

# Variation in Resin Composition of the Italian Cypress (*Cupressus sempervirens* L.) Grown in Israel\*)

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## Summary

Analysis of branchlet resin composition was used to determine the intra and inter population variability of *Cupressus sempervirens* L. trees planted during the 19th and early 20th centuries in Israel.

Resin composition of *C. sempervirens* var. *horizontalis* differs significantly from that of cv. *stricta*, whereas the composition of trees with intermediate forms tend toward the composition of cv. *stricta*.

The occurrence of different chemotypes than these which are present in the old trees of cv. *stricta* growing in Israel and the occurrence of different chemotypes in var. *horizontalis* suggests that imported seed material from Jordan, Syria, Greece as well as other unidentified sources have been used to establish the plantations. Single plus tree selection is proposed to start a breeding program concerning the resistance to cypress canker diseases because there is evidence that different chemotypes show different response to the cypress canker (SCHILLER and MADAR, unpublished data).

*Key words:* *Cupressus sempervirens*, resin composition, cypress canker.

## Introduction

Although Israel is included within the geographical range of *Cupressus sempervirens* L. (ZOHARY, 1962, 1973), no natural occurrence of this species, which could have been used as a seed source, is known to have existed in this country during the last centuries; obviously all specimens have been cut long ago, probably due to their valuable wood properties (TISCHLER, 1981). Consequently, the seed source and/or the origin of trees that were planted in the last two centuries is most likely foreign.

With the exception of only a few 200- to 400-year-old trees of *C. sempervirens* L. cv. *stricta* (var. *stricta* AITON = var. *pyramidalis* NYMAN) growing at the Temple Mount and the Bahi garden near Acer, all plantings made since the last two decades of the 19th century are mixed: trees of cv. *stricta* and var. *horizontalis* (MILL.) GORDON are growing in association with intermediate forms. Cv. *stricta* trees show a compact crown composed of erect branches, whereas the var. *horizontalis* trees grow spreading branches like those of *Cedrus* sp. or *Picea* sp.. The intermediate forms have branches growing at different acute angles to the stem. Natural forests are usually composed of var. *horizontalis* with single specimens of cv. *stricta* = var. *pyramidalis*, a form which is believed to be a mutation or a recessive homozygote (BOLOTIN, 1964; PAVARI, 1934) occurring at a minute rate. Etruscans or Romans most likely selected the *pyramidalis* form for ornamental and short rotation plantings (MAKKONEN, 1986), and spread this variety (cv. *stricta*) all around the Mediterranean.

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The cypress canker *Coryneum* (*Seiridium*) *cardinale* WAG., which has spread over the Mediterranean region of western and southern Europe, has recently been observed in Israel too (SOLLEL *et al.*, 1981, 1983). In plantations, both affected and unaffected trees of all forms are present. A program was started to improve the planting stock, the resistance to the canker disease and the establishment of seed orchards. For this breeding program more information is needed to evaluate the genetic variability within and between populations planted before the 20th century and used later as seed source for reforestation, shelterbelts and ornamental plantings. Analysis of the resin composition is widely used in research into genetic variation of conifers (SQUILLACE, 1976) and was selected, therefore, as an investigational approach also in this study.

## Materials and Methods

Most of the ornamental and forest plantations planted between 1880 to 1930 growing in different regions of the country, were used in this study. Data on type and number of trees selected at each site are given in table 1, in most cases the populations were of a small number of trees. From all the trees in gardens and from an adequate number of trees in forests, which have no sign of cypress canker yet, 200 g of sprays (branchlets, ramilli) was collected at random around the crown of each tree separately, at a height of 6 m above ground, and stored at 4 °C. The following day, 100 g of the fresh vegetative growth was ground, of which 20 g was used for cold extraction of the resin with 40 ml of analytically pure Hexane. After 5 days the mixture was passed through a micronic sieve and the Hexane was partly evaporated by N<sub>2</sub>-gas flow and concentrated to a volume of 5 ml. of this solution 3-µl. was used in the actual analysis.

A Packard-7400 gas-liquid chromatograph fitted with an flame ionization detector and a glass column 4 m in length and 2 mm in diameter, packed with 8% PEG 20 M on Chromosorb W-60/80, was used.

