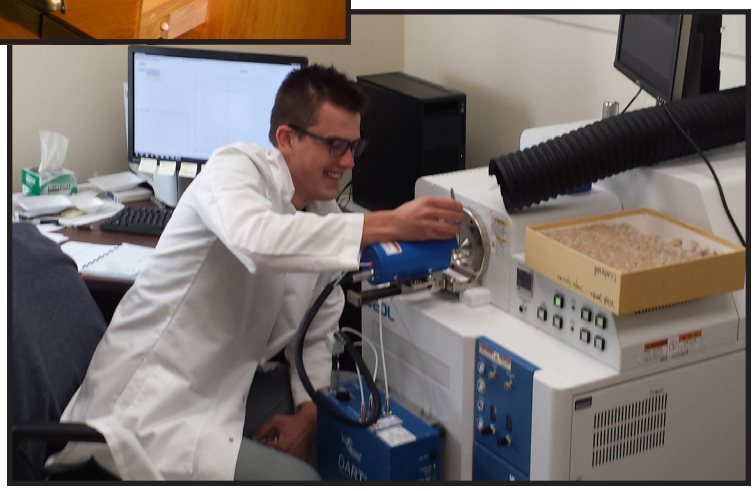
USDA
United States Department of Agriculture

Species Verification of *Dalbergia nigra* and *Dalbergia spruceana* Samples in the Wood Collection at the Forest Products Laboratory

Michael C. Wiemann
Edgard O. Espinoza



Forest Service | Forest Products Laboratory | Research Paper FPL–RP–690 | March 2017

Abstract

To evade endangered timber species laws, unscrupulous importers sometimes attempt to pass protected *Dalbergia nigra* as look-alike, but unprotected, *Dalbergia spruceana*. Wood density and fluorescence properties are sometimes used to identify the species. Although these properties are useful and do not require special equipment, they may be less reliable than direct analysis in real time–time of flight mass spectrometry (DART–TOFMS). DART–TOFMS was used to check identities of the *D. nigra* and *D. spruceana* samples contained in the wood collection of the Forest Products Laboratory (Madison, Wisconsin). Based on DART-TOFMS, voucher-backed heartwood samples of the two species are all correctly named, but some of the unvouchered samples are not.

Keywords: *Dalbergia nigra*, *Dalbergia spruceana*, caviunin, CITES, dalnigrin, DART-TOFMS, density, mass spectrometry, principal components analysis, wood anatomy, wood identification

Acknowledgments

Steve Verrill and Samuel Glass of the Forest Products Laboratory and Peter Gasson of Kew Botanic Gardens gave critical reviews that greatly improved the manuscript. Geoffrey Kite of Kew Botanic Gardens and Thomas Eberhardt of the Forest Products Laboratory also commented and suggested corrections and improvements. Barbara Hogan's careful editing improved the readability of the paper. The cover shows Regis Miller examining samples in the Madison wood collection (upper photo) and Victor Deklerck holding a wood sliver at the mass spectrometer (lower photo).

March 2017

Wiemann, Michael C.; Espinoza, Edgard O. 2017. Species verification of *Dalbergia nigra* and *Dalbergia spruceana* samples in the wood collection of the Forest Products Laboratory. Research Paper FPL–RP–690. Madison, WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory. 10 p.

A limited number of free copies of this publication are available to the public from the Forest Products Laboratory, One Gifford Pinchot Drive, Madison, WI 53726-2398. This publication is also available online at www.fpl.fs.fed.us. Laboratory publications are sent to hundreds of libraries in the United States and elsewhere.

The Forest Products Laboratory is maintained in cooperation with the University of Wisconsin.

The use of trade or firm names in this publication is for reader information and does not imply endorsement by the United States Department of Agriculture (USDA) of any product or service.

In accordance with Federal civil rights law and U.S. Department of Agriculture (USDA) civil rights regulations and policies, the USDA, its Agencies, offices, and employees, and institutions participating in or administering USDA programs are prohibited from discriminating based on race, color, national origin, religion, sex, gender identity (including gender expression), sexual orientation, disability, age, marital status, family/parental status, income derived from a public assistance program, political beliefs, or reprisal or retaliation for prior civil rights activity, in any program or activity conducted or funded by USDA (not all bases apply to all programs). Remedies and complaint filing deadlines vary by program or incident.

Persons with disabilities who require alternative means of communication for program information (e.g., Braille, large print, audiotape, American Sign Language, etc.) should contact the responsible Agency or USDA's TARGET Center at (202) 720–2600 (voice and TTY) or contact USDA through the Federal Relay Service at (800) 877–8339. Additionally, program information may be made available in languages other than English.

To file a program discrimination complaint, complete the USDA Program Discrimination Complaint Form, AD-3027, found online at http://www.ascr.usda/gov/complaint_filing_cust.html and at any USDA office or write a letter addressed to USDA and provide in the letter all of the information requested in the form. To request a copy of the complaint form, call (866) 632–9992. Submit your completed form or letter to USDA by: (1) mail: U.S. Department of Agriculture, Office of the Assistant Secretary for Civil Rights, 1400 Independence Avenue, SW, Washington, D.C. 20250–9410; (2) fax: (202) 690–7442; or (3) email: program.intake@usda.gov.

USDA is an equal opportunity provider, employer, and lender.

Species Verification of *Dalbergia nigra* and *Dalbergia spruceana* Samples in the Wood Collection of the Forest Products Laboratory

Michael C. Wiemann, Botanist
Forest Products Laboratory, Madison, Wisconsin

Edgard O. Espinoza, Deputy Director
National Fish and Wildlife Forensic Laboratory, Ashland, Oregon

Introduction

Institutional wood collections are the primary sources of research material for wood anatomy. They are essential to validate the numerous wood identifications requested by scientists, industry, and the public. The wood collection of the USDA Forest Service, Forest Products Laboratory (FPL) in Madison, Wisconsin, maintained by the Center for Wood Anatomy Research (CWAR), was started in 1910 with the opening of FPL (Miller 1999) and the hiring of the first female scientist in the Forest Service, Eloise Gerry (Wayne 2011). The collection grew slowly; most of the original samples were woods of the United States that were discards from expositions and fairs (McBeath 1978). Samples were gradually added, and in the 1940s, when it contained some 11,000 samples, newly hired curator B.F. Kukachka reorganized the collection into a system in which samples were arranged by family, genus, and species instead of by accession number. This reorganization facilitated the comparison of multiple samples of a taxon, but in the process Kukachka discarded several thousand samples that lacked voucher documentation (Miller 1999). Only two samples of *D. nigra* (Vell.) Allem. ex Benth. (MAD7017 and MAD10769; Table 1), both vouchered, survived the purge. Additional samples of *D. nigra* (some vouchered, some not) were subsequently added to the Madison collection (Table 1).

Accurate identification of *D. nigra* wood is especially important because commercial trade of the species without special permits is prohibited by the Convention on International Trade in Endangered Species (CITES) (Environment Canada 2002). Ensuring accurate identification requires knowledge of the range of variability that can be found in the species; this requires many samples for comparison.

