this fashion, three seasons of growth were simulated, resulting in seedlings which averaged 6.4 cm in height. Photosynthetic CO_2-uptake was measured during a 2 week period 2 months after the termination of the last chilling period. All seedlings had set buds at the time of measurement.

Measurements of the CO_2-exchange rate were made using the system described by Ledig and Clark (in press). The rate of CO_2-depletion between ca. 330 vpm to 280 vpm CO_2 in a closed system was measured with an infrared gas analyzer (Mine Safety Appliances Co. LIRA Model 200). Air flow rate for all measurements was maintained at 3 $1/min^{-1}$.

The light source consisted of fluomeric lamps, positioned above a glass-topped temperature cabinet which contained the seedlings. The seedlings were sealed in cylindrical (400 cc capacity) plexiglass cuvettes with silicone rubber. A water bath was interposed between the light bank and the cabinet top. Light intensity was varied by insertion of screens between the lamps and chamber or by changing the distance between the seedlings and the lamps. Intensity at the level of the plant top was measured with a Weston Model 756 illumination meter.

Two experiments were run; the first to determine the photosynthetic response to temperature, and the second the response to light. To determine the temperature reponse of photosynthesis the rate of CO_2 assimilation was measured at 0°C, 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, and 35°C. The order of measurement was 20°C, 15°C, 10°C, 5°C, 0°C, 25°C, 30°C, 35°C. Each 5°C temperature change was made over a half-hour interval, allowing gradual acclimation of the plants. Light intensity was kept constant at 2000 ft-c. The temperatures reported are air temperatures in the cuvette. Details of the temperature control apparatus appear elsewhere (Ledig and Clark, in press). Rates of CO_2 exchange were measured on 3 pots (total of 9 seedlings) for seedlings from the extremes of the elevational transect, 2,400 feet and 4,800 feet. At intermediate elevations, 2800 feet, 3800 feet, and 4300 feet, two pots (6 seedlings) per source were measured.

Light response was measured by determination of CO_2 assimilation rate at or near the following light intensities; 150 ft-c, 300 ft-c, 1000 ft-c, 2000 ft-c, 3000 ft-c, 4000 ft-c, 5000 ft-c, and 6000 ft-c while temperature was kept constant at 22 C. Determinations were made in the ascending sequence of light intensities. Rates of CO_2 exchange were measured on 2 pots (6 seedlings) for each of the elevational sources.

After CO_2 exchange rates had been determined, the needles were oven dried at 95°C and weighed. The rate of apparent or net CO_2 exchange was expressed in mg CO_2 h^{-1} g^{-1} dry weight of needle tissue.

RESULTS AND DISCUSSION

Balsam fir seedlings showed a typical unimodal response of photosynthetic CO_2-uptake to temperature, with a pronounced optimum at which CO_2-uptake was most rapid. The temperature optimum for seedlings from 2400 feet msl was ca. 23°C while that for seedlings from 4800 feet msl averaged ca. 17°C (Fig. 1). While there was considerable pot-to-pot variation within elevations, a decrease in the temperature optimum with increasing elevation of the seed source was obvious (Fig. 2). Assuming a linear change with elevation, the regression equation is:

$$\text{Temperature optimum } (^\circ\text{C}) = 29.5 - 0.0027 \times \text{elevation (ft)}.$$

That *is*, there was a 2.7°C change in the temperature optimum for photosynthetic CO_2-uptake with every 1000-foot change in elevation. The correlation coefficient ($r = -0.82$) is statistically significant at the .01 level of probability.

As climatic data were not available from Mt. Moosilauke, a comparison was made with temperature conditions at nearby Pinkham Notch at 2000 feet msl and Mt. Washington at 6262 feet msl (U.S. Department of Commerce, 1964). Mean daily maximum temperatures for June to August at these two stations showed an actual change in air temperature of 2.4°C for each 1000-foot change in elevation. The similarity between the regression coefficient for photosynthetic temperature optima on elevation and the actual change in air temperature with elevation in the White Mountains is obvious. Both the observed change in temperature optimum for photosynthetic CO_2-uptake and the change in mean daily maximum air temperature are only slightly lower than the dry adiabatic lapse rate of 3°C per 1000-foot change in elevation.