Operating conditions were 250 °C at the injector, 250 °C at the detector, and 50 °C to 230 °C at the column, with the temperature increasing at a rate of 5 °C/min. Nitrogen was used as the carrier gas at a flow rate of 25 ml/min. Peak areas as component percentages of the sum total of all peaks were calculated with a 3390A Hewlett-Packard integrator.

Principal component, discriminant and cluster analyses procedure, using the SAS package (SAS, 1985), were then conducted on data that were first transformed using the arcsin function (SHAW *et al.*, 1982).

## Results and Discussion

Between 50 °C and 230 °C, 35 peaks, *i.e.*, resin components, were visible on the chromatograph (Figure 1) of

Table 1. — *Cupressus sempervirens* L. populations and number of trees sampled of each type.

Population	Type				Total number of trees
	1	2	3	4	
Haifa (former German colony)	6	1	-	12	19
Tel Aviv (former German colony)	8	21	5	1	35
Wilhelma (former German colony)	6	14	3	3	26
Jerusalem (former German colony)	15	5	5	-	25
Waldheim A (former German colony)	13	3	-	-	16
Waldheim B (former German colony)	9	2	5	-	16
Bab al Wad (British Mandet For Dep.)	19	17	-	-	36
Wadi al Kuf (British Mandet For Dep.)	-	-	-	31	31
Qiryat Anavim (J.N.F. For Dep.)	17	4	3	-	24
Ben Shemen (J.N.F. For Dep.)	13	13	3	-	29
Mishmar ha'Eneq (J.N.F. For Dep.)	4	20	3	-	27
Nazareth A (Italian Monastery)	15	3	3	-	21
Nazareth B	25	-	3	-	28
Lod (British built railway sta.)	1	3	-	-	4
Acre (Old city)	5	-	2	-	7
Bahai Garden (by Acre)	6	-	-	-	6
Jericho (former British Agric. Res. Sta.)	5	3	3	-	11
Jericho (Jordanian Agric. Farm)	4	13	-	-	17
Miqwe Yisra'el (Jewish Agric. School)	-	-	-	9	9
Haram al Sherif (Temple Mount)	7	-	-	-	7
Kefar Tavor (Jewish settlement)	9	-	-	-	9
St. Catherina Monastery, Sinai	27	-	-	-	27
Kefar Gil'adi (Jewish settlement)	6	2	-	-	8
Mt. Scopus botanical garden	-	14	-	-	14
Rosh Pinna (Jewish settlement)	20	1	8	-	29
Mt. Tabor (Greek Orthodox Monastery)	11	-	1	-	12
Total number of trees sampled	251	139	47	56	493

Type 1. *cv. stricta*

Type 3. Intermediate form

Type 2. Var. *horizontalis*

Type 4. Trees with no form description

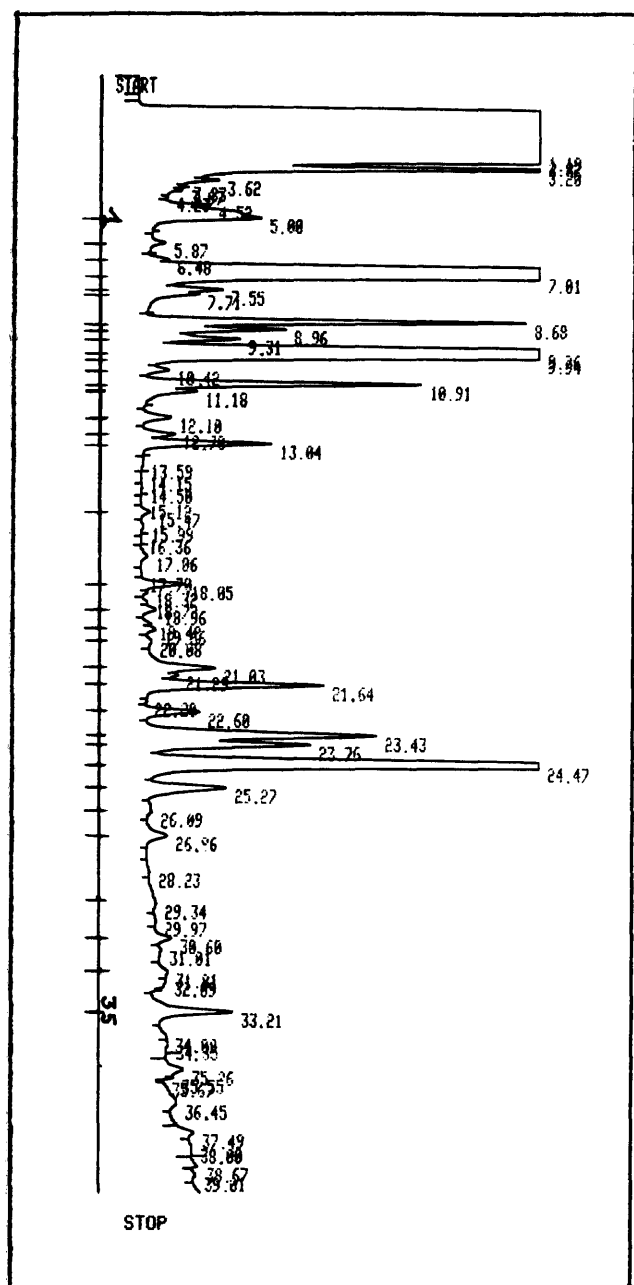
them only few were identified by comparison with pure standards. Resin composition of *C. sempervirens* L., probably of a few *cv. stricta* trees, was analysed by PIOVETTI *et al.* (1981) and PAULI *et al.* (1983). However, these researchers did not distinguish between *cv. stricta* and var. *horizontalis* in their investigations, and did not take into account the possible differences between provenances in their resin composition, as was shown to exist in American cypress species (ZAVARIN *et al.*, 1971). Twenty nine out of the 35 resin components ( $x_1-x_{29}$ ) were selected for the statistical analysis; of these, 16 components of very small variation were grouped together (=  $X_{29}$ ).

