

FLAVONOID DISTRIBUTION IN ZELKOVA

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Abstract: Each of the five species of *Zelkova* tested could be distinguished by the qualitative or quantitative distribution of flavonol glycosides. Thus, the verification of interspecific hybrids might be achieved by flavonoid analyses. Such analyses indicated that *Z. X verschaffeltii* is actually a rare hybrid between *Z. carpinifolia* and *Z. serrata*.

The genus *Zelkova* belongs to the elm family (Ulmaceae) and consists of 5 to 7 species in western and eastern Asia and on the island of Crete.

In recent years, the Japanese keaki (*Z. serrata* (Thunberg) Makino) has been widely planted as a landscape tree in the United States, largely as a supposed "replacement" for the American elm (*Ulmus americana*). Two vegetatively propagated cultivars, 'Village Green' and 'Parkview', have been introduced into the American nursery trade and seedling material has even been marketed under the confusing name "Sheerlund elm" (Amer. Forest 81(2): 15, 1975).

Zelkova carpinifolia (Pallas) K. Koch is native to the Transcaucasus and neighboring Iran. It has been widely used in Great Britain, but is rare in cultivation in the United States. The Cretan zelkova is *Z. abelicea* (Lamarck) Boissier (= *Z. cretica* Spach) and although reputed to be a small tree, some specimens may reach a height of 49 feet (8).

There are two Chinese species, *Z. sinica* Schneider and *Z. schneideriana* Handel-Mazzetti. Both are rare in cultivation in the United States and although *Z. sinica* has been recommended for greater use (5, 7), it is inferior in most respects to *Z. carpinifolia* and *Z. serrata*, because of its irregular branching habit. On the other hand, *Z. schneideriana* may be worthy of further trial. As far as we know, the only mature tree of this species in the United States is located at the U.S. Plant Introduction Station in Glenn Dale, Maryland. It was originally regarded as a *Z. serrata*, but may be easily distinguished from that species by the soft pubescence on the underside of the leaf. This

particular specimen is remarkable for its russet-red autumn leaf color which generally lasts from September 1 to early November.

There is one more species of dubious origin and identity: *Z. verschaffeltii* (Dippel) Nicholson. Although it has been in cultivation at the Royal Botanic Gardens, Kew, England since 1886 (2), Dippel was the first to classify this small tree as a zelkova in 1892. He considered it to be a variety of the Japanese species. Henry (6) suggested that the tree was a hybrid between *Z. crenata* (*Z. carpinifolia*) and *Z. cretica* (*Z. abelicea*). No wild specimens of *Z. verschaffeltii* have been observed.

Trees of *Z. carpinifolia* have been killed by Dutch elm disease in Iran (1), but no published reports on the disease susceptibility of the other species have been found. We inoculated 3 trees of diverse origins of *Z. serrata* with mixtures of aggressive strains of the Dutch elm disease fungus in 1976 and 1978, and consider the species to be highly resistant.

We were interested in developing flavonoid profiles for the various species so that we could use these chemical criteria to establish hybridity in the various progenies resulting from our planned hybridizations in the genus.

The flavonoid analyses of Giannasi (3) on hydrolyzed dried leaves were of limited value since he did not identify the glycosides present in the individual species.

Materials and Methods

Our chemical analyses were restricted to living trees in the Washington, D.C. area that we intended to use in our hybridization program. Only single trees were available in *Z. abelicea*, *Z. schneideriana*, *Z. sinica*, and *Z. X verschaffeltii*. Two trees of *Z. carpinifolia* and several trees of *Z. serrata* were also utilized.

Fresh leaves collected during the middle of the growing season were extracted by boiling for 1 hour in 70% ethanol, concentrated to about 1 ml.

of extract for each gram of leaf tissue. Preliminary chromatograms were prepared by subjecting the raw extracts to 2-dimensional ascending paper chromatography in BAW (*n*-butanol-glacial acetic acid-water, 4:1:5 (V/V) upper phase) and water. These chromatograms, after being fumed with ammonia and viewed under ultra-violet light, gave an indication of the number and behavior of the compounds likely to be found in the extract. The flavonoids were purified and isolated by repeated banding and elution of 1-dimensional runs in BAW, water, and 15% acetic acid, in whatever sequence was most appropriate for complete separation from other compounds. Purified compounds were chromatographed against certain known compounds in all 3 solvents, and occasionally in TBA (tertiary butanol-glacial acetic acid-water (3:1:1) upper phase).

