Use of wood anatomy to identify poisonous plants: Charcoal of *Spirostachys africana*

AUTHORS:
Sandra J. Lennox[1,2]
Marion Bamford[2,3]

AFFILIATIONS:
[1]School of Geosciences, University of the Witwatersrand, Johannesburg, South Africa
[2]DST–NRF Centre of Excellence in Palaeosciences, University of the Witwatersrand, Johannesburg, South Africa
[3]Evolutionary Studies Institute, University of the Witwatersrand, Johannesburg, South Africa

CORRESPONDENCE TO:
Sandra Lennox

EMAIL:
sandrajanetlennox@gmail.com

POSTAL ADDRESS:
Evolutionary Studies Institute, University of the Witwatersrand, Private Bag 3, Wits 2050, South Africa

DATES:
Received: 23 Apr. 2014
Revised: 16 July 2014
Accepted: 25 July 2014

KEYWORDS:
Middle Stone Age; Sibudu; hearth; tambotie; Euphorbiaceae

HOW TO CITE:
Lennox SJ, Bamford M. Use of wood anatomy to identify poisonous plants: Charcoal of *Spirostachys africana*. S Afr J Sci. 2015;111(3/4), Art. #2014-0143, 9 pages. http://dx.doi.org/10.17159/sajs.2015/20140143

Spirostachys africana Sond. (tamboti/tambotie) is a woodland tree that is often found near water. It has a poisonous and purgative latex. The archaeological site of Sibudu, a rock shelter in KwaZulu-Natal, has evidence, from well-preserved charcoal and seeds, of past environments and wood use from approximately 77–38 thousand years ago (ka). As their uses and environmental indicators are different, it is critical to confidently distinguish among the three anatomically similar woods of the Euphorbiaceae: *Spirostachys africana*, *Sclerocroton integerrimus* and *Shirakiopsis elliptica*. A detailed anatomical study of reference and archaeological charcoal shows that xylem vessel width increases proportionally as vessel frequency decreases, from *Spirostachys africana*, *Sclerocroton integerrimus* to *Shirakiopsis elliptica*. Crystals of calcium oxalate are present in ray cells of *Spirostachys africana*, whereas silica bodies are present in ray cells of *Sclerocroton integerrimus* and *Shirakiopsis elliptica*. Using these features, the presence of *Spirostachys africana* was confirmed amongst hearth charcoal of the Spotty Camel layer, with an age of approximately 58 ka and of the Mottled Deposit occupational layer, with an age of approximately 49 ka. The presence of this charcoal, collected from ancient fireplaces or sieved from surrounding sediments, implies that people at Sibudu understood and used this poisonous tree to their advantage. We are encouraged in this view by the presence of many *Cryptocarya woodii* leaves found on the surface of 77-ka sedge bedding at Sibudu (Wadley L et al., Science. 2011;334:1388–1391). *Cryptocarya woodii* has insecticidal and larvacidal properties and members of the Laurel family are well known for their medicinal properties.

Introduction

General introduction

Three indigenous woods – *Spirostachys africana* (tamboti), *Sclerocroton integerrimus* (duiker-berry) and *Shirakiopsis elliptica* (jumping-seed tree) – were tentatively identified from charcoal recovered from archaeological deposits at the rock shelter Sibudu. The anatomy of charcoal reference material was compared with that of the recovered charcoal in order to confidently identify these taxa in the archaeological charcoal. The identification of these woods in the Middle Stone Age at Sibudu is significant because *Spirostachys africana* is particularly poisonous. *Spirostachys africana* Sond., *Sclerocroton integerrimus* Hochst. (=*Sapium integerrimum* (Hochst.) J.Léonard) and *Shirakiopsis elliptica* (Hochst.) Esser (=*Sapium ellipticum* (Hochst.) Pax) are members of the family Euphorbiaceae, subfamily Euphorbioideae, tribe Hippomaneae A.Juss. ex Bartl. and subtribe Hippomaninae.[1]

Sibudu

Sibudu is situated on the uThongathi River, KwaZulu-Natal. It has a sequence of archaeological layers from the Middle Stone Age, dated by single-grain optically stimulated luminescence to approximately 77–38 ka.[2-6] Some of the evidence for the behaviour of the anatomically modern people who visited and lived at Sibudu includes stone tools, ochre, bone, perforated seashells and hearths[7-15], as well as evidence for the making and use of compound adhesives[16], and circumstantial evidence for snares[17] and bows and arrows[18]. There is evidence of the use of plant resources from pollen, phytoliths, seeds, nutlets, stems, charcoal and leaves excavated at Sibudu.[2,19-27]

The relevance of identifying *Spirostachys africana*

The presence of charcoal at Sibudu implies that people who visited and occupied the site burned wood.[2,4,5]

Spirostachys africana charcoal was tentatively identified from Sibudu in a previous study.[20,21] Nowadays, the wood from this tree is not used as fuel for cooking because the smoke and fumes are poisonous.[28] People who live in a particular environment for long periods develop knowledge about local resources[2,14,25,29] and the *Spirostachys africana* wood was almost certainly recognised for its toxic properties and utilised by ancient hunter–gatherers[14]. Therefore a secure identification of the archaeological charcoal is necessary in order to interpret behavioural strategies in the past.

Sclerocroton integerrimus wood anatomy is similar to that of *Spirostachys africana*.[1,20,30] Both were recorded as *Spirostachys*/*Sapium* in the scanning electron microscopy (SEM) study of charcoal from Sibudu,[20,21] so it is important to try to distinguish between the two taxa. *Shirakiopsis elliptica* wood anatomy is also similar.[1,31] As it was not in the original charcoal reference collection for Sibudu,[20] new fresh material was gathered, charred and studied.

Anthracology

Archaeological charcoal is identified by means of wood anatomy[32-35] to describe palaeoenvironments and palaeoclimate and to develop an understanding of past wood use[36-38]. Anatomical features of living or fresh woods, listed by the International Association of Wood Anatomists, accessed on InsideWood, an online database[39], can assist with charcoal identification[34,35,40] but charred reference material is more useful[41-43]. Woody taxa have been identified from charcoal assemblages from many sites elsewhere in southern Africa; a few examples are Diepkloof Rock Shelter[44] and Elands Bay Cave in the Western Cape[45] and from sites in Lesotho[46,47].