Principal components analysis was used for a further reduction of the data, the first five principal components, given in table 2, accounted for 67% of the variance. To evaluate differences in resin composition between populations and tree types, *i.e.*, *cv. stricta*, var. *horizontalis* and intermediate forms, analysis of variance and DUNCAN'S Multiple Range Tests were carried out on these five principal components. Significant differences were found in the resin composition between populations and between tree types as given in table 3. Of the five factors used, factor three which is heavily loaded by resin components  $X_1$ ,  $X_8$ ,  $X_{26}$  (Table 2) was the best discriminator between the tree types; whereas factors 2, 4 and 5 discriminate only partly between the types and factor 1 not at all (Table 3); factors 2, 3 and 1 discriminate best between the populations (Table 4). The resin composition of the trees with intermediate form tends toward the resin composition of *cv. stricta* trees and is not an intermediate composition between the two main forms. Stepwise discriminant analysis indicated that the relative amounts of the resin components  $X_1$ ,  $X_4$ ,  $X_5$ ,  $X_{13}$ ,  $X_{17}$ , and  $X_{26}$  are the

best discriminators between tree types (Table 5).

Ward's minimum variance cluster analysis (FASTCLUS procedure, SAS, 1985) on the individual trees, was used to track down possible relations within tree types between populations. Figures 2 and 3 show the clusters of *cv. stricta* and var. *horizontalis*, respectively. Seven clusters groups were decided upon, using the Eigenvalue of the covariance Matrix. The number of trees in the population examined belonging to each of the groups, *i.e.*, chemotypes (genotypes), is given in the tables below the figures. Figure 2 and the table below it show that in most populations more than one chemotype is present. In three groups, trees of an old population growing in Israel, which could have been used as a seed source, are included: group 2 with the Bahai Garden trees near Acre; groups 6 and 7 can be identified with trees of Haram al Sherif (Temple Mount, El Aqza Mosque) in Jerusalem. Group 5 includes trees of the St. Caterina's Monastery (Sinai) population. The other groups (1 and 3) could not be related. Figure 3 and the table below it show that also within most of the var. *horizontalis* populations, more than one chemotype is present. It can be seen that the population on Mt. Scopus, believed to originate from Syria includes only two chemotypes. Of interest is the fact that the Haram al Sherif, Bahai Garden, St. Catherina, Kefar Gil'adi and Mt. Scopus populations, which are very old and/or of known origin, have a small number of chemotypes. This may point to the use of single tree progenys or to a very low genetic variability within the populations.

