Effects of Hedging on Maturation in Radiata Pine: Western Gall Rust Susceptibility

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Summary

Resistance of *Pinus radiata* (radiata pine) to *Endocronartium harknessii* (western gall rust) was investigated in a clonally replicated study including several putative maturation states. Rooted cuttings from 4 heights of hedging of donor plants and from free-growing trees, together with seedlings serving as a juvenile control, were naturally infected by the fungus. Since earlier studies had shown that resistance to western gall rust was maturation related, it was hoped that incidence of the disease could be used to assess the effects of hedging height on maturation. In their 5th growing season, the 3 stocktypes could be characterized as follows; seedlings commonly had heavy infection, tree-origin stecklings had no infection and hedge-origin stecklings varied, with intermediate levels of infection. Trends of decreasing infection with increasing hedge height suggested that height of hedging may have affected maturation in at least some of the clones, but the relationship was not sufficiently strong or consistent so that infection levels could serve as a reliable index of maturation. Clonal variation was a significant component of total variation in disease susceptibility, emphasizing the potential value of selection in controlling western gall rust in plantation forestry.

Key words: Endocronartium harknessii, Pinus radiata, rooted cuttings, disease resistance, maturation.

Introduction

For several years we have been focussing research on problems related to maturation in *Pinus radiata* D. Don (radiata pine). This research has followed 2 lines: 1. The identification and monitoring of traits that are correlated with the process, or state of radiata pine maturation. 2. The possibility of managing maturation state in propagules through regeneration from seed or from cuttings of various origins. The 1st line of research has shown that traits as diverse as rooting (Libby et al., 1972; Libby and Hood, 1976), growth and form (Libby and Hood, 1976; Hood and Libby, 1978; Tufour and Libby, 1973; Bolstad and Libby, 1982), bark morphology (Dodd, 1986), wood structure (Dodd and Walker, 1988; Dodd, 1988) and disease resistance (Power and Dodd, 1984; Old et al., 1986; Zagory and Libby, 1985) are correlated with maturation in radiata pine. The 2nd line of research has shown, not only are seedlings more juvenile than rooted cuttings in the expression of these traits (Fielding, 1970), but that the latter differ in their level of maturation if taken from continually hedged plants or from free-growing trees (Libby et al., 1972; Libby and Hood, 1976; Hood and Libby, 1979; Bolstad and Libby, 1982). Concurrence that many maturation-related traits are more juvenile in cuttings from hedges than in cuttings from free-growing trees indicates that hedging arrests, or at least slows, the process of maturation in radiata pine. This led to the question of whether a series of maturation states could be obtained by allowing cutting donors to grow to different heights before hedging. To test this, a plantation of clonally replicated stock was established at the University of California Russell Reservation in 1973. As ramets reached the desired heights, they were hedged periodically at those different heights.

The effectiveness of these step-hedges in creating a series of differing maturation states is being tested in secondary rooted cuttings taken from the step-hedge donors. Power and Dodd (1984) examined levels of infection with *Dothistroma pini* among seedlings and rooted cuttings from 4 hedge-heights 2 growing seasons after outplanting. Their results showed higher levels of infection among seedlings than among rooted cuttings, but failed to identify differences in levels of infection among the cutting origins. Zagory and Libby (1985) showed that radiata pine propagated from hedged and non-hedged donor plants (differing in height by less than 2 m) exhibited a clear differential susceptibility to western gall rust (*Endocronartium harknessii* (Moore) Hiratsuka). We wondered if western gall rust susceptibility was a sufficiently sensitive and relible trait so that it could serve, among other such traits, in an index that would indicate maturation state in radiata pine. Cuttings from clonal replications of free-growing trees and 4 hedging heights, together with related seedlings (serving as the most juvenile state), were planted at 2 sites where natural inoculum was present. We report here data on western gall rust infection 4 years after outplanting, together with some data on growth and form.

