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To the Graduate Council:

I am submitting herewith a thesis written by Nancy C. Wilson Griffin entitled "Germination and Early Survival of Picea rubens Sargent in Experimental Laboratory and Field Plantings." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Science Education.

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GERMINATION AND EARLY SURVIVAL OF PICEA RUBENS
SARGENT IN EXPERIMENTAL LABORATORY
AND FIELD PLANTINGS

A Thesis
Presented to
the Graduate Council of
The University of Tennessee

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Nancy C. Wilson Griffin
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CHAPTER I

THE PROBLEM

In the Smoky Mountains Picea rubens Sarg. (red spruce) occurs at elevations which suggest that it should occur further south than it does. Whittaker (1956) describes the potential altitudinal range of the spruce-fir forest as being in the southwestern Smoky Mountains. High-elevation deciduous forests with reduced stature, and "perhaps well beyond their favorable conditions of temperature and growing season," have replaced the sub-alpine forest type. If, as has been suggested by Whittaker (1956) and Mark (1958), historical factors based on changes of climate in geological time have eliminated red spruce southward, then a modern man-made range extension today might be successful.

The objectives of the study are: (1) to determine first-year seed germination and survival of red spruce in four areas above 4000 feet in the Southern Appalachian Mountains outside the natural range of red spruce, using four seed sources; (2) to correlate effects of altitude, slope, soil and seed source with germination and survival in the field; (3) to compare red spruce seed germination on varying substrata and regulated soil pH's at different depths of planting under controlled conditions in the laboratory; (4) to compare red spruce seed germination under controlled conditions in the laboratory with field germination; (5) to determine red spruce seedling growth at varying elevations in the Smoky Mountains.

CHAPTER II

LITERATURE SURVEY

The distribution of most plant species is determined largely by such environmental factors as temperature (especially the number of days during which the temperature remains above a certain minimum), light (especially day length as related to the flowering process), and edaphic factors.

Red spruce has a distribution in eastern North America which is observed for a variety of "Boreal-type" plants. It is classified as a northern species that is restricted to areas where the climate is cool and humid. In the middle and southern parts of its range, red spruce is almost entirely limited to the upper elevations where conditions of temperature and moisture are imitative of those found within the Acadian section of the Boreal Forest. According to Little (1953), red spruce ranges from "Nova Scotia to Maine and southern Quebec, south to eastern New York, northeastern Pennsylvania, and northern New Jersey. Also south in Appalachian Mountains of western Virginia, western Maryland, West Virginia, western North Carolina, and eastern Tennessee."

In the Thornthwaite classification of 1948, Shanks (1954) locates Southern Appalachian spruce-fir in perhumid-Mesothermal climate with higher stands passing out of the classification system. Within the entire range of red spruce, average annual temperature varies from 4.5 - 12.7° C; average annual precipitation ranges from 88 - 200 cm.; frost-free period varies from 100 - 180 days, depending on latitude and local climate (Korstian, 1937).

The soil of conifers shows a decided podsolization, high acidity and leaching. High soil moisture, sometimes with poor aeration, low soil temperature and pH and a short decay season account for low micro-organismal activity resulting in a low decomposition rate of litter and subsequent deep litter (Cain, 1931; McGinnis, 1958). The rate of litter breakdown on the forest floor influences the release of nutrients to the soil and their availability to growing plants. Breakdown is affected by the litter species and the environment in which the litter is decomposing. The rate of litter breakdown is affected by altitude with a "2.4 per cent difference in rate of breakdown per 1000 ft. elevation or approximately 1 per cent per degree Fahrenheit"; however, no significant difference is seen in the regression slope between deciduous and evergreen forest (Shanks and Olson, 1961). McCracken, Shanks, and Clebsch (1962) report Sol Brun Acide soils in the spruce-fir forest of the high elevations in the Smoky Mountains are characterized by very low base status, high exchangeable Al and a high C/N level.

A comparison of mineral cycling of deciduous and evergreen vegetation types in the high altitudes of the Smoky Mountains indicates that the leaves, flowers and fruits of the deciduous vegetation contain an appreciably higher amount of Ca and K than those of the evergreen forest (Shanks, Clebsch, and DeSelm, 1961).

Red spruce is described by Murphy (1917), Korstian (1937), Westveld (1931), Hart (1959), and others as a medium-sized evergreen forest tree (about 75 feet in average height). It has a shallow root system, making it susceptible to windthrow and a thin bark with a resinous exudate.

A minimum degree of maturity, regardless of size and height, is essential before the tree may become seed-bearing. This is generally found to be between thirty to forty years of age. Under exceptional conditions red spruce may begin bearing seed at an earlier age (Murphy, 1917).

Male and female strobili are found on the same tree, but on separate twigs.. Cones, ovoid in shape, mature from September to October, at which time the seedfall begins and continues until March. Seeds are disseminated mainly by animals and wind (Murphy, 1917).

Baldwin (1934) describes the mature red spruce seed as being "very dark brown, about three mm. long, with short, broad wings rounded above the middle. The testa, or coat, consists of an outer crustaceous layer, dark brown and occasionally mottled or streaked, and an inner membranous layer, pale chestnut brown and lustrous. The pale yellow embryo is axile in a large, fleshy endosperm. The number of cotyledons varies from four to seven."

Germination involves a renewal of embryonic activity. Among the conditions necessary for seed germination are a moist, aerated substrate at a suitable temperature. Water is found to be the single most important factor in germination and establishment of red spruce within the range of favorable temperature (Baldwin, 1934). Water is necessary for the hydrolysis of the food reserves in the seed, and it allows for mechanical swelling and bursting of the seed coat (Baldwin, 1934). Following germination, seedling survival is often a matter of water availability-aeration factors (Murphy, 1917).

