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Estimation of Strength Loss and Decay Severity of *Juniperus procera* by Juniper Pocket Rots Fungus, *P. demidoffii* in Ethiopian Forests

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Abstract

A juniper pocket rot fungus, *Pyrofomes demidoffii* is a basidiomycetous fungus responsible for damage of living *Juniperus* spp. However, its effect on the residual strength and on the extent of decay of juniper's trunk was not determined in any prior studies. The purpose of this study was to study the features of *J. procera* infected by *P. demdoffii*, and to estimate the level of strength loss and decay severity in the trunk at D.B.H height using different five formulas. Infected juniper stands were examined in two Ethiopian forests through Visual Tree Assessment (VTA) followed by a slight destructive drilling of the trunk at D.B.H height. The decayed juniper tree is characterized by partially degraded lignin material at incipient stage of decay to completely degraded lignin material at final stage of decay. In the evaluated formulas, results of ANOVA showed that a significantly higher mean percentage of strength loss and decay severity were recorded in the trees of larger D.B.H categories ($p<0.001$). The strength loss formulas produced the same to similar patterns of sum of ranks of strength loss or decay severity in the trunk, but the differences varied significantly among D.B.H categories in Kruskal Wallis-test ($p<0.001$). In conclusion, the employed formulas showed similar to different degree of variability in quantification of strength loss or decay severity in the trunk. The findings of our study could be used as the baseline for further study on juniper's strength loss or decays in the trunk of *Juniperus* spp. and unequivocally helps to design the corresponding management as result of *P. demidoffii*.

Key Words: D.B.H, decay severity, *J. procera*, *Pyrofomes demidoffii*, strength loss

Introduction

Tropical forests are currently facing unprecedented species losses and limit ecosystem services including carbon sequestration as a result of unsustainable forest management, deforestation and land degradation (Rahman et al. 2017). Trees are considered as hazardous on the basis of their predicted likelihood of failure. Dunster et al. (2013) defined tree failure as the breaking of any root, branch, or stem, or the loss of mechanical support in the roots. Tree failure can be caused by several factors among which the presence of decay within the tree can be cited as the most important factor (Smiley and Fraedrich 1992; Kane et al. 2001; Kane and Ryan 2004). Wood decay fungi invade wood cells and degrade cell wall components resulting in detrimental effects on strength and other wood properties (Wagener 1963; Rayner and Boddy 1988; Schwarze et al. 1997). Wood decay fungi can also predispose trees to the risk of wind throws or limb failures (Lonsdale 2000; Råberg et al. 2005; Hickman et al. 2011) thereby affecting quality of timber

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production in forests (Oliva et al. 2011).

Among various wood decay fungi, a juniper pocket rot fungus known as *Pyrofomes demidoffii* (Lév.) Kotl. and Pouzar was the cause of a decline of *J. procera* in Ethiopia (Assefa et al. 2015; Assefa and Abate 2018). It causes a white heart rot in the trunk of living *Juniperus* spp. in several countries (Ryvarden and Johansen 1980; Gilbertson and Ryvarden 1987; Ryvarden and Gilbertson 1994; Doğan and Karadelev 2006; Dai and He 2009; Doğan et al. 2011; Assefa et al. 2015; Assefa and Abate 2018). Infected juniper is characterized by white rot with a spongy appearance (Ryvarden and Gilbertson 1994; Dai and He 2009; Assefa et al. 2015; Assefa and Abate 2018). *Pyrofomes demdoffii* causes a great damage in juniper stands under high anthropogenic pressure and/or natural injuries (Doğan and Karadelev 2006; Doğan et al. 2011; Assefa and Abate 2018). Juniper's stands with complete-die back and larger diameter at breast height were recorded to have higher disease occurrence of *P. demidoffii* (Assefa and Abate 2018).

The assessment of occurrence of wood decay fungi is important in an effort to produce well developed forest and for the timely detection of potentially hazardous situations (Luana et al. 2015). According to Schneider et al. (2008), decay occurrence as a probability, while decay extent is often quantified as a proportion (or percentage) of decay when it occurs. The assessment of decay generally requires a measurement of the extent and position of the remaining sound wood (Lonsdale 2000). There is no single tree risk assessment method that is accepted as the standard for all situations (Mattheck et al. 2006; Fink 2009; Matheny and Clark 2009; Klein et al. 2019). The common tree risk assessment methods include The International Society of Arboriculture (ISA) Tree Hazard Evaluation; United States Department of Agriculture (USDA) Forest Services community Tree Risk Evaluation Method; The ISA Tree Risk Assessment Best Management Practice (BMP) Method, Quantitative Tree Risk Assessment (QTRA) and Visual Tree Assessment method, VTA (Klein et al. 2019). Despite some prominent differences, all common assessment methods involve an assessment of the tree structure, identification of defects and subsequent evaluation of tree failure probability, an assessment of targets, and an appraisal of the potential damage caused by target impact (Matheny and Clark 1994; Mattheck and Breloer 1994; Ellison 2005; Klein et al. 2019).

