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THE EFFECTS OF TEMPERATURE TREATMENT ON PHOTOSYNTHESIS OF PITCH PINE FROM NORTHERN AND SOUTHERN LATITUDES

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Pitch pine (*Pinus rigida* Mill.) seedlings were grown at constant temperatures of either 21 C or 29 C. Photosynthetic temperature and light response curves were determined after 59, 107, and 153 days. Rates of CO_2 uptake decreased with age, and the response to changes in light and temperature became less pronounced. Growth temperatures had no effect on the photosynthetic temperature optimum, and populations from Quebec, New Jersey, and Tennessee all had the same temperature optimum and response pattern. For the Tennessee population, growth at 21 C depressed the rate of photosynthesis at all temperatures at both 59 and 107 days, but stimulated it at 153 days. The Quebec population showed no effect of premeasurement temperature at 59 or 153 days, and the New Jersey population was intermediate in this respect. Photosynthetic plasticity, defined as the difference between photosynthetic rates of seedlings grown at 21 C and those grown at 29 C, was related inversely to the degree of seasonal variation under which the populations had evolved.

Introduction

We cannot completely comprehend ecological systems without understanding the physiological adaptations of plants to fluctuating climatic conditions and their genetic adaptations to spatial variation. Two types of photosynthetic adaptation to temperature variation have been observed, individual and population differentiation. Individuals may adapt or acclimate to the temperature regime in which they find themselves by modifications in their photosynthetic temperature optimum or temperature compensation points (MOONEY and WEST 1964; STRAIN and CHASE 1966; MOONEY and SHROPSHIRE 1967; MCNAUGHTON 1973; SORENSEN and FERRELL 1973). Such individual modifications, in response to environment, are examples of phenotypic flexibility or plasticity. The mechanisms for acclimation are not well understood but apparently involve complex adjustments in the diffusion pathway and regulation of the types of enzymes produced or their proportions (MOONEY and HARRISON 1970; MCNAUGHTON 1974). The capacity of individuals to modify their temperature response may be the result of natural selection in heterogeneous environments. The perfection of such mechanisms reduces the need for population differentiation as a response to spatial heterogeneity. When acclimation responses are not possible, or possibly inefficient, then populations may adapt specifically to local conditions, resulting in genetic races that differ in their temperature optima (FRYER and LEDIG 1972). As a third alternative, populations may develop genetic mechanisms to maintain polymorphism, allowing each generation to make its own response to current environments as a result of selection of preadapted segregates.

WENT (1958) felt that acclimation, or individual adaptability, was not common in higher plants. Since then, acclimation has been well documented and is considered a nearly universal phenomenon. Moreover, there is intraspecific variability in the capacity to acclimate. Populations from mild environments show greater ability to acclimate than those from rigorous environments (MOONEY and SHROPSHIRE 1967; MCNAUGHTON 1973).

We measured the effect of temperature acclimation on photosynthesis in pitch pine seedlings from northern, southern, and mid-latitude populations to determine if pitch pine had developed temperature races and if seedlings adjusted adaptively to their temperature regime. During ontogeny, the rate of photosynthetic CO_2 uptake in pitch pine changes severalfold (LEDIG, DREW, and CLARK 1976), so we investigated temperature and light responses at three stages in ontogeny.

Material and methods

Seeds were collected from six pitch pines (*Pinus rigida* Mill.) in each of three stands. One stand represented the northern extreme of the species range, one was near the southern extreme, and one stand was centrally located (table 1). For the purposes of this study, seeds were bulked within stand, using equal numbers of seed from each of the six trees in a stand.

Seeds were stratified in damp peat for 20 days, then sown into plastic pots 7.6 cm wide × 15.2 cm deep, and covered with 1.9 cm of white quartz sand. The soil mix was loam, peat, and sand (1:1:1). Seedlings were thinned to either one, two, or three per pot shortly after germination. A commercial 20:20:20 fertilizer was applied at periodic intervals.

