Zusammenfassung

Papierchromatographische Analysen von Polyphenol-Verbindungen an Nadeln von Artbastarden der Subsektion Sylvestres erwiesen sich für die Anwendung bei Züchtungs-Programmen und anderen Untersuchungen geeignet. Die chromatographischen Muster der F, enthalten im allgemeinen alle Substanzen, die beiden Elternarten gemeinsam sind. Bei keinem Besipiel war aber das Hybridmuster eine einfache Summierung der Muster beider Elternarten. Verbindungen, die in einem der Elternarten nicht vorhanden waren, kamen aber bei vielen Bastarden vor. Diese „Bastard"-Verbindungen waren wiederum charakteristisch für andere Sylvestres-Arten. Dies weist auf eine gewisse genetische Homogenität eines polygenen Systems der Phenolsynthese innerhalb der Subsektion hin.

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Intraspecific Variation in Foliage Polyphenols[1]) of Pinus (Subsection Sylvestres)

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The use of chemical characters in systematic studies is not new, but the development and refinement of chromatographic methods have greatly aided the study of the distribution of natural organic compounds within and among plant taxa. In the genetic approach to plant taxonomy, useful information has been obtained through studies of the secondary, or intermediate, metabolites of plants such as terpenes, alkaloids, and phenols.

Phenolic compounds are especially well-suited to empirical surveys of variation at lower taxonomic levels because of their diverse chemical and physical properties and the rather restricted taxonomic distribution of individual compounds. One explanation for the extreme variability in the distribution of plant phenols is that they have evolved more recently than basic metabolites and have not been subjected to such rigorous selection pressures as sugars, amino-acids, and organic acids. But the physiological functions of most phenolic compounds are either unknown or disputed and this makes difficult the development of theories to explain the selective advantage of individual compounds.

Research on the action of plant phenols as phytoalexins in diseased plant tissue (CRUICKSHANK and PERRIN, 1964; HARE, 1966) and as regulators and inhibitors of plant growth (SHANTZ, 1966) indicates that many of these compounds are physiologically active.

Regardless of whether the genetic mechanism responsible for this variation is natural selection, random genetic drift, or mutation with subsequent reproductive isolation, plant phenols are potentially useful for studies of natural variation.

While there has been much interest in the phenolic constituents of wood, particularly their role in lignin formation and the inhibition of fungi and bacteria, there have been few systematic studies utilizing the phenolic compounds in the foliage of forest trees. Qualitative intraspecific variation in phenolic compounds from the foliage of Picea, Salix, and Populus was reported by BÖRTITZ (1962,

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1963), and DUGLE (1966) found some variation within *Betula* species. COCCIA (1967) was able to differentiate sections, species, and clones of *Populus* on the basis of paper chromatography of leaf flavonoids. Other species for which intraspecific variation in phenolic compounds has been reported include *Pseudotsuga menziesii* (CHING, *et al.*, 1965) and *Eucalyptus sideroxylon* (HILLIS and HASEGAWA, 1962; HILLIS and ISOI, 1965). HANOVER and WILKINSON (1970) observed some quantitative and qualitative variation between limited samples of geographic seed sources of three North American *Picea* species.

In *Pinus*, BÖRTITZ (1963) reported intraspecific variation in the chromatographic pattern of ultra-violet light fluorescent compounds in foliage extracts of *P. sylvestris*. Geographically related quantitative differences in heartwood phenol (pinosylvin) content were found in a survey of natural populations of *P. sylvestris* in Sweden (ERDTMAN *et al.*, 1951; ERDTMAN and MISIORNY, 1952).

The present investigation of the foliage phenolic compounds of 16 *Pinus* species had two purposes; 1) to provide an estimate of the extent and pattern of quantitative and qualitative variation within each of these species, and 2) to determine the consistency of chromatographic patterns and to construct a standard chromatogram for each species to

Table 1. — Location of provenances and natural stands of 16 *Pinus* species. Foliage samples collected from four trees per provenance or stand for analysis.

Pinus resinosa Ait.

Provenance Test - Augusta, Mich.