The Visual Tree Assessment (VTA) method is the method based on the visual inspection for diagnostic signs and symptoms of defects and tree vitality; confirmation of the defects' existence and measuring their extent; and together with assessment of internal defects with estimation of tree's residual strength (Mattheck and Breloer 1994; Leong et al. 2012; Klein et al. 2019). The importance of visual assessment of trees was stressed in several studies (Matheny and Clark 1994; Kennard et al. 1996; Gruber 2008). The visual assessment can be effective when the user has an understanding of the factors that can cause a tree to fail (Gruber 2008). The VTA method can be also used in complement to PCR methods (Nicolotti et al. 2009). Visual risk assessment techniques were found to be scientifically sound, yet practical (Hickman et al. 1995; Kennard et al. 1996; Koeser et al. 2016). Visual assessments have been accepted as an efficient and reliable method in identifying compromised trees, as compared to other trees (Kennard et al. 1996; Fink 2009; Dunster et al. 2013). According to Stenlid and Wästerlund (1986), the most widely practised method of VTA is to visually study core samples taken from the trunk using an increment borer. Despite its wide uses, some limitations of VTA method include its subjective nature and, a method of less significance when the tree had no internal decay (Smiley et al. 2011; Koeser et al. 2015; 2016; Klein et al. 2019).

Quantifying the amount of decay has been related to the probability of failure using strength loss formulas (Wagener 1963; Coder 1989; Smiley and Fraedrich 1992; Mattheck and Breloer 1994; Kane et al. 2001; Kane and Ryan 2004) or through estimation of decay severity (Terho 2009). Terho (2009) introduced decay internal grading based on decay severity of the heartwood. The detection of potentially hazardous trees may offer an opportunity to prevent the failures with a considerable reduction of associated damage to the environment, properties and people (Wagener 1963; Klein et al. 2019).

It was suggested that more concerted attention to be given for strength loss of the internal heartwood than the comparative strength of the sound wood (Wagener 1963). Recent study showed that a juniper with an advanced decay and cavities, and completely declined or dead-standing ju-

niper trees possessed a higher frequency of fruiting bodies of *P. demidoffii* (Assefa and Abate 2018). However, there is a paucity of information on the extent that such damage affects the strength loss and decay severity in the trunk of juniper infected by *P. demidoffii*. The knowledge of the residual strength and decay severity in juniper owing to *P. demidoffii* helps to make appropriate decision for management of the tree. The objectives of this study were therefore: to determine the characteristics of juniper decayed by *P. demdoffii*; to estimate the level of strength loss and decay severity in the trunk of *J. procera* infected by *P. demidoffii* using decayed core woods samples collected from juniper stands at diameter at breast height (hereafter D.B.H); and finally to compare the predictive formula/s used for quantification of strength loss in the case of *J. procera* infected by *P. demdoffii*. The findings of this study could be therefore expected to fill the research gaps with regard to the residual strength and the fate of *J. procera* heavily infected by *P. demidoffii* thereby helping the foresters to make appropriate decision before major damage by juniper pocket rot fungus is extremely high.

Materials and Methods

Study areas and field survey

Juniper trees infected by juniper pocket rot fungus were surveyed for potential hazard strength and decay severity in Adaba-Dodola and Menagesha Forests, Ethiopia. The Adaba-Dodola forest is located in South-eastern Ethiopia at latitude and longitude ranges of 6°50′-7°00′ N and 39°07′-39°22′ E, respectively (Assefa et al. 2015; Assefa and Abate 2018). The Menagesha forest is found at northwest of Addis Ababa and geographically located at latitude and longitude ranges of 8°57′-9°02′N and 38°32′-38°45′E (Assefa et al. 2015; Assefa and Abate 2018). The Adaba-Dodola forest has a maximum annual temperature of 7-24°C, a maximum annual rainfall of 1200 mm, and an altitude of 2400-3100 a.s.l. There are two rainy seasons in Adaba-Dodola forest, the longest rainy season is from June to September whereas shortest rainy season is from March to April (Assefa et al. 2015; Assefa and Abate 2018). The Menagesha forest has a maximum annual temperature of 12-16°C, a maximum annual rainfall of 1225 mm, and an altitude of 2,400-3,000 a.s.l. The rainy seasons are the same to the Adaba-Dodola forest.

Characterization of decay caused by juniper pocket rot fungus

The morphological changes and the progress of decay of lignocellulosic materials of *P. demidoffii* infected juniper were studied by using decayed wood samples recovered from three infected trees during 2012 to 2013. Wood samples from three health trees were used as control for comparison. Infected juniper trees were identified based on the presence of the typical basidiocarp of *P. demidoffii* (identified microscopically, and macroscopically) (Ryvarden and Johansen 1980; Gilbertson and Ryvarden 1987; Ryvarden and Gilbertson 1994) or based on culture from fresh basidiocarp and/or white rot of infected trees (Assefa et al. 2015; Assefa and Abate 2018).