The pots were divided between two growth chambers. Both chambers had a photoperiod of 16 h, relative humidity of 75% ± 5%, and light of circa 12,000 lx provided by fluorescent tubes supplemented with incandescent lamps. One chamber was maintained at 21 C (cool treatment) and the other at 29 C (warm treatment). These temperatures represent

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TABLE 1

PITCH PINE POPULATIONS USED IN THIS STUDY

Locality	County	State or province	Stand ID no.	N Lat.	W Long.	Elevation (m)
St. Chrysostome..........	Chateauguay	Quebec	17	45°06′	73°54′	69
Great Egg Harbor River...	Atlantic	N.J.	34	39°30′	74°46′	9
Bates Mountain..........	Blount	Tenn.	60	35°43′	83°45′	518

the range between mean maximum temperatures during the coolest months of the growing season in Quebec and the warmest months in Tennessee, sources of the northernmost and southernmost populations. Climatic data was provided by the National Weather Records Center, Asheville, North Carolina, and the Meteorological Branch, Ottawa, Canada. Tissue temperatures were not measured, but for needle-leaves of conifers heat transfer is rapid with adequate turbulence, and leaf temperature is usually close to air temperature (JARVIS 1971).

We measured CO_2 exchange according to the following schedule: (1) at 59 days after germination, five pots of three seedlings each from each population-temperature combination were sampled; (2) at 107 days, six or seven pots of two seedlings each from each combination; and (3) at 153 days, six pots (except four in one combination) of one seedling each from each combination. Actual completion of measurements required as much as ±7 days around the nominal harvest ages of 59, 107, and 153 days. Reduction of the number of seedlings per pot to two and one at the second and third periods was to prevent crowding and crown overlap.

Aboveground portions of the seedlings were sealed in Plexiglas cuvettes for measurement of CO_2 uptake. The rate of CO_2 uptake was determined over the interval of 280–320 ppm with an infrared gas analyzer (IRGA) operated in a closed system. Procedures were similar to those described by LEDIG and CLARK (1972) except the light source was a bank of 150-W cool-lux wide-flood lamps, and seedlings representing one complete replication were allowed to adjust to measurement conditions simultaneously. Solenoid valves, operated from outside the temperature-controlled system, switched seedlings from standby, during which humidified air was passed through the cuvette, to the IRGA circuit. Uptake of CO_2 was measured at 5,380, 10,760, 21,520, and 32,280 lx at 25 C to describe the light response curve.

Because previous observations had revealed no significant differences in dark respiration among pitch pine populations (including two of the populations reported here; LEDIG et al. 1976), respiration was ignored. However, we recognize that respiratory acclimation could be an important response to temperature variation. Temperature response at 10,760 lx was determined at 5, 10, 20, 25, and 35 C. The order was always lowest to highest temperature and highest to lowest light. Seedlings were allowed 20 min to adjust to a change in light or temperature before recording rate of CO_2 uptake. We found 20 min adequate to allow rates to reach equilibrium. Following each CO_2-exchange measurement, the seedlings were harvested. Roots were washed from the soil and separated from stem and leaves. Weights were determined after several days of drying at 90 C.

Because of the unequal numbers of observations, data were analyzed on a plot-mean basis with 3 sources × 3 dates × 2 premeasurement temperatures × 4 light intensities for the light response data and 3 sources × 3 dates × 2 premeasurement temperatures × 5 measurement temperatures for the temperature response series. All factors were considered fixed effects for the analysis of variance.

For convenience, we will refer to the rate of CO_2 uptake as "photosynthesis," although, strictly, we consider photosynthesis a process of photophosphorylation in which carbon fixation is merely one way of utilizing the reducing power of photochemical reactions.

