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Resin-Secretory Structures of Boswellianeglecta (Burseraceae) in Borana Administrative Zone, Southern Ethiopia

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Abstract

Boswellia neglecta is a small tree that produces commercially important oleo-resin known as frankincense. This frankincense has been used for varied purposes, and it is a source of income for rural households in southern and south-eastern Ethiopia. Despite the long history of using this species, knowledge on bark anatomy and resin-secretory structures is lacking. In this study, anatomical description of the bark including network and distribution of resin secretory structures were determined; relationship between bark thickness, and axial resin canal characteristics with tree DBH of B. neglecta were assessed in dry deciduous woodlands Borana Administrative Zone, Ethiopia. Twenty-one sampled trees were raandomly selected for this study. The network of resin-secretory structure was investigated from tangential, radial and transversal sections of bark samples using light microscopy. The average density, diameter and total area of axial resin canals were determined from transversal sections of 42 bark samples. The results revealed that the inner bark axial and radial resin canals occurred and that they are interconnected by radial resin canals. Whereas, in the wood, there were no any resin secretory structures. On average, density of functional-axial resin canals is low, reaching 0.33 mm^{-2}. Inner bark thickness linearly increases with tree diameter, but resin canal density was not varying across the inner bark. The total area of axial resin canals was highly significant ($p<0.001$) positively related to the DBH. The findings of the study will thus lead to new advances in our insights and knowledge on the resin secretory structures of B. neglecta. However, further research is required to introduce new tapping technology for a sustainable resin production.

Keywords: Axial resin canals; Boswellia neglecta; Frankincense; Resin-secretory structures

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Introduction

Several tree species, mainly in tropical and subtropical zones, produce resins (Langenheim, 1994). Although resin-producing species are widely distributed in the plant kingdom, copious amount of commercially valuable resins are produced from few families, (e.g., *Burseraceae*, *Pinaceae* and *Leguminosae*) (FAO, 1995). Resins produced from *Boswellia* and

Commiphora species are recognized as frankincense and myrrh, respectively and are used for religious ceremony (Groom, 1981; Tucker 1986a). They are also traded as a source of raw material for several industries (Lemenih and Teketay, 2003), such as pharmaceuticals (Greene, 1993; Michie and Cooper, 1991), food industries (Ford *et al*., 1992; Khan and Abourashed., 2011), and cosmetic industries (Khan &Abourashed., 2011; Tucker, 1986b).

In Ethiopia, a resin is produced by several tree species, and three types of frankincense are well known according to their origin: Tigray, Ogaden and Borana type. Tigray and Ogaden types represent frankincense produced in northern and north western as well as eastern parts of Ethiopia, respectively (Lemenih & Kassa, 2011). The Borana-type frankincense is produced from *Boswellia neglecta*growing in the southern and south-eastern part of the country (Eshete *etal*., 2005; Tadesse *et al*., 2007a). This frankincense has been used for various purposes, and it is a source of income for rural households (Eshete *et al*., 2005; Mekonnen *et al*., 2013; Worku, 2006; Worku *et al*., 2011). Currently, it is also widely traded as incense on local markets (Mekonnen *etal*., 2013; Worku, 2006).

Nowadays, in Ethiopia, the traded resins are collected both from natural oozing out of a tree in southern part of the country (*B. neglecta*) (Africa and Sahel, 2004; Tadesse *et al*., 2007a; Worku, 2006) or tapping of trees during the dry season (*B. papyrifera*) in the northern, north western and western part (Tadesse *et al*., 2007; Lemenih*et al*., 2011). Tapping is an artificial wounding of trees for resin production. It involves several incisions in the bark of the stem using a special tool, locally known as a "mengaf" or "sonke" (Eshete *et al*., 2012). However, improper tapping induces physical damage to a tree (Mengistu *et al*., 2012; Rijkers *et al*., 2006a). For example, in the last 20 years, more than 177,000 hectares of *Boswellia* forests have been destroyed in Tigray region because of improper tapping and others factors, such as over grazing, fire and conversion of forestland to agriculture (Kindeya, 2002).

The southern part of Ethiopia, predominantly the Borana Administrative Zone, is endowed with species of gum and resin producing tree species which have the potential to provide additional opportunity to improve the livelihood of the local community as well as the country (Gemedo-Dalle *et al*., 2005; Worku *et al*., 2012). Among these, *Boswellia neglecta*is ecologically adapted, commercially and socio-culturally preferred species. The species is useable as charcoal, fuelwood, construction wood, medicine, and is also well known to produce commercially important oleo-gum resin: frankincense (Eshete *et al*., 2005; Gizaw, 2006). This product is important to local livelihoods for cash income (Dalle*et al*., 2005; Worku, 2006; Worku*et al*., 2011). However, resin collection is carried out by picking of exudates from trees, which is naturally or accidently oozing out the trees (Eshete *et al*., 2005; Tadesse *et al*., 2007b). In this area, there is no well-developed tapping strategy for efficient resin production (Eshete et al., 2005; Gizaw, 2006; Mengistu *et al*., 2012).