The decrease in temperature optimum for photosynthetic CO_2-uptake with increasing elevation is in agreement with the results of most other studies

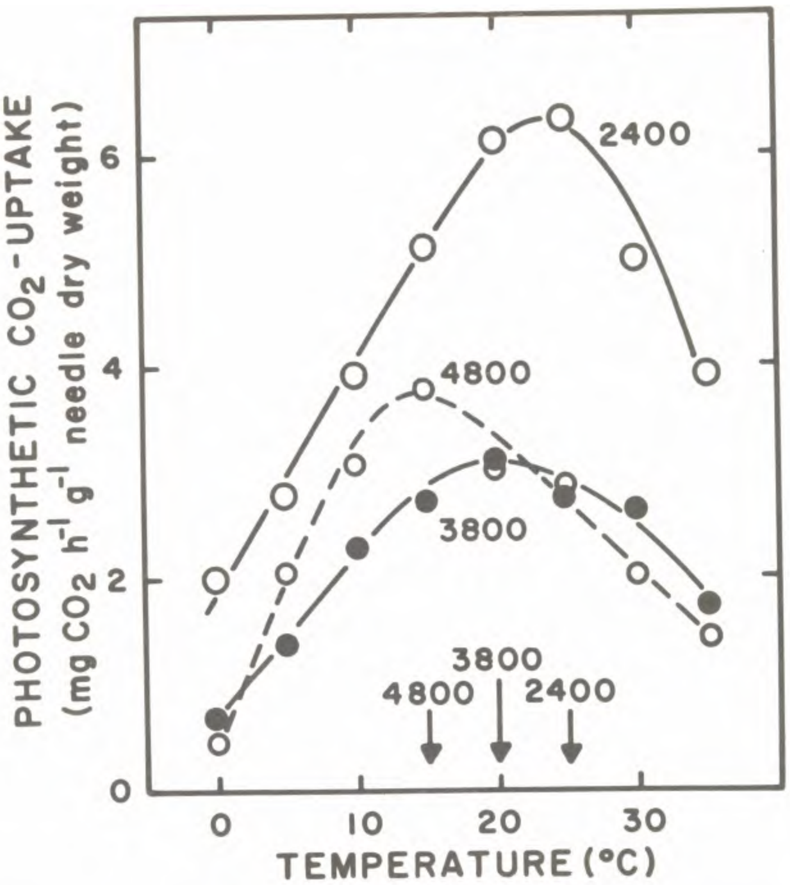

Figure 1.--Net photosynthetic CO_2-uptake of balsam fir seedlings from different elevational origins (2400, 3800, and 4800 feet msl) as a function of temperature.

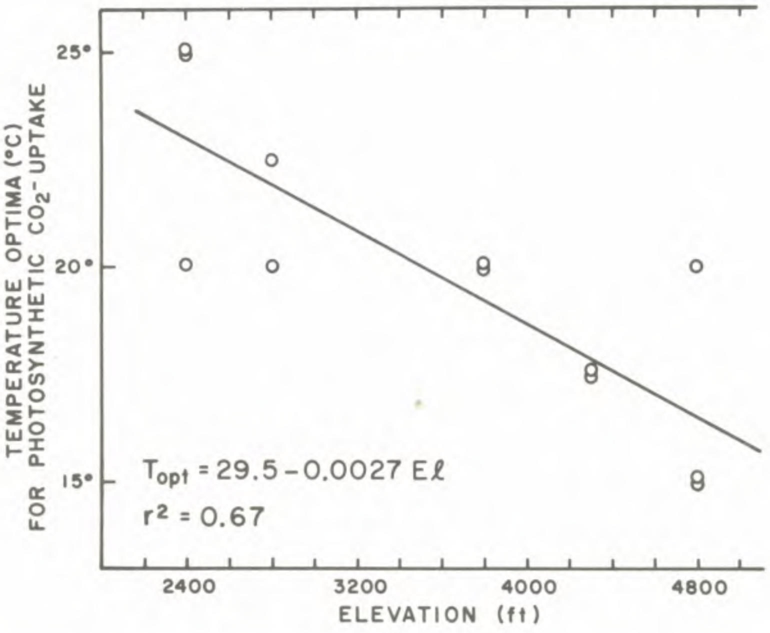

Figure 2.--Relationship of temperature optimum for net photosynthetic CO_2-uptake to elavation of origin for balsam fir seedlings.

