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Photosynthesis in black and red spruce and their hybrid derivatives: ecological isolation and hybrid adaptive inferiority

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Photosynthetic responses of black and red spruce were used to define parameters of their fundamental niches. Grown at warm temperature, black spruce had highest rates of CO₂ uptake at high light intensities, fitting it for a pioneering role, while red spruce had the lowest light compensation point, fitting it for a late successional role. Black and red spruce responded in different ways to low temperature acclimation. Low temperature, low light (12°C, 100 ft-c (1 ft-c = 10.764 lx)) acclimation stimulated CO₂ uptake in black spruce under all conditions while it depressed CO₂ uptake in red spruce relative to rates following high temperature (26°C) acclimation. Niches defined by photosynthetic responses corresponded to observed habitats in the area of sympatry of black and red spruce and demonstrated the existence of ecological isolation, a premating restriction to hybridization.

Hybrids, backcrosses, and recurrent backcrosses of black and red spruce had lower rates of CO₂ uptake than one or the other species in all light and temperature environments tested. In most cases, the hybrid had lower rates than both species. Growth depends on photosynthesis and is one component of fitness. Therefore, negative heterosis in hybrids and hybrid derivatives represents another mechanism of reproductive isolation, the postmating barrier of hybrid adaptive inferiority. Selection against hybrids should be density dependent, explaining both their widespread occurrence in habitats opened by fire or logging and their paucity in closed stands.

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Le comportement photosynthétique de l'épinette rouge et de l'épinette noire a servi à définir les paramètres de leur niche fondamentale respective. A haute température, l'épinette noire a les taux les plus élevés d'absorption du CO₂ aux intensités lumineuses élevées, ce qui la rend adaptée au rôle d'espèce pionnière; par ailleurs, l'épinette rouge a le point de compensation lumineuse le plus faible, ce qui la caractérise comme une espèce des stades tardifs de la succession. Les deux espèces réagissent différemment à l'acclimation aux basses températures. L'acclimation aux basses températures à faible intensité lumineuse (12°C, 100 ft-c) stimule l'absorption du CO₂ chez l'épinette noire dans toutes les conditions, alors que chez l'épinette rouge elle diminue l'absorption du CO₂ par rapport au taux atteint à la suite de l'acclimation aux températures élevées (26°C). Les niches définies par les réactions photosynthétiques correspondent aux habitats des deux espèces dans la région où elles sont sympatriques et elles démontrent l'existence d'un isolement écologique, soit une barrière à l'hybridation agissant avant la fécondation.

Les hybrides et les produits de rétrocroisements, et de rétrocroisements récurrents entre l'épinette noire et l'épinette rouge ont des taux d'absorption du CO₂ plus faibles que chez l'une ou l'autre des espèces parentes, et ce pour toutes les conditions de température et de lumière expérimentées. Dans la plupart des cas, les hybrides manifestent un taux plus bas que celui des deux espèces parentes. La croissance dépend de la photosynthèse et est l'une des composantes de l'adaptation. Par conséquent, l'hétérosis négative des hybrides représente un autre mécanisme d'isolement reproducteur, agissant après la fécondation, soit la barrière de l'infériorité adaptative des hybrides. La sélection contre les hybrides doit dépendre de la densité, ce qui expliquerait leur fréquence dans les habitats ouverts par le feu ou la coupe et leur rareté dans les peuplements fermés.

[Traduit par le journal]

Introduction

Since publication of Anderson's (1949) book on introgressive hybridization, some botanists have

tended to view the occurrence of hybrids as evidence of introgression. For example, when hybrids and backcrosses of black spruce (*Picea mariana* [Mill.] B.S.P.) and red spruce (*P. rubens* Sarg.) were identified in uniform garden tests established with seed of *putative* red spruce, Morgenstern and Farrar (1964) concluded that introgression was oc-

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curing. Black $\times$ red spruce have been produced with ease by controlled pollination (Wright 1955; Fowler 1968; Fowler *et al.* 1970; Morgenstern and Fowler 1969), bringing to question whether the taxa are even biological species. The case became a textbook example drawing Stern and Roche (1974) to conclude that "Ecological isolation . . . in the Acadian region . . . is weak" and "No genetic barriers exist between the species."

However, recent field investigations in the Acadian region of Canada suggested that red and black spruce were clearly distinguishable on two extreme site types, i.e., red spruce on the uplands and black spruce in bogs (Manley 1972). Gordon (1976) has reached the same conclusion based on extensive taxonomic and ecologic analysis. Of the two taxa, black spruce has the wider distribution, being transcontinental through Canada. Red spruce is restricted to New England, the Canadian Maritimes, and higher peaks in the southern Appalachians. It is sympatric with black spruce throughout the Maritimes (Fig. 1). The similarity of sympatric populations from the extreme bog and upland site types to conspecific populations from areas where red and black spruce were allopatric suggested the possibility that introgression was not occurring. Furthermore, the understory, or advanced reproduction, on sites occupied by hybrids tended to be more like one or the other parental species, suggesting an ephemeral existence for the hybrids followed by succession to black spruce in bogs and red spruce on uplands. If in fact red and black spruce coexist as two species, then (1) they must be ecologically differentiated and (2) some mechanism of reproductive isolation must operate to preserve the distinction.

