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Freezing avoidance in tropical Andean bamboos

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ABSTRACT

Frost resistance was compared in five species of different life forms in the neotropical woody bamboo genus *Chusquea* growing along a cloud forest-paramo gradient, between 2250-4010 m a.s.l. in the Venezuelan Andes. *C. multiramea* and *C. serrulata* are viny bamboos of the upper montane cloud forest-treeline ecotone (2400-2900 m); whereas *C. angustifolia C. spencei* and *C. guirigayensis* are shrublike bamboos associated to swampy low elevation paramos (2525 m) in the first case, a broad range of paramo ecosystems (2670-3600 m) in the second, and dry high elevation paramos in the third (3800-4010 m). Intercellular ice nucleation and 50% tissue injury temperatures were estimated in these five species under laboratory conditions in order to determine frost resistance mechanisms. No significant differences were observed among ice nucleation and tissue injury temperatures in any of these species, indicating that all avoid intercellular ice nucleation through supercooling (-12.3 to -10.1 ºC). We conclude that variations in supercooling capacity and freezing injury are not related to life form, habitat or elevation and that freezing temperatures are not a determining factor in the altitudinal distribution of tropical Andean bamboos.

Key words: supercooling capacity, tropical Andes, *Chusquea*, Bambusoideae, altitudinal gradients.

INTRODUCTION

Temperature is one of the main environmental factors that affect plant distribution. In the tropical Andes, life-form distribution usually responds to changing environmental conditions along altitudinal gradients, and those that reach the highest altitudes have adapted successfully to resist low temperature environments (Larcher 1995, Körner 1998). In the Venezuelan Andes, low temperature resistance mechanisms have been described for giant rosettes (Goldstein *et al*. 1985), trees (Rada *et al*. 1985, Cavieres *et al*. 2000, García-Núñez *et al*. 2004; Azócar *et al*. 2007, Rada *et al*. 2009), dwarf shrubs (Squeo *et al*. 1991, Azócar 2006), herbs (Azócar *et al*. 1988; Squeo *et al*. 1991, Azócar 2006, Azócar and Rada 2006) and a wide variety of grasses (Márquez *et al*. 2006). Above treeline limits, freezing temperatures may occur any night of the year and surviving below zero temperatures is achieved either by frost tolerance (withstanding ice formation in intercellular spaces) or avoidance (withstanding temperatures well below zero without ice formation in any of the tissues) mainly through deep supercooling (Levitt 1972; Beck *et al*. 1982, 1984, Sakai and Larcher 1987, Pearce 2001).The former has been described as a mechanism utilized in harsher environments with prolonged below zero temperatures, whilst the latter, in less severe environments (Larcher 1995, Körner 1998, Sakai and Larcher 1987). In general, freezing avoidance represents the most common mechanism exhibited by woody species of the tropical high Andes (Squeo *et al*. 1991, Azócar 2006, Azócar and Rada 2006, Rada *et al*. 1985, 2009), given that periods below 0 °C may occur any night of the year, but remain close to zero lasting only a few hours (Azócar and Rada 2006). Avoidance through supercooling in leaves is achieved in tropical Andean giant rosettes and dwarf shrubs by unique anatomical traits such as compact mesophylls with low apoplastic water contents (Rada *et al*. 1987, Azócar 2006, Azócar and Rada 2006). In other paramo woody species like *Polylepis sericea,* avoidance is achieved by the accumulation of osmotically active solutes and carbohydrates (Rada *et al*. 1985). In contrast with woody life forms, herbaceous plants including grasses, tolerate intercellular ice formation (Squeo *et al*. 1991, Márquez *et al*. 2006). Bamboos regarded as "woody grasses", are associated to a diversity of high altitude tropical ecosystems, from cloud forests to high elevation paramos (Clark 1995, Monasterio and Molinillo 2003, Clark and Ely 2011). However, how this group responds to freezing temperatures remains unknown. Temperate Japanese bamboos of the genera Sasa and Sasamorpha avoid frost formation in foliage tissues through deep supercooling, reaching freezing temperatures between -22 and -15 ºC (Ishikawa 1984, Sakai 1976, Sakai 1995, Tanaka 2002). In the tropical Andes, all of the high elevation bamboos belong to the genus Chusquea (Clark 1989, Clark 1995, Bussman 2004, Niño *et al*. 2006, Clark and Ely 2011), and the greatest species diversity may be found in Andean cloud forests, between 2400-2800 m a.s.l (Clark 1995). At higher altitudes (3000-4000 m a.s.l.), species diversity decreases markedly, and the viny life-form is replaced by a shrublike one. The *Chusquea* species that grow along the upper cloud forest-paramo ecotone are: *C. multiramea* (2200-2750 m a.s.l.), *C. serrulata* (2450-2900 m a.s.l.), *C. angustifolia* (2525-2800 m a.s.l.), *C. spencei* (2780-3650 m a.s.l.) and *C. guirigayensis* (3800-4010 m a.s.l.). The first two species are upper cloud forest viny bamboos whereas; the remaining three species are shrublike and differ in their altitudinal distribution: *C. angustifolia* grows in swampy low paramos, *C. spencei* along the treeline-paramo ecotone, and *C. guirigayensis* exclusively in high elevation paramos.

At present no studies on the effect of freezing temperatures on high altitude Andean bamboos have been conducted. Two main questions are addressed in this study: Firstly, how do Andean bamboos respond to freezing temperatures? One could expect the species of lower altitudes to be frost avoiders and those of higher elevations frost tolerant. Secondly, does freezing resistance increase in woody bamboos along the altitudinal gradient?

MATERIALS AND METHODS

Field sample collection and thermal analysis were carried out from 2008 through 2011. Fresh leaf samples were collected at different intervals, during both dry and rainy seasons, along an altitude gradient ranging from 2450 to 4010 m a.s.l., comprising upper cloud forests-treeline ecotones, low and high paramos. Samples of *C. multiramea* and *C. serrulata* were collected at 2450 m a.s.l. in Monte Zerpa cloud forest (08°38 92' N and 71°24 63' W). *C. angustifolia* was collected in Las Piñuelas at 2525 m (N 8º37'35 and W 71º24'), which constitutes the lowest paramo of the Venezuelan Andes. *C. spencei*, due to its broad altitudinal range of 930 m (Ely 2009), was collected at three different altitudes: paramos Las Coloradas (N 8°28' and W 71°57') at 2670 m, La Culata (N 08°44 99' and W 71°04.17') at 3025 m a.s.l., and La Aguada (N 8°35; and W 71°09') at 3320 m a.s.l. *C. guirigayensis*, was collected in the páramo Piedras Blancas (N 8º53' and W 70º57'), at 4010 m a.s.l. Mean annual rainfall at these five sites are: 2286 mm in Las Piñuelas, 2520 mm in Monte Zerpa, 1877 mm in Las Coloradas, 1780 mm in La Culata, 1573 mm, in La Aguada (Ely 2009) and 800-900 mm in Piedras Blancas (Kiyota 2011).

Species description

Extensive descriptions of the vegetative characters for these species may be reviewed in Clark (1989), Niño *et al*. (2004), Ely (2009), Kiyota (2011), Clark and Ely (2013). Vouchers of the five species collected are deposited in MERC, Instituto Jardín Botánico de Mérida, Faculty of Sciences, University of The Andes, Mérida, Venezuela.

Chusquea angustifolia (Soderstr. and C. Calderón) L.G. Clark. (*F. Ely et al. 2009*, v.s. 44 *MERC*). Shrub of variable height, 0.2-1.7 m high, that grows between 2520-2800 m a.s.l. Foliage leaves pubescent, somewhat sticky, lanceolate, coriaceous, blades 6-8 cm long x 0.2-5 mm wide.

Chusquea guirigayensis Niño, L.G. Clark and Dorr. (*F. Ely et al. 2009*, v.s. 43 *MERC*). Miniature shrub, typically 20-50 cm high (exceptionally 120 cm). In Mérida, this species grows between 3800-4010 m a.s.l. Foliage leaves glabrous, triangular to lanceolate, consistency markedly coriaceous, blades 1-2.5 cm long x 0.3-0.5 cm wide.

Chusquea multiramea L.G. Clark and F. Ely. spec. nov. (*F. Ely & Borregales 2006*, v.s. 15 *MERC*). Woody climber, usually 2-6 m high that grows between 2200-2700 m a.s.l. Foliage leaves glabrous, membranous, blades 6.5-8.2 cm long x 3.8-4.2 cm wide.

Chusquea serrulata Pilger. (*F. Ely et al. 2006* v.s. 18 *MERC*). Woody climber, usually 3-6 m high, that grows between 2450-2900 m a.s.l. Foliage leaves glabrous, linear, papery blades 18-30 cm long x 0.4-0.6 cm wide.

Chusquea spencei Ernst. (*F. Ely et al. 2006* v.s. 1 *MERC*). Shrub, ranging from 0.8-3 m high, growing between 2670-3650 m a.s.l. Foliage leaves glabrous, linear-lanceolate, coriaceous, blades 5-14 cm long x 0.2-0.6 cm wide.

Climate measurements

Air temperature (°C) and relative humidity (RH) data from the last 10 years were considered, as well as measurements performed at these six localities; from February to October 2008 in Monte Zerpa, La Culata and La Aguada, and from May to November 2010 in Las Piñuelas, El Molino and Piedras Blancas. Data were registered every 15 minutes with portable data loggers at each site (*HOBO, Pro Series.* Onset, Massachusetts, USA). In all of the study sites, sensors were placed at 1.5 m above ground level, protected from direct full sunlight. These were also placed at ground level in the paramos Las Piñuelas, El Molino and Piedras Blancas.

Thermal analysis

The relationship between tissue ice nucleation and injury temperatures determine frost resistance mechanisms. If these two temperatures coincide well below zero, we refer to freezing avoidance. In frost tolerant plants, ice nucleation temperatures occur close to 0°C while injury at significantly lower temperatures.

Intercellular ice nucleation temperatures were measured in fresh leaves collected at the six sites. A minimum of four trials were carried out in n=18-20 samples per species. Leaf samples were collected from these species during both the dry (January through March) and the rainy (May through September) seasons. Sampling consisted of five young culms per species of different genets collected from culms separated at a minimum distance of 10 m. Culms were cut at ground level in the field, placed in water and the ends cut again under water to avoid formation of air bubbles in xylem vessels (Lei and Koike 1998). In the lab, culms were covered with black plastic bags and allowed to rehydrate overnight. A total of five leaf samples were placed in small glass test tubes, tightly sealed with rubber stoppers in order to avoid tissue moisture changes. Copper-constantan thermocouples were inserted in the tissue samples and temperature was continuously monitored with a 5-channel data logger connected to a PC. Test tubes were immersed in a refrigerated alcohol bath (*NESLAB*, mod. RTE-111). Temperature was then lowered progressively from 5 ºC to -25 ºC at a rate of approximately 7.5 ºC/h and monitored continuously with specially designed software (*Planta-ICAE*). Intercellular ice nucleation was registered through the formation of exotherms, the result of an abrupt increase in temperature generated by heat released during the freezing process.

Determination of injury temperatures

50% Injury temperatures were determined in these five species through the electrolyte leakage method used previously by Ishikawa (1984) and later modified by Lindén (2002). Electrical conductivity (µS) was measured in leaf tissues previously submitted to decreasing low temperatures, from 5 ºC to -25 ºC, and submerged in deionized water (with an initial electrical conductivity of 0 µS). Increases in electrical conductivity resulted from electrolyte leakage due to release of potassium ions as a consequence of cell wall rupture. Tissue samples were submitted to the same frost-inducing procedure described previously. This procedure was performed in n=18-20 samples per species. At 5 ºC intervals, three tubes were withdrawn from the refrigerated bath, leaf samples removed from the tubes and placed in clean plastic containers with 15 mm3 of deionized water. The containers were then refrigerated at 6 ºC during 48 h, and electrical conductivity was measured with an ExStik digital conductimeter, mod. EC500 (*Extech Instruments,* U.S.A). After measurements were performed, complete tissues rupture was induced by submerging samples briefly in liquid nitrogen and placing them again in their respective containers. These were refrigerated again for 48 h and electrical conductivity was measured afterwards. This last measurement corresponded to the electrical conductivity of samples after 100% leakage had occurred. Tissue injury was estimated as the temperature at which 50% leakage occurred through the following equation:

$$T_{50\%} = \frac{\text{Initial Electrical Conductivity} * 100}{\text{Final Electrical Conductivity}}$$

Initial Electrical Conductivity corresponded to the temperature to which the tissue was exposed before withdrawing the tubes from the refrigerating bath, whereas Final Electrical Conductivity represented the electrical conductivity of the samples after inducing complete tissue rupture.

Data analysis

Ice nucleation temperatures were represented graphically in box plots (Sigma Plot, ver. 10.0), and average temperatures and standard deviation errors were estimated. U Mann-Whitney non-parametric tests were carried out to determine whether or not differences between intercellular ice nucleation and injury temperatures were statistically significant for each species. Kruskall-Wallis tests (*SPSS Statistics,* ver. 17.0) were performed to determine statistically significant differences regarding ice nucleation and injury temperatures among these five species.

RESULTS

Climate characteristics

Below zero temperatures occurred only above 3000 m, and were relatively infrequent at treeline limits (3025-3320 m) where they remained close to zero, being more frequent in the upper open paramo limits (3800-4010). The lowest night temperatures were registered in Páramo de Piedras Blancas followed by Páramo La Aguada (Table 1). The frequency of nights with freezing temperatures increased during the dry season (end of December through the end of March 2008) above 3000 m, representing a 10% of the total of days registered at 3000 m, 18% at 3320 m, and 41% at 4010 m.

Intercellular ice nucleation and injury temperature

Both ice nucleation and 50% injury temperatures were consistent in all of the trials. In these five species, average intercellular freezing temperatures varied between -12.1 and -10.1 ºC, whereas average 50% injury values

Table1. Average air temperatures registered at the six study sites. Maximum and minimum temperatures registered are indicated in parentheses.

Study site	Average Temperature (°C)		Average Min. temperature (°C)		Average Max. temperature (°C)	
	Soil	Air	Soil	Air	Soil	Air
Monte Zerpa (2450 m)	ID	13.02	ID	8.03 ± 0.01 (6.53)	ID	17.06 (20)
Las Piñuelas (2525 m)	13 ± 0.03	14 ± 0.03	10 ± 0.2 (6.6)	8.5 ± 0.1 (4.6)	25 ± 0.6 (32)	20 ± 0.2 (25)
Las Coloradas (2670 m)	11 ± 0.02	12 ± 0.02	7.5 ± 0.2 (4.6)	9.0 ± 0.1 (7.0)	15 ± 0.2 (19)	17 ± 0.2 (21)
La Culata (3025 m)	ID	9.25 ± 0.07	ID	4.3 ± 0.65 (-0.16)	ID	18.8 ± 0.08 (19)
La Aguada (3320 m)	ID	7.6 ± 0.1	ID	2.3 ± 1.0 (-0.68)	ID	20 ± 0.06 (24)
Piedras Blancas (4010 m)	8.6 ± 0.1	6.2 ± 0.04	1.5 ± 0.1 (-12)	2.0 ± 0.1 (-4)	27 ± 1.0 (46)	15 ± 0.4 (21)

ID: insufficient data due to technical difficulties with the loggers

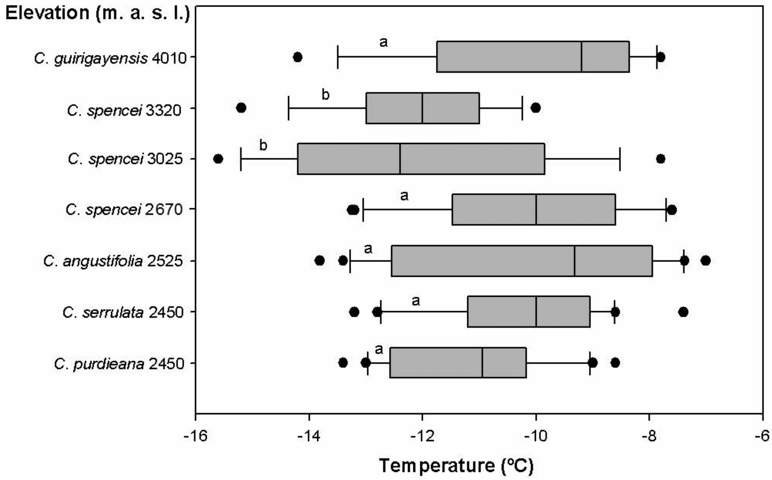

Figure 1. Relationship between ice nucleation temperatures and altitude for five *Chusquea* species along the 1560 m altitudinal gradient. Black circles represent extreme (high and low) values of ice nucleation temperatures in each case. Different letters depict significant differences ($p<0.05$).

ranged between -12.3 and -10.3 ºC (Table 2). No statistically significant differences were observed between intercellular ice nucleation temperatures (exotherm formation temperature) and injury temperatures in any of the species along the altitudinal gradient, indicating that in these five Chusquea species, intercellular ice nucleation was avoided through a moderate supercooling capacity.