In a study of the effects of seedbed preparation, Davis and Hart (1961) state that the "ratio of seed to seedling varies widely among locations. . . . To produce one seedling a year after seedfall required 5 to 70 (seeds) for spruce."

Baker (1949), Baldwin (1934) Moore (1926), Place (1955), and others suggest that mineral soil is an excellent seedbed for red spruce because it retains a sufficient amount of moisture to enable seed germination. Coniferous litter, because of its low density, fluctuates greatly in moisture content and temperature, making it difficult for seedling establishment. Moore (1917) maintains that contact with the mineral soil is unnecessary if humus has sufficient moisture. In a comparison of seed plots of red spruce on undisturbed litter and on plots cleared of litter, a delay of spring germination on uncleared plots is reported (Westveld, 1931). Murphy (1917) finds that red spruce establishes itself more easily on fir needles than on its own humus because fir needles decompose more easily. Korstian (1937), Barr (1930), Davis and Hart (1961), and others note that spruce seeds that fall on hardwood litter cannot become established because of their inability to absorb moisture from particles of organic matter; the roots of the seedlings dry out before reaching moisture. Barr (1930), working with Picea englemannii Parry (Englemann spruce) in British Columbia, shows germination in the forest to be negligible on undisturbed raw humus soil, but good on exposed mineral soil. Increased shade apparently reduces seed germination regardless of soil type.

Understory also affects seed germination. Bracken fern and grasses are considered a hindrance to spruce regeneration because of

their effect on lowering surface moisture and decreasing the illumination reaching the forest floor (Murphy, 1917; Place, 1955).

Baldwin (1934) describes temperature as a limiting factor in germination. Effective germination temperatures range from 15-30° C, the optimum being 24-28° C.

The U. S. Department of Agriculture (1948) reports that red spruce seeds have a germination capacity of 32-94 per cent. Place (1955) finds the germination of red spruce to be rather low except in rigorously cleaned samples.

Baldwin (1942) states that seeds buried in the soil begin appearing above ground in about twelve days, and that germination is nearly completed by the twentieth day. Little germination has been noted beyond the fiftieth day.

Natural reproduction of red spruce is more dependent upon factors of survival than upon germination. Place (1955) recognizes three periods in the establishment of seedlings: (1) the germination stage, which ends with the appearance of the seedling at the surface of the ground; (2) the succulent stage, which lasts for a few weeks between appearance of the seedling above ground and the hardening of the hypocotyl; (3) the juvenile stage, an indefinite period, which lasts from the hardening of the hypocotyl until the seedling is "definitely established." The latter stage varies with the rate of growth of the seedling and its micro-environment.

Because red spruce seedlings are slow-growing and have shallow root systems, they show a high rate of mortality during the first two

years of life (Pettis, 1909). Barr (1930) observes that "In experimental plots in the forest the seedlings on mineral soil suffered considerable damage from birds and insects."

Toumey (1929) describes red spruce seedling roots as being slow-growing, shallow, primary roots with extensive, rapid-growing lateral roots, making it necessary for red spruce to become established in areas where available moisture remains in the top soil.

In experiments, conducted by Place (1955), red spruce seedlings have been found to die soon after germination due to damping-off fungi, high temperature, or failure of the seedling to root properly. Summer drought is observed to be the main lethal factor because of the shallow root system (Place, 1955). Barr (1930) maintains that young seedlings are more susceptible to drought than more mature ones.

In a two year study, Place (1955) finds that the date of germination greatly influences the survival of red spruce. He states that most of the seeds which germinated in July or later succumbed to drought.

Rubner found that "seed buried at depths of 1/2 and 1 cm in sand and garden soil displayed better germination and survival than seed sown at the same depth in clay or humus," (Barr, 1930). Barr (1930) reports a higher germination of red spruce on mineral soil than on humus, but survival is almost twice as great on humus as compared with mineral soil.

The effects of pH have been demonstrated by Baldwin (1934). He finds that red spruce seed germination is higher in more acid solutions

than in neutral or alkaline ones. In most cases alkaline solutions are distinctly injurious. No optimum pH for red spruce germination has been found; substantial germination occurs between pH 2.0-6.6. Germination is poor in alkaline solutions.

Most woody plant nutrition is directly involved with the nutrition of higher fungi. The fungal mycelia associated with underground plant parts, particularly roots, form a compound structure called mycorrhiza (Daubenmire, 1959).

Gymnospermous trees generally possess ectotrophic mycorrhizae which are characterized by a fungal mantle around the root tip and mycelia between the root cells (Melin, 1953; McArdle, 1932).

McDougall (1922) describes two species of ectotrophic mycorrhiza associated with red spruce: a Cortinarius sp., producing a yellow mycelium, found in association with red spruce, balsam fir and yellow birch; and a Cortinarius sp., producing a white mycelium, found in association with red spruce, balsam fir and white pine. He describes the fungal layers on red spruce as rather thin, one- or two-branched, not in clusters, and not very compact.

McArdle's experiments (1932) show that conifer seedlings without mycorrhizae have difficulty obtaining nitrogen when it is in complex organic compounds. An analysis of seedlings in a substrate with peptones added as a source of nitrogen shows that after three years, the infected seedlings contain 8 per cent more nitrogen than the uninfected seedlings (McArdle, 1932). Mycorrhizae are thought to facilitate uptake of nitrogen and other nutrient elements (Melin, 1953).

