Studies of Genetic Variation with Five-Needle Pines in Germany

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Abstract—After 30 years, a field trial with 65 seed samples of eastern white pine (*Pinus strobus*) showed that the best growing provenances have their origin in the Appalachians south of latitude 39°, and provenances with the slowest growth in regions north of latitude 45°. Provenances from regions between 39° and 45° latitude varied greatly in their growth, even when their origins were from adjacent locations. The great interspecific and intraspecific differences of five-needle pines in resistance to the blister rust fungus *Cronartium ribicola* was demonstrated by resistance tests. Obvious racial variation in the blister rust fungus was found by a joint inoculation experiment with alternate hosts (*Ribes* and *Pedicularis*).

Key words: Eastern white pine, *Pinus strobus*, provenance trial, blister rust, growth performance, *Pinus cembra*, *P. wallichiana*, *P. peuce*, *P. parviflora*

Introduction

Europe has in contrast to North America only two native five-needle pine species. Only Swiss stone pine (*Pinus cembra* L.) is native in Germany. This species occurs in small populations in the Alpine regions of southern Germany at elevations up to 1867 m.

The second European species is the Macedonian pine (*Pinus peuce* Griseb.) of the mountainous Balkans. This species is rather slow-growing and in general of less interest for forestry practice. However, because of a relatively high tolerance against air pollutants, it is suitable for afforestation in southeastern Germany (Lattke and others 1987, Lattke 1998), where other tree species (for example Norway spruce) were severely damaged or even eliminated during recent decades.

In addition to these native species, several other five-needle pine species were introduced, mainly for ornamental purposes: The Himalayan white pine (*Pinus wallichiana* A. B. Jacks.) from Pakistan and India is grown in parks and larger gardens in the warmer climate of southwestern Germany. In other regions the trees are subject to damage from late frost, as shown in some provenance trials (for example Stephan 1974).

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The Japanese white pine (*Pinus parviflora* Sieb. et Zucc.) is a common ornamental tree species in parks, arboreta, gardens and cemeteries, where it is of interest because of its slow growth and attractive blue needle colour.

Pinus strobus L., the eastern white pine from North America, is the only five-needle pine with extensive silvicultural use in Germany. The species was introduced in Europe in 1605. It can be grown successfully under various environmental site conditions and shows good natural regeneration. The main disadvantage is the high susceptibility to blister rust disease caused by *Cronartium ribicola* J.C. Fischer.

In the following paper some results of a provenance trial with *P. strobus* will be presented. In addtion, results are summarized from resistance tests with several five-needle pine species and studies of variation in *Cronartium ribicola*.

Materials and Methods

Provenance Trials

Provenance trials with 65 seed samples from the natural range of *P. strobus* were started in 1963. Geographical data for the provenances are given in table 1. Field trials were established with 3-year-old plants on two sites in northern Germany in 1966 and 1967. Measurements and assessments of growth performance, mortality (rust and non-rust related) and the presence of stem infections as well as branch infections by blister rust were conducted in the following years. Detailed information is given in earlier papers (Stephan 1974, 1986a). The last evaluations were carried out in 1994 when the trees were 32 years old, and these data are presented here. Because of the design of the trial and the narrow space within the plots, further exact evaluations are not possible.

Resistance Test with Five-Needle Pines

The Institute for Forest Genetics at Grosshansdorf participated in the international IUFRO experiment testing resistance of white pines to *Cronartium ribicola*. This joint experiment was initiated by Bingham and Gremmen (1971). A total of 17 five-needle pine species including 76 provenances and progenies were tested by artificial inoculation (Stephan 1986b). About 10,000 pine plants were grown in containers under greenhouse conditions at temperatures above 0 °C to avoid frost damage of the frost sensitive species. For artificial inoculations, rust-infected leaves of the alternate host *Ribes nigrum* L. were used. The pine seedlings were inoculated at an age of two years. Each plant was assessed annually for the rust symptoms, beginning with needle lesions or spots, appearance of spermogonia, normal cankers and/or bark reactions, and blisters with

Table 1—Provenance trial with *Pinus strobus* at Forest District Nordhorn (Wielen Ki 26) in northwestern Germany. Provenances are listed in order of their volume under bark per ha at age 27.

