Vernicia fordii ‘Spiers’, a New Tung Tree for Commercial Tung Oil Production in the Gulf Coast Region

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Tung trees (*Vernicia fordii* Hemsl.) are native to China and were grown in the U.S. Gulf Coast region, mostly U.S. Department of Agriculture (USDA) cold hardiness zones 8 and 9, for tung oil production from 1937 to 1969 (Robb and Travis, 2013). Tung oil production ranged from northern Florida to Texas during the tung oil industry boom. Pearl River County in southern Mississippi, where the USDA—Agricultural Research Service (ARS) Thad Cochran Southern Horticultural Laboratory (TCSHL) is located, had more than 60,000 acres of tung trees in production at one time. Industrial tung oil use in the United States continues today, but raw oil is imported, mainly from South American orchards and China. There have been several attempts to revive tung tree farming in the Gulf Coast region, but yearly production is substantially affected by weather, especially, late frosts that reduce yields and high winds that damage orchards. Replacement of tung trees takes up to 5 years before new trees reach peak production. At the current time, the most prominent use of tung trees is for landscape purposes including the recent release of ‘Anna Bella’, a nutless tung tree, from the TCSHL germplasm collection (Rinehart et al., 2013).

Recent studies on the unique qualities of tung oil have revived interest in domestic tung oil production as a specialty crop. Tung oil contains high levels of eleostearic acid, a novel conjugated trienoic fatty acid that contains highly desirable chemical properties for the production of industrial products including paints, varnishes, and wood finishing products. There is also interest in domestic tung oil production as a biofuels additive (Shang et al., 2012). Companies seeking to reduce reliance on foreign imports and label products as “Made in America” are also interested in domestic production of tung oil. To that end, researchers at TCSHL evaluated the germplasm collection for potential cultivar release(s) for commercial tung oil production in collaboration with small business partners. An elite selection from the 1950s designated L266 was identified, evaluated, and released to the public.

Cultivation from orchard plantings to tung nut harvesting by field labor or mechanical methods has been extensively researched and recommended practices are still effective today (Potter and Crane, 1957). The most significant risk to tung tree cultivation is damage to the bloom from late frosts in the spring. Here we are publishing the release of *Vernicia fordii* ‘Spiers’, a late-flowering tung tree for commercial tung oil production in the Gulf Coast region.

Origin and Description

L266, a seedling of L92, was produced by open pollination in 1941 by the USDA-ARS tung tree improvement research program. It

was planted in Folsom, LA, and selected in 1954 because of its late-flowering time and superior production values. Yield was calculated from actual measures of nuts per tree in 1954, 1956, and 1957, and again in 1964 and 1968. Percent oil (w/f) was also calculated over the same time spans. Additional evaluations were published by Spiers and Kilby in 1973 from replicated plantings that were 12 and 14 years old. Yields were compared with ‘Isabel’, a popular cultivar in production at the time. Results demonstrate that L266 oil production (calculated as a percentage of whole fruit and as pounds per tree or acre) was comparable or superior to commercial cultivars and other late-flowering selections (Spiers and Kilby, 1973). Hundreds of tung trees were under evaluation from 1939 through the 1960s. Only a small fraction of these breeding lines are maintained by TCSHL in the germplasm collection (Table 1). Detailed breeding notes suggest that L266 was an elite selection that was used to develop at least 11 different superior lines that were not preserved after the breeding program was discontinued in 1970.

L266 is similar to all other genotypes in the collection using simple sequence repeat (SSR) markers developed by Xu et al. (2012). Of the 22 SSR loci developed from *Vernicia montana* ESTs, eight demonstrated polymorphism among *V. fordii* samples, but only between collection sites (Xu et al., 2012). *Vernicia fordii* samples collected from the same geographic region produced identical DNA fingerprints using these SSR markers, presumably because the loci that are cross-amplifying between species represent conserved DNA that accumulate repeat length mutations more slowly than other nonconserved regions. Therefore, these SSR loci are not useful for identifying cultivars or determining parentage, but could be used to broadly estimate the diversity of the genotypes in the collection in relation to the geographic range of the native populations in China. Our results indicate that the TCSHL collection (Table 1) likely represents a single geographic region, or narrow gene pool, since all of the samples had identical DNA fingerprints (data not shown). The single exception is the semisterile ornamental tung tree ‘Anna Bella’, which was polymorphic at five of the eight loci. This either indicates it is from a different genetic location, or more likely, that it is a hybrid between *V. montana* and *V. fordii*, which would explain its reduced fertility.