Materials and Methods

Plant Stocktypes

The 6 putative maturation states (referred to as stocktypes) are seedlings, and a set of clones with cutting-donor ramets hedged at 4 different heights (hedge origins) or allowed to grow in unrestricted tree-form (tree origins). The seedlings were related to the clones either as full-sibs or as offspring, in either case sharing half their genes.

The vegetative stocktypes were obtained as follows:

Cutting donors were planted in a step-hedge plantation at the University of California Russell Reservation in May 1973. The donors were secondary ramets from primary-ramet donors, planted nearby in 1963 and 1966. These primary ramets had been hedged to a one-meter height until 1972. In this step-hedge plantation, each clone was planted in 2 4-ramet rows; in each row 1 of the ramets was hedged at each of 0.5m, 1m, 2m and 4m heights. The 4 hedge-heights were developed by periodic hedging after the plant had attained the desired height. Additional ramets of each clone had been allowed to grow in tree-form (i.e. no hedging) after planting in 1963 (Año Nuevo, Monterey, Cambria populations) and 1966 (Guadalupe, New Zealand populations).

In the current study, cuttings were taken from a total of 12 clones from the step-hedge plantation described above: 2 clones of the Año Nuevo (A), 2 of the Monterey (M),

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1 of the Cambria (C), 2 of the Guadalupe Island (L) populations, and 4 clones of New Zealand landrace select families (Z=55x19 and NZ=7x96). Cuttings were taken in June 1978 and in January 1979 from hedge-top shoots of hedged donors at each of the 4 hedge-heights and from branch tips at 6m to 8m above ground level in the tree-form donors. Rooting techniques are described elsewhere (POWER and DODD, 1984). In order to closely match genetic origin of seedlings with that of the cutting donors, open-pollinated seeds were obtained from some of the tree-form members of the study, or from full sibs of the other clones available as stored seed. The 4 New Zealand clones were an exception to this, since our trees were not yet producing seeds and stored full-sibs of these clones were not available. Therefore we used Año Nuevo x Monterey hybrid seedlings, in recognition of the Año Nuevo and Monterey origin of the New Zealand landrace (BURDON and BANNISTER, 1985). Seeds were germinated in June 1979 so as to be similar in height (approximately 15 cm) to the rooted cuttings at the time of planting.

The rooted cuttings and seedlings were planted at 2 sites; one at the University of California Russell Reservation on 11 February 1980 and the other near the town of Philo, California, on 5 April 1980. Trees were organized in blocks of 6 randomized trees at a 3 m square spacing within blocks and 4 m spacing between blocks. Ideally each block consisted of 5 rooted cuttings of a single clone (1 from each of the 4 hedge-heights and 1 from the tree-form donor), and a related seedling. Lack of sufficient rooted cuttings of some stocktypes in some clones, and subsequent mortality, did not allow the above layout to be fulfilled throughout both plantations. In some instances, rooted cuttings of 1 donor-height were replaced by cuttings from a donor height with excess stock for that clone. In a few cases, additional seedlings were utilized to replace unavailable rooted cuttings. The final numbers of stecklings available for this study are shown in *table 5*. The plantation at Russell consisted of 56 blocks and the plantation near Philo of 68 blocks.

Measurements

Growth and form measurements and observations were made in January 1982, 2 seasons after outplanting. These included stem height, diameter at stem base (basal diameter), diameter at two thirds of stem height (top diameter) and numbers of primary branches. Heights of trees were measured for a 2nd time at Philo in June, 1983 and at Russell in May 1984, partway into their 4th and 5th growing seasons respectively.

Incidence of WGR infection was recorded in both plantations in May and June 1984, as numbers of branch galls and numbers of stem galls per tree. To standardize for tree size, numbers of stem (or branch) galls were divided by tree height to obtain numbers of galls per unit stem length.