Resins are produced in two different ways (Langenheim, 2003). The first is induced resin production, where resin is synthesized at the moment and at a site of injury as a response to wounding. The second is constitutive resin formation, where preformed resin is produced in specialized secretory structures in the root, leaves, bark or wood parts of a tree and released in case of injury (Langenheim, 2003). In some commercial species, such as *Protium copal*, this constitutive resin is produced in the bark of trees and drained by tapping (Neels, 2000). Resin is produced and stored in internal specialized secretory structures, such as resin canals, resin pockets (i.e. blisters), and resin cavities, which are common in conifers and tropical angiosperm trees (Langenheim, 2003). Nevertheless, the types of structures vary according to species (Langenheim, 2003; Nagy *et al*., 2000).

Tolera et al. (2013a) investigated in detail the wood and bark structure of *Boswellia papyrifera*in north-western Ethiopia. They, for example, found that both axial and radial resin canals that form three-dimensional network in the inner bark with axial resin canals being abundant near the cambium, but decrease in number when increasing the distance from the cambium to outwardly as a result of dilatation. In the wood, the authors noted that there are a few radial resin canals. Similarly, (Bhatt, 1987) found that both axial and radial canals in the bark of *Commiphorawightii (Burseraceae*). In a sequential study, Tolera*et al*. (2013b) showed that resin canal characteristics (resin-canal area and density of resin canals) are significantly related to tree size characteristics (DBH, leaf apices and age), but not with radial growth. These features are also closely related to resin yield. This result is supported by Blanche *et al*. (1992).

Likewise, several others studies(Fahn, 1988; Nagy *et al*., 2000; Nair, 1998; Rodríguez-García *et al*., 2014b; Zhang *et al*., 2008) have been conducted on the resin-secretory structures to understand tree morphological factors related to resin-secretory structures.For example, Ella and Tongacan(1992) pointed out that the abundance of resin canals could be closely related to tree size characteristics, such as stem diameter and crown condition. Such information is important in developing appropriate tapping techniques for individual trees to obtain a sustainable resin yield (Ella &Tongacan, 1992; Tolera, 2013*)*.

Yet, no comparable research has been done on the economical valuable resin-producing species, especially focusing mainly on *B. neglecta* which is found in southern Ethiopia. The limitation on such information is an obstacle to develop sustainable tapping technology. Taking all concern and facts into consideration, the government is now planning different activities to develop tapping technologies for various gum and resin producing species in Ethiopia.

Understanding the distribution and networking of resin-secretory structures that are responsible for synthesis, storage and transport of resins is important to develop appropriate and efficient tapping technologies (Bannan, 1936; Rodríguez-García *et al*., 2014b; Tolera *et al*., 2013). Hence, the present study was initiated to understand the bark structure and distribution of resin-secretory structures in the bark and wood of *B. neglecta* in Borana Administrative Zone, south-eastern Ethiopia.

According to our primarily observation, *B. neglecta* naturally oozed white or black resin from their bark during the dry seasons. This leads to the expectation that the resin-secretory structures, such as axial and radial resin canals do appear in the bark and probably also in the wood of *B. neglecta*. It was also hypothesized that the distribution and the density of axial resin canals exhibit directional changes throughout the inner bark as a result of dilatation, like found in B. *papyrifera* (Tolera *et al*., 2013). Accordingly, it was also expected that trees with a higher DBH have a thicker bark and contain more functional resin canals (Tolera, 2013b). Therefore, the aim of this study was to provide scientific information on bark structure for the development offrankincense collection or through the introduction of appropriate and efficient tapping techniques. The research objectives were to: (i) investigate how is the bark structured and resin canals distributed in the inner bark (and wood) of *B. neglecta, and (ii)*examine the relationships between bark thickness and resin-secretory structure with tree size.

Materials and Methods

Description of the study area

The study area is located in the Borana Administrative Zone of Oromia National Regional State, southern Ethiopia. Borana represents the majority of southern and south-eastern parts of Oromia National Regional Statewith an area of 69,373.3 square kilometres(Worku *et al*., 2012). Specifically, this research was conducted in Arero District, which is found in south-eastern part of Borana at a distance of 660 km south of Addis Ababa (Angassa & Oba, 2008). The district covers an area of 10,890 km^2, and the average annual rain-fall ranges between 400 and 600mm (Gemedo-Dalle *et al*., 2005). The rainfall distribution is bimodal whereas the main dry season occurs between December and February, and a long rainfall falling in April and May (Gemedo-Dalle *et al.*, 2005). The dominant soil types in the district are granitic and volcanic with *Acacia-commiphora* woodlands dominated by *Acacia*, *Commiphora*and *Boswellia* species (Worku *et al*., 2012). They are the main source of economical valuable oleo-gum resins (Eshete *et al*., 2005; Worku *et al*., 2011).