Morphology of basidiocarp and basidiospore

Pieces of basidiocarp material were mounted in lactophenol cotton blue, 5% KOH and Melzer's reagents and were studied with a digital microscope DM5500B (Leica Microsystems, Germany) at 1,000× magnification. Melzer's reagent was used to study amyloidity or dextrinoidity of hyphae, spores, and hymenial organs through microscopic observations. The size of basidiospores per basidiocarp was measured in Melzer's reagent. The microscopic and macroscopic characters of the basidiocarp were compared with descriptions in literatures for identification of the fungus to species level (Ryvarden and Johansen 1980; Gilbertson and Ryvarden 1987; Ryvarden and Gilbertson 1994; Dai and He 2009).

Isolation and cultural characteristics

Pieces of tissues removed from fresh basidiocarps and/or fresh white rot samples presumably be *P. demidoffii* were transferred to Petri dish containing 2% (w/v) Malt Extract Agar, hereafter MEA (20 g malt extract, 0.5 g mycological peptone, 15 g agar in 1000 ml distilled water; Oxoid, Basingstoke, UK). The inoculated plates were incubated at 25°C in the dark up to three weeks for primary isolation and the emerging fungal mycelium were subcultured on to fresh 2% MEA for further cultural studies. The isolates fungal cultures were identified to species level according to descriptions by Campbell (1938), Nobles (1958; 1965) and

Stalpers (1978).

Sampling, and study design for strength loss and decay severity analysis

Sampling was done based on the disease occurrence of *P. demidoffii* from previous study in Adaba-Dodola forest and Menagesha forest (45.7% from fruiting bodies and 34.8% from detection of fruiting bodies (Assefa and Abate, 2018). Accordingly, a total of 100 trees in the three D.B.H categories (11-50 cm, 51-90 cm and 91-150 cm) were randomly sampled from the two study areas for presence of fruiting bodies and/or white rot ascribed to *P. demidoffii*. The sampled trees were thoroughly examined for signs and symptoms of decay (such as fruiting bodies, decay, cavities and/or defects) through VTA method. Accordingly, thirty one infected juniper trees in D.B.H categories of 11-50 cm (n=10), 51-90 cm (n=13) and 91-150 cm (n=8) were detected and examined for strength loss in the trunk or for their decay severity in the trunk. The defects were recorded and the strength loss of the examined trunks was determined as suggested in several studies (Mattheck and Breloer 1994; Kennard et al. 1996; Fink 2009; Leong et al. 2012; Klein et al. 2019). To this effect, a hand-held drill (Fig. 1) of different drill bit size (0.5 mm to 3.0 mm in diameter and 10-50 cm in length size) was gently drilled into the infected juniper trees at three separate locations at D.B.H (1.3 m above the ground) until resistance of the drill bit significantly decreases when decay was encountered as it was used in prior studies (Luley et al. 2009; Leong et al. 2012). The circumference of the trees, the thickness of the sound wood and the diameter of the decayed/hollowed trees were measured at D.B.H as described by Fraedrich (1999). The thickness of the bark was subtracted to obtain the actual sound wood thickness. Moreover, visual inspection of the texture of wood core samples was conducted to identify the status of the decay (discolored, decayed or white rotted) of the infected wood.

Estimation of strength loss in the trunk

The trees' diameter (D), the diameter of the decayed or hollowed area in the tree trunk (d), and the ratio of cavity opening to trunk wood circumference (R) were entered into the Wagener's (1963), Coder's (1989) or Smiley and Fraedrich's (1992) formulas where appropriate to quantify the strength loss in the trunk (Table 1). Mattheck and Breloer's (1997) formula defined as the ratio of the thick-