Location	Latitude	Longitude	Elevation (m)
Pennsylvania	41°16'N	77°48'W	400
Pennsylvania	41°31'N	76°09'W	305
New Hampshire	43°10'N	71°47'W	120
Michigan	44°15'N	83°30'W	245
Nova Scotia	44°20'N	65°09'W	150
New York	44°29'N	74°17'W	510
Wisconsin	44°47'N	91°21'W	305
Vermont	45°12'N	72°10'W	230
Michigan	45°20'N	84°30'W	-
New Brunswick	45°58'N	64°50'W	75
Ontario	45°58'N	77°33'W	150
Wisconsin	46°00'N	83°30'W	490
Wisconsin	46°16'N	91°34'W	325
Manitoba	49°05'N	95°55'W	350
Quebec	54°08'N	73°16'W	20

Pinus nigra Arn.

Provenance Test - Wooster, Ohio

Location	Latitude	Longitude	Elevation (m)
Greece	37°15'N	22°33'E	-
Turkey	37°15'N	28°30'E	975
Spain	37°55'N	3°00'W	1130
Greece	39°12'N	26°30'E	700
Turkey	39°25'N	28°09'E	915
Greece	39°51'N	21°23'E	1070
Spain	40°10'N	1°45'W	1040
Greece	40°12'N	22°05'E	1465
Turkey	40°30'N	32°40'E	1310
Greece	40°44'N	24°45'E	700
Greece	41°17'N	23°55'E	855
France	43°45'N	3°30'W	610
Yugoslavia	43°51'N	19°32'E	1220
Crimea (USSR)	46°00'N	34°00'E	-
Austria	48°10'N	16°15'E	490

Pinus sylvestris L.

Provenance Test - Augusta, Michigan

Location	Latitude	Longitude	Elevation (m)
Greece	39°54'N	21°12'E	1370
Turkey	40°30'N	32°42'E	1495
Spain	40°48'N	4°00'W	1495
Georgia (USSR)	41°48'N	43°30'E	1585
Yugoslavia	43°54'N	19°24'E	915
Italy	46°00'N	11°12'E	760
Italy	46°18'N	11°18'E	1005
Austria	47°12'N	11°18'E	915
Hungary	47°42'N	16°36'E	305
Czechoslovakia	48°54'N	20°30'E	825
Czechoslovakia	49°06'N	13°18'E	670
Germany	49°06'N	8°06'E	150
Germany	49°24'N	7°36'E	395
Germany	50°18'N	12°12'E	1890
Germany	50°54'N	13°42'E	550
Germany	50°54'N	14°18'E	305
England (Planted)	51°12'N	0°48'E	215
Siberia	52°24'N	117°42'E	610
Poland	53°42'N	20°30'E	185
Siberia	54°00'N	94°00'E	150
Sweden	55°54'N	14°06'E	30
Scotland	57°06'N	4°54'W	185
Sweden	58°48'N	14°18'E	120
Ural Mountains	58°48'N	60°48'E	915
Sweden	60°54'N	13°24'E	460
Sweden	62°30'N	15°42'E	215

Pinus mugo Turra

Provenance Test - Amance, France

Location	Latitude	Longitude	Elevation (m)
France	43°00'N	0°05'E	-
Germany	48°00'N	8°00'E	-
	Natural Stands		
Italy (6 stands)	45°01' to 45°08'N	25°33' to 34°15'E	105 to 2180
France (Vosges)	48°09'N	6°57'E	
Czechoslovakia	50°45'N	15°45'E	1370

Pinus halepensis Mill.

Provenance Test - Rome, Italy

Location	Latitude	Longitude	Elevation (m)
Italy	40°30'N	17°10'E	6
Italy	42°25'N	12°35'E	403
Italy	44°20'N	8°30'E	5

Provenance Test - Leghorn, Italy

Algeria	36°00'N	2°00'E	550
Spain	37°55'N	2°55'W	1068
Italy	40°30'N	17°10'E	6
Italy	42°35'N	12°40'E	403
France	43°30'N	5°30'E	-