Statistical Methods

Since numbers of galls included zeros and were skewed in this direction, data were transformed by adding 0.5 and taking square roots. These transformed variables showed marginal departure from normality (P=0.045 stem galls, P=0.049 branch galls) using the Wilks-Shapiro statistic. The continuous variables height, numbers of primary branches and the 2 stem-diameter measurements did not depart significantly from normality. In view of the minor departures from normality, all the above variables were further treated by statistics assuming normality. Sources of variation for transformed stem-gall and branch-gall data, 2nd year stem height, stem diameters and numbers of primary branches were examined by an unbalanced design analysis of variance (ANOVA), partitioning variation by plantation, plant origin, clone and interaction between clone and plant origin. For this purpose seedlings (for the youngest origin) were grouped with their related clone. The second set of height data was analyzed separately for each plantation since measurements were taken at different times. Variance components for the different sources of variation were computed. Comparisons among the stocktypes were tested by TUKEY's studentized range test for continuous variables.

Comparisons of untransformed gall frequencies on stems and branches were made among stocktypes using the non-parametric χ^2 contingency table analysis.

Table 1. — **Means of growth and form of different** stocktypes of radiata pine.

STOCK TYPES		Seedlings	Hedges 0.5m	Hedges 1m	Hedges 2m	Hedges 4m	Trees
Philo							
2nd year stem height	(m)	0.65a	0.46b	0.48b	0.46b	0.37b	0.38b
4th year stem height	(m)	1.54a	1.15b	1.22ab	0.96bc	0.72c	0.85bc
Basal diameter	(cm)	1.12a	0.98ab	0.93b	0.93b	0.82b	0.83b
Top diameter	(cm)	0.56a	0.48ab	0.45b	0.47ab	0.46ab	0.42ab
Primary branches		21.29a	7.40b	7.19b	7.56b	5.19b	5.00b
Russell							
2nd year stem height	(m)	1.19a	1.11a	1.14a	1.00a	1.07a	1.08a
5th year stem height	(m)	2.74a	2.70a	2.85a	2.43a	2.72a	2.84a
Basal diameter	(cm)	2.33a	2.11ab	2.19ab	1.98ab	1.91b	1.87b
Top diameter	(cm)	1.35a	1.28a	1.28a	1.16a	1.18a	1.27a
Primary branches		30.19a	17.17b	16.93b	14.84bc	12.08c	9.81c

Note: stocktypes **with same letter are not significantly different** (0.05; Tukey's Multiple Range Test).

Table 2. — Analysis of variance and variance components for growth and form of different stocktypes of radiata pine.

	SOURCE	DF	M.S.	ESTIMATED COMPONENT(%)	P>F
2nd year					
Stem Height					
	Plantation (P)	1	41.70	61.71	0.0001
	Stocktype (S)	5	0.56	1.76	0.0001
	Clones (C)	18	0.42	4.50	0.0001
	SxC	40	0.11	1.15	0.1559
	Residual	434	0.09	30.89	
4th year					
Height at Philo					
	Stocktype (S)	5	3.28	21.77	0.0001
	Clones (C)	18	0.70	8.51	0.0011
	SxC	37	0.33	5.25	0.2163
	Residual	133	0.27	64.46	
5th year					
Height at Russell					
	Stocktype (S)	5	0.74	0.30	0.5964
	Clones (C)	18	1.93	12.63	0.0176
	SxC	35	0.51	0.00	0.9900
	Residual	153	1.00	87.07	
Basal diameter					
	Plantation (P)	1	145.77	62.60	0.0001
	Stocktype (S)	5	1.83	1.80	0.0001
	Clones (C)	18	1.35	4.50	0.0001
	SxC	40	0.31	0.00	0.5283
	Residual	399	0.32	31.10	
Top diameter					
	Plantation (P)	1	67.73	69.00	0.0001
	Stocktype (S)	5	0.27	0.30	0.0433
	Clones (C)	18	0.40	2.70	0.0001
	SxC	40	0.13	0.40	0.3198
	Residual	398	0.12	27.60	
Number of Primary branches					
	Plantation (P)	1	6046.87	13.80	0.0001
	Stocktype (S)	5	4444.90	30.40	0.0001
	Clones (C)	18	459.62	8.40	0.0001
	SxC	40	95.30	0.70	0.3315
	Residual	399	87.42	46.70	