Intercellular ice nucleation temperatures did not decrease linearly along the altitude gradient in this genus (Figure 1); nor did they vary significantly between the two viny cloud forest species (*C. multiramea* and *C. serrulata*); nor amongst the latter and the paramo shrublike species growing at the lower and upper limits of this gradient (*C. angustifolia* at 2520 m, the genets of *C. spencei* at 2670 m, and *C. guirigayensis* at 3800-4010 m). However, significant differences were observed between the genets of *C. spencei* growing at lower and upper limits of its distribution range (2670 m vs 3025-3320 m), as well as between the genets of *C. spencei* growing above 3000 m and the remaining four species (Figure 1, Table 2).

DISCUSSION

Chusquea is distinguished as the genus with the greatest diversity regarding species, habitats, life forms and altitudinal distribution in tropical Andean ecosystems (Clark 2001), yet above 3000 m, species diversity decreases markedly. These variations in species abundance suggest that freezing temperatures could be a determining factor in woody bamboo distribution in tropical mountain ecosystems. We had initially assumed that the bamboos growing below

Table 2. Average intercellular ice nucleation and 50% injury temperatures measured in these five *Chusquea* species. Maximum and minimum temperatures are indicated in parenthesis.

Species/ elevation (m a.s.l.)	Intercellular ice nucleation temperature (°C)	50% Injury temperature (°C)
C.multiramea (2450 m)	(-9.0) -11.0 ± 0.3^a (-13.4)	(-10.8) -11.5 ± 0.3^a (-12.4)
C. serrulata (24500 m)	(-9.0) -10.5 ± 0.3^a (-13.0)	(-9.6) -10.1 ± 0.4^a (-11.9)
C. angustifolia (25250 m)	(-7.4) -10.8 ± 0.2^a (-13.8)	(-11.4) -12.3 ± 0.3^a (-13.1)
C. spencei (26700 m)	(-7.6) -10.6 ± 0.4^a (-13.3)	(-8.4) -10.2 ± 0.4^a (-13.7)
C. spencei (30250 m)	(-7.9) -12.1 ± 0.5^b (-15.6)	(-9.8) -12.0 ± 0.2^b (-13.5)
C. spencei (33200 m)	(-10.4) -12.0 ± 0.3^b (-15.0)	(-9.8) -12.2 ± 0.3^b (-15.9)
C. guirigayensis (4010 m)	(-8.0) -10.3± 0.4^a (-14.2)	(-9.0) -10.6 ±0.3^a (-12.0)

Different letters depict significant differences ($p<0.05$).

treeline limits (3000 m), which are not exposed to freezing temperatures, such as the viny species (*C. multiramea* and *C. serrulata*) and the genets of *C. angustifolia* and *C. spencei* of low paramo ecosystems (2520 and 2670, respectively) were altogether devoid of freezing resistance mechanisms in which case, ice nucleation and injury temperatures should have occurred very close to 0 ºC, as described for other woody species of the upper cloud forest-paramo ecotone (Cavieres *et al*. 2000). Nevertheless, all five species avoided intercellular ice formation through a moderate supercooling, regardless of their life-form, habitat, plant height, foliage leaf size and consistency. Supercooling capacity values were higher in these *Chusquea* species than those reported for other paramo woody species (Rada *et al*. 1985, 2009; Cavieres *et al*. 2000), and comparable to those reported for giant rosettes of the genus *Espeletia* (Goldstein *et al*. 1985, Rada *et al*. 1987). Another unexpected result was that neither intercellular ice nucleation, nor 50% injury temperatures varied significantly between the species situated at the upper and lower limits of the cloud forest-paramo gradient (2450 and 4010 m). Only *C. spencei* presented a slight increase in its supercooling capacity with increasing altitude, as the differences between the genets growing below and above 3000 m suggest. A possible explanation for the relatively uniform supercooling capacity observed in these five species is that after the last glaciation, cloud forest and paramo boundaries suffered repeated displacements, with paramo ecosystems descending as low as 2000 m (Van der Hammen 1974, 1988, Van der Hammen 2000, Salgado-Labouriau *et al*. 1977, 1992). In addition, minimum air temperatures have also increased considerably during the last decades, reducing the frequency of nocturnal frosts, as recent microclimate studies indicate (Monasterio and Reyes 1980, Azócar and Rada 2006, Azócar 2006, Ely 2009, Kiyota 2011).

Our results suggest that neither plant height nor elevation necessarily condition freezing resistance mechanisms in this group. *C guirigayensis* with the smallest height and growing at the highest elevations responds to freezing temperatures in the same manner as the other species studied. Freezing resistance mechanisms differ among herbaceous tussock grasses and strongly lignified grasses such as bamboos, regardless of whether they grow in temperate (Ishikawa 1984, Tanaka, 2002, Ashworth and Pearce 2001, Liu and Osborne 2008) or tropical climates (Márquez *et al*. 2006). Tussock grasses of the Venezuelan paramos tolerate extracellular freezing, with intercellular ice nucleation temperatures between -6.3 and -3 °C, and 50% injury of foliage tissues occurring between -18 and -9.8 °C (Márquez *et al*. 2006). Tussock grasses are subjected to lower temperatures and for longer intervals; due to their proximity to the ground where the temperatures are lowest in the air-soil gradient, therefore foliage tissues are exposed to freezing temperatures in their early development, in contrast with woody bamboos, in which developing organs are protected from extreme temperatures by thick culm leaves until they have reached maturity (Ely 2009). In the Venezuelan paramos, where freezing air temperatures typically remain close to 0 °C and last for only a few hours , a moderate supercooling capacity combined with the protection of developing organs should be sufficient to impede the formation of intercellular frost in foliage tissues.

These results support the studies conducted in the Japanese species of *Sasa* and *Sasamorpha*, which differ from these *Chusquea* species in their greater supercooling capacity (-22 to -15 ºC, Sakai 1976, 1995, Ishikawa 1984), which likely evolved as an adaptation to the seasonal winters (Tanaka 2002). Based on these results we also conclude that the altitudinal distribution of *Chusquea multiramea, C. serrulata*, *C. angustifolia*, *C. spencei* and *C. guirigayensis* in the Venezuelan Andes is not conditioned by freezing temperatures, but more likely by other environmental factors not taken in account in the present study.

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Figure 2. *Chusquea multiramea*

Figure 3. *Chusquea spencei*

Figure 4. *Chusquea guirigayensis*

Figure 5. *Chusquea spencei*

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Effect of rainwater harvesting on distribution of water and nutrients in soil influencing growth of *Dendrocalamus strictus* in degraded hills in Rajasthan, India

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ABSTRACT

Use of rainwater harvesting (RWH) and perennial vegetation in restoring degraded land improve soil carbon and nutrients, but subsequent increase in drainage may influence carbon and nutrient distribution in soils and plant growth. A three year study was conducted in the plots distributed in <10%, 10-20% and >20% slopes and have contour trench (CT), gradonie (GD), Box trench (BT), V-ditch (VD) rainwater harvesting treatments and a control, to monitor changes in soil water, soil properties and concentrations of SOC, NH_4-N, NO_3-N, PO_4-P and their distribution in 0-40 and 40-80 cm soil layers. Slope gradient and soil texture influenced soil water storage and soil properties. Intra and inter annual distributions of rainfall and corresponding increase/decrease in soil water content showed positive correlation to SOC and soil nutrients. Greater soil water and nutrients in <10% slope promoted height and shoot numbers in planted *Dendrocalamus strictus* L., whereas greater silt and clay content in the soil of >20% slope favoured SOC and NH_4-N accumulation and supported growth in >20% as compared to 10-20% slope. Effects of RWH in facilitating deep soil storage was greater on soil water, NO_3-N and dissolved salts (EC) than on SOC, NH_4-N and PO_4-P, but the effects of CT was significant ($p < 0.05$) resulting in highest growth of *D. strictus* in this treatment. V-ditch supported distribution of water, dissolved salts and NO_3-N availability in upper soil layer and promoted shoot numbers during monsoon, but faster depletion of soil water with advancement of summer lead to greater shoot mortality in this treatment. Contour trench facilitated storage of organic carbon (carbon sequestration) and water in deeper soil layers, favouring growth of *D. strictus* during dry period. However, regular mortality in shoots of *D. strictus* during December to June emphasizes to increase water harvesting furthermore to promote shoot survival and growth that help restore degraded hills effectively.

Keywords: Degraded hills, restoration, soil water and nutrients distribution, soil layers

Abbreviations:

EC	electrical conductivity
RWH	Rainwater harvesting
SOC	Soil organic content,
SWC	Soil water content
CT	contour trench
GD	gradonie
BT	box trench
VD	V-ditch

INTRODUCTION

Water and nutrients are essential components of increasing land productivity, but management of these are determined by the need of soil and water conservation particularly in dry areas (Van der Zaag 2012). Though methods of water and nutrient conservation including irrigation and water harvesting are age old practices (Reij *et al.* 1996, Thomas 1997, Mutunga *et al.* 2001, Mati 2005), however very little differences are there between soil and water conservation and rainwater harvesting (RWH) technologies in enhancing land productivity. Soil and water conservation reduces water losses by surface runoff and evaporation, whereas RWH maximizes water storage in soil and help enhance productivity (Critchley & Siegert 1991). The common methods of soil and water conservations and rainwater harvesting are level contour bunds, grass strips, cutoff drains, hill terracing, graded bench terraces, contour trench, V-ditches, underground tanks, open pans and ponds, spate irrigations, mulching and various tillage systems. Wiyo *et al.* (2000) observed reduction in surface runoff and increase in soil water (i.e., greater soil water in fine-textured soils as compared to coarse-textured soils) under tied-ridging. Studies on the interaction of soil water and nutrients (Brouwer & Bouma 1997, Fox & Rockström 2003) emphasize the importance of assured water availability to improve nutrient uptake in crops. However, increased drainage with RWH may increase leaching of plant nutrients out of the root zone reducing crop yield and has environmental implications too (Barron 2004, Branca *et al.* 2012).

Risk of total crop failure can be minimized by increasing additional capacity of soil water storage through RWH (Temesgen *et al.* 2009). Evidences of increasing dry spells and its frequent occurrence in the face of climate variability gives emphasis to enhance water control/harvesting (Enfors & Gordon, 2008; Shongwe *et al.* 2009). Better management of water through rain water harvesting help improve current farming practices (Rockström 2003, Jha & Lalnunmawia 2004), whereas use of RWH and afforestation in restoration programme stabilize hillslopes, reduces soil erosion, improves terrestrial and aquatic habitats, enhances stream aesthetics, and care for water quality (Dattilo & Rhoades 2005). Perennial species like bamboos and *Vetiveria zizanoides* have been observed better for soil erosion control and streambank stabilization (Rocheleau *et al.* 1988, Yanhui & Yongmin 1995, Anon. 2012, Pande *et al.* 2012). Though, negative effects of bamboo on tree seedlings have also been reported (Caccia *et al.* 2009, Wang *et al.* 2012), but bamboo grooves intercropped with fodder grass found the most effective in soil erosion control (Arunachalam & Arunachalam 2002, Deng *et al.* 2003). Characteristic features of bamboo in controlling soil erosion are its extensive fibrous roots and inter connected rhizome, dense foliage and high amount of leaf litter providing cover to the soil surface (Ben-zhi et.al. 2005). Though depends on the species, but high growth and biomass in bamboo can be achieved by increased soil water and nutrients availability through RWH. *Dendrocalamus strictus* L has been reported quite promising in degraded sites (Dhruva Narayana 1993) and flourishes in places with an annual rainfall between 700 mm and 5000 mm. Whereas, application of rainwater harvesting (RWH) may enhance growth of *D. strictus* in lower rainfall zone, the extensive root system enhancing root surface may increase the rhizosphere microbial biomass that help enrich soil fertility (Singh & Singh 1999, Singh 2009) and organic matter content (Lin *et al.* 2004, Tong 2007).

It is hypothesized that RWH would enhance the growth of *D. strictus*, but a combination of these two factors (i.e., RWH and plantation of *D. strictus*) may influence soil nutrient partitioning and soil carbon content in different soil layers under increased drainage and resource utilization pattern by *D. strictus*. Therefore, objective of this work was to assess the impact of RWH on the survival and growth of *D. strictus* and a combined effect of RWH and *D. strictus* plantation on soil carbon and nutrient build up in different soil layers during the restoration process of degraded hills.

MATERIALS AND METHODS

Site characteristics

Study was conducted during 2008-2011 in a mixed plantation done in 2005 that covers about 17 ha area encompassing degraded hills of varying size, slope and aspects (Singh, 2009). The site is located 17 km south -west of district head quarter Banswara (23°32'28.2" N and 74°26'30.3" E), Rajasthan. Air temperature varied widely between the months of a year ranging from 4 °C in January to 42 °C in May. Mean minimum annual temperature during 2005 to 2010 ranged from 18.7 °C in 2008 to 20.1 °C in 2006, whereas mean maximum annual temperature ranged from 32.8 °C in 2008 to 34.3 °C in 2010 (Table 1). Average

Table 1. Rainfall, number of rain days and mean annual maximum and minimum temperatures in different years near the experimental site.

Year	Rain days	Rainfall during monsoon (June-October) and annual (mm) Mean Temp.(°C)								
		June	July	Aug.	Sept.	Oct.	Total	Annual	Max	Min.
2005	42	82.6	290.0	169.1	483.5	-	1025.2	1026.7	33.2	19.8
2006	63	148.4	648.6	630.3	489.1	44.0	1960.4	2266.0	33.1	20.0
2007	44	102.0	232.0	565.0	254.0	-	1153.0	1391.0	33.5	19.0
2008	29	101.0	192.3	129.5	74.0	-	496.8	562.5	32.7	18.6
2009	30	69.0	457.0	333.0	-	-	859.0	859.0	33.9	19.0
2010	39	3.0	93.0	427.0	71.0	5.0	599.0	636.0	34.3	19.9
Av.	41.2	84.3	318.8	375.7	228.6	8.2	1015.6	1123.5	-	-

annual rainfall is about 960 mm. However, years 2008, 2009 and 2010 received 563 mm, 859 mm and 636 mm rainfall in 29, 30 and 39 rainy days, respectively (Table 1). Hillslopes were categorized in to steep (>20%), medium (10-20%) and gentle (<10%). Crystalline gravels and pebbles of varying size occupied the steep slope areas, whereas light textured sandy loam soils of shallow depth was available at medium slope. Loamy to clay loam soils with varying depth were available in gentle slope area. In June 2005, soil pH ranged between 6.34 and 7.02. Concentrations of soil organic carbon (SOC) and available ammonium nitrogen (NH_4-N), nitrate nitrogen (NO_3-N) and phosphate phosphorus (PO_4-P) were 0.76%, 22.15 mg kg^{-1}, 2.50 mg kg^{-1} and 4.51 mg kg^{-1}, respectively. Dominant vegetation in steep slope, medium slope and gentle slopes were *Lantana camara, Prosopis juliflora* with occasional *Lantana camara* and *Prosopis juliflora* and *Lantana camara* bushes, respectively.

Experimental design

Seventy-five plots each 700 m^2 area were in three slope categories, i.e. <10%, 10-20% and >20% covering almost all aspects. Each plot had individual boundary of trench (45 x 45 cm^2 cross section area) cum bund and rainwater harvesting (RWH) structure of 30 running meter length. Contour trench (CT), gradonie (GD), Box trench (BT) and V-ditch (VD) RWH structures were excavated at different contour levels to harvest run-off. There was a control without rainwater harvesting structure that make a sum of five RWH treatments (Singh 2009). Contour and Box trenches were 45 cm x 45 cm in cross section area. In this contour trenches were continuous in nature whereas box trench were 15 intermittent trenches of 2 m length. Gradonie and V-ditch were 1800 cm^2 cross section area, but the difference in these structures was a vertical cut of 30 cm height-downside of the slope in VD and upside of the slope in GD (to reduce velocity of surface run-off water). The excavated soil was always kept downside of the dugout to make a 'berm' of 40 cm height. A mixed plantation of *Acacia catechu, Azadirachta indica, Emblica officinalis, Holoptelia integrifolia* and *Zizyphus mauritiana* and *Dendrocalamus strictus* planted in August 2005 was used under the study. Five rainwater harvesting treatments laid in five replicates and distributed in three slope categories resulted in 75 observation plots.

Observations recording

Soil texture and initial soil nutrients anlyses were done for the soil samples collected in 0-40 cm soil layer in June 2005. To monitor soil water and nutrient distribution in different soil layers, soil samplings from June 2009 onwards was done in 0-40 and >40 cm soil layers (up to 80 cm depending upon soil availability). The collected soil samples were dried and passed to a 2 mm sieve for separation of course and fine earth (soil) fraction. Soil pH and electrical conductivity (EC) were determined in 1:2 soil water ratio (Jackson 1973). Walkley & Black (1934) method was followed for SOC determination. Available NH_4-N, NO_3-N and PO_4-P (Olson's extraction) were determined following standard procedures (Jackson, 1973; Cataldo *et al.*, 1975; Baruah and Barthkumar, 1999) by using uv-vis-spectrophotometer

Model Shimadzu-1650PC. For soil water content (SWC) determination, collected soil samples from the vicinity of the plants were put immediately into polyethylene bag to avoid water loss during transport. Soil water content was estimated by oven drying of the sample at 110 °C for a constant weight. Plant height and number of shoot (culm) of *D. strictus* were measured in June and December of each year to monitor seasonal growth, i.e. monsoon and spring and mortality due to increased soil water stress during December to June.