Habits, habitats and uses of the three woods

Spirostachys africana is a medium-sized, hardwood, deciduous tree, 10–18 m tall, and grows in woodland and valley bushveld. Often found in dense stands; in warm, dry areas along rivers and drainage lines; in poorly drained brackish and clay soils; or near underground water, tambotie is distributed from KwaZulu-Natal to Tanzania.[48] *Sclerocroton integerrimus* is a small- to medium-sized, hardwood, deciduous tree, 2–10 m tall, and grows in coastal thicket, on forest margins and in wooded grassland.[48] *Shirakiopsis elliptica* is a medium to tall, softwood, deciduous tree, 12–20 m tall. It grows in wooded ravines and is common at the canopy edge of evergreen forests and as a canopy tree in swamp forests.[48] The timber of the three trees is similar.[49]

The bark, wood, stems and leaves of *Spirostachys africana* contain poisonous milky latex[28] which is used as a purgative in small doses[50]. This fish and arrow poison causes conjunctivitis when in contact with the eyes[28] and urticaria and blistering when in contact with the skin[28]. A triterpenoid (C_{30}) isolated from the bark showed significant inhibition of the bacteria *Staphylococcus aureus, Salmonella typhi, Vibrio cholera, Escherichia coli* and *Shigella dysenteriae*, explaining the traditional use in several African countries against diarrhoea and dysentery.[50-55] Cytotoxic and genotoxic activities have been reported.[28,52]

The cytotoxins in latex are phorbol esters, which are terpenoids.[28,54] *Spirostachys africana* is classified as an extremely hazardous Class IA cellular poison according to the four toxicity classes recognised by the World Health Organization. This measure of poisoning is based on an LD_{50} determination in rats, that is, less than 5 mg of plant material ingested per kilogram body mass killed 50% of the population.[28] (Poisons kill in minute amounts, toxins are less toxic than poisons and toxicants are toxic in high concentrations only.[50]) Phorbol esters affect mucous membranes in the skin and the alimentary tract.[28] The latex and bark have antimicrobial, anthelminthic and larvacidal properties and are effective against ailments of the digestive tract, skin, reproductive system and respiratory tract.[50-55]

Spirostachys africana wood is hard and heavy with a beautiful close grain coppery brown colour, impervious to insect attack and weathering.[48] When burned, the wood gives off a sweet odour, which can cause headaches, nausea and diarrhoea.[28,55] The Venda use *Spirostachys africana* smoke to fumigate their huts against wood-boring and other insects (Anonymous reviewer, 2014, written communication of personal observation, July 03).

Sclerocroton integerrimus has clear latex which is suspected of being poisonous and is used as a mouthwash to relieve toothache and coughs.[56] Fruit is used to make ink and as a source of tannin. Fallen fruit and leaves are eaten by antelope and stock animals.[48]

Shirakiopsis elliptica is considered very poisonous and is used as a drastic purgative in West Africa.[57] The rough bark has sparse, scattered depressions and clear latex. Bark latex is added to arrow poison, *ouabain*, from the East African *Acokanthera schimperi* and is used as bird lime and for body markings.[57] *Acokanthera oppositifolia* (Bushman's poison), distributed along eastern and northern parts of South Africa, is amongst the *Acokanthera* species which are known to be sources of extremely toxic arrow poisons.[28] Various parts of the *Shirakiopsis elliptica* tree are used in folk medicine in Africa, in dermatology and gastroenterology, particularly as an anthelminthic.[57]

Wood anatomy

A detailed study of the wood anatomy of *Spirostachys africana*, *Sclerocroton integerrimus* and *Shirakiopsis elliptica* has been done to distinguish among these taxa and to clearly identify *Spirostachys africana* in charcoal from Sibudu. Modern reference material was collected specifically for this project, charred in a furnace and supplemented with data from InsideWood and the literature. As outlined below, the three woods share similar features but there are several useful distinguishing characteristics.

Methods

Material

Comparative reference collection

A wood sample of *Spirostachys africana* from a Southern African Forestry Department woodblock stored in the Department of Archaeology, University of the Witwatersrand, was carbonised and studied as a reference of anatomical features (SJL 103; Table 1, Figure 1a–c). Inferred archaeological *Spirostachys africana* charcoal from Ndondondwane Iron Age site[58] in KwaZulu-Natal was used as intermediate reference material (NDO; Table 1, Figure 1d). Reference wood samples and voucher herbarium specimens of *Sclerocroton integerrimus* (SJL 88, Figure 1e) and *Shirakiopsis elliptica* (SJL 67, Figure 1f) were identified by local botanists and were collected *ex hort* in Durban and on a farm near Port Edward, KwaZulu-Natal (coordinates 31.04615°S, 30.16886°E) for a study of anatomical features (Table 1).

Archaeological specimens

Sibudu archaeological charcoal was examined from squares of occupational layer Spotty Camel (SPCA), which has not been dated but lies between layers with ages of 61.3 ± 2.0 ka and 56.2 ±1.9 ka, and from Mottled Deposit (MOD) at approximately 49.7 ± 1.8 ka.[1,3-6] These layers were chosen because *Spirostachys*/*Sapium* charcoal was previously recorded from them by Allott[20,21].

Methods

Reference woodblocks were charred in a LENTON 0861 muffle furnace (Lenton, Hope, UK) for 3.5 h at 350 °C at the Palaeosciences Centre, University of the Witwatersrand. Archaeological charcoal was identified by comparing it with reference material, using standard techniques.[20,21,41-43] Charcoal blocks were viewed from three planes by means of stereomicroscopy (Olympus SZX16, Münster, Germany) and reflective and polarised light microscopy (Olympus BX51) at magnifications of 100x, 200x and 500x. Characteristic anatomy was digitally photographed using Olympus Stream Essentials® image analysis software with extended focal image capability. Anatomical features according to the International Association of Wood Anatomists' list[35,39] were recorded for the comparative reference material and archaeological specimens. Identifications were also confirmed against published reference material.[1,32,33,39,49]

Useful distinguishing features

Prismatic crystals occur in *Spirostachys africana* ray and parenchyma cells, whereas silica bodies are absent in this species.[1,30,59] Silica bodies occur in *Sclerocroton integerrimus*[2,60] and *Shirakiopsis elliptica*[1,31] ray cells, whereas crystals are absent from ray cells[40]. Crystals are occasionally visible in the parenchyma cells of *Sclerocroton* and *Shirakiopsis.*[2]

Prismatic crystals are not common in wood anatomy and their occurrence may be sporadic.[35] Features such as crystals and silica bodies are therefore useful attributes and are listed as an anatomical feature when commonly observed. Prismatic crystals are solitary, rhombohedral or octahedral crystals of calcium oxalate which are birefringent (produce a rainbow effect) under polarised light[35] and appear shiny in charcoal specimens.

Silica bodies are spheroidal or irregularly shaped particles of silicon dioxide which are non-birefringent (do not produce a rainbow effect) under polarised light[35] and appear opaque. Silica is present in ray cells in African *Sapium* species in aggregates, which often fill the entire cell lumen, or as grains or small dark dots in Asian *Sapium* species such as

