Re-examination of the volatile leaf oils of *Juniperus flaccida, J. martinezii, and J. poblana*

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ABSTRACT

The composition of the volatile leaf oils (chiefly terpenoids) of *Juniperus flaccida, J. martinezii, J. poblana, J. poblana* var. *decurrens* and affiliated *J. poblana* from Nayarit are reported. All of the taxa's oils are dominated by α-pinene (16.6 - 65.0%). However, divergence is evident in the oils of *J. martinezii* and the juniper from Nayarit as well as *J. poblana*, Oaxaca. The number of unique compounds in the oil of the aff. *J. poblana* from Nayarit support its recognition as a distinct taxon. However, DNA sequences, morphology and ecological data are needed to determine the taxonomic status of the Nayarit junipers.
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KEY WORDS: *Juniperus flaccida, J. martinezii, J. poblana, J. poblana* var. *decurrens, Cupressaceae*, Nayarit juniper, terpenes, leaf essential oil, morphology.

The flaccid foliage *Juniperus* of Mexico consist of three species: *J. flaccida* Schlecht. with large (9-12 mm diam.), multi-seeded [(4-)-6-10-(13)] cones; *J. poblana* (Martínez) R. P. Adams (formerly *J. flaccida* var. *poblana* Martínez) with very large (9-15 mm diam.), multi-seeded [(4-)-6-10-(13)] cones and *J. martinezii* Pérez de la Rosa with small seed cones (5-7 mm), 1-2 seeds per cone and foliage somewhat drooping but branchlets tips erect (Adams, 2014; Pérez de la Rosa, 1985). *Juniperus martinezii* is quite distinct in its morphology, but the other two taxa differ little in morphology, with *J. flaccida* having radial branching and seed cones tan to brownish purple, whereas *J. poblana* has distichous foliage in vertical planes like *Thuja/Platycladus*, and not very flaccid (Zanoni and Adams, 1976, 1979; Adams, 2014) with bluish-brown seed cones. Each of these taxa has leaf margins that are hyaline and nearly entire, with either a few small teeth or merely a wavy margin (Adams, 2014). However, their DNA clearly places them in the serrate leaf margined *Juniperus* species of the western hemisphere with teethed margins secondarily lost (Adams, 2014).

Juniperus flaccida, J. martinezii and *J. poblana* have been treated as varieties of *J. flaccida*, until DNA sequencing of nrDNA (ITS) and trnC-trnD (Adams et al., 2006) revealed that *J. flaccida* varieties are not monophyletic and they recognized *J. f.* var. *martinezii* as *J. martinezii* and *J. f.* var. *poblana* as *J. poblana*. More recently, Adams and Schwarzbach (2013) published a detailed phylogeny of the serrate junipers of the western hemisphere based on nrDNA and four cp genes. They found *J. flaccida* (var. *flaccida*) in a group with *J. standleyi* and *J. poblana* (*J. f.* var. *poblana*) in a well-supported sister group relationship. Likewise, *Juniperus martinezii* (*J. f.* var. *martinezii)* grouped with *J. durangensis* supported by high branch support. Their work appears to solidify support for the recognition of *J. martinezii* and *J. poblana.*

Recently, samples were collected from a new population of aff. *J. poblana* in Nayarit. Samples of *J. poblana* from the type locality (Amozoc) and a population of *J. flaccida* from Coahuila were also

collected. Preliminary DNA analysis revealed the Nayarit junipers grouped with *J. poblana* (authors, unpublished).

The composition of the volatile leaf oils of *J. flaccida* and *J. poblana* (as *J. f.* var. *poblana*) were first reported by Adams, Zanoni and Hogge (1984). The composition of the leaf oil of *J. martinezii* was reported by Adams, Pérez de la Rosa and Cházaro (1990). Recently, Adams and Zanoni (2015) have reported on a re-examination the leaf oils of *J. flaccida, J. martinezii and J. poblana* using modern FID-GC quantitation methods.

The purpose of this paper is to report on the volatile leaf oils of the Nayarit junipers as well as the oil of *J. poblana* from the type locality (Amozoc) and *J. flaccida* from Coahuila.

MATERIALS AND METHODS

Specimens examined:

J. flaccida, Adams 6892-6896, 23 km e of San Roberto Junction on Mex. 60, Nuevo Leon, Mexico;

J. flaccida, Reserva Ecologica Municipal de Sierra y Cañon de Jimulco, 25° 07' 38" N, 103° 16' 15" W., 2118 m, 17 Jan 2017, Torreon, Coahuila, Mexico, Coll. *Manuel Rodríguez Munoz et al. #1,2,3,4,5*, Lab Acc. *Adams 15203 - 15207*;

J. martinezii, Adams 5950-5952, 8709, 40 km n of Lagos de Moreno on Mex. 85 to Amarillo, thence 10 km e to La Quebrada Ranch, 21° 33.08' N, 101° 32.57' W, Jalisco, Mexico;

J. poblana var. *decurrens, R. P. Adams 11926, 11927, 11928*, small trees, to 5 m tall, with strong central axis, foliage very, very, weeping, common, about 2 km s of Valle de Topia. All leaves decurrent, and prickly and are not merely juvenile leaves. 25° 14' 11" N; 106° 26' 55.7" W, 1818 m, 30 Jun 2009, Durango, Mexico;

J. poblana, *Adams 6868-6870*, 62 km s of Oaxaca, Mexico on Mex. 190.

J. poblana, uncommon young trees (saplings) 2 m, in oak woodland dominated by *Quercus resinosa*, Mexico, Nayarit, Mpio. El Nayar, SW of Mesa del Nayar on road to Ruiz, Km 86.8; S of bridge of arroyo del Fraile, E of El Maguey, 22° 10' 08" N, 104° 43' 51" W, 1150 m, 19 Jan. 2016, Coll. *M. S. Gonzalez-Elizondo and M. Gonzalez-Elizondo 8381 with L. López, A. Torres Soto;* Lab Acc. *Adams 14896*

J. poblana, large, single stemmed trees, foliage long and pendulous, abundant trees, up to 25 m high, on strongly rocky slope, forest of *Juniperus-Clusia* with elements of mesophytic forest (*Magnolia*) and tropical forest (*Bursera, Opuntia, Pilosocereus purpusii*) as well as *Agave attenuata* and *Yucca jaliscensis*, Mexico, Nayarit, Mpio. El Nayar, SW of Mesa del Nayar on road to Ruiz; NE of El Maguey, 22° 07'40" N, 104° 47' 47" W, 1430 m, 19 Jan. 2016, Coll. *M. S. Gonzalez-Elizondo and M. Gonzalez-Elizondo 8379a,b,c,d, with L. López, A. Torres Soto;* Lab Acc. *Adams 14897-14900,*

J. poblana, growing in a *J. poblana* - oak forest. Amozoc de Mota, just S of town. 19° 01' N, 98° 01' W, 2300 m. Date 15 Dec 2016 Mpio. Amozoc State: Puebla, Mexico, Coll. *L. Caamano A and Allen Coombes 10172,10173,10174,10180,10181*, Det. Socorro Gonzalez, Lab Acc. *Adams 15208 - 15212*

Voucher specimens are deposited at BAYLU and CIIDIR when applicable.

Fresh, air dried leaves (50-100 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at 20 °C until analyzed. The extracted leaves were oven dried (100 °C, 48 h) for determination of oil yields.

Oils from 4-5 trees of each taxon were analyzed and average values reported. The oils were analyzed on a HP 5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica

capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software. PCO (Principle Coordinates Ordination) (Veldman, 1967) was performed on the similarity matrix (Adams, 1975).

RESULTS AND DISCUSSION

Analyses of the volatile leaf oils of *J. p.* var. *decurrens*, *J. poblana* (Amozoc, Nayarit and Oaxaca), *J. flaccida* (Coahuila and Nuevo Leon) and *J. martinezii* are given in Table 1. The oil of *J. poblana* (Amozoc, type locality) is dominated by α-pinene (41.9%) with moderate amounts of β-pinene (3.9%), myrcene (4.5%), limonene (3.5%), β-phellandrene (3.5%), (E)-nerolidol (2.7%) and manool oxide (1.8%). The leaf oil of *J. p.* var. *decurrens* is dominated by α-pinene (53.2%) with moderate amounts of β-pinene (5.3%), myrcene (5.6%), δ-2-carene (1.5%), δ-3-carene (2.5%), limonene (3.2%), β-phellandrene (3.1%). terpinolene (1.0%), (E)-caryphyllene (1.1%) and germacrene D (1.5%). Its oil is most similar to that of *J. poblana* from Amozoc.

The leaf oil of *J. poblana* from Oaxaca is dominated by α-pinene (41.3%) and δ-3-carene (10.7%), with moderate amounts of β-pinene (2.9%), myrcene (3.7%), δ-2-carene (3.5%), limonene (5.6%), β-phellandrene (3.7%). linalool (2.5%), piperitone (1.8%) and elemol (1.8%).

The oil of aff. *J. poblana* from Nayarit is quite distinct and is dominated by α-pinene (41.9%) and germacrene D (12.1%) with moderate amounts of γ-cadinene (6.1%), β-pinene (3.4%), myrcene (3.7%), β-phellandrene (2.3%), (E)-caryophyllene (3.0%), (E)-nerolidol (3.3%), epi-α-cadinol (3.0%) and epi-α-muurolol (3.0%). The oil also contains ten (10) compounds unique to the taxa studied: β-bourbonene, α-muurolene, α-cadinene, germacrene D-4-ol, trans-muurol-5-en-4-α-ol, salvial-4(14)-en-1-one, pentadecanal, octadecane, hexadecanal and hexahydrofarnesyl acetone.

The oil of *J. flaccida* from Coahuila is dominated by α-pinene (32.4%), δ-3-carene (18.1%) and manoyl oxide (10.4%) with moderate amounts of δ-2-carene (2.3%, β-pinene (2.9%), myrcene (3.7%), limonene (2.7%), β-phellandrene (4.0%), terpinolene (2.4%) and abietatriene (1.0%).

The leaf oil of *J. flaccida* (Nuevo Leon) is similar to that from Coahuila as, it too, is dominated by α-pinene (65.0%) with moderate amounts of β-pinene (4.8%), myrcene (4.3%), limonene (3.5%), β-phellandrene (3.4%), linalool (2.9%) and manool oxide (3.0%), but it contains no δ-3-carene.

The oil of *J. martinezii* is quite distinct with major components being α-pinene (16.6%), sabinene (10.4%) and camphor (11.1%) and moderate amounts of β-pinene (1.4%), myrcene (3.6%), limonene (1.8%), β-phellandrene (5.3%), linalool (2.8%), γ-terpinene (1.8%) and terpinen-4-ol (6.1%). It also contain seven (7) unique compounds: cis-sabinene hydrate (0.6%), p-cymenene (0.7%), karahanaenone (1.3%), linalool acetate (0.4%), neo-iso-3-thujanyl acetate (0.8%), an aromatic phenol (KI 1320, 0.5%), and an unknown diterpene (KI 1978, 0.6%).

To determine the overall similarities of the oils of these taxa, 35 components (Table 2) were coded and pair-wise similarity measures computed. The resulting similarity matrix was factored and yielded six (6) eigenroots accounting for: 34.4, 23.2, 13.9, 11.4, 10.9 and 6.1% of the variance (100%). Interestingly, the eigenroots asymptote after five roots, implying there are six entities of variation among the seven taxa. From the PCO (Fig. 1) these six entities appear to be: *J. martinezii*; *J.* aff. *poblana*, Nayarit; *J. flaccida* (Coah, Nuevo Leon); *J. poblana*, Amozoc; *J. poblana* var. *decurrens*; and *J. poblana*, Oaxaca.

The first coordinate separated aff. *J. poblana*, Nayarit and *J. martinezii* from all other taxa (Fig. 1). Their oils are quite distinct, so this is not surprising. The second coordinate separated did not clearly separate taxa, but combined with coordinate three, they ordinate several groups.

