Segregation Patterns of Isoabienol in Pinus sylvestris (L.)

By R. Gref, D. Rudin and D. Lindgren

Department of Forest Genetics and Plant Physiology,
Swedish University of Agricultural Sciences,
S-901 83 UMEÅ, Sweden

(Received 27th April 1984)

Summary

The isoabienol content in needles following controlled crosses in *Pinus sylvestris* L. was investigated. The number of trees with or without isoabienol segregated often in agreement with a monohybrid pattern, although several irregularities occurred. For outcrosses an excess of segregating families was found.

Key words: Scots pine, inheritance, isoabienol, gas chromatography.

Zusammenfassung

Es wurde der Isoabienolgehalt der Nadeln bei kontrollierten Kreuzungen von *Pinus sylvestris* untersucht. Die Anzahl Bäume mit und ohne Isoabienol spalteten häufig in Übereinstimmung mit einem monohybriden Muster auf, auch wenn verschiedene Unregelmäßigkeiten vorkamen. Bei Fremdungen war die Zahl der aufspaltenden Familien übermäßig hoch.

Introduction

Isoabienol is the main diterpene alcohol in Scots pine needles (Ekman *et al.*, 1977). It belongs to the labdane type diterpenoids, which are widely distributed in conifers. It is one of the major components in the petroleum ether extract of Scots pine needles. There is so far no information regarding the possible biological role of isoabienol.

Gref (1981) found that there is a distinct difference for Scots pine between trees with isoabienol (0.6—1.0 per cent of the dry needle weight) and trees without isoabienol. The frequency of different types of isoabienol changed between populations in a way indicating a clinal variation. Thus it seems to be a distinct either/or character of a type where Mendelian segregation patterns often have been found. Isoabienol seems to be physiologically stable, polymorphism seems to be common and it is rather easy to study; thus it may be a valuable additional tool in genetic studies, and to judge the limits of such applications a study of its inheritance is necessary.

Mendelian segregation patterns have been found in Scots pine for isozymes (Rudin, 1975, 1977; Yazdani and Rudin, 1982). Monoterpenes have also been studied and a segregation pattern with an evident influence of Mendelian nature was found (Hiltunen, 1975, 1976; Yazdani *et al.*, 1982).

Material and Methods

Plant materials

Plant materials from four different sources were used for this study. *Table 1* and 2 give information about the different materials. Mature needles were always used, the samples were stored cool and reduced as quickly as possible to −20° C, and then analysed within 6 months.

Material 1. Needles from trees originating from selfings and crosses were collected at Sävar nursery (63° 50' N, 20° 15' E, Alt. 5 m) in April and November 1978. The needles were immediately transported to the laboratory and stored at −20° C for up to two weeks until the extractions were performed. This study is available in a preliminary form (Gref and Rudin, 1981). In a preliminary study parental clones were analyzed in order to find clones containing or lacking isoabienol.

Material 2. Needles from 5-year-old pine seedlings originating from full sib families growing in Johannisfors nursery, north of Umeå (63° 50' N, 20° 15' E, Alt. 8 m). These samples were collected in August 1977. This material was described and published by Gref (1981).

Material 3. Needles from four mature grafted clones of northern Swedish origin. The clones were growing as 15 year-old grafts at Tenö NE of Stockholm (59° 20' N, 18° 15 E, Alt. 5 m). This material was collected in May and September 1976.

Material 4. Needles from parental clones were collected at the seed orchard E468 Tjuttorp (58° 48' N, 15° 51' E, Alt. 60 m.) in November 1982 on grafts.

Certain of the plus tree clones at Tjuttorp were selfed and crossed. A field trial was sown 1969 and planted in the autumn 1971, Hägersdal (60° 10' N, 17° 29' E, Alt. 50 m., IDL 14911) around 40 km north of Uppsala. Needles were collected in November 1982. Only progenies originating from selfing were used in this investigation. The same needles have also been used for a study of the inheritance of resin acids. These families have also been used for a study of phenolic compounds of Scots pine needles. (O. Theander, Swedish University of Agricultural Sciences, Uppsala, pers. comm.).