Original trees, including L266 that were selected in the mid-1900s, were clonally propagated by bud grafting to evaluate production traits. However, during preservation of the tung germplasm collection after 1970, some selections were propagated by seed and may not reflect the original genotypes. ‘Spiers’ represents an incremental improvement in delayed flowering; however, it may be possible to make salutatory improvements in flowering and other horticulturally important traits by using diverse germplasm in future breeding. Unfortunately, diverse

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genetic material is not found in the remaining tung tree collection at Poplarville, MS.

Outstanding Characteristics and Uses

Flowering time for L266 was estimated to be 2 weeks later than commercial cultivars (Spiers and Kilby, 1973). Bloom data from replicated plantings in Folsom, LA, that were evaluated from 1954 to 1964 in the month of March support these results. Recent observations suggest that the range of bloom times in the germplasm collection is only 16 d long (Fig. 1). On average, L266 blooms on day 11, which is not the latest flowering selection in our collection, but similar or later than previous USDA cultivars such as ‘Folsom’, ‘Isabel’, and ‘Gahl’. Because of the narrow range in bloom time, we are releasing L266 for clonal propagation by bud grafting instead of

a seed propagated line. While 80% to 95% of the seed from open-pollinated mother plants are expected to perform true to type, the additional genetic variability incurred during seed propagation may affect late flowering performance of L266. Clonal propagation will ensure that late flowering and oil production traits will be fully expressed.

Bumble bee (*Bombus impatiens*) mediated seed production of L266 was used for the oil analyses reported here (Fig. 2). The results of the analysis of lipid content and composition in ‘Isabel’ (L2) and L266 are shown in Table 2. The fatty acid compositions of total extractable seed lipids, dehulled lipid meat, and extracted oils were determined for both cultivars. The composition of the oil samples closely reflected that seen in the other samples; therefore, only the oil fatty acid profiles in extracted oil are shown in Table 2. On the basis of the average of

two replicates, the total oil content of L266 was slightly lower than that in L2, but the difference was not statistically significant (unpaired *t* test, *P* = 0.0628). However, there were some interesting differences in fatty acid composition observed between the two cultivars. Oleic acid was nearly twice as high in L2 as in L266 (9.2% vs. 5.5%) and linoleic acid was also significantly increased in L2 (8.1% vs. 6.0%). Most importantly, α-eleostearic acid (ESA) content is significantly higher in L266 compared with L2 (81.4% vs. 75.0%). The explanation for the increase in ESA in L266 oil is not yet known, but could either reflect higher levels of expression of *FADX*, the gene that converts linoleic acid to ESA in developing tung seeds (Dyer et al., 2002), or other enzymes downstream of *FADX* in the oil biosynthetic pathway, such as glycerol-3-phosphate acyltransferases (Gidda et al., 2011) or diacylglycerol acyltransferases (Shockey et al., 2006). Regardless of the explanation, this finding, if consistently replicated under field conditions, is significant: ESA is the unique component that provides the chemical utility (and therefore, the value) to tung oil. An increase of 6% ESA content, combined with the late-flowering trait present in L266, is likely to produce larger average yields of seeds that contain higher levels of ESA, thus easily overcoming the very small difference in total oil yield observed here.

Lipids were extracted from cracked, dehulled mature seed samples from cultivars L2 (Isabel) and L266 (Spiers) that were freeze dried for ≈3 d. Total oil content was determined from coarsely chopped seed meat by Soxhlet extraction (Tecator Soxtec System HT 1043; Foss, Eden Prairie, MN) using a 17:1 (v/w) ratio of petroleum ether to dry weight seed tissue. For oil composition, 5 μL of oil was combined with one 2.3-mm chrome steel beads (BioSpec Products, Bartlesville, OK) and 150 μL of sodium

Table 1. Tung (*Vernicia fordii*) germplasm at the U.S. Department of Agriculture (USDA)—Agricultural Research Service Thad Cochran Southern Horticultural Laboratory in Poplarville, MS.