Statistical analysis

All data were analyzed using SPSS version 8.0 statistical package. Plant height and number of shoots, shoot mortality, and mean annual increment data were analyzed using a two-way ANOVA. Data of soil organic carbon, pH, electrical conductivity (EC) and NH_4-N, NO_3-N, PO_4-P were analyzed separately for each soil layer, where above-mentioned parameters were considered as the dependent variables. Hillslopes and rainwater harvesting treatments were the fixed factors. Since SWC determined repeatedly six times from December 2008 to June 2011 in 0-40 and 40-80 cm soil layers, these data were analyzed using repeated measure ANOVA separately for each layer. Differences in SWC between 0-40 and 40-80 cm soil layers were tested for significance using 'Paired T-test'. Percent SWC data were also analyzed using a two-way ANOVA after square root transformation (Sokal & Rolf 1981). Duncan Multiple Range Tests (DMRT) was applied to group homogeneous subsets of slope and RWH treatments at $P < 0.05$ levels. To obtain relations between rainfall, SWC, soil properties and growth variables of *D. strictus*, Pearson correlation coefficients were calculated and regression equations were developed to relate SWC and soil organic carbon.

RESULTS

Soil water content

Soil water content indicated ample seasonality with its highest value in December 2010 and lowest value in June 2009. SWC was always greater ($p < 0.01$) in December than in June. Season x slope interaction was significant ($p < 0.05$) and average SWC was highest ($p < 0.05$) in <10% slope in December 2008 to June 2010 and in >20% slope in December 2010 to June 2011. The lowest SWC was observed in 10-20% slope throughout the study (Table 2). SWC did not differ between <10% and >20% slopes for 40-80 cm soil layer. As compared to SWC value in 10-20% slope, respective increase in SWC in >20% and <10% slopes were 31% and 51% in 0-40 cm soil layer and 34% and 49% in 40-80 cm soil layer. SWC did not differ ($p > 0.05$) among RWH treatments, but it was highest in BT and lowest in the control plots. However, DMRT indicated highest SWC in BT treatment ($p < 0.05$) in December 2008 in 0-40 cm soil layer and in CT treatments in June 2010 and June 2011 in both 0-40 cm and 40-80 cm soil layers. SWC was greater in 40-80 cm soil layer in all observations except in June 2009 and 2011. BT plots showed highest SWC in both soil layers in <10% slope. In 10-20% slope, SWC was highest in CT in 0-40 cm and in GD plots in 40-80 cm soil layers, whereas in >20% slope it was highest in VD plots in 0-40 cm and BT plot in 40-80 cm soil layers. When averaged for December and June separately, difference in SWC between RWH treatments was not significant ($p > 0.05$) in December, but varied significantly ($p < 0.05$) in June (highest in CT/BT plots). SWC was highest in BT plots in December and in CT plots in June. GD and VD plots showed greater SWC than the control plots in December and lesser SWC than the control plots in June.

Soil properties and nutrients

Infiltration rate and soil texture observed in 2007 indicated highest infiltration rate (12.2 mm min^{-1}) in 10-20% slope and lowest (7.7 mm min^{-1}) in <10% slope. Silt and clay fraction were highest and lowest in >20% slope and 10-20% slope, respectively, whereas sand fraction was highest in 10-20% slope (Supplementary Table 1). Soil pH, EC and SOC, NO_3-N and PO_4-P varied ($p < 0.01$) between years (Table 3). Year x slope interaction was significant ($p < 0.05$) for pH and SOC in both soil layers, PO_4-P in 0-40 cm and NO_3-N in 40-80 cm soil layers. In June 2008, SOC was highest in <10% slope, but soil

Table 2. Soil water content and its distribution in soil layers influenced by slope gradient, rainwater harvesting structures and growing plantations during post monsoon (December) and pre monsoon (June) period.

Slope	RWH Treat	Soil	2008	2009		2010		2011
		Layer	December	June	December	June	December	June
<10%	C	0-40	4.95±0.78	1.25±0.22	4.61±0.75	1.34±0.14	4.45±0.51	2.16±0.08
		40-80	5.47±1.17	0.90±0.18	4.49±0.65	1.52±0.16	5.94±1.08	1.89±0.08
	CT	0-40	7.73±0.43	0.78±0.18	4.47±0.42	1.75±0.06	5.76±1.22	2.3±0.27
		40-80	8.65±0.86	1.12±0.40	4.57±0.49	2.04±0.13	5.63±2.15	2.41±0.32
	GD	0-40	4.18±1.26	0.47±0.13	4.56±0.45	0.89±0.15	5.09±1.04	2.02±0.29
		40-80	3.70±1.10	0.82±0.29	4.42±0.70	0.97±0.09	4.97±1.06	1.60±0.14
	BT	0-40	8.15±0.41	1.47±0.42	5.09±0.83	1.59±0.30	6.86±1.04	1.59±0.19
		40-80	7.95±0.53	0.99±0.19	4.83+0.71	1.91±0.27	7.22±1.08	1.52±0.28
	VD	0-40	6.04±0.57	0.86±0.26	5.67±0.70	1.01±0.14	6.76±1.18	1.31±0.11
		40-80	7.22±0.66	0.89±0.26	5.14±0.56	1.08±0.07	5.21±0.64	1.05±0.16
10-20%	C	0-40	2.58±0.35	0.88±0.38	3.34±0.19	0.75±0.13	3.74±1.10	0.94±0.17
		40-80	1.76±0.68	0.45±0.15	3.67±0.33	0.78±0.09	4.20±0.94	1.17±0.21
	CT	0-40	3.30±0.38	0.66±0.21	4.83±0.61	1.19±0.04	5.20±1.18	1.55±0.24
		40-80	4.18±0.55	0.47±0.12	4.53±0.58	1.53±0.10	4.83±1.66	1.34±0.22
	GD	0-40	2.79±0.35	0.47±0.16	5.19±0.57	0.68±0.12	4.60±0.88	1.30±0.15
		40-80	3.50±0.83	1.01±0.53	5.21±0.55	0.74±0.11	6.72±1.83	1.00±0.11
	BT	0-40	3.32±0.54	0.60±0.15	3.01±0.11	0.88±0.12	4.53±0.90	2.00±0.35
		40-80	3.82±0.77	0.57±0.21	3.51±0.58	1.33±0.19	4.27±1.15	1.69±0.40
	VD	0-40	1.70±0.47	0.27±0.11	3.29±0.25	0.68±0.08	3.51±0.90	1.98±0.26
		40-80	1.72±0.59	0.38±0.11	3.49±0.35	0.87±0.17	4.44±0.94	1.66±0.35
>20%	C	0-40	3.90±0.75	0.58±0.17	3.58±1.20	0.82±0.11	6.01±1.60	2.18±0.11
		40-80	3.82±0.77	0.68±0.20	3.72±0.94	1.34±0.14	6.72±1.38	2.33±0.15
	CT	0-40	3.52±0.55	0.72±0.24	3.40±0.84	1.30±0.08	6.67±2.07	2.32±0.16
		40-80	3.55±0.95	0.59±0.16	3.88±0.74	1.54±0.12	8.56±2.39	2.22±0.21
	GD	0-40	4.08±0.45	0.74±0.32	3.68±0.40	0.98±0.16	7.01±1.04	2.22±0.13
		40-80	4.01±0.61	0.55±0.17	3.50±0.48	1.17±0.15	6.92±1.08	2.41±0.26
	BT	0-40	3.74±0.59	0.52±0.12	5.01±1.03	0.90±0.16	6.23±2.12	2.31±0.21
		40-80	3.79±0.64	0.65±0.30	5.09±1.04	1.29±0.17	8.15±2.94	2.58±0.11
	VD	0-40	4.13±0.41	0.55±0.15	5.21±0.88	1.25±0.12	6.03±1.76	1.61±0.26
		40-80	3.88±0.77	0.69±0.36	5.38±0.91	1.37±0.11	6.30±1.51	1.44±0.30

pH, EC (Supplementary Table 2) and NH_4-N, NO_3-N and PO_4-P were highest in >20% slope (Table 4). In both June 2009 and 2010, SOC and NH_4-N were highest in >20% slope, whereas pH, EC and NO_3-N and PO_4-P concentrations were highest in <10% slope in both soil layers. However, NH_4-N in 40-80 cm soil layer and soil pH in both the soil layers showed their highest values in 10-20% slope. Among RWH treatments, soil pH, EC, and NO_3-N concentration were highest in VD treatment, whereas concentrations of SOC, NH_4-N and PO_4-P were highest in CT treatment. In the most of the observations, soil pH was lowest in CT plots. While considering soil layers, SOC ($P<0.001$), NH_4-N ($p < 0.01$), PO_4-P ($p < 0.01$) and soil pH were greater in 0-40 cm soil layer, whereas NO_3-N and EC were highest in 40-80 cm soil layer in all years. Control plots indicated greater NO_3-N concentration in 0-40 cm soil layer than in 40-80 cm soil layer in both 2009 and 2010, whereas NH_4-N was greater in 40-80 cm soil layer (a reverse trend as observed in RWH plots).

Plant growth

In June 2008, plants of *D. strictus* were taller in <10% slope as compared to the other slopes and maintained the pattern throughout the study period. Plants were shorter in height in >20%

Table 3. F values of Repeated Measure ANOVA for soil water status and soil physico-chemical properties analysed separately for 0-40 cm and 40-80 cm soil layers.

Variables	Soil layer	Tests of within-subjects effects				Tests of between subjects effects		
		Season/Year (Y)	Y × Slope (S)	Y × Treatment (T)	Y × S × T	Slope	RWH treatment	S ×T
Soil water	df	5	10	20	40	2	4	8
	0-40	155.2***	6.65**	0.78NS	0.34NS	13.48**	1.32NS	0.88NS
	40-80	121.82**	6.55**	1.05NS	1.62*	9.63**	1.76NS	1.20NS
Degree of freedom		3	6	12	24	2	4	8
Soil pH	0-40	77.19**	5.08**	1.36NS	1.39NS	2.69*	0.94NS	1.95NS
	40-80	11.84**	2.53*	0.61NS	1.73NS	4.35*	1.46NS	1.79NS
Soil EC (dSm-1)	0-40	15.79**	1.10NS	0.67NS	0.99NS	2.31NS	2.64*	0.71NS
	40-80	11.28**	0.74NS	0.74NS	1.80NS	2.41NS	1.11NS	1.17NS
SOC (%)	0-40	14.03***	2.77*	0.65NS	0.89NS	4.51*	0.27NS	0.54NS
	40-80	17.83**	6.21**	1.31NS	0.60NS	6.44**	0.38NS	0.35NS
NH4-N	0-40	0.81NS	1.93NS	0.61NS	0.63NS	1.65NS	1.26NS	0.31NS
	40-80	0.25NS	1.65NS	1.31NS	0.91NS	3.55*	0.59NS	0.56NS
NO3N	0-40	15.71**	2.00NS	0.46NS	0.80NS	2.21NS	0.29NS	1.09NS
	40-80	5.13*	5.42**	0.45NS	0.34NS	3.14NS	1.15NS	0.66NS
PO4-P	0-40	14.23**	5.87**	1.54NS	2.29*	1.15NS	0.74NS	0.88NS
	40-80	22.91**	1.47NS	1.61NS	0.38NS	4.82*	1.75NS	1.16NS

Table 4. Soil nutrients influenced by natural slope gradient, rainwater harvesting devices and soil layers in degraded hills in Rajasthan. Values are mean± SE of five replications.

Slope	RWH Treatment	Soil layer	NH_4-N (mg kg^{-1})			NO_3-N (mg kg^{-1})			PO_4-P (mg kg^{-1})		
			2008	2009	2010	2008	2009	2010	2008	2009	2010
S1	Control	0-40	13.2±2.1	10.6±1.6	12.0±1.6	2.7±0.5	5.3±0.9	7.0±0.5	12.0±1.7	14.7±0.5	14.3±0.5
		40-80		10.6±0.9	11.9±0.7		5.6±1.3	6.8±1.1		11.7±0.7	12.2±0.7
	Contour Trench	0-40	14.8±2.8	13.3±2.5	14.3±2.4	4.7±1.2	5.5±0.4	7.1±1.3	14.9±2.0	16.6±1.8	17.3±1.7
		40-80		12.9±2.4	13.6±1.8		4.9±0.6	5.7±1.2		13.6±0.8	15.2±1.1
	Gradonie	0-40	14.8±0.9	15.0±0.7	15.5±1.2	5.4±0.9	6.0±0.8	5.8±0.9	18.2±2.3	17.6±1.7	17.9±1.5
		40-80		14.1±0.9	14.4±0.6		7.8±0.9	7.8±1.3		14.5±1.9	15.1±1.0
	Box Trench	0-40	15.5±2.0	13.2±1.9	12.7±1.6	5.4±1.0	6.2±0.8	8.3±2.3	13.1±1.5	14.8±1.5	16.7±1.5
		40-80		12.9±2.1	10.6±1.9		6.5±0.9	9.1±2.7		12.1±1.1	14.7±1.4
	V-ditch	0-40	16.3±2.9	12.7±2.0	12.9±1.9	4.3±0.9	6.4±1.1	6.9±1.0	14.7±1.0	16.7±1.4	18.1±0.9
		40-80		12.7±1.3	12.4±1.8		7.0±1.3	8.3±1.2		13.3±0.8	15.9±0.6
S2	Control	0-40	13.6±1.7	11.8±1.6	13.3±1.4	3.0±0.5	5.0±1.2	5.9±1.2	16.7±3.5	13.7±1.6	14.1±1.3
		40-80		12.9±1.0	12.3±0.9		4.9±1.0	6.5±1.2		12.2±1.7	12.6±1.4
	Contour Trench	0-40	15.6±3.7	15.3±1.0	17.0±0.7	3.3+0.5	4.5±0.9	5.4±1.1	16.3±2.5	15.4±1.0	16.3±0.9
		40-80		14.2±0.9	14.2±0.8		6.0±1.2	6.2±1.2		12.9±0.6	14.2±0.8
	Gradonie	0-40	12.7±1.1	15.1±1.7	16.3±1.5	3.0±0.7	4.7±0.8	5.8±0.9	17.3±1.5	13.1±0.7	13.9±1.0
		40-80		13.3±1.5	14.3±1.5		5.1±1.7	6.2±1.5		11.2±0.5	12.1±1.6
	Box Trench	0-40	12.1±3.1	16.3±1.0	16.6±2.4	4.4±1.0	6.4±1.5	8.0±2.2	14.3±2.0	15.6±0.8	17.6±0.5
		40-80		14.6±1.5	15.7±1.9		5.1±1.1	7.4±1.8		13.2±0.6	14.9±0.9
	V-ditch	0-40	13.9±1.7	14.1±1.1	14.2±1.9	4.5±0.5	4.7±0.6	4.9±1.0	14.9±2.3	14.9±0.7	16.6±0.9
		40-80		14.4±1.0	15.2±2.5		5.6±1.0	7.1±0.9		12.0±0.8	13.1±0.7
S3	Control	0-40	14.2±2.4	15.1±1.4	14.2±1.4	5.5±0.9	5.0±0.8	5.6±1.1	16.6±1.1	15.0±0.9	16.4±1.1
		40-80		14.8±1.6	13.7±1.7		5.5±1.0	4.7±0.9		11.6±0.8	12.0±0.3
	Contour Trench	0-40	17.1±2.3	14.2±0.9	15.8±1.6	4.2±0.8	5.7±0.6	5.6±0.6	20.6±3.1	15.2±0.5	16.5±0.2
		40-80		15.1±0.8	15.4±0.7		6.0±0.9	5.3±1.0		12.2±0.8	12.8±0.8
	Gradonie	0-40	13.8±1.5	13.2±0.9	16.0±1.2	6.4±2.3	5.4±1.0	5.4±0.7	16.0±2.1	14.4±1.2	15.2±1.3
		40-80		15.1±1.1	15.1±1.1		6.3±0.8	6.0±0.9		11.3±0.8	11.1±0.7
	Box Trench	0-40	15.1±3.9	15.8±0.7	16.2±0.8	3.9±0.6	4.3±0.7	3.5±0.5	15.3±1.1	13.8±0.9	15.5±0.6
		40-80		14.4±1.2	13.9±0.5		5.5±1.2	5.4±0.8		12.6±0.6	13.0±0.5
	V-ditch	0-40	14.5±1.8	15.4±1.8	17.2±0.6	4.2±0.4	6.5±0.6	4.2±0.8	17.3±1.6	15.4±0.8	15.8±0.6
		40-80		13.9±1.2	13.7±1.0		6.5±0.5	4.6±0.7		11.8±0.6	13.7±0.6

Table 5. Average growth and mean annual increments of 65 months old plants of *Dendrocalamus strictus* plants influenced by natural slope gradient and rainwater harvesting devices in degraded hills in Rajasthan. Values are mean± SE of five replications.