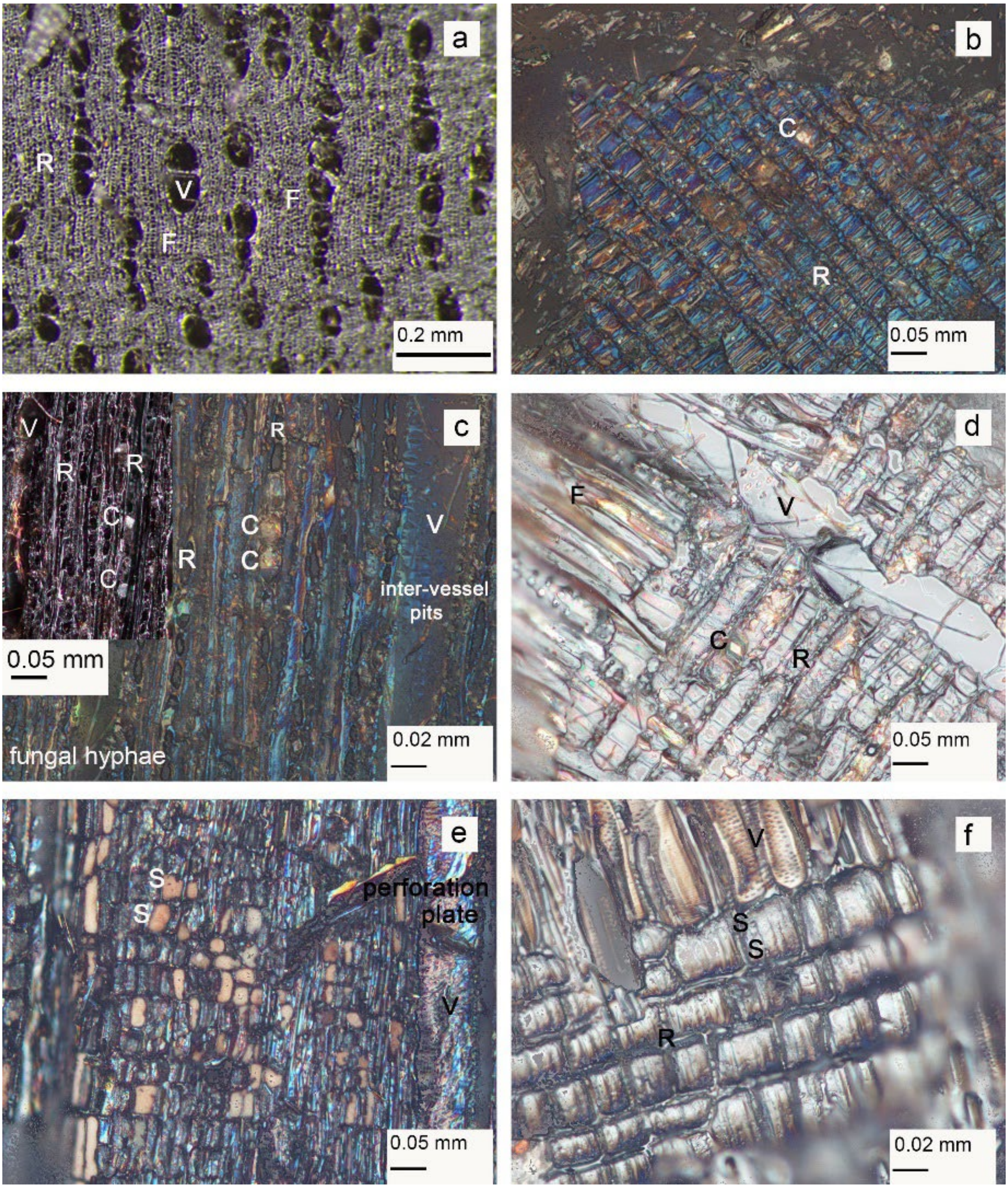

Figure 1: Diagnostic characteristics of charcoal reference material: (a–c) *Spirostachys africana* (SJL 103), (d) *Spirostachys africana* (NDO), (e) S*clerocroton integerrimus* (SJL 88) and (f) *Shirakiopsis elliptica* (SJL 67). (a) In transverse section, *Spirostachys africana* has many small vessels (V) in long lines. Vessels are occasionally in pairs. The shiny cell contents may be resin[2] or gum[30]. These fibres (F) are thin-walled and regular. (b) The radial longitudinal section has crystals labelled (C) in the ray cells, which shine under polarised light. Ray cells (R) are mixed; the procumbent cells are as high as the square cells and occasionally there are upright cells in the margins. The rays are low. (c) The tangential longitudinal section has frequent, uniseriate rays. The prismatic crystals of calcium oxalate in ray cells which distinguish *Spirostachys* shine under polarised light. Vessels with alternate inter-vessel pits occur and the vessel walls are birefringent under polarised light. The insert shows these ray crystals at a lower magnification. (d) Inferred *Spirostachys africana* reference material from Ndondondwane, KwaZulu-Natal (NDO) has prismatic, rhombic, crystals in the ray cells which shine under non-polarised light, in radial longitudinal section. (e) In S*clerocroton integerrimus* reference material*,* SJL 88, there are silica bodies labelled (S) in the ray cells. These are spheroidal or irregularly shaped particles which are opaque under polarised light. The ray cells are heterocellular, mixed procumbent, square and upright in radial longitudinal section. The cell walls of the vessels, rays and fibres are birefringent in polarised light. (f) In *Shirakiopsis elliptica* reference material, SJL 67, the silica bodies (S) in ray cells are granular, dark spots. Rays are heterocellular with upright and square cells seen in radial longitudinal section. These inter-vessel pits are alternate.

Sapium luzonicum.[40] The arrangement (aggregated, irregularly shaped or globular) or surface (smooth or verrucose) may be diagnostic in certain groups and needs to be recorded in a description.[35]

Results and discussion

Figure 1 illustrates reference charcoal of the three taxa. Figure 1d, *Spirostachys africana* (NDO), is of archaeological charcoal and is therefore an interpreted identification. Figure 2 illustrates the identified archaeological charcoal from Sibudu. Table 1 summarises the charcoal anatomy of the modern reference and archaeological material. Table 2 lists the most useful diagnostic features for identifying the three species, the environmental conditions required by the trees and the medicinal and other uses for their wood.

Table 1: The anatomical features of modern and archaeological charcoal specimens of *Spirostachys africana, Sclerocroton integerrimus* and *Shirakiopsis elliptica*

Plant species	Charcoal specimen	Vessel radials	Vessel diameter	Vessel frequency	Ray width in cells	Heterocellular ray body cells	Ray marginal cells	Parenchyma	Ray crystals or silica bodies
Spirostachys africana	SJL 103	l	s	a	1	p,s,u	–	da	c
	NDO	l	s	a	1	p,s,u	–	da	c
	MOD C6a 39	l	s	a	1	p,s,u	(s)	d	c
	SPCA D5c 51	l	s	a	1	p,s,u	(s)	d	c
	SPCA B4b 62	l	s	m	1	p,s,u	–	d	c
	SPCA B4b 67	l	s	m	1	p,s,u	–	d	c
Sclerocroton integerrimus	SJL 88	s	m	m	1	p,s,u	–	da	s
	MOD E3d 08	s	m	m	1	p,s	(u)	d	s
	SPCA B4b 65	l	m	m	1	p,s	–	d	s
	SPCA B4b 66	l	m	m	1	p,s,u	–	d	s
	SPCA B4c 07	l	m	m	1	s,u	–	d	s
	SPCA B4c 14	l	m	m	1(–2)	s,u	–	d	s
	SPCA D5a 27	l	m	m	1	s,u	–	d	s
Shirakiopsis elliptica	SJL 67	s	l	f	1(–2)	s,u	–	d	s
	MOD C6a 46	s	m	m	1(–2)	p,s	u	dc	s
	SPCA B4c 45	s	m	m	1(–2)	p,s	u	d	s