The supply of *D. nigra* samples at FPL was replenished by the transfer of Yale University's Samuel J. Record collection (55,000 wood samples) in 1969 (Stern 1973; Miller 1999) and the Chicago Field Museum collection (20,000 wood samples) in 1971 (Williams 1971; Miller 1999). Some of the transferred samples were voucher-backed although most were not, and some were duplicates of samples already in the collection in Madison. The samples from the Field Museum were integrated into the Madison collection (MAD), although the herbarium vouchers remained at the Field Museum (Williams 1971). The wood collection from Yale (SJR) has been kept separate, and its herbarium vouchers were transferred to Madison; they are now in the herbarium of the University of Wisconsin (WIS), kept as a separate collection. Information pertaining to the SJR wood samples is kept in card catalogues in the CWAR, and information pertaining to the MAD wood samples can be found online (FPL 2017). The CWAR now has about 30 samples of *D. nigra*, which is fortunate because species protected by CITES (CITES 2016), such as *D. nigra*, would be difficult to replace. For unvouchered samples to be useful, however, their identity must be verified.

Gasson and others (2010) compared the anatomy of *D. nigra* with other species of the genus *Dalbergia* with which it might be confused. For the Brazilian species, they concluded that it could be distinguished from *D. decipularis* Rizzini & Matt. based on color, from *D. cearensis* Ducke based on vessel frequency, and from *D. miscolobium* Benth. based on ray frequency. However, no anatomical characters could consistently separate *D. nigra* from *D. spruceana* Benth.

Separation of *D. nigra* from *D. spruceana* based on physical properties was the subject of a report by Miller and Wiemann (2006), and separation based on physical properties and mass spectrometry is the subject of this one. Miller and Wiemann (2006) used samples from the FPL collection to find physical characteristics that could be used to distinguish *D. nigra* from the look-alike species *D. spruceana*, which has no CITES restrictions. The physical properties used to separate the two species were

Table 1—*Dalbergia nigra* and *Dalbergia spruceana* samples in the Forest Products Laboratory wood collection[a]

Collection	No.	Species label	Location notes	Voucher	Sample notes
MAD	7017	*D. nigra*	Bahia	WIS	Collected by H.M. Curran, 6; botanical material identified by Dr. Blake; =SJR4146
MAD	10769	*D. nigra*	—	WIS	Collected by H.N. Whitford, 76; received from Smithsonian Institute, 3/6/1928; =SJR3273
MAD	13091	*D. nigra*	Espirito Santo	unknown	Brazilian Forest Service 6; Nov. 1949
MAD	18588	*D. spruceana*	Rio Juruá, Amazonas	NY	Collected by B.A. Krukoff, 4921, 1933; Fourth expedition to Brazil, received from Syracuse
MAD	21010	*D. nigra*	Brazil	—	From Fred C. Ness, National Rifle Association of America, Washington, DC, June 24, 1935; received from Field Museum in 1971
MAD	23334	*D. nigra*	Brazil	—	Gift Set 57 from Instituto Florestal de Sao Paulo; received from Field Museum in 1971
MAD	31954	*D. nigra*	Brazil	—	Colombian Exposition, 1893; received from Field Museum in 1971, #01050
MAD	31955	*D. nigra*	Brazil	—	Colombian Exposition, 1893; received from Field Museum in 1971, #01132
MAD	31956	*D. nigra*	Brazil	—	Colombian Exposition, 1893; received from Field Museum in 1971, #01133
MAD	31957	*D. nigra*	Brazil	—	Collected by Hildebrand, 17; received from Field Museum in 1971, #621818
MAD	31958	*D. nigra*	Rio de Janeiro	F, RB	Collector unknown, 19; received from Field Museum in 1971, #622879
MAD	31968	*D. spruceana*	Brazil	WIS	Collected by A. Ducke, 150; received from Field Museum in 1971, #614338; =SJR22610
SJR	550	*D. nigra*	—	—	Veneer
SJR	1430	*D. spruceana*	Lower Amazon	—	Brewer collection, 192; identified by S.J. Record
SJR	1442	*D. nigra*	Lower Amazon	—	Brewer collection, 231; identified by S.J. Record, April 26, 1941
SJR	3107	*D. nigra*	—	—	Donated August 1918 by Mr. Hargreaves of Alves Vasconcellos & Co., Rio de Janeiro, to H.N. Whitford; identified by S.J. Record, Oct. 1940
SJR	3222	*D. nigra*	Espirito Santo	—	Collected by H.N. Whitford, 42, July, 1918 at the mill of the Société Forestière et Industrielle de Collantina; identified by S.J. Record, Aug. 6, 1939
SJR	3273	*D. nigra*	Espirito Santo	WIS	Collected by H.N. Whitford, 76 at Collantina, July 1918; identified by S.J. Record, Dec. 1, 1940; =MAD10769
SJR	3301	*D. nigra*	Escura, Minas Gerais	—	Trade sample collected by H.N. Whitford, 1, 1918; identified by S.J. Record, Dec. 1, 1940
SJR	3302	*D. nigra*	Escura, Minas Gerais	—	Trade sample collected by H.N. Whitford, 2, 1918; identified by S.J. Record, Dec. 1, 1940
SJR	3303	*D. nigra*	Minas Gerais	—	Trade sample collected by H.N. Whitford, 3, 1918
SJR	3508	*D. nigra*	Pedras Pretas, Bahia	—	Collected by H.M. Curran, 370, 1918
SJR	3509	*D. nigra*	Pedras Pretas, Bahia	WIS	Collected by H.M. Curran, 370A, 1918; identified by Samuel J. Record, 1938
SJR	3525	*D. nigra*	Jequie, Bahia	—	Collected by H.M. Curran, 386, 1918; identified by Samuel J. Record, July, 1938
SJR	4014	*D. spruceana*	Pará	—	Museu Goeldi, sample 12
SJR	4146	*D. nigra*	Rio Grongogy, Bahia	WIS	Collected by H.M. Curran, 6; =MAD7017

Table 1—*Dalbergia nigra* and *Dalbergia spruceana* samples in the Forest Products Laboratory wood collection[a]—continued