Slope	Treat	Height (cm)			Number of culm (cm)		
		June 2008	June 2011	MAI	June 2008	June 2011	MAI (Tiller)
S1	Control	256.0±22.2	479.3±33.3	59.7±17.5	3.8±0.3	4.3±0.5	0.2±0.01
	Contour Trench	331.4±20.0	557.4±23.7	54.6±5.4	4.0±0.1	5.0±1.3	0.3±0.21
	Gradonie	279.9±16.1	485.3±35.5	63.9±6.0	3.3±0.5	4.6±0.5	0.4±0.22
	Box Trench	327.1±19.7	484.6±34.5	46.3±6.9	3.8±0.2	4.3±0.5	0.2±0.18
	V-ditch	372.4±23.8	502.3±33.6	47.7±6.0	4.3±0.6	4.9±0.4	0.2±0.12
S2	Control	270.7±25.8	461.5±39.9	55.2±10.6	3.3±0.5	4.7±0.2	0.5±0.10
	Contour Trench	286.6±32.4	494.1±14.3	71.1±13.9	3.7±0.3	5.0+0.6	0.4±0.18
	Gradonie	268.3±35.9	416.7±47.7	52.9±16.9	3.9±0.7	3.9±0.5	0.0±0.00
	Box Trench	354.5±21.5	387.7±50.2	48.6±6.8	4.2±0.2	4.3±0.4	0.0±0.00
	V-ditch	297.8±41.1	438.8±29.3	55.4±7.0	3.8±0.1	5.1±1.3	0.5±0.35
S3	Control	182.5±42.7	314.0±94.1	43.4±25.1	2.4±+0.2	2.4±0.4	0.0±0.00
	Contour Trench	302.7±5.9	436.5±61.4	55.7±17.7	2.6±0.5	5.1±0.6	0.9±0.37
	Gradonie	281.2±12.1	498.8±36.2	72.5±9.6	3.1±0.2	3.0±0.8	0.0±0.02
	Box Trench	261.7±45.7	528.4±45.9	75.7±10.8	2.7±0.3	3.7±0.5	0.3±0.12
	V-ditch	309.9±49.5	495.3±22.9	65.1±18.4	2.8±0.3	3.9±0.5	0.4±0.07
Two way ANOVA			F values				
Slope (S)		7.85*	2.40NS	0.52NS	11.22**	1.74NS	0.18NS
Treatment (T)		3.52*	1.84NS	0.29NS	0.53NS	1.68NS	1.48NS
S x T		1.17NS	1.04NS	0.81NS	0.73NS	0.72NS	1.24NS

Table 6. Average mortality in culms of *Dendrocalamus strictus* plants influenced by natural slope gradient and rainwater harvesting devices in degraded hills in Rajasthan. Values are mean± SE of five replications.

Slope	RWH Treatment	Dec 2008 to June 2009	Dec 2009 to June 2010	Dec 2010 to June 2011
S1	Control	0.2±0.1	1.5±0.6	0.4±0.2
	Contour Trench	0.1±0.01	1.5±1.0	0.7±1.3
	Gradonie	0.3±0.2	5.5±2.2	0.9±0.3
	Box Trench	0.4±0.2	3.3±0.8	1.0±0.4
	V-ditch	0.5±0.3	1.3±0.4	2.4±0.7
S2	Control	0.0±0.0	1.7±0.7	1.8±0.3
	Contour Trench	0.1±0.1	1.4±0.5	1.6±0.3
	Gradonie	0.7±0.4	2.4±0.4	2.4±0.4
	Box Trench	0.3±0.2	2.9±1.3	2.5±1.4
	V-ditch	0.7±0.5	0.8±0.7	2.0±0.6
S3	Control	0.2±0.2	0.6±0.3	1.1±0.7
	Contour Trench	0.4±0.2	0.8±0.3	2.3±0.5
	Gradonie	0.5±0.2	2.2±1.2	2.4±0.2
	Box Trench	0.7±0.5	3.2±2.7	0.9±0.5
	V-ditch	0.3±0.2	1.9±0.3	2.0±0.6
Two way ANOVA		F values	F values	F values
Slope (S)		0.25NS	0.84NS	2.54NS
Treatment (T)		0.26NS	2.34NS	0.81NS
S x T		0.69NS	0.50NS	0.72NS

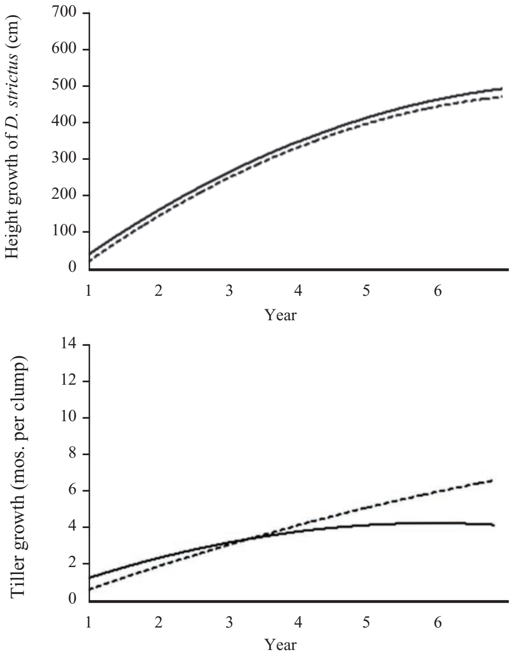

Figure 1. Height (top) and number of tillers (down) growth of *Dendrocalamus strictus* during restoration of degraded hills under rainwater harvesting. Solid line indicates growth pattern in December, whereas dotted lines indicates growth pattern in June.

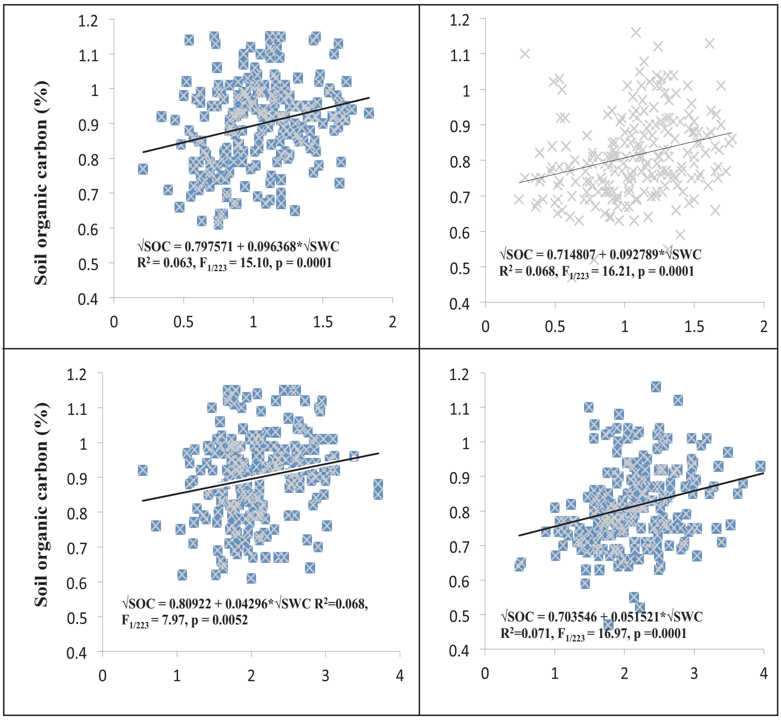

Figure 2. A linear relationship between soil water content (%) and soil organic carbon (%) across the slope, rainwater harvesting structures and years. Descriptions of the indent of both the panels (left and right) are same on the Y-axis.

slope up to December 2009, but outperformed in latter period (June 2010 to June 2011) as compared to that in 10-20% slope (Table 5). Plant height in June 2011 differed significantly ($p < 0.05$), when plants were taller in CT plots and shorter in control than in the other plots. Number of shoots per clump ranged from 2.4 to 4.3 with an average value of 3.5 numbers per clump across the slope and RWH treatments in June 2008. Number of shoots did not vary in June 2011 due to both slopes and RWH treatments. Plant height and number of shoots increased, but the variation in number of shoots was high. Number of shoots was highest in December but decreased under soil water stress towards June (Figure 1). The lowest shoots number was in June 2008 and highest in December 2009 (6.5 shoots per clump), when shoots were highest in GD (7.5 shoots per clump) among RWH treatments and in <10% slope (7.2 shoots per clump) under slope gradient. Shoot mortality was highest during December 2009 to June 2010 (Table 6). The highest shoot mortality was in <10% slope during 2008-09 (0.2 number per clump) and 2010-11 (1.1 number per clump), whereas it was highest in 10-20% slope during 2009-10 (1.8 number per clump). Among the RWH treatments, mortality was lowest in the control plots, whereas the highest mortality was recorded in BT plots in 2008-09, in Gradonie plots in 2009-10 and in VD plots in 2010-11.

Correlations

Rainfall was positively correlated to SWC in June ($r = 0.223$, $p < 0.01$, $n = 225$) in 40-80 cm soil layer) and soil organic carbon ($r = 0.2239$ and 0.210 in 0-40 and 40-80 cm soil layer, respectively, $p < 0.01$). Positive correlations were also observed between soil organic carbon in June and SWC ($r = 0.213$ in 0-40 cm and $r = 0.238$ in 40-80 cm soil layers, $p < 0.01$) in same year (June) and December of previous year ($r = 0.176$ in 0-40 cm and $r = 0.231$ in 40-80 cm soil layers, $p < 0.05$). Soil available PO_4-P was correlated to SWC in June 2009 in both 0-40 ($r = 0.343$, $p < 0.05$) and 40-80 cm ($r = 0.225$, $p < 0.05$) soil layers. Height of *D. strictus* in December 2008 ($r = 0.333$, $p < 0.01$), December 2010 ($r = 0.302$, $p < 0.05$) and June 2011 ($r = 0.301$, $p < 0.05$) showed positive correlation with SWC and number of culm per clump ($r = 0.327$, $p < 0.05$). Number of shoots per clump in June of a year ($r = 0.341$ to 0.849, $p < 0.01$) showed positive relation to number of shoots per clump in the December of previous year. Mortality during December to June showed a negative correlation to SWC in 0-40 cm ($r = -0.279$, $p < 0.05$) and 40-80 cm ($r = -0.247$, $p < 0.05$) soil layers. Height of *D. strictus* followed sigmoid pattern ($R^2 = 0.871$ in December and $R^2 = 0.899$ in June, $p < 0.001$) of growth in both December and June. However, growth in number of culm per clump showed positive power relationships ($R^2 = 0.712$ in December and $R^2 = 0.483$, $p < 0.001$) with age of the plantation (Figure 1). A linear relation was observed between SWC and soil organic carbon both in 0-40 and 40-80 cm soil layers in December as well as in June (Figure 2).

DISCUSSION

Soil water dynamics

Rainfall during monsoon period of June to September enhanced SWC in December; however increase in evaporative demand with increase in temperature towards summer (June) resulted in a decline in SWC by June. Studies (Li *et al.* 2004, Wang *et al.* 2010) indicates that deep-rooted shrubs/trees and shallow-rooted annuals influence water use as well as the spatial distribution of soil water. Extended period of rainfall had greater influence on soil water storage indicated by greater SWC in December 2010 than in December 2009 (Lodge & Brenan 2009). This is indicated by a positive correlation between SWC in December and rainfall ($r = 0.223$, $p < 0.01$). However, soil texture i.e., sand content also influenced SWC shown by the lowest ($p < 0.05$) SWC in 10-20% slope with greater sand fraction as compared to the soils of other slopes. Reduced run-off loss and greater infiltration of water into the soil in <10% slope and greater coarse fraction present on the soil surface restricted surface runoff and facilitated water infiltration during rain enhancing SWC in these slopes (Van Wesemael *et al.* 1996, Katra *et al.* 2008, O'Geen 2012). Spatial heterogeneity in silt and clay content (greater in >20% than in 10-20% slope) also influenced SWC between the slopes (Vogel 2000, Singh 2012) and appeared to be a dominant factor that made the effect of RWH treatments on SWC non-significant in almost 50% observations. However, the effect of BT (December 2008) and CT treatments (June 2010 and 2011) was significant in enhancing SWC in both 0-40 cm and 40-80 cm soil layers. Though absence of RWH affected SWC in control plots, but the effects of growing plants and the companion vegetation also affected SWC. Greater storage capacity of CT and BT structures as compared to GD and VD structures facilitated water storage in deeper soil layer that remained available for a longer period of time as indicated by higher SWC in CT/BT treatments area in June also (James *et al.* 2003). However, highest SWC in VD and GD treatments in December 2009 was the impact of relatively greater rainfall for an extended period, which facilitated distribution of soil water in upper soil layers in these treatments (Singh *et al.* 2010). Soil water depletion due to growing vegetation and plants along with increased evaporative demands resulted in greater decline in SWC in VD/GD treatments as compared to CT/BT treatments. However, soil water depletion is also related to distribution of plants/vegetation roots in different soil layers (Singh *et al.* 1998, Wang *et al.* 2010).

Changes in soil properties

Rainwater harvesting and afforestation improved soil conditions -decrease in soil pH and increase ($p < 0.01$) in SOC, NO_3-N and PO_4-P concentrations. Growing vegetation and turnover of the above- and belowground biomass positively influenced soil improvement (Dabney *et al.* 2001), but inter annual variations in the above-mentioned soil variables were most likely the influence of rainfall and corresponding variations in SWC. This was indicated by decreases in SOC (and some observations of EC) in 2009 and 2011 (with rainfall of 496 mm and 599 mm in 2008 and 2010, respectively) as compared to SOC in 2008 (1153 mm in 2007) and 2010 (859 mm in 2009), respectively. A linear increase in SOC with SWC and positive correlations between soil organic carbon and soil water content ($r = 0.226$, $p < 0.01$) of same month (June) and December of previous year ($r = 0.201$, $p < 0.05$) support this inference. Significant ($p < 0.05$) year x slope interactions for soil pH and SOC in both soil layers and PO_4-P in 0-40 cm and NO_3-N in 40-80 cm soil layer indicated rainfall dependent distribution of these soil variables among the slopes, where accumulation (though run-off) of soil nutrients and dissolved salts were greater in <10% slope. However, concentrations of silt and clay also played an important role in carbon accumulation in >20% slope similar to the observation recorded for grasslands (Fornara & Tilman 2008) and boreal forests (Hollingsworth *et al.* 2008). In the study of Saha *et al.* (2009) the soil particles of <53 µm size accumulated higher amounts of carbon as compared to the 250-2000 µm and 53-250 µm size classes. Effect of RWH treatments was evidenced by greater values of soil pH, EC and NO_3-N concentration in VD treatment and those of SOC, NH_4-N and PO_4-P in CT treatment. The most interesting is the distribution of salts and nutrients indicating greater values ($p < 0.01$) of SOC, NH_4-N, PO_4-P and soil pH in 0-40 cm soil layer and those of NO_3-N and EC in 40-80 cm soil layer. Such differentiation in nutrient distribution was influenced by the water infiltration under RWH and nutrient mobility. It was substantiated by observed reverse trend in NO_3-N (greater concentration in 0-40 cm than in 40-80 cm soil layer) and NH_4-N (greater in 40-80 cm soil layer) concentrations in the control plots as compared to RWH treated plots. Though concentrations of NH_4-N and NO_3-N increases with increase in planting time, NH_4-N concentration remained higher in upper soil layer than the deeper soil layer, whereas NO_3-N concentration increased with increasing soil depth (Liu *et al.* 2010).