Seed-book no.	County, state	Latitude N	Longitude W	Altitude (m)	Height (m) age 27	Dbh (cm) age 32	m^3/ha age 27
3862	Newaygo, MI	43°43'	85°55'	262	13.30	26.00	321.8
3851	South Carolina	34°39'	82°55'	533	13.50	30.35	319.7
3874	Clearfield, PA	41°00'	78°27'	—	12.80	—	307.7
3800	Henderson, NC	35°20'	82°30'	671	13.03	22.00	304.9
3850	Kentucky	37°00'	87°00'	—	13.25	29.00	285.9
3812	Garrett, MD	39°30'	79°25'	707	12.95	21.40	279.7
3871	Garrett, MD	39°30'	79°25'	707	12.70	27.67	281.2
3829	Carroll, VA	36°42'	80°52'	780	13.13	29.00	275.7
3803	Schoharie, NY	42°45'	74°25'	274	12.60	—	272.8
3839	Buncombe, NC	35°28'	82°32'	655	13.00	28.50	274.4
3861	Manistee, MI	44°16'	86°03'	213	13.05	26.70	269.9
3857	McKean, PA	41°42'	78°55'	457	12.78	22.35	272.0
3818	Washington, MD	39°41'	78°14'	194	12.78	27.25	269.0
3875	Warren, NY	43°37'	73°44'	305	13.35	21.85	267.2
3825	Saratoga, NY	43°00'	73°43'	152	12.95	28.50	260.8
3827	Dunn/Polk, WI	45°00'	91°19'	366	12.35	26.10	260.1
3804	Berkshire, MA	42°30'	73°14'	274	12.80	24.40	258.0
3872	Pike, PA	41°10'	75°00'	335	12.38	27.50	251.5
3837	Oconee, SC	34°50'	83°10'	457	12.95	21.75	246.5
3876	Warren, NY	43°41'	73°41'	396	12.55	26.25	244.4
3810	Middlesex, CT	41°38'	72°30'	—	13.03	26.00	240.8
3842	Quebec, QC	46°55'	71°31'	168	12.95	24.50	239.9
3823	Litchfield, CT	41°58'	73°13'	390	12.58	26.25	238.8
3809	Hillsborough, NH	43°06'	71°55'	262	12.05	23.30	241.3
3838	Wytha, VA	37°00'	81°15'	762	12.65	19.25	237.5
3801	Greenbrier,WV	38°59'	80°09'	686	12.50	25.15	231.8
3808	Chittenden, VT	44°27'	73°12'	91	12.28	25.50	236.1
3802	Strafford, NH	43°08'	70°57'	31	12.53	25.07	232.5
3821	Somerset, PA	39°47'	79°02'	640	12.95	24.00	228.9
3820	Somerset, PA	39°47'	79°02'	640	12.08	26.00	234.2
3824	Litchfield, CT	41°58'	73°13'	408	12.40	25.75	234.2
3815	Allegany, MD	39°40'	78°28'	239	12.50	23.40	226.9
3853	Schoharie, NY	42°45'	74°25'	305	12.63	24.00	226.8
3870	Strafford, NH	43°08'	70°56'	18	12.10	23.05	227.7
3822	Tucker, WV	39°10'	79°35'	503	12.38	26.57	227.6
3819	Garrett, MD	39°42'	79°08'	678	13.23	24.93	228.5
3830	Carroll, VA	36°37'	80°53'	780	12.75	22.90	233.0
3835	Sauk, WI	43°30'	89°55'	305	12.18	25.15	227.4
3805	Juneau, WI	43°35'	90°00'	210	12.00	23.00	217.7
3806	Chittenden, VT	44°28'	73°09'	290	12.15	24.67	216.6
3814	Preston, WV	39°33'	79°29'	777	11.63	22.00	208.9
3833	Garrett, MD	39°33'	79°21'	756	12.48	16.00	213.3
3828	Coerthier, QC	46°17'	73°25'	213	12.30	25.00	207.4
3855	Addison, VT	44°07'	73°13'	122	12.30	24.03	206.4
3834	Garrett, MD	39°25'	79°24'	701	12.00	23.50	201.8
3811	Garrett, MD	39°30'	79°25'	707	12.38	24.20	201.7
3836	Sauk, WI	43°30'	89°55'	305	12.25	22.15	199.0
3843	Sawyer, WI	46°00'	91°25'	—	12.95	22.25	194.3
3844	Renfrew, ON	45°57'	77°27'	160	11.50	23.00	194.2
3807	Chittenden, VT	44°28'	73°09'	122	12.23	23.85	195.0
3841	Lake, MN	48°02'	91°36'	402	12.15	22.50	188.4
3877	Essex, NY	44°20'	73°46'	229	12.05	21.70	181.7
3845	Sunbury, N.B.	46°22'	66°11'	122	11.50	26.75	175.5
3848	LaSalle, IL	41°19'	88°59'	155	11.90	23.73	173.4
3840	York, ME	43°22'	70°53'	122	11.83	20.33	169.8
3854	Todd, MN	46°21'	94°12'	405	12.58	21.70	170.1
3826	Itasca, MN	47°19'	93°34'	397	12.13	21.10	166.0
3846	Quebec, QC	46°57'	71°31'	305	12.25	24.00	147.5
3813	Preston, WV	39°33'	79°29'	786	11.75	21.50	139.7
3847	Manitoba, MN	54°00'	100°00'	—	11.40	20.90	130.1
3856	Ogle, IL	41°57'	89°23'	221	11.00	20.00	119.5
3831	North Carolina	—	—	—	12.75	27.00	—
3852	Wisconsin	—	—	—	12.73	26.90	—
3849	Michigan	—	—	—	12.50	26.33	—
3832	North Carolina	—	—	—	11.70	21.37	—
Average					12.47	24.23	228.4