Results

Morphology

Stem Height:

Under the more favorable conditions at Russell, 2nd-year plant heights (January, 1982) were significantly greater than at Philo (*Table 1*) and this component dominated the ANOVAs, accounting for 62% of total variance (*Table 2*). Clonal and stocktype differences were both highly significant in 1982, accounting for about 5% and 2% of total variance, and 12% and 5% of variance excluding that due to plantations. By the 4th growing season (mid-1983), seedlings were significantly taller than the stecklings at Philo, with the low-hedged steckling stocktypes taller than those from higher hedges (*Table 1*). At Philo, stocktype accounted for 22% of total variance and clones 9%, both being highly significant. In contrast, fifth-season (May 1984) height at Russell was essentially uniform among stocktypes, with clones accounting for 13% and stocktypes less than 1% of the total variance. We suggest that the pattern of growth among the stocktypes at Philo reflects a differential response to stress among seedlings and stecklings, (and among different stocktypes of the stecklings) that may be related to their maturation states.

Stem Diameters:

Consistent with the more favorable growth conditions at Russell, both basal and top stem diameters were greater at this site (*Table 1*). Clonal variation was highly significant for both, and stocktype differences were highly significant for basal diameter and marginally ($p=0.04$) significant for top diameter (*Table 2*). Seedlings averaged larger than any steckling stocktype for both of these traits at both sites (*Table 1*).

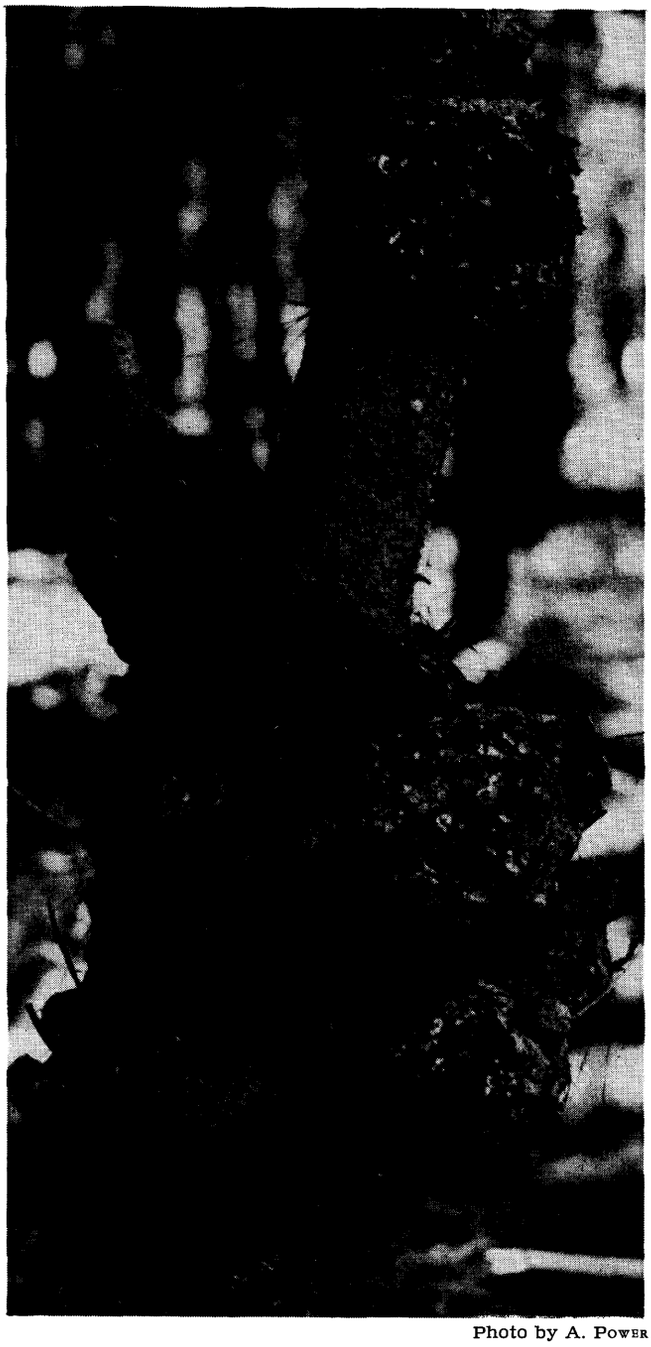

Photo by A. Power

Figure 1. — Galls formed by western gall rust (*Endocronartium harknessi*) on radiata pine.