Our objectives were to measure certain niche dimensions of red and black spruce from sympatric populations and to determine the nature of their reproductive isolating mechanisms. To accomplish this, habitat preference and hybrid occurrence were studied throughout the Canadian Maritimes; seed set, germination, and seedling abnormalities were quantified for crosses within species and between and among species and their hybrid derivatives; survival and growth of species and hybrids were recorded in experimental plots under red and black spruce stands and in controlled environments; the frequency of natural hybridization was scored in seed collected at species ecotones; and photosynthetic responses of parental and hybrid types to light and temperature were measured. Only photosynthetic responses are reported here. Other aspects of hybridization between red and black spruce will be published later.

Materials and Methods

Material

Using a hybrid index based on 19 leaf, twig, bud, cone, and crown characteristics (Manley 1969, 1971), individuals were selected that corresponded to black spruce, red spruce, an F_1 , and the two backcross types. These were coded as 0, 100, 50 and 25 and 75, respectively. The trees were located on or near the Acadia Forest Experiment Station, New Brunswick, Canada, and originated from natural reproduction. Intraspecific and interspecific crosses, backcrosses, and recurrent backcrosses were made using standard techniques. Female strobili were isolated with nonwoven pollination bags ca. 3 weeks before anthesis. Pollen was collected by removing catkins prior to pollen shed and forcing dehiscence in the laboratory. At anthesis, pollen was introduced into the pollination bag with an atomizer. Cones were collected and seed was extracted in the fall. Resulting progeny were classified according to the expected mean hybrid index of the cross. For example, the cross of a black spruce backcross $\times$ putative F_1 (25 $\times$ 50) is expected to yield a progeny with a mean hybrid index of 37.5 under a model of completely additive inheritance. This index is termed the expected progeny index. Nine possible combinations were produced (0, 12.5, 25, 37.5, 50, 62.5, 75, 87.5, and 100) representing intraspecific crosses of black spruce at one extreme and red spruce at the other. The crossing scheme is illustrated in Table 1. Similar crosses were made in each of 4 years, using different parents each year.

Light Response

Seed was germinated in petri dishes at room temperature (20°C) and seedlings were transplanted into pots containing 3 in. (1 in. = 2.54 cm) of peat over 2 in. of a 1:1:1 sand:loam:peat mixture with a 1-in.-layer of gravel on bottom. There were three seedlings per pot. Pots were placed in the greenhouse on January 1 and grown under natural daylight supplemented by incandescent lighting to provide a photoperiod of 15 h.

After 3 months, 4 pots for each of the nine progeny types were used for CO_2 exchange measurements, for a total of 36 pots. All seedlings were active at the time of measurement. During the measurement of CO_2 exchange, seedlings were sealed in Plexiglas cuvettes using silicone rubber, and all three seedlings in the pot were measured as a unit. CO_2 exchange rates were measured in the dark and at 100, 200, 500, 1000, and 3500 ft-c (1 ft-c = 10.764 lx). Temperature during measurement was maintained at 21°C, corresponding to the mean diurnal temperature in the greenhouse during the growing period. Measurements were made with an infrared gas analyser (IRGA) in a temperature-controlled system similar to that described by Ledig and Clark (1972) which allowed all nine progeny types to become simultaneously adjusted to new conditions as light and temperature were varied. Humidity was not closely controlled but dew point was monitored continuously and maintained at high levels. For each change in light, seedlings were allowed to adjust ca. 0.5 h before measurement. Exit air temperatures were recorded by Cu-Co thermocouples and were within $\pm 1^\circ C$ of nominal. Air flow rate was 3 L/min, which corresponded to a calculated minimum air speed of 48 cm/min in the cuvettes used. However, a hot-wire anemometer indicated a highly turbulent flow varying from 0.3 to over 1.81 km/h within the cuvette. The light source consisted of 150-W Cool-lux wide flood lamps, and intensity was adjusted with cheesecloth screens. The basic measurement was the rate of CO_2 depletion or enrichment measured between 330 and 280 ppm by volume CO_2 in a closed system.

Immediately after measurement, seedlings were separated into leaves, roots, and stems and oven-dried at 30°C. Rates of apparent CO_2 exchange were calculated on the basis of milligrams CO_2 per hour per gram dry weight of leaf tissue.