Growth of *D. strictus*

Rainwater harvesting and its conservation effects on soil water and nutrients influenced height growth and number of shoots of *D. strictus* positively. Increased availability of soil water and nutrients and favourable environmental conditions during monsoon not only enhanced the number of shoots but the height growth also (Marion & Everett, 2006). Rainfall during June to September/October and corresponding increase in SWC particularly in upper soil layer promoted tillering in *D. strictus* increasing number of shoots as observed by the highest number of shoots in December 2009 (6.5 number per clump). Relatively greater amount of soil water and nutrients in <10% slope as compared to the other slopes was due to spatial redistribution of surface runoff resulting in higher nutrients and soil water availability on lower slope positions that contributed to plant growth (Tsui *et al.* 2004, Yong *et al.* 2006). The increased concentrations of SOC, SWC and NH_4-N in >20% slope in latter period (June 2010 to June 2011) as a result of rainwater harvesting promoted growth of *D. strictus*, which outperformed in >20% as compared to the growth in 10-20% slope. It was similar to the observed responses in tropical tree seedlings towards added nutrients (Santiago *et al.* 2012). Greater silt and clay content and corresponding increase in soil water retention in >20% slope than in 10-20% slope was also responsible for better growth of *D. strictus* in >20% slope. In absence of RWH and consequently less SWC in the control plots was the main factor responsible for the lowest number of shoots and height growth in *D. strictus*. The plants in the CT treatment showed the highest growth variables of *D. strictus* because of greater ($p < 0.05$) SWC in this treatment as height growth of *D. strictus* was positively correlated to SWC in December 2008 ($r = 0.333$, $p < 0.01$), December 2010 ($r = 0.302$, $p < 0.05$) and June 2011 ($r = 0.301$, $p < 0.05$). Despite of significant ($p < 0.05$) increase in number of shoots in December as compared to June same year, increased mortality with increase in soil water stress during December to June resulted in a non-significant difference in number of shoots between June 2011 and June 2008. A negative correlation between number of shoot mortality and SWC ($r = -0.263$, $p < 0.05$) indicates the negative impact of SWC on increasing bamboo productivity and emphasize the importance of increasing the quantity of RWH structure further to meet the water requirement of growing shoots. The highest mortality in 10-20% slope in 2009-10 also suggests to enhance soil water and to reduce the mortality in number of shoots. Interestingly the mortality was greater where number of shoots was greater ($r = 0.341$ to 0.849, $p < 0.01$). For example lowest mortality was in the control plots with the lowest number of shoot, whereas highest mortality was in BT plots in 2008-09, in GD plots in 2009-10 and in VD plots in 2010-11, which had highest number of shoots.

CONCLUSIONS

Harvesting of rainwater help improve soil condition by facilitating vegetation growth and nutrient turnover. However, low rainfall and consequently SWC affected SOC and soil nutrients in June 2009 and 2011 than in June 2008 and 2010, respectively. Effect of RWH was such that it caused a greater accumulation of NO_3-N and dissolved salts in 40-80 cm soil layer (than the accumulation of SOC, NH_4-N and PO_4-P) as compared to 0-40 cm soil layer. V-ditch promoted accumulation/distribution of water, dissolved salts and availability of PO_4-P and NO_3-N in upper soil layer that facilitated enhanced tillering, but faster depletion of soil water towards June leads to mortality in shoots in this treatment. Greater concentrations of soil water, SOC, NH_4-N and PO_4-P in CT treatment promoted height growth and reduced shoot mortality. Natural slopes and available soil influenced soil water storage and soil properties, thus greater soil water and nutrients accumulated in <10% slope from higher slopes through runoff promoted height growth and increased number of shoots in *D. strictus*. The increased SOC, NH_4-N and greater silt and clay content in the soil of >20% slope resulted in enhanced plant growth in >20% slope than in 10-20% slope. Contour trench facilitated deep soil storage of organic carbon (carbon sequestration) and water for its use in growth of *D. strictus* during dry period. However, regular mortality in shoots of *D. strictus* during December to June indicates that there is need to increase quantity of rainwater to promote shoot survival and growth, restore degraded hills and improve local livelihoods.

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Supplementary Table 1. Infiltration rate (mm minute^{-1}) and soil texture in different slope categories in degraded hills in Rajasthan. Values are mean± SE of 16, 15 and 16 replications in <10%, 10-20% and >20% slopes, respectively.

Variables		Slope category		
		<10%	10-20%	>20%
Infiltration (mm min^{-1})		7.65±1.68	12.17±2.35	11.47±2.03
Soil	Silt (%)	8.88±0.46	8.00±0.59	9.71±0.46
	Clay	10.50±0.67	8.33±0.57	10.71±0.67
	Sand	80.25±1.22	83.67±1.13	79.59±1.01

Supplementary Table 2. Soil physico-chemical properties influenced by natural slope gradient, rainwater harvesting devices and soil layers in degraded hills in Rajasthan. Values are mean± SE of five replications.

Slope	Treat	Soil layer	Soil pH				Soil EC ($mScm^{-1}$)				Soil organic carbon			
			2008	2009	2010	2011	2008	2009	2010	2011	2008	2009	2010	2011
S1	Control	0-40	7.14±0.08	6.62±0.11	6.76±0.09	6.36±0.12	0.18±0.03	0.22±0.08	0.21±0.04	0.32±0.09	0.98±0.07	0.89±0.08	0.86±0.05	0.82±0.07
		40-80		6.64±0.09	6.61±0.23	6.49±0.12		0.22±0.01	0.29±0.11	0.38±0.08		0.62±0.16	0.83±0.13	0.65±0.12
	CT	0-40	6.75±0.04	6.74±0.11	6.62±0.18	6.25±0.06	0.21±0.02	0.25±0.02	0.13±0.01	0.22±0.07	1.00±0.10	0.83±0.07	0.88±0.03	0.85±0.02
		40-80		6.61±0.04	6.62±0.18	6.32±0.08		0.23±0.02	0.15±0.02	0.27±0.01		0.68±0.10	0.76±0.13	0.73±0.04
	Gradonie	0-40	6.89±0.05	6.68±0.12	6.40±0.16	6.22±0.13	0.15±0.03	0.22±0.01	0.19±0.05	0.40±0.11	0.84±0.18	0.73±0.17	0.86±0.15	0.79±0.11
		40-80		6.67±0.11	6.54±0.25	6.37±0.08		0.20±0.02	0.14±0.06	0.41±0.09		0.83±0.28	0.83±0.18	0.64±0.10
	BT	0-40	6.89±0.12	6.71±0.22	6.81±0.19	6.51±0.10	0.18±0.02	0.22±0.02	0.27±0.09	0.32±0.08	0.78±0.09	0.73±0.08	0.90±0.08	0.82±0.02
		40-80		6.71±0.22	6.73±0.14	6.53±0.08		0.20±0.03	0.12±0.02	0.26±0.06		0.75±0.21	0.75±0.11	0.74±0.14
	V-ditch	0-40	6.91±0.08	6.37±0.11	6.78±0.21	6.48±0.15	0.24±0.05	0.19±0.09	0.21±0.01	0.36±0.05	0.86±0.08	0.76±0.13	0.86±0.07	0.77±0.10
		40-80		6.28±0.14	6.75±0.12	6.38±0.16		0.20±0.02	0.22±0.04	0.28±0.06		0.79±0.15	0.77±0.13	0.73±0.05
S2	Control	0-40	6.80±0.06	6.10±0.04	6.46±0.22	6.35±0.12	0.14±0.02	0.16±0.01	0.10±0.02	0.26±0.07	0.70±0.06	0.56±0.07	0.73±0.10	0.65±0.22
		40-80		6.10±0.02	6.46±0.26	6.38±0.14		0.16±0.01	0.16±0.05	0.19±0.06		0.35±0.21	0.57±0.17	0.55±0.05
	CT	0-40	6.80±0.11	6.11±0.09	6.45±0.08	6.35±0.05	0.18±0.02	0.19±0.01	0.11±0.02	0.33±0.08	0.61±0.10	0.62±0.14	0.74±0.16	0.79±0.11
		40-80		6.21±0.09	6.43±0.13	6.38±0.07		0.19±0.01	0.12±0.03	0.38±0.05		0.60±0.23	0.78±0.24	0.71±0.07
	Gradonie	0-40	6.74±0.08	6.23±0.13	6.48±0.17	6.39±0.11	0.22±0.04	0.18±0.01	0.16±0.06	0.20±0.05	0.76±0.09	0.55±0.05	0.85±0.10	0.71±0.04
		40-80		6.21±0.08	6.41±0.21	6.44±0.05		0.16±0.01	0.13±0.04	0.16±0.02		0.45±0.12	0.77±0.22	0.52±0.03
	BT	0-40	6.71±0.17	6.61±0.17	6.46±0.21	6.49±0.13	0.22±0.02	0.21±0.02	0.11±0.03	0.21±0.04	0.74±0.04	0.70±0.09	0.93±0.10	0.84±0.07
		40-80		6.75±0.14	6.54±0.17	6.52±0.11		0.20±0.02	0.10±0.02	0.21±0.04		0.52±0.08	0.78±0.13	0.65±0.05
	V-ditch	0-40	7.14±0.20	6.59±0.15	6.60±0.22	6.42±0.13	0.23±0.04	0.13±0.02	0.28±0.10	0.28±0.07	0.87±0.10	0.76±0.11	0.83±0.95	0.85±0.06
		40-80		6.62±0.06	6.44±0.34	6.43±0.08		0.16±0.01	0.38±0.21	0.19±0.04		0.29±0.16	0.53±0.15	0.58±0.05
S3	Control	0-40	7.02±0.05	6.52±0.02	6.39±0.07	6.36±0.09	0.22±0.02	0.17±0.01	0.20±0.06	0.37±0.06	0.86+0.13	0.78±0.16	0.95±0.10	0.90±0.11
		40-80		6.37±0.06	6.54±0.18	6.44±0.11		0.16±0.01	0.13±0.04	0.27±0.08		0.64±0.22	0.68±0.26	0.74±0.11
	CT	0-40	6.88+0.11	6.47±0.18	6.43±0.09	6.33±0.11	0.26±0.03	0.17±0.01	0.10±0.02	0.23±0.05	0.96±0.06	0.89±0.07	1.11±0.10	0.92±0.05
		40-80		6.19±0.15	6.29±0.07	6.34±0.10		0.18±0.02	0.21±0.08	0.27±0.11		0.45±0.06	1.22±0.25	0.75±0.06
	Gradonie	0-40	6.93±0.14	6.70±0.06	6.55±0.11	6.45±0.08	0.21±0.04	0.22±0.01	0.10±0.03	0.25±0.10	0.81±0.08	0.62±0.12	1.08±0.24	0.92±0.13
		40-80		6.62±0.21	6.54±0.15	6.44±0.05		0.22±0.02	0.10±0.04	0.22±0.06		0.71±0.22	1.06±0.33	0.70±0.08
	BT	0-40	7.02±0.03	6.38±0.03	6.34±0.17	6.33±0.05	0.23±0.03	0.24±0.04	0.14±0.03	0.22±0.07	0.81±0.08	0.82±0.15	0.93±0.08	0.94±0.10
		40-80		6.43±0.06	6.35±0.09	6.32±0.11		0.21±0.02	0.31±0.16	0.30±0.08		0.61±0.15	1.13±0.18	0.74±0.08
	V-ditch	0-40	6.89±0.08	7.12±0.08	6.31±0.10	6.38±0.04	0.26±0.03	0.32±0.03	0.33±0.04	0.36±0.08	0.73±0.09	0.78±0.07	1.03±0.04	1.00±0.07
		40-80		6.32±0.06	6.34±0.19	6.37±0.07		0.29±0.02	0.26±0.03	0.30±0.07		0.67±0.13	1.12±0.30	0.79±0.02

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Influence of Environmental Conditions on the DBH of *Guadua angustifolia* Kunth (Poaceae: Bambusoideae) in the Colombian Coffee Region.

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ABSTRACT

The *Guadua angustifolia* Kunth is an American bamboo, native of Central America and northern South America, where it is important in their economies, tourism landscape and environment. The amount of biomass is key to the profitability of agribusiness of this grass, being therefore the height and the diameter of the culms essential parameters in that industry. In this work, 21 sites in the coffee zone of Colombia, considered the best area for growing this plant species, were chosen to measure the diameter of 625 culms at 1.5 m height (DBH) and the results were correlated with the following variables: distance to the surface water, height above sea level, average temperature, relative humidity and average annual rainfall level in the sampled sites. Statistical analysis showed that the DBH is independent of the average annual rainfall level and relative humidity, but showed a slight dependence with temperature, height above sea level. In this paper, the results of the DBH are compared with those of other authors.

Keywords: *Guadua angustifolia* Kunth, Diameter at breast height DBH, weather conditions, forestry, agribusiness.

RESUMEN

La *Guadua angustifolia* Kunth es un bambú americano de gran importancia económica, paisajística y ambiental de Centro América y del norte de Sur América. La cantidad de biomasa es clave para la rentabilidad de la agroindustria de esta gramínea, siendo por lo tanto el diámetro de los culmos el parámetro esencial en esa industria. En este trabajo se tomaron las medidas del diámetro a la altura del pecho (DAP) en 625 Guaduas, pertenecientes a 21 rodales de la zona del eje cafetero de Colombia, considerada la zona óptima para la siembra de esta especie vegetal y se correlacionaron con las variables: distancia al lecho de agua, altura del sitio sobre el nivel del mar, temperatura promedio, humedad relativa promedio y nivel de pluviosidad anual en los rodales muestreados. El análisis estadístico arrojó independencia a las variables distancia al lecho de agua, pluviosidad y humedad relativa, y mostró una ligera dependencia con las variables temperatura y altura sobre el nivel del mar. En este trabajo se comparan los resultados del DAP con los de otros autores.

Palabras Claves: *Guadua angustifolia* Kunth, diámetro a la altura del pecho, condiciones climáticas, silvicultura, agroindustria.

INTRODUCTION

Of the 1,040 species of bamboo that exist in the world, the *Guadua angustifolia* Kunth (*G. angustifolia*), is selected among the 20 that make up “priority species of bamboo”. The *G. angustifolia* for its wide spectrum of

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applications has become one of the world's important bamboos and remains as the most useful of Latin America. It is native of Colombia, Venezuela and Ecuador. This American bamboo is an excellent sustainable resource, self-renewable, of fast growing, versatile, lightweight, flexible, durable and easy to use. These features show that the bamboo is a forest species that offers a wide range of benefits (Cruz, 2009).

The economic importance of *G. angustifolia* is manifested in the construction industries and furniture building. Although it is not a timber, it has huge advantages over timber species such as durability, strength, and lightness. Moreover, it is a crop with low maintenance, regenerates itself, high yields per hectare and exploitation in short periods. It is also used by many farmers to obtain international environmental bonds or certificates of low carbon footprint. We must also highlight the importance of *G. angustifolia* in environmental conservation, because it is considered as protector of soils, contributor of organic matter, erosion controller and host of wildlife. Without forgetting, of course, the cultural and landscape importance of that species.

It should be mentioned that the *G. angustifolia* has a high geographical distribution due to its adaptability to various biophysical conditions, that is, different climates and soil conditions. However, their growth and development is not always equal due to variations of these conditions, even in the same grounds, where there could be good, fair or poor sites for growth of *G. angustifolia* (Castaño y Moreno, 2004).

Although *G. angustifolia* is found in different regions of Colombia, it is in the coffee zone of the Andean region where *G. angustifolia* is best developed and where the larger forests exist. It is, then, within that area in Quindío and North of the department of Valle del Cauca where there are the most exuberant forests of the country and where the prevailing climate and soil conditions are optimal for the development of the species (Cruz, 2009).

Different authors have addressed the issue of optimal environmental conditions for growth in thickness and height of the *G. angustifolia*. Cruz (2009) reports that the *G. angustifolia* in Colombia grows in optimal conditions between 900 and 1,600 meters above sea level, in a range of precipitation of 2,000mm to 2,500 mm / year, relative humidity ranging between 75% and 85% and the optimum range of temperature oscillating between 20°C and 26°C. Camargo *et al.* (2007) carried out detailed zoning of *G. angustifolia* resource in the coffee zone, Tolima and Valle del Cauca and reported the following optimal ranges: for height of 1,200m to 1,500 m above sea level, temperature of 19.5°C to 22.5°C, relative humidity of 75% to 85% and precipitation in a range from 1,800mm to 2,600 mm / year. Castaño and Moreno (2004) report on their work "Guadua for all, its cultivation and use", the optimal ranges of the factors affecting the growth of bamboo, such as: altitude of 600m to 2,000 m; temperature of 20°C to 26°C, precipitation of 1,800mm to 2,500 mm / year and relative humidity of 75% to 85%. Agudelo and Toro (1994) conducted an investigation to determine the limiting factors in the development of *Guadua angustifolia* Kunth, in the mountainous area of the department of Valle del Cauca, reporting that the best development of the species occurred in sites with altitudes between 1,300 and 1,500 meters above sea level, annual rainfall of 1200mm to 1700mm with loam and clay soils. Riaño *et al.* (2002) report that optimum growth is reached between 500m and 1,500 m of altitude over sea level, rainfall of 1,200mm to 2,500 mm per year, temperatures between 18°C and 24°C, and 80% to 90% relative humidity, also in the department of Valle del Cauca.

The round and hollow culm of *G. angustifolia* is a constraint to produce goods for the construction industry and furniture, for example laminate products, where there is much waste of raw materials. (Mahdavi *et al.* 2012, De Flander 2009) According to Held and Manzano, in the Guadua-Bamboo Project in 2004, the diameter of the bamboo is an important quality criterion for the 88.2% of a survey respondent, within the production chain of bamboo. Also, they write that in the building industry, 50% of the bamboo is used like *"esterilla"* (vertical sections of the culm forming a mat), where the diameter is significant (Held and Manzano 2004). Since bamboo can be considered as a good substitute for wood

(Vogtländer J. *et al.* 2010), the authors found the best environmental conditions that have influence on the diameter of the *G. angustifolia*, in order to produce the raw material for the construction and furniture industries, at least in the Colombian coffee region.