Because the seed source and date of planting of the different populations is unknown, and dating by ring analysis, for the knowledge of the planting year, is misleading (LIPHSCHITZ *et al.*, 1981; LEV-YADUN, 1986), canonical



discriminant analysis was used to reveal possible relations between populations. Table 6 shows the first four canonical coefficients, their Eigenvalues and cumulative proportions. An average of the canonical components was calculated for every population, Table 7 summarizes the grouping of the populations, Sixteen populations out of 28 could be combined into five groups. The first group includes populations, (forests) planted by the Forest Department of the Jewish National Fund, suggesting that the same seed sources were used to establish these populations, including the Haram al Sherif population, which might have been the main seed source of the other populations. The third group is composed of the populations from northern Israel, among them the old trees of the Bahai Garden near Acre, which could also have been used as a seed source. The fourth group consists of three populations from the centre of the country — two Templar settlements (19th Century German colonies in Palestine) and the third a forest planted by the Forest Department of the British Government. The fifth group includes only two populations: Rosh Pinna and Mt. Tabor (Greek Orthodox Monastery). The sixth group, a non-group, consists of populations which differ significantly in their resin composition and are therefore not related; this may be an indication that they have originated from very different seed sources. Three of these populations are known to originate from very different geographical regions: the Kefar Gil'adi cv. *stricta* population is known to be from France (N. HOROWITZ, personal communication); the Mt. Scopus population is known to have been established by the late Prof. M. ZOHARY after a visit to Syria; the population from the Jordanien farm near Jericho was established with seedlings said to be of Jordanian origin; and the St. Catherian's population is said to originate from Mt. Athos, Greece.

If it is true that the trees of cv. *stricta* at the Italian Monastery in Nazareth are the progeny of the Haram al sherif population, or that the Mishmar ha'Emeq population is related to the Ben Shemen and Qiryat Anavim

Figure 1. — An integrator chromatogram of *Cupressus Semper-virens* L. branchlet resin composition. Peak No: 4 =  $\alpha$ -pinene; 7 =  $\beta$ -pinene; 10 = 3-carene; 11 = myrcene; 12 =  $\alpha$ -terpinene; 13 = limonene; 14 =  $\beta$ -phellandrene; 15 =  $\gamma$ -terpinene; 24 = caryophyllene.

Table 2. — Principal Components Analysis.

Components	Principal component factors				
	1	2	3	4	5
X <sub>1</sub>	-0.309	0.529	-0.482	-0.072	0.159
X <sub>4</sub>	-0.642	0.327	-0.082	-0.014	-0.100
X <sub>5</sub>	0.599	0.184	-0.387	-0.111	0.021
X <sub>7</sub>	-0.622	0.487	0.059	-0.163	-0.026
X <sub>8</sub>	0.359	-0.099	0.467	-0.618	0.178
X <sub>10</sub>	0.485	-0.490	-0.265	-0.114	0.043
X <sub>13</sub>	0.461	0.324	0.342	-0.244	0.205
X <sub>15</sub>	0.509	-0.596	-0.090	0.031	-0.370
X <sub>17</sub>	0.698	-0.376	-0.329	0.116	-0.061
X <sub>19</sub>	0.534	-0.130	-0.204	0.198	0.640
X <sub>23</sub>	0.623	0.326	0.299	0.087	-0.346
X <sub>24</sub>	0.532	0.388	0.318	0.361	-0.096
X <sub>26</sub>	-0.153	-0.331	0.575	0.480	0.262
X <sub>29</sub>	0.460	0.560	-0.188	0.328	0.016
Eigenvalue	3.774	2.211	1.515	1.030	0.862
Cumulative	0.269	0.427	0.535	0.609	0.671

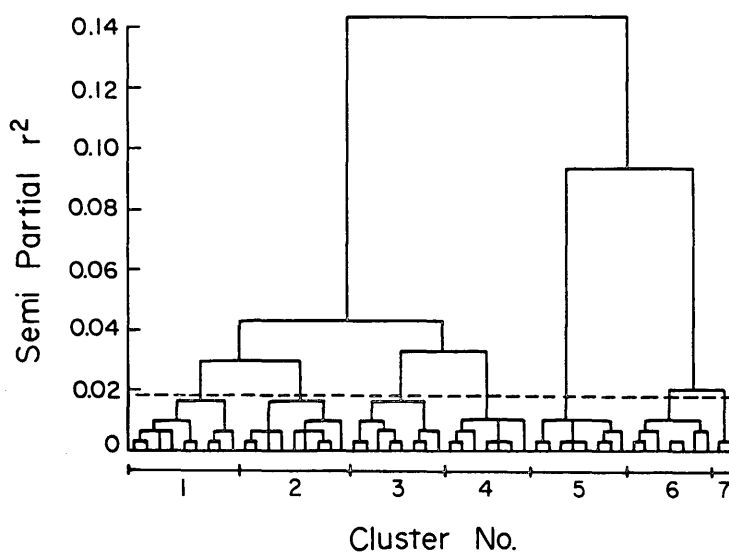
Table 3. — Averages of Principal components Factors for different tree types and Analysis of Variance (F test) imposed on Factors 1 to 5 (Table 2) to discriminate among the tree types. Means not sharing the same letter are significantly different at P = 0.05 by DUNCAN'S Multiple range test.