Notes:
Vessel radials: short (s), 1–3 vessels; or long (l), >4 vessels
Vessel diameter: small (s), <50 µm; medium (m), 50–100 µm; large (l), 100–200 µm
Vessel frequency: few (f), 5–20 per mm²; medium (m), 20–40 per mm²; abundant (a), 40–100 per mm²
Heterocellular ray body cells: procumbent (p); square (s); upright (u)
Ray marginal cells: square (s); upright (u); (bracketed = occasional)
Parenchyma: diffuse (d) or diffuse-in-aggregate (da); crystals (c)
Ray crystals (c) or silica bodies (s)

Attributes common to Spirostachys*,* Sclerocroton *and* Shirakiopsis

Vessels are commonly arranged in long, radial multiples ($\geq$4). Perforation plates are simple. Inter-vessel pits are alternate and polygonal; medium (8–10 μm) in *Spirostachys africana* [1,30,49], medium to large (8–10 μm to 11–16 μm) in *Sclerocroton integerrimus*[60] and large (11–16 μm) in *Shirakiopsis elliptica*[31]. Vessel-ray pits are bordered and similar to inter-vessel pits in size and shape in *Spirostachys africana* and *Shirakiopsis elliptica;* rounded or angular, with much reduced borders, in *Sclerocroton integerrimus*. Fibres are non-septate, with simple to minutely bordered pits. Fibres are short in *Spirostachys africana*; in *Sclerocroton integerrimus,* they are medium length and regularly arranged. *Shirakiopsis elliptica* fibre length varies from short to long (Figure 1).

Diffuse parenchyma occurs in *Spirostachys africana.* Parenchyma which is diffuse-in-aggregate (SJL 103, Figure 1a), or in narrow bands or lines which are up to three cells wide, may be observed as a variation.[1,30,49] Parenchyma which is diffuse or diffuse-in-aggregate is difficult to see. In *Sclerocroton integerrimus* parenchyma is diffuse-in-aggregate and/or there are narrow bands or lines up to three cells wide.[60] *Shirakiopsis elliptica* parenchyma is diffuse and/or diffuse-in-aggregate, with the variation of occasionally occurring in narrow bands or lines up to three cells wide.[31] Axial parenchyma strand length is either 4 or 8 cells per parenchyma strand in all three woody taxa.

Rays are exclusively uniseriate, commonly heterocellular, with procumbent, square and upright cells mixed throughout the ray (SJL88, Figure 1e), although this pattern varies within and between the three woody taxa (Table 1; SJL 103, Figures 1a–c; NDO, Figure 1d). *Shirakiopsis elliptica* reference charcoal ray cells are upright and square (SJL 67, Figure 1f). Very long rays occasionally occur in *Shirakiopsis elliptica* where two rays meet end to end, and are visible in both reference material (SJL 67) and archaeological material (SPCA B4c 45).[1,31,40]

Rays are frequent, with up to 12 observed per millimetre. Laticifers – thin, radial tubes carrying latex which occasionally occur in Euphorbiaceae wood – were absent from the charcoal examined.[1,35]

Distinguishing attributes of reference material

The charcoal of the three species differs in vessel size and frequency as well as in the presence or absence of crystals or silica bodies in ray cells. The differences in vessel size and pattern among the wood of the three species are recorded in a photographic study of endgrain woodblocks of Euphorbiaceae.[49]

Spirostachys africana has several to many, small to medium vessels in long radial lines.[1,30,49] Charcoal reference, SJL 103, (Figure 1) and interpreted reference material, NDO, vessels are narrow (30–50 μm), at a frequency of between 40–100 vessels per mm^2 (an average of 80/mm^2) and vessels are arranged in long radial lines (radial multiples $\geq$ 4). The prismatic crystals in ray cells are birefringent, appearing shiny under reflected polarised light. Shape may vary in different material, from clearly rhombic in the archaeological specimen – which is an interpreted reference for *Spirostachys africana* (NDO in Figure 1d) – to irregular but shiny in the modern wood forestry block reference material (SJL 103 in Figure 1b and 1c). Crystals are visible in the SEM images recorded by Allott[20]. Silica bodies are absent from ray cells. The vessel inclusions are either resin[1] or gum[30]. Resin occurs in the heartwood.[1] Similar comparative images of *Spirostachys africana* have been recorded by Allott[20], Ilic[32] and Kromhout[33].

Sclerocroton integerrimus has few, medium to large vessels in radial lines.[1,60] Charcoal reference material, SJL 88, vessels are narrower than 100 μm, at a frequency of 20–40 vessels per mm^2, and are arranged in short radial lines of two to four vessels. The silica bodies are non-birefringent and opaque under polarised light (SJL 88, Figure 1e).[35] The silica bodies present in ray cells are spheroidal, irregularly shaped particles[35] arranged in aggregates which often fill the entire cell lumen[40]. These silica bodies are visible in the SEM images recorded by Allott[20]. Prismatic crystals are absent from ray cells.