Collection	No.	Species label	Location notes	Voucher	Sample notes
SJR	4230	*D. nigra*	—	—	Donated to C.M. Richards by a missionary, August, 1920; identified by Samuel J. Record, Dec. 1, 1940
SJR	4296	*D. nigra*	Catete, Bahia	—	Donated by Henry J. McCall to E.C.M. Richards of NY, 1920; identified by S.J. Record, July 1938
SJR	4452	*D. nigra*	—	—	Donated by the J. H. Monteath Co., NY
SJR	5900	*D. nigra*	—	—	Donated by C.H. Pearson, NY
SJR	5990	*D. nigra*	—	—	Donated by W.W. Rowlee, Cornell; identified by S.J. Record
SJR	6001	*D. nigra*	—	—	Donated by W.W. Rowlee, Cornell; identified by S.J. Record
SJR	22610	*D. spruceana*	Brazilian Amazon	WIS	Collected by A. Ducke, 150; received January 14, 1933; =MAD31968
SJR	32586	*D. nigra*	—	—	Donated by the J. H. Monteath Co., NY, June 13, 1936; identified by S.J. Record
SJR	36063	*D. nigra*	Espirito Santo	—	Donated by Paulo F. Souza, Ministerio da Agricultura, December 9, 1938
SJR	38188	*D. nigra*	—	—	Donated by W.J. Hutchinson, NY, June 1, 1940
SJR	38189	*D. nigra*	—	—	Donated by W.J. Hutchinson, NY, June 1, 1940
SJR	38248	*D. spruceana*	Pará	—	Donated by W.J. Hutchinson, NY, from Ministerio da Agricultura, Serviço Florestal, Rio de Janeiro, June 1940
SJR	53033	*D. nigra*	Minas Gerais	WIS	Collected by Col. H.S. Irwin, 2025; 4.5-cm bark-covered branch or small stem

[a]Samples labelled MAD are from the Madison collection, and samples labelled SJR are from the Samuel J. Record collection. The collection location, voucher, and sample notes give the information included on the Center for Wood Anatomy Research website or in collection files (for the MAD samples), on the note cards maintained in the Center for Wood Anatomy Research (for the SJR samples), or from direct observation of the samples. The location of the herbarium vouchers for the samples that have them are the University of Wisconsin in Madison (WIS), New York Botanical Garden (NY), Field Museum of Natural History, Chicago (F), or Jardim Botânico do Rio de Janeiro (RB). An equal sign before a sample number in the Sample Notes column indicates that a duplicate is also included in the MAD or SJR collection.

air-dry density and fluorescence under ultraviolet (UV) light. They concluded that the density of air-dry (6%–7% moisture content) samples of *D. nigra* was less than 1 g/cm^3, whereas the density of *D. spruceana* was greater than or equal to 1 g/cm^3. Water extract of *D. nigra* did not fluoresce, whereas water extract of *D. spruceana* had a blue fluorescence. Ethanol extract of *D. nigra* had greenish-blue fluorescence, and ethanol extract of *D. spruceana* had blue fluorescence.

In the spectrometric method that we used for this study, direct analysis in real time–time-of-flight mass spectrometry (DART–TOFMS), ions are created, detected, and analyzed using a mass spectrometer. The method is described in more detail in Cody and others (2005, 2012) and Espinoza and others (2015), but essentially, the molecules in a wood sample that is held in an ion stream transfer protons to the chemical compounds that are encountered in the sample, producing characteristic protonated signals, which are directed to the TOFMS. The method requires only that a sliver of wood be placed between the DART and the TOFMS inlet for a few seconds, which allows molecules to be removed from the wood surface and drawn into the mass spectrometer. Advantages of DART are that it is used in open air, does not require difficult sample preparation, needs no radioactive components, and gives instant results (Cody 2013; Cody and Dane 2010; Cody and others 2005; Harris and others 2011). It has been used previously to separate American species of *Dalbergia* (Lancaster and Espinoza 2012; Espinoza and others 2015).

The DART–TOFMS spectra are then analyzed statistically using multivariate analyses such as principal component analysis (PCA). In its simplest form, PCA attempts to explain data structure by projecting data onto perpendicular axes, such that the variability of the data along the first axis is maximized, and each succeeding axis accounts for the greatest amount of residual variance. In this way, it attempts to approximate masses of data by relatively few explainable components (Johnson and Wichern 2007). Sometimes a mass of data cannot be simplified in this way. In such a case, the structure might be simplified by mapping the data onto coordinate axes that are obtained by a nonlinear transformation. Kernel principal component analysis

(KPCA) is a technique that can perform such a transformation, thereby revealing structure that is not revealed by simple PCA and extracting features that are useful for classification and pattern recognition (Baudat and Anouar 2000; Hastie and others 2009; Schölkopf and others 1999).

Materials and Methods

A list of all *D. nigra* and *D. spruceana* samples at FPL was compiled using the online database (FPL 2017) for the MAD samples or by searching the card catalogues for the SJR samples. The list of samples is given in Table 1, although not all the samples could be found. All samples that could be found of the two species were assembled for examination and measurement.

For the samples for which physical properties were not previously reported, density was measured as weight in grams divided by volume in cubic centimeters and volume was measured by water displacement or with a ruler (or calipers in the case of veneer thickness). The samples were all at the ambient equilibrium moisture content (EMC) of the wood collection. When original densities were measured, EMC was 6% to 7% (Miller and Wiemann 2006). In 2012, the wood collection was moved from the fourth floor to the first floor of the main building at FPL. To see if the new location had a significant effect on the moisture content of the wood samples, EMC of a selection of eight woods was determined by weighing, drying at 103 °C, then reweighing. The EMC range of these eight woods was 7% to 9%.

Water and ethanol fluorescence were measured as described in Miller and Wiemann (2006). Each fluorescence sample was prepared by placing a few shavings in a small vial, adding 2 or 3 mL of water or ethanol, shaking the vial vigorously, and observing the color of the extract under UV illumination, using a 100-W, 2-A, long-wave (340–380 nm) professional UV lamp.

Heartwood samples identified as either *D. nigra* or *D. spruceana* were analyzed using DART–TOFMS. This was done by holding an approximately 1-mm-thick, 2- to 4-cm-long wood sliver, split in the longitudinal direction from a sample block, in the gas stream with no further sample preparation. Although sapwood samples were included in the report by Miller and Wiemann (2006), their exudations require that the mass spectrometer be cleaned frequently. Therefore, results from sapwood samples are not included in this study. *Dalbergia* samples that are entirely sapwood are usually of no commercial value, although it is common to find artifacts that include both sapwood and heartwood.

The method used is the same as that described in Espinoza and others (2015). In summary, mass spectra were acquired using a DART–SVP ion source (IonSense, Saugus, Massachusetts) coupled to a JEOL AccuTOF TOFMS (JEOL USA, Peabody, Massachusetts) in positive ion mode. Spectra were obtained across the mass-to-charge ratio (m/z) range of 60 to 1000 at one scan per second. A mass calibration standard of polyethylene glycol 600 (Ultra, Kingstown, Rhode Island) was run between samples. Software from TSSPro3 (Shrader Analytical Labs, Detroit, Michigan) and Mass Mountaineer (RBC Software, Peabody, Massachusetts) was used to export files, perform statistical analyses, and plot results.

Results and Discussion

Table 1 shows the 33 samples labelled *Dalbergia nigra* and the six labelled *Dalbergia spruceana* that are listed as being in the CWAR collection. Two of these could not be found (SJR3508 and SJR4146), two were all sapwood (samples 20 and 34), and one was a 4.5-cm-diameter sapwood branch (sample 33).

Figure 1 is a heat map for *Dalbergia* heartwood samples. Caviunin, with m/z of 375 (374.346; ILDIS and CHCD 1994b), is prominent in *D. nigra* but faint or absent in *D. spruceana*, and elemicin, with m/z of 208 (208.257; ILDIS and CHCD 1994b), is present in *D. spruceana* but not in *D. nigra*.