Number of Primary Branches:

Variation in numbers of primary branches was considerable in 1982. Although 14% of this variation was attributable to greater numbers of branches in the faster growing trees at Russell (*Tables 1 and 2*), highly significant levels of variation were attributable to clones (30%) and to stocktype (8%). Seedlings had significantly more primary branches than any of the steckling stocktypes. A weak trend for decreasing numbers of branches through increasing hedge heights could be observed at Philo, and a strong and consistent trend developed at Russell.

Interactions between clones and stocktypes were not statistically significant for all 6 growth and form traits, and were near 0 for 5 of them (*Table 2*).

Western Gall Rust

Typical galls caused by western gall rust (WGR) infection on radiata pine are shown in *figure 1*. By the 5th growing season, WGR infection was common throughout both plantations. The design of the experiment into blocks in which each of the stocktypes was included (with randomization of stocktype within each block), minimized confounding due to non-uniform spread of the disease through the plantation.

Mean numbers of stem and branch galls, and numbers of galls per unit stem length are shown for the 6 stocktypes at Russell and Philo in *table 3*. The partition of variation on the transformed data is reported in *table 4*. Although trees at Philo were shorter than at Russell, they had significantly more stem galls than at Russell, but generally fewer (no significant difference) branch galls (*Tables 3* and *4*). Both Russell and Philo had highly significant differences in numbers of stem and branch galls among stocktypes and among clones (*Table 4*). Whereas clones accounted for a higher proportion of variance in stem-gall numbers, stocktypes accounted for more of the variance in branch-gall numbers.

Based on numbers of both stem and branch galls, or on numbers of stem and branch galls per unit stem length, the stocktypes could be divided into 3 groups: Seedlings, hedge-origin stecklings and tree-orgin stecklings. At both Philo and Russell, average numbers of galls were without exception most on seedlings, absent on tree-origin stecklings, and intermediate on hedge-origin stecklings (*Table 3*). These 3 divisions were supported by χ^2 contingency table analyses (*Table 3*). The differences among hedge-origin stocktypes were not statistically significant. However, trends towards lower levels of infection with increasing hedge-height can be observed for some trait/location combinations whereas the opposite trend did not occur. It is particularly evident in the Philo data (*Table 3*). For all measures in *table 3*, the sum of 0.5m plus 1m stocktypes was greater than the sum of 2 m plus 4 m stocktypes.

The highly significant component of variance associated with clones further confirms previously reported differences in resistance among clones (Zagory and Libby, 1985). Since branch-gall numbers tended to be more sensitive to the hedge-heights than stem-gall numbers, the former were used to further investigate clonal differnces. A clone-by-clone investigation of galls per unit length, for the 2 plantations combined, identified 3 groups (*Table 5*). The first group included 5 apparently highly resistant clones (A74, A02 C91, L56 and L57). The A02, A74, L56 and L57 clones are from 2 of the more resistant populations, but C91 is from one of the most susceptible populations (Old et al., 1986). The 2 remaining groups were identified after calculating correlation coefficients between branch-gall numbers or branch gall numbers per unit stem length and the 5 steckling stocktypes (*Table 6*). For this purpose, steckling stocktypes were given numerical values of 1 to 5 for increasing donor height. Group 2 included 2 clones (Z11 and NZ1) that had low ($r=0.01$ and 0.04) statistically nonsignificant positive correlations between branch galls and donor height. The 3rd group included the remaining 4 clones (Z9, NZ4, M17 and M15) that had statistically significant correlations (in the range 0.35 to 0.41) between branch galls and donor height.