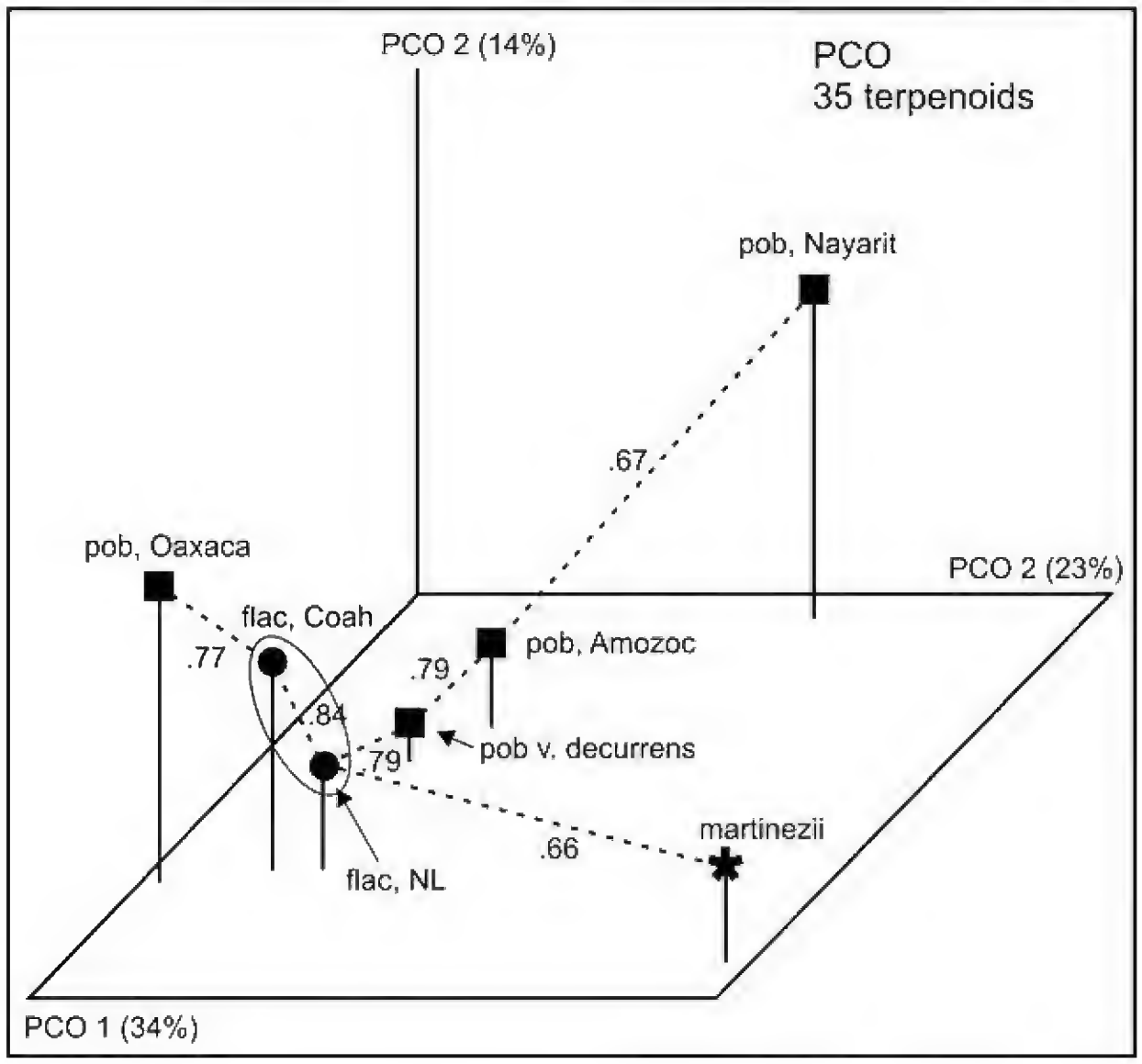

Figure 1. PCO based on 35 terpenoids. The dashed lines are the minimum spanning network. The numbers next to the dashed lines are the similarity between taxa. NL = Nuevo Leon.

It seems unusual that the oil of *J. p.* var. *decurrens* is equally similar to that of *J. poblana*, Amozoc and that of *J. flaccida*, NL (Nuevo Leon) (Fig. 1). It is also surprising that the oil of *J. poblana*, Oaxaca is most like that of *J. flaccida*, Coah. (Fig. 1). As far as the volatile leaf oils are concerned, the oil of *J. poblana*, Oaxaca is not typical, but considerably different from other *J. poblana* oils (Fig. 1).

In summary, the oils of these taxa are dominated by α-pinene (16.6 - 65.0%) and are generally similar. However, divergence is evident in the oils of *J. martinezii* and the juniper from Nayarit. The number of unique compounds in the oil of the putative *J. poblana* from Nayarit support its recognition as a distinct taxon. Additional DNA, morphology and ecological data are needed (in progress) to determine the taxonomic affinity of the Nayarit junipers.

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Table 1. Leaf essential oil composition for aff. *J. poblana* (Nayarit, *Adams 15034*) compared with *J. poblana* var. *poblana* (Amozoc, *Adams 15214*), *J. poblana* var. *decurrens* (*Adams 11932*), *J. poblana* (Oaxaca, *Adams 6868-6872*), *J. flaccida*, Coah. (*Adams 15213*) *J. flaccida*, Nuevo Leon (NL, *Adams 6892*) and *J. martinezii* (*Adams 5974*) based on FID gas chromatography and GCMS identification. Those compounds that appear to distinguish the Nayarit juniper or group Nayarit with *J. poblana* and *J. p.* var. decurrens are in boldface. Thirty-five (35) compounds marked with an asterisk (*) were used in PCO.

KI	Compound	aff. *pob* Nayarit	*poblana* Amozoc	*pob.* var. *decurrens*	*poblana* Oaxaca	*flaccida* Coah.	*flaccida* NL	*mart.*
921	tricyclene	t	t	t	t	t	0.2	0.6
924	α-thujene	t	t	t	t	t	t	0.6
932*	**α-pinene**	**26.3**	**41.8**	**53.2**	**41.3**	**32.4**	**65.0**	**16.6**
945	α-fenchene	-	t	0.1	0.5	-	t	-
946	camphene	0.3	0.5	0.5	0.5	0.6	0.6	0.7
953	thuja-2,4-diene	t	0.1	t	t	0.3	t	0.1
961*	verbenene	0.3	0.5	0.1	1.9	1.3	1.3	0.2
969*	**sabinene**	**0.1**	**t**	**-**	**t**	**0.2**	**0.2**	**10.4**
974	**1-octen-3-ol**	**t**	**t**	**0.1**	**t**	**-**	**-**	**-**
974*	β-pinene	3.4	3.9	5.3	3.2	2.9	4.8	1.4
988*	myrcene	3.7	4.5	5.6	4.3	3.7	4.3	3.6
1001*	**δ-2-carene**	**0.9**	**1.5**	**1.2**	**3.5**	**2.3**	**-**	**-**
1002	α-phellandrene	0.2	0.2	0.1	0.2	t	0.1	1.0
1008*	**δ-3-carene**	**0.9**	**0.7**	**2.5**	**10.7**	**18.1**	**-**	**-**
1014	α-terpinene	t	t	t	0.3	t	t	1.0
1020	p-cymene	0.1	0.2	t	0.2	t	0.1	1.8
1024*	limonene	1.6	3.5	3.2	5.6	2.7	3.5	1.8
1025*	β-phellandrene	2.3	3.5	3.1	3.7	4.0	3.4	5.3
1032	(Z)-β-ocimene	t	t	0.1	t	t	t	t
1044	(E)-β-ocimene	1.0	1.3	1.8	0.8	0.7	1.5	0.4
1054	γ-terpinene	t	t	0.1	0.1	t	0.2	1.8
1065	**cis-sabinene hydrate**	**-**	**-**	**-**	**-**	**-**	**-**	**0.6**
1067	cis-linalool oxide (furanoid)	-	-	-	-	t	0.1	-
1086	terpinolene	0.6	0.9	1.0	1.9	2.4	0.5	0.8
1089*	**p-cymenene**	**-**	**-**	**-**	**-**	**-**	**-**	**0.7**
1094	96, 109,43,152, C10-OH	0.1	0.2	-	0.9	0.5	1.0	1.8
1095	linalool	0.5	1.1	0.7	2.5	1.1	2.9	2.8
1111	6-camphenol	-	-	-	-	0.3	-	-
1112	3-m-3-buten-me-butanoate	-	-	-	-		0.2	-
1114	endo-fenchol	t	0.2	0.1	t	t	-	-
1118	cis-p-menth-2-en-1-ol	0.2	0.2	0.1	0.4	0.3	0.1	0.5
1122	α-campholenal	0.3	1.2	0.1	0.3	0.4	0.3	0.4
1135	trans-pinocarveol	0.4	1.1	-	0.4	0.4	0.3	0.8
1136	trans-p-menth-2-en-1-ol	-	-	0.2	0.4	-	-	-
1141	camphor	0.3	1.8	0.3	0.4	0.6	0.5	11.1
1141	trans-verbenol	-	-	-	-	-	-	-
1145	camphene hydrate	0.2	0.6	0.2	0.4	0.2	0.4	1.3
1148	citronellal	-	-	-	t	0.7	0.2	-
1154*	**karahanaenone**	**-**	**-**	**-**	**-**	**-**	**-**	**1.3**
1153	myrtenyl, methyl ether	-	0.2	-	-	-	-	-
1155	iso-isopulegol	0.2	0.5	-	0.5	0.2	0.1	-
1155	isomer - p-mentha-1,5-dien-8-ol	-	-	-	-	0.5	-	-
1160	p-mentha-1,5-dien-8-ol	-	1.2	-	0.6	0.7	-	1.0
1165	borneol	0.4	-	0.7	t	t	0.7	-
1172	cis-pinocamphone	t	0.3	0.1	t	0.2	0.2	0.3
1174	terpinen-4-ol	0.2	0.2	0.2	0.3	0.5	0.3	6.1
1178	naphthalene	t	-	0.4	-	-	-	t
1179	p-cymen-8-ol	t	0.2	t	t	0.2	t	0.5

KI	Compound	aff. *pob* Nayarit	*poblana* Amozoc	*pob.* var. *decurrens*	*poblana* Oaxaca	*flaccida* Coah.	*flaccida* NL	*mart.*
1186	α-terpineol	0.6	0.5	0.9	0.8	0.6	0.4	0.7
1195	myrtenol	t	-	-	t	t	0.1	t
1195	myrtenal	t	1.0	-	0.4	-	-	0.1
1195*	**methyl chavicol**	**0.7**	t	**0.8**	**t**	**t**	**-**	**-**
1200	terpene alcohol, 95,121,139,154	-	0.9	-	0.4	-	-	-
1200	trans-dehydrocarvone	-	-	-	-	0.2	-	0.6
1204	verbenone	0.2	0.6	t	0.4	0.3	t	0.5
1215	trans-carveol	0.1	0.8	-	0.2	0.2	0.1	-
1218	endo-fenchyl acetate	-	t	0.1	-	-	-	-
1223	citronellol	t	t	-	t	0.3	0.1	-
1232	thymol, methyl ether	t	-	0.1	-	-	-	-
1235	trans-chrysanthenyl acetate	t	-	-	-	0.1	-	0.5
1239	carvone	-	0.2	-	t	t	-	-
1249*	piperitone	0.1	0.9	0.1	1.8	0.8	0.2	0.9
1254	**linalool acetate**	**-**	**-**	**-**	**-**	**-**	**-**	**0.4**
1255	4Z-decenol	-	-	-	-	0.3	0.2	-
1284	bornyl acetate	0.6	1.2	0.8	0.2	0.1	0.4	1.8
1289	trans-sabinyl acetate	-	-	-	-	-	-	0.1
1289*	**neo-iso-3-thujanly acetate**	**-**	**-**	**-**	**-**	**-**	**-**	**0.8**
1289	thymol	-	-	-	-	0.1	-	-
1291	trans-verbenyl acetate	-	0.1	-	t	-	-	-
1292	(2E,4Z)-decadienal	0.1	-	-	-	-	0.1	-
1298	trans-pinocarvyl acetate	-	0.1	-	-	-	-	-
1315	(2E,4E)-decadienal	-	-	-	-	t	0.1	-
1320*	**aromatic phenol 149,91,77,164**	**-**	**-**	**-**	**-**	**-**	**-**	**0.6**
1324	myrtenyl acetate	0.1	0.5	-	-	-	-	-
1345	α-terpinyl acetate	t	-	-	-	t	-	0.2
1345	α-cubebene	t	t	-	-	t	0.1	0.3
1374	**α-copaene**	**0.2**	**t**	**-**	**-**	**-**	**-**	**-**
1387	**β-bourbonene**	**0.2**	**-**	**-**	**-**	**-**	**-**	**-**
1389	**β-elemene**	**0.1**	**t**	**-**	**-**	**-**	**-**	**-**
1396	duvalene acetate	0.1	0.1	0.3	-	t	-	-
1403	methyl eugenol	t	0.3	0.3	-	t	0.1	-
1417*	**(E)-caryophyllene**	**3.0**	**1.3**	**1.1**	**0.5**	**0.3**	**0.2**	**0.1**
1448	cis-muurola-3,5-diene	0.1	-	0.2	-	-	-	-
1451	trans-muurola-3,5-diene	0.2	-	-	-	-	-	0.2
1452*	**α-humulene**	**0.6**	**0.1**	**-**	**-**	**-**	**-**	**-**
1465	cis-muuola-4(14),5-diene	-	0.1	-	-	-	-	-
1475	trans-cadina-1(6),4-diene	0.2	-	0.1	-	-	-	0.3
1484*	**germacrene D**	**12.1**	**1.8**	**1.5**	**0.4**	**0.5**	**0.1**	**-**
1493	trans-muurola-4(14),5-diene	0.7	-	0.1	-	-	-	0.7
1493	epi-cubebol	0.6	-	-	-	-	-	0.5
1500	α-muurolene	0.5	-	-	-	t	-	-
1513*	**γ-cadinene**	**6.1**	**0.1**	**0.2**	t	**t**	**-**	**-**
1514	cubebol	-	-	0.4	-	-	-	1.1
1521	trans-calamenene	-	-	-	-	-	-	0.5
1522	δ-cadinene	1.7	0.2	0.4	t	t	-	0.4
1528	zonarene	-	-	0.1	-	-	-	0.1
1533	trans-cadina-1,4-diene	-	-	-	-	-	-	t
1537	α-cadinene	0.2	-	-	-	-		-
1548*	**elemol**	**0.5**	**0.7**	**-**	**1.8**	**0.2**	**0.1**	**1.0**
1555*	**elemicin**	**t**	**0.8**	**0.4**	t	**t**	**-**	**-**
1561*	**(E)-nerolidol**	**3.3**	**2.7**	**0.9**	**0.4**	**t**	**-**	**-**
1574	**germacrene D-4-ol**	**0.4**	**-**	**-**	**-**	**-**	**-**	**-**
1582	caryophyllene oxide	1.0	0.8	0.8	0.3	0.3	0.2	0.3
1587*	**trans-muurol-5-en-4-α-ol**	**0.5**	-	**-**	**-**	-	**-**	**-**
1594	**salvial-4(14)-en-1-one**	**0.2**	-	**-**	**-**	-	**-**	**-**