Table 2 gives the air-dry density, fluorescence response, and DART–TOFMS results of each sample tested. The identity of each sample for each test is also given, in plain font when it is the same as that listed by the wood collection data (Table 1), in bold when it is different, or as a question mark when the test was inconclusive.

The results of the KPCA are plotted in Figure 2. Only the first two principal components (PC1 and PC2) are plotted, but they are sufficient to separate the data into interpretable groups. Samples with PC1 values greater than 0 were *D. nigra*, and samples with PC1 values less than –0.4 were *D. spruceana*. Two samples, 15 and 29, had PC1 values of –0.17 and –0.25, respectively. Therefore, it was not clear to which species they should be assigned (Table 2).

The results of the PCA are plotted in Figure 3. As in Figure 2, only the first two principal components (PC1 and PC2) are plotted. Visually, the separation provided by the PCA is better than that provided by the KPCA. In the PCA case, after samples 12, 27, and 28 were corrected (we believe) to *D. spruceana*, the *D. nigra* samples all had PC1 values of less than 1.3, whereas the *D. spruceana* samples all had PC1 values of greater than 4.7.

For KPCA, the first principal component accounted for 20% of the variability in the data, the second accounted for another 11%, and a third accounted for 9%. For PCA, the first principal component accounted for 33% of the variability in the data, the second accounted for another 15%, and the third accounted for another 9%. Based on the proportions of the data explained by the principal

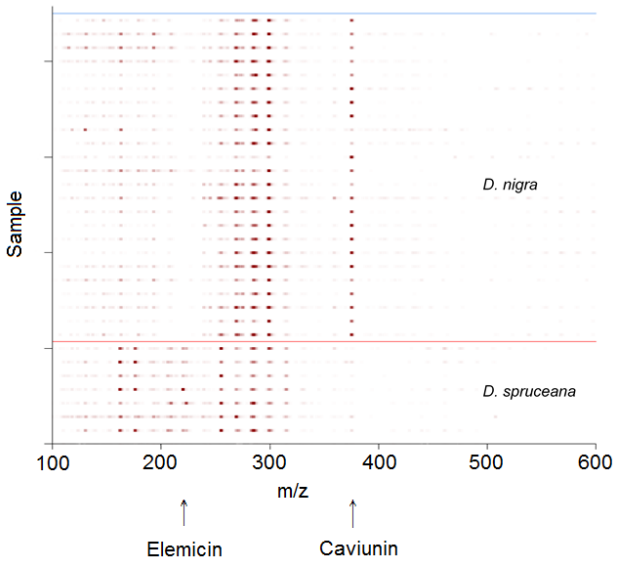


Figure 1. Graphical representation (heat map) of the spectral results comparing the heartwood of *Dalbergia nigra* (24 samples, between blue line and red line) with that of *Dalbergia spruceana* (seven samples, below red line). The abscissa, m/z, is the mass-to-charge ratio of the compound detected (its molecular mass divided by its electrical charge), and the ordinate contains the individual samples. The compound elemicin, indicated by the arrow on the left, is prominent in *D. spruceana* but faint or absent in *D. nigra*. The compound caviunin, indicated by the arrow on the right, is prominent in *D. nigra* but faint or absent in *D. spruceana*.

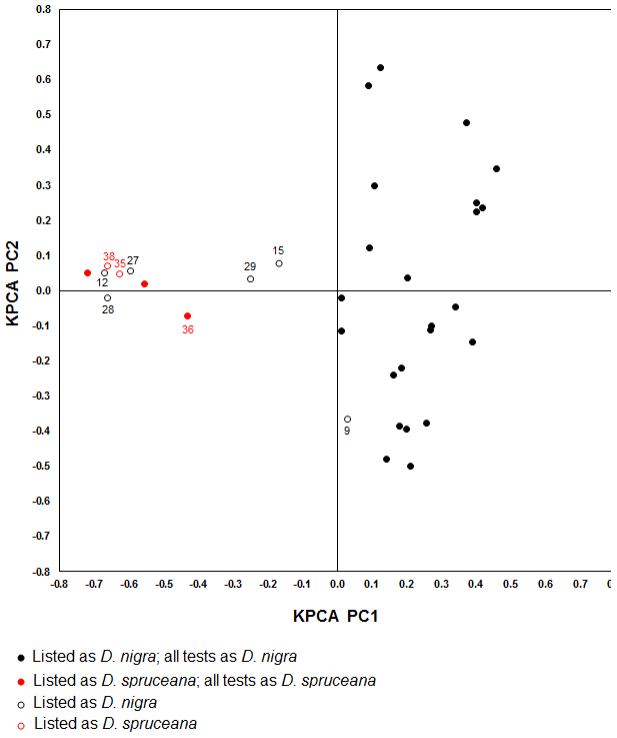


Figure 2. Values of the first two principal components (PC1 and PC2) extracted using kernel principal component analysis (KPCA) for each of the *Dalbergia* samples analyzed using direct analysis in real time–time of flight mass spectrometry (DART–TOFMS). Numbers above or below open circles refer to sample numbers in Table 2.

components and by comparison of the separations seen in Figures 2 and 3, we concluded that the kernel approach did not result in a better separation of the two species.

Most of the samples were judged, on all tests, to be as originally identified (Table 2). Some, however, were not. Three unvouchered samples, 12, 27, and 28, labelled as *D. nigra*, were all identified as *D. spruceana* by density, water fluorescence, ethanol fluorescence, and DART–TOFMS (Table 2). Therefore, we concluded that they were misidentified when they were added to the SJR wood collection (Table 2). They were all trade (nonvouchered) samples identified by Samuel J. Record based on wood anatomy (Table 1). Sample 27 (SJR5990) was also tested by Kite and others (2010) and found to lack dalnigrin but to contain pseudobaptigenin, also indicating that it is not *D. nigra* but rather *D. spruceana*.

Sample 9, labelled *D. nigra* and also unvouchered, had the density of *D. spruceana* but the water fluorescence, ethanol fluorescence, and DART–TOFMS results, including the prominent peaks of caviunin and dalnigrin, characteristic of *D. nigra* (Table 2; Fig. 4). Miller and Wiemann (2006) concluded that this sample was *D. spruceana*, based mainly on density, although they recognized that it had the fluorescence of *D. nigra*. The sample came from the Field Museum and has a collector's number (Hildebrand 17; Table 1), but it is unknown if it was identified based on floral or vegetative material or was a trade sample identified only on wood characteristics. It has the mixture of vasicentric, aliform, diffuse-in-aggregates, and apotracheal banded parenchyma that is characteristic of the genus *Dalbergia*. A comparison of the spectrum of the sample with the spectra of two vouchered samples of *D. nigra* (samples 1 and 10; Fig. 4) shows that their spectra are very similar. Therefore, we concluded that sample 9 is in fact *D. nigra* and that the conclusions of Miller and Wiemann (2006) (that it was misidentified) were incorrect.