Few reports have been found concerning red spruce outside its natural range. Wright (1955) reports that "Since 1939, the New York state nursery at Lowville, New York, has attempted to grow red spruce five different times. All sowings were nearly complete failures, despite the facts that Lowville is only a few miles away from native red spruce stands and that Norway and white spruce are successfully grown in the nursery. Most of the red spruce mortality occurred after the first summer. The exact cause is unknown."

Hanlon (1964) reports two red spruce plantations in North Carolina beyond the species' natural range. One is located in Rocky Bald Gap between Wine Spring Bald and Rocky Bald near Wayah Bald in Macon County. The other plantation is located just south of Cullowhee Gap near the Macon-Jackson County line, North Carolina. Vincent (1964) reports a forty year old spruce plantation adjacent to Brasstown Bald in Towns County, Georgia. The origin of the seedlings is not known. T. S. Seely (Hanlon, 1964) reports, "We have several red spruce plantations on the Sherwood management area dating back to 1942. There are also a few spots of virgin spruce in the management area." Mark Holst (1964) reports provenance tests of spruce seed sources including Southern Appalachia ones in twenty-six localities in Canada. Results are not available. Red spruce is occasionally planted at low elevations as an ornamental but does not escape, suggesting either lack of fertile seed, lack of germination, or early death of the seedlings.

Planting of forest tree seeds outside their natural ranges has been a subject of research for nearly two centuries. Wakeley (1963)

maintains that inadequate research, contradiction of former studies and genetic variation in plants complicates the picture as far as explaining successful plant establishment outside the species' natural range. Langlet (1959) states that, "The risk of a seed transfer from a native stand to a site with a different temperature, climate, and a different day-length must be judged in relation to the variability of the species."

Fowler and Dwight (1964) report provenance differences in stratification requirements of white pine seeds. Findings from seven periods of stratification of white pine seeds from eleven provenances indicate that seeds from northern sources require considerably less stratification than seeds from southern sources. "Highly significant correlations were found between average germination of seeds from different provenances and the latitude and mean January temperature of their origins, for the stratification periods tested."

Plantings of six seed sources of loblolly pine were made in the TVA nursery at Clinton, Tennessee. After five years, Thor and Brown (1962) report that survival of seedlings from all six sources was excellent.

Seedlings of Pinus strobus L., P. resinosa Ait., Picea glauca (Moench) Voss and P. mariana Mill. from Wisconsin and Minnesota have been transplanted to the Olustee Experimental Forest in northern Florida. Growth comparisons of the species under normal and long photoperiods made between outplantings in Wisconsin and Florida reveal that the four northern conifers moved to the warm, humid climate of northern Florida did survive and grow. The natural photoperiod of Florida results in

dwarfing of the seedlings, but seedlings subjected to a 20 hour day-length in Florida are comparable or larger in diameter than those of the northern populations. This relationship holds for top length as well.

Minckler (1950) reports one-year old loblolly and shortleaf pine seedlings transplanted to southern Illinois--outside their natural ranges--showing a mean survival from all sources of more than 95 per cent at the end of the first year's growth.

Failure of establishment from seed plantings outside their natural range has been reported for several tree species. Santamour (1963) shows in provenance testing of three ecotypes of Fraxinus pennsylvanica Marsh. (green ash) seeds planted northeast of their natural range that a high mortality results in the southern seedling sources. In the Southwide Pine Seed Source Study, Wakeley (1963) reports: twenty races of ponderosa pine, planted in northern Idaho, grew successfully for nine years. A colder-than-average winter caused 100 per cent mortality of the pines. Two attempts in establishing longleaf pine by seed transplants north and west of its range have been unsuccessful. Likewise, three attempts in establishing shortleaf pine by seed transplants to Pennsylvania have not succeeded. Wakeley (1963) concludes that the further a seed is moved in any direction from its natural range, the greater the risk of environmental extremes exceeding the genetic tolerances of the organism moved.

CHAPTER III

METHODS AND MATERIALS

Three series of experimental plantings were incorporated into the study. They were the field plantings, laboratory seed plantings, and field seedling plantings. For purposes of this study, the criteria for germination has been the emergence of the seedling above ground.

Field Experiments

This study consisted of a comparison of embryo germination and seedling survival in four areas of the Southern Appalachian Mountains. Two hundred seeds of red spruce were selected from four different sources: (1) the Adirondack Mountains (obtained through a commercial source--the Herbst Brothers Seed Company of New York); (2) the Richland-Balsam Mountains, North Carolina; (3) Roan Mountain, Tennessee; and (4) the Smoky Mountains, Tennessee and North Carolina.

Seeds from the latter three sources were collected from fallen cones in the areas during February and March, 1964.

The cones were air-dried for an average of seventy-two hours, after which the bracts were removed and the large, well-formed seeds selected and de-winged. Viable seeds were few from Roan Mountain, Richland-Balsam Mountains, and the Smoky Mountains, since most of the cones contained empty seeds or otherwise imperfectly developed ones. About one seed in every three cones was viable. A large quantity of cones from each area (a bushel or more) was required to obtain two hundred viable seeds. The paucity of viable seeds was attributed to poor seed production as well

as to the lateness of the collection date. Moore (1917), Baldwin (1942), and others stated that good seed crops of red spruce occur periodically, usually every three to eight years, with very poor crops in the interim. Dissemination of many of the seeds had no doubt occurred before the collecting date. Hart (1959) mentioned that a bushel of cones yields seventeen to twenty-four ounces of clean seeds.