aeciospores. Rust related and non-rust related mortality was recorded. During the experiment all plants were kept in greenhouses until they reached the age of eight to nine years. The white pine plants were not exposed to further natural infections.

Studies on Race Differences of *C. ribicola*

The objective of these studies was to investigate the extent of pathogenic variation in *C. ribicola* in ability to infect alternate host species. Therefore, joint inoculation experiments were carried out with *Ribes nigrum* L. (*Saxifragaceae*) and *Pedicularis resupinata* L. (*Scrophulariaceae*) in Germany and South Korea. Various cultivars of *R. nigrum* are grown in Germany and are the main alternate hosts of the white pine blister rust fungus. *Pedicularis resupinata* is native in eastern Asia and is also an alternate host plant of *C. ribicola*. Both alternate host species or cultivars were grown in Germany as well as in South Korea, and inoculated with the respective *C. ribicola* aeciospores in both countries. Further details of the materials and methods are given in the paper of Stephan and Hyun (1983).

Results

Provenance Trial with *Pinus strobus*

Height growth (age 27), stem diameter at breast height (1.3 m) (age 32) and calculated total volume under bark (m^3/ha) (age 27) are shown as an example for the trial at Wielen (Ki 26), northwestern Germany, in table 1. The averages for height growth at age 27 varied between 11 m and 13.5 m, for diameter at age 32 from 16 cm to about 30 cm, and for volume at age 27 from 119.5 m^3/ha to 321.8 m^3/ha.

The performance of the provenances at the two test sites was very similar. The provenances differed significantly in their growth performance at different ages. Traits are correlated negatively with provenance latitude (for correlation coefficients see Stephan 1974). Trees from provenances in the southern Appalachians south of 39° latitude grew well under the conditions in northern Germany (fig. 1). Provenance samples from the regions north of latitude 45° grew poorly. Provenance samples from areas between 39° and 45° latitude showed great variation in growth rate.

Differences between provenances in blister rust infection could be observed. As the trees were mostly infected at the lower part of the stems or branches, it could be assumed that they were obviously infected already as young plants in the nursery. The range in rust infection was relatively low and varied from 2 percent and 7 percent between the two test sites, but from 0 percent to 25 percent between provenances. There was a weak correlation (r = 0.32) among provenances over the two test sites. A correlation between origin of the provenances and infection could not be found. One provenance from Maryland and one from Quebec had no rust infected trees after seven years in the field. On the contrary, 25 percent trees of a provenance from Wisconsin were rust infected.