Natural stands

Israel	31°48'N	35°06'E	671
Israel	32°35'N	35°09'E	305
Israel	32°45'N	35°02'E	488
Israel	33°00'N	35°29'E	732
Algeria	36°51'N	2°56'E	396
Turkey	36°54'N	30°56'E	49
Turkey	37°37'N	35°31'E	244
Italy	40°30'N	17°00'E	15
Greece	40°36'N	22°59'E	9
France	43°37'N	1°27'E	-
Yugoslavia	44°02'N	15°20'E	52
Yugoslavia	44°09'N	15°22'E	30
Italy	44°20'N	9°20'E	100
Italy	44°25'N	8°55'E	352

Pinus heldreichii Christ

Natural Stands

Location	Latitude	Longitude	Elevation (m)
Greece	39°48'N	21°16'E	1403
Yugoslavia	43°30'N	17°50'E	760 to 1280

Pinus pinaster Ait.

Provenance Test - Bordeaux, France

Location	Latitude	Longitude	Elevation (m)
Portugal	39°45'N	8°50'W	30
Portugal	--	--	575
Corsica	42°18'N	9°52'E	--
France	44°22'N	0°52'W	75
France	45°30'N	1°00'W	25
France	45°32'N	1°00'W	15
France	45°40'N	1°05'W	11
France	45°45'N	1°12'W	18

Provenance Test - Leghorn, Italy

Morocco	34°08'N	4°00'W	--
Algeria	37°00'N	6°30'E	425
Spain	40°50'N	4°10'W	--
Spain	41°00'N	4°30'W	885
France	43°15'N	6°40'E	183
Italy	43°20'N	10°30'E	3
Italy	43°55'N	10°25'E	183
Italy	44°20'N	9°20'E	--
France	45°00'N	1°10'W	45
Italy	45°45'N	13°25'E	3

Natural Stands

Italy	43°35'N	11°20'E	245
Italy	44°15'N	9°25'E	170
Italy	44°25'N	8°55'E	250

Pinus brutia Ten.

Natural Stands

Location	Latitude	Longitude	Elevation (m)
Turkey	36°54'N	30°56'E	45
Greece	40°46'N	24°37'E	120

Pinus pinea L.

Natural Stands

Location	Latitude	Longitude	Elevation (m)
Turkey	36°54'N	30°56'E	45
Greece	40°39'N	23°01'E	260
Italy	43°20'N	10°30'E	3
Italy	43°45'N	11°20'E	170

be used in further investigations of species relationships and hybridization in the Subsection *Sylvestres*.

Materials and Methods

Samples of one-year-old foliage were collected from trees of 16 species growing in provenance tests or natural stands *(Table 1)*. The samples of natural populations of European and Asian species were collected by cooperators in the countries of origin. *Pinus pinea*, while not a member of the Subsection *Sylvestres*, was included in the study because it is the only other European species of the Subgenus *Pinus*. Four trees per population were sampled, and the foliage was dried prior to shipment and storage.

Prior to analysis, samples were dried at 45° C for 72 hours in a forceddraft oven. The extraction technique was a modification of the method of HANOVER and HOFF (1966). Eight grams of oven-dried tissue was cut into approximately 1 cm lengths and homogenized in 100 ml of ethyl ether for four minutes in a Waring semi-micro blender. The homogenized mixture was transferred to a 250 ml Erlenmeyer flask and placed on a rotary motion shaker. A preliminary extraction test established a schedule of three successive decantings and replacement with 50 ml of fresh ether at 12 hour intervals. After 48 hours, the extraction of ether-soluble phenolic components was complete.

Following ether extraction and vacuum filtration, the tissue was extracted with n-butanol by the same procedure for 48 hours. The tissue was then extracted with cold, distilled water for 48 hours to obtain water-soluble components.

Thus, three fractions were obtained for analysis — simple phenols (ether), polyphenols (n-butanol), and tannins (water). Virtually all of the chlorophyll and other extraneous substances such as waxes, fats, terpenes, and alkaloids were removed in the ether fraction.

Quantitative tests for phenols and tannins

The modified FOLIN-DENIS method (SWAIN and HILLIS, 1959) was used for the determination of total phenols and tannins. For the determination of simple phenol and polyphenol content (ether and n-butanol fractions) the extracts were filtered, diluted to 250 ml, and a 0.5 ml aliquot of this diluted to 10 ml with water in a colorimetric tube. To this was added 0.5 ml of the FOLIN-DENIS reagent, followed by a thorough mixing. After exactly three minutes, 1.0 ml of saturated sodium carbonate solution was added. The tube was shaken again and allowed to stand for one hour, at which time absorption at 725 mμ was determined on a BAUSCH and LOMB Spectronic-20 colorimeter.