Tree types	Number of trees	Factor no.				
		1	2	3	4	5
No description	56	0.113 A	-0.288 C	0.838 A	-0.334 B	-0.164 B
cv. <i>stricta</i>	251	-0.066 A	0.102 AB	-0.268 C	-0.064 AB	-0.088 AB
Var. <i>horizontalis</i>	139	0.031 A	-0.150 BC	0.162 B	0.243 A	0.194 A
Intermediate	47	-0.045 A	0.286 A	-0.159 C	-0.036 AB	0.040 AB
Total	493					
F		0.99	5.03	26.61	5.74	3.20
PR > F		0.395	0.0018	0.0001	0.0007	0.0232

population, then we must conclude that there is a strong interaction between the genotype and the site factors. The Mishmar ha'Emeq population is growing on very deep and moist brown rendzina where as the Ben Shemen and Qiriat Anavim populations grow on much less favorable site conditions, the phenotypic expression within cv.

*stricta* or var. *horizontalis* is strongly affected by ecological factors (HOFMANN, 1943; MAYER, 1983).

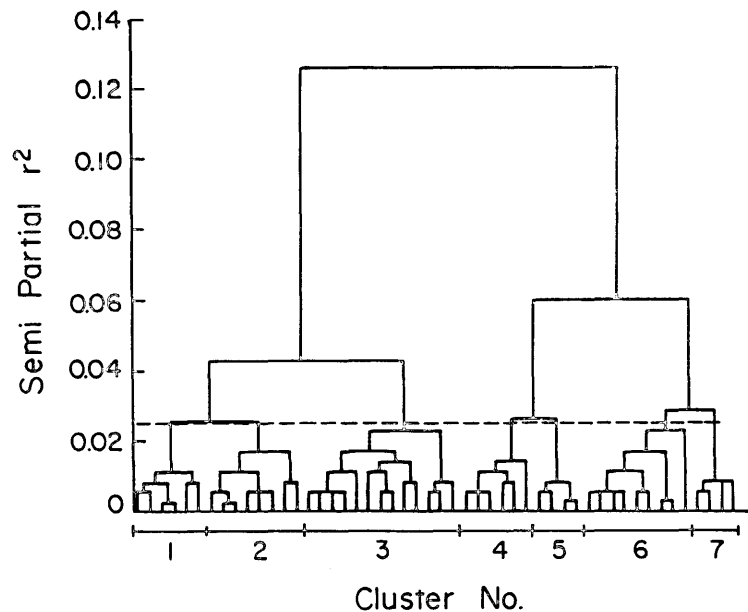
The occurrence of more chemotypes within the cv. *stricta* planted populations than within the few old trees growing at holy places in Israel (Figure 3; cluster 2, 6, 7), the relatively numerous chemotypes in var. *horizontalis* (Fig-



Number of trees of each population in the cluster groups

Population	Total number of trees	Cluster No.						
		1	2	3	4	5	6	7
Haifa	1	-	-	-	1	-	-	-
Tel Aviv	22	1	3	11	-	-	-	-
Wilhelma	14	1	3	2	-	-	8	-
Jerusalem	5	-	-	5	-	-	-	-
Waldheim A	3	-	-	3	-	-	-	-
Waldheim B	2	-	-	2	-	-	-	-
Bab al Wad	17	-	3	10	1	1	2	-
Qiryat Anavim	4	-	3	1	-	-	-	-
Ben Shemen	13	-	3	8	-	1	1	-
Mishmar ha'Emeq	20	-	-	16	2	1	1	-
Nazareth A	3	-	-	-	1	2	-	-
Lod	3	-	-	-	-	1	2	-
Jericho (Agric. Res.)	3	-	1	-	-	-	1	1
Jericho (Jord. Farm)	13	-	-	6	-	-	-	7
Kefar Gil'adi	2	-	2	-	-	-	-	-
Mt. Scopus	14	14	-	-	-	-	-	-

Figure 2. — Ward's minimum variance cluster analysis (by Fastclus procedure) of var. *horizontalis*.