Name	Cultivar	Dates of original tree	Pedigree
BR221		Planted 1962	(L14 × open, F578) × (<i>Vernicia montana</i> × <i>V. fordii</i>)
F554		Chance seedling from northern Florida, in 1941	Unknown
F732		Evaluated in Capps, FL in 1942	Unknown
F742		Unknown	Unknown
F744		First evaluations in 1966 in Compass Lake, FL	F732 × open
F757		Unknown	Unknown
F765		Unknown	Unknown
L47	Folsom	Isabel, LA	Chance seedling
L51	Gahl	Planted 1932, selected 1938 in Isabel, LA	Chance seedling
L152		Planted 1940, selected 1945 in Folsom, LA	L51 × open
L2	Isabel	Planted 1921, selected 1938 in Isabel, LA	Unknown
L266	Spiers	Planted 1941, selected 1954 in Folsom, LA	L92 × open
L273		Evaluated in 1960 in Isabel, LA	L94 × open
L274		Planted 1941, selected 1954 in Folsom, LA	L94 × open
L438		Planted 1956, selected 1960 in Bush, LA	(G46 × F99, L324) × open
L449		Planted 1956, selected 1962 in Bush, LA	(L94 × open, L273) × open
L452		Unknown	Unknown
L508		Planted 1956, selected 1963 in Bush, LA	(L92 × open, L266) × open
L91		Planted 1933, selected 1939 in Isabel, LA	Unknown
L92		Planted 1933, selected 1939 in Isabel, LA	Unknown
L94		Planted 1933, selected 1939 in Isabel, LA	Unknown
LNG		Unknown	Unknown
M147		Planted 1949, selected 1958 in White Sands, MS	L2 × open
M174		Planted 1958, selected 1960 in Lucedale, MS	L47 × (L92 × open, L307)
M185		Planted 1940, selected 1963 in Bass, MS	Unknown
M200		Planted 1956, selected 1962 in White Sands, MS	(L14 × open, F578) × (L94 × open, L278)
M289		Planted 1962	F732 × open
TSN01	AnnaBella	Released by USDA for ornamental use, semisterile	Suspected hybrid between <i>V. fordii</i> and <i>V. montana</i>

Fig. 1. Tung (*Vernicia fordii*) germplasm collection in bloom at the Thad Cochran Southern Horticultural Laboratory field plots in McNeil, MS, in March during peak bloom on 12 Mar. 2009.

Fig. 2. Bumble bee mediated self-pollination to produce seed from *Vernicia fordii* L266 for oil analysis.

Table 2. Comparison of oil content and oil fatty acid composition in tung (*Vernicia fordii*) cultivars L2 (Isabel) and L266 (Spiers) using two replicate samples labeled A and B.

	L2			L266		
	A (%)	B (%)	Avg (%)	A (%)	B (%)	Avg (%)
Oil content	59.70	60.50	60.10	58.20	58.60	58.40
Oil composition						
Palmitate*	2.50	2.60	2.60	2.10	2.20	2.10
Stearate	3.00	3.00	3.00	2.60	2.60	2.60
Oleate*	9.40	9.00	9.20	5.50	5.50	5.50
β -Linoleate	0.90	0.90	0.90	1.10	1.10	1.10
Linoleate*	8.10	8.20	8.10	6.00	6.10	6.00
Linolenate	0.10	0.10	0.10	0.00	0.00	0.00
α -Eleostearate*	74.90	75.20	75.00	81.30	81.50	81.40
β -Eleostearate	0.90	0.90	0.90	1.10	0.80	0.90
Parinarate	0.20	0.20	0.2	0.2	0.20	0.20

Significant differences between L2 and L266 were determined by unpaired *t* test and are indicated by *.