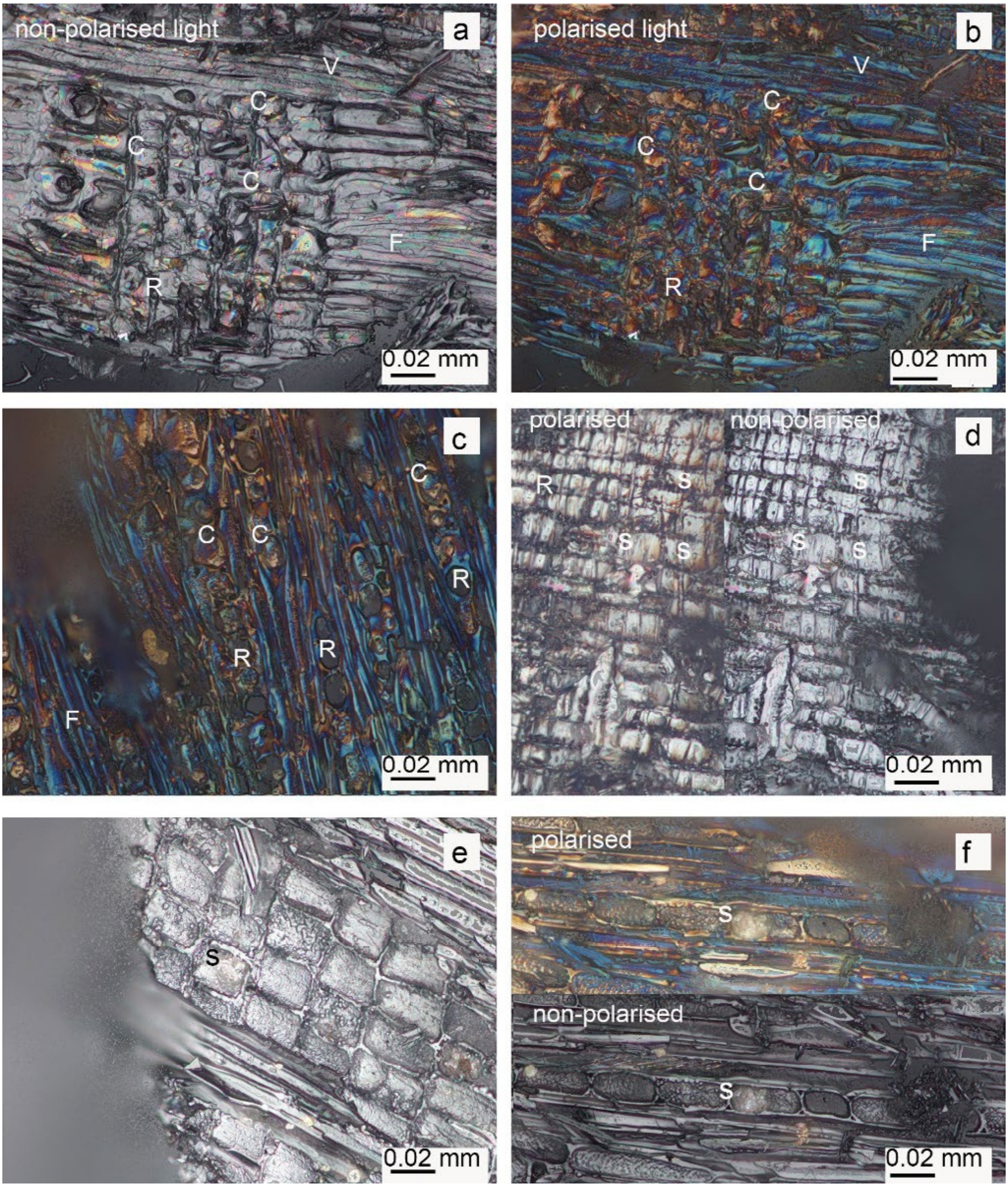

Figure 2: Diagnostic characteristics of archaeological charcoal: (a–c) *Spirostachys africana,* (d) *Shirakiopsis elliptica* and (e,f) *Sclerocroton integerrimus.* (a) In *Spirostachys africana*, SPCA B4b 62, there are prismatic crystals (C) in the procumbent ray cells (R), under non-polarised light. (b) These appear shiny under polarised light in radial longitudinal section, matching those of the *Spirostachys africana* SJL 103 reference material. The vessel (V), fibre (F) and ray cell (R) walls are also birefringent under polarised light. (c) In *Spirostachys africana,* SPCA D5c 51, tangential longitudinal section, the ray cell walls are cut away and the crystals underneath are present and shiny, matching those of the *Spirostachys africana* SJL 103 reference material when magnified 500x. (d) In *Shirakiopsis elliptica* archaeological material, MOD C6a 46, silica bodies (S) are present as grains and dots in ray cells and appear opaque under both polarised and non-polarised light. Rays are heterocellular, with mixed upright, square and procumbent cells, in radial longitudinal section. (e) In *Sclerocroton integerrimus* archaeological charcoal, SPCA B4b 66, the silica bodies in the ray cells are spheroidal or irregularly shaped and opaque in non-polarised light in radial longitudinal section and in both polarised (f) and non-polarised light in tangential longitudinal section.

Shirakiopsis elliptica has very few, large to very large vessels in radial lines.[1,31] Charcoal reference material, SJL 67, (Figure 1f) vessels are wider than 100 μm, at a frequency of fewer than 10 vessels per mm^2, and are arranged in short radial lines. Silica bodies occur as grains or small dark dots in Asian *Sapium* species.[41] In our reference material, the silica bodies are inconspicuous, prismatic crystals are absent from ray cells with occasional crystals observed in the parenchyma and tyloses commonly occurring in vessels.[1,31]

Spirostachys *and* Sclerocroton *archaeological charcoal*

Spirostachys africana was identified in charcoal from Sibudu from MOD square C6a and from SPCA squares B4b and D5c. Specimens MOD C6a 39 and SPCA 62 have as many as 110 vessels per mm^2, small (20–30 μm) to medium (50–100 μm) in size and arranged in radial multiples $\geq$4 (long radial lines); rays are uniseriate, frequent and heterocellular, with mixed procumbent, square, upright cells. Prismatic crystals occur in ray cells and shine under polarised light (Figure 2a–c).

Sclerocroton integerrimus charcoal was identified from MOD square E3d and from SPCA squares B4b, B4c and D5a. Few (30–50 vessels per mm^2), medium-sized (50–100 μm) vessels occur in radial multiples $\geq$4. Silica bodies occur as aggregates which partially fill the ray cell lumen and are opaque under both non-polarised as well as polarised light (SPCA B4b 66; Figure 2e). Variation was observed in vessel arrangement and ray cell pattern (Table 1).[1,60]

Shirakiopsis elliptica charcoal was identified from MOD square C6a. Specimen MOD C6a 46 has many (40 vessels per mm^2), medium ($\pm$50 μm) vessels in radial lines. Specimen SPCA B4c 45 has as many as 50 large (100-μm) vessels per mm^2. Occasionally two uniseriate rays meet, forming rays which are jointly longer than 1 mm. Silica bodies in ray cells appear as inconspicuous grains or small, dark dots (MOD C6a 46; Figure 2). The diffuse parenchyma occasionally contains crystals.[1]

Summary of the characteristic diagnostic features of each species

The detailed anatomical study of reference material enables the identification of these taxa based on the occurrence of crystals in *Spirostachys africana* and silica bodies in *Sclerocroton integerrimus* and *Shirakiopsis elliptica* ray cells[1] as well as on vessel size classes and frequency of vessels. These diagnostic characteristics are compared in Table 2.

The vessel size of *Spirostachys, Sclerocroton* and *Shirakiopsis* increases proportionally as vessel frequency decreases, from *Spirostachys africana*, with the smallest and most numerous vessels, to *Sclerocroton integerrimus*, then to *Shirakiopsis elliptica* with the largest and fewest vessels.

A comparison of anatomical features

No clear differentiation in anatomical features among these three taxa under investigation could be found, thus necessitating this study. There is some variation in vessel width and frequency, ray cell type, the absence or presence of tyloses in vessels, of laticifers in rays and of gum or resin deposits in vessels among different published accounts.[1,30,31,33,49,59]

Relative abundance of crystals may vary. As wood is inherently variable, some features are well defined in some samples, but poorly defined or absent in other samples of the same species.[35] There are no quantitative criteria for 'common' in the list of the International Association of Wood Anatomists. Comments on relative frequency are therefore added to descriptions.[35]

The crystals of calcium oxalate are birefringent under polarised light; however, some cell walls, especially lignified cell walls, are also birefringent (Anonymous reviewer, 2014, personal observation, written communication, July 03). Structures such as xylem vessels with birefringent cell walls have a rainbow sheen in the reference material (SJL 103) and in the inferred reference material (NDO). Birefringent crystals are visible in *Spirostachys africana* reference material in the

Table 2: Comparing the wood anatomy, environment and uses of *Spirostachys, Sclerocroton and Shirakiopsis* (Euphorbiaceae)