Seed viability was estimated from size, color, and fulness. Microscopic examination of several seeds and preliminary germination tests on moist filter paper confirmed the presence of viable-looking and germinable embryos.

The viable seeds from each source were separated into groups of fifty, placed in labeled glass containers, and stratified. Stratification entailed storing the seeds at 6.6° C for thirty days in a moist room which was aerated. Eckerson (1913) and others have shown after-ripening to be a process in which the seed progresses from a dormant condition to one of readiness for germination.

Plantings were made in early May at selected sites. Site location data are as follows:

Big Blac planting. Site located at 4100 feet in Harlan County, Kentucky, on the southwestern edge of Grassy Gap in a wooded area approximately six miles northwest of Appalachia, Virginia, on Kentucky state highway 160. At the six-mile point, the site is then located about 3000-3500 feet out a dirt road toward the U. S. federal radar station.

Cumberland Mountain planting. Site located at 3200 feet in Bell County, Kentucky, about 250 feet southwest of the highest point in the Cumberland Mountains National Historic Park.

Smoky Mountains planting. Site located at 5160 feet in the Smoky Mountains National Park approximately 100 feet below the Indian Gap parking area, or approximately 1.5 miles southwest of Newfound Gap on the park road to Clingman's Dome.

Wayah Bald planting. Site located at 5200 feet in Macon County, North Carolina, approximately nine miles west of Franklin, North Carolina, on the Wayah (mountain) road leading to Wayah Bald. Proceeding west, one turns to the right at Wayah Gap on the ridge road and continues until reaching the first cabin located 150 feet below the summit. Facing the summit at this point, the site lies 75-100 feet off the road to the left.

Brasstown Bald planting. Site located at 5200 feet in Towns County, Georgia, approximately five miles southwest of Hiwassee, Georgia, on Georgia state highway 66. Proceeding southwest from Hiwassee, one turns right onto the summit road and continues until reaching the area 150 feet below the summit. Facing the summit at this point, the site lies 20-25 feet off the road to the left.

Altitude, slope and amount of canopy cover were criteria used in the selection of each site. Plot descriptions and a list of plant species occurring there are included in Tables I and II.

Planting design involved use of a 15 x 25 foot plot marked every 3 x 5 feet with a six-inch aluminum stake. At each corner of every stake, two seeds were planted at 1/2 inch depth. The seed sources were planted with the Adirondack source upper left, Clingman's Dome source upper right, Roan Mountain source lower left, and Richland-Balsam source lower right.

TABLE I
PLOT DESCRIPTION

Site ¹	Latitude ²	Elevation (ft.)	Facing Direction	Slope		Position
				Per Cent		
Big Black Mt.	37°28'	4100	SSE	4		crest of ridge top
Cumberland Mt.	37°01'	3200	NW	25		about 250 ft. below summit
Smoky Mts.	36°02'	5160	SSW	20		about 100 ft. below Indian Gap
Wayah Bald	35°20'	5200	NW	10		about 150 ft. below summit
Brasstown Bald	35°22'	5200	SE	25		about 100 ft. below summit

TABLE I (continued)

Site ¹	Character of mineral soil	Litter Character	Litter depth(inches)	Depth to rock(inches)	Canopy closure (per cent)
Big Black Mt.	sandy	oak L&F ³	$\frac{1}{4}$ -1	6-12	80
Cumberland Mt.	sandy	oak L&F	$\frac{1}{2}$ -1	4-9	60-80
Smoky Mts.	silty with humus	spruce L, F. H	$\frac{1}{2}$ -1 $\frac{1}{2}$	6-12	90
Wayah Bald	silty	oak-birch	$\frac{1}{2}$ -1	6-12	80
Brasstown Bald	silty	oak L,F	$\frac{1}{2}$ -2	3-9	60-80

¹See pp. 13-14 for detailed site location data.

²From Bartholomew, 1957.

³Hoover and Lunt, 1952.

TABLE II
SPECIES DISTRIBUTION

Arboreal Layer	Big Black	Cumberland	Smoky	Wayah	Brasstown
<i>Abies fraseri</i>			x		
<i>Acer pennsylvanicum</i>				x	
<i>A. rubrum</i>		x			x
* <i>A. saccharum</i>	x			x	
<i>A. spicatum</i>				x	
* <i>Amelanchier arborea</i>	x			x	x
<i>A. laevis</i>				x	
* <i>Betula alleghaniensis</i>			x		
<i>B. lenta</i>				x	
<i>Carya sp.</i>		x			
* <i>Crataegus sp.</i>	x				
<i>Fagus grandifolia</i>				x	
* <i>Liriodendron tulipifera</i>		x			
* <i>Picea rubens</i>			x		
* <i>Pinus virginiana</i>		x			
<i>Prunus serotina</i>	x				
<i>Quercus alba</i>	x				x
* <i>Q. rubra</i>				x	x
<i>Robinia pseudo-acacia</i>		x			
<i>Sassafras albidum</i>		x			
<u>Shrub Layer</u>					
<i>Amelanchier sp.</i>					x
<i>Castanea dentata</i>	x				
<i>Carya sp.</i>					x
<i>Fraxinus pennsylvanica</i>					x
<i>Hydrangea arborescens</i>		x			
<i>Kalmia latifolia</i>		x			x
<i>Picea rubens</i>			x		
<i>Pinus strobus</i>					x
<i>Quercus alba</i>	x				x
<i>Rhododendron calendulaceum</i>	x				x
<i>R. catawbiense</i>					x
<i>R. maximum</i>		x			
<i>Rubus sp.</i>	x	x	x	x	
<i>Smilax bona-nox</i>	x				
<i>Vaccinium simulatum</i>					x
<i>Viburnum sp.</i>		x			