Selection of species for the study

Boswellia neglecta S. Moore (of the family *Burseraceae*) is a small deciduous tree/shrub that is widely distributed in Ethiopia, Kenya, Somalia, Tanzania and Uganda (Vollesen, 1989). In Ethiopia, the species widely occurs in *Acacia-Commiphora*woodlands of south and south-east of the country (Lemenih *et al*., 2011; Worku *et al*., 2012). *B. Neglecta*grows up to a height of 6 meters and a stem diameter of 30 centimetre with thick grey-brown bark (Rijkers *et al*., 2006a). During the dry season, white or black resin oozes from the bark of *B. neglecta*but the white resin changes to black at a time of hot season (Worku, 2006). The resin contributes to local livelihoods for cash income and medicinal value (Mengistu *et al*., 2012; Rijkers *et al.*, 2006a;Worku, 2006).Despite resin is a highly important commodity, appropriate and efficient production system has not yet been developed in Ethiopia (Lemenih, 2011; Tadesse *et al*., 2007b).

Data collection and sampling techniques

Sample trees were selected from 12 quadrants, each measuring 50x50m laid along three transect lines in woodland dominated by *B. neglecta*. The quadrants were drawn from a square grid of 500m between the successive quadrants and same distance (500m) between the transect lines. The direction of transect lines and the distance to the first quadrant were selected randomly. To select the sample trees, stem diameter of all individual trees of *B.*

neglecta were measured and coded at breast height (DBH) i.e., at 1.3 m above ground using diameter tape in each quadrant. After measurement, trees were distributed in differentDBH classes (1=5-7cm, 2=7.1-9 cm, 3= 9.1-11, 4=11.1-13 and 5= 13.1-15, 6=15.1-17, 7= ≥ 17.1) based on their stem diameter. Then, three trees per diameter class were sampled randomly. The method for random sampling used in the study was random number tables. First, the diameters of all individual trees of B. neglecta were measured and assign a sequential number from each diameter class in the sample quadrants. Then, three sampled trees were chosen from random number tables in each diameter class. Therefore, a total of 21 sampled trees were selected for the study.

Bark and wood sampling size/procedure

Two bark samples per tree were taken from the opposite side at breast height with a Trephor tool (size: 140 mm long and 5 mm in diameter) from a sampled of 21 *B. neglecta* trees, i.e. three sample trees for each diameter class were sampled. For this study, 42 bark samples were collected. Investigation of resin-secretory structures in wood of *B. Neglecta* was done on samples from stem discs of seven trees.

Icro-Thin Section Preparation

Micro-thin sections of 50-µm thickness were prepared from transversal sections of all 42 bark samples (i.e. three barks per diameter class), and tangential and radial thin sections were prepared from seven bark samples (i.e. one bark sample per diameter class randomly selected) by using a sliding microtome (type: G. S. L.I light-weight microtome). And also, micro-thin sections (50 µm thickness) were prepared from transversal, tangential and radial sections of seven stem discs to check the presence/absence of resin-secretory structures in the wood of *B. neglecta*. The thin sections were stained with a mixture of Astra blue and safranin (150mg Astra blue, 40mg safranin and 2 ml acetic acid in 100 ml distilled water) for 15 minutes for microscopic observations and to distinguish functional (i.e. living) tissues from non-functional (i.e. dead) tissues (Schweingruber*et al*., 2006). Next, the stained micro-thin sections were cleared by distilled water, and subsequently the water droplets were dehydrated by glycerin solution.

Examination of resin-secretory structures

The type, network, distribution and characteristics of resin-secretory structures in the bark as well as presence/absence of resin-secretory structures in the wood were investigated from transversal, tangential and radial sections of the micro- thin sections of bark and wood samples using a light microscope (Leica DM 2500) with magnification ranges from 1.25 to 40x. Digital images of resin-secretory structures, such as, axial and radial resin canals were taken from 42 transversal, 7tangential and 7radial micro-thin sections using a light microscope attached with Leica camera.

The resin canals were classified as functional and non-functional resin canals according to the techniques illustrated by (Tolera *et al*., 2013). Functional and non-functional resin canals were distinguished according to the presence or absence of lignification of the cell wall of epithelial cells by using light microscopy. If a resin canal was encircled by non-lignified cell walls of epithelial cells, it was considered as functional resin canal; if a resin canal was encircled by lignified cell wall of epithelial cells, it was considered as non-functional resin canal.

Determination of Bark Cross-Sectional Area

The width and thickness of the inner bark of each bark sample as well as total bark thickness (inner + outer bark) were measured and averaged for the two bark samples per tree following the method applied by Tolera *et al*. (2013) using Image J software. The inner bark-area of individual bark samples was determined from the width and thickness (inner bark area = tangential width of the bark sample × thickness of the bark). The bark cross-sectional area at the sampling height (DBH) of each sample tree was estimated from the total cross-sectional area of the sample tree and the average bark thickness as calculated from the two samples. This means that bark cross-sectional area = total cross-sectional area - its cross-section- area of wood (assume circular shape at point of sampling). Where by cross-sectional area of wood= ((DBH/2)- average bark thickness)$^2 \times \pi$.