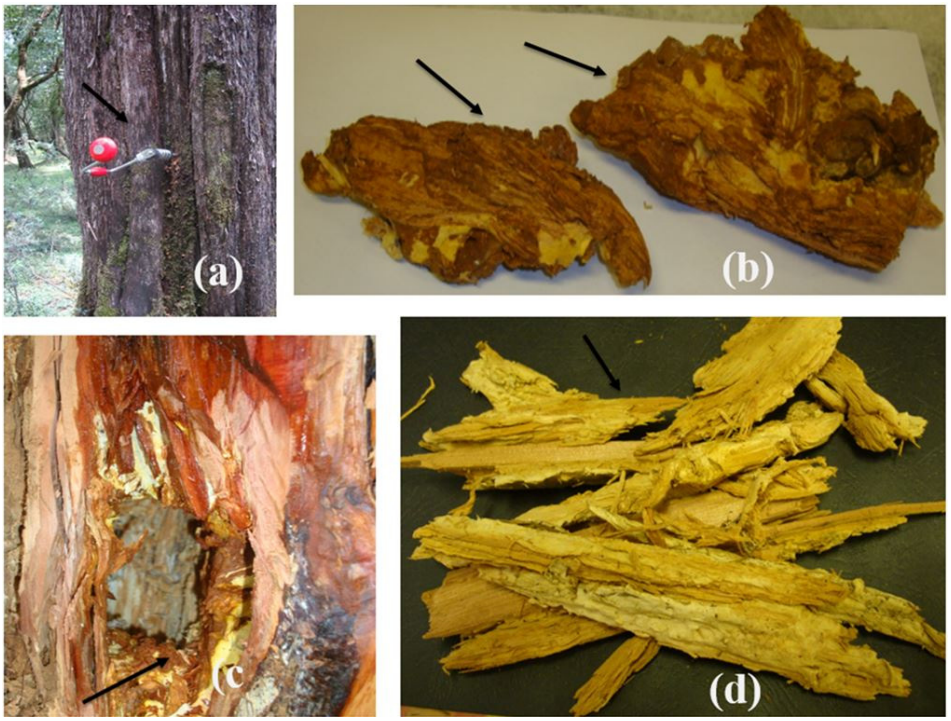

Fig. 1. The progress of decay in trunk of juniper trees decayed by *P. demidoffii* in Menagesha and Adaba-Dodola forests, Ethiopia. (a) Undecayed trunk with hand held drill in place (arrow), Menagesha forest; (b) hollowed trunk containing partially degraded lignin and cellulose (arrow), Adaba-Dodola forest; (c) decayed wood containing partially degraded lignin (brown) and cellulose fibers (white), and dried fungal mycelia felts (arrows), Adaba-Dodola forest; (d) selective delignification of lignin, and cellulose fibers intermixed with fungal mycelia felts (arrows), Adaba-Dodola forest.

ness of sound wood remaining in a stem (t) to the radius of the stem (R) was used to quantify the hazard status of the sampled trees. The threshold value for each formula to declare the hazard status of decayed trunk was illustrated in Table 1. The formula/s that produced a reproducible hazard status of juniper trees decayed by *P. demidoffii* was identified and suggested for analysis of strength loss in related *Juniperus* species by *P. demidoffii*.

Estimation of decay severity in the trunk (at D.B.H height)

The infected juniper's trees were drilled in the trunk at D.B.H in three random places as mentioned hitherto. The diameter of the tree (D), and the average thickness (t) of the sound wood (excluding the bark thickness) were measured. The diameter of the defect area (cavities, hollow or decayed) was calculated as the diameter of the tree at D.B.H height (D) minus the average thickness (t) of the sound wood. The disease severity in the decayed area, cavities or hollowed areas in the trunk were therefore calculated using proportion of areas of decay in cross-sectional area outlined in several studies (Kennard et al. 1996; Terho and Hallaksela 2008; Terho 2009; Smiley et al. 2012; Frank et al. 2018) as:

$$\text{Decay proportion} = \frac{\text{Area of decay in the trunk at D.B.H}}{\text{Wood Area at the D.B.H}} \times 100$$

where decay proportion is the ratio of decay in the trunk at D.B.H height [π [$(D\text{-}t)^2$] to wood area [π (D^2)$\times$100]. The decay severity in the trunk was interpreted using Terho's (2009) decay grading in the trunk as moderate decay, poor decay and severe decay (Table 1).

Data analysis

Analysis of data was conducted using Statistica 7 (Stat Soft, Inc. 2004) as appropriate. The percentage of strength loss in the trunk due to juniper pocket rot fungus was separately calculated by Wagener (1963), Coder (1989), Smiley and Fraedrich (1992) and Mattheck and Breloer (1997) formulas as indicated in Table 1. The mean of three measurements were used to quantify strength loss in the trunk. Decay severity was calculated using Terho (2009) decay severity assessment ranges and mean of three measurements were recorded. In all cases, data was expressed as a mean$\pm$SE. The significant difference among the means was analyzed by Factorial ANOVA and Tukey HSD test was used for multiple comparisons and significant at $p < 0.05$. The sum of ranks of strength loss or percentage of decay severity in the trunk among different D.B.H categories was analyzed using Kruskal-Wallis ANOVA test, H. All

Table 1. Threshold values of strength loss in the or percentage of areas of trunk decay using different formulas and assumption

Wagener's (1963)	Coder's (1989)	Smiley and Fraedrich's (1992)	Mattheck and Breloer (1997)	Terho (2009)
$=d^3/D^3$	$=(d^4/D^4)$	$=(d^3+R(D^3-d^3))/D^3\times100$	$t/R<0.3$ a hazard	• Moderate decay ($<$70% cross-sectional area) and sound wood complete • Poor decay ($<$70% cross-sectional area) and decay extends to disc margin • Crown decline/sever decay ($>$70% cross-sectional area) and decreased vitality of crown
$>$33% loss in I, hazard	20%$\leq$loss in $I\leq$44%=caution; $>$44% loss in I=hazard	33% loss in I, hazard		

d is the diameter of the decayed or hollow area in the tree trunk; D is the diameter of the tree; R is the ratio of cavity opening to stem circumference (Fraedrich's) (Smiley and Fraedrich's); t is the thickness of sound wood remaining in the stem; R is the stem radius (Mattheck and Breloer). Threshold values and interpretation are reviewed in Kane et al. (2001) and Kane and Ryan (2004); Terho (2009) percentage of areas of trunk decay defined by.

samples with the sum of ranks originated from the different distribution were considered as significant ($p < 0.05$). All ANOVA were preceded with a Shapiro–Wilk test and a Levene test to check the normality of the distributions and the homogeneity of the variances. Pearson correlation was used to correlate formulas used to quantify the strength loss or decay severity of *Juniperus procera* caused by *P. demidoffii*.