The pure compounds were hydrolyzed, to remove sugars, by boiling in 2N HCL for 1 hour, and the hydrolyzed product chromatographed in Forestal solvent (acetic acid-HCL-water, 30:3:10) with quercetin control. Sugars were analyzed by ascending paper chromatography in iso-propanol-water (4:1) with glucose control. Ultra-violet absorption spectra in 95% ethanol were determined before and after hydrolysis. Spectral shifts with a variety of additives (sodium

acetate, boric acid, aluminum chloride) were recorded. The methods of analyses and standards for comparison are included in Harborne (4).

Flavonoid compounds found in the various species are given in Table 1. Each species appeared to have a unique qualitative and quantitative flavonoid distribution pattern that would allow verification of hybridity in young seedling material. Unfortunately, we were never able to attempt these tests. Our controlled pollinations of *Z. serrata* X *Z. carpinifolia* and *Z. schneideriana* X *Z. serrata* failed to produce a single viable seed.

Our analyses did, however, shed some light on the possible origin of *Z. X verschaffeltii*. *Zelkova serrata* was unique among good species in producing the flavonol quercetin 7-glucoside. An unidentified UV-absorbing spot (R_f 0.72 BAW, 0.43 water, 0.64 in 15% acetic acid) was only found in *Z. carpinifolia*. The chromatographic profile of *Z. X verschaffeltii* was virtually a composite of these 2 species and contained both of the assumed species-specific compounds. Thus the chromatographic evidence would suggest that *Z. X verschaffeltii* is a hybrid between *Z. carpinifolia* and *Z. serrata*.

Z. serrata was first introduced into England in 1861, and *Z. verschaffeltii* has been cultivated since 1886. (2). It may well have been that some

Table 1. Distribution of flavonoids in *Zelkova* species; (+) indicates significant amounts and (tr) indicates trace amounts.

Species	Myrcetin				Quercetin				Kaempferol Dark Spot	
	3-rham-3-gluc-noside	3-rutin-3-glucoside	3-glucor-3-glucor-onide	3-gluc-3-glucor-onide	3-rutin-3-glucor-onide	3-glucor-7-glucoside	7-gluc-3-glucoside	3-glucoside		
<i>abelicea</i>	+	tr	tr	+	0	tr	tr	0	0	0
<i>schneideriana</i>	0	+	0	+	+	0	tr	0	tr	0
<i>sinica</i>	+	tr	0	+	tr	0	+	0	0	0
<i>carpinifolia</i>	0	+	0	0	+	0	0	0	+	+
<i>serrata</i>	0	0	0	0	0	+	0	+	0	0
<i>X verschaffeltii</i>	0	+	0	0	+	tr	0	+	+	+

of the *Z. serrata* trees reached sexual maturity before 1886 and crossed with *Z. carpinifolia* already in cultivation. The fact that *Z. X vershafeltii* is really a clone would indicate that such a cross might be a rare event, and our experience would tend to substantiate this hypothesis.

Literature Cited

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ABSTRACTS

FERGUSON, J.J. and J.A MENGE. 1982. **How and why to add mycorrhizal fungi to plants in the field.** Am. Nurseryman 156(6): 67-71.

Soil fumigation is required in some states for producing nursery crops. Methyl bromide, a commonly used fumigant, destroys many soil-borne pests, but concentrations used in most field applications can also destroy indigenous mycorrhizal fungi. Nursery trees that are grown in low-nutrient soil following such fumigation are often stunted. Although many methods have been used to inoculate plants with these mycorrhizal fungi in field and greenhouse trials, few inoculation methods are acceptable for large-scale commercial application. The purposes of this research were (1) to determine the efficacy of various vesicular-arbuscular mycorrhizal formulations and field application methods for both directly seeded and transplanted citrus in fumigated nursery soils, and (2) to evaluate mechanical field inoculation methods.

SCHUDER, D.L. 1982. **Insect resistance in woody ornamentals.** Plants and the Landscape 5(3): 59-61.

The recognition and utilization of insect resistance in woody ornamentals is still in its infancy, but the potential for its use is unlimited. Resistance in plants may be defined as any factor or group of factors which deter insect attack. The sources of resistance to insects have been classified as (a) non-preference resulting from lack of one or more preferred factors in the host plant, (b) antibiosis or the adverse effect of the plant on the insect, and (c) tolerance of the plant to insect damage. The latter may be the ability to withstand the insect attack or to repair tissues and recover from attack. Selection for insect resistance has been gaining renewed emphasis since biochemical studies have shown that chemical factors in plants are responsible for the feeding habits of insects.