Number of trees of each population in the cluster groups

Population	Total number of trees	Cluster No.						
		1	2	3	4	5	6	7
Haifa	6	-	2	1	-	-	2	1
Tel Aviv	8	2	-	5	-	1	-	-
Wilhelma	6	1	-	3	1	1	-	-
Jerusalem	15	-	6	8	7	-	1	-
Waldheim A	13	-	-	-	-	-	8	5
Waldheim B	9	-	-	-	-	-	6	3
Bab al Wad	19	1	5	10	3	-	-	-
Qiryat Anavim	17	1	6	9	-	1	-	-
Ben Shemen	13	-	6	3	-	-	3	1
Mishmar ha'Emeq	4	1	2	-	-	-	1	-
Nazareth A	15	-	-	-	-	-	15	2
Nazareth B	25	13	6	3	2	-	1	-
Acre	5	-	3	1	-	-	-	1
Bahai Garden	6	-	5	-	-	-	-	1
Jericho (Agric. Res.)	5	1	-	-	4	-	-	-
Jericho (Jord. Farm)	4	3	-	1	-	-	-	-
Haram al Sherif	7	-	-	-	-	-	5	2
Kefar Tavor	9	-	-	2	-	3	2	-
St. Catherina Monast.	27	-	-	-	-	27	-	-
Kefar Gil'adi	6	-	-	-	6	-	-	-
Mt. Tabor	11	1	5	4	1	-	1	-
Rosh Pinna	20	2	6	6	4	-	1	-

Figure 3. — Ward's minimum variance cluster analysis (by Fastclus procedure) of *cv. stricta*.

Table 4. — Summary table of Stepwise Discriminant Analysis of Principal Components Factors to best discriminate between populations.

Step	Variable	Partial $r^2$	F	PR > F
1	Factor 2	0.560	13.07	0.0001
2	Factor 3	0.434	13.86	0.0001
3	Factor 1	0.404	12.26	0.0001
4	Factor 4	0.262	6.40	0.0001
5	Factor 5	0.227	5.16	0.0001

ure 2), and the occurrence of intermediate forms and chemotypes, imply the use, at least partly, of imported seed from different seed sources to establish these popu-

lations which subsequently served as a seed source for further plantings. Within *cv. stricta* populations (Figure 3), trees of French and Greek origin (clusters 4 and 5,

Table 5. — Stepwise Discriminant Analysis between components for best identification of the tree types (D = 3, 490).

Step	Variable entered	Partial r <sup>2</sup>	F	PR > F
1	X <sub>1</sub>	0.1353	26.811	0.0001
2	X <sub>26</sub>	0.0430	7.689	0.0001
3	X <sub>17</sub>	0.0409	7.278	0.0001
4	X <sub>13</sub>	0.0319	5.609	0.0010
5	X <sub>4</sub>	0.0204	3.538	0.0146
6	X <sub>5</sub>	0.0221	3.837	0.0099
7	X <sub>24</sub>	0.0146	2.515	0.0566
8	X <sub>23</sub>	0.0181	3.116	0.0256
9	X <sub>10</sub>	0.0139	2.384	0.0673
10	X <sub>8</sub>	0.0152	2.604	0.0504

Table 6. — The standardized canonical coefficients.

Components	Can. 1	Can. 2	Can. 3	Can. 4
X <sub>1</sub>	1.4839	-0.3551	-0.1804	0.4268
X <sub>4</sub>	-0.0664	-0.2096	0.1506	-0.1082
X <sub>5</sub>	0.2039	-0.2914	0.0786	-0.0629
X <sub>7</sub>	0.1199	0.3459	0.1548	0.0304
X <sub>8</sub>	0.1078	0.1095	-0.7725	0.3363
X <sub>10</sub>	0.0436	0.1970	-0.3231	0.2924
X <sub>13</sub>	0.1564	-0.0286	-0.3723	0.5649
X <sub>15</sub>	-0.2522	-0.0217	0.0755	0.1350
X <sub>17</sub>	-0.3636	-0.3900	0.7395	0.6041
X <sub>19</sub>	-0.0925	-0.0090	-0.1190	0.0869
X <sub>23</sub>	0.0711	0.7194	0.0350	-0.2396
X <sub>24</sub>	0.1259	0.1349	-0.1053	-0.1451
X <sub>26</sub>	-0.0187	0.0656	-0.3791	-0.2163
X <sub>29</sub>	0.2660	0.6323	0.4293	-0.0915
Adjusted canonical correlation	0.828	0.707	0.673	0.522
Eigenvalue	2.435	1.176	0.932	0.468
Cumulative proportion	0.398	0.591	0.743	0.820

Table 7. — Grouping of populations by canonical discriminant analysis.