Figure 4 shows prominent peaks for three *D. nigra* samples (1, 9, and 10), corresponding to the phenolic compound dalnigrin and isoflavone caviunin, which are abundant in the heartwood of *D. nigra* but not *D. spruceana* (ILDIS and CHCD 1994a; Kite and others 2010). Although Kite and others (2010) reported that dalnigrin is unique to *D. nigra*, the sample of *D. spruceana* also showed a smaller peak at the same m/z value. This peak probably corresponded to a different compound with a similar m/z, and chromatography is needed to differentiate between such isomers that cause confusion in interpretation of the DART–TOFMS results.

Table 2—Heartwood samples identified as *Dalbergia nigra* or *Dalbergia spruceana* using density, water fluorescence, ethanol fluorescence, or DART[a]

Sample number	Wood collection number	Density		Water fluorescence		Ethanol fluorescence		DART, PCA		DART, KPCA	
		Value (g/cm^3)	Species from density	Color	Species from fluorescence	Color	Species from fluorescence	Projection onto PC1	Species by DART, PCA	Projection onto PC1	Species by DART, KPCA
Samples labelled *Dalbergia nigra*											
1	MAD7017[b]	0.96	*D. nigra*	none	*D. nigra*	greenish blue	*D. nigra*	–5.2349	*D. nigra*	0.2580	*D. nigra*
2	MAD10769[c]	0.79	*D. nigra*	none	*D. nigra*	greenish blue	*D. nigra*	–1.4813	*D. nigra*	0.4026	*D. nigra*
3	MAD13091	0.76	*D. nigra*	none	*D. nigra*	greenish blue	*D. nigra*	0.5032	*D. nigra*	0.1792	*D. nigra*
4	MAD21010	0.85	*D. nigra*	none	*D. nigra*	greenish blue	*D. nigra*	–5.4438	*D. nigra*	0.4029	*D. nigra*
5	MAD23334	0.97	*D. nigra*	none	*D. nigra*	greenish blue	*D. nigra*	–2.3060	*D. nigra*	0.0131	*D. nigra*
6	MAD31954	0.82	*D. nigra*	none	*D. nigra*	greenish blue	*D. nigra*	–3.8667	*D. nigra*	0.4600	*D. nigra*
7	MAD31955	0.86	*D. nigra*	none	*D. nigra*	greenish blue	*D. nigra*	–2.3705	*D. nigra*	0.4201	*D. nigra*
8	MAD31956	0.88	*D. nigra*	none	*D. nigra*	greenish blue	*D. nigra*	–1.6756	*D. nigra*	0.3909	*D. nigra*
9	MAD31957[d]	1.01	***D. spruceana***	none	*D. nigra*	greenish blue	*D. nigra*	–2.3950	*D. nigra*	0.0288	*D. nigra*
10	MAD31958	0.66	*D. nigra*	none	*D. nigra*	greenish blue	*D. nigra*	–1.8438	*D. nigra*	0.1856	*D. nigra*
11	SJR550[e]	0.75	*D. nigra*	none	*D. nigra*	greenish blue	*D. nigra*	–4.9774	*D. nigra*	0.2692	*D. nigra*
12	SJR1442	1.11	***D. spruceana***	blue	***D. spruceana***	blue	***D. spruceana***	5.0931	***D. spruceana***	–0.6711	***D. spruceana***
13	SJR3107	0.84	*D. nigra*	none	*D. nigra*	greenish blue	*D. nigra*	–2.7503	*D. nigra*	0.2107	*D. nigra*
14	SJR3222	0.82	*D. nigra*	none	*D. nigra*	weak greenish blue	*D. nigra*	0.3578	*D. nigra*	0.1989	*D. nigra*
15	SJR3273[f]	0.81	*D. nigra*	none	*D. nigra*	greenish blue	*D. nigra*	1.2837	*D. nigra*	–0.1687	?
16	SJR3301	0.84	*D. nigra*	none	*D. nigra*	greenish blue	*D. nigra*	–3.0526	*D. nigra*	0.0139	*D. nigra*
17	SJR3302	0.93	*D. nigra*	none	*D. nigra*	greenish blue	*D. nigra*	–2.5575	*D. nigra*	0.2716	*D. nigra*
18	SJR3303	0.80	*D. nigra*	none	*D. nigra*	greenish blue	*D. nigra*	–0.2419	*D. nigra*	0.2022	*D. nigra*
19	SJR3508	0.80	*D. nigra*	none	*D. nigra*	greenish blue	*D. nigra*	—	—	—	—
20	SJR3509[g]	0.72	*D. nigra*	—	—	—	—	—	—	—	—
21	SJR3525	0.79	*D. nigra*	none	*D. nigra*	greenish blue	*D. nigra*	–3.3547	*D. nigra*	0.3732	*D. nigra*
22	SJR4146[h]	0.90	*D. nigra*	none	*D. nigra*	greenish blue	*D. nigra*	—	—	—	—
23	SJR4230	0.88	*D. nigra*	none	*D. nigra*	greenish blue	*D. nigra*	–1.5035	*D. nigra*	0.1065	*D. nigra*
24	SJR4296	0.81	*D. nigra*	none	*D. nigra*	greenish blue	*D. nigra*	–2.2327	*D. nigra*	0.3417	*D. nigra*
25	SJR4452	0.98	*D. nigra*	none	*D. nigra*	greenish blue	*D. nigra*	–2.2954	*D. nigra*	0.0893	*D. nigra*
26	SJR5900	0.85	*D. nigra*	none	*D. nigra*	greenish blue	*D. nigra*	–5.0023	*D. nigra*	0.0927	*D. nigra*
27	SJR5990[i]	1.01	***D. spruceana***	blue	***D. spruceana***	blue	***D. spruceana***	9.9405	***D. spruceana***	–0.5949	***D. spruceana***
28	SJR6001	1.06	***D. spruceana***	blue	***D. spruceana***	blue	***D. spruceana***	8.5320	***D. spruceana***	–0.6631	***D. spruceana***
29	SJR32586[j]	0.79	*D. nigra*	none	*D. nigra*	yellow-green	?	1.2226	*D. nigra*	–0.2489	?
30	SJR36063	0.93	*D. nigra*	none	*D. nigra*	greenish blue	*D. nigra*	–2.5146	*D. nigra*	0.1427	*D. nigra*
31	SJR38188	0.83	*D. nigra*	none	*D. nigra*	greenish blue	*D. nigra*	–0.9718	*D. nigra*	0.1611	*D. nigra*
32	SJR38189	0.92	*D. nigra*	none	*D. nigra*	greenish blue	*D. nigra*	–3.0590	*D. nigra*	0.1254	*D. nigra*
33	SJR53033[k]	—	—	—	—	—	—	—	—	—	—
Samples labelled *Dalbergia spruceana*											
34	MAD18588[g]	0.80	***D. nigra***	—	—	—	—	—	—	—	—
35	MAD31968[l]	0.86	***D. nigra***	blue	*D. spruceana*	blue	*D. spruceana*	8.4813	*D. spruceana*	–0.6261	*D. spruceana*
36	SJR1430	1.00	*D. spruceana*	weak blue	*D. spruceana*	blue	*D. spruceana*	4.7688	*D. spruceana*	–0.4311	*D. spruceana*
37	SJR4014	1.00	*D. spruceana*	blue	*D. spruceana*	blue	*D. spruceana*	6.2695	*D. spruceana*	–0.5540	*D. spruceana*
38	SJR22610[m]	0.85	***D. nigra***	blue	*D. spruceana*	blue	*D. spruceana*	8.0297	*D. spruceana*	–0.6626	*D. spruceana*
39	SJR38248	1.05	*D. spruceana*	blue	*D. spruceana*	blue	*D. spruceana*	6.6490	*D. spruceana*	–0.7198	*D. spruceana*