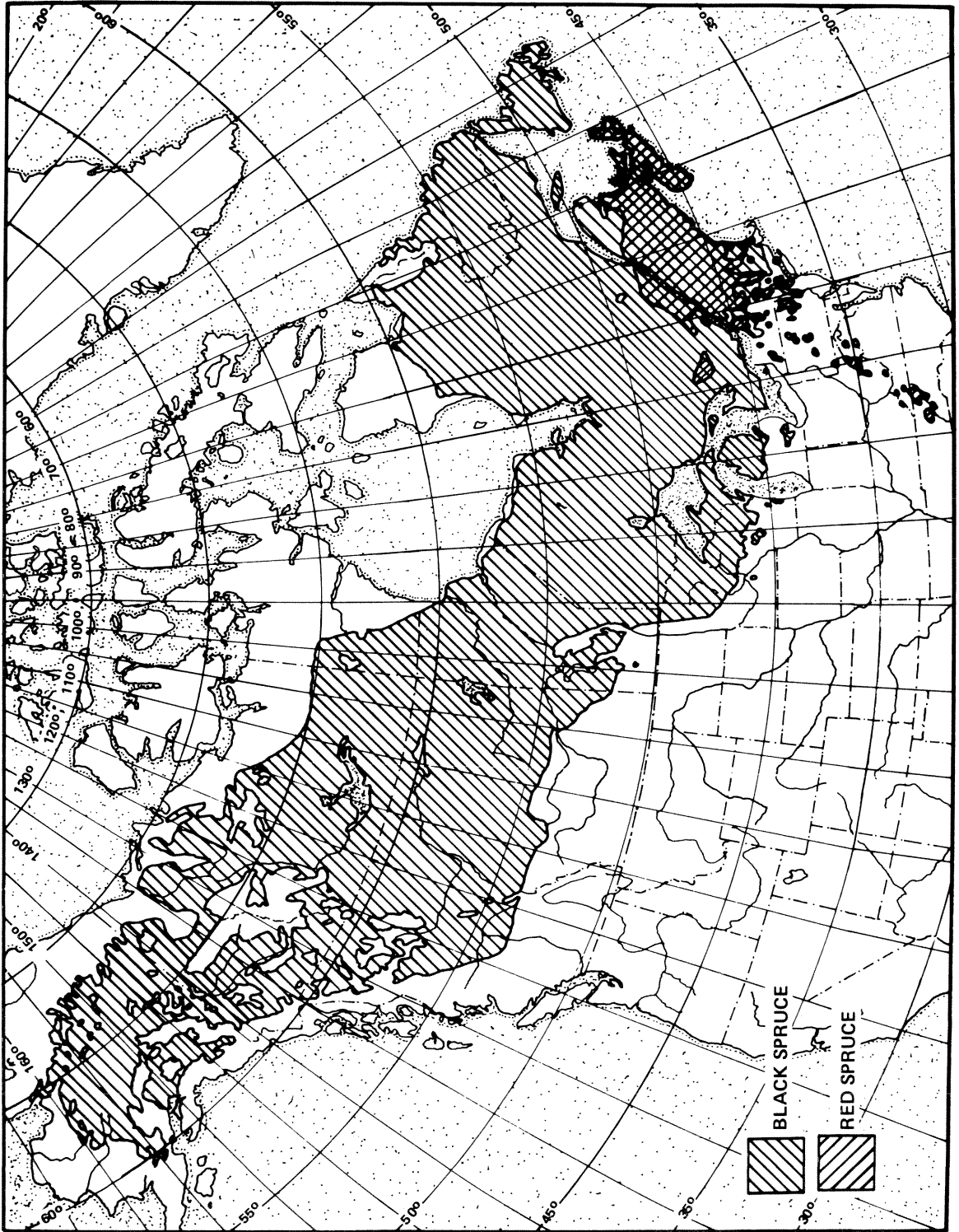


FIG. 1. Range of black and red spruce. The species are sympatric over extensive areas in the Canadian Maritimes and northern New England.

TABLE 1. Crossing design for black and red spruce and their putative natural hybrids and backcrosses showing expected mean hybrid index of progeny

Series 1, hybrid index of female trees	Series 2, hybrid index of male trees				
	0*	25	50	75	100
0*	0†	12.5	25	37.5	50
25	12.5	25	37.5	50	62.5
50	25	37.5	50	62.5	75
75	37.5	50	62.5	75	87.5
100	50	62.5	75	87.5	100

*Parents were assigned hybrid index values based on a morphological scale using 19 characteristics (see Manley (1971) for the hybrid index). 0 represents black spruce, 25 a backcross to black spruce, 50 an F₁ hybrid, 75 a backcross to red spruce, and 100 a red spruce. There were three series of parents; each group of five trees (0, 25, 50, 75, 100) represented a series. Series 1 was crossed with series 2. Series 2 males served as females in crossing series 2 with a third series, series 3 (not shown). Series 3 was then crossed with series 1 so that there were three replications of each type of cross.
†Progeny produced by controlled crossing. The hybrid index value appearing for the progeny was determined by averaging the hybrid index of each parent, i.e., 0 × 0 = 0 and 0 × 25 = 12.5. The progeny hybrid index value is referred to as expected hybrid index.