Results and Discussion

Characteristics of decayed juniper by P. demidoffii

Field assessment of internal decay in the *P. demidoffii* infected trees was examined in natural forest at Adaba-Dodola (South-eastern Ethiopia), and Menagesha forest (central Ethiopia) during 2012 to 2013. The morphological characteristics and progress of decay in the examined juniper tree is illustrated in Fig. 1. The healthy (uninfected) trunk of juniper exhibited sound wood with strong thickness and resistance to drilling (Fig. 1a). The infected trunk is characterized by wood containing partially degraded lignocellulosic material (at incipient stage of decay) to completely degraded lignin material (at advanced decay). Early decay is characterized by a brown to light-yellow color whereas an advanced decay is characterized by a white rot with abundant mycelial felts in the decayed wood (Fig. 1b, c). In selective delignification, lignin is degraded earlier in the decay process than cellulose or hemicellulose (Blanchette et al. 1997; Schwarze et al. 1997; 2000; Schwarze 2007). Therefore, *P. demidoffii* undertakes the selective delignification of lignocellulosic materials of juniper, similar to many white rot fungi and pathogenic wood decay fungi such as *Armillaria mellea*, *Ganoderma* spp., *Heterobasidion annosum*, and others (Schwarze 2007). In our study, *P. demidoffii* caused a decay that was limited to the tree's cross-section (especially low toward the sapwood). But, a more extensive type of decay was created in vertical columns of the heartwood than toward the sapwood (Fig. 1c). In completely decayed trees, decay was characterized by a white rot intermixed with fungal mycelia (Fig. 1c, d). Fungal wood decay in many conifers also produced similar patterns of decay in the infected host trees (Wagener 1963). Terho et al. (2007) found that the extensiveness of horizontal decay in the stem (toward the cambium) is more important than the vertical extensiveness of the decay column in determining breakage hazard. As the loss of cross-sectional areas increased, the calculated loss of heartwood to cause an equivalent magnitude of stress was almost twice as large as cut area of sapwood (Smiley et al. 2012). As opposed to our study, wood decay fungi such as *Cerrena unicolor* (Bull.) Murrill, *Ganoderma applanatum* (Pers.) Pat., *Ganoderma lipsiense*, *Inonotus obliquus* (Pers.) Pilát, *Kretzchmaria deusta* (Hoffm.) P. Martin, and *Phellinus igniarius* (L.) Quél produce horizontal decay that extends into the cambium thereby causing the greatest potential for stem breakage (Terho and Hallaksela 2005; 2008; Terho et al. 2007).

Visual assessment of decay and internal decay analysis

In this study, I conducted visual inspection of indicators of decay of juniper (by *P. demidoffii*) followed by internal decay analysis using a slightly destructive method (using hand-held drill) (Fig. 1a). The importance of the VTA method in detection of decay and estimating the residual strength status of many tree species were documented (Matheny and Clark 1994; Hickman et al. 1995; Kennard et al. 1996; Mattheck and Breloer 1997; Mattheck et al. 2006; Gruber 2008; Fink 2009; Matheny and Clark 2009; Nicolotti et al. 2010; Leong et al. 2012; Luely 2012; Koeser et al. 2016; Klein et al. 2019). Fruiting bodies, cavities, bulges, and cracks are the visual indicators of decay presence (Pokorny 2003; Luely 2012; Koeser et al. 2016). The presence of fruiting bodies in the infected living trees further confirms the extension of decay (Wagener and Davidson 1954; Terho and Hallaksela 2008; Terho 2009; Assefa and Abate 2018). The VTA method is of paramount importance in detection of decay especially when there a significant internal decay (Smiley et al. 2011; Koeser et al. 2015; 2016). It also provides a clear understanding on the tree failure as a result of fungal sporocarps and other anomalies in living trees of many species (Pernek et al. 2013). Koeser et al. (2017) reported that the likelihood of failure ratings derived from visual assessments is less variable as compared to assessments informed by advanced assessment technologies. The importance of both resistograph and portable drill for detection of decay in standing was also well reviewed (Johnstone et al. 2010). Decay indicators that are accompanied by advanced assessment techniques provide a repeatable decay assessment process

(Luely 2012).

Quantitative assessment of strength loss

Different formulas/ models (Wagener 1963; Coder 1989; Smiley and Fraedrich 1992; Mattheck and Breloer 1997) were used for analysis of strength loss in the trunk of infected juniper. Using Wagener (1963), Coder (1989), and Smiley and Fraedrich (1992) formulas, the mean percentage of strength loss in the trunk of the decayed juniper's trees were indicated in Table 2. There was a considerable variation in means of strength loss among the interactions of formulas/models and D.B.H categories (Two Way ANOVA, $F_{6, 112}=12.24$, $p<0.001$) (Tables 2 and 3) although the difference at D.B.H of 10-50 cm and 51-90 cm were not significantly varied among all formulas ($p > 0.05$) (Table 2).