MATERIALS AND METHODS

Field of Study

For the development of the present investigation different *G. angustifolia* farms were selected within the *G. angustifolia* optimal growth regions, in terms of altitude above sea level, average temperature, rain precipitation and relative humidity located in the different sites in the department of Quindío, in the municipalities of Buga, and Cartago in the department of Valle del Cauca, and in the municipality of the Virginia in the department of Risaralda, all within the central Andean region of Colombia. These places are considered to have the best conditions for planting the *G. angustifolia*. The working area was limited by the following coordinates: latitude between 3° 52' and 4° 52' and longitude between 75° 36' and 76° 17'.

This study evaluated a total of 21 *G. angustifolia* plantations, located in height above sea level from 920 m to 1,835 m. The 2l “guaduales”, were selected according to the following characteristics: easy access by land, wide differences in the altitudinal range, and the availability and support of the owners of such properties. In each of the stands selected for the development of this study randomly mature culms were chosen, in which we proceeded to measure its circumference at breast height (CBH), and then find the diameter (DBH), using the cylindrical shape of the culm.

Specifically, for each sampling site, it was recorded the following information: location (city, village and farm), sampling date, height above sea level and geographical coordinates using a GPS (Global Positioning System), distance to the surface water using a decameter, when it was available, but when that distance was more than 100 m, the information was provided by the farmer. The average temperature and average relative humidity also was taken during the sampling stage, measured with a hygrometer. However, these data only served to be compared with data tabulated by the various agencies. The average temperature, rain precipitation and relative humidity were obtained through the official web sites of CRQ (2010), GIS Quindío, Cenicafé, Agustín Codazzi Institute of Colombia and from different municipalities where samples were taken, as well as from work performed by Ingeominas (Ruiz, 2004).

Data Processing

The data for each individual sample were entered into arrays and processed statistically through a multiple linear regression model with variable selection, using the statistical package Statgraphics. The analysis was used to determine the relationship between the DBH of *G. angustifolia* and the different environmental variables (humidity, rainfall, temperature and altitude) and the distance to the surface water. Finally, graphs were made using the graphing software OriginPro 7.

RESULTS AND DISCUSSION

The 21 sites were discriminated geographical by location as shown in Table 1. The values of average DBH, distance to the surface water and altitude above sea level were measured at the farm, while the environmental conditions were taken from the various documents of the government agencies.

Figure 1 shows the measured values for all *G. angustifolia’s* DBH at different sampling sites as a function of the distance to the surface water. A linear regression to the data was performed and the results are shown in Table 2. Such regression is plotted in Figure 1. Figure 2 shows the same data but averaged DBH per site and depending on the height above sea level. Table 3 shows the values of mean temperature; mean relative humidity and annual precipitation for each site according to the data found in official documents and published works.

Statistical analysis

In Table 2, the regression coefficient R shows a high dispersion of points. This regression yielded the following fit equation:

$$\text{DBH (cm)} = 11{,}5341 - 0{,}0016{*}D \quad (1)$$

Table 1. DBH Measurements by site

Department	Municipality	Place of sampling	Latitude	Longitude
Quindío	Armenia	Botanical museum "Cedro Rosado"	4° 32 N	75º 40` W
Quindío		Town El Caimo	4° 28` N	75° 41` W
Quindío	Calarcá	Farm "La colonia" (village Calle Larga)	4° 26 N	75° 88 W
Quindío		Farm “El Cedral” (village Quebrada Negra)	4° 26 N	75° 46 W
Quindío		Farm "La sorpresa" (village La Soledad)	4° 25 N	75° 43 W
Quindío	Circasia	Farm “La Siberia” (village La Pola)	4° 35 N	75° 41 W
Quindío	Córdoba	Farm "Petaluma" (village Rio Verde)	4° 24 N	75° 43 W
Quindío	Filandia	Farm “El Portugal” (village Buenavista)	4° 40 N	75° 41 W
Quindío		Rural school (village Buenavista)	4° 40 N	75° 40 W
Quindío		Farm “La Sonora” (village Buenavista,)	4° 40 N	75° 40 W
Quindío	Montenegro	Farm "El Aguacatal" (village Pueblo Rico)	4° 33 N	75° 45 W
Quindío	Quimbaya	Reserve "El Ocaso" (village El Laurel)	4° 34 N	75° 51 W
Quindío		Village la Unión	4° 36 N	75° 44 W
Quindío		Farm “La Mejorana” (village la Unión)	4° 36 N	75° 44 W
Quindío		Farm “Santa Anita” (village Santa Ana)	4° 36 N	75° 54 W
Quindío	Salento	Farm "La Linda" (village Canaán)	4° 36 N	75° 36 W
Quindío		Farm “las Brisas” (village Palestina)	4° 37 N	75° 36 W
Quindío		Agricultural institute (village San Juan)	4° 34 N	75° 38 W
Risaralda	La Virginia	Farm "Villa Eliza"	4° 52 N	75° 55 W
Valle	Buga	Farm “Vértigo” (Quebradaseca)	3° 52 N	76° 17 W
Valle	Cartago	El Prado (city limits)	4° 45 N	75° 55 W

The slope of the linear equation (1) is very close to zero, which means that there is only a very slight dependence of DBH with distance (D) from the point where the water is. The high dispersion of points does not allow us to state that Equation (1) is a valid equation for all cases.

We performed statistical analysis of the data using multiple regressions with the program Statgraphics Plus 5.0, in which the dependent variable was DBH and the independent variables were: distance to the surface water, height above sea level, average temperature, relative humidity and average annual rainfall level in the sampled sites. Results are shown in Table 4, in which only those values where p-value is less than 0.05, are shown. Since the p-value in the ANOVA table is less than 0,05, there is a statistically significant relationship between the variables at the 95,0% confidence level. From this analysis, it was concluded that the DBH is independent of the following variables: distance to the surface water, relative humidity and annual rainfall, while possessing dependence with the temperature (T) and the height above sea level (H), as follows:

$$\text{DBH (cm)} = 0.2924*T + 0.0040*H \quad (2)$$

The R-Squared statistic indicates that the model as fitted explains 94,7487 % of the variability in DBH.

This equation is understood, to be valid only in the temperature range between 18°C and 27°C and in the range of heights above sea level between 920 m and 1,835 m. To determine the influence of extreme values on the equation, an analysis of standardized skewness and kurtosis was performed, also to determine if the sample obeys a normal distribution. Values of these statistics outside the range of -2 to +2 indicate significant departures from normality, which tends to nulll the statistics of the standard deviation. However, in all cases, the values of standardized skewness and kurtosis are within the expected range for normally distributed data.

As an exercise to check the validity of the equation (2), it was applied to the data reported by other authors. Table 5 presents the results and their comparison with our measured data on similar sites of temperature and height above sea level. In the case of Camargo *et al.* (2007), errors were calculated using equation

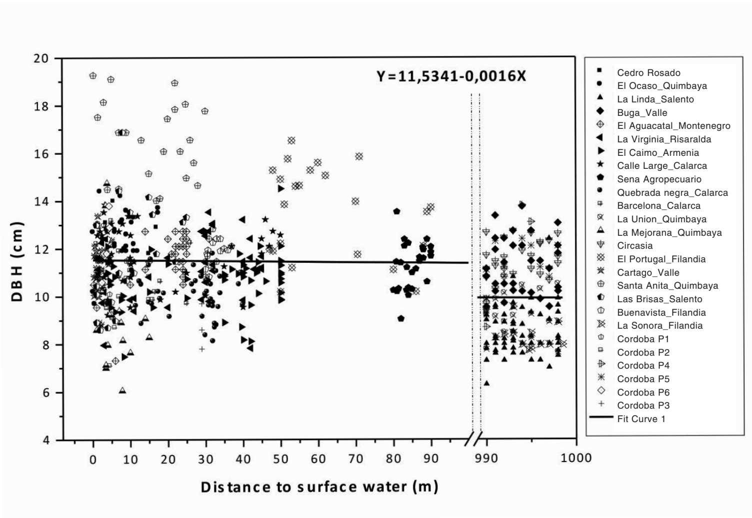

Figure 1. DBH values for all *G. angustifolia* measures depending on the distance to the surface water. Different symbols correspond to different sampling sites. Solid line corresponds to a fit with a linear regression.

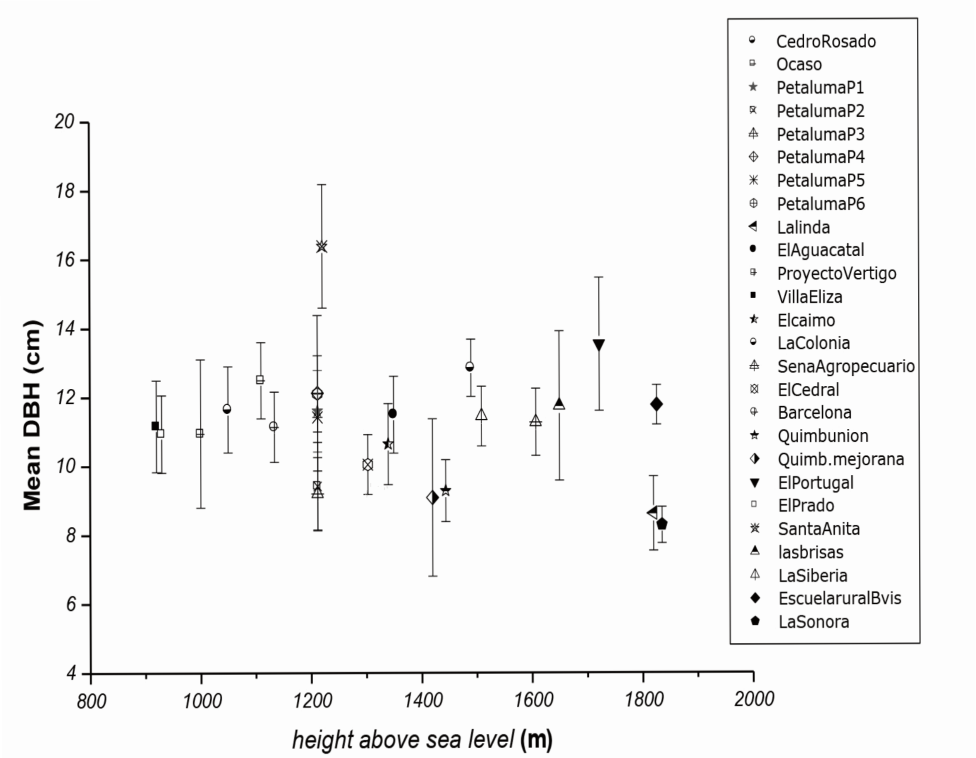

Figure 2. Average values of DBH, with its error bar (standard deviation) for different sampling sites depending on the height above sea level of the site.

Table 2. Linear regression to the data of Figure 1.

Equation	y = a + b*x	Value	Standard Error
a	Intercept	11,5341	0,09103
b	Slope	-0,0016	1,78045E-4

Adj. R-Square=0,1171

Table 3. Environmental data of the sample sites, from other sources.

Site	Mean temperature (C)	Annual precipitation (mm/año)	mean relative humidity %
Muesum Cedro Rosado	20[a]	3,467.8[a]	70[g]
Reserve El Ocaso	23.5[b]	1,850.0[b]	76[g]
Farm Petaluma	25[a]	2,273.1[a]	85[i]
Farm La Linda	18[c]	3,628.1[a]	82.8[i]
Farm El Aguacatal	21.5[b]	2,500.0[b]	79[c]
Buga	24[d]	1,458.0[d]	70[d]
Farm Villa Eliza	27[e]	1,750.0[e]	73[e]
El Caimo	21[c]	2,050.0[b]	76[f]
Farm La Colonia	22.5[b]	2,050.0[b]	76[f]
Agricultural institute	19.5[b]	3,100.0[b]	82.8[i]
Farm El Cedral	21[c]	2,350.0[f]	85[i]
Farm La Sorpresa	21[f]	2,171.0[f]	76[f]
Village La Unión	21[c]	2,050.0[b]	79.3[f]
Farm La Mejorana	21[c]	2,050.0[b]	79.3[f]
Farm La Siberia	20.5[b]	2,800.0[b]	86[i]
Farm El Portugal	20[f]	2,460.0[f]	79.3[f]
Farm Santa Anita	21[c]	2,050.0[b]	79.3[f]
Farm Las Brisas	19.5[b]	3,100.0[b]	82.8[i]
Cartago	24[h]	1,300.0[h]	70[h]
Rural School Buenavista	20.1[f]	2,460.0[f]	79.3[f]
Farm La Sonora	20.1[f]	2,460.0[f]	79.3[f]

[a] Meteorological data Autonomous Regional Corporation of Quindío (pers. comm.)
[b] Ingeominas
[c] www. serviciaf.igac.gov.co
[d] www.guadalajaradebuga-valle.gov.co
[e] www.lavirginia-risaralda.gov.co
[f] www.cenicafe.org
[g] www.uniquindio.edu.co
[h] www.valledelcauca.gov.co
[i] www.crq.gov.co/news/251-crq-entrega-i

Table 4. Results of multiple regression with the dependent variable DBH and the independent variables: distance to the surface water, height above sea level, average temperature, relative humidity and average annual rainfall level.

Parameter	Standard Estimate	T Error	Statistic	P-Value
Temperature	0,2924	0,0647	4,5156	0,0000
Height above sea level	0,0040	0,0010	3,7614	0,0005

Adj. R-Square=0,1171

Table 5. Comparison between DBH data reported by several authors, calculated with our equation (2) for those authors and those obtained by us under similar conditions of average temperature and altitude.

Reference	Site	Temp Altitude	DBH ref (cm)	DBH ref with equation (2) (cm)	DFBH Our values (cm)	Our Site	Temp/ Altitude
Camargo *et al.* 2007	Risaralda	26°C 900 m	11.6	11.20 ± 1.11	11.16 ± 1.33	La Virginia Risaralda	27°C 920 m
Camargo *et al.* 2007	Armenia	19.5°C 1,500 m	11.1	11.7 ± 1.04	12.85 ± 0.83	Cedro Rosado Armenia	20°C 1,490 m
Camargo *et al.* 2007	Armenia	22.5°C 1,200 m	11.1	11.38 ± 1.04	10.64 ± 1.18	El Caimo Armenia	21°C 1,340 m
Camargo *et al.* 2007	Quimbaya	22.5°C 1,500 m	11.1	12.58 ± 1.04	9.28 ± 0.90	La Unión Quimbaya	21°C 1,444 m
Camargo *et al.* 2007	Quimbaya	22.5°C 1,500 m	11.1	12.58 ± 1.04	9.09 ± 2.28	La Mejorana Quimbaya	21°C 1,420 m
Camargo *et al.* 2007	Quimbaya	22.5°C 1,500 m	11.1	12.58 ± 1.04	16.38 ± 1.79	Santa Anita Quimbaya	21°C 1,339 m
Camargo *et al.* 2007	Montenegro	22.5°C 1,200 m	11.1	11.38 ± 1.04	11.49 ± 1.11	El Aguacatal Montenegro	21,5°C 1,350 m
Camargo *et al.* 2007	Quindío	22.5°C 1.200 m	10.7	11.38 ± 2.31	12.49 ± 1.11	El Ocaso Quimbaya	23,5°C 1,110 m
Perea *et al.* 2003	San Agustín	18°C [a]1,730 m	12.18	12.18	13.53 ± 1.93	El Portugal Filandia	20°C 1,722m
Perea *et al.* 2003	Pitalito	20°C [b]1,318 m	12.11	11.12	10.05 ± 0.87	El Cedral Calarcá	21°C 1,303 m
Perea *et al.* 2003	La Plata	23°C [c]1,050 m	10.96	10.92	11.64 ± 1.25	La Colonia Calarcá	22,5°C 1,050 m

[a] www.sanagustin-huila.gov.co

[b] www.pitalito-huila.gov.co

[c] www.laplata-huila.gov.co

*errors were calculated between the maximum and the minimum of temperature and height above sea level

(2) for heights above sea level and the maximum and minimum temperatures given by the authors, per site. The error was calculated by the difference of DBH for these two temperatures. For comparison, it also added the mean value of DBH obtained experimentally by the authors. In the case of Perea *et al.* (2003), the data of average temperature of the sites were found on the web pages referenced below. We can conclude that within the margins of error, the equation works quite well.

Authors such as Castaño and Moreno (2004) argue that the factors to be evaluated should be temperature and rainfall, due to the strong influence of these two factors on the development and growth of bamboo. However, for us the rainfall within the working range of 1,300-3,600 mm / year, does not influence the DBH.

Von & Fu (2001), pointed out that in mosso bamboo, the most important factors for growth in height and diameter of bamboo are the availability of water, sunlight and soil nutrients. While for us, the DBH is not dependent on the proximity to the source of water or rainfall, but yet we interpret that groundwater in the soil is important for the growth of *G. angustifolia*. Sunlight and soil nutrients were not analyzed in this investigation, but yet, as we work only in the region of optimal growth of *G. angustifolia*, we can say that due to the geographical location of the coffee region in the tropics and the characteristic of volcanic soils, it ensures good water availability, adequate solar incidence and a land rich in nutrients.