Fig. 3. (A) Mature tung nuts from 'Spiers' and (B) dried tung nuts from 'Spiers' before processing.

methoxide (0.5 M in methanol; Sigma-Aldrich, St. Louis, MO) in a 2-mL centrifuge tube and mixed for 1 min with Disruptor Genie bead beater (Scientific Instruments, Bohemia, NY). After a 15-min incubation at room temperature, 200 μ L of saturated NaCl solution and 500 μ L of heptane were added. The vial was mixed again for 1 min on the Disruptor Genie and centrifuged to separate the phases. The heptane was injected into a gas chromatograph (Model 6890; Agilent, Santa Clara, CA) equipped with a 30-m-long capillary column (Supelco SP-2380, 0.20- μ m film thickness; Supelco, Bellefonte, PA) and a flame ionization detector. The injector and detector temperatures were held constant at 220 °C and the oven was increased from 160 to 200 °C at 4 °C/min and then held at 200 °C for 10 min. Individual fatty acid methyl esters were identified by comparison of retention times to previously characterized peaks derived from tung oil and other commercially available fatty acid methyl ester standards (Supelco).

In all other aspects, L266 performs like a typical *V. fordii* tree with a deciduous,

upright growth habit with a symmetrical, oval canopy. Mature heights reach 9.0 m with 8.0-m width after 25 years growth. Tung nut production begins 3 to 5 years after planting and nut production is expected for 30 years. Bark is smooth and gray [Royal Horticultural Society (RHS) N200B] (Royal Horticultural Society, 1986). New stem growth is rapid and light green (RHS 144A). Leaves are alternative, simple, and dark green (RHS 146A) on adaxial leaf surface and lighter green on abaxial leaf surface (RHS 147B) at maturity. Leaves are pointed, ovate-cordate, on long stalks with red glands at the top of each petiole. Most leaves are unlobed, heart shaped, and emerge just after flowering. Emerging leaves are green (RHS 144A) on adaxial leaf surface and lighter green on abaxial leaf surface (RHS 146C).

Inflorescences are terminal, solitary, and consist of multibranched corymbiform thyrsoid panicles. These panicle cymes or clusters consist of 50 to 80 flowers containing both staminate and pistillate flowers. Flower buds are set during the previous season and may be affected by vigor and/or freeze damage during winter. Late frosts during flowering cause damaged flowers to drop off the tree and significantly reduce nut production. After self-fertilization or cross-pollination, ovaries swell and fruits develop into large (8.0-cm-diameter) tung nuts that ripen in late September (Fig. 3). Fully ripe tung nuts weigh an average 88.7 g in September before falling to the ground in October (Fig. 3). Trees are softwood and susceptible to damage from high winds.

Culture

More than 20 years of evaluation at three sites document that 'Spiers' has vigor; broad soil adaptability; environmental tolerance to heat, sun, and drought; and disease and insect resistance. The growth habit and fruit production qualities of 'Spiers' are comparable or better than historic cultivars such as Folsom, Isabel, and Ghal. 'Spiers' is easily propagated by bud grafting in early spring or late summer (Potter and Crane, 1957). Rootstocks can be grown from cultivated seed sources or 1-year-old seedlings collected from the wild. Leaders are cut back in late

spring and bare root or potted whips can be planted in the winter or early spring of the next year.

Under production conditions, plants should mature to $\approx$ 3.5 m high and 1.5 m wide with full flowering by 4 years with recommended soil amendment and fertilizer (Potter and Crane, 1957). Trees are expected to be less than 9.0 m tall at maturity with an umbrella canopy providing dense shade. 'Spiers' has not been tested outside the Gulf Coast region, which is USDA cold hardiness zones 8 and 9.

Availability

'Spiers' was released by the USDA Agricultural Research Service and is not patented. It may be propagated and sold freely. TCSHL can also supply budwood to farmers wishing to propagate. 'Spiers' will be used by entrepreneurs, small businesses, and farmers that are interested in reviving domestic tung oil production in the Gulf Coast region of the United States. A new tung oil mill has been established by Gulf Coast Tung Oil, LLC in Tallahassee, FL, by Greg Frost. There is also interest in domestic production for use as a biofuels additive, which would benefit from high eleostearic acid content found in 'Spiers'.

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