Results

The rate of CO_2 uptake at 32,280 lx and 25 C decreased in a nearly linear fashion with age (fig. 1), and seedlings became less responsive to environmental conditions as they aged. Differences among populations were most pronounced at 59 days following treatment at 21 C (fig. 1). All populations responded in the same way to light and measurement temperatures, so data are bulked in figures 2 and 3. At 59 days, photosynthesis increased rapidly with increase in light to a saturation point between 10,760 and 21,520 lx (fig. 2). At 107 days, the rate of increase in response to increasing light was less rapid, and by 153 days there was almost no response to light within the range covered. Temperature response was also most pronounced at 53 days, and the response curve became progressively flatter up to 153 days (fig. 3). The rate of fall-off in photosynthetic rate from 25 to 35 C was greatest at 59 days and least at 153 days. The maximum rate of photosynthesis was near 25 C at 59 and 107 days but increased to higher values (between 25 and 35 C) at 153 days.

The effect of premeasurement temperature differed between 59- and 107-day-old seedlings on the one hand and 153-day-old seedlings on the other (figs. 2, 3). In general, rates of photosynthesis in young

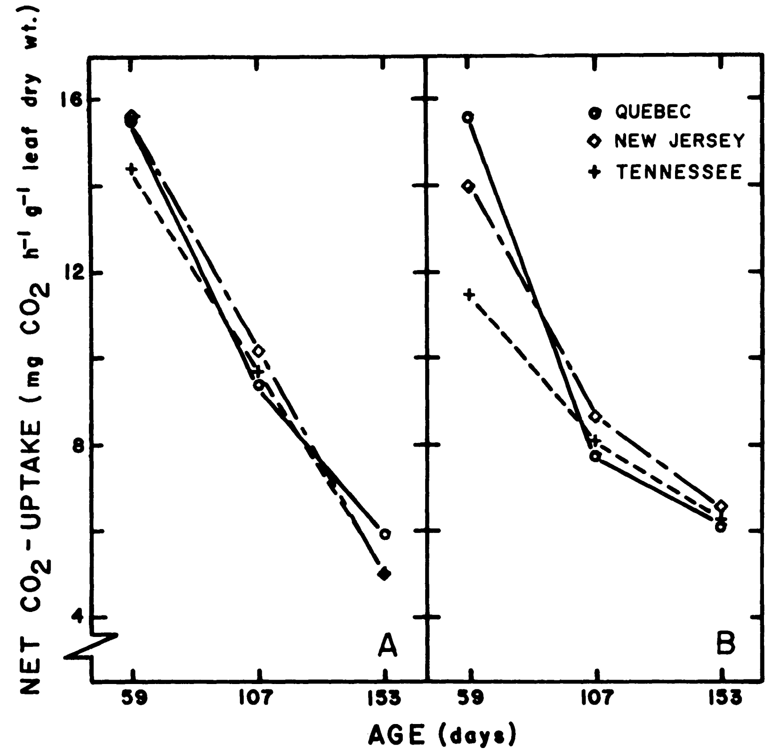

FIG. 1.—The rate of photosynthetic CO_2 uptake at 32,280 lx and 25 C decreased with age in pitch pine, including populations originating from the northern and southern extremes of the range (N = 6). Seedlings grown at: *A*, 29 C; *B*, 21 C.

seedlings were depressed by growth at cool temperatures relative to the rates of seedlings grown at warm temperatures. For 153-day-old seedlings, rates of photosynthesis were higher among those grown at cool temperatures. However, there was a statistically significant interaction of population with premeasurement temperature; that is, not all populations responded the same. The effect of cool treatment on the southern population was to depress maximum photosynthetic rates (i.e., the rate of CO_2 uptake at 25 C, 21,520 or 32,280 lx) 20% at 59 days and stimulate rates 20% at 153 days, while rates for the northern population were scarcely changed (2% and 4%, respectively). The mid-latitude population was intermediate. All populations were affected to the same extent at 107 days (see fig. 1). The extent of change is an index of plasticity: the absolute difference between cool- and warm-grown seedlings in maximum rate of CO_2 uptake (rates at 25 C and 21,520 or 32,280 lx). The difference in rates of CO_2 uptake between cool- and warm-grown seedlings was inversely related to the degree of seasonality at a population's origin ($r = -0.98$, nonsignificant because of the small number of populations, N = 3; table 2). We used the standard deviation computed among the January–December mean monthly maximum temperatures as an index of seasonality.