Temperature and Light Acclimation

Seed was germinated and seedlings were transplanted three per pot as described above. Replicated pots were placed in growth chambers under two light intensities, 100 and 2000 ft-c, at each of two temperatures, 12 and 26°C (a 2 × 2 factorial). Green nylon screens were used to reduce light to the 100 ft-c level. One hundred foot candles simulates conditions under a red spruce canopy and was believed marginal, at best, for black spruce. The cool temperature was expected to favor black spruce and the warm temperature, red spruce.

After 3 months, CO₂ exchange rates were measured. Four pots of seedlings were selected from each of the nine progeny types from each of the four growth-chamber conditions, for a total of 144 pots. There was significant nanism among the hybrids and hybrid derivatives; therefore, only apparently normal seedlings were used for measurements of CO₂ exchange. All seedlings had set buds except those grown at 2000 ft-c, 26°C. CO₂ exchange rates were measured in a factorial arrangement over four temperatures (12, 16, 21, and 26°C) at two light intensities (2000 and 100 ft-c). Each CO₂ exchange measurement could be classified by progeny type, light under which the seedlings were grown, temperature under which they were grown, light at which they were measured, and temperature at which they were measured. Data were analyzed as a 9 × 2 × 2 × 2 × 4 factorial with four replications. In the following description, light and temperature regimes under which the seedlings were grown will be referred to as acclimation conditions and light and temperature under which CO₂ exchange rates were measured will be referred to as measurement conditions. Methods were the same as those described above for the light response experiment.

Results

Light Response

Differences among species and hybrids and their interactions with light intensity were statistically significant. Red spruce was apparently light saturated at 1000 ft-c while the rate of photosynthesis in black spruce continued to increase up to 3500 ft-c, the highest intensity tested (Fig. 2). Logan and Pollard (1971) reported light saturation for excised

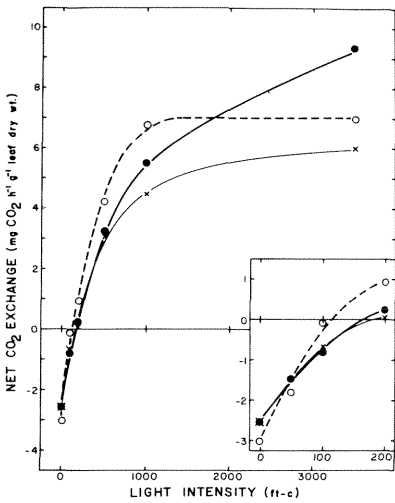


Fig. 2. Light response curves of photosynthetic CO₂ uptake at 21°C; means for seedlings of black spruce (●), red spruce (○), and their F₁ hybrid (×). Inset shows points near light compensation in expanded scale. Backcrosses, recurrent backcrosses, and other hybrid derivatives are omitted for clarity. n = 4.

twigs of black spruce at 4000 ft-c. The light compensation point was ca. 110 ft-c in red spruce and nearly twice as high (195 ft-c) in black spruce. Some red spruce maintained net photosynthesis at light even as low as 75 ft-c. Photosynthetic rate in the F₁ hybrid was always lower than in one or the other parent and above 500 ft-c it was lower than in either parent. Nevertheless, the shape of the light response curve for hybrids was apparently intermediate. The light response curves of backcrosses and recurrent backcrosses tended to approach those of the recurrent parent, either red or black spruce. Nearly identical results were obtained in preliminary experiments performed in a different year using crosses among different series of parents.

All hybrid derivatives, including even recurrent backcrosses, had lower rates of photosynthesis than one or the other of the parental types at each light intensity tested. Hybrid inferiority in photosynthetic rate may have been even more pronounced if nanistic individuals were included in the results.

Temperature and Light Acclimation

All interactions and main effects were statistically significant. The temperature optimum for CO₂ uptake was higher at 2000 than at 100 ft-c (Fig. 3). An increase in the temperature optimum usually accompanies an increase in light intensity (e.g., Scott and Menalda 1970). Seedlings acclimated at 26°C had a higher optimum temperature than seed-