KI	Compound	aff. *pob* Nayarit	*poblana* Amozoc	*pob.* var. *decurrens*	*poblana* Oaxaca	*flaccida* Coah.	*flaccida* NL	*mart.*
1627	1-epi-cubenol	0.9	-	0.7	-	-	-	1.0
1630*	**γ-eudesmol**	**-**	**-**	**-**	**0.5**	**t**	**-**	**t**
1638*	**epi-α-cadinol**	**3.0**	**0.1**	**0.8**	**t**	**t**	**-**	**-**
1638	epi-α-muurolol	3.0	0.1	0.8	t	t	-	-
1644	α-muurolol	0.3	-	-	t	-		-
1649*	**β-eudesmol**	**-**	**0.3**	**-**	**0.5**	**t**	**-**	**0.3**
1652	α-eudesmol	-	0.4	-	0.5	t	-	0.3
1652*	**α-cadinol**	**1.7**	**0.5**	**0.8**	**t**	**t**	**-**	**-**
1685*	**germacra-4(15),5,10-trien-1-al**	**1.0**	**0.6**	**0.8**	**0.2**	**t**	**-**	**-**
1688	shyobunol	-	-	-	-	t	-	-
1759	benzyl benzoate	-	-	t	-	0.2	-	-
1712	**pentadecanal**	**0.2**	**-**	**-**	**-**	**-**	**-**	**-**
1800	**octadecane**	**0.1**	**-**	**-**	**-**	**-**	**-**	**-**
1814	**hexadecanal**	**0.1**	**-**	**-**	**-**	**-**	**-**	**-**
1840	**hexahydrofarnesyl acetone**	**0.1**	**-**	**-**	**-**	**-**	**-**	**-**
1933	**cyclohexadecanolide**	**0.3**	**0.1**	**t**	**-**	**t**	**t**	**-**
1958*	**iso-pimara-8(14),15-diene**	**-**	**-**	**-**	**-**	**t**	**0.1**	**1.0**
1959*	**hexadecanoic acid**	**1.8**	**1.1**	**0.3**	**-**	**-**	**-**	**-**
1978*	**diterpene,43,81,147,243**	**-**	**-**	**-**	**-**	**-**	**-**	**0.6**
1987*	**manoyl oxide**	**1.6**	**1.8**	**0.6**	**0.3**	**10.4**	**3.0**	**1.0**
2009	epi-13-manool oxide	-	-	-	-	t	t	-
2055*	abietatriene	0.9	1.2	0.1	0.2	1.0	0.3	0.8
2056	manool	t	-	0.1	-	-	t	-
2087	abietadiene	-	-	-	-	-	-	2.3
2105	iso-abienol	0.3	0.6	t	t	0.1	t	-
2107	phytol, isomer	t	0.6	t	-	-	-	-
2184	sandaracopimarinal	-	-	-	-	0.2	t	-
2256	methyl sandaracopimarate	-	-	-	-	0.1	t	-
2264*	**diterpene,43,55,271,286**	**0.3**	**0.5**	**0.8**	**0.4**	**t**	**-**	**-**
2314	trans-totarol	t	t	-	-	0.3	t	t
2331*	trans-ferruginol	0.6	1.1	0.2	0.1	0.4	t	-

KI = Kovats Index (linear) on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Table 2. Thirty-five compounds used for PCO analysis. Note the patterns of the divergence of the Nayarit aff. *J. poblana* and *J. martinezii* in their oil components.

KI	Compound	*aff. pob* Nayarit	*poblana* Amozoc	*pob.* var. *decurrens*	*poblana* Oaxaca	*flaccida* Coah.	*flaccida* NL	*martinezii*
932*	α-pinene	26.3	41.8	53.2	41.3	32.4	65.0	16.6
961*	verbenene	0.3	0.5	0.1	1.9	1.3	1.3	0.2
969*	sabinene	0.1	t	-	t	0.2	0.2	10.4
974*	β-pinene	3.4	3.9	5.3	3.2	2.9	4.8	1.4
988*	myrcene	3.7	4.5	5.6	4.3	3.7	4.3	3.6
1001*	δ-2-carene	0.9	1.5	1.2	3.5	2.3	-	-
1008*	δ-3-carene	0.9	0.7	2.5	10.7	18.1	-	-
1024*	limonene	1.6	3.5	3.2	5.6	2.7	3.5	1.8
1025*	β-phellandrene	2.3	3.5	3.1	3.7	4.0	3.4	5.3
1195*	methyl chavicol	0.7	t	0.8	t	t	-	-
1089*	p-cymenene	-	-	-	-	-	-	0.7
1154*	karahanaenone	-	-	-	-	-	-	1.3
1249	piperitone	0.1	0.9	0.1	1.8	0.8	0.2	0.9
1289*	neo-iso-3-thujanly acetate	-	-	-	-	-	-	0.8
1320*	aromatic phenol 149,91,77,164	-	-	-	-	-	-	0.6
1417*	(E)-caryophyllene	3.0	1.3	1.1	0.5	0.3	0.2	0.1
1452*	α-humulene	0.6	0.1	-	-	-	-	-
1484*	germacrene D	12.1	1.8	1.5	0.4	0.5	0.1	-
1513*	γ-cadinene	6.1	0.1	0.2	t	t	-	-
1548*	elemol	0.5	0.7	-	1.8	0.2	0.1	1.0
1555*	elemicin	t	0.8	0.4	t	t	-	-
1561*	(E)-nerolidol	3.3	2.7	0.9	0.4	t	-	-
1587*	trans-muurol-5-en-4-α-ol	0.5	-	-	-	-	-	-
1630*	γ-eudesmol	-	-	-	0.5	t	-	t
1638*	epi-α-cadinol	3.0	0.1	0.8	t	t	-	-
1649*	β-eudesmol	-	0.3	-	0.5	t	-	0.3
1652*	α-cadinol	1.7	0.5	0.8	t	t	-	-
1685*	germacra-4(15),5,10-trien-1-al	1.0	0.6	0.8	0.2	t	-	-
1958*	iso-pimara-8(14),15-diene	-	-	-	-	t	0.1	1.0
1959*	hexadecanoic acid	1.8	1.1	0.3	-	-	-	-
1978*	diterpene,43,81,147,243	-	-	-	-	-	-	0.6
1987*	manoyl oxide	1.6	1.8	0.6	0.3	10.4	3.0	1.0
2055*	abietatriene	0.9	1.2	0.1	0.2	1.0	0.3	0.8
2264*	diterpene,43,55,271,286	0.3	0.5	0.8	0.4	t	-	-
2331*	trans-ferruginol	0.6	1.1	0.2	0.1	0.4	t	-

Comparison of hydrocarbon yields in cotton from field grown vs. greenhouse grown plants.

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ABSTRACT

Four accession of cotton (SA-1181, 1403, 1419, and 2269) were grown both in field conditions and a greenhouse to compare the environmental effects on leaf biomass, % yield of hydrocarbons (HC), and total HC (g HC /g leaves) under natural and controlled (protected) conditions. Leaf biomass was similar but higher in two field cultivated accessions. All four accessions produced higher % HC yields under field conditions, with greenhouse plants producing lower yields ranging from 20.6 to 46.0% as much HC as found in naturally grown plants. Total HC yields (g HC / g 10 leaves) were even lower in the greenhouse with yields being only 19.7 to 39.1% as high as from field grown plants. Overall, the environmental component to the yield of free HC in cotton leaves was a major factor, with the genetic component contributing to less than half (46%) of the HC yield. This trend corresponds to literature reports of large induction of defense chemicals in cotton upon attack by herbivores and diseases. The same pattern has been found in sunflowers and is discussed in regards to cotton. Ontogenetic variation in HC for SA-2269 showed HC yields in leaves remained at a constant, low level from bud to flowering, then increased rapidly as bolls matured. Published on-line **www.phytologia.org** *Phytologia 99(3): 200-207 (Aug. 8, 2017).* ISSN 030319430.

KEY WORDS: Cotton, *Gossypium* spp., yields of pentane extractable leaf hydrocarbons, petrochemicals, liquid fuels

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Many plant species protect themselves from herbivory by a response to an attack (see Karban and Myers, 1989 for a review). It appears that early research on plant defensive chemicals focused on constitutive (or stored) chemicals such as terpenoids, tannins and aromatic metabolic compounds derived from the shikimic acid pathway (Pare and Tumlinson, 1998). But, more recently, greater focus has been on inducible plant defenses (Chen 2008; Pare and Tumlinson, 1997, 1998; Turlings, et al. 1995). Turlings et al. (1995) published a seminal paper entitled "How caterpillar-damaged plants protect themselves by attracting parasitic wasps". They showed that plants injured by herbivores emit chemical signals that attract and guide the herbivores' natural enemies to the damaged plants. Thus, indirectly, injured plants send out a "SOS" signal for help against herbivores. Pare and Tumlinson (1997) nicely documented this phenomenon in a series of experiments on cotton using beet army worms and mechanical damage to leaves.

Chen (2008) discusses that some constitutive chemicals may be increased to even higher levels after insect attack. The present research (herein) is concerned with total extractable hydrocarbons for alternative fuels and chemical feedstocks from cotton leaves.

In a seminal paper, Stipanovic, Bell and Benedict (1999) reviewed the defensive role of pigment gland constituents in cotton. Cotton gland constituents (sesquiterpenoids, gossypol, and gossypol derivates, etc.) are a constitutive defense resource for cotton resistance to insects and diseases. Stipanovic, Bell and Benedict (1999) also discussed that these gland constituents can be rapidly synthesized in response to pathogens.

Opitz, Kunert and Gershenzon (2008) examined the response of stored (constitutive) terpenoids in cotton subjected to mechanical damage, herbivory and jasmonic acid treatments. They found that terpenoid levels increased successively from control to mechanical damage, herbivory, and jasmonic acid treatments. In addition, they reported that herbivory or mechanical damage in older leaves led to terpenoid increases in younger leaves. Higher terpenoid yields were achieved by two methods: 1. increased filling of existing glands and 2. the production of additional glands. The composition of the terpenoid mixture did not significantly differ in response to herbivore, mechanical damage or jasmonic acid treatments.

Recently we reported (Adams et al. 2017a) on the yields of pentane extractable hydrocarbons (HC) from leaves of thirty USDA germplasm cotton accessions (Hinze et al. 2016), grown with supplemental underground drip irrigation at College Station, TX. We discovered % HC yields were very high in four accessions with 11.34, 12.32, 13.23 and 13.73 % HC. Per plant HC yields varied from 0.023 to 0.172 g/ g leaf dry weight (DW). The correlation between % HC yield and average leaf DW was non-significant (0.092), suggesting that breeding for increased HC and plant biomass may be possible.

In addition, Adams et al. (2017a) conducted an ontogenetic study of a commercial cotton cultivar, (FiberMax 1320), grown under dryland conditions. They reported the dry weight of leaves reached a maximum at the 1st flower stage, and then declined as bolls opened. However, % pentane soluble hydrocarbon yields continued to increase throughout the growing season (due to the decline of leaf DW). It seems likely that as the bolls mature and seed are filled, carbohydrates from the leaves are catabolized and sugars are transported to the bolls for utilization. Per plant HC yields increased from square bud stage to 1st flower, remained constant until 1st boll set, then declined at 1st boll-opened stage. This seems to imply that most of the HC are not catabolized and converted to useable metabolites for filling bolls and seeds.

The evolution of modern cotton (*Gossypium* spp.) encompasses an improbable series of events that involved transoceanic, long-distance dispersal with hybridization involving two diploids, one from the Old World and one from the New World, forming the modern cultivated allo-tetraploid, *G. hirsutum* (Wendel and Grover, 2015).

Although there are several papers on the conversion of cotton field stubble to liquid fuels (see Putun, 2010; Putuan et al., 2006; Akhtar and Amin, 2011 and references therein), there are no reports on the environmental versus genetic nature of the production of total extractable HC in cotton.