Measurement of resin-canal characteristics

Resin-canal characteristics were measured from transversal thin sections made from the two bark samples collected per tree. All axial resin canals were counted and their diameters were measured across the inner bark by using Image analysis software (Image J version 1.44p).

Average resin-canal density, diameter and total area of axial resin canals were measured from 21 study tree samples (i.e. 2 bark samples per tree) of *B. neglecta,* following the methods showed by Tolera, (2013b*)*. The average density of axial resin canals per inner-bark area (mm^{-2}) was calculated by dividing the total number of axial resin canals by the measured inner bark area of the sample. For each tree, an average resin-canal density is calculated from the measurements collected from the two samples. Likewise, the average diameter of the axial resin canals was determined for each sample tree and averaged for the two bark samples. Average diameter of axial resin canals was used to estimate the surface area of a single axial resin canals; area = $\pi\ (d/2)^2$ where d is a representative of the average resin canal diameter for each bark sample. Total resin-canal area of each sample tree was calculated by multiplying the total number of axial resin canals and average surface area of single axial resin canals.

Data Analysis

Descriptive statistics was used to describe the bark structure, network and distribution of resin-secretory structures in the bark and wood of *B. neglecta*. Pearson's correlation analysis was also used to determine the relationship between bark thickness, and axial resin canals characteristics with tree DBH. A test of the normal distribution was performed using Shapiro-Wilk. SPSS (PASW 19.0 for Windows statistical software package) was used for the analysis. The results were presented in Tables and Graphs.

Results of The Study

This section presents an anatomical description of the bark of *B. neglecta* including the network of resin-secretory structures and distribution of resin canals throughout the inner bark. In the second part, the relationship between axial resin canals and tree size (DBH) *of B. Neglecta*in the Borana Administrative Zone was presented.

Bark thickness, bark-anatomical features and resin-secretory structures of Boswellia neglecta

The average bark thickness of *B. neglecta*was 12.88 mm (sd=3.4) with a range of 7.98-22.08 mm at breast height. This was the equivalent of the total tree radius of 20 % (Table 1). The bark of *B. Neglecta*consists of two bark layers, defined as inner and outer bark layer (Fig. 1). There were differences in bark thickness between the inner and outer bark layers. The average thickness of the inner bark was about 11.05 mm (sd=3.19), it covers about 87.51 % of the total bark and the range was between 6.64 to 20.15 mm (Table 1). Fig. 1 showed an overview bark, whereas a closer view on the inner bark was given in Fig. 2. The inner bark located adjacent to the vascular cambium composed of living tissues and was characterized by the occurrence of multiseriate tangential bands of sieve tubes, sclerenchyma fibers and axial parenchyma cells in a regular pattern, which are crossed by phloem rays (Fig. 1).

With increasing distance from the cambium, rays get discontinues and the regular pattern of alternating sieve tubes and sclerenchyma layers is less obvious (Fig. 2). Resin canals are imbedded in the parenchyma bands. In most observed trees, parenchyma cells get enlarged and form wedges pattern called dilatation (Fig. 1).

The degree on the presence of dilatation varies between trees, but tends to be more obvious in trees with thicker inner bark. Nevertheless, the transition between the part of inner bark which is not affected by dilatation and the part affected by dilatation was not distinct.

The outer bark layer consists of a periderm and is hence formed as secondary protective tissue. The periderm is a multi-layered tissue system consisting of a (usually large) phellem part with regular cells. The outer bark of *B. neglecta* is much thinner than the inner bark, on average, 1.62 mm (sd=0.71) with a range of 0.23-2.93 mm.

From Resin Canals to Resin-Secretory System

In the wood of *B. neglecta,* neither resin canals nor axial, nor radial were observed. In the inner bark of B. *neglecta*, axial and radial resin canals (in the rays) are present and can be studied on transversal and tangential sections of the inner bark, respectively. Figure 2 showed that on the transversal sections, axial resin canals were scattered throughout the inner bark, surrounded by a single layer of epithelial cells, and imbedded in multi-seriate sheaths of axial parenchyma.

Close to the vascular cambium, most of the axial resin canals are encircled by non-lignified epithelial cells (appearing blue in the thin-section as stained by astra blue). Often resin canals appear arranged in tangential rows, with between two to five canals visible on the cross section, which has a tangential width of about 3mm (Fig. 2). Inner bark parts at large distance from the cambium sometimes contains axial canals embedded by lignified epithelial cells indicated by red colour). However, only few lignified resin canals were detected in 11 trees. From the tangential sections, it became obvious that axial resin canals are connected with radial resin canals, a phenomenon called anastomosis, and resulting in a three-dimensional network of resin canals. Figure 3c illustrated how axial resin canals split up and join neighbouring axial resin canals or radial resin canals. The average lumen diameter of the functional axial resin canals is 0,08mm (sd=0.02) and ranges from 0.05-0.12mm. The average density of functional axial resin canals was calculated to be 0.33 (sd=0.11), with a range of 0.15-0.73resin canals mm^{-2} (Table 1).