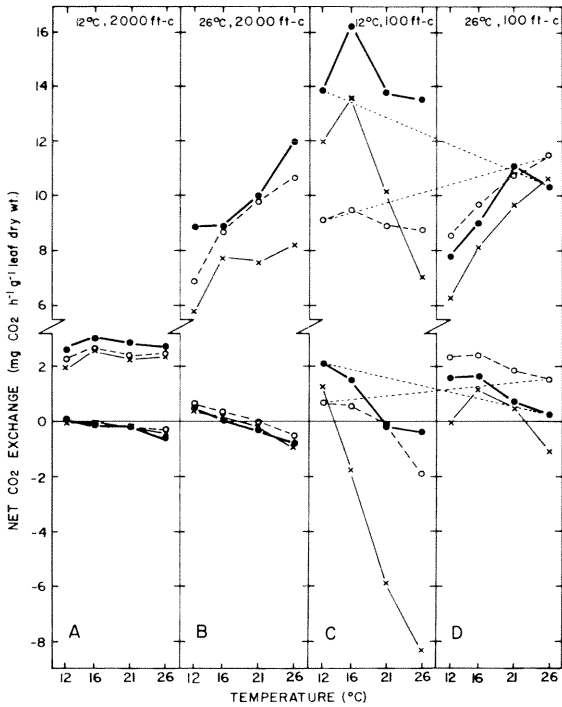


FIG. 3. Mean photosynthetic CO_2 uptake in relation to measurement temperature at 2000 ft-c (upper curves) and 100 ft-c (lower curves) for seedlings of black spruce (●), red spruce (○), and their F_1 hybrid (×). Backcrosses, recurrent backcrosses, and other hybrid derivatives are omitted for clarity. Seedlings were acclimated for 3 months in controlled environments at (A) 12°C , 2000 ft-c; (B) 26°C , 2000 ft-c; (C) 12°C , 100 ft-c; and (D) 26°C , 100 ft-c. In C and D, dotted lines connect photosynthetic rates measured at the same temperatures at which the seedlings were grown (after the graphic method of Christophersen (1967) and Billings *et al.* (1971)). Adjustment to warm temperature was positive for red spruce and negative for black spruce grown at 100 ft-c. In Christophersen's (1967) terminology, compensation for red spruce was "inverse" and varied from "supraoptimal" at 2000 ft-c to "partial" at 100 ft-c for black spruce. Following acclimation at 2000 ft-c (A and B), all responses were "inverse" when measured at 2000 ft-c and "partial" when measured at 100 ft-c. Note the general inferiority of the hybrid. $n = 4$.

lings acclimated at 12°C (a reasonable response). Measured at 2000 ft-c, the temperature optimum of seedlings acclimated at 26°C was at least 26°C , except for black spruce which had an apparent optimum at 21°C when grown at 100 ft-c. The suggestion of a lower temperature optimum for black than for red spruce (Fig. 3D) is compatible with the boreal distribution of black and temperate distribution of red spruce. When acclimated at 12°C , the temperature optimum measured at 2000 ft-c was ca. 16°C for all crosses at light intensities of either 100 or 2000 ft-c. Some change in the optimum temperature often accompanies changes in acclimation temperature (e.g., Mooney and West 1964; Strain

and Chase 1966; Sorensen and Ferrell 1973), but for these spruce, the shift was nearly complete. That is, there was a 10°C shift in optimum for a 16°C change in acclimation temperature.

Neither red nor black spruce responded to acclimation light as a sun species. In general, seedlings grown at 100 ft-c had higher rates of CO_2 uptake measured at 2000 ft-c than those actually grown at 2000 ft-c, especially for seedlings grown at 12°C . The one exception is that rates for black spruce differed little for seedlings grown at 26°C and 100 ft-c versus 2000 ft-c (Figs. 3B, 3D). One explanation is that seedlings grown at higher intensity had greater leaf area than seedlings at 100 ft-c, so greater self-shading may have occurred. Self-shading reduces the light intensity received by a leaf and thus reduces photosynthetic rate. Unfortunately, it is difficult to measure CO_2 exchange on single needle leaves of spruce, so this hypothesis was not tested. Physiological adaptation to low light was obvious in seedlings grown at 100 ft-c. For example, black spruce grown at low light demonstrated net photosynthesis at 100 ft-c, a value below its compensation intensity when grown under full light in the greenhouse (Figs. 3C, 3D). However, the compensation intensity of red spruce also decreased so that when grown at 26°C and either 2000 or 100 ft-c or at 12°C and 2000 ft-c (Figs. 3A, 3B, 3D), the photosynthetic rate of black spruce at 100 ft-c was lower than that of red spruce, confirming expectations based upon the light response curves for greenhouse-grown seedlings (Fig. 2). That is, black spruce grown at 26 or 12°C and 100 ft-c was frequently superior to red spruce when measured at 2000 ft-c but still inferior at 100 ft-c, although the differences were so slight they were not statistically significant.

However, results for seedlings acclimated at 12°C and 100 ft-c showed a remarkable reversal (Fig. 3C). After exposure to low temperature, black spruce was superior in photosynthetic rate to red spruce even under conditions to which red spruce seemed best adapted otherwise (low light, high temperature). This is the most interesting result of the acclimation experiment and its possible significance will be discussed below.

The photosynthetic response curves reveal that hybrids and hybrid derivatives did not have lower photosynthetic rates than *both* species under all conditions. However, they did have lower rates than *at least one* of the parental types under virtually all conditions. Hybrid inferiority was most pronounced after growth at 26°C , particularly when CO_2 uptake was measured at 2000 ft-c. Hybrid de-

rivatives were intermediate or nearly intermediate after growth at 2000 ft-c and measurement at 100 ft-c, conditions that resulted in little differentiation between parental species.