The purpose of this paper is to report on changes in HC production in field cultivated cotton compared with cotton grown in a greenhouse. In addition, data is reported on ontogenetic variation in HC production in cotton accession SA-2269.

MATERIALS AND METHODS

Plant Materials:

Commercial, cultivated cotton

FiberMax 1320, dryland, dark, loam soil, JP TeBeest Farm, 36° 25' 0.6" N, 101° 32' 17.3" W, 3258 ft., Oslo, TX, avg. annual rainfall, 19.3". The eight (8) lowest growing, non-yellowed mature leaves were collected at random from each of 10 cotton plants, at square bud, 1st open flower, 1st boll, and 1st boll completely opened stages. The leaves were air dried in paper bags at 49° C in a plant dryer for 24 hr or until 7% moisture was attained.

HC yields of 4 high yielding HC cotton accessions grown in a greenhouse

Four accessions (Acala SJ-1,SA-1181; 3010, SA-1403; China 86-1, SA-1419; TM 1, SA-2269) were grown the USDA-ARS Plant Stress and Germplasm Development Research Center, Lubbock, TX. Acala SJ-1,SA-1181; 3010, SA-1403; China 86-1, SA-1419; TM 1, SA-2269 cotton seeds were planted into 27 cm diameter pots containing Sunshine Mix #1 soil (Sun Gro Horticulture Distributors Inc., Bellevue, WA). Three seeds were planted per pot and pots were placed on benches in a greenhouse set to provide a 31/27°C day/night cycle. Plants were thinned to one plant per pot and grown throughout the experiment. 430 W high-pressure sodium lights (P. L. Light Systems, Beamsville. ON Canada) were used to maintain a 16/8 h photoperiod. Nutrients were maintained by daily application with Peters Excel fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, OH) through the automated watering system.

For comparison, the HC yields and leaf DW from the greenhouse study were compared with data from these four accessions (Acala SJ-1,SA-1181; 3010, SA-1403; China 86-1, SA-1419; and TM 1, SA-2269) that were cultivated at the USDA-ARS Southern Plains Agricultural Research Center, College Station, TX, during the summer of 2016 (see Materials and Methods, Adams et al. 2017).

Leaves were ground in a coffee mill (1mm). Three grams of air dried material (7% moisture) was placed in a 125 ml, screw cap jar with 20 ml pentane. The jar was sealed, then placed on an orbital shaker for 18 hr. The pentane soluble extract was decanted through a Whatman paper filter into a pre-weighed aluminum pan, and the pentane was evaporated on a hot plate (50°C) in a hood. The pan with hydrocarbon extract was weighed and tared.

The shaker-pentane extracted HC yields are not as efficient as soxhlet extraction, but much faster to accomplish. To correct the pentane yields to soxhlet yields, one sample was extracted in triplicate by soxhlet with pentane for 8 hrs. All shaker extraction yields were adjusted to oven dry wt (ODW) by a correction factor (CF) of 1.085. For the cultivated cotton from Oslo, TX, the shaker yields were corrected by the increased soxhlet extraction efficiency (CF = x1.56). For the Lubbock accessions, the soxhlet CF was x1.31 and for the accessions grown at College Station, the soxhlet CF was x1.69.

Statistical analyses (means, variance, standard deviation, standard error of mean) were performed by use of EasyCalculation (https://www.easycalculation.com/statistics/standard-deviation.php)

RESULTS

Biomass and hydrocarbon (HC) yields in greenhouse vs. field grown data for four accessions (Table 1) shows that the leaf biomass is similar, but HC yields (as % HC yields) are considerably lower in greenhouse conditions.

Table 1. Comparison of leaf biomass and HC yields for greenhouse versus field grown cotton.

	Greenhouse grown, Lubbock, TX bolls maturing			Field grown, College Station, TX flowering and with bolls		
Accession	DW for 10 lvs/plant, 2 std err.	% HC yield, 2 std err.	HC g/ 10 lvs DW, 2 std err.	DW for 10 lvs/plant	% HC yield	HC g/ 10 lvs DW
SA-1181	9.86 g 1.543	4.25 %, 0.355	0.419 g, 0.0552	9.62 g	12.32 %	1.19 g
SA-1403	11.38 g 1.230	4.18 % 0.336	0.476 g 0.0420	14.63 g	9.08 %	1.33 g
SA-1419	11.50 g 3.243	2.73 % 0.600	0.340 g 0.157	13.10 g	13.23 %	1.73 g
SA-2269	11.93 g, 0.966	4.50%, 0.543	0.537 g, 0.0782	12.44 g	11.09 %	1.38 g

Similar leaf biomass was observed for SA-1181 and SA-2269, but larger leaf biomass was obtained from field grown SA-1403 and SA-1419 (Fig. 1) than from greenhouse grown plants.

In contrast, large differences were found in the % yields of HC (Table 1, Fig. 2). The ratio of % yields in greenhouse / field grown varies as: SA-1181 - 34.5%; SA-1403 - 46.0%; SA-1419 - 20.6%; and SA-2269 - 40.5%. In spite of the robust growth achieved in the greenhouse, these accessions yielded only 20.6% to 46.0% as much as when cotton was field cultivated and exposed to natural challenges in the environment.

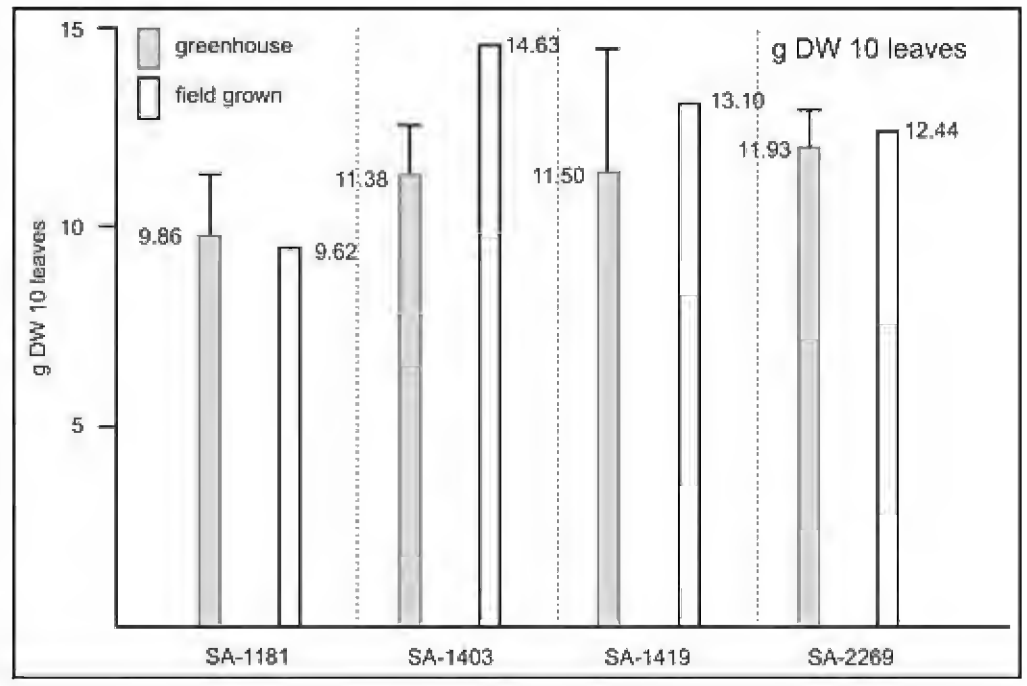

Fig. 1. Comparison of g DW of 10 leaves for greenhouse vs. field grown plants for four cotton accessions (at College Station, 2016). Note: standard error of the mean could not be obtained for the 2016 data, as leaves from plants were combined for each accession)

The lower % yield of SA-1419 (Fig. 2) in protected conditions (i.e., greenhouse) seems to imply the genotype is particularly affected by insects, diseases, water stress, etc. that apparently induced increased HC production in the field (13.23% vs. 2.73% greenhouse). This suggests that higher HC yields might be induced by applying stresses, and some may be inducible with the right growth regulators, etc. But, it also is a note of caution that the farmer may be at risk of producing low HC yields when 'ideal' growing conditions occur.

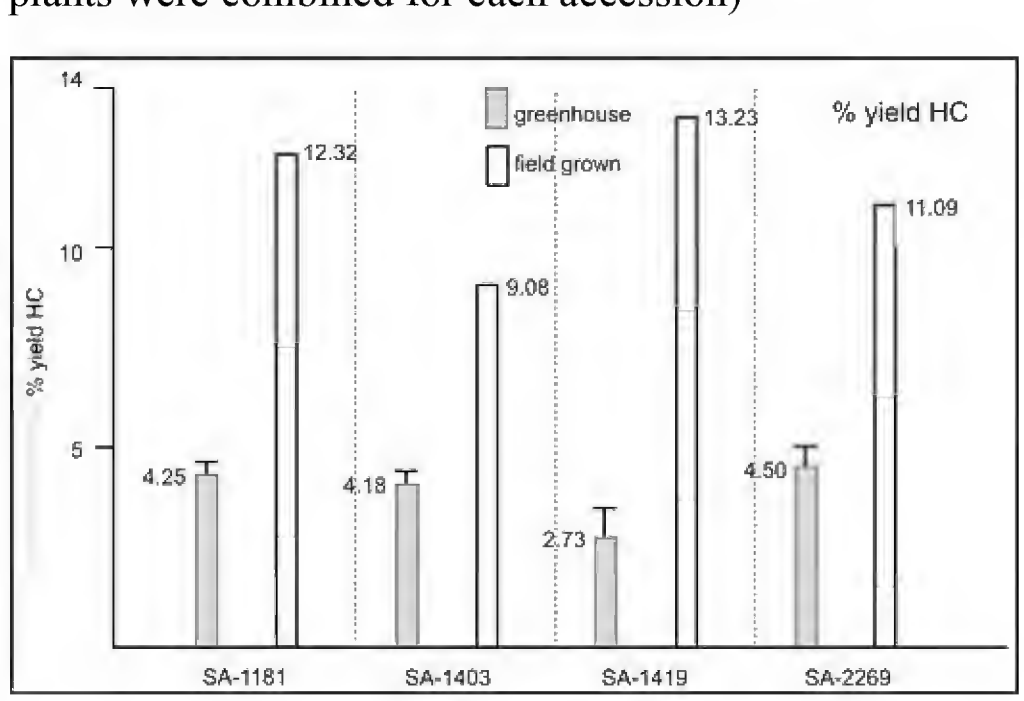

Fig. 2. Comparison of % yield of HC from greenhouse vs. field grown plants (at College Station, TX, 2016).

The trend seen for the g HC / 10 leaves data (Table 1, Fig. 3) is very similar to that seen for the % HC yields (Table 1, Fig. 2). Again SA-1403 was very low in greenhouse plants (only 19.7% as much as field grown). The g HC / 10 leaves yields from greenhouse / field grown varies from 19.7% to 39.1%. As with the % yield data, total harvestable HC might be greatly increased by the application of growth regulators (cf. methyl jasmonate, salicylic acid, etc.) or other agents that induce the synthesis of HC in leaves.

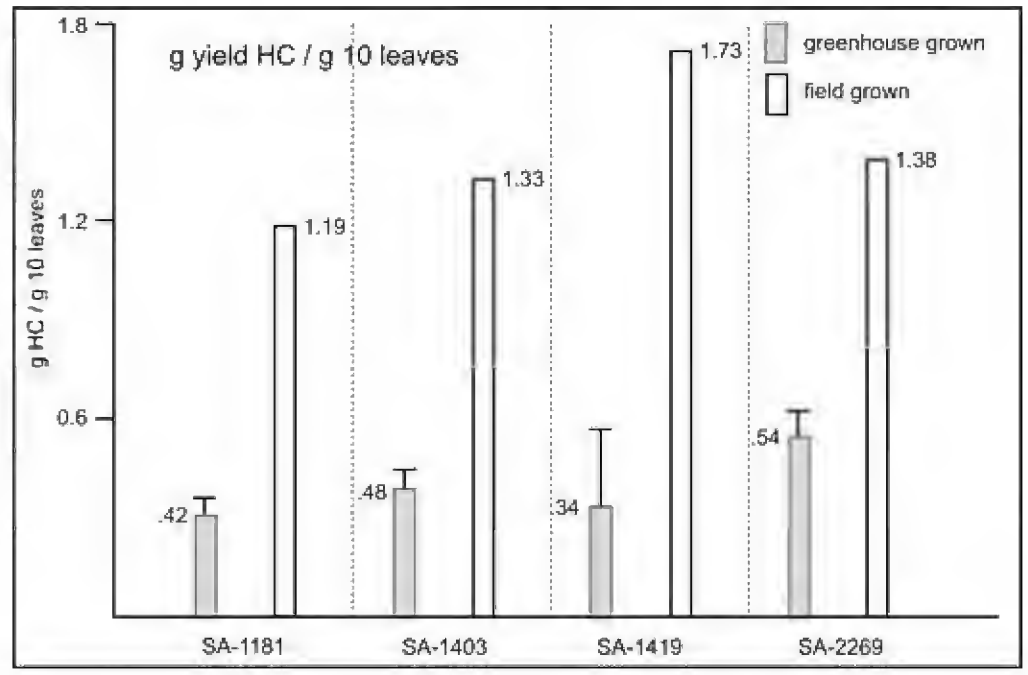

Fig 3. Comparison of g HC / 10 leaves from greenhouse vs. field grown plants (at College Station, 2016).