TABLE II (continued)

Herbaceous Layer	Big Black	Cumberland	Smoky	Wayah	Brasstown
<i>Abies fraseri</i>					x
<i>Acer rubrum</i>					x
<i>Achillea millefolium</i>	x				x
<i>Actaea</i> sp.				x	
<i>Amphicarpa bracteata</i>	x				
<i>Anemone</i> sp.	x				
<i>Ascyrum hypericoides</i>	x				
<i>Aster lateriflorus</i>	x				
<i>Cacalia atriplicifolia</i>	x				
<i>Carex pennsylvanica</i>			x		x
<i>Chrysopsis mariana</i>					x
<i>Cirsium</i> sp.	x				
<i>Collinsonia canadensis</i>	x				
<i>Coreopsis major</i>					x
<i>Dryopteris</i> sp.	x		x		
<i>Duchesnia indica</i>					x
<i>Erigeron pulchellus</i>					x
<i>Erythronium</i> sp.	x				
<i>Eupatorium purpureum</i>	x				
<i>Eupatorium</i> sp.	x				
<i>Fragaria virginiana</i>	x	x			
<i>Gentiana</i> sp.	x				x
<i>Geranium maculatum</i>	x				
<i>Geranium</i> sp.		x			
<i>Gillenia trifoliana</i>	x				x
<i>Houstonia purpurea</i>	x				x
<i>H. caerulea</i>				x	
<i>Lysimachia quadrifolia</i>	x				x
<i>Oenothera biennis</i> var. <i>hirsutissima</i>					x
<i>Oxalis stricta</i>	x				
<i>Panicum</i> sp.	x				
<i>Pedicularis canadensis</i>	x	x			
<i>Picea rubens</i>			x		
<i>Pilea</i> sp.					x
<i>Podophyllum peltatum</i>	x	x			
<i>Polygonatum biflorum</i>	x				
<i>Polystichum</i> sp.		x			
<i>Potentilla canadensis</i>	x	x			
<i>P. pumila</i>					x
<i>Prenanthes</i> sp.	x			x	
<i>Ranunculus abortivus</i>	x				
<i>R.</i> sp.				x	

TABLE II (continued)

Herbaceous Layer	Big Black	Cumberland	Smoky	Wayah	Brasstown
<i>Senecio smallii</i>			x		x
<i>Silene virginica</i>	x				
<i>Smilacina racemosa</i>	x				x
<i>Solidago</i> sp.	x			x	x
<i>Stellaria</i> sp.		x			
<i>Teucrium</i> sp.					x
<i>Thelypteris novaboracensis</i>	x			x	
<i>Tovara virginiana</i>	x				
<i>Vaccinium simulatum</i>	x				
<i>Viola sororia</i>	x			x	
<i>V. spp.</i>	—	x	—	x	—
Total species no.	40	19	8	17	31

*Present in overstory.

The upper left corner of the plot was marked in each site with a 4-1/2 foot iron stake, except the Smoky Mountain site, which was marked in the lower right corner.

Field quadrats were marked as discretely as possible to thwart tourists as well as to maintain an undisturbed ecosystem.

Observations of germination and survival of the seeds were made on an average of once every four weeks from June to September, 1964.

Laboratory Experiments

Laboratory experiments of germination and survival were conducted using 4650 red spruce seeds from the Adirondack source. Artificial seedbeds were set up in perforated plastic trays and plantings for each experiment were in triplicate. Effects of watering, planting depth, substrate type and soil pH were tested.

Effects of watering were determined by comparing sets watered with tap water--distilled water and Hoagland's solution--distilled water.

Studies of the effects of planting depth were carried out to determine if there was a optimal depth for germination; seeds were planted 1/4, 1/2, and 1-inch beneath the soil surface.

Murphy (1917) described the qualities of a good seedbed as having good water infiltration and aeration, where the soil packs around the seedling but offers little mechanical resistance to germination. Laboratory experiments were conducted to compare germination in various mineral soils with that in spruce-humus and in sand.

A laboratory study of comparative germination was conducted using pH values of 4.0, 6.0, and 8.0 of mineral soil. CaCO_3 , HCl , and NaOH

were used in regulating the soil pH. The pH of the unadjusted control plantings remained at 4.4. Periodic remeasurements of pH were made to determine if pH readjustment was necessary.

Elevational Transplants

Certain red spruce seeds which germinated in the laboratory were transplanted in greenhouse soil on June 23, 1964, to the Smoky Mountains at about 4000, 5000, and 6000 foot elevations. Transplants grew undisturbed until October 3, 1964, when they were removed with their roots from the soil and dry weights obtained. See Table III for transplant location data.

TABLE III
DESCRIPTION OF TRANSPLANT AREAS

	Sites		
	4000 ft.	5000 ft.	6000 ft.
Mileage from Sugarland Visitor Center	9.1 mi.	12.6 mi.	18.1 mi.
Stand type	spruce	spruce	spruce-fir, yellow birch
Per cent slope	4	25	4
Soil litter type	spruce-beech	spruce-fir, birch	spruce-fir, birch
Litter depth in inches	0- $\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$ - $\frac{1}{2}$

CHAPTER IV

RESULTS

In the present study, per cents of embryo germination and seedling survival of red spruce were compared. Data from several kinds of plantings were analyzed and appear in the accompanying tables.