Two observations were impressive. (1) There was no interaction of measurement temperature with populations; all three populations had the same temperature optimum and response pattern. (2) There was no effect of acclimation on the photosynthetic temperature response pattern; premeasurement treatment at 21 C did not shift the optimum to lower values than treatment at 29 C.

Discussion

Because of the significant population × acclimation × age interaction, differences among populations can only be discussed in relation to levels of the other variables. Such interactions were also prominent for CO_2 uptake in Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco), and just as for pitch pine, population differences were most pronounced in early ontogeny and after exposure to cool temperatures (SORENSEN and FERRELL 1973).

An age-related decline in photosynthesis was previously observed for seedlings from two of these populations (LEDIG et al. 1976) and seems to be characteristic of conifers in general (KRUEGER 1963; LEDIG and PERRY 1969; SORENSEN and FERRELL 1973). Previously, we found that under constant conditions the decrease in photosynthetic rate continued only to circa 121 days after germination and then remained stable or increased slightly for another 60 days (LEDIG et al. 1976). As the seedling grows, self-shading within the crown increases, but it is unlikely that the decline in CO_2 uptake was the result of shading because it was particularly obvious at saturation intensities, that is, when light was not limiting. Some possible explanations are: (1) progressive senescence of lower leaves, (2) increased respiration as a result of increasing amounts of stem tissue, (3) increased diffusion resistance resulting from accumulation of wax in stomatal pores (HANOVER 1974), and (4) decreasing photosynthetic capacity in the sequentially produced leaf types, cotyledonary, primary, and secondary (BOURDEAU and MERGEN 1959; WRIGHT 1970). Probably all were contributing causes.

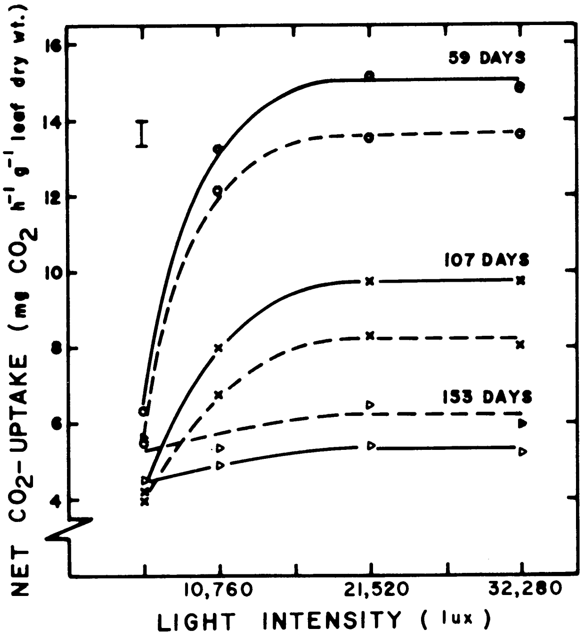

FIG. 2.—Photosynthetic light response curves for pitch pine at 25 C (N = 18; mean of three populations), showing the decrease in photosynthetic rate and in light response with age for seedlings grown at 29 C (———) or 21 C (---------). Bar indicates least significant difference for $t_{.01}$.

The decreased response of older seedlings to light suggested that the decline in photosynthesis was related to stomatal aging. It is well documented that the stomatal pores of mature conifer needles are almost completely occluded with wax (HANOVER 1974). The buildup of waxes may diminish the effectiveness of stomata in controlling gaseous exchange. The decrease in temperature response in pitch pine could suggest that limitations at the carboxylation step were also involved. However, stomatal resistance may be so high that it masks response at later steps in the "diffusion" pathway. Less pronounced age effects were noted in Douglas-fir seedlings and cattail (*Typha latifolia* L.) leaves (MCNAUGHTON 1973; SORENSEN and FERRELL 1973).