This same trend of lower HC yields was reported in greenhouse reared sunflowers (Adams et al. 2017b). Leaves and seeds from the same plant were collected from native *H. annuus* from Gruver, TX (GT), Lake Tanglewood, TX (LT) and Salt Lake City, UT (SLC) when the flowers had 10% to 30% disk flowers present. The seeds from each population were germinated and plants grown in the greenhouse at Oklahoma Panhandle State University, Goodwell, OK. The greenhouse grown progeny from the three natural populations had much less leaf biomass (Fig. 4, upper).

Just as seen for cotton, % yield HC was much lower in the greenhouse grown progeny (Fig. 4, center): GT - 45.6%; LT - 55.6%; SLC 78.3%. The SLC population appears to be much less affected by local environmental factors, than the populations at GT and LT.

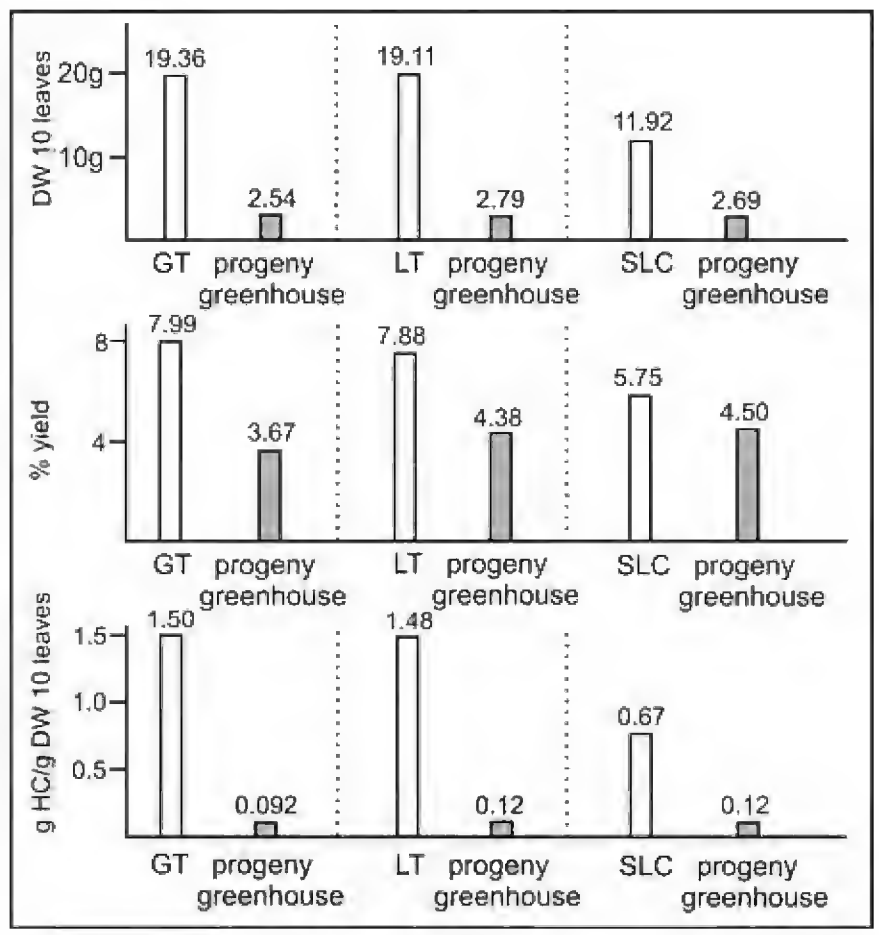

Fig. 4. Comparison of natural sunflowers versus greenhouse grown progeny (Adams et al. 2017b)

The g HC / g DW 10 leaves is similar to the % yield data (Fig. 4. lower), but with a more extreme reduction of HC (i.e, greenhouse / natural = GT - 6.1%; LT - 8.1%; SLC - 17.9%).

So, for at least two genera (*Gossypium* and *Helianthus*), there is a much reduced production of leaf HC when plants are greenhouse grown and removed from naturally occurring plant stresses that induce defense chemicals.

In addition to a study of the effects of greenhouse growth versus natural environment growth, it is of interest to examine ontogenetic variation for the production of HC in the leaves of cotton. Two cotton accessions: SA-2269 (greenhouse grown) and FiberMax 1320 (field grown, see Adams et al. 2017b) were sampled at several growth stages: bud, flowering, bolls maturing and, for FiberMax 1320, bolls open. Comparison of SA-2269 and FiberMax 1320 for leaf biomass, % HC yields and g HC / 10 leaves is presented in Table 2 and Figure 5.

Table 2 Ontogenetic variation in pentane soluble hydrocarbon (HC) yields from four cotton accessions grown at the USDA greenhouse, Lubbock, TX compared to HC yields from FiberMax 1320 field grown at Oslo, TX (Adams et al. 2017b).

Accession, collection growth stage	DW for 10 lvs/ plant, 2 std err.	% HC yield, 2 std err.	Range of yields (%)	HC g/ 10 lvs DW, 2 std err.
SA-2269, Greenhouse, Lubbock, TX, bud stage	5.79 g, 0.718	2.91%, 0.336	(2.20-3.93%)	0.163 g, 0.0419
SA-2269, Greenhouse, Lubbock, TX, flowering with some small bolls	11.08 g, 1.228	2.98%, 0.270	(2.32-3.69)	0.335 g, 0.0596
SA-2269, Greenhouse, Lubbock, TX, bolls maturing	11.93 g, 0.966	4.50%, 0.543	(3.22, 5.63)	0.538 g, 0.0782
FiberMax 1320, Oslo, TX square bud	6.85 g, 0.64	4.05%, 0.30	(3.31 - 4.56)	0.275 g, 0.032
FiberMax 1320, Oslo, TX 1st flower	9.53 g, 0.78	6.05%, 0.70	(4.78 - 7.84)	0.564 g, 0.106
FiberMax 1320, Oslo, TX 1st boll set	7.86 g, 0.72	6.99%, 0.62	(4.95 - 8.28)	0.550 g, 0.068
FiberMax 1320, Oslo, TX bolls open, seeds maturing	5.54 g, 0.57	8.02%, 0.50	(6.65 - 8.90)	0.474 g, 0.054

Both SA-2269 and FiberMax 1320 increase their leaf biomass from bud to flowering stages (Table 2, Fig. 5, upper). However, SA-2269 continued to increase leaf biomass to the bolls stage (Table 2, Fig. 5, upper). It should be noted that FiberMax 1320 was sprayed with a growth regulator between flowering and boll set, whereas the plants grown in the Lubbock greenhouse were not sprayed with a growth regulator.

The % HC yields for both SA-2269 and FiberMax 1320 continued to increase with maturity (Table 2, Fig. 5, middle). Whereas, FiberMax 1320 had a linear increase, SA-2269 yields were constant from bud to flowering, and then showed a large increase from flowering to bolls (Fig. 5, middle).

Due to the interaction of leaf biomass and % HC yields, FiberMax 1320 and SA-2269 had quite different patterns in their g HC / g 10 leaves data (Fig. 5, lower). FiberMax 1320 reached a maximum at flowering with a plateau from flowering to bolls, whereas SA-2269 had a linear increase from bud to flowering to bolls maturing (Fig. 5, lower). The curve for FiberMax 1320 should be viewed with caution, because it was sprayed with a growth regulator between flowering and boll set.

It is interesting to note that the natural populations of sunflowers with the highest % HC (Gruver, TX and Lake Tanglewood (Amarillo, TX) are in high wind areas and that Karban and Myers (1989) suggested that wind may maintain a high level of defensive chemicals. No appreciable amount of wind was present in either the cotton or sunflower greenhouse environments. In addition, the greenhouse plants were not significantly damaged by insects, except white flies that did minimal leaf damage.

These data on HC yields with maturity are encouraging, yet, illustrate the need for more detailed research to more clearly elucidate the changes in HC production in cotton in natural versus greenhouse (controlled) environments. Additional research is ongoing to further investigate the leaf biomass, % HC yields and g HC / leaves under stress conditions such different irrigation regimes (well- and limited irrigation) under a drip surface system and different developmental stages.

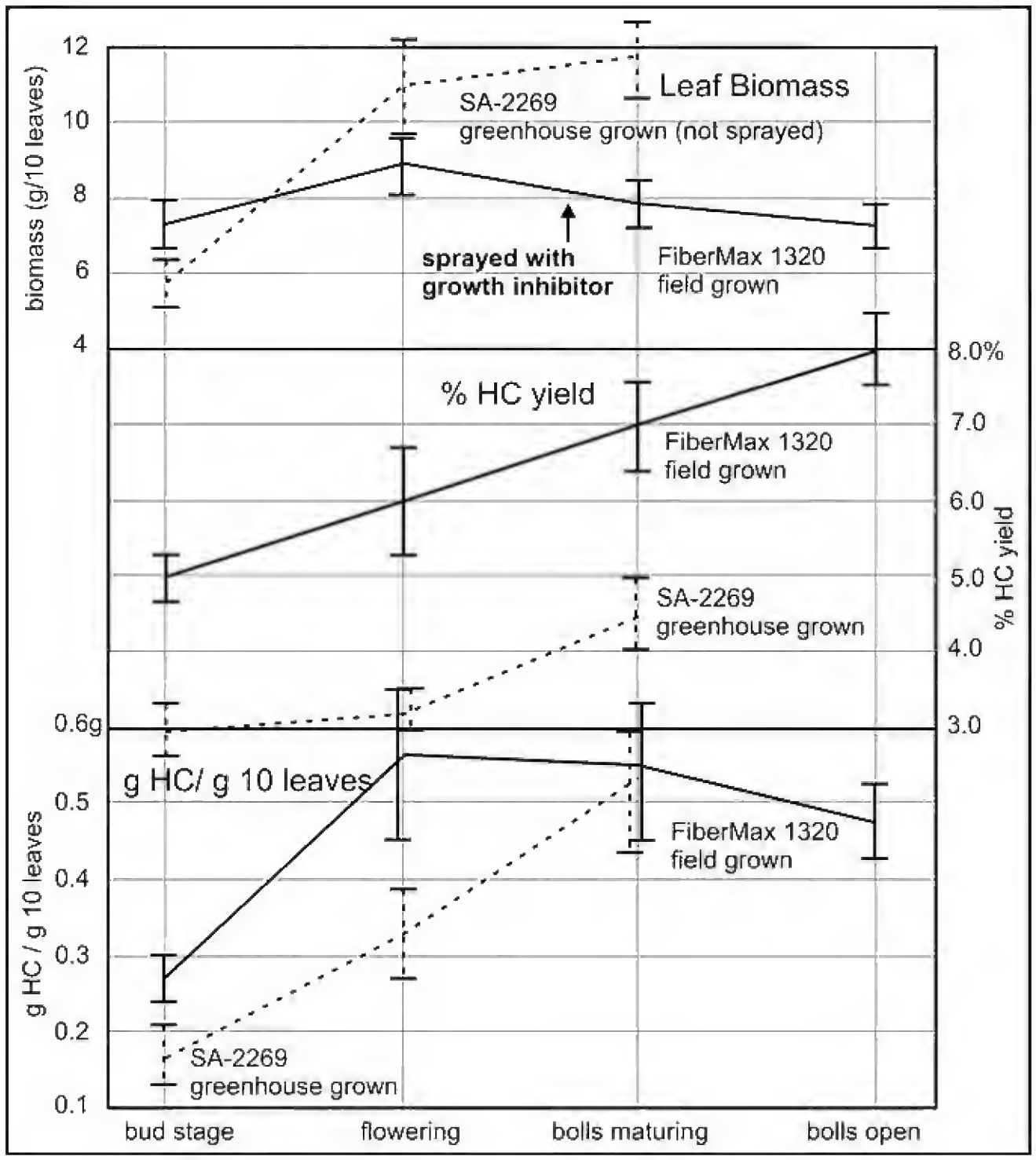

Fig. 5. Comparisons of SA-2269 and FiberMax 1320 for their leaf biomass, % HC yields, and g HC/ 10 leaves at various stages of maturity.