The experiments revealed that seeds of red spruce do germinate under field conditions outside their natural range. With the exception of the Wayah Bald planting, the Adirondack seed source showed a consistently higher germination rate than others. Field plantings on Big Black Mountain and Brasstown Bald showed similar germination per cents for two seed sources. Germination in the Adirondack seed source was similar on the Cumberland Mountain and Wayah Bald sites (Table IV). Two of the sources--Richland Balsam and Roan Mountain--failed to germinate at Wayah and Brasstown Bald environments.

Field survival appeared to be a function of original germination, i.e., higher germination resulted in higher rates of survival. After four to five months, representatives from all seed sources were surviving in at least some of the plots. The Adirondack source had the highest average survival (Table IV).

A comparison of results of germination between stratified and non-stratified plantings of the Adirondack source on different substrates in the laboratory may be seen in Tables V and VI.

Germination rate was lower in the case of the one-inch plantings as compared to plantings at 1/4 and 1/2 inches (Table VII). Plantings

TABLE IV
GERMINATION AND SURVIVAL OF RED SPRUCE SEEDS FROM
FOUR GEOGRAPHIC SOURCES PLANTED ON FIVE SITES
IN THE SOUTHERN APPALACHIAN MOUNTAINS
(50 SEEDS PLANTED PER SITE)¹

Planting Site	Source				Mean
	Adirondack	Roan Mt.	Balsam	Smoky Mt.	
	<u>Germination</u>				
Big Black Mt.	9	3	4	8	6
Cumberland Mt. ²	2	-	1	-	1.5
Smoky Mts.	15	2	2	1	5
Wayah Bald	1	0	0	3	1
Brasstown Bald	8	0	0	7	3.75
Mean	7.0	1.25	1.4	4.75	
	<u>Survival</u>				
Big Black Mt.	0	3	3	0	1.5
Cumberland Mt.	0	-	0	-	0.0
Smoky Mts.	9	0	0	1	2.5
Wayah Bald	0	0	0	1	0.25
Brasstown Bald	4	0	0	3	1.75
Mean	2.6	0.75	0.6	1.25	

¹Survival refers to those seedlings remaining alive by September 30, 1964; 5 months after planting.

²Only two sources were planted in the Cumberland Mountains.

TABLE V
GERMINATION OF SEEDS PLANTED AT TWO DEPTHS
WITH AND WITHOUT A COLD TREATMENT¹

Planting depth in inches	Cold				No Cold				n ^o t ⁿ
	A	B	C	$\bar{x}$	A	B	C	$\bar{x}$	
<u>Mineral Soil</u>									
.25	6	7	4	5.6	13	8	10	10.3	2.76
.50	8	8	18	11.3	10	12	15	12.3	.289
<u>Humus</u>									
.25	11	9	11	10.1	8	9	9	8.6	-1.99
.50	7	12	12	9.6	10	4	6	6.6	-1.23

¹Replications of 25 Adirondack source seeds germinating in the greenhouse in Smoky Mountains source mineral soil and spruce forest humus.

TABLE VI
GERMINATION OF SEEDS PLANTED IN TWO SUBSTRATES
WITH AND WITHOUT COLD TREATMENT¹

Substrate	Cold				No Cold			
	A	B	C	$\bar{x}$	A	B	C	$\bar{x}$
Milled Moss	25	17	12	18	12	12	6	10
Sand	0	0	1	0.3	11	8	11	10

¹Replications of 50 Adirondack source seeds germinated in new wing constant temperature laboratory planted at $\frac{1}{4}$ -inch depth. Mosses were from the Smoky Mountains spruce stand at Indian Gap.

TABLE VII
GERMINATION AND SURVIVAL OF SEEDS IN TWO SOILS
AT THREE PLANTING DEPTHS¹

Planting depth in inches	<u>Brasstown Bald Soil</u>				<u>Wayah Bald Soil</u>				"t"
	A	B	C	$\bar{x}$	A	B	C	$\bar{x}$	
	<u>Germination</u>								
.25	14	10	17	13.6	15	20	19	18.0	-3.48
.50	14	13	17	14.3	21	11	24	18.6	1.65
1.00	1	0	0	0.3	9	9	8	8.6	
	<u>Survival</u>								
.25	3	1	6	3.3	8	15	15	12.6	5.34
.50	7	4	5	5.3	10	4	15	9.6	2.06
1.00	0	0	0	0.00	6	0	2	2.6	

¹Replicates of 50 Adirondack source seed, not cold treated
grew in the new wing constant temperature laboratory.

at 1-inch depth showed reduced survival during the four to five month observation period over those planted at 1/4 and 1/2 inch depths (Table VII).

Differences in means for both germination and survival of the laboratory plantings of Adirondack seeds in Wayah and Brasstown Bald soil appeared to be significant above the 95 per cent level of probability (as indicated by "t" test values) except in the case of germination at the 1/2 inch depth where the level of probability dropped to 86 per cent. Thirty to sixty day survival differences were observed between the two soil types. Survival per cents in the laboratory ranged from 0-30 per cent for Wayah Bald and from 0-14 per cent for Brasstown Bald (Table VII).

The "t" test values comparing means between pH's of a control lot (control pH 4.4) and three manipulated lots (pH 4.0, 6.0, 8.0) indicated that the germination differences were significant (probabilities were above 95 per cent--Table VIII).

The "t" tests of red spruce germination in sand watered with Hoagland's solution or with distilled water showed no significant difference between the means of the two experimental groups (Table IX). Survival over a two to threemonth period was higher in the case of the distilled water set (Table IX). However, in germination Adirondack source seeds in spruce forest mosses, water source had no effect on survival (Table X).