Table 3. — Means of stem and branch gall numbers, raw data and standardized by stem height and χ^2 probabilities of differences between stocktypes of radiata pine.

	RUSSELL					PHILO				
Stocktype	(n)	SG	BG	SG/HT	BG/HT	(n)	SG	BG	SG/HT	BG/HT
Seedlings	57	0.33	12.51	0.17	4.48	61	0.70	5.46	0.51	4.22
Hedges										
0.5	37	0.05	1.32	0.03	0.50	30	0.47	2.53	0.51	2.15
1	37	0.16	2.68	0.05	0.73	30	0.27	2.17	0.20	1.48
2	34	0.06	1.68	0.03	0.46	35	0.37	1.71	0.43	1.27
4	36	0.08	1.42	0.04	0.46	28	0.11	0.75	0.18	1.03
Trees	11	0.00	0.00	0.00	0.00	10	0.00	0.00	0.00	0.00
$P(\chi^2)$ s		<0.01	<0.01	0.02	<0.01		0.02	<0.01	0.02	<0.01
$P(\chi^2)$ h		0.66	0.74	0.37	0.46		0.56	0.28	0.44	0.33
$P(\chi^2)$h*t		0.29	0.10	0.64	0.12		0.16	0.11	0.56	0.21

χ^2 probabilities show comparisons among all stocktypes (S), among hedge stocktypes (H), and between hedges combined as a single group versus trees (h*t). SG = number of stem galls, BG = number of branch galls, HT = height, n = number of observations.

Table 4. — Analysis of variance of square root transformed stem and branch galls of different stocktypes of radiata pine 4 years after planting.

SOURCE	df	M.S.	ESTIMATED COMPONENT (%)	P>F
STEM GALLS				
Plantation (P)	1	1.37	9.50	0.0001
Stocktype (S)	5	0.2	2.80	0.0053
Clones (C)	18	0.19	9.80	0.0001
SxC	38	0.06	0.00	0.5561
Residual	306	0.05	77.90	
BRANCH GALLS				
Plantation (P)	1	1.20	0.00	0.1599
Stocktypes (S)	5	12.64	21.45	0.0001
Clones (C)	18	2.95	13.74	0.0001
SxC	38	0.61	0.13	0.4584
Residual	306	0.61	64.68	

Table 5. — Clonal means for stem and branch gall numbers per unit stem height of *Pinus radiata* pine stecklings.

	PHILO			RUSSELL		
CLONE	(n)	SG/HT	BG/HT	(n)	SG/HT	BG/HT
A74	3	0.000ab	0.000 abc	2	0.000 a	0.000 ab
AO2	9	0.000ab	0.000 ab	11	0.000 a	0.000 a
C91	12	0.104ab	0.000 a	18	0.013 a	0.000 a
L56	9	0.000ab	0.000 ab	10	0.000 a	0.000 a
L57	13	0.000a	0.000 a	14	0.000 a	0.020 a
M15	18	0.046a	0.373 a	23	0.055 a	0.865 ab
M17	10	0.000ab	0.066 ab	18	0.034 a	0.082 a
NZ1	15	0.340ab	0.618 ab	20	0.021 a	0.095 a
NZ4	14	1.186b	2.238 abc	5	0.179 a	2.501 b
Z11	14	0.173ab	3.631 bc	14	0.056 a	0.074 a
Z9	16	0.965ab	5.215 c	20	0.072 a	2.032 b

Clones with the same letter are not significantly different (0.05 level; Tukey's Multiple Range Test). Note that clone A74 with only 3 and 2 replicates could not be statistically separated from other clones in spite of a complete absence of galls on its 5 ramets.

Table 6. — Correlation coefficients between branch gall numbers and stocktypes (seedlings excluded).