Table 1: Characteristics of bark and axial resin canals of *B. neglecta* trees in Borana Administrative Zone, south Ethiopia (N = 21)

Variables	Mean ± SD	Min – Max
DBH in (mm)	120 ± 41.33	61-185
Inner-bark thickness in (mm)	11.26 ± 3.19	6.64-20.15
Outer–bark-thickness (mm)	1.62 ± 0.71	0.23-2.93
Total bark thickness (mm)	12.88 ± 3.40	7.98-22.08
Inner-bark-cross-section- area (mm^2)	4079.88 ± 2281.82	1206.56-9876.92
Bark cross-section-area in (mm^2)	4728.06 ± 2552.06	1378.67-10955.39
Average density of axial canals number in (mm-2)	0.33 ± 0.11	0.15-0.73
Average diameter of resin canal in (mm)	0.08 ± 0.02	0.05-0.12
Total number of resin canals	1540.62 ± 972.90	463.79-4131.25
Total resin-canal-area in inner bark of tree (mm^2)	7.69 ± 5.26	1.32-17.82

SD - Standard deviation; Min – minimum; Max – maximum; N = Number of trees

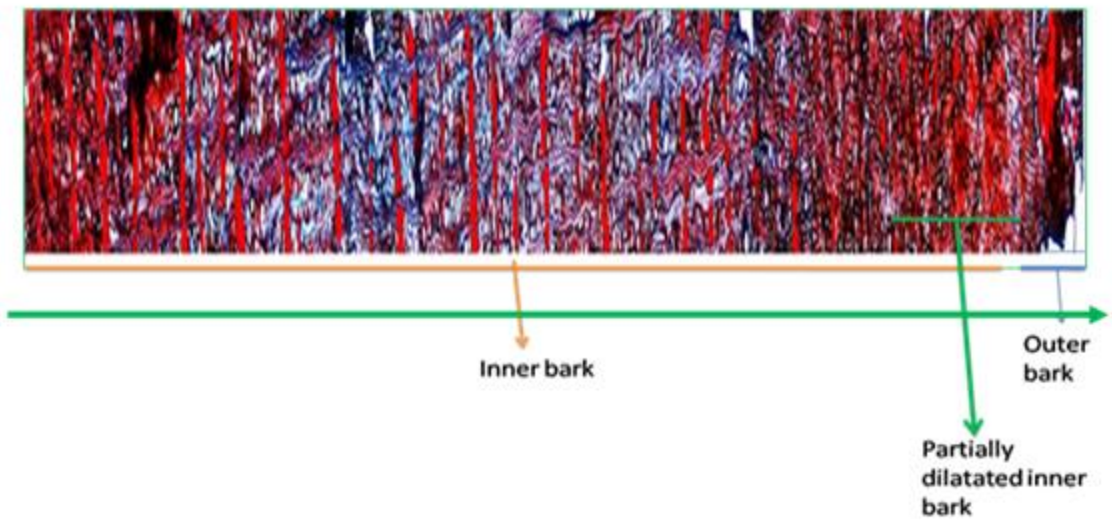

Figure 1: Transversal sections through the bark of *B. neglecta*. The cambium is located left. The inner bark layer forms the major part of the bark; however, the outer bark layer is much thinner (see also other texts for wood anatomical description of *B. neglecta*).

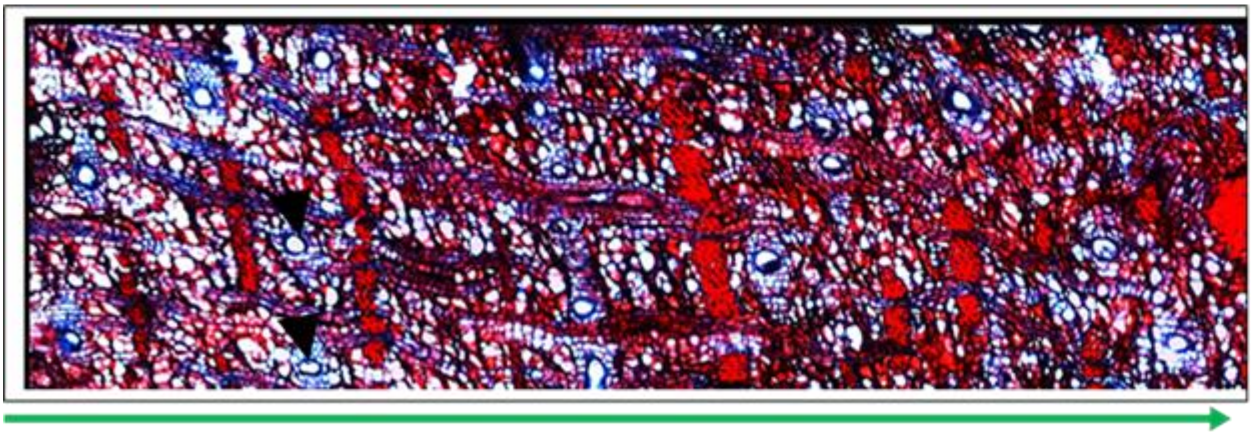

Figure 2: Microscopic view of the inner bark and resin-secretory structures of *B. neglecta*: The green arrow points the inner bark structures that arises from nearly cambium towards the outer bark; some resin canals indicated by black arrow heads.