The depression of photosynthetic CO_2 uptake by growth at cool temperatures (e.g., 21 C relative to 29 C in the present case) is apparently a rather general phenomenon. It was observed in rice (*Oryza sativa* L.), Douglas-fir, six species of shrubby perennials, and cattail (MOONEY and WEST 1964; MOONEY and SHROPSHIRE 1967; MCNAUGHTON 1973; SORENSEN and FERRELL 1973; KISHITANI and TSUNODA 1974). Exceptions were several species of winter-active, desert perennials which were stimulated by low temperatures (STRAIN and CHASE 1966).

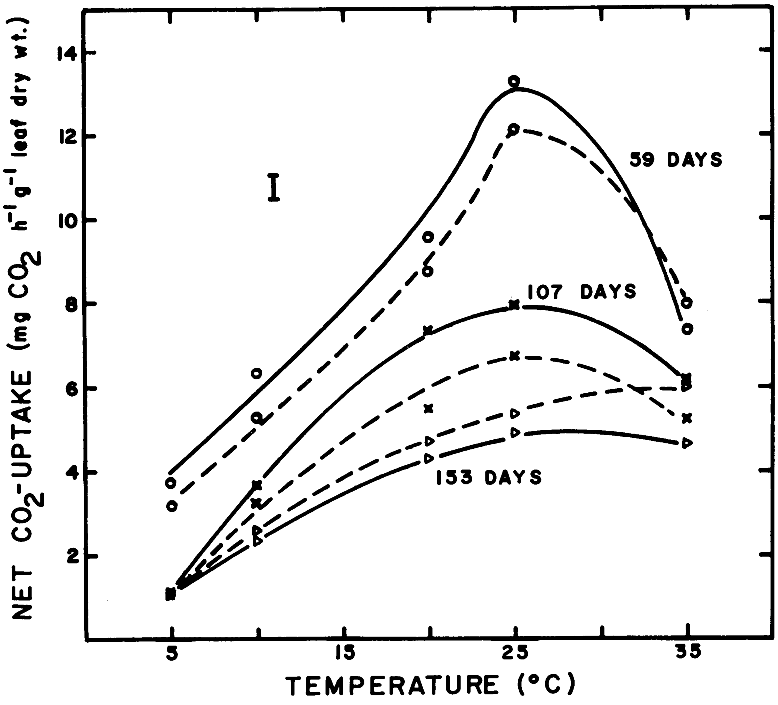

FIG. 3.—Temperature response curves for pitch pine at 10,760 lx (N = 18; mean of three populations), showing the decrease in photosynthetic rate and in temperature response with age for seedlings grown at 29 C (———) or 21 C (---------). Bar indicates least significant difference for $t_{.01}$.

TABLE 2

RELATIONSHIP BETWEEN SEASONAL VARIABILITY AT THE ORIGIN AND MAGNITUDE OF PHOTOSYNTHETIC RESPONSE (PLASTICITY) TO PREMEASUREMENT TEMPERATURE IN PITCH PINE SEEDLINGS FROM THREE POPULATIONS

Population	Seasonality index[a] (°C)	Plasticity index[b] (mg CO_2 h^{-1} g^{-1} leaf dry weight)
St. Chrysostome, Quebec	11.68	1.10
Great Egg Harbor River, N.J.	9.46	1.47
Bates Mountain, Tenn.	8.26	1.89

[a] Standard deviation among mean monthly maximum temperatures.

[b] Absolute difference between cool- and warm-acclimated seedlings in photosynthetic rate at light saturation and 25 C.