	Spirostachys africana[2,30,59]	***Sclerocroton integerrimus***[2,60]	***Shirakiopsis elliptica***[2,31]
Wood anatomy: vessel size and frequency, cell inclusions	Small to medium vessels, 50–100 μm wide Several (20–40/mm^2) to many (40–100/mm^2) vessels	Medium (50–100 μm wide) to large (100–200 μm wide) vessels Few vessels, 5–20/mm^2	Large (100–200 μm wide) to very large ($\geq$200 μm wide) vessels Very few vessels, $<$5/mm^2
	Prismatic crystals are present in ray cells. Parenchyma may contain prismatic crystals.	Silica bodies are present in ray cells. Parenchyma may contain prismatic crystals.	Silica bodies are present in ray cells. Parenchyma may contain prismatic crystals.
Timber	The hard, heavy wood has contrasting light sap wood and dark heartwood, with an attractive lustre when polished and has therefore been used as a replacement for sandalwood. It is used in furniture, for staves, beads and bangles and in construction as rafters. The sawdust is poisonous, as is the wood if burnt for fuel, causing conjunctivitis, nausea and food poisoning.[28,55]	The hard, durable wood is used as timber for general purposes, in construction and for furniture.[56]	The tough, soft, light, white wood is used to make instruments, burnt as firewood and charcoal, but not used as rafters when used in construction as it is susceptible to insects.[57]
Phytochemistry and uses	Phorbol esters (terpenoids) classify latex in bark, wood, stems and leaves as an extremely hazardous, Class 1a, cellular poison (LD_{50} =5 mg/kg).[28,50,61] Isolated terpenoids have antibacterial properties.[28] A very drastic purgative, the bark and milky latex are used to treat alimentary tract infections. Latex is used to treat tooth decay and eye infections. Used as fish and arrow poison, latex causes conjunctivitis, or a severe contact dermatitis. Bark is used for skin ailments, and headaches. The fragrant woodblocks are an insect repellent.[62] Smoke is inhaled for treatment of respiratory infections.[28,55]	Toxic tetracyclic triterpenic cucurbitacins have been extracted from root bark of *Sclerocroton cornutus* from West and Central Africa.[56] Suspected of being poisonous, the clear latex is used as an antiseptic against toothache and coughs. The fruits were formerly used to make a black ink and are used for tanning.[56]	Tannins and alkaloids have been extracted from the whole plant. Bark extracts have moderate antimicrobial activity against *Campylobacter jejuni* which causes food poisoning.[57] Considered very poisonous and a very drastic purgative, the clear latex is added to arrow poison and used as bird lime.[57]

tangential longitudinal section in which the cell walls are cut away. The outline of these crystals is visible, differentiating these crystals from ray cell walls in the radial longitudinal section. Under non-polarised light, the crystals in the inferred reference material match those seen in SJL 103 and those usually seen in wood[35] and charcoal[44]. The crystals seen in archaeological specimens of *Spirostachys africana* match those found in reference material SJL 103 ray cells observed in radial and tangential longitudinal sections at high magnification.

The *Spirostachys africana* crystals are magnified to 500x the original size and they are clearly visible. In SEM studies of charcoal, energy dispersive x-ray spectrometry analysis capabilities may be used to distinguish between crystals of calcium oxalate and silica, such as the crystals found in *Searsia undulata* (Namaqua kuni-bush), *Cassine peragua* (spoon-wood) and *Gymnosporia buxifolia* (spike-thorn) from archaeological charcoal at Diepkloof Rock Shelter.[44]

The variation in anatomical features between the charcoal and fresh wood or between charcoal made from modern wood and archaeological charcoal are because of the natural variation occurring in biological material affected by the sample origin (twig or trunk) or habitat.[49] Quantitative variation may be a result of the shrinkage and distortion which occurs during the formation of charcoal.[36,38]

We have supplemented the charcoal wood anatomy descriptions with those from fresh woods from the InsideWood database as more detail usually is visible in fresh wood. Comparative sizes rather than measurements are used in anthracology because the anatomy may be distorted by shrinkage, vitrification, diagenesis, and fragmentation and powdering.[36,38,41]

Conclusion

The charcoal anatomy of *Spirostachys africana, Sclerocroton integerrimus* and *Shirakiopsis elliptica* enables these species to be distinguished by vessel arrangement, size and frequency, as well as by the presence or absence of crystals or silica bodies in ray cells.

Spirostachys africana has narrow, frequent vessels; prismatic crystals are present in ray cells. *Sclerocroton integerrimus* has wider, less frequent vessels. Of the three species under comparison, *Shirakiopsis elliptica* has the widest and least frequent vessels. Silica bodies are present in ray cells of *Sclerocroton integerrimus* and in *Shirakiopsis elliptica* as small grains and dots while prismatic crystals are absent from ray cells. The silica bodies of *Sclerocroton integerrimus* are aggregates of irregularly shaped silicon dioxide particles which often fill the entire cell lumen and appear opaque under polarised light. The silica bodies of *Shirakiopsis elliptica* appear as grains or small, dark dots.

Spirostachys africana was identified amongst hearth charcoal of the SPCA layer in squares B4b and D5c, with an age of approximately 58 ka; and in charcoal of the MOD layer in square C6a, with an age of approximately 49 ka. This find confirmed the use of *Spirostachys africana* wood at Sibudu rock shelter. *Sclerocroton integerrimus* charcoal occurred in SPCA in squares B4b, B4c and D5a and in MOD in square E3d. *Shirakiopsis elliptica* charcoal was found in SPCA B4c and in MOD in square C6a.

Sclerocroton integerrimus, Shirakiopsis elliptica and poisonous *Spirostachys africana* wood was deliberately burned by people at Sibudu who utilised natural resources. *Sclerocroton integerrimus* timber is a hard, heavy, durable wood. *Shirakiopsis elliptica* timber is soft, light, and suitable for making implements. *Spirostachys africana* wood is a hard, durable wood, with poisonous properties.

Many of the present day uses of these woods were not applicable during the Middle Stone Age, but these species may have been selected for making wooden implements and for firewood. *Spirostachys africana* wood is a skin irritant and it seems unlikely that this wood would have been worked by hand to make implements. Nor does it seem likely that the poisonous wood was used for domestic fires to cook food. It seems more likely that *Spirostachys* was deliberately selected for its toxic or insecticidal properties, perhaps so that its smoke would fumigate insects from the camp in Sibudu.

Acknowledgements

The National Research Foundation (South Africa) and the Palaeontological Scientific Trust and its Scatterlings Projects (PAST) are thanked for providing funding. S.J.L. thanks the ESI for equipment and her PhD supervisors for enabling this research and for helping in countless ways. Dr Christine Sievers and the late Tony Abbott helped to collect the modern wood reference material. Archaeologists working at Sibudu under the leadership of Professor Lyn Wadley, University of the Witwatersrand, collected the charcoal during several excavations over several years. A donation of modern wood from the Larry Leach Herbarium and Department of Biodiversity, University of Limpopo, extended the charcoal reference collection. Access to the microscopes in the Geology Department, University of Limpopo, assisted the study of *Shirakiopsis* silica bodies*.* The anonymous reviewers are thanked for improving this paper.

Authors' contributions

S.J.L. was responsible for the experimental work and wrote the manuscript; M.K.B. supervised the research, helped with charcoal identification and assisted with writing the manuscript.