Discussion

Photosynthesis and Fitness

We argue that photosynthetic rate is indicative of fitness in tree seedlings. While fitness depends on many factors, for plants in general and trees in particular, fitness in part is dependent on growth rate under conditions of interspecific and intraspecific competition for light and water. In *Pinus taeda*, the number of seedlings per acre may vary from 900 to 12 000 but will reduce to only 150 trees per acre at maturity (Allen 1961). In *Eucalyptus* spp., competition may reduce cohorts from 200 000 to 10 per acre by the time that senescent stands are ready for reproduction (Barber 1965). And in *Fagus silvestris*, 500 000 trees per hectare at age 10 may be reduced to 400 at age 100 (Assman 1970). A population of 7000 to 20 000 spruce per acre at 10 years of age will be reduced at maturity to 680. Because trees grow to tremendous size, there must be a complementary reduction in numbers before maturity. Therefore, there is ample opportunity for the most rapidly growing, favored by chance or by genotype, to overtop others in their cohort. In terms of MacArthur's (1968) model, the dominants may reduce the light resource below the minimum required for survival of the suppressed individuals. On the other hand, if the resource was not reduced below minimal requirements, the suppressed individuals could survive and reach maturity upon senescence of the overstory.

Growth, in turn, is closely related to photosynthesis, a sensitive measure of a plant's adaptation to environmental factors. In the present experiment, dry weight of seedlings was correlated with the rate of CO_2 uptake measured at the same light and temperature under which the seedlings had grown (Fig. 4). All correlations among crosses within environments were positive, ranging from 0.47 to 0.87, and the correlation over all environments (12°C, 100 ft-c; 12°C, 2000 ft-c; 26°C, 100 ft-c; 26°C, 2000 ft-c) and crosses was 0.87. The relationship between rate of CO_2 uptake and growth has been demonstrated for several other tree species (e.g., Fryer and Ledig 1972; Ledig and Botkin 1974).

Ecological Isolation

Black and red spruce have different fundamental niches in the Hutchinsonian sense. These niches apparently correspond to natural habitats found in

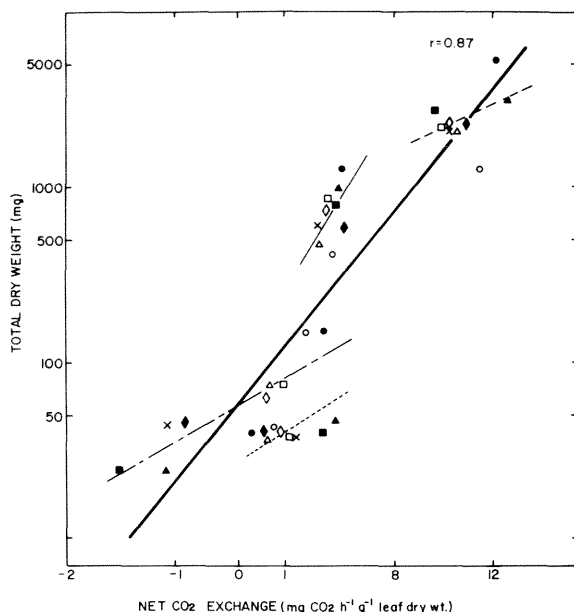


FIG. 4. The relationship of total seedling dry weight in four growth-chamber environments to photosynthetic CO_2 uptake measured at the same light intensity and temperature. Thin lines represent regressions within environments ($r = 0.47$ to 0.87), and the thick line represents the regression over all environments. Logarithmic scales were used for illustration because dry weights spanned three orders of magnitude. Symbols for expected progeny indexes: 0, $\bullet$; 12.5, $\blacktriangle$; 25, $\blacklozenge$; 37.5, $\blacksquare$; 50, $\times$; 62.5, $\square$; 75, $\diamond$; 87.5, $\triangle$; 100, $\circ$. $n = 4$.

the Canadian Maritimes and the result is ecological isolation. Though ecological isolation is based on microgeographic separation, it is the result of genetic differences in environmental response and, therefore, is a form of reproductive isolation. Response to temperature acclimation may also relate to the allopatric separation of black and red spruce, black spruce ranging into cooler climates in Canada and red spruce extending southward in the eastern United States. Of course, ecotypic differentiation may also occur within species.

Provided that an adequate source of seed is available, black spruce usually dominates sites opened by fire or logging, and this may be related to its ability to utilize high light intensities for photosynthesis, while red spruce is light saturated at 1000 ft-c. Black spruce has other adaptations favoring its role as a colonizer of disturbed habitats, e.g., partially serotinous cones that remain closed after maturation but open following fire and light seed suited to dispersal into openings (Fowells 1965).

We hypothesize that even though red spruce may be overtopped and suppressed by black spruce, on

warmer sites it could maintain itself in the shade of the dominants because of its lower light compensation point at moderate temperatures. Eventually, the initial overstory of black spruce would be replaced by red spruce, which can reduce the light resource below the level required for survival of black spruce reproduction, a phenomenon observed in the field. Red spruce is known as a climax or subclimax species (Fowells 1965).