[a]Density and fluorescence from Miller and Wiemann (2006) or measured. Species determined from physical property tests or spectroscopic method in italic font when it is the same as sample label and in bold italic when it is different. A question mark indicates that the test did not suggest a species. Projections onto principle component 1 (PC1) axis are based on direct analysis in real time (DART) results that extract three axes (PCA, principal component analysis; KPCA, kernel principal component analysis).
[b]Sample from same tree as SJR4146.
[c]Sample from same tree as SJR3273; 20% sapwood.
[d]20% sapwood; spectrograph with huge caviunin peak.
[e]Veneer.
[f]Sample from same tree as MAD10769; 80% sapwood.
[g]100% sapwood.
[h]Sample from same tree as MAD7017; sample not tested by DART.
[i]10% sapwood.
[j]Ethanol fluorescence test indeterminate.
[k]Bark-covered; 100% sapwood; sample not tested.
[l]Sample from same tree as SJR22610; 30% sapwood.
[m]Sample from same tree as MAD31968; 40% sapwood.

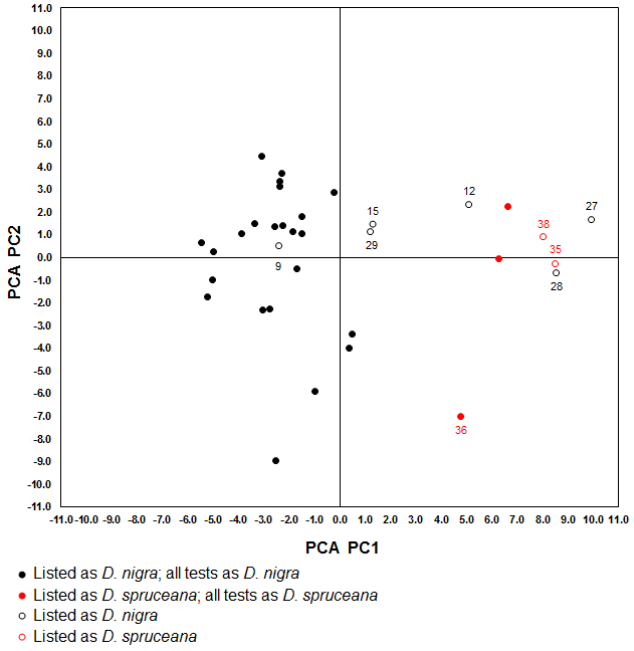


Figure 3. Values of the first two principal components (PC1 and PC2) extracted using principal component analysis (PCA), for each of the *Dalbergia* samples analyzed using direct analysis in real time–time of flight mass spectrometry (DART–TOFMS). Numbers above or below open circles refer to sample numbers in Table 2.

Sample 29, an unvouchered sample labelled *D. nigra*, had a yellow–green ethanol extract fluorescence; this color was not found in any other sample. Density, water extract fluorescence, and PCA all pointed to *D. nigra*, but the KPCA PC1 value was intermediate between the values for *D. nigra* and *D. spruceana* (Table 2; Fig. 2). Inconsistent fluorescent results are not uncommon. Miller (2007) found variability in the presence, color, and intensity of surface fluorescence in many of the 1400 fluorescent species that he tested. For water and ethanol extract fluorescence, Wiemann and Ruffinatto (2012) found differences in color and intensity among samples of *D. stevensonii* Standl. and *D. tucurensis* Donn. Sm., even when pairs of samples were collected from the same tree. A near infrared spectroscopy comparison of sample 29 with samples of *D. nigra* and five other species of *Dalbergia* determined it to be *D. nigra* (Pastore 2017).

The other sample with inconclusive KPCA results was sample 15. However, all other tests point to it being correctly identified as *D. nigra*. Therefore, we concluded that the natural variability of the KPCA accounts for the lack of a clear separation.

Samples 15 and 29 both plotted near each other in Figures 2 and 3 (KPCA and PCA, respectively), their densities are similar (0.81 and 0.79, respectively; Table 2), and their PCA PC1 values are closer to those of *D. nigra* than *D. spruceana*. Therefore, we concluded that they are both *D. nigra*.

Samples 34, 35, and 38 were all vouchered samples labelled as *D. spruceana* (Table 1), but their densities indicated that they were *D. nigra* (Table 2). Sample 34 was entirely sapwood; therefore it was not tested by fluorescence or DART–TOFMS. Samples 35 and 38 came from the same