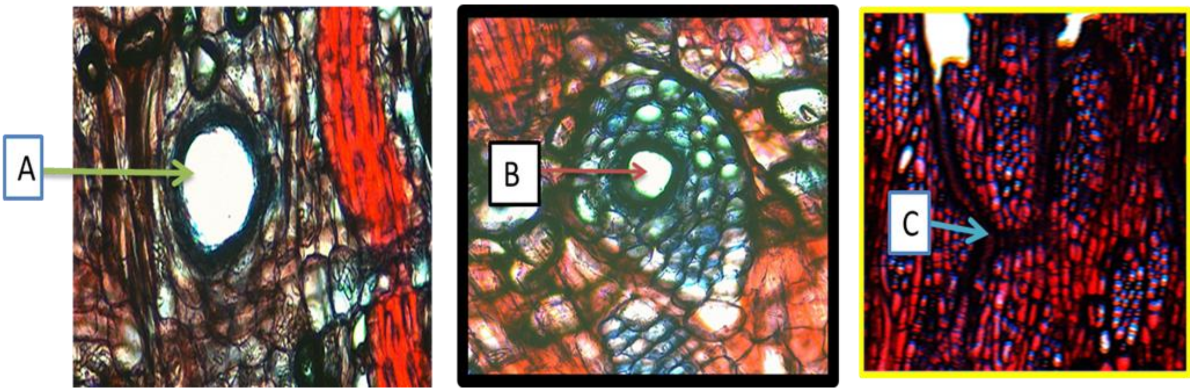

Figure 3: Resin-secretory system in the inner bark of *B. neglecta:* Left, cross-section of inner bark with the arrow (A) indicating a resin canal surrounded by non-lignified epithal cells (dark blue) and parenchyma cells; middle, tangential section showing a resin canal in a ray encircled by non-lignified epithelial cells (B); right, tangential section illustrating connection between axial – and possible radial - resin canals called anastomosis (C).

Distribution of Axial Resin Canals Across the Inner Bark

In the inner bark mainly functional resin canals were detected. The average density of functional axial resin canals per $mm^2$was 0.33 with a range of between 0.15-0.73 per mm^2.The average density of non-functional axial resin canals was considerably lower and amounted to be 0.06 per mm^2 and ranged between 0 and 0.12 numbers per mm^2. Functional axial resin canals were evenly distributed across the inner bark. It has to be mentioned that the general low number of observations made it difficult to indicate trends. There was however, no obvious and systematic change in resin-canal density across the inner bark. Non-functional axial resin canals only occured with increasing a distance from the cambium.

Relationship between bark thickness, and axial resin canals characteristics with tree DBH

Inner bark thickness was significantly related to tree diameter (r=0.677, p<0.001), meaning that inner bark size increased with tree size (Table 2). The average density and diameter of axial resin canals in the inner bark were found to be not significantly related to inner bark thickness, r=-0.076, r=0.107 (p>0.05), respectively (Table 2). However, the total area of axial resin canals in bark cross-sectional area was significantly and positively correlated with DBH, r=0. 795, (p<0.001) (Table 2).

Table 2: Pearson correlation coefficient (r) on resin canal and selected tree characteristics of *B. neglecta* (N=21)

Variables	Inner-bark thickness in(mm)	Axial resin canal density mm^{-2}	Diameter of axial resin canal in (mm)	Total resin canal area in (mm^2)
Inner-bark thickness in(mm)		-0.076	0.107	
DBH (mm)	0.677***			0.795***

* Correlation is significant with P<0.05, **Correlation is significant with P<0.01
*** Correlation is significant with P<0.0001) as indicated in Table 2.