Wood density of the provenances was investigated separately and varied only slightly, ranging from 0.257 to 0.290 g/cm^3 with an average of 0.271 g/cm^3.

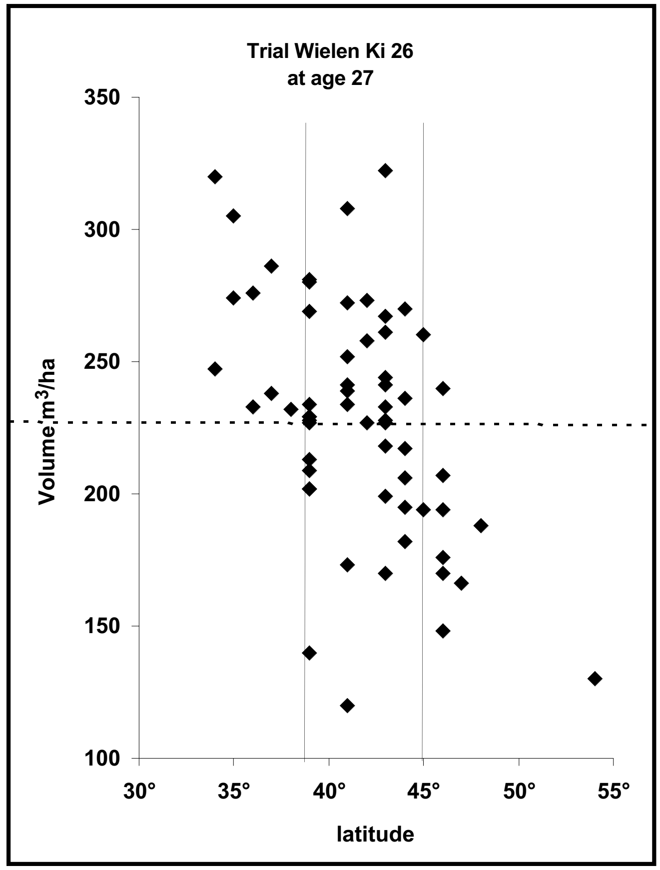

Figure 1—Volume under bark (m^3/ha) at 27 years of 61 provenances of eastern white pine in relation to geographic latitude at one provenance trial (Ki 26) site location at Wielen, northwestern Germany.

Differences Among Five-Needle Pines in Blister Rust Resistance

There was wide genetic variation among pine species, provenances and progenies in the reaction after artificial inoculation with basidiospores of *C. ribicola* (table 2). Generally, European and Asian pines remained uninfected or were less infected by the rust fungus on the basis of needle lesions, percent of trees with stem symptoms and mortality rate, than the extremely susceptible North American pines. Particularly *P. cembra*, *P. armandii* and *P. pumila* showed neither needle symptoms nor stem symptoms. Large differences of percent rust infected trees existed among seed samples within the Asian species *P. parviflora* and *P. wallichiana* (table 2). Among the North American pine species, four *P. aristata* provenance samples had the lowest percentage of tree with stem cankers (mean of 66 percent).

Progenies from crosses between selected rust-free parents from two of the North American species had some trees with heavy infection, but in general they had fewer infected trees than did trees in provenances of their respective pine species six and a half years after inoculation (see *P. lambertiana* and *P. monticola* in table 2). A few years later, however, these canker-free progenies were also heavily infected by blister

Table 2—Blister rust infection (percent of trees with cankers) of 8- and 9-year-old white pines about 6 years after artificial inoculation with *Cronartium ribicola*.