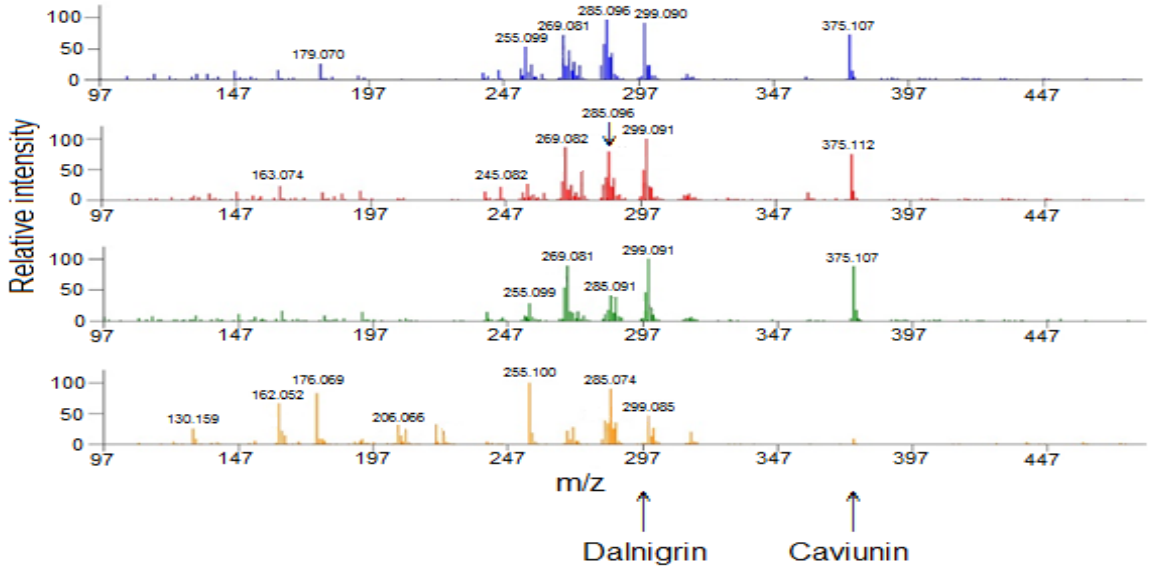


Figure 4. Comparison of direct analysis in real time–time of flight mass spectrometry (DART–TOFMS) mass spectrum of unvouchered *Dalbergia nigra* sample 9 (top, blue) with the spectra of two vouchered *D. nigra* samples, sample 1 (second from top, red) and sample 10 (third from top, green), and vouchered sample 35 of *D. spruceana* (bottom, yellow). Each peak corresponds to an ion that was detected in the wood sample. The abscissa, m/z (mass-to-charge ratio), is the molecular weight of an ion divided by its charge number, and the ordinate is the relative signal intensity of the ions. The compounds dalnigrin and caviunin, found in the *D. nigra* samples, are indicated by the labelled arrows. In *D. spruceana*, two much smaller peaks are found at the same m/z values, but these are not dalnigrin or caviunin (Kite and others 2010).

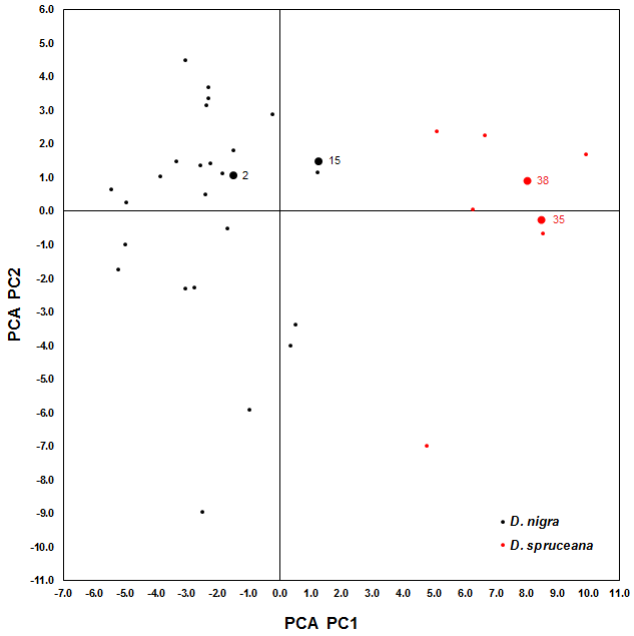


Figure 5. Principal components map for the principal components analysis (PCA) based on the direct analysis in real time–time of flight mass spectrometry (DART–TOFMS) spectra; PCA PC1 is the first principal component, PCA PC2 the second. Large symbols and labels indicate the paired (MAD and SJR) *Dalbergia* samples. Black symbols are for *D. nigra* samples, and red symbols are for *D. spruceana*. Numbers refer to sample numbers in Table 2.

tree. Their densities were almost the same (0.86 and 0.85, respectively) and corresponded to the typical density of *D. nigra* (Table 2). But their blue water and ethanol fluorescence indicated that they were *D. spruceana*. Their principal components projections, indicated by open red circles on the left side of Figure 2 (KPCA) and the right side of Figure 3 (PCA), were clearly in the *D. spruceana* area. Therefore, we concluded that they were low-density samples of *D. spruceana*.

Comparison of matching samples from the MAD and SJR collections (Table 2) gives an idea of the similarity of DART–TOFMS results that can be expected within a tree. Figure 5 shows the PCA PC1 and PC2 coordinates of all of the samples. *D. nigra* samples 2 and 15 and *D. spruceana* samples 35 and 38 are indicated by large circles. The coordinates of the two *D. spruceana* samples are very close, but those of *D. nigra* are more widely separated. Both sets of samples contain sapwood, but the size and shape of the blocks resulted in the slivers for the DART–TOFMS being sampled differently with respect to the heartwood–sapwood transition. In both *D. spruceana* samples, slivers were taken about 4 cm from the transition. For the *D. nigra* samples, however, a sliver was taken about 3 cm from the transition in sample 15 (right side of the vertical axis in Fig. 5) but only about 1 cm from the transition in sample 2 (left side of the vertical axis in Fig 5). Thus, it is possible that a difference in the chemical composition of the heartwood because of age might be a factor in the results from the DART–TOFMS analysis. Hillis (1999) states that amounts and types of extractives vary across the heartwood zone because of change in rate of conversion of precursors to extractives, change in solubility with age, and changes in hydrolysis, polymerization, and pH with time.

All heartwood samples determined to be *D. nigra* contained caviunin (m/z = 375 on the heat map; Fig. 1), but only three of the *D. spruceana* heartwood samples showed traces of caviunin (samples 36, 38, and 35). Caviunin is reported to be found in *D. nigra* wood (Gottlieb and Magalhães 1961) and *D. spruceana* sapwood (Cook and others 1978), as well as in the wood of *D. barretoana* Hoehne, *D. inundata* Benth., and *D. villosa* (Benth.) Benth. from Brazil (ILDIS and CHCD 1994a), *D. paniculata* Roxb. from Asia (Adinarayana and others 1971), the root and bark of the shrub *D. spinosa* Roxb. from Asia (ILDIS and CHCD 1994a), and the heartwood of *D. tucurensis* Donn. Sm. (Espinoza and others 2015). Two of the *D. spruceana* samples with traces of caviunin contained sapwood (samples 35 and 38). The third sample (sample 36) was a trade sample cut from a piece of shaped molding, without sapwood. It is possible that proximity to sapwood may be the reason for caviunin in samples 35 and 38, although the slivers tested were heartwood. The presence of caviunin in sample 36 is unexplained; however, its water extract fluorescence was weak blue rather than the blue color typical of *D. spruceana* (Table 2) and its projection onto PCA PC1 and PC2 (4.769, –6.996) (Table 2; Fig. 3) showed it as an outlier compared with the other samples of *D. spruceana*. It was not an outlier when compared using KPCA, but it plotted closer to the *D. nigra* samples than any other sample of *D. spruceana* (–0.431, –0.071; Table 2; Fig. 2). It is possible that the sample is neither *D. nigra* nor *D. spruceana*.

Conclusions

If a heartwood sample of *Dalbergia* is known to be either of the Brazilian look-alike species *D. nigra* or *D. spruceana*, CITES-protected *D. nigra* can usually be distinguished from unprotected *D. spruceana* based on density, water fluorescence, and ethanol fluorescence. However, in some cases, these physical characteristics give erroneous or ambiguous results. Based on the criteria of Miller and Wiemann (2006), density seemed anomalously high in four samples labelled *D. nigra* (9, 12, 27, and 28), density seemed anomalously low in three samples labelled *D. spruceana* (34, 35, and 38), and the fluorescence color of the ethanol extract of one sample of *D. nigra* (29) was completely different from that found in any other sample. DART–TOFMS confirmed the identity of all but three of the samples (12, 27, and 28). Therefore, we concluded that

the physical property differences found in the others were caused by interspecific variability.