DISCUSSION

Bark and Resin-Secretory Structures of B. Neglecta

The bark thickness of *B. neglecta* was about 12.83 mm at breast height, which was equivalent of the total tree radius of 20% (Table 1). It consists of two bark layers, defined as inner and outer bark layer. The average thickness of the inner bark was 11.05 mm; and it covers about 87.51 % of the total bark. This number is lower than that of *B. papyrifera* (i.e. the average bark thickness of inner bark which was 17.2 reported by Tolera*et al*. (2013)). The outer bark of *B. neglecta* was much thinner than the inner bark, on average, 1.62 mm. In *B. neglecta*, larger tree has relatively thicker bark and more bark cross-sectional area. The same features were also shown in *B. Papyrifera* (Tolera, 2013). The present study revealed about axial and radial resin canals in the inner bark of *B. neglecta*. On the transversal sections, axial resin canals were laid unevenly throughout the inner bark, lined by a thin layer of epithelial cells (Fig. 1). But, a few axial resin canals that are encircled by thick-walled epithelial cells occurred particularly in close to the outer bark, which may show the non-functionality of epithelial cells (Oven & Torelli, 1999). The overall total average number of axial resin canals at cross-sectional area of sampled tree was 1540.62 and the overall mean density of axial resin canals was about 0.36 per mm^2. These numbers are lower than that of *B. Papyrifera* (i.e. the average total number of axial resin canals was 9887 and average density of axial resin canals was 0.86 per mm^2) as it was reported by Tolera (2013b). This difference could be due to the genetic and micro-site variations (Langenheim, 2003). As resin synthesized and accumulated in specialized secretory structures (Langenheim, 2003), the difference in resin canal number and density between the two Boswellia species may show the difference in abilities to produce and store resin.

This result of the study showed that both axial and radial resin canals are present on the tangential sections of inner bark, with the axial resin canals connection by the radial resin canals. This may indicate how resin flows in the bark of Boswellia trees (Tolera *et al.*, 2013). However, in the wood, the result showed that there are no any types of resin-secretory structures. Incontrast, in other species, for instance, in the wood of *Pinus balepensis,* both axial and radial canals were abundantly found, with the axial canals connected by the radial (Werker & Fahn, 1969). Although axial and radial resin canals are interconnected in the bark of *B. neglecta*, it is not clear how the canal network of the bark is connected to the wood, but there is a continuity of the ray tissues. In the wood of *P. pinasters*, (Wu & Hu, 1997) study's result, however, indicated that radial and axial resin canals connected by anastomosis. Likewise, in *B. Papyrifera(Tolera et al., 2013)*study, the result clearly showed that axial and radial resin canals form a three-dimensional network in the nearly cambium (intact zone), and radial resin canals were provided to connect the canal network of bark to wood.

Distribution of Axial Resin Canals Across the Inner Bark

In this study, it was a hypothesized that the distribution of axial resin canals in density exhibit directional changes throughout the inner bark as a result of dilatation, like changes found in *B. papyrifera*. Tolera*et al*. (2013) found a gradient in resin-canal density throughout the inner bark with a higher density of axial resin canals close to the vascular cambium and a decrease in resin canal number with increasing distance from the cambium as a result of dilatation. Although dilatation was also observed in *B. neglecta*, it was less intense than in *B. papyrifera*, where the authors could even separate the inner bark into three sections: intact, partly dilatated and strongly dilatated (Tolera*et al*., 2013). The inner bark of B. *neglecta*exhibits less secondary changes in outer parts, even in thick-bark trees, and the occurrence of dilatation is limited which results in a more even distribution of resin canals, which moreover occur in much lower density in comparison to *B. Papyrifera* (Fig.3A). The slight variation in density of axial resin canals in *B. negleca* can be explained by the limited number of canals, which were revealed in this study. In addition, such uneven distribution may be the result of seasonal variation of resin-canal production (Langenheim, 2003). Internal and external factors, such as radial growth rate, wind, pressure, temperature, precipitation, and photoperiod may not only influence the structure of the wood of *B. neglecta* (Mokria, unpublished data) but also influence the distributions of resin canals in the bark (Fahn&Zamski, 1970; Wimmer&Grabner, 1997; Zamski, 1972). For example, in *Pinus abies* and *Pinus sylvestris*, density and distribution of axial resin canals were influenced by climatic conditions (Wimmer&Grabner, 1997). More axial resin canals producedunder above-normal temperatures and drought stress. Similarly, axial resin canal density of *Pinus taeda*responds to climatic variation (Blanche *et al*., 1992).

Axial resin canals surrounded by lignified epithel cells have been found in the inner bark with increasing distance from the cambium. The occurrence of these possibly non-functional axial canals in the outer parts of the inner bark may be an effect of physiological age (Rosner & Hannrup, 2004). Another more likely factor is the effect of dilatation on the epithelial cells (Bannan, 1936; Tolera *et al*. 2013). Dilatation has been observed in *B. neglecta* though to a much less extent than in *B. papyrifera*. The degree of dilatation can strongly vary between species. Heavy dilatation has been occurred on the bark of several mainly thick-barked tree species, for instance in *Quercus faginea* Lam (Teresa *et al*., 2013). Similarly, dilatation was also heavily occurred on the outside inner bark of *Q. Robur* (Trockenbrodt, 1994) and *Q. cerris var. cerris*(Şen *et al*., 2011). The formation of dilatation is due to increasing tangential strain as increasing of tree stem diameter (Evert and Eichhorn, 2006; Quilhó *et al*., 2013). Dilatation can strongly affect the structure of the bark

(Teresa *et a*l., 2013). For instance, resin canals and alternating zones pattern were influenced by dilatation growth in *Anginondifforme* (L) species (Kotina *etal*., 2012). Diseases and disturbance of resin canals towards outside could be an effect of dilatation (Kolalite *et al*., 2003; Tolera *et al*., 2013).