The analytical procedure for the tannins was similar, except that the extract was diluted to 500 ml and a 0.25 ml aliquot of this diluted to 25 ml with water.

Chromatography of polyphenols

Prior to chromatography, extracts of polyphenols were evaporated to dryness on a Buchler Rotary Evapo-mix and redissolved in 1.0 ml of n-butanol. Compounds were separated on Whatman 3MM filter paper (46 × 57 cm sheets) by two-dimensional descending chromatography. A template was used to mark the point of application and the margins of the folds. Extracts were applied with a micropipet in 10 μl increments to a total of 120 μl.

The solvent combination employed was the organic (upper) phase of n-butanol: acetic acid : water (4 : 1 : 5 v/v) followed by an aqueous solution of sodium formate (10 g HCOONa : 200 ml water : 2 ml formic acid). Development times were approximately 18 hours in the first solvent and 4.5 hours in the second.

Results and Discussion

Preliminary experiments indicated that environmental and seasonal factors influenced the total polyphenol and tannin content of *Pinus* foliage. As a result, only *P. sylvestris*, *P. nigra*, and *P. resinosa*, which had been sampled from provenance tests, could be analyzed for total polyphenol and tannin content. Environmental factors did not

Table 1.

(Continued from Table 1)

Pinus densiflora Sieb. and Zucc.

Natural Stands

Location	Latitude	Longitude	Elevation (m)
Korea	37°15'N	127°00'E	45
Korea	37°45'N	127°10'E	55

Nursery - Tokyo, Japan

Japan	37°40'N	140°30'E	455
Japan	37°50'N	141°10'E	305
Japan	39°00'N	141°20'E	185
Japan	40°50'N	141°20'E	120

Pinus thunbergiana Franco

Natural Stand

Location	Latitude	Longitude	Elevation (m)
Korea	37°15'N	127°00'E	45

Nursery - Tokyo, Japan

Japan	32°30'N	130°30'E	450
Japan	35°00'N	138°30'E	305
Japan	35°30'N	140°20'E	305

Pinus insularis Endl.

Natural Stands

Location	Latitude	Longitude	Elevation (m)
Philippines	8°16'N	125°02'E	750
Philippines	14°08'N	125°04'E	670
Philippines	16°30'N	120°40'E	1500
Philippines	16°38'N	121°22'E	900
Thailand	16°50'N	101°50'E	915

Pinus merkusii Jungh. and De Vriese

Natural Stands

Location	Latitude	Longitude	Elevation (m)
Philippines	15°30'N	120°00'E	90
Thailand	16°50'N	101°50'E	915
Thailand	17°45'N	100°45'E	1005

Pinus luchuensis Mayr.

Natural Stands

Location	Latitude	Longitude	Elevation (m)
Ryukyu Islands	24°50'N	125°19'E	30
Ryukyu Islands	25°16'N	124°15'E	75
Ryukyu Islands	26°14'N	127°44'E	120

Pinus tabulaeformis Carr.

Planted - Maple, Ontario

Location	Latitude	Longitude	Elevation (m)
Manchuria	45°20'N	126°30'E	-
Unknown source	Planted - Norfolk, Connecticut		

Pinus hwangshanensis Hsia

Planted - Maple, Ontario

Location	Latitude	Longitude	Elevation (m)
China	33°45'N	113°00'E	-

Table 2. — Comparison of the 95% confidence intervals for polyphenol content between *Pinus sylvestris* and *P. nigra*, and *P. resinosa*.

Term	*Pinus sylvestris*		*Pinus nigra*		*Pinus resinosa*	
	DF	Variance component	DF	Variance component	DF	Variance component
Seed source	24	.3154 < .6409 < 1.4003	14	.3477 < .9271 < 2.4970	15	.0054 < .0208 < .1042
Error	75	.5014 < .6741 < .9549	45	.3482 < .5061 < .8028	48	.0592 < .0852 < .1330

modify the chromatographic patterns of polyphenols, however, i. e., there were no effects on the presence or absence of individual compounds. Therefore, chromatograms of all samples, whether grown in provenance tests or natural stands, could be validly compared and analyzed for evidence of intraspecific variation.