References

1. Mennega AMW. Wood anatomy of the subfamily Euphorbioideae. A comparison with subfamilies Crotonoideae and Acalyphoideae and the implications for the circumscriptions of the Euphorbiaceae. IAWA J. 2005;26:1–68. http://dx.doi.org/10.1163/22941932-90001601
2. Wadley L, Sievers C, Bamford M, Goldberg, P, Berna F, Miller C. MSA bedding construction and settlement patterns at Sibudu, South Africa. Science. 2011;334:1388–1391. http://dx.doi.org/10.1126/science.1213317
3. Jacobs Z, Roberts RG, Galbraith RF, Deacon HJ, Grün R, Mackay A, et al. Ages for the MSA of southern Africa: Implications for human behaviour and dispersal. Science. 2008;322:733–735. http://dx.doi.org/10.1126/science.1162219
4. Wadley L. Partners in grime: Results of multi-disciplinary archaeology at Sibudu Cave. South Afr Humanit. 2006;18:315–341.
5. Wadley L. MIS 4 and MIS 3 occupations in Sibudu, KwaZulu-Natal, South Africa. S Afr Archaeol Bull. 2013;68:41–51.
6. Wadley L, Jacobs Z. Sibudu Cave: Background to the excavations, stratigraphy and dating. South Afr Humanit. 2006;18:1–26.
7. Backwell L, d'Errico F, Wadley L. MSA bone tools from the Howiesons Poort layers, Sibudu Cave, South Africa. J Archaeol Sci. 2008;35:1566–1580. http://dx.doi.org/10.1016/j.jas.2007.11.006
8. Bentsen SE. Size matters: Preliminary results from an experimental approach to interpret MSA hearths. Quatern Int. 2012;270:95–102. http://dx.doi.org/10.1016/j.quaint.2011.09.002
9. Bentsen SE. Using pyrotechnology: Fire-related features and activities with a focus on the African Middle Stone Age. J Archaeol Res. 2014;22(2):141–175. http://dx.doi.org/10.1007/s10814-013-9069-x
10. d'Errico F, Vanhaeren M, Wadley L. Possible shell beads from the MSA layers of Sibudu Cave, South Africa. J Archaeol Sci 2008;35:2675–2685. http://dx.doi.org/10.1016/j.jas.2008.04.023
11. Hodgskiss T. An investigation into the properties of the ochre from Sibudu, KwaZulu-Natal, South Africa. South Afr Humanit. 2012;24:99–120.
12. Sievers C, Wadley L. Going underground: Experimental carbonization of fruiting structures under hearths. J Archaeol Sci. 2008;35:2909–2917. http://dx.doi.org/10.1016/j.jas.2008.06.008
13. Wadley L. Some combustion features at Sibudu, South Africa, between 65,000 and 58,000 years ago. Quatern Int. 2012;247:341–349. http://dx.doi.org/10.1016/j.quaint.2010.10.026
14. Wadley L. Two 'moments in time' during MSA occupations of Sibudu, South Africa. South Afr Humanit 2012;24:79–97.
15. Hall G, Wadley L, Woodborne S. Past environmental proxies from the Middle Stone Age at Sibudu, Kwazulu-Natal, South Africa. J Afr Archaeol. 2014;12(1):7–24. http://dx.doi.org/10.3213/2191-5784-10246

16. Wadley L, Hodgskiss T, Grant M. Implications for complex cognition from the hafting of tools with compound adhesives in the MSA, South Africa. Proc Natl Acad Sci USA. 2010;106:9590–9594. http://dx.doi.org/10.1073/pnas.0900957106

17. Wadley L. Were snares and traps used in the MSA and does it matter? A review and a case study from Sibudu, South Africa. J Hum Evol. 2010;58:179–192. http://dx.doi.org/10.1016/j.jhevol.2009.10.004

18. Lombard M, Phillipson L. Indications of bow and stone-tipped arrow use 64 000 years ago in KwaZulu-Natal, South Africa. Antiquity. 2010;84:635–648. http://dx.doi.org/10.1017/S0003598X00100134

19. Allott LF. Changing environments in oxygen isotope stage 3: Reconstructions using archaeological charcoal from Sibudu Cave. S Afr J Sci. 2004;100:179–184.

20. Allott LF. Palaeoenvironments of the MSA at Sibudu Cave, KwaZulu-Natal, South Africa: An analysis of archaeological charcoal [PhD thesis]. Johannesburg: University of the Witwatersrand; 2005. p. 54–96.

21. Allott LF. Archaeological charcoal as a window on palaeovegetation and wood-use during the MSA at Sibudu Cave. South Afr Humanit. 2006;18:173–201.

22. Bruch AA, Sievers C, Wadley L. Quantification of climate and vegetation from southern African MSA sites – An application using late Pleistocene plant material from Sibudu, South Africa. Quaternary Sci Rev. 2012;45:7–17. http://dx.doi.org/10.1016/j.quascirev.2012.04.005

23. Miller CE, Sievers C. An experimental micromorphological investigation of bedding construction in the MSA of Sibudu, South Africa. J Archaeol Sci. 2012;39:3039–3051. http://dx.doi.org/10.1016/j.jas.2012.02.007

24. Sievers C. Seeds from the MSA layers at Sibudu Cave. South Afr Humanit. 2006;18:203–222.

25. Sievers C. Sedges from Sibudu, South Africa: Evidence for their use. In: Fahmy AG, Kahlheber S, D'Andrea C, editors. Windows on the African past. Current approaches to African archaeobotany. Proceedings of the Sixth International Workshop on African Archaeobotany; 2009 June 13–15; Cairo, Egypt. Frankfurt: Afrika Magna Verlag; 2011. p. 9–18.

26. Sievers C. Sedges as bedding in MSA Sibudu [PhD thesis]. Johannesburg: University of the Witwatersrand; 2013.

27. Renaut R, Bamford MK. Results of preliminary palynological analysis at Sibudu Cave. South Afr Humanit. 2006;18:235–240.

28. Wink M, Van Wyk B-E. Mind altering and poisonous plants of the world. Pretoria: Briza; 2008. Cunningham AB. Applied ethnobotany, people, wild plant use and conservation. London: Earthscan Publishers; 2001.

29. Cunningham AB. Applied ethnobotany, people, wild plant use and conservation. London: Earthscan Publishers; 2001.

30. Mugabi P, Gasson PE, Wheeler EA. Spirostachys africana Sond. anatomical description. In: InsideWood [homepage on the Internet]. c2004 [cited 2013 Aug 14]. Available from: http://insidewood.lib.ncsu.edu

31. Louppe D, Détienne P, Wheeler EA. Shirakiopsis elliptica (Hochst.) Esser. anatomical description. In: InsideWood [homepage on the Internet]. c2004 [cited 2013 Aug 23]. Available from: http://insidewood.lib.ncsu.edu

32. Ilic J. CSIRO atlas of hardwoods. Melbourne: Crawford House Press in Association with the CSIRO; 1991. p. 209.

33. Kromhout CP. `n Sleutel vir die mikroskopiese uitkenning van die vernaamste inheemse houtsoorte van Suid-Afrika [A key for the microscopic identification of prime indigenous woods of South Africa]. Bulletin 50. Pretoria: South African Department of Forestry; 1975. Afrikaans.