Dry weights of seedlings transplanted to the Smoky Mountains pointed to a greater assimilation rate of materials at the 5000 foot elevation (Table XI). Many factors, including local conditions of

TABLE VIII
GERMINATION AND SURVIVAL OF ADIRONDACK
SOURCE SEEDS IN MANIPULATED pH¹

Check pH				Manipulated pH											
4.4				4.0				6.0				8.0			
A	B	C	$\bar{x}$	A	B	C	$\bar{x}$	A	B	C	$\bar{x}$	A	B	C	$\bar{x}$
<u>Germination</u>															
22	20	17	19.6	12	14	15	13.6	13	11	12	12	2	5	2	3
<u>Survival</u>															
22	19	15	18.6	10	12	13	11.6	13	7	11	10.3	0	1	0	0.3
<u>"t" Values (germination)</u>															
				<u>4.0</u>				<u>6.0</u>				<u>8.0</u>			
4.4				-35.3				-5.28				-14.82			
<u>"t" Values (survival)</u>															
				<u>4.0</u>				<u>6.0</u>				<u>8.0</u>			
4.4				-5.00				-4.85				--			

¹Replicates of 50 Adirondack source seeds were planted at 1/4 inch depth in mineral soil in which the pH was manipulated using CaCO₃, NaOH, and HCl. Seeds had no cold treatment, sets maintained at 25° C in the Percival growth chamber.

TABLE IX
GERMINATION AND SURVIVAL OF SEEDS WATERED WITH
HOAGLAND'S SOLUTION OR DISTILLED WATER¹

Treatment	Sets				"t"
	A	B	C	$\bar{x}$	
<u>Germination</u>					
Hoagland's solution	16	17	8	13.6	0.456
Distilled water	19	19	9	15.6	
<u>Survival</u>					
Hoagland's solution	13	10	3	8.6	5.71
Distilled water	19	17	8	14.6	

¹Replicates of 50 Adirondack source seeds planted at $\frac{1}{4}$ -inch depth in lake sand; sets maintained at 25° C in Percival growth chamber.

TABLE X
GERMINATION AND SURVIVAL OF SEEDS PLANTED
IN FOREST MOSSES AND WATERED WITH
TAP OR DISTILLED WATER¹

Treatment	Sets			
	A	B	C	$\bar{x}$
	<u>Germination</u>			
Distilled water	18	14	6	12.6
Tap water	16	8	7	10.3
	<u>Survival</u>			
Distilled water	14	8	6	9.6
Tap water	14	6	7	9.0

¹Replicates of 50 Adirondack source seeds, no cold treatment, planted $\frac{1}{4}$ -inch depth in moss pads from the Smoky Mountains spruce forest; maintained in the new wing laboratory constant temperature 25° C.

TABLE XI
 WEIGHT OF GREENHOUSE GERMINATED SEEDLINGS TRANSPLANTED
 TO THREE ELEVATIONS IN THE SMOKY MOUNTAINS
 COMPARED WITH A CONTROL SET
 NOT TRANSPLANTED¹

	<u>Elevation (thousands of feet)</u>			Control
	<u>4</u>	<u>5</u>	<u>6</u>	
Number of seedlings	18	36	15	16
Mean weight	3.97	4.86	3.54	6.43

¹Data in grams, dry weight.

water availability, soil fertility and soil temperature undoubtedly played a role in influencing net assimilation over any one period of time. No definite results involving assimilation rate at different elevations could be reached particularly at the early critical stages of development of red spruce seedlings,

CHAPTER V

DISCUSSION

Seed germination and seedling survival of red spruce were a function of the interaction of climatic, edaphic, and biotic factors. Germination at the various field planting sites was highest in Adirondack seed. This was probably a function of professional seed selection and machine culling rather than something intrinsic about the source itself. Fowler and Dwight (1964) suggested from work with white pine that seed from more northern latitude populations may have a less prolonged stratification requirement. Among the Southern Appalachian sources germination was best from the larger Smoky Mountain population. Survival, as noted later, was approximately proportional to germination and in the field planting was very low (0.6 to 2.6 per cent). The similarity of germination and survival percentages does not make a strong case for probability of genetic races in this species. However, the use of more seed sources from a wider geographic range might well have revealed their existence.

Average germination by site (1 to 6 per cent) was thought to be a function of aspect rather than degree of slope, slope position, soil depth or mechanical character, litter character, floristic composition of the community overstory or understory, elevation, or latitude. It was noted that the highest germination was on south facing planting sites.

Average survival by site was best in the Smoky Mountains under a spruce-fir canopy. The narrow range in germination percentages (0 to 2.5), however, suggests that more time is required for effects to be fully felt. Approximately one-third to one-half of germinated seeds survived.

Several seedlings disappeared between observation periods. Field observations strongly suggested both mechanical and animal damage may be factors in determining seedling survival in particular areas.

The rather heavy fall of branch-litter in the Smoky Mountains plot could have been a factor in reducing seedling number. Over a thirty-five to forty day period, seedling number dropped from twelve to nine. The occasional discovery of a broken seedling seemed to indicate that branch fall was involved. This statement would not be applicable in the case of the other plots. Hart (1959) reported that small seedlings are subject to this general kind of damage, especially in smothering and crushing by litter.

Pettis (1909) suggested that bird and insect damage is common to seedlings. Birds are often attracted to seedlings before they have lost their seed coats. No estimate of bird or insect damage in the field was made, although it is probable that animals had an effect upon seed germination and seedling survival by either removing the seeds or destroying the seedlings.