Red and black spruce exhibit a complex pattern of acclimation to temperature. According to the classification of Christophersen (1967), the pattern of adjustment varied from inverse to supraoptimal depending on the light intensity at which seedlings were acclimated. In contrast, adjustment of arctic and alpine ecotypes of *Oxyria digyna* was ideal (complete compensation) or inverse (Billings *et al.* 1971; however, these authors apparently interpreted Christophersen's categories differently than we and cited their results as illustrations of ideal and partial compensation). The most obvious difference between red and black spruce occurred when they were grown at 100 ft-c. The response to high temperature acclimation in black spruce was strongly negative (varying from supraoptimal to partial compensation in Christophersen's terminology) at both 100 and 2000 ft-c, but the slope of adjustment in red spruce was positive, demonstrating inverse compensation. The terms are unfortunate, implying inferior or superior adjustment, but this depends on the points of view, e.g., supraoptimal adjustment of photosynthesis to high temperatures is disadvantageous in plants moved to a warm temperature regime and a species with ideal compensation may still be inferior to one with partial compensation if its rate of photosynthesis is lower. Based on the significant interaction between species and acclimation temperature, black spruce is adapted, in the evolutionary sense, to cool climates and red spruce to warm climates. This agrees with the boreal distribution of black spruce and temperate distribution of red spruce (Fig. 1).

We propose for further testing under field conditions a hypothesis which provides a physiological explanation for the habitat divergence of red and black spruce. Topography is gentle in those parts of the Maritimes where black and red spruce are sympatric. Black spruce bogs are separated from adjacent red spruce uplands by elevations of 3–6 m and occasionally up to 45 m. There is not sufficient difference in elevation to have an appreciable effect on adiabatic cooling. Rather, bogs and stream beds are much cooler because they are sinks for cold air drainage during the night. Based on results in other

species, some degree of temperature acclimation can occur within 24 h (Mooney and Harrison 1970), though the rate of response was not tested for black and red spruce. Black spruce reacts to cool temperatures by a positive adjustment in its photosynthetic rate, but red spruce has a negative adjustment. Exposure to cool night temperatures may have the effect observed under controlled conditions. That is, black spruce seedlings may become capable of net photosynthesis under marginal light intensities while red spruce experiences net loss of CO₂. Therefore, black spruce would be the climax type in cool environments such as bogs. Because bog stands break up rapidly, light is perhaps 30% of full sunlight (Gordon 1976), a situation to the advantage of black spruce but not red spruce. Additional factors in the separation of black and red spruce may be differential tolerance of flooded and acidic soils. However, acclimation response to cool temperatures (Fig. 3C) may be sufficient to explain the predominance of black spruce in bogs.

Hybrid Adaptive Inferiority

The most notable aspect of the results was the high degree of negative heterosis revealed by the hybrid. Obviously, the adaptive gap between red and black spruce was too broad to be spanned by hybridization. Hybrids suffered from the recombination of noncoadapted systems. In three separate experiments involving different growth conditions and crosses among different sets of parents, the F₁ and intermediate hybrid derivatives averaged photosynthetic rates 25% lower than the biparental mean (Fig. 5). Hybrids also exhibited negative heterosis for growth, to be reported later.

Intraspecific hybridization is often associated with positive heterosis for yield, but there are few reports of photosynthesis in hybrids. For intervarietal hybrids, positive heterosis was reported for *Zea mays* (Heichel and Musgrave 1969), while in *Phaseolus vulgaris*, the rate of photosynthesis in the F₁ was lower than the midparental mean (Izhar and Wallace 1967). Hybrids of sun and shade ecotypes in *Solidago virgaurea* were heterotic for photosynthetic rate as a result of their low stomatal resistance (Holmgren 1968). The interspecific hybrid, *Eucalyptus fastigata* × *robertsonii*, had a higher rate of CO₂ uptake, higher light saturation, and lower light compensation point than either parent (Brittain and Cameron 1973). Photosynthesis in natural hybrids of *Phlox maculata* and *P. glaberrima* was either intermediate or superior to that of the parents over a range of environmental conditions, and the photosynthesis–respiration ratio was

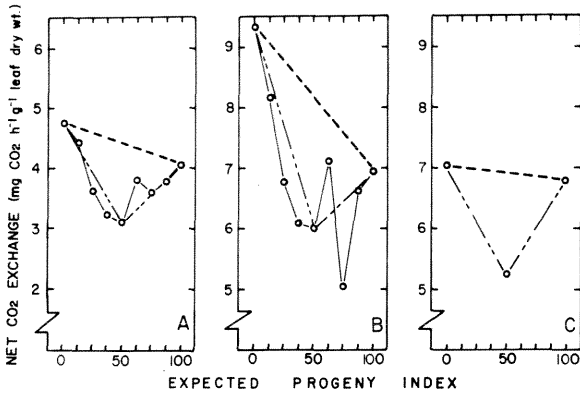


FIG. 5. Negative heterosis for CO₂ uptake in hybrids and hybrid derivatives of black and red spruce. Based on a completely additive model of inheritance, hybrid values should fall on the broken line connecting black spruce (expected progeny index 0) and red spruce (expected progeny index 100). Deviation below the line is a measure of negative heterosis. In general, the greater the admixture of black and red spruce genes, the greater the negative heterosis, and even recurrent backcrosses (expected progeny indexes 12.5 and 87.5) appeared inferior to their recurrent parent. (A) Mean of CO₂ exchange rates over all acclimation and measurement conditions for seedlings grown in a growth chamber in a temperature and light acclimation experiment ($n = 132$). (B) CO₂ exchange rates at 3500 ft-c for greenhouse-grown seedlings ($n = 4$). (C) CO₂ exchange rates at 3000 ft-c for seedlings grown in growth chambers at 26°C day and 15°C night temperatures and 1000 ft-c. $n = 5$ to 9.