In all decay assessment models/formulas, the D.B.H height was selected because there is a positive correlation between tree's D.B.H and discoloration by wood decay fungi in previous study (Vasiliauskas and Stenlid 1998); and similar study also used the D.B.H height for decay assessment (Luely et al. 2009). Higher mean percentage of strength loss in the trunk was observed in the infected trees of larger D.B.H categories in all decay assessment formulas (Table 2). Studies reported that trees with larger D.B.H to have a high probability of failure or damage (Mattila and Nuutinen 2007; Luely et al. 2009; Kane et al. 2015). Earlier studies also indicated the existence of linear age-D.B.H relationships among *J. procera* trees from Menagesha forest, Ethiopia (Couralet et al. 2005; Sterck et al. 2010). Therefore, the chance breakage of infected juniper trees of smaller D.B.H category seems unlikely in our study. In some *Juniperus* species such as *Juniperus poly-*

Table 2. Factorial ANOVA results of mean percentage of strength loss or decay severity of trunk of juniper trees (n=31) infected by *P. demidoffii* at Adaba-Dodola forest and Menagesha forest Ethiopia

D.B.H categories (cm)	Wagener's (1963) formula	Coder (1989) formula	Smiley and Fraedrich (1992) formula	Mattheck and Breloer (1997) formula	Terho (2009) assumption of decay
11-50 (n=10)	35.24±1.89 e	25.03±1.69 ac	23.30±2.08 a	0.42±0.025 b	49.75±1.7 a
51-90 (n=13)	37.93±0.93 e	27.50±0.89 ac	24.21±1.08 a	0.38±0.011 b	52.36±0.85 a
91-150 (n=8)	65.22±4.88 f	57.05±5.65 d	56.09±5.95 d	0.16±0.029 b	74.87±3.75 b
Total (n=31)	44.10±2.66	34.33±2.29	32.15±3.06	0.34±0.022	57.33±2.0

In ANOVA test, means followed by the same letter(s) in all rows and column of Wagener (1963), Coder's (1989), Smiley and Fraedrich (1992) and Mattheck and Breloer (1997) were not significantly different at $p=0.05$ in Two way factorial ANOVA; In Terho's (2009) assumption of decay, it was analyzed by One way ANOVA separately from other formulas.

Table 3. Strength loss or decay severity analysis of variance (factorial ANOVA results)

Decay risk assessment	Difference source	Sum of squares	df	Mean square	F value	p-value
Strength loss[a]	DBH	12371.5	2	6185.8	106.534	0.001
	Decay formulas	35850.6	3	11950.2	205.811	0.001
	DBH*decay formulas	4265.1	6	710.9	12.243	0.001
	Error	6503.2	112	58.1		
	Total	56669.6	123			
Decay severity[b] (among decay categories and D.B.H categories)	D.B.H	563.03	2	281.51	29.84	0.001
	Decay categories	919.85	2	459.92	48.75	0.001
	D.B.H×decay categories	49.03	1	49.03	5.197	0.001
	Error		25	9.43		
	Total		31			

Strength loss[a] and Decay severity[b] among decay categories and D.B.H were evaluated using two way ANOVA.

carpos, it was found that the wood properties including wood density, mechanical properties of wood such as modulus elasticity (MOE) and modulus rupture (MOR) were significantly higher at D.B.H height as compared to 50% and 75% of the height of the tree (Kiaei et al. 2015). The importance of rot of internal wood in affecting the extent of strength loss of the trees was suggested in the earlier studies (Wagener 1963).

Using Wagener (1963) formula, trees at all D.B.H categories were classified as a hazardous tree (Table 2). Using Coder (1989) formula, most of the infected juniper trees (D.B.H of 11-90 cm) were interpreted as the trees with higher likelihood of losing strength in the sound wood (Table 2). However, the strength loss of trees in the D.B.H of 91-150 cm was beyond the threshold ($>44\%$) and such trees could be classified hazardous tree. Using Smiley and Fraedrich (1992) formula, the strength loss of juniper's trunks in D.B.H categories of 11-50 cm (25.03 ± 1.69 cm) and 51-90 cm (27.5 ± 0.89 cm) were below the threshold (33%) to be designated as a hazardous tree. But in the D.B.H of 91-150 cm, the trees were designated as a hazardous tree. Wagener (1963) suggested that the maximum allowable loss of one third of the initial strength corresponded to a heartwood loss of 70% (measured by the diameter of decay). Ciftci et al. (2014), however, explained the limitations of Wagener (1963) formula as follows. Both stem cross-sections and areas of decay are not always perfectly circular; bark thickness is not considered; trees are not equivalent to defect-free specimens used to determine wood properties are part of the tree. Kane and Ryan (2004) also suggested that Wagener (1963) formula was significantly less accurate in quantifying loss in *I*STEM than Smiley and Fraedrich (1992) formula, a result consistency with our study. It was suggested that Wagener and Coder, and Smiley and Fraedrich equations are all based on decay and cross-section having the same center (Kane et al. 2001; Kane and Ryan 2004). In addition, the Smiley and Fraedrich (1992) formula accounts for trunk cavities (Kane and Ryan 2004) and it is better than Wagener (1963) and Coder (1989) as it accounts for trunk cavities (Kane et al. 2001; Kane and Ryan 2004; Liang and Fu 2012).