Camargo (2006) mentions that although several aspects such as productivity of the stands, the behavior of *G. angustifolia* in different environments and the interactions between management and environmental factors, such as soil and climate, have not been completely defined, we can say that even now, not knowing the complete picture of all factors that influence the growth and development of *G. angustifolia* in terms of height and DBH, this work contributes to add valuable information in the study of the variable DBH.

CONCLUSIONS

It is concluded that *Guadua angustifolia's* DBH is independent of the following variables: distance from the source of water, relative humidity and annual precipitation but dependent of the temperature and the height above sea level, as it is indicated by the equation: DBH (cm) = $0.2924*T + 0.0040*H$, valid at least in the range of heights above sea level between 920 m and 1,835 m and temperatures between 18°C and 27°C. The average DBH for the 21 sites evaluated in this work was 11.23 cm.

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Assessing the Effectiveness of Neem Seed Oil-Treatment for Split-Bamboo (*Bambusa vulgaris*) against *Pycnoporus sanguineus* Based on Tensile Strength Properties

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ABSTRACT

Information on tensile strength properties of bamboo culms is limited in literature. These mechanical properties for treated bamboo culms, particularly with organic preservatives, are not readily available, hence this study was conducted to contribute to efforts aimed at increasing data in this regard by assessing the effectiveness of mechanically extracted neem (*Azadirachta indica* A. Juss) seed oil as a preservative for split-bamboo (*Bambusa vulgaris* Schrad. ex J.C. Wendl.) against a basidiomycete known as *Pycnoporus sanguineus* (L. ex Fr.) Murr. based on these mechanical properties. Harvested bamboo stems were converted to test samples in conformity with modified ASTM D-143 and treated by soaking two sets differently in the oil at an ambient room temperature of 25 ± 2°C for 24 hours (A) and 60°C for 4 hours (B) with samples not treated with the oil as control. The oil-treated (soaked and heat treated) and those that were not oil-treated (control samples) were all inoculated with cultured *P. sanguineus*, incubated for 12 weeks and then subjected to tensile strength tests. Results obtained showed that Modulus of Rupture was lower for control (117.70 ± 1.01 Nmm^{-2}) but higher for A (139.79 ± 1.20 Nmm^{-2}) and B (126.11 ± 0.91 Nmm^{-2}). However, Modulus of Elasticity was higher for control (2,425.94 ± 22.11 Nmm^{-2}) and lower for A (1,821.74 ± 26.18 Nmm^{-2}) and B (1,886.59 ± 19.09 Nmm^{-2}). Mean values for Energy at Maximum Load, Load at Yield and Extension at Yield respectively roughly followed the trend obtained for MOR. The data obtained were statistically analysed using basic descriptive tools, ANOVA and Fishers' Least Significant Difference as a follow-up test to compare means ($P < 0.05$).

Keywords: Bamboo, Neem, Vegetable oil, Basidiomycetes, Mechanical properties, Tensile strength

INTRODUCTION

It is a well known fact that some wood and other lignocellulosic materials have little or no inherent natural durability against agents of biodegradation such as certain insects, micro-organisms, among others. This poor natural durability is the main reason why this category of lignocellulosic materials is susceptible to easy destruction by these biodeterioration agents when left unprotected. Consequently, these materials are protected from these agents of biodeterioration by using various means such as treating and preserving them with chemical preservatives in such ways as to increase and/or enhance their service life, among other reasons.

Stems/culms of bamboos, long identified for multi-purpose uses, have been noted to belong to the class of lignocellulose that requires preservatives for protection against easy destruction by biological agents. These stems or culms, like many other lignocellulosic materials, are used for different purposes by a wide range of end users based on series of application motives. One of such reasons for employing bamboo stems in some applications is their excellent strength/mechanical properties. For instance, many of the bamboo species possess culms with high tensile strength properties (Kumar *et al.*, 1994; Ghavami, 2005; Erakhrumen, 2009; 2012a), with some of them noted to possess tensile strength similar to that of steel in some cases (Ghavami, 2005).

However, when harvested bamboo stems without protection are attacked by agents of biodeterioration, some of their properties that are negatively affected are the strength/mechanical properties (INBAR, 1998; Erakhrumen, 2012a; 2012b), among which are the tensile strength and associated properties, partly because when sclerenchyma fibres that are mainly responsible for these strength properties in bamboos are attacked by micro-organisms, such as fungi, strength/mechanical properties have been observed to reduce considerably. Nevertheless, protecting lignocellulosic materials such as bamboo from easy destruction by these biological agents using synthetic chemical preservatives are increasingly receiving criticisms worldwide (Erakhrumen, 2012a; 2012b).

The core arguments against most synthetically-produced preservatives worldwide are mainly linked with their observed negative impacts on the environment and life forms (Onuorah, 2000; Cooper, 2001; Hale, 2003) coupled with the concerns for their cost implications as this is relatively increasing particularly in the developing countries such as Nigeria (Erakhrumen, 2012a; 2012b; 2012c). There is therefore the need for sustainable alternative means of realising the objective of protecting bamboo against agents of biodeterioration in cost effective and environmentally benign ways. One of such ways that has been suggested is the use of plant derived extracts.

An example of such extracts is vegetable oil that contains substances that are innocuous to the environment and life forms but noted to be useful in controlling these agents of biodegradation (Erakhrumen, 2012b; 2012c). For instance, researches have shown that oil from neem (*Azadirachta indica* A. Juss) seed have some anti-microbial properties (Parveen and Alam, 1993; Locke, 1995; Puri, 1999). These earlier reported studies concerning neem seed oil (NSO) collectively served as a basis for studies such as Erakhrumen, (2011). This study by Erakhrumen, (2011) was particularly carried out to evaluate some physical and chemical properties of mechanically extracted NSO, in line with its likely potentials for treating and preserving lignocellulosic materials.

Nevertheless, up to the present, these reported anti-microbial properties of NSO have not been applied/exploited in treating/protecting/preserving bamboo stems/culms against biodegradation in Nigeria and West Africa in general (Erakhrumen, 2012b). In line with the reported anti-microbial properties of NSO, this study was therefore carried out to assess the effectiveness of this oil in treating the most prevalent bamboo species in south-western Nigeria known as *Bambusa vulgaris* Schrad. ex J.C. Wendl. against a fungus, also a basidiomycete, known as *Pycnoporus sanguineus* (L. ex Fr.) Murr.

Tensile strength parallel to the longitudinal axis, (i.e., the maximum tensile stress sustained in the direction parallel to the axial plane) and other associated properties, obtained for split samples of this bamboo species were used for the assessment of the effectiveness of this oil. It is noteworthy that tensile strength is a strength/mechanical property of most wood and other lignocellulosic materials with relatively few data in literature (Green *et al.*, 1999). This property has also been observed to be less investigated for bamboos' stems/culms (Erakhrumen, 2009).

MATERIALS AND METHODS

Sourcing of bamboo culms

The bamboo culms that were converted and experimented upon in this study were wildly sourced from Isale-Togun Forest, Lanlate, Ibarapa, Oyo State, Nigeria (latitude 7° 36' N and longitude 3°27' E) in the month of October, 2008. This area is located in between the humid and sub-humid tropical climate zones. The mean annual rainfall ranges from 1,117.10 to 1,693.30 mm. The harvested matured culms had no known age or history of management. It is on record that no quantitative parameters have been presently established to identify the different growth stages of a bamboo culm for adequate harvesting purposes (Londoño *et al.*, 2002).

In order to ensure minimal influence of age, lack of management, and other variables on the results from the research, only matured culms with mean circumferential length of 300 mm at the second node above ground were harvested and cross cut in such a way that only the basal culm portion of 3,000 mm length were removed and placed in jute bags with nylon lined inner surface to avoid contamination from the soil. All the harvested culms in the bags were transported to and stored for 14 days in the wood workshop of the Department of Forest Resources Management, University of Ibadan, Ibadan, Nigeria, for conversion to the test specimens in compliance with the different test standards.

Sourcing of neem seeds

The ripe neem seeds from which oil was mechanically extracted in this study were obtained from *A. indica* trees on the University of Ibadan campus located on the northern edge of the city of Ibadan, Nigeria (latitude 7° 20' N and longitude 3° 50' E) of about 10.4 square kilometres. Ibadan lies at 200 m above sea level with a humid tropical climate (27°C average), a March – October rainy season (1,250 mm) followed by a mild dry season. Collection of the seeds was done by placing clean nylon sheets around the stems of *A. indica* trees in such a way that it covered a substantial cross sectional area of the crown in order to directly collect the seeds as they fall.

The neem seeds were sourced in the months of June to early August of 2008. The seeds obtained were thoroughly washed using deionised water to remove dirt and other impurities and then air dried in an open space with regular movement for aeration to ensure proper drying, a method also applied by Soetaredjo *et al.*, (2008), to reduce the moisture content (MC) for proper crushing and to facilitate high oil volume recovery during mechanical extraction. The seeds were stored in a nylon lined jute bag and kept away from the reach of organisms such as rodents and other animals that can consume the seeds and also to prevent contamination, and daily air dried with proper monitoring to prevent damage as a result of possible moisture fluctuations.

Conversion of bamboo culms to test samples

The harvested bamboo culms were carefully sawn in the longitudinal direction with circular and vertical breakdown sawing machine into strips. Each strip was planed on both the inner and outer surface, using a planing machine, in order to obtain the bamboo timber with mean thickness of 5 ± 0.5 mm for the tests. Bamboo timber, according to Chand *et al.*, (2006) is the part between the bamboo skin and the pith. Bamboo skin is the outermost part of cross-section of stem wall, where no vascular bundles are seen, while pith is the part of stem wall next to bamboo cavity, and this also does not contain vascular bundles (Chand *et al.*, 2006).

The strips were first conditioned in the laboratory for 14 days and then oven-dried at 103 ± 2°C to constant weight. They were removed from the oven afterwards, allowed to cool in a dessicator to the ambient temperature and stabilised in the laboratory for 24 hours. The mean MC of split-bamboo samples after stabilisation was 11.76% prior to NSO-treatment. They were then converted to test specimens with dimension 25 mm (tangentially) x 200 mm (longitudinally) x 5 mm (radially), as shown in Figure 1, in conformity with slightly modified ASTM D 143-94 owing to the nature of this bamboo species.

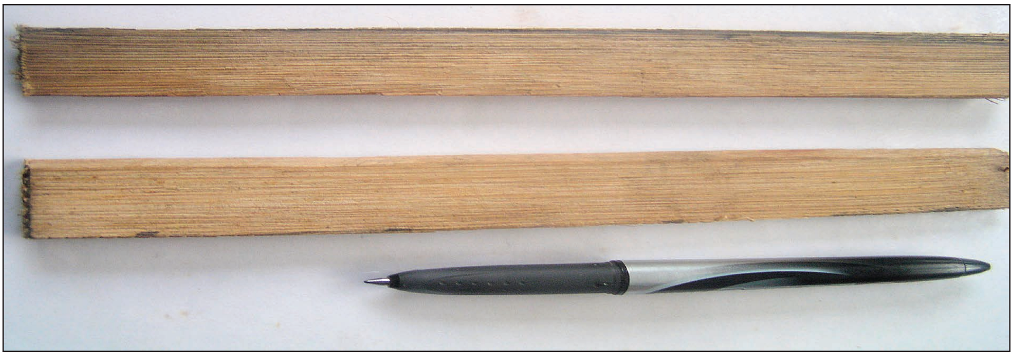

Figure 1: Pictorial representation of split-bamboo samples for testing tensile strength properties

This sample dimension was used in conducting this experiment because it was impossible to obtain *B. vulgaris* with culm thickness, in the radial plane, of up to or more than 25 mm irrespective of age and position along the culm. It is also noteworthy that presently there are still various methods of determining many of the physical and mechanical properties of bamboo stems (Kumar *et al.*, 1994; Erakhrumen and Ogunsanwo, 2009). For instance, mechanical properties of bamboo have been investigated either with full size specimens (Espiloy *et al.*, 1986; Shukla *et al.*, 1988; Sattar *et al.*, 1994) or small size/ split-bamboo specimens (Abd.Latif *et al.*, 1991; Lee *et al.*, 1994; Wahab *et al.*, 2006).

Moisture content determination for split-bamboo samples

Test specimens of dimensions 20 mm (tangentially) x 60 mm (longitudinally) x 5 mm (radially) were used to determine MC based on oven-dry method in accordance with AST

$$MC = [(W_m - W_o)(W_o)-1] \times 100 \qquad [1]$$

Where: MC = moisture content; W_m = weight of split-bamboo specimens before oven-drying (kg); and W_o = weight of split-bamboo specimens after oven-drying to constant weight (kg).

Mechanical extraction of neem seed oil

There are several methods of extracting oil from different seeds including those of neem, such as mechanical pressing, supercritical fluid extraction, solvent extraction, among others (Puri, 1999). Mechanical extraction is the most widely used method to extract oil from neem seeds (Fasina and Ajibola, 1989; Puri, 1999), since this method is effective for seeds containing 30 to 70% oil (Ketaren, 1986). However, the oil obtained with this method may not be highly priced, since it is considered turbid and contains a significant amount of water as compared to those obtained by supercritical fluid extraction and solvent extraction (Liauw *et al.*, 2008).

In order to mechanically extract NSO, the seeds obtained from the wild were decorticated, separating the kernel from the shells and dirt, and then air-dried. Dried kernels were carefully pulverised into smaller particles using a seed grinder ensuring no significant loss of seeds' oil. Mechanical extraction of oil was performed by cold pressing the pulverised seeds using an oil expeller at a maximum pressure of 31.03 MPa (31.03 Nmm^{-2} or 4500 psi). Mechanical extraction was performed at this pressure until the oil stopped flowing.

This method of oil extraction is expected to allow for easy adoption by most of the target end-users such as the potential and existing cottage and small-scale forest industries interested in adding value to bamboo resource particularly in the rural areas of developing countries like Nigeria where the most investment is needed and the generation of employment is the most difficult (FAO, 2001). These rural areas in the developing countries are where people living below poverty line are concentrated (Canagarajah, 1998). The mechanical oil extraction have several advantages compared to the other methods, such as simple equipment and low investment, low operating cost, and that the oil does not undergo a solvent separation process, among others (Fasina and Ajibola, 1989).

Treatment of split-bamboo samples with the extracted neem seed oil

The split-bamboo samples to be treated with NSO and those for control were earlier stabilised in the laboratory to MC of 11.76%. The stabilised samples to be oil-treated were subjected to two NSO-treatment regimes through (1) soaking a set of samples in oil at ambient room temperature of 25 ± 2°C for 24 hours and (2) soaking another set in hot oil at 60°C for 4 hours, removed from the oil afterwards and allowed to cool in a dessicator at an ambient room temperature of 25 ± 2°C.

These methods of NSO-treatments were also adopted in the experiments reported by Erakhrumen, (2009), Erakhrumen and Ogunsanwo, (2009), Erakhrumen and Ogunsanwo, (2010), Erakhrumen, (2012a), Erakhrumen, (2012b) and Erakhrumen, (2012c). The maximum heat treatment temperature of 60°C was adopted in this research because strength and stiffness values used in practice for most lignocellulosic materials are valid for temperatures below 60°C (Homan and Jorissen, 2004).

Sourcing, identification, and culturing of fungal isolates

Knowledge of bamboo fungi is limited and it is mostly in recent years that mycologists have catalogued fungi on bamboo. More than 1,100 species of fungi have thus, been described or recorded world-wide from bamboo and these include *ca.* 630 ascomycetes, 150 basidiomycetes and 330 mitosporic taxa (100 coelomycetes and 230 hyphomycetes). Most of the fungal species are saprobes found on decaying bamboo culms, although, pathogens and endophytes have also been recorded in literature (Hyde *et al.*, 2002).

In order to obtain fungal species that attack *B. vulgaris*, a decaying culm of this bamboo species found on the forest floor where the bamboo samples for this research were sourced was obtained and taken to the Laboratory of the Department of Forest Resources Management, University of Ibadan, Ibadan, Nigeria, where samples of the microbes present were obtained and later identified at the Pathology Laboratory of the Department of Botany and Microbiology, University of Ibadan, Ibadan, Nigeria, as predominantly a white rot fungus known as *Pycnoporus sanguineus* (L. ex Fr.) Murr. This fungus has been observed to attack many bamboo species including *B. vulgaris* (Suprapti, 2010).

A nutrient medium of potato dextrose agar (PDA) in deionised water was prepared in line with the method adopted by Erakhrumen, (2012b). First, 35 grammes of the PDA was mixed with 1 litre of water in conical flask and then homogenised. After homogenising, 4cl of the PDA was poured into McCartney bottles and sterilised by autoclaving at a pressure and temperature of 0.1 N mm^{-2} and 120°C respectively for a period of 20 minutes. The culture bottles in which the test fungus was eventually cultured was first completely sterilised and the prepared PDA was poured into it and covered with an air tight non-infected lid in preparatory for fungal inoculation.