Quantitative comparisons

Analysis of variance for total polyphenol and total tannin content in *P. sylvestris, P. nigra,* and *P. resinosa* yielded significant differences among geographic seed sources for both variables in all three species. Subsequent application of the TUKEY test to the source means isolated differences between individual seed sources, but patterns of variation could not be established when the data were plotted over geographical, altitudinal, or climatic factors. *Pinus nigra* was a possible exception to this statement because Spanish sources were lowest in polyphenol and tannin content while Greek and Turkish sources were generally the highest.

When the results for the three species are compared, it is evident that *P. resinosa* is much more homogeneous than the other two species. This is apparent from a comparison of the 95% confidence intervals of the variance components for polyphenol content *(Table 2).*

Qualitative comparisons

A total of 117 different compounds were separated on the chromatograms of the 16 species examined. One compound (d-catechin, compound 75) was identified to serve as a reference for the Rf values of other spots. Standard chromatograms for each species were constructed from these data (THIELGES, 1969). Chromatographic data for the compounds discussed in this study are presented in *Table 3.*

In 14 of the species, a sufficient number of seed sources or populations were sampled to permit analyses of intraspecific variation. Discrepancies in the species data (intraspecific variation) are summarized in *Table 4,* where the presence or absence of individual compounds in divergent populations of these 14 species are recorded. The data presented in this table represent results that were valid after rechromatography at higher applications of foliage extract.

With the exception of *P. nigra,* the species sampled from provenance tests *(P. sylvestris, P. resinosa, P. halepensis,* and *P. pinaster)* were much more uniform than those species in which samples were collected from natural stands, despite the fact that a much larger number of seed sources from a wider geographic range were sampled in the provenance tests. The variant populations of *P. halepensis* were both sampled from natural stands. The relative homogeneity of chromatographic patterns among the morphologically-diverse *P. sylvestris* seed sources indicates the lack of correlation of morphological and chemical variation within this species.

The presence of four additional compounds in the Greek and Turkish sources of *P. nigra* from the Aegean region coincide with the area occupied by the variety *'aegea'* discussed by FUKAREK (1958). These four compounds (16, 36, 42, and 43) are found consistently in *P. sylvestris, P. hale-*

Table 3. — Chromatographic data for phenolic constituents of foliage associated with intraspecific variation in *Pinus* species.

Compound Number	Rf (BAW)	Rf (HCOONa)	UV Color Untreated	UV Color NH_3	Color in Sulfanilic Acid
3	.15	.02	DV	DV	—
4	.20	.00	Y	Y	—
5	.20	.24	Y	Y	—
12	.24	.18	Y	Y	—
16	.25	.57	Y	Y	Y
23	.28	.44	Y	Y	—
31	.33	.01	Y	Y	—
35	.34	.11	DV	DV	—
36	.34	.35	Y	Y	—
39	.36	.00	Y	Y	—
41	.37	.08	Y	Y	—
42	.37	.58	Y	Y	Y
43	.38	.43	Y	Y	Y
61	.47	.91	—	R	DBr
69	.51	.68	BG	BG	—
*75	.55	.32	LB	LB	Y
79	.56	.67	BG	BG	—
82	.57	.75	BG	BG	—
91	.62	.84	B	B	—
101	.70	.35	B	B	—
113	.79	.55	—	—	Y

* Identified as d-catechin by co-chromatography.
Color abbreviations: B = blue; LB = light blue; BG = blue-green; DBr = dark brown; R = red; Y = yellow; DV = dark violet.

Table 4. — Summary of intraspecific variation in the chromatographic pattern of foliage polyphenols for 14 *Pinus* species.