The DART–TOFMS method, using PCA, is reliable, based on results from this study and the study by Espinoza and others (2015). PCA of the spectra confirmed the identity of all MAD samples tested by this method (ten *D. nigra* and one *D. spruceana*). For the SJR samples, the method confirmed the identity of 16 *D. nigra* and four *D. spruceana* samples but determined that three samples labelled *D. nigra* were, in fact, *D. spruceana*.

Kukachka's decision to discard doubtful samples improved the accuracy of the collection, but the retention of all samples by Yale means that we now have more samples available of a now-restricted species (*D. nigra*). Under the assumption that all tested samples are either *D. nigra* or *D. spruceana*, it also added three reliably identified samples of under-represented *D. spruceana*.

References

Adinarayana, D.; Radhakrishniah, M.; Rao, J.R. 1971. The occurrence of caviunin in *Dalbergia paniculata* Leguminosae. Current Science (Bangalore). 40: 602.

Baudat, G.; Anouar, F. 2000. Generalized discriminant analysis using a kernel approach. Neural Computation. 12: 2385-2404.

CITES. 2016. Convention on international trade in endangered species of wild fauna and flora. Appendix 1. https://www.cites.org/eng/app/appendices.php (Sept. 1, 2016).

Cody, R.B. 2013. What is the opposite of Pandora's box? Direct analysis, ambient ionization, and a new generation of atmospheric pressure ion sources. Mass Spectrometry 2, Special Issue. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3810099/.

Cody, R.B.; Dane, A.J. 2010. Direct analysis in real time ion source. In: Meyers, R.A., ed. Encyclopedia of analytical chemistry. Hoboken, NJ: John Wiley & Sons, Ltd. (DOI: 10.1002/9780470027318.a9012).

Cody, R.B.; Dane, A.J.; Dawson-Andoh, B.; Adedipe, E.O.; Nkansah, K. 2012. Rapid classification of white oak (*Quercus alba*) and northern red oak (*Quercus rubra*) by using pyrolysis direct analysis in real time (DART™) and time-of-flight mass spectrometry. Journal of Analytical and Applied Pyrolysis. 95: 134-137.

Cody, R.B.; Laramée, J.A.; Durst, H.D. 2005. Versatile new ion source for the analysis of materials in open air under ambient conditions. Analytical Chemistry. 77: 2297-2302.

Cook, J.T.; Ollis, W.D.; Sutherland, I.O.; Gottlieb, O.R. 1978. Pterocarpans from *Dalbergia spruceana*. Phytochemistry. 17: 1419-1422.

Environment Canada. 2002. CITES identification guide – Tropical Woods. Ottawa, Canada: Environment Canada.

Espinoza, E.O.; Wiemann, M.C.; Barajas-Morales, J.; Chavarria, G.D.; McClure, P.J. 2015. Forensic analysis of CITES-protected *Dalbergia* timber from the Americas. IAWA Journal. 36: 311-325.

FPL. 2017. Search MADw collection. https://www.fpl.fs.fed.us/search/madw_request.php. Madison, WI: USDA Forest Service, Forest Products Laboratory. (Feb. 22, 2017).

Gasson, P.; Miller, R.; Stekel, D.; Whinder, F.; Ziemińska, K. 2010. Wood identification of *Dalbergia nigra* (CITES Appendix I) using quantitative wood anatomy, principal components analysis and naïve Bayes classification. Annals of Botany. 105: 45-56.

Gottlieb, O.R.; Magalhães, M.T. 1961. Chemistry of Brazilian Leguminosae. II. Isolation and structure of caviunin. Journal of Organic Chemistry. 26: 2449-2453.

Harris, G.A.; Galhena, A.S.; Fernandez, F.M. 2011. Ambient sampling/ionization mass spectrometry: applications and current trends. Analytical Chemistry. 83: 4508-4538.

Hastie, T.; Tibshirani, R.; Friedman, J. 2009. The elements of statistical learning. Second edition. New York: Springer Science. 745 p.

Hillis, W.E. 1999. The formation of heartwood and its extractives. In: Romeo, J.T., ed. Phytochemicals in human health protection, nutrition, and plant defense. New York, Boston, Dordrecht, London and Moscow: Kluwer Academic: 215-253. Chapter 9.

ILDIS and CHCD 1994a. Phytochemical dictionary of the Leguminosae, Volume 1: Plants and their constituents. London: Chapman and Hall. 1051 p.

ILDIS and CHCD 1994b. Phytochemical dictionary of the Leguminosae, Volume 2: Chemical constituents. London: Chapman and Hall. 573 p.

Johnson, R.A.; Wichern, D.W. 2007. Applied multivariate statistical analysis. Sixth edition. Upper Saddle River, NJ: Pearson Prentice Hall. 773 p.

Kite, G.C.; Green, P.W.C.; Veitch, N.C.; Groves, M.C.; Gasson, P.E.; Simmonds, M.S.J. 2010. Dalnigrin, a neoflavonoid marker for the identification of Brazilian rosewood (*Dalbergia nigra*) in CITES enforcement. Phytochemistry. 71: 1122-1131.

Lancaster, C.; Espinoza, E. 2012. Analysis of select *Dalbergia* and trade timber using direct analysis in real time and time-of-flight mass spectrometry for CITES enforcement. Rapid Communications in Mass Spectrometry. 26: 1147-1156.

McBeath, L.W. 1978. Eloise Gerry, a woman of forest science. Journal of Forest History. 22: 128-135.

Miller, R.B. 1999. Xylaria at the Forest Products Laboratory: past, present and future. In: Maes, F.; Beeckman, H., eds. Wood to survive. Tervuren: Royal Museum for Central Africa: 243-254.

Miller, R.B. 2007. Fluorescent wood of the world. In: Flynn Jr., J.H., ed. A guide to more useful woods of the world. Madison, WI: Forest Products Society. 358 p.

Miller, R.B.; Wiemann, M.C. 2006. Separation of *Dalbergia nigra* from *Dalbergia spruceana*. Res. Pap. FPL-RP-632. Madison, WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory.

Pastore, T.C.M. 2017. Personal communication. Environmental analyst, Brazilian Forest Service, Forest Products Laboratory, Brasilia, Brazil.

Schölkopf, B.; Smola, A.J.; Müller, K.-R. 1999. Kernel principal component analysis. In: Advances in kernel methods. Cambridge, Massachusetts and London, United Kingdom: MIT Press: 327-352.

Stern, W.L. 1973. The wood collection—what should be its future. Arnoldia. 33: 67-80.

Wayne, T.K. 2011. American women of science since 1900. Santa Barbara, CA: ABC-CLIO.

Wiemann, M.C.; Ruffinatto, F. 2012. Separation of *Dalbergia stevensonii* from *Dalbergia tucurensis*. Res. Pap. FPL-RP-665. Madison, WI: U.S. Department of Agriculture, Forest Service, Forest Product Laboratory.

Williams, L.O. 1971. Field Museum's wood collection. Taxon. 20: 824-825.