Extraction and analysis

1 g of fresh pine needles was homogenized in a Sorvall Omnimixer with 50 ml petroleum ether (b.r. 40°—60°) containing methylheptadecanoate as an internal standard. The mixture was then extracted in an ultrasonic bath at room temperature for 2 hours. The mixture was filtered and the extract was evaporated to dryness on a rotary evaporator. The dried extract was redissolved in 1 ml of diethylether-methanol (9:1 v/v) and methylated with diazomethane.

The gas chromatographic (GC) analyses were performed with a Varian 2700 gas chromatograph equipped with a flame ionization detector (FID). The chromatograph was equipped with a glass wall-coated open tubular (WCOT) capillary column (45 m × 0.3 mm) coated with SE-30 stationary phase and the column was connected to the injector via an inlet splitter. The operating conditions were: injector and detector temperature 280°C, column isothermally at 240° C, carrier gas nitrogen at a column flow rate of 1.2 ml/min, split ratio 1:50. The area under each peak was determined by means of a Varian CDS 111C Chromatography Data System. The quantitative determination of isoabienol was based on peak area relative to methylheptadecanoate. The identities of the eluted compounds were confirmed with GC-mass spectrometry.

Table 1. — Segregation ratios of selfings.

			Offspring		
Parent	Material	Parent type 5)	+	0	P(3:1)
E 3001	4)	0	0	10	
E 3003	4)	0	0	10	
E 3004	4)	0	0	10	
H 1000	4)	+	5	4	0.17
H 1007	4)	+	6	4	0.22
H 1008	4)	0	0	10	
H 1010	4)	+	1	9	0.000
H 1011	4)	+	7	3	0.47
S 3004	4)	0	0	10	
S 3006	1)	+	3	1	0.68
S 3244	1)	0	6	1	0.87
W 1015	4)	0	0	10	
W 1031	4)	0	0	10	
W 3059	4)	0	0	10	
W 3123	4)	0	0	10	
W 3124	4)	0	0	10	
W 6009	1)	0	0	8	
			28	130	

1), 2), 3), 4) refers to material origin according to "Material and methods".

Results

Analytical

Isoabienol could be quantified with good reproducibility and was well resolved from methylated resin acids in the extracts. The amount of isoabienol did not seem to change during storage.

Most trees with isoabienol had around 1 per cent of the dry needle weight, while a few values below 0.5 were found. All trees with more than 0.1 per cent were regarded as "with", and those with less as "without".

Table 2. — Segregation ratios in outcrossings.

			Segregation			
Parents	Material	Type 5)	+	0	P(3:1)	P(1:1)
S 3006 x W 6009	1)	+ x 0	4	6	0.20	0.38
S 3006 x S 3244	1)	+ x 0	5	10	0.001	0.15
S 3244 x S 3059	1)	0 x 0	1	4	0.02	0.19
T 6007 x S 3059	1)	+ x 0	5	0	0.76	1.00
Y 4014 x Z 4004	2)		5	5	0.08	0.62
Y 4016 x Z 4040	2)		6	4	0.22	0.83
Y 4501 x Y 4017	2)		7	3	0.47	0.95
Y 4506 x Z 4000	2)		5	5	0.08	0.62
Z 2017 x Z 2011	2)		6	4	0.22	0.83
Z 3001 x Z 4040	2)		4	6	0.02	0.38
Z 4001 x Z 4040	2)		7	3	0.47	0.95
Z 4002 x Z 2016	2)		5	5	0.08	0.62
Z 4003 x Y 4510	2)		4	6	0.02	0.38
Z 4001 x X 4400	3)		7	3	0.47	0.95
Z 4001 x Y 4017	3)		6	4	0.22	0.83
Z 4001 x Z 4004	3)		4	6	0.02	0.38
Z 4020 x Z 4500	3)		4	6	0.02	0.38
Y 4501 x Z 4040	3)		5	5	0.08	0.62
Y 4506 x Z 4000	3)		7	3	0.47	0.95
Y 4509 x Y 4510	3)		5	5	0.08	0.62
Y 4014 x Z 2078	3)		5	5	0.08	0.62
			107	98		

1), 2), 3), 4) refers to "Material and methods".