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Using a Drone (UAV) to Determine the *Acer grandidentatum* (bigtooth maple) Density in a Relic, Isolated Community

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ABSTRACT

Relic populations of *Acer grandidentatum* (bigtooth maple, Sapindaceae) are found in isolated, deep canyons in central Texas. These populations are challenging to get to and boundaries are hard to identify from ground level. Other woody species in these canyon communities include *Juniperus ashei* (ashe juniper), *Prunus serotina* (black cherry), *Quercus laceyi* (Lacey oak), *Q. buckleyi* (Texas red oak), *Tilia caroliniana* (Carolina basswood), *Aesculus pavia* (red buckeye) and other mostly deciduous species. The density of *A. grandidentatum* and associated species in a series of these isolated communities were measured using the quadrat procedure. However, this was a sample, not the whole community. The density of *A. grandidentatum* overstory trees based on the ground survey using the quadrat procedure was 788 ± 964 plants/ha and in the understory there were 176 ± 110 juvenile *A. grandidentatum* plants/ha. A UAV or drone was employed to survey the complete canyon community including the *A. grandidentatum* population that was under study. This was completed in the fall of 2016 when the deciduous plants were in full autumn colors so the limits of these deciduous communities would be more visible from the air. The boundaries of the *A. grandidentatum* communities in the canyon were digitally outlined on a computer and the total area was estimated. Total area of the *Acer* communities was 3.81 ha (9.41 ac). Using this area, the predicted number of *A. grandidentatum* overstory trees was 3,002 while the number of juveniles was 681 in this isolated, relic, canyon community. The total number of most other overstory and understory woody species present in these communities would also increase by a factor of 3.81.
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KEY WORDS: populations, central Texas, unmanned aircraft system, UAS, rare species, overstory, understory.

The use of drones, UAV's (unmanned aerial vehicles) or UAS's (unmanned aircraft systems) is a relatively new technology to assess and survey plant or animal species, including populations or communities difficult to approach, detect or observe, as well as identifying insect infestations, drought effects and inspecting areas with populations that are hard to reach. In addition, using a drone has minimal impact on sensitive habitat and causes little disturbance to easily agitated or hypersensitive species (Ogden 2013; Hodgson et al. 2016; Christie et al. 2016; Cruzan et al. 2016). Drones could be used to find and identify unreported communities and populations of relatively rare species.

Acer grandidentatum (bigtooth maple, Sapindaceae) populations in central Texas have been studied for several years. Juvenile plants are sensitive to herbivory (Nelson-Dickerson 2011; Nelson-Dickerson and Van Auken 2016) and they appear to be shade plants (Nelson-Dickerson and Van Auken 2017) having reduced CO_2 uptake in low light conditions, but not as low as sun plants in shade. In addition, their niche seems to be deep, sheltered, limestone canyons with some plants in these central Texas canyons establishing approximately 400 years ago (Van Auken et al. 2017). However, only population samples from these communities have been measured, never the entire communities.

Woodland and forest population dynamics are difficult to understand, probably because of the long lives of the woody species present. A glimpse of the past can be gained by looking at size or age distributions, but looking into the future is a guess. A favorite quote is "Prediction is very difficult, especially about the future.", Niels Bohr, Danish physicist, 1885-1962. Previously, size and age structure of several different species including central Texas *A. grandidentatum* populations were examined to try and determine potential success of the species in the future (Ryniker et al. 2006; Van Auken et al. 2007, 2017; Van Auken and Bush 2013). Most of the species examined turned out to be early successional species (shade intolerant) requiring a disturbance to continue in the same community in the future. *Acer grandidentatum* is however a shade tolerant woody plant, capable of growing in mature woodland or forest understories (Nelson-Dickerson and Van Auken 2017).

There are anecdotal reports indicating that *A. grandidentatum* populations in central Texas are not being replaced (BCNPSOT 2010; Heidemann 2011). There is some evidence that suggests high populations of white-tailed deer (*Odocoileus virginianus*) are a cause of lack of *A. grandidentatum* recruitment (Nelson-Dickerson and Van Auken 2016). But, this is not known for all populations of *A. grandidentatum* in central Texas and this could be a temporal or cyclic phenomenon, reoccurring at unknown, but long temporal intervals.

In addition, estimating population composition of low-density species, species with discontinuous distributions, or future populations of long-lived species has proved troublesome, time consuming and expensive. Use of drones could reduce the time and expense of studying populations or communities as indicated above.

PURPOSE

The purpose of this study was to determine the area covered and the number of overstory and understory *A. grandidentatum* plants in "Tin Cup Canyon", a steep, isolated, central Texas canyon community. To do this we used drone or UAV technology. With success, drone technology could be used in the remaining parts of the Albert and Bessie Kronkosky State Natural Area to determine the total area covered by *A. grandidentatum* and the number of plants present in the entire Natural Area.

METHODS

Previously, a ground level, quadrat survey of five adjacent, but isolated *A. grandidentatum* communities in the Edwards Plateau Physiographic region of central Texas was completed (Figure 1, approximately 29° 44' 25'' N, 98° 50' 18'' W, Van Auken et al. 2017). The communities are located in deep, isolated, limestone canyons (Figure 2) of the 1520 ha (=3757 ac) Albert and Bessie Kronkosky State Natural Area (=ABK throughout), specifically in the "Tin Cup Canyon" (Van Auken et al. 2017).

Domestic grazing was the main industry of the general area including the Natural Area, but in 1998 a 2.4 m high deer fence was constructed and domestic grazing was halted (Carpenter and Brandimarte 2014). The elevation of the study area is 484-614 m a. m. s. and canyon bottom *Acer grandidentatum* communities have relatively deep calcareous silty clay soil (Mollisols over limestone bedrock, SCS 1977). Mean annual temperature is approximately 18.3°C, with a range from near 0.7°C in January to 34.1°C in August, and is highly variable. Mean annual precipitation is 72.4 cm/year with very little in July and August and highly variable with May and September being wettest (World Climate 2011).

Density of overstory *A. grandidentatum* trees and six other species with the highest density found in these communities was determined and will be presented. However, the total area of each community in the previous study was not determined. In the current study the density previously calculated was used for the seven high density species present in the deciduous community (Van Auken et al. 2017). The area

of each community was estimated based on a drone aerial survey, and the mean density of the five deciduous communities previously determined was used to calculate the number of each overstory and understory species in the canyon deciduous communities.

The canyon study areas were first surveyed by conducting a reconnaissance flyover in November 2014 as the canyon deciduous canopy started to change color. Field conditions and site accessibility allowed ground surveying the deciduous woodlands containing the *A. grandidentatum* woody plant population in five adjacent but separate canyon sites beginning in November 2015 and continuing through June of 2016 using the quadrat method (Van Auken et al. 2005).

The number of 25 m^2 quadrats varied in each of the *A. grandidentatum* communities due to site conditions and topography. Adequate sampling was determined by examining species and density stabilization curves but is not presented. There were a total of 223 quadrats or 0.56 ha sampled in the *Acer* communities. All plants greater than 137 cm in height and 3 cm basal diameter were considered trees and part of the overstory. They were identified (Correll and Johnston 1979; USDA 2016) and counted. Five 1 m^2 sub-quadrats were established in each of the 25 m^2 quadrats to measure understory woody plants (one in each corner and one near the center). All woody plants less than 137 cm in height and/or 3 cm basal diameter were identified and counted as seedlings or juveniles. Identity, density, relative density, basal area, and relative basal area were calculated for each overstory species and identity and density was determined for the understory species within each community (Van Auken et al. 2017). Next, means were determined, but only mean species density and relative density are presented here for overstory and understory woody species.

In order to visually distinguish the entire area of the *A. grandidentatum* community, we observed the change of color of the trees. At the point in time when the deciduous species colors could be easily distinguished from other species present the drone survey was started. Airborne Aerial Photography was engaged to fly a DJI Phantom 4 quadcopter (Figure 3) at an altitude of 96.3 m (=316 feet AGL [above ground level] from the point of liftoff) in an east – west pattern across the "Tin Cup Canyon" area of the ABK Preserve (Figure 4). Flight conditions were within the range allowed by a Texas Parks and Wildlife drone operating permit (issued to Justin Moore). This allowed orthoimagery coverage of the entire canyon area previously sampled using the quadrat method on the ground (Figure 5). Cloud cover was 100% reducing the possibility of shadow casts and image stitching errors. Airborne time was just under 12 minutes to cover 18 ha (= 46 acres at a resolution of 4.064 cm or 1.6 inches per pixel). The camera ISO was set at 400 and shutter speed at 1/240 sec.

The captured imagery was then uploaded to Drone Deploy for stitching and then exported as a georeferenced TIFF image. The TIFF was then imported to ArcGIS desktop software. Using the canopy color as a guide, the *A. grandidentatum* communities were outlined to create polygons (Figure 6). Area was calculated using the ArcGIS measurement tools on the resultant polygons. The ground sample areas were measured, then summed to get the actual total deciduous community area in ha, and was multiplied by the specific plant density in plants/ha to determine the number of plants of each species in the entire canyon deciduous woodland community.

RESULTS

The locations of the five deciduous woodland communities sampled in our previous study are identified on the drone photograph (Figure 6). The number of 25 m^2 quadrats examined in the "Tin Cup" deciduous woodland communities was 24-85, with an average of 44.6 quadrats/transect or community (Table 1). Total area sampled was 5,575 m^2 or 0.56 ha with an average area of 0.112 ha/transect or community. The area of the deciduous woodlands estimated from the drone photographs was 1,637-17,089 m^2 (Table 1). Total area of the deciduous woodlands was 38,070 m^2 or 3.81ha.

From the previous study, seven overstory species were found with high density or relative density including the highest that was *A. grandidentatum* (Table 2). Density of *A. grandidentatum* was 788 ± 965 plants/ha or 52% of the total community density of 1,343 ± 987 plants/ha. *Juniperus ashei* had the second highest relative density at 25%, with the other five major species found in these communities having relative densities between one and six percent (Table 2). The number of overstory *A. grandidentatum* plants or trees expected overall in these deciduous communities was 3,002 (Table 2). The number of most of the other species would be expected to increase by the same amount, 3.81 times, which is the total area of the deciduous communities based on the calculation from the drone pictures.

For the understory seven species were found in the previous study with fairly high density or relative density including *A. grandidentatum* (Table 3). Density of understory or juvenile *A. grandidentatum* plants was 176 ± 110 plants/ha or 13% of the total community understory density of 1,407 ± 987 plants/ha. This was the fourth highest relative density (Table 3). *Juniperus ashei* had the seventh highest relative density at 5%, with the other five major species found in these communities having equal or higher densities and relative densities (Table 3). The number of understory *A. grandidentatum* plants or juveniles expected overall in these deciduous communities was 671 (Table 3). The number of most of the other species would be expected to increase by the same amount, 3.81 times, which is the total area of the deciduous communities based on the calculation from the drone pictures.

DISCUSSION

After a literature review, this report seems to be the first study in Texas to determine plant community area using drone technology. It is also the first study to use community area determined from drone derived photographs and ArcGIS software to estimate the number of plants of certain species within a specific community. This unmanned aircraft technology has been used in other areas and in many types of studies including a wide range of applications in applied work and population surveys of both plants and animals (Ogden 2013; Hodgson et al. 2016; Christie et al. 2016; Cruzan et al. 2016).

Drones have been used to monitor insect pests in crops and forests (see Ogden 2013). They have been used to locate small mammal burros (pygmy rabbits) as well as following and counting sandhill crane populations. Animal populations difficult to access and count such as manatees, other marine mammals, as well as various nesting bird populations have been observed using drone technology. Others have used drones to try to reduce the illegal killing of large endangered animals. Plant ecological studies can be done with drones as well and can be similar to what has been done with animals (see Cruzan et al. 2016). However, the species or populations may not seem as spectacular. Habitat maps have been made with relatively small effort but with minimal habitat destruction, especially for marsh or wetland habitats. Drone aerial surveys can be done at various times of the growing season to discern species using seasonal phenology. Diseases can be followed using spectral analysis as can community disturbances and restoration.

Using unmanned aircraft or drones can reduce the high cost of field studies by 90 – 94% or by 10-16.7 times (Ogden 2013). There are suggestions that drone use increases collection efficiency, cost effectiveness and accuracy over ground methods (Hodgson et al. 2016). Regardless, drone or aircraft collected information must be ground-truthed. For the current study, drone flight time was 12 minutes to photograph 18 ha. It took a team of three approximately 30 days (90 man days) in the field to get to the study site in the canyon and count and measure the overstory and understory woody plants in 0.56 ha. These times do not consider man-hours working with the drone photographs or entering field data into the computer and calculating ecological parameters. However, it certainly suggests the efficiency of using drone collected data compared to ground based field work.

Size of native populations are basically unknown, thus it is not really possible to know the correctness or accuracy of a specific procedure to determine a specific population size. However, it is feasible to compare the variance of populations reported by different investigators using the same or different procedures. Lower variance has been reported for a number of population studies when comparable drone counts were examined (Hodgson et al. 2016). Standard deviation calculated for total density or individual species density was high in the current study, probably because of large size differences in the communities examined.

Different personnel have not been used to examine drone derived photographs or ArcGIS measurements to determine the area covered by the deciduous population or compared variance associated with these types of measurements, but this will be considered in the future. It is still difficult to determine the edge of the deciduous woodland community as can be noted by examining Figure 4. *Juniperus ashei* trees and some of the deciduous species inter-digitate at the edge of the upland *Juniperus* community and the deciduous woodland where *A. grandidentatum* is found. In addition, some of the deciduous species of the genus *Quercus* had apparently not changed colors when the current drone study was carried out. This potential error was minimized by drawing limiting lines through the middle of these inter-digitations.

In the future, drone technology will be fused with GIS remote sensing procedures to identify the species in these woodlands from each other to reduce variance and to better estimate population density. This procedure has been used in other studies and can certainly be used in these central Texas remote, relic populations.