CORRELATION COEFFICIENTS	r	Prob.
NZ1	0.01	0.96
Z11	0.04	0.84
NZ4	0.40	0.05
M17	0.37	0.05
M15	0.35	0.02
Z9	0.41	0.01

Discussion

The original objective of this study was to investigate the strength of the relationship of donor heights and various traits sensitive to maturation state. When the plantation became infected by western gall rust, this presented the opportunity to investigate whether WGR infection could be used as part of an assay of maturation state. It is now clear that a test of this size, with substantial mortality, is insufficient to establish a clear relationship, or lack thereof, between hedge height and maturation state. Neither a relationship nor its absence can be clearly rejected based on data obtained. Using WGR infection as a single and clear indicator of maturation state is not feasible. The variability among ramets within stocktypes and clones, as indicated by the large residuals in *table 2,* and the frequent departure from wholly consistent trends in *table 3*, make WGR infection unreliable as a sensitive stand-alone assay of maturation state. However, the consistency of the comparison between low hedges (0.5m and 1 m) and high hedges (2m and 4m) in *table 3* indicate that WGR infection is affected by hedge height, thus providing some suggestion that hedge height does affect maturation state.

The segregation of the stocktypes into 3 groups (seedlings, hedge-origin stecklings and tree-origin stecklings) with regard to WGR infection is consistent with, and extends, the earlier results of Zagory and Libby (1985), in which tree-origin stecklings were more resistant than hedge-origin stecklings. One interpretation of our data might suggest an indirect relationship between WGR infection and maturation state due to differences in branch number. Seedling plants had many more branches than did stecklings, clearly providing greater exposure to inoculum. However, closer examination of the data indicates that this does not fully explain the difference. Among the steckling stocktypes, branch numbers of stecklings classes (*Table 3*). When comparing the seedlings to hedged donors (*Table 1*), yet infection levels were qualitatively as well as quantitatively different in these two steckling classes (*Table 3*). When comparing the seedlings to hedged stecklings, adjustment of WGR infection by branch number (primary branch numbers from *Table 1* and branch galls from *Table 3*) brought seedling infection to similar levels as hedge-origin stecklings. However, at Russell, even after such a standardization, seedling infection was 2.5 times to 5 times, higher than that of hedge-origin stecklings. Some care is needed in interpreting these data since 2 growing seasons had elapsed between counts of primary branches and counts of galls. Nevertheless, we favor the interpretation that maturation-related resistance is not simply an indirect result of the more branchy habit c juvenile origins. Further work to elucidate the mechanisms of maturation in resistance to western gall rust is currently underway.

Unfortunately, rooting data for the cuttings used in this study, which might provide a sensitive indicator of maturation state of the donors (Libby et al., 1972), was lost before it could be analyzed. Our growth and form data, although providing evidence of differences between seedlings and stecklings, generally failed to indicate strong trends among hedging heights. The donor hedges themselves had been variably weakened by heavy WGR infection at Russell Reservation, which may have resulted in continuing differences in health among the rooted cuttings, contributing to the lack of resolution among hedge heights. Furthermore, because of mortality at various stages in the study, a balanced experimental design and analysis was not possible. Thus, although a relationship between hedging height and WGR rust infection is indicated, to provide an unambiguous answer, larger sample sizes with more careful screening of donor stock will be needed.

Our data indicate clonal differences in susceptibility to WGR, with clones falling into 1 of 3 groups, highly resistant clones, susceptible clones with no correlation between branch infection and donor height and susceptible clones with significant correlations between branch infection and donor height.

The difference between the 2 latter groups may indicate that some clones are more sensitive to maturation state as it affects WGR rust resistance than others. However, the low values for clone x stocktype interaction (*Table 4*) does not support this suggestion, nor do the results reported by Zagory and Libby (1985), where clonal rankings for western gall rust susceptibility were nearly identical at 2 maturation states.

This study on variation in resistance to western gall rust supports the earlier results of Zagory and Libby (1985). Good opportunities exist for clonal selection for disease resistance. If combined with the use of cuttings of more mature origin, substantial benefits in disease resistance can be expected.