greatest for individuals with hybrid indices closest to the F₁ (Hadley and Levin 1969). An F₁ hybrid of *Gossypium barbadense* and *G. hirsutum* had higher rates of photosynthesis than parental species (Elmore *et al.* 1967).

In the black and red spruce complex, individuals closest in genotype to the F₁ had lowest photosynthetic rates, and even recurrent backcrosses were inferior when compared with their recurrent parent (Fig. 5). We know of only one other example of negative heterosis for photosynthesis, the cross between *Atriplex rosea* and *A. patula*, C₄ and C₃ species, respectively (Björkman *et al.* 1970; Björkman, Nobs, and Berry 1971; Björkman, Percy, and Nobs 1971). In the case of *A. rosea* and *A. patula*, negative heterosis might be expected because these species have unique leaf anatomies associated with different metabolic pathways for CO₂ reduction. Red × black spruce hybrids are apparently the first example of negative heterosis for CO₂ uptake in species with the same photosynthetic pathway, suggesting that there may be more variation in the organization of the photosynthetic system (including components attributable to diffusion and to light interception) than is customarily acknowledged. Certainly, negative heterosis indicates the red and black spruce

genomes have diverged to the point where they are incompatible, i.e., morphological or physiological systems in the hybrids are uncoordinated. Hybrid inviability or adaptive inferiority is also revealed by lower germinative capacity, higher incidence of embryo and seedling abnormalities, and lower survival than parental species when seeded or planted in stands of red or black spruce (Manley and Ledig, unpublished).

Selection against hybrids will be density dependent, not absolute. Hybrids were not so unfit that they were incapable of survival, at least in the absence of parental competition and competition from other species. However, the denser the reproduction, the more intense the competition and the less chance of survival for hybrids and hybrid derivatives compared with black or red spruce. Manley (1972) originally hypothesized that hybrids and hybrid derivatives were associated with an intermediate or "hybridized habitat" created by fire or logging. Apparently, it is not the intermediate nature of the habitat that is critical to hybrid survival but the lack of competition, a conclusion also reached by Gordon (1976). There were no environments in which hybrids were superior to parental types. Anderson's (1949) notion of habitats "hybridized" by disturbance should be considered more critically, as Grant (1971) suggested. In fact, even Anderson (1956), who popularized the term, recognized that freedom from competition, not habitat intermediacy, was the critical factor when hybridization was observed.

The occurrence of extensive hybrid swarms of black and red spruce is understandable in view of the large-scale disturbances by fire and logging in the Canadian Maritimes. Loggers invariably leave a few red spruce because of small size or poor form. Chance factors act to spare isolated red spruce from wildfires. As the scattered remnants of red spruce mature, they are flooded with pollen from the pockets of black spruce remaining in bogs which were never logged because of the small size of black spruce and the difficulties in harvesting poorly drained sites and which were seldom burned because of the high water table (see Gordon (1976) who also suggested this scenario). Because red spruce pollen will be rare, hybrids will occur and will suffer lessened disadvantage in a competition-free habitat. If the disturbed area is large enough, the hybrids may mature and backcross or intercross, eventually reforesting the site. Judging from observations in the Maritimes (Manley 1972; and also noted by Gordon (1976)), as succession proceeds on the uplands, pure red spruce become more frequent in the understory,

perhaps because they can tolerate lower light intensities than the hybrids, and hybrids are selectively eliminated. In undisturbed forest with closed canopies and high leaf-area index, hybrid types are eliminated as they arise, for similar reasons. Bogs are rarely disturbed, but in bogs, we hypothesize that hybrid types would be eliminated in competition with black spruce because of its positive response to cool temperatures and perhaps other factors as yet unevaluated. Our ideas regarding the ecological role of red and black spruce in sympatry and the nature of isolating barriers between the species are substantially the same as those of Gordon (1976), although our conclusions were reached independently.

Both ecological isolation and hybrid adaptive inferiority are reproductive barriers between black and red spruce. The Dobzhansky (1951) hypothesis suggests that postmating barriers, such as adaptive inferiority, should result in selection for premating barriers, the subject of future reports. Whether introgression occurs is still debatable, but if it does, at least it has not resulted in the breakdown of species isolation. Earlier conclusions (Stern and Roche 1974) that "No genetic barriers exist between the species" are untenable.

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