Based on Mattheck and Breloer (1997) formula, the t/R ratio of the examined trees was not hazardous for D.B.H in the ranges of 11-90 cm ($t/R>0.3$), but hazardous for trees with D.B.H of 91-150 cm (below $t/R<0.3$ cm). Statistically, the t/R ratio was not however significantly varied among the D.B.H categories ($p>0.05$) (Table 2). Smiley and Fraedrich (1992) formula also revealed the same results with Mattheck and Breloer's (1997) in our analysis. However, the inferiority of Smiley and Fraedrich (1992) formula to Mattheck and Breloer (1997) in describing hazard status of trees was described in earlier study (Kane and Ryan 2004). Studies also indicated that Mattheck and Breloer (1997) formula was principally the best method when the decay center and the stem centers are different, but Wagener (1963), Coder (1989) and Smiley and Fraedrich (1992) were the method of choice when decay centre and stem center are located at the same site (Kane et

Table 4. Sum of ranks of hazard analysis (Kruskal Wallis-test) of decayed trunk of juniper trees (n=31) infected by *P. demidoffii* at Adaba-Dodola forest and Menagesha forest Ethiopia

D.B.H categories (cm)	Wagener (1963) formula	Coder (1989) formula	Smiley and Fraedrich (1992) formula	Mattheck and Breloer (1997) formula	Terho (2009) assumption of decay
11-50 (n=10)	101	101	111	220	101
51-90 (n=13)	175	175	165	240	175
91-150 (n=8)	220	220	220	36	220
Kruskal Wallis H (2, N=31)	18.029	18.026	18.15	17.43	18.02
p-value	<0.001	<0.001	<0.001	<0.001	<0.001

Smiley and Fraedrich (1992) designates Bartlett's formula; In Kruskal Wallis-test, sum of ranks originated from the same category of loss of strength of trunk in the same column (trunk decay formula) was significantly different at $p=0.05$ using Statistica Software. In Terho (2009) assumption of decay, the Sum of Ranks was analyzed separately from other formulas.

al. 2001; Kane and Ryan 2004; Liang and Fu 2012). Kane and Ryan (2004) also reported that when the trees are even designated as hazardous based on Mattheck and Breloer (1997) formula, they could not be in immediate danger of failure as a result of presence of branching and protection from adjacent forest trees.

Using the Kruskal Wallis-test, the sum of ranks of strength loss in the trunk of the examined juniper trees originated from different D.B.H categories were significantly varied among strength loss formulas ($p < 0.001$) (Table 4). For each D.B.H category, Wagener formula, and Coder formula produced the same sum of rank patterns (Table 4). Smiley and Fraedrich (1992) and Mattheck and Breloer (1997) formula, however, produced different patterns of sum of ranks with the above formulas. This shows that the strength loss of juniper's trunk infected by *P. demidoffii* is affected by the D.B.H categories of the sampled trees, a result that corroborate with related previous studies (Mattila and Nuutinen 2007; Luely et al. 2009; Kane et al. 2015).

Decay severity in the trunk

The mean percentage of decay severity in the trunk of juniper varied significantly among D.B.H categories (One Way ANOVA, $F_{2, 28}=29.84$, $p < 0.001$) (Tables 2 and 3) and was ranged from 49.76±1.73 to 74.87±3.75 (Table 2). According to LeMay (1993), the use of percent decay area at breast or stump height showed a substantial successfully improvement of estimation of decay of many tree species. The highest decay severity was noted in juniper stands of the largest D.B.H categories (90-150 cm). Recent study indicated that a juniper with higher D.B.H categories was characterized by a higher disease occurrence of *P. demidoffii* (Assefa and Abate 2018). The D.B.H also affected the survival rate of pine trees infested with pine wood nematode (PWN) (other than fungi), and more survival was noted in trees with smaller D.B.H than larger D.B.H, a finding which corroborate our study (Ha and Lee, 2017). Using resistograph sampled trees of many tree species at D.B.H height, it was indicated that the incidence and severity of decay were higher in trees with larger D.B.H (Luley et al. 2009), in agreement with our study. An increase in D.B.H to height ratio was highly correlated with age of trees and older trees harbor broken branches and wounds that serve as entry points for wood decay microorganisms (Giroud et al. 2008; Frank et al. 2018). The high rate of susceptibility of heartwoods or sapwoods of older trees to decay was due to decline of the amount of fungicide or fungistatic substances (Scheffer and Cowling 1966; Eisner et al. 2002).