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Genetic Variation of Pinus sylvestris from Spain in Relation to Other European Populations

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Summary

Seven populations of Scots pine (*Pinus sylvestris* L.) from the natural distribution of the species in Spain were investigated isoenzymatically at 11 enzyme loci, and compared with 16 populations from northern and eastern Europe at seven loci. Populations from Spain are genetically differentiated in relation to the frequencies of genes and genotypes. The similarity between the 7 populations was studied and agreed to a significant extent with taxonomical subdivisions of Scots pine on the Iberian peninsula. The Spanish and the northern and eastern European populations form 2 very distinct and heterogeneous groups according to the genetic similarity. In comparison with the other European populations the Spanish material showed a slightly higher number of alleles per gene locus, a higher number of genotypes, and a slightly lower degree of heterozygosity. In the Spanish populations five alleles were detected, which are not present in the other European populations, whereas in the northern and eastern European populations only 2 alleles were found, which were not present in the Spanish material.

Key words: Pinus sylvestris, genetic variation, allozymes, heterozygosity.

Introduction

The genetic structure and the geographical variation of populations of *Pinus sylvestris* L. in Europe were formed by many different factors. In central and eastern Europe besides the climatic and environmental conditions the post-glacial migration of the species from refugia in south-western, southern and south-eastern Europe have had the most important influence (WULF, 1943; GODWIN, 1956; LANGLET, 1959). Also man's activity changed the "original" gene pool of *Pinus sylvestris* populations by cutting, reforestation and more recently by effects of air pollution, which is especially dramatic in central and eastern Europe.

Populations of *P. sylvestris* from the Iberian peninsula have had their own and different history compared to the rest of Europe. They are considered as relics of the tertiary period and represent "old" gene pools. Today, *P. sylvestris* inhabits disjunct, isolated areas separated by long distances, as a consequence of edaphic and climatic factors (PRAVDIN, 1969). The Spanish Scots pine stands are marginal populations of the natural range of the species in south-western Europe. Therefore, *P. sylvestris* populations from Spain represent a unique material highly valuable for genetic studies, also in connection with gene conservation programs. But they are also very interesting from the taxonomical point of view, because they represent different ecotypes or varieties of the species (SVOBODA, 1953; GAUSSEN, 1960; NICOLAS and GANDULLO, 1969).

Unfortunately, data concerning isoenzymatic differentiation of Scots pine as related to geographic and racial aspects and also our knowledge about the genetic variation of *P. sylvestris* populations and especially of populations from the Iberian Peninsula remain still largely incomplete (for review see PRUS-GLOWACKI, 1991; MÜLLER-STARCK et al., 1992). Therefore, we investigated the genetic variation and geographic differentiation of indigenous, mountainous populations of 7 isolated provenance regions in Spain and compared several genetic parameters of these populations with 16 populations from eastern and northern Europe.

Materials and Methods

For the isoenzyme study open pollinated cones of seven populations of *P. sylvestris* from Spain were collected. The seeds of each of the populations (21 to 25 trees from each population) were put together and used as random samples. From each population 40 to 112 embryos from germinated seeds were isolated and analysed.

As reference, 16 populations from eastern and northern Europe were chosen from provenance trials (Lubien IUFRO 1937, Kórnik 1968) and from four natural stands in Poland. For these isoenzyme analyses winter buds of the trees (29 to 51 from each population, mostly 30) were used.

The data for the origin of the populations are given in *table 1* and *figure 1*. Edaphic and climatic conditions for the main Scots pine areas in Spain were described by ALIA et al. (1991) and PARDOS and STEPHAN (1988).

In the Spanish material the variability of 11 enzyme loci were analysed: Alcohol dehydrogenase (ADH) E.C. 1.1.1.1, Diaphorase (DIA) E.C. 1.6.99, Fluorescent esterase (FEST) E.C. 3.1.1.1, Glutamate dehydrogenase (GDH) E.C. 1.4.1.2, Glutamate oxaloacetate transaminase (GOT), 2 loci, E.C. 2.6.1.1, Malate dehydrogenase (MDH), 2 loci, E.C. 1.1.1.37,

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