(e.g. Mooney et al., 1964; Mooney and Billings, 1961; Mooney and Shropshire, 1967; Duncan and Hesketh,(1968) although Milner and Heisey 1964) failed to find any differences in temperature optima among altitudinal races of Mimulus cardinalis Dougl. However, most previous investigations have compared only two extreme populations or have confounded the elevational complex gradient with geographic gradients. To our knowledge, this study is the first to compare CO2 -uptake in plants originating from populations located along a single slope and to conclusively demonstrate a gradual genetic change in the temperature optimum with elevation. Our results suggest that balsam firs at different elevations are closely adapted by natural selection to the local temperature regime.

The magnitude of phenotypic plasticity was not tested in this study, but might conceivably contribute to an even finer adjustment of individuals to local and yearly temperature fluctuations. In Oxyria there was a marked genotypic effect on temperature optimum for photosynthesis, but the effect of environmental regime prior to testing was even greater (Billings and Godfrey, 1968). Temperature optimum was easily changed by altering the temperature at which the plants were grown.

There appeared to be no significant difference among seedlings from different elevations in photosynthetic response to light intensity. Most seedlings reached light saturation between 3000 and 4000 ft-c (Fig. 3). A similar light saturation point was reported for foliage of mature balsam fir (Clark, 1961).

Because of the filtering effect of the atmosphere, light intensity is expected to be greater at the top of a mountain than at the base. Races of Mimulus cardinalis are apparently adapted to such a gradient; high elevation races require a higher light intensity for saturation of the photosynthetic mechanism than low elevation races (Milner and Hiesey, 1964). Why is there no difference in light response among balsam fir seedlings native to different elevations? One explanation may be found in the ecology of the species. Balsam fir generally regenerates under heavy shade, where the effects of variation in sunlight would be considerably diluted, particularly at the seedling stage. Thus, there may be no altitudinal variation in selective pressure for adaptation to light conditions. Secondly, there may actually be little difference in light intensity among high and low elevations in the White Mountains; cloud cover at the higher elevations may negate any potential increase in radiation density. A third possibility is that variation among elevations in light response may be present at other temperatures but not at 22°C, the temperature used in the present experiment. Temperature is known to have an effect on the light saturation and compensation points (e.g. Milner and Hiesey, 1964). Finally, the number of measurements in the present experiment is very small, and genetic differences among elevations may be obscured by genetic variation within elevations. Further work will be required to determine the true status with respect to light adaptation in altitudinal populations of balsam fir.

The present study indicates that there are physiological races in trees adapted to environmental factors associated with altitudinal zones, the elevational complex gradient. These results are also in agreement with the phenotypic evidence of Myers and Bormann (1963) and our own data (unpublished) that var. phanerolepis is a high elevation extreme of Abies balsamea, and that complete intergradation exists between var. balsamea and var. phanerolepis.