Group No.	Populations
1	Qiryat Anavim, Ben Shemen, Mishmar ha'Emeq
2	Haram al Sherif, Waldheim A, Waldheim B, Nazareth (Italian Monastery)
3	Bahai Garden (near Acre), Nazareth, Kefar Tavor
4	Tel Aviv, Wilhelma, Bab al Wad.
5	Mt. Tabor (Greec Orthodox Monastery), Rosh Pinna.
6	<u>Populations which could not be grouped:</u> Jerusalem, Wadi al Kuf, Acre (Old city), Jericho (British Agric. Res. Sta.), Jericho (Jordanian farm), Miqwe Yisra'el, St. Catherina Monastery, Kefar Gil'adi, Mt. Scopus (Botanical garden), Lod.

respectively) are present together with trees of unknown origin (clusters 1 and 3), whereas within var. *horizontalis* populations (Figure 2) trees of Syrian and Jordanian origin (clusters 1; 3 and 7, respectively) are present

together with trees of unknown provenances (clusters 2, 4, 5, 6).

In conclusion, past use of imported seed was shown from the occurrence and distribution of chemotypes which

do not occur in the old tree population growing in Israel. Unfortunately, most chemotypes could not be related to specific seed source.

Thus, the establishment of seed orchards must start with a test for genetic stability of the chemotypes and with the selection of single plus trees of var. *horizontalis*, cv. *stricta* and intermediate forms, which also display resistance to the cypress canker. So far, no resistant seed source (population) was revealed in our study or in research abroad (Grasso and Raddi, 1979).

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## Geographic Variation of Green Ash in the Western Gulf Region<sup>1)</sup>

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#### Abstract

At age 12, four green ash plantations containing 27 open-pollinated families from east Texas, southeast Louisiana, and southwest Arkansas were evaluated. Significant differences were indicated for height and specific gravity among the provenances; provenances from the western edge of the species' range had the slowest growth. Significant family within provenance differences existed for all traits except survival.

Family heritabilities for height, diameter and volume were moderate ( $h^2 = 0.56, 0.56$  and  $0.57$ , respectively); family heritability for specific gravity was high ( $h^2 = 0.89$ ). Expected genetic gains for these traits from the combined analysis were 5.9%, 9.0%, 7.0% and 10.8%, respectively. Coefficients of genetic prediction between height, diameter and volume were nearly as large as family heritabilities; however, a weak negative relationship existed between diameter or volume and specific gravity.

Regressions of yield deviations on seed source latitude and longitude accurately predicted the performance of

sampled provenances. However, the uneven provenance distribution made interpolation of performance to other provenances questionable. The regression of yield deviations on seed source and plantation latitude or longitude were also able to locate the optimum latitudes and longitudes for seed collection.

*Key words:* *Fraxinus pennsylvanica*, heritability, genetic gain.

#### Introduction

Green ash (*Fraxinus pennsylvanica* MARSH.) possesses suitable silvical characteristics for both plantation management and genetic improvement (KELLISON, 1971). The high specific gravity and low moisture content of green ash wood enable it to produce a higher fiber yield per unit volume than sweetgum (*Liquidambar styraciflua* L.), sycamore (*Platanus occidentalis* L.) or cottonwood (*Populus deltoides* BARTR.). This partially compensates for the slower growth rate of green ash relative to these faster growing species (JETT and ZOBEL, 1974). Additionally, green ash is better suited to imperfectly drained soils and will thrive on sites unsuited for sycamore and cottonwood (KELLISON *et al.*, 1979).

The majority of geographic variation studies on green ash have been established in New England and the Plains states where the species has shown excellent survival and growth in windbreaks (READ, 1958). These studies indicated significant geographic differences for growth, phenologi-

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