Species	Divergent Population(s)	Compounds Present	Compounds Absent
P. sylvestris	Germany (5 provenances) and Hungary		91
	Scotland, England (planted)		39
P. nigra	Aegean (E. Greece and Turkey — 5 provenances)	16, 36, 42, 43	
P. resinosa	No qualitative differences		
P. pinaster	No qualitative differences		
P. halepensis	Italy	23, 101	
	Turkey		75
P. brutia	Turkey	4, 5, 12	75
P. mugo	France (Vosges)	35, 41	
	Italy (6 natural stands)	31, 69	
P. heldreichii	No qualitative differences		
P. densiflora	Japan		82
P. thunbergiana	Japan		82
P. insularis	Philippines (4 natural stands)	3, 113	
P. merkusii	Thailand (2 natural stands)	61, 79, 82	
P. luchuensis	No qualitative differences		
P. pinea	Turkey		75

pensis, and *P. pinea*. It is possible that the occurrence of these compounds in the Aegean sources of *P. nigra* is indicative of natural hybridization between *P. nigra* and these other species, all of which occur sympatrically in the Aegean area. PRAVDIN (1963) discussed the possibility of hybridization between *P. nigra* and *P. sylvestris* at the end of the Tertiary. This cross has been accomplished by controlled pollination (BENEA, *et al.*, 1963; DUFFIELD, 1952; WRIGHT and GABRIEL, 1958), and natural hybrids between *P. nigra* and *P. sylvestris* have been reported (VIDAKOVIĆ, 1958). Artificial hybridization between *P. nigra* and *P. halepensis* has also been successful (VIDAKOVIĆ, 1963) and a natural hybrid between these species has been reported (SVOBODA, 1940). In support of this hypothesis, foliage samples of the progeny of the artificial crosses *P. nigra* × *sylvestris* and the reciprocal were found to contain compounds 16 (10 of 12 hybrids), 36 (6 of 12 hybrids), and 42 (7 of 12 hybrids).

Assuming the variation of individual compounds in other species to be valid (i. e., not artifacts), the hypothesis of hybridization can be extended. Compound 23, found in two of the *P. halepensis* populations from Italy, was a normal constituent of the foliage of all the other European pines investigated, with the exception of *P. heldreichii*. Compounds 4, 5, and 12, normal components of *P. halepensis*, were also present in foliage samples of a population of *P. brutia* from an area of Turkey where active natural hybridization between *P. brutia* and *P. halepensis* has been reported (KAYACIK, 1954). The two French populations of *P. mugo* were the only ones of this species containing compounds 35 and 41, both of which are consistently present in *P. sylvestris* foliage. Natural hybridization between *P. mugo* and *P. sylvestris* is known to occur and, in fact, a hybrid population from France was included in the analyses. Compounds 35 and 41 were present in these hybrids and also in the progeny of a controlled backcross to *P. sylvestris*. These results suggest natural hybridization and introgression between *P. mugo* and *P. sylvestris* in the French (Vosges) populations. The occurrence of compounds 61, 79, and 82 in the Thailand populations of *P. merkusii* may be indicative of ancient hybridization with other species of the Asian mainland, several of which contain these three compounds.

In several instances, compounds found in certain populations were 'unique', i. e., they were not found in any other samples analyzed in this study. This was the case for compound 101 in *P. halenpensis* (Italy), compound 3 in *P. insularis* (Philippines), and compounds 31 and 69 in *P. mugo* (Italy). Whether the limited occurrence of these compounds represents biochemical variants of the species or are the result of interspecific hybridization cannot be fully ascertained. Studies in pine (THIELGES, 1972) and other taxa (ALSTON and TURNER, 1963) have shown that new compounds (hybrid substances) are often found in interspecific hybrid progenies. *Pinus mugo* compounds 31 and 69 were not found in either the F_1 hybrid or backcross progenies with *P. sylvestris*, however, (THIELGES, 1972), and it seems possible that the Italian populations may contain these two 'unique' compounds as a result of mutation, response to localized selection pressure, or random genetic drift and are valid chemical variants of *P. mugo*.

The variation patterns of compounds 75 and 82 were geographically correlated. Compound 82 was not found in the Japanese populations of *P. densiflora* and *P. thunbergiana*. Compound 75 was absent from foliage samples of the Turkish populations of *P. halepensis*, *P. brutia*, and *P. pinea*. It is difficult to imagine that mutation or random deviations in opposite directions for the genes involved in the synthesis of these compounds has occurred simultaneously in local populations of two or more species, and the data suggest that response to local selection pressures, affecting all species in the area and maintained by subsequent reproductive isolation, may be responsible for this variation pattern.