The white rot fungus that was obtained from the decaying culm was first cultured and again sub-cultured, in order to obtain pure culture, in sterilised Petri dishes on PDA until the nutrient was completely covered by the mycelium of the cultured fungal species. The prepared PDA in the culture bottles were then inoculated with the pure culture of the fungal species and also allowed to be completely covered by the mycelium of the pure cultured fungus. Studies have shown that white rot fungi are generally more virulent than brown rot fungi, for instance, in hard wood species (Eaton and Hale, 1993). This observation was also made concerning some bamboo specimens in a study by Leithoff and Peek, (2001).

Infection of split-bamboo samples with fungal isolates

The oil-treated split-bamboo samples i.e., soaked and heat treated with dimensions 25 mm (tangentially) x 200 mm (longitudinally) x 5 mm (radially) to be infected with the fungal species were sterilised by wrapping them in aluminium foil and placed in an autoclave at a pressure and temperature of 103°C and 0.1Nmm^{-2} respectively for 10 minutes and then placed aseptically into the culture medium in chemically clean amber glass culture bottles with dimension 300 mm (length) x 50 mm (breadth) x 200 mm (height) in which there were cultures of actively growing test fungal species. All the split-bamboo samples to be inoculated with the cultured fungal species of *P. sanguineus* in the laboratory test were placed such that they came in contact with the aerial mycelium of the fungus and not the culture medium itself.

The culture bottles were then properly covered with Teflon lined lids also made of glass ware to prevent external contamination particularly from the surrounding air. Ten split-bamboo test samples for each treatment (soaked and heat treated) and those not treated with oil (control) were so infected. The control test samples were first wrapped in aluminium foil and sterilised in the oven with a temperature of 103 ± 2°C for 2 hours and allowed to cool in a dessicator before being introduced to the test fungus. The bottles together with their contents were left in an incubating room with ambient temperature of 25 ± 2°C and relative humidity of 65 ± 5% and monitored for twelve weeks (84 days) in line with slight modifications to methods described by Leithoff and Peek, (2001) and Luna *et al.*, (2004).

Evaluation of tensile strength and other related properties for split-bamboo

The tests for tensile strength and associated properties were carried out using split-bamboo test samples with specimens dimension of 25 mm (tangentially) x 200 mm (longitudinally) x 5 mm (radially). The oil-treated and control specimens were subjected to these tests after they have all been exposed to isolates of *P. sanguineus* for 12 weeks. The tests for tensile strength and associated properties along the specimens' longitudinal axis were carried out using a computer controlled Instron® 3363 Universal Testing Machine (UTM) at a constant cross-head speed of 4.00 mm min^{-1}, introducing the tension load to the longitudinal plane of the test samples with their fibres assumed to be orientated parallel to the longitudinal axis.

Most bamboo cells are axially orientated at the internodes (Liese, 1985). Cellulose fibres are aligned along the length of the bamboo providing maximum tensile flexural strength and rigidity in that direction (Lakkad and Patel, 1980). In addition, this assumed fibre orientation is also based on the knowledge that bamboo is much simpler constructed than wood with their anatomical construction appearing rather uniform making the differences among the about 1,200 species appear relatively small (Liese, 1992; Liese, 1998; Liese, 2003). The UTM used for the tests was located at the Material Testing Laboratory of the Centre for Energy Research and Development, Obafemi Awolowo University, Ile-Ife, Nigeria.

Statistical analyses

The data obtained for tensile strength and other associated properties evaluated for both the oil-treated and control split-bamboo samples after exposure to fungal attack were subjected to basic descriptive statistical analyses such as mean and standard deviation. Analyses of variance (ANOVA), was employed in analysing the data for statistical significant variation ($P < 0.05$) while Fishers' Least Significant Difference (LSD) was applied as a follow-up test to compare means ($P < 0.05$).

RESULTS AND DISCUSSION

The values obtained for tensile strength and associated properties, shown in Table 1, revealed that mean value for MOR obtained for control samples was 117.70 Nmm^{-2} while they were 139.79 Nmm^{-2} and 126.11 Nmm^{-2} for samples soaked in oil at ambient room temperature of 25 ± 2°C for 24 hours and samples soaked in hot oil at 60°C for 4 hours respectively. The mean values obtained for MOE were 2,425.94 Nmm^{-2} for control, 1,886.59 Nmm^{-2} for samples soaked in hot oil at 60°C for 4 hours and 1,821.74 Nmm^{-2} for samples soaked in oil at ambient room temperature of 25 ± 2°C for 24 hours.

The tabulated mean values in Table 1 showed that MOR was lower for control samples than those for samples treated with NSO at the two temperature regimes. Mean value for MOR was highest for samples soaked in oil at ambient room temperature of 25 ± 2°C for 24 hours followed by that for samples soaked in hot oil at 60°C for 4 hours. Conversely, the pattern

Table 1. Mean values obtained for selected tensile strength and other related properties for the oil-treated and control split-bamboo samples subjected to fungal attack

Treatment	MOR (Nmm^{-2})	MOE (Nmm^{-2})	Energy at Maximum Load (kJ)	Load at Maximum (Zero Slope, N)	Extenssion at Yield (Zero Slope, mm)
Control	117.70 ± 1.01	2,425.94 ± 22.11	0.12 ± 0.01	2,1211 ± 102.74	7.63 ± 1.11
Samples soaked in oil at ambient room temperature of 25 ± 2°C for 24 hours	139.79 ± 1.20	1,821.74 ± 26.18	0.13 ± 0.01	2,3265 ± 93.09	8.41 ± 1.10
Samples soaked in hot oil at 60°C for 4 hours	126.11 ± 0.91	1,886.59 ± 19.09	0.13 ± 0.01	2,2011 ± 97.77	9.02 ± 1.13

Values are means for 10 test samples per treatment

Table 2. Summary of ANOVA results for data obtained for the selected mechanical properties evaluated for treated and control split-bamboo samples

Source of variation	Selected properties	(F-cal)	(F-tab)
Treatment	MOR	1190.21*	3.35
	MOE	446.99*	
	Energy at Maximum Load	2.71ns	
	Load at Yield	2115.07*	
	Extension at Yield	1106.17*	

*denotes significance, ns denotes not significant ($p < 0.05$)

was reversed in the mean value for MOE as control samples had mean value that was higher followed by that for samples soaked in hot oil at 60°C for 4 hours while samples soaked in oil at ambient room temperature of 25 ± 2°C for 24 hours had the lowest MOE mean value.

Mean value for Energy at Maximum Load was slightly lower for control samples (0.12 kJ) but was the same for samples soaked in oil at ambient room temperature of 25 ± 2°C for 24 hours (0.13 kJ) and samples soaked in hot oil at 60°C for 4 hours (0.13 kJ). Load at Yield was also lower for control samples (2, 1211 N) compared with values obtained for samples soaked in oil at ambient room temperature of 25 ± 2°C for 24 hours (2, 3265 N) and samples soaked in hot oil at 60°C for 4 hours (2, 2011 N). Extension at Yield was also lower for control samples (7.63 mm) in comparison to values obtained for samples soaked in oil at ambient room temperature of 25 ± 2°C for 24 hours (8.41 mm) and samples soaked in hot oil at 60°C for 4 hours (9.02 mm).

Result of ANOVA (Table 2) revealed that variations in the evaluated data for all the properties except Energy at Maximum Load for tensile strength were statistically significant. Fisher's LSD (Table 3) revealed that the mean values for MOR were significantly different for all the treatment regimes. Table 3 also showed that mean values for MOE for samples soaked in oil at ambient room temperature of 25 ± 2°C for 24 hours and those soaked in hot oil at 60°C for 4 hours were not statistically significantly different but both were significantly different from mean value obtained for control samples.

Data obtained for Energy at Maximum Load were not statistically significantly different for control samples and those for samples soaked in oil at ambient room temperature of 25 ± 2°C for 24 hours and samples soaked in hot oil at 60°C for 4 hours. However, the data obtained for both Load at Yield and Extension at Yield were statistically significantly different for all the treatments.

As earlier stated, studies have shown that unprotected wood and other lignocellulosics

Table 3. Fisher's Least Significant Difference of pair of means for the selected mechanical properties evaluated for treated and control split-bamboo samples

Treatment	MOR (Nmm^{-2})	MOE (Nmm^{-2})	Energy at Maximum Load (kJ)	Load at Maximum (Zero Slope, N)	Extenssion at Yield (Zero Slope, mm)
Control	117.70a	2,425.94a	0.12a	2,1211a	7.63a
Samples soaked in oil at ambient room temperature of 25 ± 2°C for 24 hours	139.79b	1,821.74b	0.13a	2,3265b	8.41b
Samples soaked in hot oil at 60°C for 4 hours	126.11c	1,886.59b	0.13a	2,2011c	9.02c

Means with the same superscript in the same column are not significantly different ($p < 0.05$)

with poor natural durability are easily degraded by micro-organisms such as fungi. These organisms are able to accomplish this through different means that mostly lead to cell wall degradation. Cell wall degradation by fungi can be achieved, for instance, when their colonising hyphae, most especially those of white-rot types, are able to develop in parenchyma, fibre and vascular cell lumina including growing through pits and within intercellular spaces in the parenchyma tissues thereby subjecting parenchyma and fibre cells to widespread degradation (e.g. Wahab *et al.*, 2005).

These micro-organisms are able to accomplish this after they have been able to decompose the lignin in order to gain access to the cellulose and hemicelluloses that are embedded within the lignin matrix (Hammel, 1997) thereby weakening the cell wall and thus, reducing most of the strength properties of such ligno-cellulosic material (Erakhrumen, 2012b). This is likely to be the pattern of degradation of lignocellulosic matrix by *P. sanguineus*, a white-rot fungus, identified to be a lignivorous (lignin-degrading) fungus in some wood species (Luna *et al.*, 2004).

However, there is the need for studies aimed at determining the pattern(s) of degradation of bamboo culms by this and other fungal species (Erakhrumen, 2012b) owing to the fact that little is still presently known about the decay patterns of bamboos by fungi (Cho *et al.*, 2006). Irrespective of this, the result obtained from tensile strength's MOR (Table 1) revealed that the oil-treated samples, i.e., those soaked in oil at ambient room temperature of 25 ± 2°C for 24 hours and in hot oil at 60°C for 4 hours had higher values than those for control. It is noteworthy that MOR and MOE are mostly determined from bending rather than from axial tests.

The MOR reflects the maximum load carrying capacity of a member and is proportional to maximum moment borne by the specimen. The mechanical property known as MOR is an accepted criterion of strength, although it is not a true stress because the formula by which it is computed is valid only to the elastic limit while elasticity implies that deformations produced by low stress are completely recoverable after loads are removed. When loaded to higher stress levels, plastic deformation or failure occurs. The three Moduli of Elasticity are the elastic moduli along the longitudinal, radial, and tangential axes of lignocellulosic materials, although, data for MOE along radial and tangential axes are not extensive in literature (Green *et al.*, 1999).

These mean values obtained in this study imply that in terms of MOR, the control samples had higher susceptibility to the cultured fungal species as compared to the oil-treated ones, meaning that the oil-treatment might have had negative influence on certain intrinsic/extrinsic conditions and/or processes that would have aided *P. sanguineus* in bio-degrading the oil-treated split-bamboo specimens to the extent of significant reduction in MOR as observed in control split-bamboo samples. This observation is supported by results obtained for some chemical properties of NSO in a study by Erakhrumen, (2011) which related these properties to this oil's anti-microbial properties recorded in literature (e.g. Locke, 1995; Puri, 1999). Similar trend regarding MOR was also obtained is another study by Erakhrumen, (2012b).

On the other hand, the values obtained for MOE was higher for control samples in comparison with the oil-treated samples. This trend which indicated a reduction in this property in treated split-bamboo samples might likely be as a result of chance or might also be as result of influence of the oil and perhaps its temperature on this mechanical property and not necessarily that the control samples resisted the fungal attack more than the oil-treated samples or that the cultured fungal species was able to reduce this mechanical property in the NSO-treated split-bamboo samples. Reductions in strength/mechanical properties for lignocellulosic materials at high treatment temperature have been variously documented in literature (Leithoff and Peek, 2001; Wahab *et al.*, 2006; Kumar, 2007; Manalo and Acda, 2009; Erakhrumen, 2009; Erakhrumen and Ogunsanwo, 2010; Erakhrumen, 2012a; 2012b).

In addition, mean values for Energy at Maximum Load which indicated the energy expended in bringing the split-bamboo samples to failure by the UTM during the test showed that the treated samples had slightly higher values than the untreated ones irrespective of the recorded negative influence of high temperature on strength properties of lignocellulose in literature as earlier highlighted. Mean values for Load at Yield and Extension at Yield also followed similar pattern as Energy at Maximum Load. The likely implication of the mean values for Load at Yield is that oil-treated split-bamboo samples were able to withstand comparatively slightly higher tensile load than those for control samples on the longitudinal plane during the test.

In like manner, Extension at Yield, which is the strain caused by the tensile load within the elastic limit for split-bamboo samples, was higher for oil-treated split-bamboo samples than for the control samples. This may therefore mean that the mean values for MOR are consistent with those obtained for Energy at Maximum Load, Load at Yield and Extension at Yield while those obtained for MOE did not appear so. Thus, the lower mean MOE values obtained for the oil-treated split-bamboo samples may not necessarily be a true reflection of the influence of NSO on the activities of the cultured fungal species as earlier stated.

The results of statistical analyses conducted at 5% probability level and tabulated in Table 2 showed that there was statistically significant variation in the data obtained for all the evaluated properties except those for Energy at Maximum Load. This was examined by subjecting the data obtained for all the evaluated properties for control samples, samples soaked in oil at ambient room temperature of 25 ± 2°C for 24 hours and samples soaked in hot oil at 60°C for 4 hours to ANOVA. Subsequently, the results of Fisher's LSD of pair of means for the evaluated properties tabulated in Table 3 showed that there was statistically significant difference in the values of MOR for control, samples soaked in oil at ambient room temperature of 25 ± 2°C for 24 hours and samples soaked in hot oil at 60°C for 4 hours.

The LSD also showed that there was no significant difference in MOE values for samples soaked in oil at ambient room temperature of 25 ± 2°C for 24 hours and samples soaked in hot oil at 60°C for 4 hours but there was a significant difference in mean MOE values between control and samples soaked in oil at ambient room temperature of 25 ± 2°C for 24 hours and samples soaked in hot oil at 60°C for 4 hours. Fisher's LSD also showed that statistical significant difference did not exist among the data obtained for Energy at Maximum Load evaluated for control, samples soaked in oil at ambient room temperature of 25 ± 2°C for 24 hours and samples soaked in hot oil at 60°C for 4 hours.

However, there was statistical significant difference in the data obtained for Load at Yield and Extension at Yield evaluated for control, samples soaked in oil at ambient room temperature of 25 ± 2°C for 24 hours and samples soaked in hot oil at 60°C for 4 hours. The likely implications of the results of statistical analyses were that the NSO-treatment had significant influence on the biodegradation ability of this fungal species including the mechanical properties obtained after the incubation period based on the F-values obtained for ANOVA. The LSD showed that based on MOR values obtained for oil-treated and untreated samples, fungal attack was not uniform, and at the same time, efficacy of the oil treatment was not similar for the oil-treated samples.

The trend of LSD values obtained for MOR in this study was similar to that for static bending MOR by Erakhrumen, (2012b). However, the MOE values obtained indicated that the efficacy of the oil treatment was similar for the oil-treated samples as also observed by Erakhrumen, (2012b) for static bending MOE for split-bamboo samples subjected to the same NSO-treatments and exposed to fungal attack. In addition, the LSD obtained for Energy at Maximum Load showed that oil-treatment did not significantly lead to variation in this property after exposure to fungal attack while oil treatment had significant influence on mean values for Load at Yield and Extension at Yield after exposure to fungal attack.

CONCLUSION

The results presented in this article as obtained from the described study showed that neem seed oil, that has been reported in literature to possess some anti-microbial properties, can be applied as a preservative for bamboo stems against fungal infestation and degradation. The use of this oil in this regard is very pertinent to the recent global intensified efforts aimed at developing environmentally benign organic preservatives for non-durable lignocelluloses. Apart from this, owing to the present levels of technological development and economic realities in many developing countries such as Nigeria, it is expected that the use of this oil will serve as a cost effective means of protecting bamboo and other non-durable lignocelluloses from infestation and biodegradation by agents of biological degradation.

Applying this oil for this purpose is also expected to encourage both potential and existing cottage and small-scale forest industries including other users that are interested in adding value to bamboo, particularly for income generation. However, need still exists for intensified efforts towards bringing to tolerable level the variability in the values of mechanical properties of interest perhaps as a result of this oil-treatment. In like manner, there is also the need for intensified efforts toward understanding how reduction in strength properties of interest can be prevented or also brought to a tolerable level at high oil-treatment temperature regimes. This implies that further studies aimed at balancing the use of this or other vegetable oil at high temperature for increase resistance to biodegradation and stability of strength properties of interest are necessary. Similar studies using other methods of preservative application for this oil are also necessary.

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