5) + = with isoabienol, 0 = without isoabienol. The phenotype of the parents of the crosses not indicated are not known.

Segregation ratios

The segregation ratios in the different families are presented in *Table 1* for selfings and *Table 2* for crosses. The probability that as many or more without should be found if the segregation ratio is binomially distributed is indicated. A probability close to 0 or close to 1 is improbable. (However, some care should be taken when transferring the figures to confidence levels).

Discussion

The main subject of the discussion will be to what extent a simple Mendelian monohybrid pattern can explain the data observed. That it is not a complete explanation is evident. Thus the "main model" discussed is that genotypes AA and Aa give phenotype + and genotype aa phenotype 0. The frequency of the hypothetical A may be designed p.

Correspondence between parents and progeny

S 3244 shows a strange behaviour. Its own phenotype is "0" although its selfed progeny segregates 6 +: 1,0 and S 3244 × S 3059 also segregates +, when it should not. A possibility might be that S 3244 is genetically Aa, and the classification as "0" is a mistake or some environmental effect. This fits to the data following selfing *(Table 1)* and the cross S 3244 × S 3059 *(Table 2)*. However, this assumption is not compatible with the excess of "0" following the cross S 3006 × S 3244 *(Table 2)*. In other families parent and offspring match reasonable well, and probably S 3244 can be regarded as a case where several genes interact in a complicated way. Another possibility is mistakes in identifications, thus what is assumed to be S 3244 may sometimes be something else.

Segregation ratios in individual families

In segregating families a monohybrid segregation may either be 3:1 (Aa × Aa), the only possibility following selfing, or 1:1 (Aa × aa). The selfed families segregates according to expectation with exception of H 1010, which shows a highly significant deficit of "+". It is however not to surprising to find some strange segregations after selfing, e.g. linkage with lethals may cause distortions. All full sib families could be fitted to either 3:1 or 1:1 segregation patterns.

Distribution of family types

		Segregation	
Families	only +	+ and 0	only 0
Selfed	0	6	11
Outcrossed	1	20	0

There seems to be a deficit of families with only +-types (the only one found is probably a non-identified segregating one). It is peculiar that only 1 possible AA parent (T6007) is found among 41 tested.

A possible difference between selfed and outcrossed may be partly explained by the more northern origin of the latter and a corresponding higher value of p in more northern populations (cf. the clinal variation suggested by GREF, 1981).

The expected proportions assuming Hardy-Weinberg equilibrium and monohybrid inheritance would be:

	Only +	+ and 0	only 0
Selfed	p^2	$2p(1-p)$	$(1-p)^2$
Outcrossed	$p^2(2-p^2)$	$4p(1-p)^2$	$(1-p)^4$

For selfing a calculated $p = 6/[2 \cdot (6 + 11)] = 0.176$ gives a good fit to obtained. For outcrossing, no p-value satisfactory explains the high frequency of segregating progenies.

The Hardy-Weinberg equilibrium may be violated. Trees in a mature forest are sometimes found to be more heterozygous than expected. Plustrees as used in this study are selected for their good performance and may be more heterozygous than expected from gene frequencies. Some epistatic action might be able to cause distortion like the observed.

Overall segregations

For selfings 27 +: 13 0 is found in segregating progenies, if the obvious deviator H 1010 is excluded, and this is in acceptable agreement with expected 3:1.

For outcrosses 107:98 is found, which is in acceptable accordance with an expected mixture of 1:1 and 3:1.

Concluding Remarks

Despite several such factors as:
(1) The deficit of individuals giving only offspring "+" and
(2) The strange behaviour of S 3244 and H 1010

which rules out that simple monohybrid inheritance is a general rule, there is still the general accordance of individual segregations with expection and the many cases where selfed parents 0 gave exclusively progeny 0 strongly indicates that in many genetic environments the character behaves as a simple dominant Mendelian one.

Although good fit is found in many families, still significant exceptions are found, and this emphasises the risks in assuming the general validity of simple Mendelian segregation just because it is found in some crosses, as is frequently done.

That such a complicated character as isoabienol often behaves as a simple Mendelian character demonstrates that forest tree breeding should probably not assume all valuable genes to behave according to the rules of quantitative genetics.