It was not possible to obtain samples from as wide a range of populations in some species as in others, but the results of the study indicated a generally discrete and consistent chromatographic pattern for each species. These data were used as the basis for further comparisons between species (THIELGES, 1969) and for studies of the inheritance of polyphenolic compounds (THIELGES, 1972) in the Subsection *Sylvestres*.

The existence of intraspecific variation in total polyphenols and tannins and in individual compounds could prove to be of value as an indirect screening procedure for early selection if these traits are linked to other characters that are more difficult to measure.

Summary

Quantitative differences in foliage polyphenols and tannins were found among seed sources of *P. sylvestris*, *P. nigra*, and *P. resinosa* collected from provenance tests. The variation patterns did not correspond to major geographic features or climatic factors. Based on previous experimental results, it is probable that some of the observed variation was due to differential responses of seed sources to the environmental conditions of the plantation site (seed source-environment interactions). *Pinus resinosa* exhibited a lesser degree of variation than did the other two species.

The results of chromatography indicated only minor variation in basic chromatographic pattern between populations of any of the species. Chemical variation was not related to patterns of morphological variation, except in the case of the Aegean sources of *P. nigra*. Where qualitative intraspecific differences were found it was suggested that these provide evidence for natural hybridization. The absence of the same compound in two or more species from the same geographic area suggests that this is the result of geographically restricted selection pressures and has been maintained by reproductive isolation of these populations.

Zusammenfassung

Bei den Polyphenolen und den Tanninen der Benadelung wurden quantitative Unterschiede zwischen den Saatgut-Herkünften von *Pinus silvestris, P. nigra* und *P. resinosa* gefunden. Die Variationsmuster korrespondierten aber nicht mit geographischen oder klimatischen Faktoren. Die beobachtete Variation wird vielmehr auf die unterschiedliche Reaktion der Herkunft auf die Umweltbedingungen am Pflanzort zurückgeführt (Herkunft-Umwelt-Interaktion). *Pinus resinosa* zeigte eine geringere Variation als die beiden anderen Kiefernarten.

Bei allen Species fand sich zwischen den Populationen nur eine geringe Variation der chromatographischen Grundmuster. Die chemische Variation stand in keinem Zusammenhang mit der morphologischen Variation. Eine Ausnahme bildete nur eine Herkunft von *P. nigra* aus der Ägäis, deren Verhalten auf das Vorhandensein natürlicher Hybridisation zurückgeführt werden kann.

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Resistance of Eastern White Pine (Pinus strobus L.) Provenances to the White-Pine Weevil (Pissodes strobi Peck.)[1])

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Introduction

Observation of damage caused by the white pine weevil *(Pissodes strobi* Peck.) in existing provenance studies in the Northeastern States offered a potentially quick, easy method of locating eastern white pine *(Pinus strobus* L.) that are resistant to attacks by this important insect. If resistant sources were found in our plantings, and if they were adapted to other sites in the white pine region *(Fig. 1)*, it is probable that this species would again become an important component of the reforestation programs in the Northeastern States, the Lake States, and southeastern Canada.

This paper reports the results of a three-year study of weeviling in a planting that contains 27 provenances of eastern white pine. The planting is completely randomized in each of 24 blocks. The results show that the amount of leader damage varied from 71—100 percent among the different provenances after the 1970 attack period. There was no correlation between weevil damage and latitude of seed source or average tree vigor.

In most cases, previous studies of the influnce of seed source on weevil damage in estern white pine lacked replication and included only a few sources (Pauley *et al.* 1955, Wright and Gabriel 1959, Trefts 1960, and Connola 1966). One exception was a study by Soles and Gerhold (1968), who placed 3-year-old white pine seedlings from 80 provenances in cages with a predetermined number of adult weevils. Weeviling of trees at this age inducated provenance differences at the 0.05 level of probability, but no extrapolations were made to larger trees. Another exception was a report on 10-year-old trees by Fowler and Heimburger (1969). Weevil damage was so slight on all

[1]) This publication reports research involving pesticides. It does not contain recommendations for their use nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

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