Relationship Between Bark Thickness, And Axial Resin Canals Characteristics with Tree DBH

In this study, the relationships between resin canal (average number, density, diameter and total area of axial resin canal) characteristics in bark cross-section and selected tree (DBH) characteristics was assessed for 21 *B. neglecta* sampled trees. Inner bark thickness has increased with tree size (DBH). Total area of axial resin canals was highly significant ($p<0.001$) and positively related to the DBH (Fig. 4. D). This is to mean that tree with larger stem DBH had a more total area of axial resin canals on the cross-sectional bark of *B. neglecta*. The fact that the bark of larger trees contains more resin-producing canals is supported by earlier results (Lorio & Sommers, 1986; Novick *et al*., 2012) which suggest that larger trees protect themselves by thicker bark with more active resin canals. However, this means that large trees can mobilize the carbon stocks for development and maintenance of an extended secretion network formed by axial and radial canals. Likewise in *Pinus pinaster*, (Rodríguez-García *et al*.(2014a) suggest that trees with better height and diameter growth would allocate their carbon budget to the defence system by increasing secretory structure formation, such as resin canals. Gebrehiwot (2003) also stated that higher resin canals from larger trees may result from larger photosynthetic carbon- acquisition capacities. As resin canal density and size did not significantly decrease through the bark, this means that larger trees are better to tap. But if the inner barks get thicker, the number of resin canals with lignified epithal cells increases. Meaning that there might be limited intact and inner bark cross-sectional area, this can be efficiently tapped. As the maximum DBH of (180 mm) exceeds in terms of the number of functional axial resin canals, the presence of these most likely non- functional resin canals is not assumed to influence bark yield.

Although total area of axial resin canals linearly scales with the stem diameter, the average density and diameter of axial resin canals can vary a lot per tree and was not significantly related to bark thickness in *B. neglecta* as revealed in the present study.

For other species, however, average density of axial resin canals was correlated positively with the stem diameter as well as with bark cross-sectional area, for example for *B. Papyrifera*(Tolera *et al*., 2013). For *Pinus taeda*, it was reported that density and diameter of resin canals were influenced by age and radial-growth rate (DeAngelis *et al*., 1986).

Implications for The Future

Sample Size

This study has been conducted as a follow-up study of the research by Tolera et al. (2013a) on *B. papyrifera*. I used a comparable sampling technique and took two bark samples per tree with a tangential width of 3 mm. As the density of resin canals was considerably lower in *B. neglecta* (average: 0.33/ mm^2) in comparison to *B. papyrifera* (average: 0.86 /mm^2), I could only measure a limited number of resin canals in the inner bark. This made it difficult to define a gradient in resin-canal density across the inner bark and quantify the degree and variation in dilatation between trees. Hence, a greater number of bark samples should be measured to verify the results of the present study.

Three-Dimensional Network of Resin Canals

Although axial and radial resin canals interconnections are recognized, a more comprehensive study of the degree of interconnection between radial and axial resin canals throughout the inner bark based on consecutive tangential thin section is necessary. This will also enable to detect which part of the resin-secretory system in the inner bark is actually functional and where disruptions occur. This could also verify that resin canals with lignified epithal cells are non-functional.

Tree Characteristics Related to Structure of Secretory System

In this study, resin-secretory structures were related to inner bark thickness (and bark-cross sectional area), which in turn are strongly related to tree size (DBH). Other tree characteristics, such as tree age and leaf area, should be included as both of them are considered to have important effecton resin-secretory structures.

Resin yield

The relationship between resin yield and resin canal characteristics as well as tree characteristics are interesting to come up with selection traits and formulation of silvicultural guidelines to introduceappropriate and efficient tapping strategies and techniques for *B. neglecta*. Thus, future study should address this topic.

Conclusions and Recommendations

In this study, bark structure, the characteristics of resin canals and the network of the resin-secretory structures in *B. neglecta* trees were described. The results showed that axial and radial resin canals are present in the inner bark, and are interconnected by radial canals. The average density of axial resin canals is lower, i.e.0.33 per mm^2, than what was found in*B. papyrifera*, another frankincense-bearing species. Inner bark thickness linearly increases with the increase in tree diameter, but resin canal density did not vary across the inner bark. It can be assumed that larger diameter trees contain much more resin- producing canals than do smaller trees with thinner bark. This suggests that tapping of larger trees will result in higher yield.

Given the current socioeconomic condition of frankincense in Ethiopia, these results can help formulate appropriate and efficient tapping strategies and techniques that in turn help to optimize frankincense production for rural livelihood improvements as well as to boost the economic development of the country. Moreover, further research is required to introduce new tapping technology for a sustainable resin production in the study site and elsewhere in Ethiopia

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