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Table 1. Transect or community number, quadrats sampled, area sampled m^2 and ha, total deciduous community in m^2 estimated from drone photographs and the sum of the area sampled using the drone photographs of the deciduous community (m^2 and ha).

Transect number	**Number of quadrats**	**Area (m^2) Sampled**	**Area (ha) Sampled**	**Area (m^2) estimated from drone photographs**
1	44	1,100	0.11	1,637
2	34	850	0.09	7,137
3	36	900	0.09	7,324
4	85	2,125	0.21	17,089
5	24	600	0.06	4,883
Total	223	5,575	0.56	38,070 m^2 = 3.81 ha

Table 2. Overstory species names, mean density (number/ha) ± SD and relative density of seven highest density species found in a previous study of the deciduous woodland community and the number of plants of each species predicted in the total community using drone photographs (3.81 ha x density/ha of the species in the previous community study).

Scientific name	**Density**	**Relative Density**	**Number Predicted**
Acer grandidentatum	788 ± 965	52	3,002
Juniperus ashei	299 ± 195	25	1,139
Prunus serotina	76 ± 70	6	290
Quercus laceyi	58 ± 27	6	221
Aesculus pavia	32 ± 48	4	122
Tilia caroliniana	23 ± 51	1	88
Quercus buckleyi	18 ± 22	1	69
Other species (7)	49	5	187
TOTAL	1,343 ± 987	100	5,118

Table 3. Understory species scientific names, mean density (number/ha) ± SD, relative density of seven of the highest density species found in a previous study of the deciduous woodland community and the number of plants of each species predicted in the total community using drone photographs (3.81 ha x density/ha of the species in the previous community study).

Scientific name	**Density**	**Relative Density**	**Number Predicted**
Quercus buckleyi	289 ± 288	21	1,101
Prunus serotina	229 ± 201	16	872
Quercus laceyi	227 ± 223	16	865
Acer grandidentatum	176 ± 110	13	671
*Smilax bono-nox***	140 ± 113	10	533
Aesculus pavia	73 ± 76	5	278
Juniperus ashei	65 ± 33	5	248
Other species (15)	209	14	796
TOTAL	1,407 ± 987	100	5,364

**woody vine

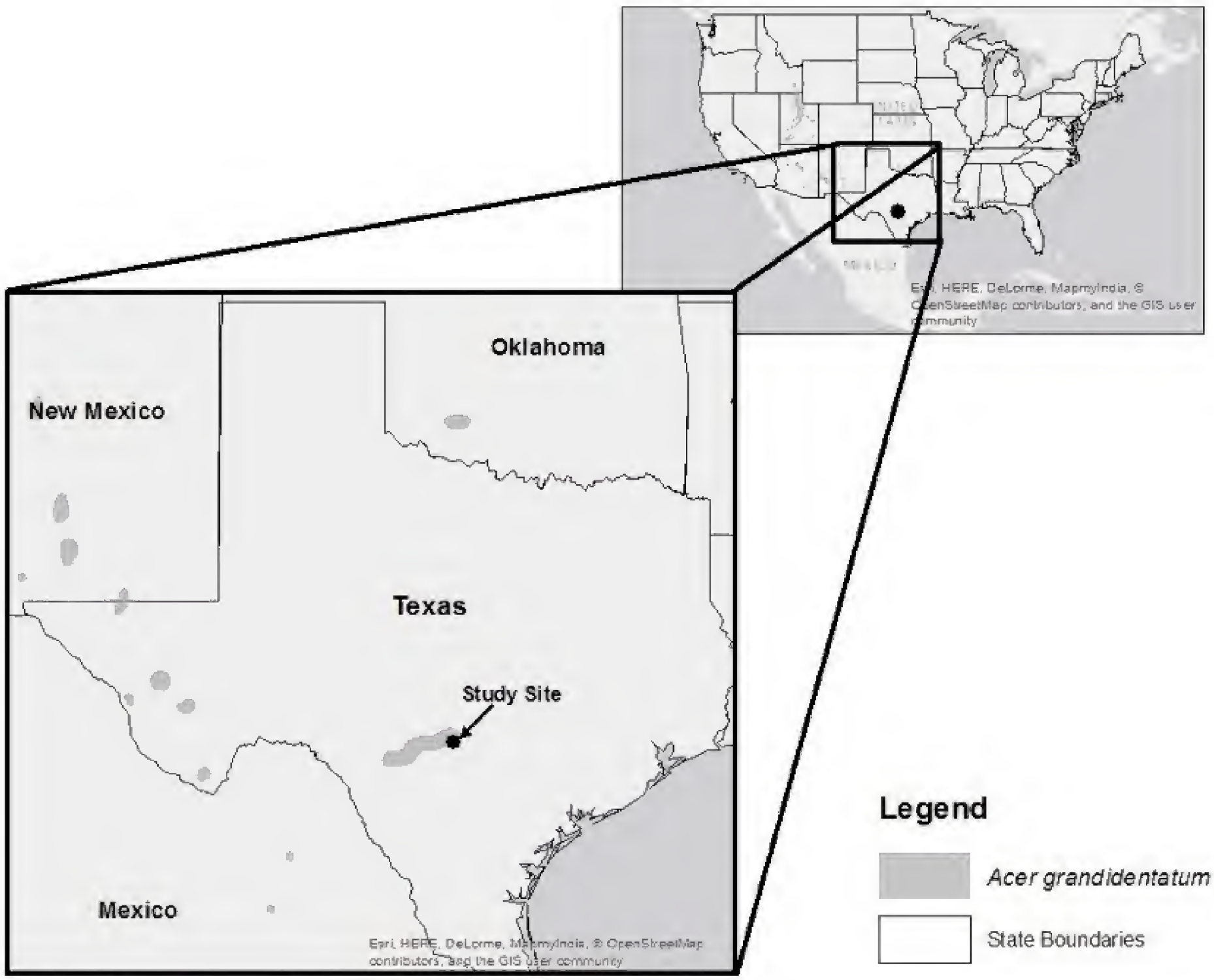

Figure 1. Distribution of *Acer grandidentatum* in Texas and western North America, with the blow-up showing the approximate location of the study site in central Texas. The arrow and the dot is the approximate location of the Albert and Bessie Kronkosky State Natural Area, in the Edwards Plateau Physiographic region of central Texas, approximately 29° 44’ 25’’ N, 98° 50’ 18’’ W.

Figure 2. Photograph (upper) shows an overview of "Tin Cup Canyon" and the understory (lower) of several *Acer grandidentatum* trees in one of the community's surveyed. Note few or no understory juvenile woody plants.

Figure 3. Photograph of the drone (DJI Phantom 4 quadcopter) in the ground transportation (ATV, top left side) and on the landing pad prior to takeoff (top right). Middle photograph shows the crew and the ATV and the photograph was taken from the drone. The bottom photograph shows the landing pad (orange circle) taken from the drone with the authors and transportation on the hilltop adjacent to the "Tin Cup Canyon".

Figure 4. Oblique aerial view (upper photograph) of the study area, mostly "Tin Cup Canyon". Photograph was taken from the drone at an altitude of approximately 100 m on November 15, 2016 showing fall colors of the deciduous community in the canyon. The lower photograph is a close up view of the edge of the trees in the deciduous woodland. The arrow points out a *Juniperus ashei* tree.

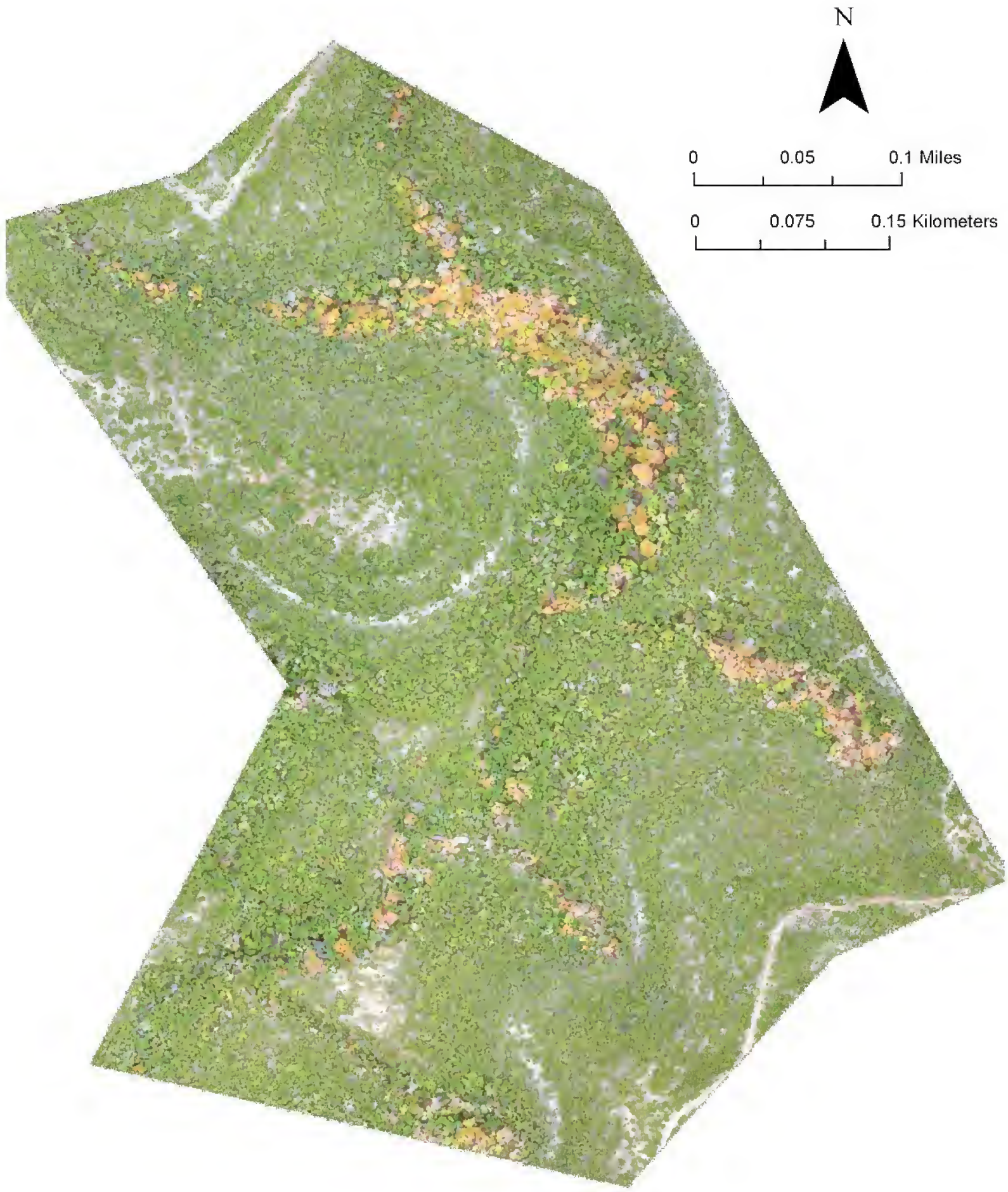

Figure 5. Aerial view of the overall study area from the drone. The deciduous communities are in fall color and at the bottom of "Tin Cup Canyon".

Figure 6. Study area from the drone at approximately 96.3 m (316 ft. AGL). Deciduous communities are outlined in orange with each transect in black and numbered.

A New Senegalia, (*S. alexae*, Fabaceae: Mimosoideae) from Panama, Brazil, and Peru.

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ABSTRACT

Senegalia alexae Seigler & Ebinger (Fabaceae, Mimosoideae) is described. This new species is illustrated and compared to its most probable related species. Published on-line www.phytologia.org *Phytologia 99(3):221-225 (Aug 8, 2017).* ISSN 030319430.

Key Words: *Acacia* s. l., Fabaceae, Mimosoideae, *Senegalia* s. s., sp. nov.

During the past six years the authors have described ten species (Seigler et al. 2012b, 2013b, 2014; Seigler 2014; Seigler and Ebinger 2014, 2015; Ebinger 2017), and two probable hybrids (Seigler et al. 2012a, 2013a) as new to *Senegalia* Rafinesque. Also, from recent morphological and genetic studies we conclude that the genus *Senegalia* was polyphyletic as previously constituted (Seigler et al. 2017). Nine species were removed and placed in the genera *Parasenegalia* Seigler & Ebinger (seven species) and *Pseudosenegalia* Seigler & Ebinger (two species). After these changes, there are approximately 93 species, including four named hybrids, of *Senegalia* in the New World Tropics, 68 in Africa, 45 in Asia, and two in Australia (Maslin et al. 2003a, 2003b; Maslin 2015); of these, eight species occur in two or more areas.