Among the evaluated junipers, ten trees (32.2%), seventeen trees (54.8%) and four trees (13%) displayed moderate decay, poor decay and severe decay, respectively (Supplementary Table 1). The decay severity considerably varied among the decay categories (moderate, poor and severe) and D.B.H categories (Two Way ANOVA, $F_{1, 25}=5.19$, $p < 0.001$) (Fig. 2; Table 3). Juniper trees in the D.B.H categories of 11-50 cm and 51-91 cm exhibited both moderate and poor type of decay. However, there was no significance difference in disease severity among poor type of decay at D.B.H of 11-50 cm, moderate and poor type of decay at D.B.H at 51-91 cm ($p > 0.05$) (Fig. 2). Severe type of decay was not detected in D.B.H categories of 11-50 cm and 51-90 cm (Fig. 2). Juniper trees at D.B.H of 90-150 cm, however, exhibited poor and severe type of decay and produced the highest mean percentage decay severity compared to other D.B.H categories (Fig. 2). Using Terho (2009) decay severity grading, the sum of ranks demonstrated by Kruskal Wallis test also revealed a significant difference in disease severity among D.B.H categories (Table 4). It was indicated that stands with larger diameter were susceptible to decay of different types (Terho 2009; Assefa and Abate

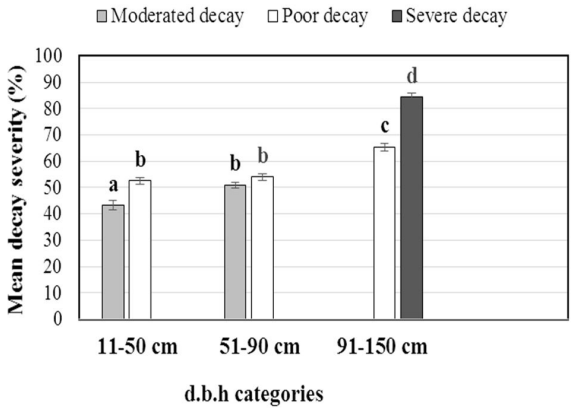

Fig. 2. Decay severity (Mean±SE) of juniper trees of different D.B.H categories using Terho' (2009) formula. Means followed by the same letter(s) in the column of the same category were not significantly different from each other at $p < 0.05$ in Factorial ANOVA using Tukey's HSD test.

Table 5. Pearson correlation coefficient of loss of strength or areas of decay assessment formulas of decayed trunk of juniper trees (n=31) infected by *P. demidoffii* at Adaba-Dodola forest and Menagesha forest Ethiopia

Risk assessment formulas	Wagener (1963)	Coder (1989)	Smiley & Fraedrich (1992)	Mattheck & Breloer (1997)	Terho (2009)
Wagener	1.00				
Coder	0.999	1.00			
Smiley & Fraedrich	0.996	0.997	1.00		
Mattheck & Breloer	-0.976	-0.965	-0.964	1.00	
Terho	0.999	0.995	0.993	-0.986	1.00

Wagener's (1963), Coder's (1989), Smiley and Fraedrich (1992), and Mattheck & Breloer's (1997) formulas assess loss of strength of trunk; Tehro's (2009) assess the areas of decay in the trunk.

2018).

Analysis using Pearson correlation coefficient showed that the strength loss and decay severity in *J. procera* by *P. demidoffii* demonstrated by Wagener (1963), Coder (1989), Smiley and Fraedrich (1992), and Terho (2009) formulas were significant and positively correlated with each other (Pearson Correlation coefficient > 0.96, $p < 0.01$) (Table 5). Therefore, all of these formulas revealed the integrity of the juniper tree could be fairly harmed by the prevailing decay by juniper rot fungus, *P. demidoffii*. But, the negative correlation between individual strength loss formula and Terho's (2009) disease severity assumptions to Mattheck and Breloer (1997) formula need further explanation.

This study was limited to assessment of strength loss and decay severity of juniper infected by juniper pocket rot fungus, *P. demidoffii*. The study was conducted by using visual assessment of tree followed by a slightly destructive method. Other tree risk assessment methods were not utilized. In this study, we used limited number of living juniper trees in order to avoid further spread of the disease into infected trees during removal of decayed wood samples. It could have been better and more realistic if large number of infected trees was sampled. The other limitations in this study was the use of hand held drill which might highly challenging especially when trees with larger D.B.H was samples despite the use of drill bit with different size.

In conclusion, juniper decayed by *P. demidoffii* is characterized by a partial degradation of lignocellulosic materials (at incipient stage of decay) followed by a complete degradation of lignin (at final stage of decay). The VTA followed by detection of internal decay in the trunk was used to determine the hazard status of juniper trees infected by *P. demidoffii* especially when advanced methods are not in place. Strength loss in juniper decayed by *P. demidoffii* was significantly higher among trees with higher D.B.H categories. The accuracy of formulas used to quantify the strength loss in the trunk of juniper (by *P. demidoffii*) could be in the order of Wagener's (1963) $\leq$ Coder's (1989) $<$ Smiley and Fraedrich's (1992) $<$ Mattheck and Breloer's (1997). More decay severity was also evident in juniper of higher D.B.H categories. For future studies, detailed studies of hazard status of infected juniper involving the entire length of the juniper tree should be conducted by using methods with high accuracy and reproducibility. Juniper trees especially with poor to severe decay status and larger D.B.H categories have to be assessed routinely for the presence of decay and structural defects caused by juniper pocket rot fungus. More importantly, before deciding to remove the decayed trees, empirical data on the level of decay, the hazard status and the probable outcomes of host-fungus interactions should be considered.

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