Dense stands of ferns and grasses on the Wayah Bald plot may have been involved in the relatively low germination in the area. The heavy shade and great amount of moisture used by the ferns and grasses may have prevented the establishment of seedlings.

The paucity of available seed made variable site planting in the field impractical.

Of interest equal to absolute germination of the planted seed and

survival of the seedlings were the environmental factors which influence these. Some of these are explained in the laboratory experiments.

Germination per cents comparable with those reported in the literature (Baldwin, 1934; Place, 1955) were obtained on a variety of substrata. Data in general confirmed the evidence of other workers, that there was an increase in germination rate on mineral over organic substrata. Toumey (1929), Baldwin (1934), and McArdle (1932) maintained that soil particle size, moisture retention, fungi, and minerals played an important part in seed germination and survival.

Preliminary results from seed planting tests suggest, at least, that the problem is more complex than might otherwise be supposed. A significant difference in germination can be shown to exist between stratified and nonstratified lots where seeds were planted at 1/4-inch depth. This significance is of the same general order of magnitude (90-98 per cent level of probability) regardless of the substrate type used. The level of significance between the cold and no cold groups drops to below that usually accepted by workers where planting was at 1/2-inch depth irrespective of the substrate involved. As the germination per cents were not consistent viewed either from the standpoint of pre-planting treatment or substrate type, detailed conclusions must await further study. The strongest suggestion from these experiments was that a shallow planting at 1/4-inch depth was more conducive to germination than deeper plantings. Reduced germination at greater depths might be attributed to the limited food reserve in the seed, aeration, and/or mechanical impediment to growth. Planting depth as a factor in

spruce seedling mortality has been observed previously by Rubner (cited in Barr, 1930).

In the case of seeds germinating in sand culture, no significant difference (as indicated by "t" test values) was observed between one group watered with Hoagland's solution and another with distilled water. The same statistical test did indicate, however, a significant difference in survival comparing the same two groups. Perhaps even "normal" nutrient concentrations of such elements as calcium were toxic in this organism.

Germination of the Adirondack seed source in soil with manipulated pH suggested that the optimal pH lies between 4.0 and 6.0. The fact that pH 4.4 (unmanipulated) had highest germination and survival among the 4.0, 4.4, and 6.0 groups suggests that manipulation itself lowered rates of germination. The actual causes underlying such observations are not known, but probably are related to aeration and suffocation.

Evidence, based on similarity in germination between field and controlled greenhouse plantings, indicated that red spruce seeds used in this study have a germination capacity and first year survival comparable with those in data reported by such workers as Place (1955), Murphy (1917), Pettis (1909), and Barr (1930). This may be further supported from germination studies of fifty Adirondack seeds planted above the Smoky Mountains seed plot at 1/4-inch depth, and laboratory germination of Adirondack seeds dropped on the surface of spruce forest feather mosses. Of the fifty field planted seeds, 38 per cent germinated and 12 per cent were surviving September 30, 1964, (five months later). Laboratory germination of seeds dropped on spruce forest feather mosses

ranged from 12-36 per cent, and survival rates ranged from 12-28 per cent.

Dry weight increases of elevational transplants cannot be accepted, as suggestive of better conditions for seedling growth at 5000 feet, but because of the other micro-environmental variables involved and the limited number of seedlings used, the work should be repeated.

CHAPTER VI

CONCLUSIONS

Conclusions are:

1. Red spruce seeds germinate under field conditions outside their natural range.
2. Field survival is proportional to original germination.
3. No significant difference is found in germination and survival on various substrata between stratified and non-stratified seeds.
4. Germination and survival of red spruce is favored by shallow plantings in the 1/4-1/2-inch range. Results of "t" tests indicate that the differences in mean for both germination and survival of the laboratory plantings of Adirondack seeds in Wayah and Brasstown soil in the laboratory are significant above the 95 per cent level of probability, except in the 1/2-inch depth where the level of probability drops to 86 per cent. No definite conclusions can be drawn at present regarding differences in response of Adirondack source seed germinated in the laboratory on varying soil substrata..
5. Optimal pH for red spruce seed germination is between 4.0-6.0.
The mechanism of the pH effect upon germination is not known.
6. Similar germination per cents are obtained when comparing field with controlled laboratory plantings.

CHAPTER VII

SUMMARY

To determine some of the factors influencing first-year germination and survival of red spruce, seed plantings from four sources of red spruce were made in four areas of the Southern Appalachian Mountains outside and one within the species' natural range; comparative studies of effects of soil types, soil pH and planting depths on germination were carried out in the laboratory; and dry weights of elevational transplants of red spruce seedlings were obtained.

Experimental studies showed that red spruce does germinate and survive for one growing season outside its natural range.

Similar germination percentages were obtained both in the laboratory and in field experiments.

Data suggest that germination was increased on mineral soil.

Red spruce germination was lower in seeds planted at 1-inch depth compared with germination in seeds planted at 1/4 or 1/2-inch depth.

Seedling survival but not per cent germination was greater in distilled watered versus Hoagland solution watered preparations.

Maximum germination in manipulated soil pH plantings was obtained in soils having a pH from 4-6. However, even "normal" ion concentrations had adverse effects.

Dry weight of seedling transplants at various elevations in the Smoky Mountains were suggestive of optimal growth conditions at 5000 feet, but variable micro-environmental conditions between sites and paucity of seedlings make conclusions tentative and expanded trials desirable.

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