Representatives of both the Old and New World members of *Senegalia* are trees, shrubs, or lianas, armed with prickles, and lack paired stipular spines. The prickles usually are scattered but in some species are grouped in twos or threes, usually at or near the nodes. They also occasionally occur in lines and in a few uncommon examples are fused together into lines. Prickles commonly occur on the petiole and rachis. Leaves are bipinnately compound with 1 to 50+ pairs of pinnae; the pinnae have 1 to 80+ pairs of leaflets. The usually small leaflets are mostly linear to oblong, not exceeding 10 mm in length, but a few species have leaflets that are lanceolate to oblanceolate and exceed 100 mm in length. The petiole and rachis have sessile or stipitate glands in variable positions, though sometimes the glands become specialized and are of variable shape. The structure and shape of the glands is normally consistent within a species and of diagnostic importance. The mostly 5-merous (rarely 4-merous) flowers are campanulate, actinomorphic, synsepalous, sympetalous, with numerous stamens (usually 40 to 100), the filaments are mostly not fused and are attached to a more or less tubular or campanulate nectar disc located on the receptacle of the flower surrounding the base of the mostly stipitate ovary. Inflorescences are globose heads or cylindrical spikes occurring solitary or in small clusters in the leaf axils, or grouped into complex axillary or terminal pseudo-racemes or pseudo-panicles. The legumes are oblong or broadly linear that mostly separate into two valves at maturity. A few species have tardily dehiscent or indehiscent fruits, and some fruits separate into one-seeded loments. The 6 to 20 uniseriate seeds are mostly strongly flattened and have a well-developed pleurogram.

During the course of our work on *Senegalia,* an undescribed species was noted from herbarium material from the state of Madre de Dios in central Peru. Later, we observed another specimen of this new

species from Huánuco, Peru. More recently we have seen specimens from northwestern Brazil and the Darién region of Panama. Material from Panama (*Gentry & Clewell 6928*) is of poor quality, but appears to be a member of this taxon. *Senegalia alexae* is clearly distinct and is here proposed as a new species.

Senegalia alexae Seigler & Ebinger, sp. nov. TYPE: Peru, Madre de Dios: Manú, Parque Nacional Manú, Cocha Juárez, Rio Manú, 400 m, Arbol 10 m, flores blancas, (fl), 3-5 May 1987, *P. Núñez, A. M. Lees & S. D. Wright 8004* (holotype: MO). (Figure 1).

Diagnosis. *Senegalia alexae* Seigler & Ebinger is superficially similar to *S. aristeguietana* (L. Cárdenas) Seigler & Ebinger, a species with an overlapping range (Seigler and Ebinger 2012), but may easily be distinguished by having scattered yellow, erect hairs to 0.8 mm long on the twigs, petiole, rachis, and rachilla. It also differs from *S. aristiguietana* by having shorter petioles (13-25 mm vs. 25-55 mm), fewer pinna pairs (8 to 16 vs. 15 to 30), and smaller flowers (3.5-4.5 mm vs.4.5-6.5 mm). *Senegalia alexae* and *S. aristeguietana* are closely associated with members of the *S. amazonica* species group.

Liana or **tree** to 15 m tall; bark not seen; twigs dark purplish brown, not flexuous, terete to ridged, puberulent and with scattered yellowish hairs to 0.8 mm long; short shoots absent; prickles purplish brown throughout to slightly darker near apex, flattened, straight to mostly recurved, woody, 1-4 x 1-6 mm at the base, puberulent at least below, persistent, scattered along the twig, petiole, and rachis. **Leaves** alternate, 80-150 mm long; stipules light to dark brown, lanceolate, symmetrical, flattened, straight, herbaceous, 3.5-4.8 x 0.8-1.4 mm near the base, usually glabrous, early deciduous; petiole shallowly adaxially grooved, 13-25 mm long, puberulent and with scattered yellow hairs to 0.8 mm long; petiolar gland solitary, located mostly near the lower third of the petiole, sessile, oblong, 0.8-2.4 mm long, apex flattened to slightly bulbous when young, becoming more bulbous and wrinkled when mature, glabrous; rachis adaxially grooved, 50-130 mm long, puberulent and with scattered yellow hairs to 0.8 mm long, an orbicular gland 0.5-1.2 mm across between the uppermost 7 to 11 pinna pairs, apex depressed, glabrous; pinnae 8 to 16 pairs/leaf, 25-65 mm long, 6-11 mm between pinna pairs; paraphyllidia 0.3-0.8 mm long, commonly absent; petiolule 1-2 mm long; leaflets 39 to 59 pairs/pinna, opposite, 0.6-1.1 mm between leaflet pairs, linear, 3.5-7.1 x 0.8-1.3 mm, glabrous or nearly so on both surfaces, lateral veins usually not obvious, 1 vein from the base, base oblique, truncate on one side, margins ciliate, apex acute, midvein submarginal. **Inflorescence** a densely 23- to 38-flowered globose head 8-11 mm across, in terminal pseudo-paniculate clusters, the main axis to 35 mm long; peduncles 6-12 x 0.3-0.5 mm thick, densely puberulent; receptacle not enlarged; involucre absent; floral bracts spatulate, 0.4-0.7 mm long, puberulent, early deciduous.

Flowers sessile, white; calyx 5-lobed, 0.8-1.3 mm long, puberulent; corolla 5-lobed, 1.4-1.9 mm long, puberulent, lobes one-quarter the length of the corolla; stamens 50 to 80, stamen filaments 3.5-4.5 mm long, distinct; anther glands present; ovary pubescent, stipe to 1.1 mm long. **Legumes** straight, flattened, not constricted between the seeds, oblong, 100-150 x 18-26 mm wide, coriaceous, transversely striated, puberulent, eglandular, dehiscent along both sutures; stipe 3-6 mm long; apex obtuse, not beaked. **Seeds** not seen.

Conservation Status: This rare species is known from only six widely scattered collections (nine specimens) in northwestern South America and adjacent Panama. Because the wet tropical forest habitat for *Senegalia alexae* is rapidly disappearing, and because there are few collections of *Senegalia alexae*, this taxon should probably be listed as threatened (IUCN 2017).

Distribution and ecology: Wet tropical forests in alluvial soil and disturbed second growth forest from sea level to 500 m in northwestern Brazil (Amazonas), eastern Panama (Darién) and in central and southeastern Peru (Huánuco and Madre de Dios).

Phenology: May-June (Brazil and Peru), December (Panama).

Etymology: *Senegalia alexae* is named for Ms. Alexa Musgrove, the artist who prepared the drawings used to illustrate many of the *Senegalia* and *Vachellia* species we have studied.

Representative Specimens: PANAMA: Darién: Río Pirre near town of Pirre, 27 Dec 1972, *A.Gentry & A.Clewell 6928* (F, GH, MO, NY). (vine on label). **BRAZIL:** Amazonas: Bocca do Tejo, May 1901, *E. Ule 5480* (HBG). **PERU:** Huánuco: Panguana, Río Yuyapichis floodplain, 74°56'W, 9°37'S, 4 Jun 1983, *F.G.Seidenschwarz 5417* (F). (tree to 12 m tall, 20 cm dbh. on label). Madre de Dios: Cocha Cashu Biological Station, Manu National Park, floodplain, 71°22'W, 11°52'S, 400 m, 14 Aug 1983, *A.Gentry 43575* (MO). (canopy liana on label). Tambopata, Cuzco Amazonico, mature floodplain, 69°3'W, 12°5'S, 200 m, 20 Jun 1989, *O.Phillips, P.Núñez & N.Jaramillo 520* (MO). (liana with a dbh of 12-20 mm on label).

DISCUSSION

Senegalia alexae is included in a group of species morphologically similar to *S. amazonica*. The presence of a solitary, glabrous, sessile, oblong petiolar gland mostly located on the lower third of the petiole, that usually becomes bulbous and wrinkled when mature, indicates a relationship to these species. Members of this group of species have inflorescences with globose heads or cylindrical spikes occurring in complex axillary or terminal pseudo-racemes or pseudo-panicles. The taxa of the *S. amazonica* species group with flowers in globose heads are keyed below.

Key to members of the *Senegalia amazonica* species group with flowers in globose heads.

a. Prickles fused in continuous lines along twig ridges (se. Brazil)..... *S. serra*
a. Prickles scattered or in lines but not fused to each other.
b Petiolar gland mostly volcano-shaped, inflorescence 20-27 mm across (ne. Brazil) ..... *S. globosa*
b. Petiolar glands flat to globose to bulbous, not volcano-shaped; inflorescence 9-18 mm across.
c. Pinnae 2 to 4 pairs/leaf; leaves less than 100 mm long (se. Brazil) ..... *S. pteridifolia*
c. Pinnae 8 to 30 pairs/leaf; leaves mostly longer (Mexico to n. South America)
d. Petiole 13-25 mm long, puberulent and with scattered yellowish hairs to 0.8 mm long ..... *S. alexae*
d. Petioles 25-125 mm long, glabrous to pubescent, lacking long, scattered yellowish hairs.
e. Leaflets 0.5-1.2 mm wide, 50 to 90 pairs/pinna ..... *S. aristeguietana*
e. Leaflets 1.3-4.0 mm wide, 20 to 45 pairs/pinna.
f. Pinnae 8 to 13 pairs/leaf; inflorescence 13-18 mm across ..... *S. membranacea*
f. Pinnae 11 to 24 pairs/leaf; inflorescence 9-12 mm across ..... *S. croatii*

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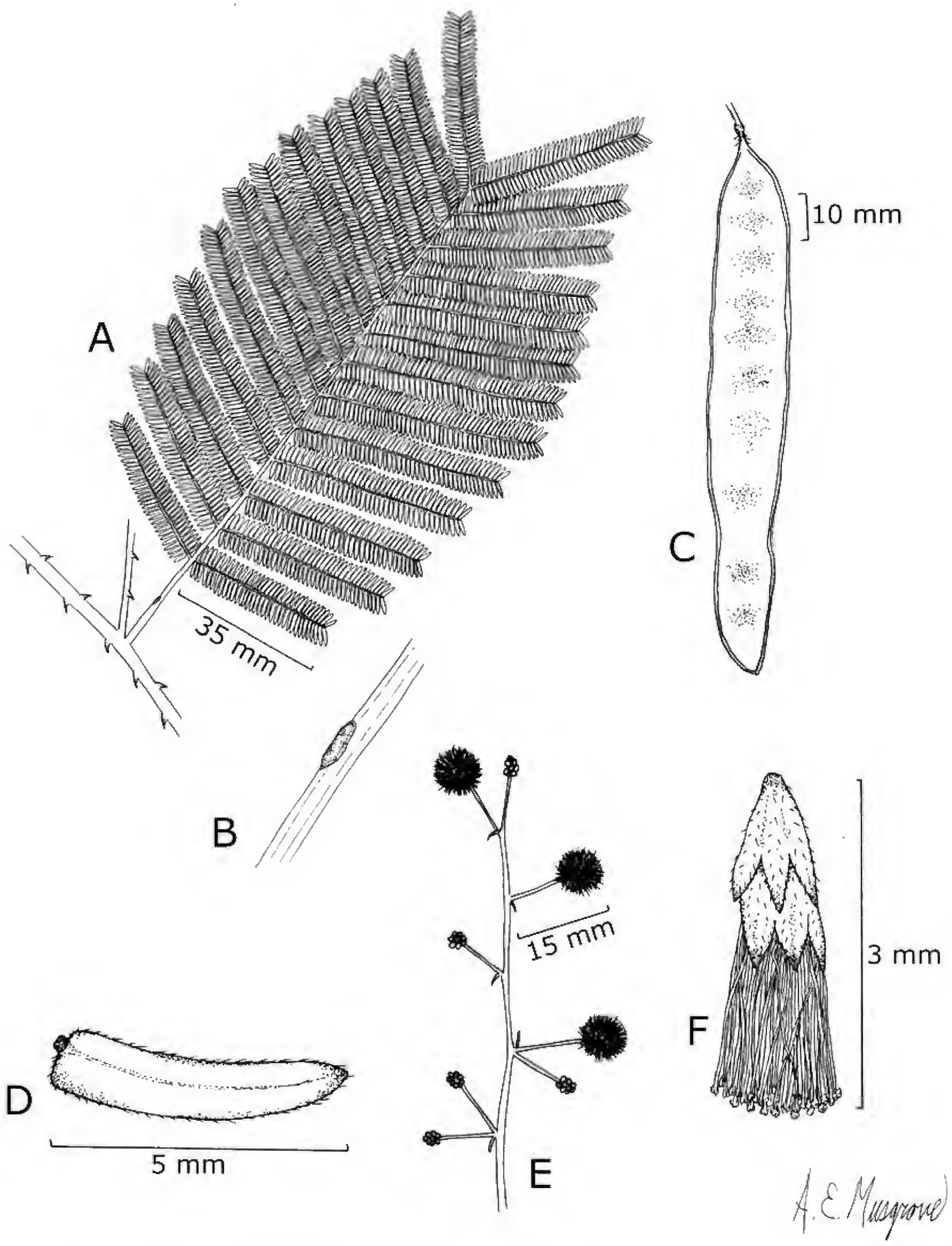

Figure 1. *Senegalia alexae* Seigler & Ebinger. A. Leaf. B. Petiolar gland. C. Fruit. D. Leaflet (abaxial view). E. Stem with pseudoinflorescences. (A, B. *A. Gentry, 43575*, MO; C - F. *G. Seidenschwarze, 5411*).