White pine species	No. of provenances/progenies	Blister rust attack (%) Mean (provenances)	Blister rust attack (%) Mean (species)
Europe			
Pinus cembra	1	0	0
P. peuce	6	0-30	22
Asia			
P. armandii	2	0	0
P. pumila	1	0	0
P. sibirica	1	—	17
P. parviflora	3	0-67	22
P. koraiensis	3	18-29	23
P. wallichiana	5	17-60	40
P. morrisonicola	1	—	40
North America			
P. aristata	4	50-94	66
P. strobiformis	4	75-95	88
P. balfouriana	4	74-100	90
P. lambertiana	6	88-100	97
— R-progeny [a)]	1	—	76
P. albicaulis	2	94-100	97
P. flexilis	4	97-100	98
P. monticola	6	93-100	99
— R-progenies [a)]	10	85-100	97
P. strobus	8	98-100	100
P. strobus (Germany)	4	60-88	71

[a)] R-progenies = F_1 and F_2 progenies from controlled crosses between parent trees resistant in North America

rust and subsequently died. Canker development in these progenies seemed to require more time. Natural infection was excluded as all trees were grown in greenhouses far away from alternate host plants.

Interestingly, the four seed samples of *P. strobus* populations grown in Germany and used in the inoculation experiment were obviously more tolerant to the German blister rust race than were autochthonous samples of North American *P. strobus* (table 2).

Genetic Variation Within *Cronartium ribicola*

Alternate hosts of the white pine blister rust fungus were inoculated simultaneously with aeciospores of the fungus in a joint experiment in Germany and South Korea. In the German trial only *Ribes nigrum* and in the Korean trial only *Pedicularis resupinata* were infected, although in both countries the respective other alternate host was also inoculated. Urediniospores and teliospores were formed after infection only on the leaves of *R. nigrum* in Germany and *P. resupinata* in South Korea. The respective other host plant species remained uninfected. Therefore, one can assume that the *C. ribicola* types used in both countries differed in their pathogenicity. Differences between various *C. ribicola* samples regarding the size of aeciospores and urediniospores could not be found.

Discussion

Pinus strobus is the most important species among the white pines of interest for forestry uses in Germany (Ritter 1978, Stratmann 1988, Waldherr 2000). The first plantation was established in southwestern Germany around 1770 (Stratmann 1988). Growth performance and natural regeneration are superior compared to the native Scots pine (*P. sylvestris* L.). The main problem is its high susceptibility to blister rust, presenting an obstacle to its otherwise desirable use as a main tree species for silviculture. First observations of the blister rust disease are known from Estonia (northeastern Europe) around 1854. Thirty years later the fungus had reached the Atlantic Ocean in western Europe and had caused tremendous losses of *P. strobus* afforestations. Therefore, around 1930 growing of eastern white pine was prohibited. Later the prohibition was again canceled and instructions for the afforestation of *P. strobus* were given. To avoid most severe losses it is recommended that eastern white pines be planted in mixture with other tree species and at a greater distance than at present from villages and plantations, where the alternate host *Ribes nigrum* is cultivated. As evidenced by the lower blister rust infection of the German land race (compared to the North America provenances) some natural selection for *C. ribicola* resistance may be occurring. Further investigation of the potential for developing more resistance in the German land race may be warranted.

There is a wide intraspecific variation in *P. strobus*, as shown by provenance trials. The results of the German trials agreed very well with those in the United States of America, Australia and New Zealand (Genys and others 1978). In Germany, southern provenances from the Appalachians are of particular interest. They seem to be very well adapted to climatic and other site conditions, but, unfortunately, resistant progenies of *P. strobus* are not available yet.

The resistance tests clearly showed that the German blister rust race used for artificial inoculations was more aggressive than the race used in western North America (Idaho), since progenies of rust resistant parents of *P. lambertiana* and *P. monticola* were also heavily infected (table 2). Our results were in a generally good agreement with French results, but differed from the American test results (Delatour and Birot 1982, Stephan 1986a). This may demonstrate similarity within the European blister rust fungus, but differences from the North American fungal type.

Race differences have also been found between the German and South Korean blister rust fungus, and a wider pathogenic variation of *C. ribicola* can be assumed in eastern Asia, for example in Korea and Japan (Stephan and Hyun 1983). These areas can be considered as the main gene centers, where host and pathogen coexisted during long periods. Because of the common coevolution tolerance of the host as well as virulence of the parasite are there in a dynamic equilibrium (Leppik 1970).

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