Acknowledgements

Fonden för skogsvetenskaplig forskning has supported the study financially. The authors are grateful to Dr. Gavin Moran, CSIRO, Division of Forest Research, Canberra, Australia, for helpful discussions.

Literature Cited

Ekman, R., Sjöholm, R. and Hannes, K.: Isoabienol, the principal diterpene alcohol in *Pinus sylvestris* needles. Acta chem. Scand. Ser. B **31**: 921—922 (1977). — Gref, R.: Variation in isoabienol content in *Pinus sylvestris* needles. Can. J. Bot. **59**: 831—835 (1981). — Gref, R. and Rudin, D.: Inheritance of isoabienol in *Pinus sylvestris* L. A pilot study. Intern rapport nr 41. Inst. för skoglig genetik och växtfysiologi, Sveriges Lantbruksuniversitet (1981). — Hiltunen, R.: Variation and inheritance of some monoterpenes in Scots pine (*Pinus sylvestris* L.). Planta Med. **28**: 315—323 (1975). — Hiltunen, R.: On variation, inheritance and chemical interrelationsships of monoterpenes in Scots pine (*Pinus sylvestris* L.). Ann. Acad. Sci. Fenn. Ser. A. IV Biol. **208**: 1—54 (1976). — Rudin, D.: Inheritance of glutamat-oxalat-transaminases (GOT) from needles and endosperms of *Pinus sylvestris*. Hereditas **80**: 296—300 (1975). — Rudin, D.: Leucine-amino-peptidases (LAP) from needles and macrogametophytes of *Pinus sylvestris* L. Inheritance of allozymes. Hereditas **85**: 219—226 (1977). — Yazdani, R. and Rudin, D.: Inheritance of fluorescence esterase and -galactosidase in haploid and diploid tissues of *Pinus sylvestris* L. Hereditas **96**: 191—194 (1982). — Yazdani, R., Rudin, D., Aldén, T., Lindgren, D. Harbom, B. and Ljung, K.: Inheritance pattern of five monoterpenes in Scots pine (*Pinus sylvestris* L.). Hereditas **97**: 261—272 (1982).

Genetic Variation in the Time of Transition from Juvenile to Mature Wood in Loblolly Pine (Pinus taeda L.)

By J. A. Loo, C. G. Tauer and R. W. McNew

Graduate student, Department of Forestry
Associate Protessor, Department of Forestry,
and Professor, Department of Statistics,
Oklahoma State University,
Stillwater, Oklahoma 74078, USA

(Received 8th May 1984)

Abstract

Wood samples were collected from a 25-year old loblolly pine progeny test in east Texas. Specific gravity and tracheid length were determined for two-ring segments from the pith to ring 22. Values for each property were plotted against age to determine the age of transition from juvenile to mature wood. The mean ages of transition were 11.45 and 10.30 years for specific gravity and tracheid length respectively. There was no correlation between the age of transition for specific gravity and tracheid length. Narrow sense heritabilities estimated on a family mean basis for age of transition of each property were sufficiently high to suggest moderate gains are possible. Genetic correlations between the age of transition for each character and height and diameter of the trees at age 20 were negative, suggesting that fast growth may be related to early age of transition. Genetic and phenotypic correlations between age of transition and whole core specific gravity and tracheid length were negative.

Key words: specific gravity, tracheid length, juvenile wood, mature wood, loblolly pine, heritability, transition age.

Zusammenfassung

In einem 25 Jahre alten *Pinus taeda*-Nachkommenschaftstest in Ost-Texas wurden Holzproben entnommen und das spezifische Gewicht sowie die Tracheiden-Länge jeweils für 2 Ring-Segmente vom Kern bis zu Ring 22 bestimmt. Diese Werte wurden für jede Eigenschaft dem Alter nach aufgezeichnet, um den Übergang vom Holz der Jugendphase zu demjenigen der Altersphase zu bestimmen. Das Mittel des Überganges lag für das spezifische Gewicht

---

Journal article No. J4447 of the Okla. Agric. Exp. Stn., Oklahoma State University, Stillwater, OK 74078. Research supported in part by the McIntire-Stennis Forestry Research Program.