34. Neumann K, Schoch W, Détienne P, Schweingruber FH. Woods of the Sahara and the Sahel, an anatomical atlas, character list. Bern: Haupt; 2001. p. 54–58.

35. Wheeler EA, Baas P, Gasson PE, editors. IAWA list of microscopic features for hardwood identification by an IAWA Committee. IAWA Bull. 1989;10:219–332. Available from: http://iawa-website.org

36. Chrzazvez J, Théry-Parisot I, Fiorucci G, Terral J-F, Thibaut B. Impact of post-depositional processes on charcoal fragmentation and archaeobotanical implications: Experimental approach combining charcoal analysis and biomechanics. J Archaeol Sci 2014;44:30–42. http://dx.doi.org/10.1016/j.jas.2014.01.006

37. February EC. Archaeological charcoal and dendrochronology to reconstruct past environments of southern Africa. S Afr J Sci. 2000;96:111–116.

38. Théry-Parisot I, Chabal L, Chrzavzez J. Anthracology and taphonomy, from wood gathering to charcoal analysis: A review of the taphonomic processes modifying charcoal assemblages, in archaeological contexts. Palaeogeogr Palaeocl. 2010;291:142–153. http://dx.doi.org/10.1016/j.palaeo.2009.09.016

39. Wheeler EA. InsideWood - A web resource for hardwood anatomy. IAWA J. 2011;32:199–211.

40. Richter HG, Dallwitz MJ. Commercial timbers: Descriptions, illustrations, identification, and information retrieval [homepage on the Internet]. c2000 [updated 2009 June 25; cited 2013 Aug 14). Available from: http://delta-intkey.com.

41. Gonçalves TAP, Marcati CR, Scheel-Ybert. The effect of carbonisation on wood structure of Dalbergia violaceae, Stryphnodendron polyphyllum, Tapirira guianensis, Vochysia tucanorum, and Pouteria torta from the Brazilian Cerrado. IAWA J. 2012;33:73–90. http://dx.doi.org/10.1163/22941932-90000081

42. Hubau W, Van den Bulcke J, Kitin P, Mees F, Van Acker J, Beeckman H. Charcoal identification in species-rich biomes: A protocol for Central Africa optimised for the Mayumbe forest. Rev Palaeobot Palyno. 2012;171:164–178. http://dx.doi.org/10.1016/j.revpalbo.2011.11.002

43. Hubau W, Van den Bulcke J, Kitin P, Brabant L, Van Acker J, Beeckman H. Complimentary imaging techniques for charcoal examination and identification IAWA J. 2013;34:147–168. http://dx.doi.org/10.1163/22941932-00000013

44. Cartwright CR. Identifying the woody resources of Diepkloof Rock Shelter (South Africa) using scanning electron microscopy of the MSA wood charcoal assemblages. J Archaeol Sci. 2013;40:3463–3474. http://dx.doi.org/10.1016/j.jas.2012.12.031

45. Cartwright C, Parkington J. The wood charcoal assemblages from Elands Bay Cave, south-western Cape: Principles, procedures and preliminary interpretation. S Afr Archaeol Bull. 1997;52(165):59–72. http://dx.doi.org/10.2307/3888977

46. Esterhuysen AB, Mitchell PJ. Palaeoenvironmental and archaeological implications of charcoal assemblage from Holocene sites in western Lesotho, Southern Africa. Palaeoeco A. 1996;24:203–232.

47. Tusenius ML. The study of charcoal from some southern African archaeological contexts [MA thesis]. Stellenbosch: Stellenbosch University; 1986.

48. Coates-Palgrave M. Keith Coates Palgrave trees of southern Africa. 3rd ed. Cape Town: Struik; 2002. p. 519–522.

49. Westra LYT, Koek-Noorman J. Wood atlas of the Euphorbiaceae. IAWA J. 2004(suppl. 4):17,21,24,102,104.

50. Paulsen B, Ekeli H, Johnson Q, Norum KR. South African traditional medicinal plants from KwaZulu-Natal: Described 1903–1904 by Dr Henrik Greve Blessing. Oslo: UNIPUB; 2012.

51. Rodin RJ. The ethnobotany of the Kwanyama Ovambos. Monographs in Systematic Botany from the Missouri Botanical Garden. vol. 9. St. Louis: Missouri Botanical Garden; 1985.

52. Beach RA, Gansho N, Flesche J, Scott C, Khumalo NP. Possible drug reaction, eosinophilia and systemic symptoms (dress) syndrome in an infant from ingestion of Spirostachys africana complicated by measles co-infection. S Afr J Child Health. 2010;4:112–113.

53. Grace OM, Prendergast HDV, Jäger AK, Van Staden J. Bark medicines used in traditional healthcare in KwaZulu-Natal, South Africa: An inventory. S Afr J Bot. 2003;69:301–363.

54. Mathabe MC, Hussein AA, Nikolova RV, Basson AE, Meyer JJM, Lall N. Antibacterial activities and cytotoxicity of terpenoids isolated from Spirostachys africana. J Ethnopharmacol. 2008;116:194–197. http://dx.doi.org/10.1016/j.jep.2007.11.017

55. Watt JM, Breyer-Brandwijk M. The medicinal and poisonous plants of southern and eastern Africa, being an account of their medicinal uses, chemical composition, pharmacological effects and toxicology in man and animal. 2nd ed. London: Livingstone; 1962.

56. Schmelzer GH. Sclerocroton cornutus (Pax) Kruijt & Roebers. In: Schmelzer GH, Gurib-Fakim A, editors. PROTA (Plant Resources from Tropical Africa) [homepage on the Internet]. c2007 [cited 2013 Aug 14]. Available from: http://www.prota4u.org

57. Schmelzer GH. Shirakiopsis elliptica (Hochst.) Esser. In: Schmelzer GH, Gurib-Fakim A, editors. PROTA (Plant Resources from Tropical Africa) [homepage on the Internet]. c2007 [cited 2013 Aug 24]. Available from: http://www.prota4u.org

58. Greenfield HJ, Fowler KD, Van Schalkwyk LO. Where are the gardens? Early Iron Age horticulture in the Thukela River Basin of South Africa. World Archaeol. 2005;37:307–328. http://dx.doi.org/10.1080/00438240500095496

59. Kribs DA. Commercial foreign woods on the American market. New York: Dover Publications; 1968. Available from: http://insidewood.lib.ncsu.edu

60. Mennega AMW. Sclerocroton integerrimus anatomical description. In: Wood anatomy of the subfamily Euphorbioideae. A comparison with subfamilies Crotonoideae and Acalyphoideae and the implications for the circumscriptions of the Euphorbiaceae. IAWA J. 2005;26:1–68. Available from: http://insidewood.lib.ncsu.edu/description.5 http://dx.doi.org/10.1163/22941932-90001601

61. Van Wyk BE, Van Heerden F, Van Oudtshoorn, B. Poisonous plants of South Africa. Pretoria: Briza; 2002.

62. Venter F, Venter JA. Making the most of indigenous trees. Pretoria: Briza; 1996.