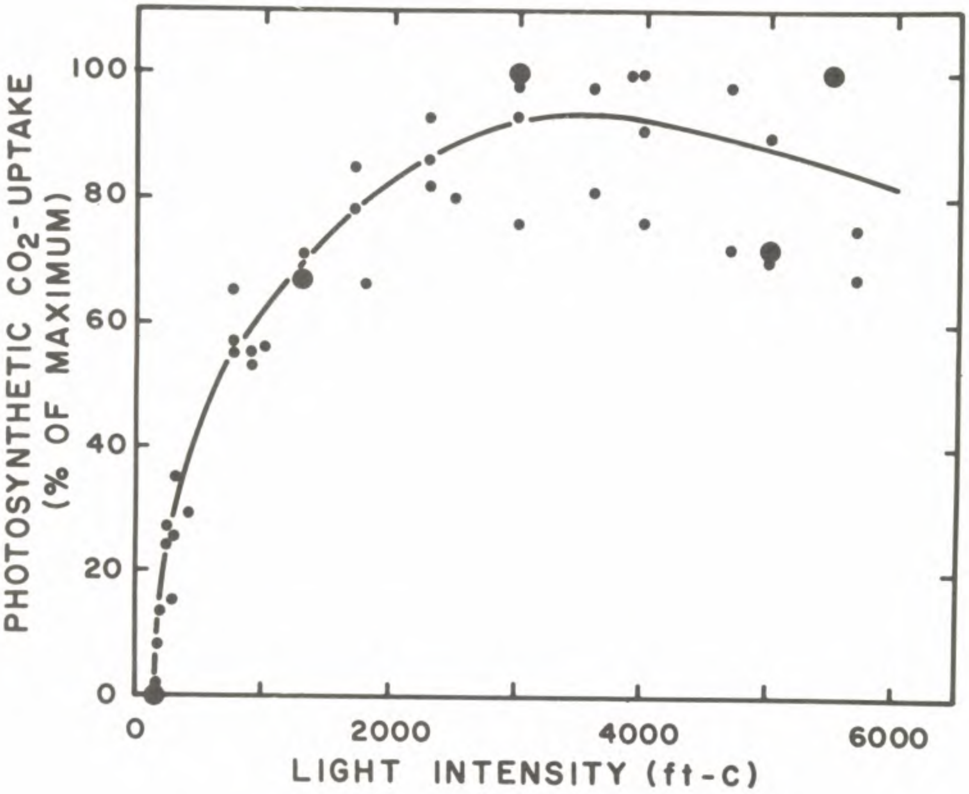

Figure 3.--Net photosynthetic CO_2-uptake of balsam fir seedlings as a function of light (large dots represent two observations, small dots one).

Maintenance of the high degree of population differentiation observed over such a short distance on Mr. Moosilauke was to us somewhat remarkable. Considerable gene flow between elevations within such a population might be expected. It is also probable that gene migration into the krummholz region is high because the krummholz is more restricted in area than the lower elevational populations and because trees at the high elevation are relatively less fecund. However, differences in flowering phenology, either genetically or environmentally controlled, may greatly reduce gene flow between all but adjacent subpopulations (e.g. Strand, 1957). In any case, selection pressures have been strong enough to result in local differentiation in balsam fir, despite migration.

APPLICATION TO FOREST TREE BREEDING

A useful tool for the genetic improvement of trees is selection among geographic races of a species based on provenance testing. However, intensive sampling along more compact environmental gradients, as are found in mountainous terrain, is not often included in provenance testing. Yet, there is a growing body of evidence to suggest that in many trees the genetic differentiation within attitudinal transects is as great and perhaps greater than that found among geographic regions. For example, differences in photosynthetic temperature response among provenances of white pine (Pinus strobus L.) which extended from North Carolina to Canada were very weak (Bourdeau, 1963), compared to the differences reported here for balsam fir. The present study and that of Irgens-Moller (1957) and Hermann and Lavender (1968) in Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco.) show that physiological variation with altitude does occur in forest trees. Tree improvers must give serious consideration to altitudinal variation in the East (where three biomes may be traversed in a few thousand feet of elevation) as well as in the Western Mountains. The successful transfer of seed sources up or down mountain slopes may be extremely limited. Future work should seek to determine whether there are populations or individuals that are plastic enough to succeed in a variety of elevational zones.

The results for balsam fir indicate that laboratory measurements of characteristics such as the rate of photosynthetic CO_2-uptake can provide a rapid means of uncovering population differentiation and determining the response of seed sources to climatic factors. Productivity-oriented parameters, such as photosynthesis and phenology, are of obvious value in studies of growth and development. For example, in the present study, dry weight growth of seed sources at 27°C in the greenhouse was related to their rate of net CO_2-uptake at 27°C. We believe that such intensive, short-term studies provide the tree breeder with a rapid means for defining limits on seed transfer and for mapping collection zones in detail. For the population biologist, altitudinal transects provide a source of variation useful for genecological studies of selection and adaptation.

SUMMARY

Balsam fir seedlings grown from seed collected at 2400, 2800, 3800, 4300, and 4800 feet msl on the southeast slope of Mt. Moosilauke, New Hampshire, were grown under uniform conditions in the greenhouse at a day temperature averaging 27°C. Photosynthetic temperature optima, as measured by net CO_2-uptake at 2000 ft-c, decreased with elevation of the source from 23°C for the 2400-foot source to 17°C for the 4800-foot source, a decrease of 2.7°C per 1000 feet. The results indicate a. close adaptation to the estimated temperature gradient along the altitudinal transect. Light intensity at the saturation point seemed to be 3000-4000 ft-c (22°C) for all sources. Light response should be studied in more detail.

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