In pitch pine, the Quebec population was less affected by cool temperature than southern populations. The difference between rates of CO_2 uptake of warm-grown seedlings compared with cool-grown seedlings (table 2) varied among populations at the 5% level of significance, according to analysis of variance. Likewise, rates of CO_2 uptake in cattail from the mild Central Valley of California were depressed by acclimation at cool temperatures, but cattail from Quebec was scarcely affected (McNaughton 1973). In rice also, varieties from temperate regions were less affected by low temperature conditioning than varieties from the tropics (Kishitani and Tsunoda 1974). Coastal populations of knotweed (*Polygonum bistortoides* Pursh.) exhibited a greater shift in photosynthetic temperature response as a result of acclimation than high altitude populations (Mooney and Shropshire 1967). In both cattail and knotweed, shifts in temperature response curves were adaptive. It may be significant that cool-climate populations of cattail are more plastic than mild-climate populations in activity of certain key enzymes (McNaughton 1969). Perhaps enzyme induction permits cool-climate populations to maintain a stable metabolic rate in spite of temperature fluctuations while the more rigid enzymatic system of warm-climate populations results in more "plastic" or less homeostatic metabolism as revealed by CO_2 exchange.

The most significant observation for pitch pine was the lack of adaptive response to temperature. Some species adapt to local climatic conditions by evolving populations differing in temperature optima or temperature compensation points (Krueger and Ferrell 1965; Fryer and Ledig 1972; Sorensen and Ferrell 1973), but not pitch pine. Northern and southern populations of pitch pine originating from areas with very different thermal regimes (8 C difference in annual mean maximum temperature) had the same pattern of photosynthetic temperature response.

Individuals of other species adjust to the temperature in which they are grown by adaptive plasticity. For example, black spruce (*Picea mariana* [Mill.] B.S.P.), red spruce (*P. rubens* Sarg.), and their hybrids had a temperature optimum of 26 C when grown at 26 C but a temperature optimum of 16 C when grown at 12 C, a 0.7° change in optimum per 1° change in premeasurement temperature (Manley and Ledig, in preparation). In Douglas-fir, the optimum temperature was shifted circa 0.4° for a shift of 1° in premeasurement temperature (Sorensen and Ferrell 1973). But pitch pine seedlings had the same temperature optimum whether they were grown at 21 or 29 C. Nor was there evidence of individual differences in temperature optima in pitch pine as observed, for example, within populations of balsam fir (*Abies balsamea* [L.] Mill. [Fryer and Ledig 1972]).

Nevertheless, there was genetic variation among pitch pine populations in response to premeasurement temperatures. Though temperature and light curves were unchanged, photosynthetic rates of the southern population depended upon temperature during growth, suggesting a lack of physiological homeostasis, leading to an apparently nonadaptive plasticity in rates of CO_2 uptake. On the other hand, rates for the northern population were stable in the face of varied thermal histories, suggesting a better buffering ability against environmental fluctuation. Stability may be related to selection in variable environments. Northern latitudes are climatically more variable than southern. For example, monthly variation in mean maximum temperature is greatest in Quebec and least in Tennessee, so homeostatic ability to adjust to temperature change without loss of productivity would be most important for pitch pine from Quebec.

It is possible that results on seedlings may not reflect the situation in mature trees. However, behavior of the seedling stage is very critical. Seedlings must cope with environmental fluctuation without the benefit of substantial reserves from previous years. The opportunity for natural selection among seedlings is greater than at later stages; first-year mortality in sugar maple (*Acer saccharum* Marsh.), beech (*Fagus grandifolia* Ehrh.), and yellow birch (*Betula alleghaniensis* Brit.) was 42%, 59%, and 93%, respectively, at Hubbard Brook, New Hampshire (Forcier 1975). Because of the importance of natural selection in early ontogeny, adaptive responses are likely to be most pronounced in first-year seedlings.

Obviously, a great deal more research is needed to understand the photosynthetic system of pitch pine as well as that of other wild plants, particularly perennials. While pitch pine, balsam fir, and the red and black spruces discussed above are all physiologically C_3-species and taxonomically conifers, they exercise different mechanisms for coping with the temperature component of their environments. In balsam fir, temperature races evolved (Fryer and Ledig 1972); in spruce, there was a pronounced adjustment of the temperature optimum in response to the thermal regime (Manley and Ledig, in preparation); in pitch pine, neither tendency was obvious, yet populations differed in their ability to maintain a uniform photosynthetic rate in contrasting thermal regimes. Apparently, the organization and control of some components of the photosynthetic system differ markedly even within the physiological category of C_3-species.

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